

UNIVERSITY OF BIRMINGHAM

EVALUATION OF PROPHYLACTIC CEFUROXIME DOSE IN OBESE PREGNANT WOMEN UNDERGOING CAESAREAN SECTION

By

HANADI HASSAN A. ALRAMMAAL

A thesis submitted to the University of Birmingham for the degree DOCTOR OF PHILOSOPHY

The Institute of Clinical Sciences
School of Pharmacy
College of Medical and Dental Sciences
University of Birmingham

March 2022

UNIVERSITY OF
BIRMINGHAM

University of Birmingham Research Archive

e-theses repository

This unpublished thesis/dissertation is copyright of the author and/or third parties. The intellectual property rights of the author or third parties in respect of this work are as defined by The Copyright Designs and Patents Act 1988 or as modified by any successor legislation.

Any use made of information contained in this thesis/dissertation must be in accordance with that legislation and must be properly acknowledged. Further distribution or reproduction in any format is prohibited without the permission of the copyright holder.

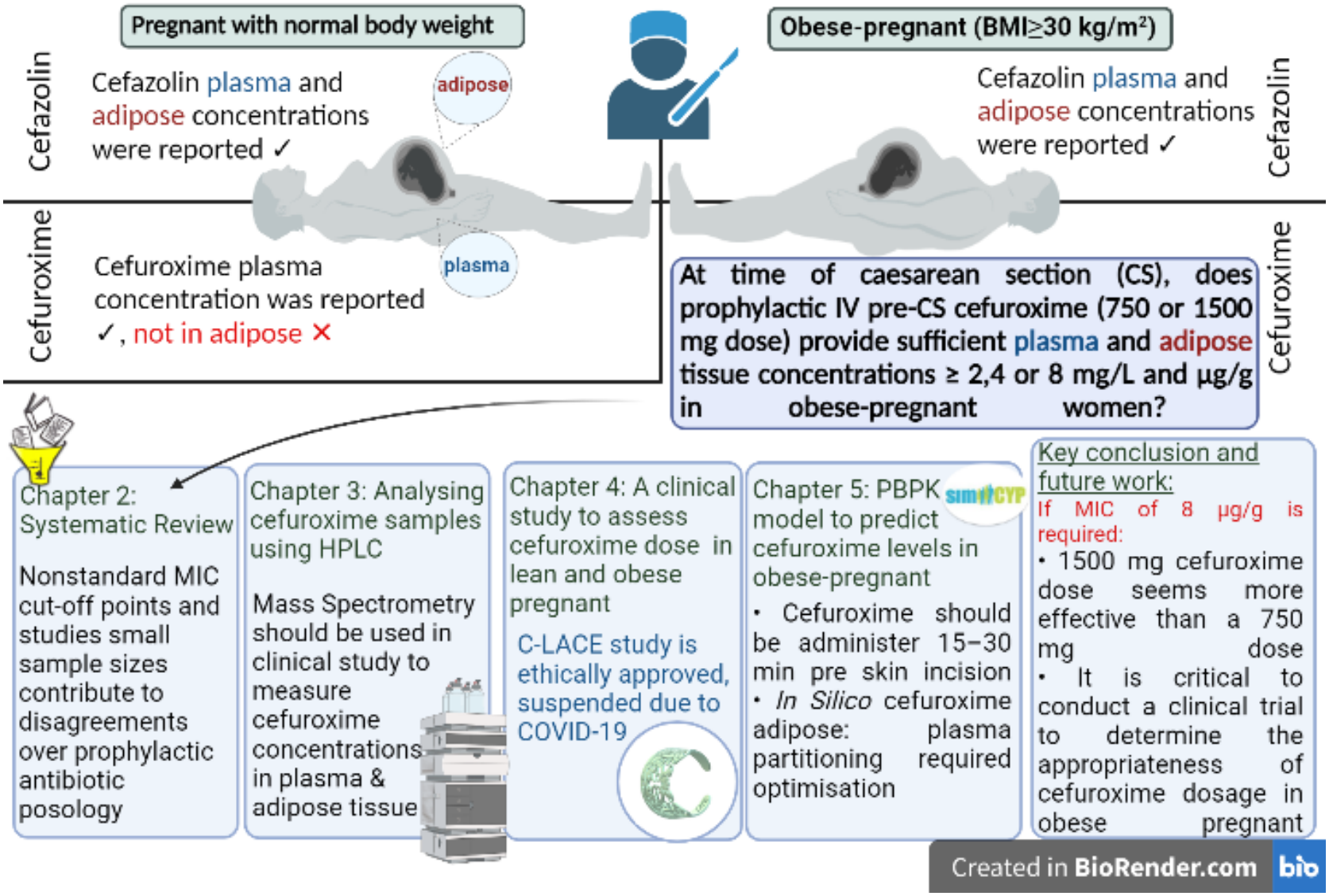
Abstract

Cefuroxime and cefazolin are used intravenously pre–caesarean section (CS) to prophylactically prevent or decrease post–surgical infections. Cefazolin pharmacokinetics (PK) has been evaluated in obese–pregnant women; while cefuroxime posology, appropriateness of doses, has not been assessed in this population. The aim of the current project was to investigate the appropriateness of cefuroxime dose given to obese–pregnant women at time of CS. Available cefazolin PK data in an obese-pregnant population was utilised to guide investigation of cefuroxime PK in an obese–pregnant population. This project comprised of four work packages: (1) Systematic review to identify information regarding cefazolin PK and rate of infection when cefazolin was given pre–CS in obese–pregnant women, and a systematic review to identify information on cefuroxime PK and rate of infection when cefuroxime is administrated to lean– or obese–pregnant at CS; (2) A research study was developed to clinically measure cefuroxime concentrations in plasma and adipose tissue in lean– and obese–pregnant (this study was approved by ethical authorities, and currently is suspended due to COVID-19 pandemic); (3) A partial validation of an analytical method to measure cefuroxime concentrations in plasma and adipose tissue samples using High Performance Liquid Chromatography; and (4) A physiologically based pharmacokinetic (PBPK) obese–pregnant model was utilised to predict cefazolin and cefuroxime disposition in such population.

At around 1–1.5 hours post cefuroxime 1500 mg dose, reported clinical cefuroxime plasma concentrations ranged within 8.7–57 mg/L in lean–pregnant women (4 articles, n=108 women); while a dose of 750 mg attained a mean plasma concentration of 14.9 mg/L at around 1 hour. There is no publicly available information of cefuroxime adipose

tissue concentration in a lean–pregnant population. To the best of our knowledge, there is no clinical information regarding cefuroxime plasma or adipose concentrations in obese–pregnant women. Information from both cefuroxime PK systematic review in lean–pregnant and PBPK modelling proposed the following: (1) A dose of 1500 mg cefuroxime appears therapeutically more appropriate than 750 mg dose for obese–pregnant women undergoing CS, if a minimum inhibitory concentration of 8 mg/L is required for up to 2 hours post dose; and (2) giving cefuroxime 15–30 minutes before skin incision is superior to current guidelines (15–60 minutes before skin incision). A clinical study is required to measure cefuroxime concentrations in plasma and site of incision to assess the appropriateness of cefuroxime dose in obese-pregnant population and to use these data to optimise the obese–pregnant PBPK model.

Graphical Abstract



Dedication

"In the name of Allah, the Entirely Merciful, the Especially Merciful"

Quran, Surah Al-Fatihah –1:1

Who is—exalted be His remembrance—generously accorded me with endless blessings.

To whom I, delightfully, followed his order "Recite, and your Lord is the Most Generous - Who taught by the pen - Taught man that which he knew not. No! [But] indeed, man transgresses Because he sees himself self-sufficient."

Quran, Surah Al-'Alaq– 96:3-7

There are no words better to describe my feeling to your greatness better than of this poem:

"I have a God —exalted be His remembrance— when I am in His vastness,

in my bowing, in my prostration, I ascend the top of my descent"
Prince Abdul Rahman bin Musa'id Al Saud

To Mother, **Hind Hendy**, the angelic soul in life… and beyond. Your prayers, support and affections were my favourite life save-guard. Indeed, you are the hidden solider and the backbone of our family.

To Father, **Hassan Alrammaal**, who I lost very early, yet I feel your presence in my soul, brain and imaginations.

For everything, you both taught me and everything I will teach, may Allah gratefully award both of you.

Brothers, **Waleed, Wael and Abdulrahman**, I would never lean or bend with your presence by my side.

To Sister, **Hadeel**, to the soft tweet and light breeze in my life. The blessing I had, that will forever keep my soul young.

To Husband, **Abdulrahman Yamani,** for the forever love that I cannot hide it or deny it, even if I want.

To son**, Bassam Yamani,** the day I have started this work, you were 7 months old, and now you are almost 5 years. You were the reason why I kept going when I felt like giving up. Just seeing you smiling after a long, hard day defrosts everything and gave me powers for next day.

To **friends**, the joy of life. We have lived it together, and for that, we shall forever bounded.

Thank you all, Hanadi Alrammaal 26/02/2022

Acknowledgments

Conducting this project while overseas was a pleasant experience; yet, it was a rough ride. I do acknowledge all who supported my progression in such journey.

Saudi Arabia and Umm al Qura University: The effort and financial support by my beloved country, sponsor and colleagues were the spine of this project.

To all supervisors, I have been pleased to meet and work with who are at the "top" of their field. The comfort and delight feelings of having you as supervisors while dealing with the current project challenges are much like a child, seeing a rainbow after a heavily rainy day. Each supervisor has his/her unique perspective, togetherness of you is perfect, as the rainbow.

Prof Hannah Batchelor: I can, delightfully, write pages and pages of what I have learned, and still learning, from you. I do appreciate the opportunity you gave me by accepting me as a student and the unlimited academic and emotional support you offered. I have learned a very important lesson; it is the simplest fact, yet, the hardest to apply: if you have responsibilities as a researcher, mother, daughter or a wife, at the end we are human, and we should take care of our delicate humanity too.

Dr Hsu Chong: Thank you for the knowledge you offered; but more, thank you for the friendship. Thank you for building the confidence in me, you always considered me much more than a junior researchers. Ethics can be written to guide research, you showed me the asset of it.

Dr Victoria Hodgetts Morton, Prof John Marriott and **Dr Katie Morris:** your braveness to "adopt" me was magical. Thank you for your absolute academic and moral support to continue this project. I know and hope that this is not the end of our collaborations; rather, a start.

Dr Khaled Abduljalil: You were the "missing piece" and the top cherry of the current project. You have taught me how to find the roots of problems, and start from there. Thank you for your patience.

Gabor Dravavolgyi and Dr Gopal Pawar: You have offered knowledge, advices, effort, and time to this project, when it was not compulsory to do so. Such a great contributions forever will be appreciated.

Certara UK Limited (Simcyp Division): "granted free access to the Simcyp Simulator through an academic licence (subject to conditions).". Thank you for the generous contribution.

University of Birmingham: I am very proud that my name will be, hopefully, accompanied by yours. Hope you would be proud too. Thank you for the facilitations, and the sweet–sour memories.

Navigation to thesis:

The structure of the current thesis is an 'alternative format' (found at: https://intranet.birmingham.ac.uk/as/studentservices/graduateschool/documents/public/rsa/alternative-format-thesis-guidelines.pdf). Manuscript/s (published or in publishable format) produced from this project can be used in the thesis. Due to manuscripts' words-restriction, a coherent introduction and general discussion were written. The reference style used is Vancouver. As per University of Birmingham guidelines, the page number of published manuscripts should not be included in thesis pagination sequence. Also it is permitted to have "some duplication since each publication-style chapter will have self-contained components that will overlap with parts of the other sections of the thesis."

List of journal articles published directly from this project:

Alrammaal HH, Batchelor HK, Morris RK, Chong HP. Efficacy of perioperative cefuroxime as a prophylactic antibiotic in women requiring caesarean section: A systematic review. Eur J Obstet Gynecol Reprod Biol. 2019 Nov;242:71-78. doi: 10.1016/j.ejogrb.2019.08.022. Epub 2019 Aug 31. PMID: 31569027. Which can be found at: https://pubmed.ncbi.nlm.nih.gov/31569027/.

Alrammaal HH, Batchelor HK, Chong HP, Hodgetts Morton V, Morris RK. Prophylactic perioperative cefuroxime levels in plasma and adipose tissue at the time of caesarean section (C-LACE): a protocol for a pilot experimental, prospective study with non-probability sampling to determine interpatient variability. Pilot Feasibility Stud. 2021;7(1):54. doi:10.1186/s40814-021-00794-3. Which can be found at: https://pilotfeasibilitystudies.biomedcentral.com/articles/10.1186/s40814-021-00794-3

Alrammaal HH, Abduljalil K, Hodgetts Morton V, Morris RK, Marriott JF, Chong HP, et al. Application of a Physiologically Based Pharmacokinetic Model to Predict Cefazolin and Cefuroxime Disposition in Obese Pregnant Women Undergoing Caesarean Section. Pharmaceutics [Internet]. 2022 May 30;14(6):1162. Which can be found at: http://dx.doi.org/10.3390/pharmaceutics14061162

Alrammaal involved in conceptualisation, methodology, formal analysis, validation, investigation, writing (original draft, review and editing) and visualization of published work.

Contents Listing

Table of Contents

Chapter 1: Introduction, Rationale and Aims 1

1.1 Background 2

1.1.1 Pharmacokinetics of drugs during pregnancy and labour in the scope of physiological and hormonal changes.....4

1.1.2 Obesity in pregnancy 9

1.1.3 Caesarean section complications in obese pregnant women 13

1.1.4 Post caesarean section infections in obese pregnant women.....15

1.1.5 Preventive measures to decrease post Caesarean section infection in obese pregnant women.....17

1.1.6 Physiologically based Pharmacokinetics 23

1.2 Hypothesis 57

1.3 Aims.....58

1.4 Objectives 60

Chapter 2: Systematic Review to Evaluate Pharmacokinetics of Cefuroxime and Cefazolin in Obese–Pregnant Women Undergoing Caesarean Section and Rate of Surgical Site Infection 62

2.1 Efficacy of perioperative cefuroxime as a prophylactic antibiotic in women requiring caesarean section: a systematic review.....71

2.1.1 Abstract.....72

2.1.2 Introduction 73

2.1.3 Methods.....75

2.1.4 Results.....77

2.1.5 Discussion.....87

2.1.6 Strengths of the study.....89

2.1.7 Study limitations 89

2.1.8 Conclusions.....89

2.1.9 Funding.....90

2.1.10 Contribution to Authorship.....90

2.1.11 Declaration of Competing Interest 90

2.1.12 Acknowledgment.....90

2.1.13 Appendix A. Supplementary data 90

2.2 Cefazolin pharmacokinetics and rate of infection post caesarean section: Systematic review and Meta-analysis 67

2.2.2 Methods.....67

2.2.3 Results....70
2.2.4 Discussion....84
2.2.5 Conclusion....93
2.3 Narrative review of eligible studies published since last update of systematic review 93
2.3.1 Cefuroxime pharmacokinetics and post caesarean section infection....93
2.3.2 Cefazolin pharmacokinetics and post caesarean section infection....94
Chapter 3: Partial Validation to Quantify Cefuroxime in Blood and Adipose Tissue using High Performance Liquid Chromatography....96
3.1 Introduction....97
3.2 Aims and objectives....99
3.2.1 Aims....99
3.2.2 Objectives....99
3.3 Methods....100
3.3.1 Optimising HPLC conditions....100
3.3.2 Linearity (Calibration curve)....102
3.3.3 Carry-over....102
3.3.4 Lower limit of detection and lower limit of quantification in solution....102
3.3.5 Matrix effect....103
3.3.6 Accuracy....103
3.3.7 Stability....105
3.3.8 Octanol/water partition coefficient (LogP) of cefuroxime: Shake-flask method 106
3.3.9 Selection of internal standard....106
3.4 Results....107
3.4.1 Optimising HPLC conditions....107
3.4.2 Linearity (Calibration curve)....108
3.4.3 Carry-over....108
3.4.4 Lower limit of detection and lower limit of quantification in solution....108
3.4.5 Matrix effect....109
3.4.6 Accuracy....110
3.4.7 Stability....111
3.4.8 Octanol/water partition coefficient (LogP) of cefuroxime: Shake-flask method 112
3.4.9 Selection of internal standard....113
3.5 Discussion....118
3.6 Limitations and optimisations....120

3.7 Conclusion 122

Chapter 4: Clinical Study to Assess the Pharmacokinetics of Cefuroxime and its Prophylactic Efficacy in Lean and Obese Pregnant Women after Caesarean Section (C-LACE)..... 123

4.1 Prophylactic Perioperative Cefuroxime Levels in Plasma and Adipose Tissue at the Time of Caesarean Section (C-LACE): A Protocol for a Pilot experimental, prospective study with non-probability sampling to determine interpatient variability..... 1

4.1.1 Abstract..... 2

4.1.2 Background {6a}..... 4

4.1.3 Methods/Design..... 6

4.1.4 Discussion..... 20

4.1.5 Trial Status..... 22

4.1.6 List of Abbreviations..... 22

4.1.7 Declaration..... 22

4.2 Pathway of C-LACE study and logistics at Birmingham Women's Hospital..... 126

4.2.1 Before requirement of participant into C-LACE study..... 126

4.2.2 Acronym and logo of C-LACE study..... 126

4.2.3 The anticipated preparations and results of C-LACE..... 126

4.3 Summary of anticipated data cleaning and management plan..... 132

4.4 COVID-19 pandemic disruption on conducting C-LACE study..... 132

4.4.1 Suspension of C-LACE study and current status..... 132

Chapter 5: Application of Physiologically-Based Pharmacokinetics Cefazolin and Cefuroxime Models in Healthy, Pregnancy and Obese Models and Development of a Combined Pregnancy and Obese Physiologically-Based Pharmacokinetics Model..... 134

5.1 Application of a Physiologically Based Pharmacokinetic Model to Predict Cefazolin and Cefuroxime Disposition in Obese Pregnant Women Undergoing Caesarean Section. 137

5.1.1 Abstract..... 138

5.1.2 Introduction..... 139

5.1.3 Materials and Methods..... 142

5.1.4 Results..... 151

5.1.5 Discussion..... 166

5.1.6 Conclusion..... 176

Chapter 6: Anticipated Results from C-LACE Study, General Discussion, Conclusion and Application..... 178

6.1 Anticipated cefuroxime concentrations results from C-LACE study..... 179

6.2 General discussion..... 183

6.3 Applications..... 194

6.4 Conclusion..... 196

6.5 Future work and recommendations 196
Chapter 7: Appendix.....200
7.1 Appendix of Chapter 2 “Systematic Review to Evaluate Pharmacokinetics of Cefuroxime and Cefazolin in Obese–Pregnant Women Undergoing Caesarean Section and Rate of Surgical Site Infection”.....201
7.1.1 Appendix of the published study “Efficacy of perioperative cefuroxime as a prophylactic antibiotic in women requiring caesarean section: A systematic review:...202
7.1.2 Appendix of Cefazolin pharmacokinetics systematic review and Meta-analysis and rate of infection post Caesarean section.....213
7.2 Appendix of Chapter 4 “Observational Study to Assess the Pharmacokinetics of Cefuroxime and its Prophylactic Efficacy in Obese Pregnant women after Caesarean Section (C-LACE)” 223
7.2.1 Appendix of the published Protocol “Prophylactic Perioperative Cefuroxime Levels in Plasma and Adipose Tissue at the Time of Caesarean Section (C-LACE): A Protocol for a Pilot experimental, prospective study with non-probability sampling to determine interpatient variability.” 223
7.2.2 Study Protocol and additional forms related to C-LACE study.....238
7.2.3 Additional forms related to C-LACE study.....251
7.2.4 Ethical approval and paperwork related to C-LACE study.....264
7.3 Appendix of Chapter 5 “Observational Study to Assess the Pharmacokinetics of Cefuroxime and its Prophylactic Efficacy in Obese Pregnant women after Caesarean Section (C-LACE)” 291
7.3.1 Application of Physiologically Based Pharmacokinetic Model to Predict Cefazolin and Cefuroxime Disposition in Obese Pregnant Women. 291
Chapter 8: List of References 306

List of Figures

Figure 1.1: General disposition of an intravenous drugs during pregnancy illustrated as compartmental physiologically based pharmacokinetic model.....5
Figure 1.2: Cross-sectional schematic of abdominal wall and the classifications of surgical site infection according to the Centers for Disease Control and Prevention 16
Figure 1.3: Building and applications of physiologically based pharmacokinetic (PBPK) modelling to predict the effects of numerous intrinsic and extrinsic elements on drug Pharmacokinetics and Pharmacodynamics 25
Figure 1.4: The Minimal physiologically based pharmacokinetic distribution model within simcyp 27
Figure 1.5: Full physiologically based pharmacokinetic distribution model within simcyp 29
Figure 1.6: Perfusion-limited distribution of drug to tissue 30
Figure 1.7: General schemes of Methods 1,2 and 3 used in Simcyp® to predict tissue : plasma partition coefficient 35
Figure 1.8: The permeability-limited kidney model (Mech Kim) incorporated into Simcyp® model.....41
Figure 1.9: The renal transporters available in the Mech Kim model within simcyp 42
Figure 1.10: The Sim–pregnancy physiological-based pharmacokinetics model (A), coupled with the multi-compartment placenta model (B) 44
Figure 1.11: Graphical gestational changes of foeto placental volume (A), plasma volume (B), red blood cells volume (C) and adipose volume (D) in Sim–Pregnant physiological-based pharmacokinetics model.....48
Figure 1.12: Graphical gestational changes of cardiac output (A), percentages of blood flows to foeto-placenta (B), kidney (C) and adipose (D) in Sim–Pregnant physiological-based pharmacokinetics model.....49
Figure 1.13: Graphical gestational changes of extracellular (A) and intracellular (B) water in Sim–Pregnant physiological-based pharmacokinetics model.....51
Figure 1.14: Graphical gestational changes of albumin (A) and alpha-1-acid glycoprotein (B) levels in Sim–Pregnant physiological-based pharmacokinetics model.....51
Figure 1.15: Graphical gestational changes of glomerular filtration rate (A) activates of hepatic CYP1A2 (B), CYP3A4 (C) and CYP2D6 (D) in Sim–Pregnant physiological-based pharmacokinetics model.....53
Figure 2.1: Flow diagram of full text (A) Cefuroxime Pharmacokinetics studies and (B) that were screened, assessed for eligibility 83
Figure 2.2: Reported Cefuroxime concentration in maternal plasma after an intravenous dose of 1500 mg or 750 mg in pregnant women underwent caesarean section. 85
Figure 2.3: Flow diagram of cefazolin (A) Pharmacokinetics and (B) surgical site infections studies that were screened, assessed for eligibility.....72
Figure 2.4: Illustration of sub grouping analysis of cefazolin pharmacokinetics systematic review.....74
Figure 2.5: Meta-analysis of cefazolin initial total adipose tissue concentrations in obese–pregnant women (BMI ≥30 kg/m^2).....78
Figure 2.6: Meta-analysis of cefazolin initial total adipose concentrations in obese–pregnant women with a BMI of 30–30.9 kg/m^2 vs. ≥40 kg/m^2 when given (A) 2 g or (B) 3 g dose of cefazolin.....79
Figure 2.7: Meta-analysis of studies reported 2 g vs. higher doses of cefazolin initial total adipose concentrations in obese–pregnant women 83
Figure 3.1: Cefuroxime chromatograms when using different mixtures of mobile phase 107

Figure 3.2: Calibration curve of cefuroxime solution using the mobile phase 0.05 M potassium dihydrogen phosphate buffer (adjusted to pH 2.5): acetonitrile (85:15) at a detection wavelength of 274 nm.....108
Figure 3.3: Adipose tissue effect on Cefuroxime chromatogram.....110
Figure 3.4: Effect of methanol on cefuroxime peak when used as liquid extraction from adipose tissue.....111
Figure 3.5: Stability of Cefuroxime in solution, frozen adipose tissue and after extraction (A), cefuroxime chromatogram in extraction stability studies (B), and extracted UV spectrum at a retention time of 19 and 25 minutes (C).....112
Figure 4.1: Logo of C-LACE study.....126
Figure 5.1: Summary of study optimization, evaluation and application of physiologically-based pharmacokinetics models in population of interest.....149
Figure 5.2: Simulated and observed cefazolin time concentrations curves in non–obese and obese subjects required surgical procedures.....153
Figure 5.3: Simulated and observed plasma time concentrations curves of cefazolin in different population.....156
Figure 5.4: Simulated and observed adipose tissue time concentrations curves of cefazolin in different population.....157
Figure 5.5: Simulated and observed cefuroxime time concentrations curves in overweight subjects.....159
Figure 5.6: Simulated and observed plasma time concentrations curves of cefuroxime in different population.....163
Figure 5.7: Simulated and observed adipose tissue time concentrations curves of cefuroxime in different population. 164
Figure 5.8: Simulated plasma, unbound adipose interstitial fluid and total adipose concentrations-time curves of cefuroxime (750 and 1500 mg doses) in obese–pregnant population. 165
Figure 6.1: Cefazolin and cefuroxime penetration to adipose extracellular and intracellular segments.....186
Figure 7.1: Poster presentation of cefuroxime systematic review at the Annual Academic Meeting held at the Royal College of Obstetricians and Gynaecologists.....201
Figure 7.2: part of CFZ-PK meta-analysis presented as a poster abstract at the Annual Academic Meeting of Royal College of Obstetricians and Gynaecologists.....202
Figure 7.3: Pregnancy (Maternal) PBPK model coupled with MechKiM model.....297

List of Tables

Table 1.1: Summary of Cytochrome P450 pregnancy-related changes 8
Table 1.2: Classification of body state according to Body mass Index.....10
Table 1.3: Summary of several complications associated with obesity in pregnancy.....12
Table 1.4: Summary of the complications associated with caesarean section in obese pregnant 14
Table 1.5: Reported pharmacokinetic parameters of cefazolin and cefuroxime in different populations of interest 21
Table 1.6: Summery of Methods (1,2 and 3) used to predict tissue partition coefficient within Simcyp® 36
Table 1.7: Comparison of Methods 1 vs. Method 2, and Method 2 vs. Method 3 performance in prediction K_p and V_{ss}.....38
Table 1.8: Facts and assumptions lead to the generation of current project hypothesis 58
Table 2.1: Summary of the data extracted from the eligible studies of Cefuroxime pharmacokinetics systematic review. 84
Table 2.2: Summary of extracted data from Cefuroxime post Caesarean section infection systematic review. 86
Table 2.3: Summary of the data extracted from the eligible studies of cefazolin pharmacokinetics systematic review. 73
Table 2.4: Summery of cefazolin pharmacokinetics systematic review empirical analysis of lean–pregnant women (BMI 20–29.9 kg/m^2) vs. obese–pregnant (BMI ≥30kg/m^2) when given diffrent doses of cefazolin (2, 3 or 4 g) 74
Table 2.5: Reported percentages of cefazolin samples under the minimum inhibitory concentration of 4 or 8 mg/L or µg/g in the eligible studies of CFZ-PK systematic review.....75
Table 2.6: Summary of extracted data from cefazolin post caesarean section infection systematic review. 82
Table 2.7: Dose efficiency in the highlight of reported means (or medians) and individual values of cefazolin adipose tissue concntrations 90
Table 3.1: Previously described method and sample preparation to quantify cefuroxime in human plasma and adipose tissue and changes made to the current method 99
Table 3.2: Chemical structure, LogP and chromatogram of proposed internal standard and cefuroxime in solution*.....114
Table 4.1: Most common bacteria causative of infection post Caesarean Section and the minimum inhibitory concentration range/Breakpoint.....8
Table 4.2: Summary of the C-LACE study sequence and methodology.....11
Table 4.3: Schedule of assessments and associated paperwork.....14
Table 5.1: Reported pharmacokinetic parameters of cefazolin and cefuroxime in different populations.....141
Table 5.2: Physiochemical properties and pharmacokinetics values of cefazolin and cefuroxime for healthy subjects used in compounds building using simcyp 20.....145
Table 5.3: Summary of study designs used for cefazolin Physiologically Based Pharmacokinetic model building.....146
Table 5.4: Summary of study designs used for cefuroxime Physiologically Based Pharmacokinetic model building.....147
Table 5.5: Observed vs simulated area under the curve, serum concentration and clearance of cefazolin.....154
Table 5.6: Observed and simulated adipose tissue concentrations of cefazolin and simulated time above MIC_{90}.....155

Table 5.7: Observed vs simulated area under the curve, serum concentration and clearance of cefuroxime 161
Table 5.8: Observed and simulated adipose tissue concentrations of cefuroxime and simulated time above MIC_{90}.....162
Table 5.9: Time above the minimum inhibitory concentration of plasma and free plasma (2, 4 and 8 µg/mL) cefuroxime concentrations of different cefuroxime doses given virtually to obese–pregnant and morbidly obese–pregnant.....170
Table 6.1: Percentages of cefuroxime penetration to adipose tissue compared to cefuroxime systematic plasma concentrations.....180
Table 6.2: Predicted cefuroxime adipose concentrations via direct extrapolation from reported plasma concentrations in lean–pregnant undergoing caesarean section (16% and 100%), and via PBPK modelling and predicted effect of body weight on cefuroxime adipose tissue concentrations.....182
Table 7.1: Cefuroxime pharmacokinetics and infection systematic reviews' inclusion and exclusion criteria.....204
Table 7.2: ClinPK Statement checklist scoring system for the studies assessed in the cefuroxime pharmacokinetics systematic review. Adopted from (269) 205
Table 7.3: ClinPK quality assessment result of cefuroxime pharmacokinetics systematic review 206
Table 7.4: Risk of bias assessment of cefuroxime post caesarean section systematic review by the Cochrane Effective Practice and Organisation of Care checklist.....206
Table 7.5: Quality assessment of studies included in cefuroxime post caesarean section infection systematic review by Joanna Briggs Institute Critical Appraisal Checklist for Randomized Controlled Trials 207
Table 7.6: Summary of the terms used for the cefuroxime pharmacokinetic and infection systematic review in MEDLINE database. 208
Table 7.7: the PRISMA Statement checklist for current study 211
Table 7.8: Cefazolin pharmacokinetics and infection systematic reviews' inclusion and exclusion criteria.....215
Table 7.9: Summary of the terms used for the cefazolin pharmacokinetic and surgical site infection systematic review in the MEDLINE database.....216
Table 7.10: ClinPK quality assessment result of cefazolin pharmacokinetics systematic review 219
Table 7.11: Risk of bias assessment of cefazolin post caesarean section infection systematic review by the Cochrane Effective Practice and Organisation of Care checklist 220
Table 7.12: Quality assessment of studies included in cefazolin post caesarean section infection systematic review by Joanna Briggs Institute Critical Appraisal Checklist for Randomized Controlled Trials 220
Table 7.13: the PRISMA Statement checklist for cefazolin systematic review and meta-analysis 221
Table 7.14: Tissue blood flow rates in female subjects in Simcyp simulator.....299
Table 7.15: Simulated time of cefazolin plasma and free plasma above minimum inhibitory concentration required to inhibit the growth of 90% of organisms 300
Table 7.16: Simulated time of cefuroxime plasma and free plasma above minimum inhibitory concentration required to inhibit the growth of 90% of organisms 301
Table 7.17: Simulated time of cefuroxime plasma, free plasma and adipose tissue concentrations above minimum inhibitory concentration required to inhibit the growth of 90% of organisms (2, 4 and 8 µg/mL or µg/g) post dose of 750 mg and 1500 mg, and dose efficacy when given cefuroxime 30 min or 60 min pre CS of 1 hour. 303

Table 7.18: Simulated percentages of Obese– and morbidly Obese–pregnant subjects achieving plasma, free plasma and interstitial fluid adipose tissue concentrations above minimum inhibitory concentration (2, 4 and 8 µg/mL or µg/g) at 1.5 hours and 2 hours post dose of 750 mg and1500 mg 305

List of Abbreviations

$[AP-]_t$	Concentration of acidic phospholipids in tissue
$[PR]_t$	Concentration of extracellular tissue protein (can be albumin or lipoprotein) in tissue
ABC	Adenosine triphosphate-binding cassette.
AT	Adipose tissue
AUC	Area under the curve
BMI	Body mass index
BSA	Body surface area
C_{EW}	Extracellular water concentrations
CFX	Cefuroxime
CFZ	Cefazolin
CG	Cockcroft-Gault
C_{IW}	Intracellular water concentrations
CL	Clearance
CL_{PD}	Passive diffusion clearance
CL_{PDF}	Passive diffusion in fetal-placental side
CL_{PDM}	Passive diffusion in maternal placental side
C_{max}	Maximum concentration
C_p	Plasma concentration
CS	Caesarean section
C_t	Concentration at time point
DAD	Diode-array detector
$D_{v\,o:w}$	Olive oil:buffer partition coefficient
EHC	Enterohepatic recirculation
EMA	European Medicine Agency
EPOC	Cochrane Effective Practice and Organisation of Care
EW	Extracellular water
F	Fractional tissue volume
f_{AP}	Volume fraction of Acidic phospholipids
FDA	Food and Drug Administration
f_{NL}	Volume fraction of neutral Lipids
f_{NP}	Volume fraction of neutral phospholipids
*f*T>MIC	Time a free fraction concentration above the minimum inhibitory concentration
f_u	Fraction unbound in plasma
f_{ut}	Fraction unbound in tissues
GA	Gestational age
GCP	Good Clinical Practice
GD	Gestational diabetes
GFR	Glomerular filtration rate
HCRW	Health and Care Research Wales
HPLC	High-performance liquid chromatography
HRA	Health Research Authority
HT	Hypertension

IRAS	Integrated Research Application System
IS	Internal standard
IV	Intravenous
IW	Intracellular water
J	Flux
JBI	Joanna Briggs Institute Critical Appraisal Tools
Ka_{AP}	Affinity constant of a drug for acidic phospholipids
K_{AP}	Association constant with Acidic phospholipid
Ka_{PR}	Affinity constant of a drug for extracellular tissue protein
K_D	Equilibrium dissociation constant
K_p	Tissue plasma partition coefficient
K_{pu}	Unbound tissue plasma partition coefficient
$K_{p\ uu,\ uu}$	Ratio of unbound unionized concentration in tissue to that in plasma
LLOD	Lower limit of detection
LLOQ	Lower limit of quantification
LogP	Partition coefficient
MA	Meta-analyses
MATE	Multi-drug and toxin extrusion protein
MDR (P-gp)	Multi-drug resistance glycoprotein
MDRD	Modification of diet in renal disease
Mech Kim	Mechanistic permeability-limited kidney model
MIC	Minimum inhibitory concentration
min	Minutes
MRP	Multi-drug resistant associated proteins
MS	Mass spectrometry
NHS	National Health Service
NL	Neutral Lipids
NP	Neutral phospholipids
OAT	Organic anion transporter
OCT	Organic cation transporter
PBS	Phosphate buffer saline
PD	Pharmacodynamics
pH_{EW}	pH of extracellular water
pH_{IW}	pH of intracellular water
PI	Principal investigator
PIS	Participant Information Sheet
PK	Pharmacokinetics
PO	Oral dose
$P_{o:w}$	Octanol:buffer partition coefficient
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
$P_{t:p}$	Tissue partition coefficients
PTC-PGK	Proximal tubule cells per gram of kidney
$P^{unbound,ionised}$	Passive permeability coefficient of unbound, ionised molecules
$P^{unbound,unionised}$	Passive permeability coefficient of unbound, unionised molecules
RAF	Relative activity factor
REF	Relative expression factor

RT	Retention time
Q_{kidney}	Arterial blood flow rate to kidney
Q_{urine}	Urinal flow
Q_{vein}	Vein blood flow rate
SD	Standard deviation
SLC	Solute carrier
SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials
SR	Systematic review
SSI	Surgical site infections
$t_{1/2}$	Elimination half-life
T>MIC	Time a concentration above the minimum inhibitory concentration
TID	Three times per day
t_{max}	Time to maximum concentration
V_d	volume of distribution
V_{nlp}	Fractional volume of neutral lipids in plasma
V_{nlt}	Fractional volume of neutral lipids in tissue
V_{pht}	Fractional volume of phospholipids in tissue
V_{ss}	Volume of distribution at steady state
V_{wt}	Fractional volume of water in tissue
WHO	World Health Organisation

.

Chapter 1: Introduction, Rationale and Aims

1.1 Background

Ethical considerations restrict clinical studies in pregnancy, which ultimately limits knowledge of drugs' pharmacokinetics (PK) and pharmacodynamics (PD) in pregnant women. A review revealed that studies evaluating the use of drugs in pregnancy are either focused on infectious diseases (one-third of studies), or on antepartum indications (one-third of studies), or on acute labour and delivery issues (1).

Consideration of adequate PK parameters is critical for prescribing drugs in pregnant women (posology), i.e.: dosage form, dose, dose frequency and route of administration (2). Clinical guidelines for pregnant women are established using basic PK principles, observational studies or predictions (2). Insufficient knowledge of PK and PD in pregnant women increases the risk of sub- or supra-therapeutic dosing, resulting in either inadequate control of the condition or a harmful effect of the drug to the mother and foetus (3).

Other conditions during pregnancy may affect PK and PD of drugs. Within obstetric practice, obesity is a frequently occurring risk factor; across United Kingdom (UK), around 16–21% of the antenatal population are obese (4, 5). Obesity during pregnancy can lead to the need for operational delivery, i.e. Caesarean section (CS) (5).

The rate of CS among an obese–pregnant population of Body Mass Index (BMI) ≥30 Kg/m^2 is significantly higher compared to normal-body weight (6). The rate of post-CS infection is higher among obese–pregnant women compared to those who are not obese; obesity is considered as major risk factor for infection in CS (7). There are various measures to decrease and/or prevent post-CS infections. A single intravenous (IV) dose of antibiotic is recommended pre-CS to decrease and/or prevent post-CS infections; several studies have investigated the efficacy of different prophylactic

antibiotics in CS (8). Cefazolin is used widely in the United States (US); while in the UK, cefuroxime is the most commonly used antibiotic in CS (9, 10). Several studies have investigated cefazolin plasma and adipose tissue (site of incision) concentrations and rate of post-CS infection in obese–pregnant women to gain an understanding of optimal dosing (10-16). However, to the best of our knowledge, no clinical study to date has evaluated the PK and PD of cefuroxime in obese–pregnant women who have had CS.

Limited information of drugs' PK and PD in obese–pregnant women, alongside the rising requirement for the systematic rationalisation of the use of drugs and/or their doses, call for an urgent attention to the need for predicting the clinical PK outcomes for these women. Data for PK and PD for such an ethically controlled population can be predicted using physiologically based pharmacokinetic (PBPK) principles. There are different simulator programmes that enable prediction of PK data for a specific population; Simcyp®, for example, is a sophisticated PBPK programme, capable of predicting pharmacokinetics in a range of populations.

The introduction chapter consist and discuss several domains. This chapter:

- Discusses the PK of drugs considering the physiological and hormonal changes during pregnancy;
- Reviews some PK parameters of certain drugs used in pregnancy;
- Discusses obesity and its complications in pregnancy, focusing on post-CS infection.
- Reviews PK of cefazolin and cefuroxime;
- Explains the use of PBPK approach and PBPK models within Simcyp®;

- Demonstrates hypothesis, aims and objectives of this project.

1.1.1 Pharmacokinetics of drugs during pregnancy and labour in the scope of physiological and hormonal changes

1.1.1.1 Elements influencing drug pharmacokinetics in pregnancy

Pharmacokinetics of drugs, i.e. absorption, distribution, metabolism and elimination, determines a drug's plasma/tissue concentration, which is the core indicator of a drug's desired effect or, occasionally, their side effects (2). Different factors are known to affect a drug's pharmacokinetics during pregnancy. These factors are commonly physiological or hormonal, causing changes at the beginning of the first trimester and developing in a linear fashion until the end of the third trimester (2, 3). A major change in the distribution of drugs is the addition of a foetal compartment as shown in Figure 1.1.

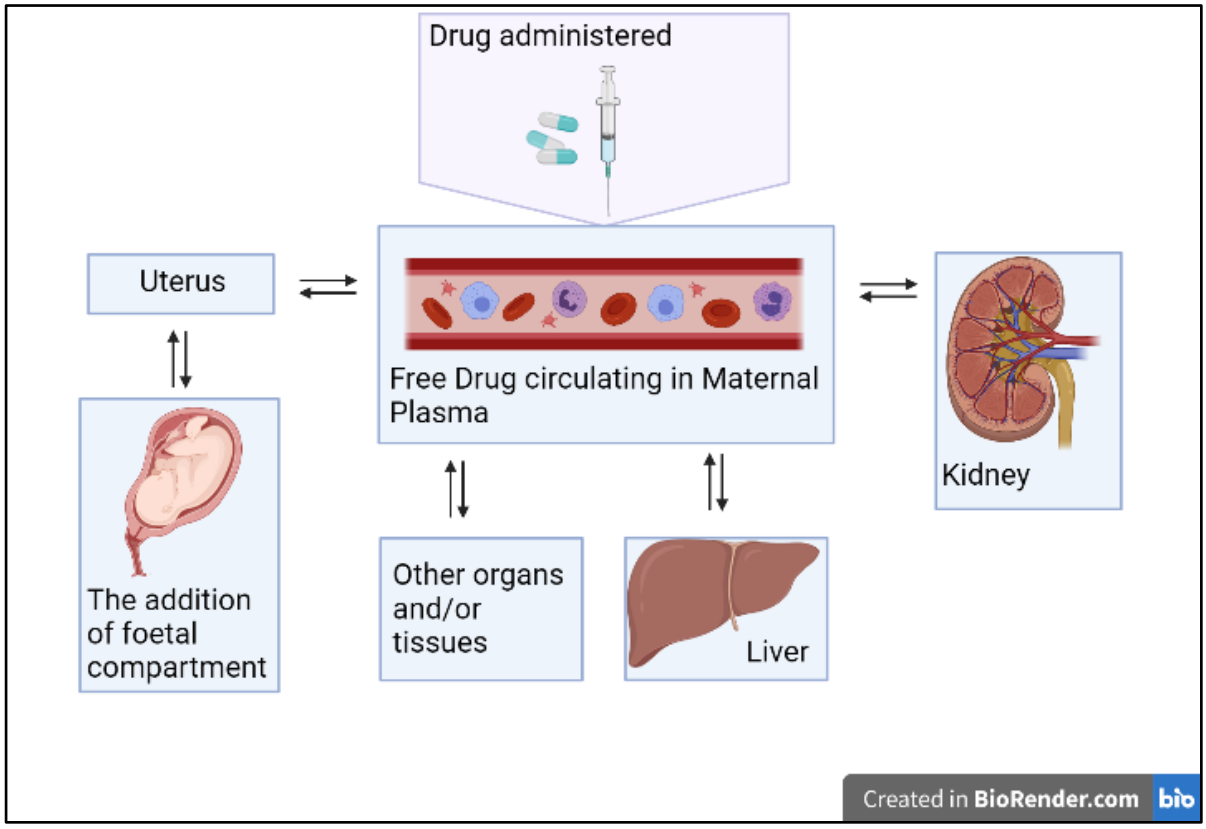


Figure 1.1: General disposition of an intravenous drugs during pregnancy illustrated as compartmental physiologically based pharmacokinetic model

1.1.1.2 Impact of pregnancy on absorption of orally administered drugs

Physiological changes in pregnancy slows down small intestine transit and gastric emptying by approximately 30–50%, this is largely mediated by increased physiological progesterone (2, 17). These changes may reduce the maximum concentration (C_{max}) of an orally administered drug and increase the time of maximum concentration (t_{max}), mainly in the third trimester (2, 3). In addition, due to increased mucus production and decreased secretion of hydrogen ion $(H)^+$, the gastric pH level will rise, resulting in an increase of weak acid ionization, this would affect the solubility and dissolution of weak acid in higher pH (2). The increased weak acid ionization is likely to cause a reduction in weak acids absorption compared to weak bases (2). The changes of C_{max} and t_{max} may be of lesser importance in repeated dosing of drugs.

However, their alteration becomes critical when they are taken as a single dose or in anticipation of a rapid onset of action, reducing the effectiveness of orally administered drugs (2). Many drugs are taken orally for their analgesic and antiemetic effects during pregnancy, especially when a rapid decrease in pain or nausea and vomiting is desired (2, 3). However, this, may affect a drug's absorption and concentration, leading to the recommendation of administering single-dosed drugs in the evening when the likelihood of vomiting is low (3).

1.1.1.3 Distribution

The total body weight increases during pregnancy. This is mainly due to a corresponding expansion in the intravascular and extravascular volume of water, the water contents in body increases up to 8 Litres (2). For hydrophilic (unbound) drugs, this produces a larger volume of distribution, while a rising body fat may increase the volume of distribution (V_d) of lipophilic drugs (18). The change in V_d mainly affects the drug's C_{max} and elimination half-life ($t_{1/2}$), Equation (1.1) and (1.2). Because an increase in the V_d of a drug may decrease its desired concentration and elongate the $t_{1/2}$, a higher dose may initially be required to achieve therapeutic drug plasma concentration (18, 19).

$$C_p^0 = \frac{D}{V_d} \quad \textbf{(1.1)}$$

$$t_{1/2} = \frac{0.693 \times V_d}{CL} \quad \textbf{(1.2)}$$

Where C_p^0 is Plasma concentration at zero time (mg/L), D is Dose (mg), V_d is Volume of distribution (L), $t_{1/2}$ is half-life (h) and CL is Clearance (L/h).

Foetal compartments cause blood perfusion to the uterus to increase, resulting in a corresponding rise in the apparent V_d. At term, the blood flow to uterus increases from 50 to 500 ml/min, suggesting a 10-fold increase (19). Certain drugs that are not bound to plasma proteins, are non-ionised and have a small particle size (< 500 Daltons) may cross the placenta to the foetal compartment (18).

Another gestation-specific alteration that affects V_d during pregnancy is the decrease in plasma concentration of proteins and/or binding affinity, which ultimately affects the drug-protein binding and free drug concentration in plasma (18-20). This will have a clinical effect, particularly in the case of drugs with high protein binding (18-20). A reduction in albumin concentration from 4.2 g/dL in non–pregnant women to 3.6 g/dL in the second trimester during pregnancy may indirectly attenuate the clinical effect of many drugs by impacting on their V_d (20). A recent systematic analysis of pharmacokinetics–induced changes in pregnant women, compared to non–pregnant women, revealed a significantly consistent increase in V_d in 19 drugs in 67 different studies (21). This illustrates the great impact of gestation-related alteration of a drug's V_d and its potential for an altered clinical effect.

1.1.1.4 Metabolism

Drugs are metabolised through phase I and phase II reactions, where oxidative metabolic enzymes have a role in drug metabolism, commonly known as cytochrome P450 (CYP 450). Table 1.1 summarises the pregnancy-induced changes of the most fundamental metabolising enzymes in the liver. As shown, the enzymes' activities can increase or decrease in pregnancy, which may ultimately increase or decrease the drug plasma/tissue concentration, affecting its efficacy and safety (19).

Table 1.1: Summary of Cytochrome P450 pregnancy-related changes

Enzyme	Pregnancy related change	Examples of Potential CYP substrate used in pregnancy	References
CYP3A4	Increased	Citalopram, Darunavir	(22)
CYP2D6	Increased	Alprenolol, codeine	(23)
CYP2C9	Increased	Phenytoin	(24)
CYP2C19	Decreased	Proguanil	(25)
CYP1A2	Decreased	Paracetamol, propranolol	(26)
UGT1A4	Increased	Lamotrigine	(20)
UGT1A1/9	Increased	Acetaminophen	(27)
NAT2	Decreased	Caffeine	(28)

Adopted from (19), with permission from Elsevier

1.1.1.5 Elimination

During pregnancy, the effective renal plasma flow increases by 50–85%, resulting in an increase of 40–65% of glomerular filtration rate (29). This has a great impact on medicines cleared by the kidneys. Increased elimination rate of certain antibiotics (ampicillin, cefuroxime and cefazolin) and cardiovascular drugs (atenolol and digoxin) have been reported (30, 31). This can be explained by an increase in the activity of some renal transporters, resulting in increasing renal secretions (18, 32). Nevertheless, the overall results is not certainly reduction in observed plasma concentration; as other gestational changes may counteract clearance changes (30).

1.1.1.6 Foeto-placental compartment

Pregnancy is unique as it has the foeto-placental compartment, where drugs may distribute. It comprises the placenta, foetal and amniotic fluid. Discussions pertinent to this area are extensive, and range from drug metabolism by placenta or foetal tissue to drug accumulation within this compartment. In general, the compartment's role in

transporting drugs is not passive, as previously discussed (1.1.1.3 Distribution). However, It is generally acknowledged that all drugs consumed by the mother during pregnancy will be exposed to the foetal compartment (18).

A recent systematic review has identified a gap in knowledge about PK in pregnancy and its corresponding clinical effects on the mother and foetus (21). There is uncertainty about whether alterations in the PK of drugs affect drug efficacy or lead to a side effect. It is far from accurate clinical practice to predict or extrapolate an outcome from a certain population to the pregnant population, without considering the impact of physiological changes. Certain conditions in pregnancy may affect the physiology of pregnant women, lessening the possibility of predictions or extrapolations. Certain serious medical conditions can have a great impact on the status of pregnancy and that of PK and PD of drugs. Certain common conditions, such as obesity, have received comparatively limited attention from medical experts, leading to an underestimate of their impact on drugs' PK and PD. The next section focuses on obesity and its complications during pregnancy.

1.1.2 Obesity in pregnancy

Obesity in pregnancy is defined as having a BMI at the first antenatal appointment of 30 kg/m^2 or more (33). Table 1.2 presents classes of normal and abnormal body state, as measured by BMI according to the World Health Organisation (WHO) (34). In UK, one in 5 pregnant women are obese (BMI: 30–34.99 Kg/m^2) and one in every 15 cases of pregnancy is classified as class II (BMI: 35.00–39.99 Kg/m^2) or class III (BMI: ≥40 Kg/m^2) obesity (35, 36). An epidemiological study that evaluated the risk factors for

maternal death between 2003 and 2005 reported obesity as a risk factor, as 28% of pregnant women who died in this study were obese (36, 37).

Table 1.2: Classification of body state according to Body mass Index

Class	Body Mass Index (Kg/m²)
Underweight	< 18.50
Normal range	18.50 – 24.99
Overweight	≥25.00
Pre–obese	25.00 – 29.99
Obese class I	30.00 – 34.99
Obese class II	35.00 – 39.99
Obese class III	≥40.00

1.1.2.1 Physiological alteration in obese pregnant and non–pregnant subjects and its impact on pharmacokinetics and pharmacodynamics of drugs

Obesity in adults results in numerous physiological changes, which can affect drug disposition. It was thought that obesity did not affect the absorption of drugs. However, recent studies suggest that the contrary is the case (38). The most influential impact of obesity on drug disposition involves a change in V_d (38). This is a result of an increase in both body fat and lean body weight; where the body subsequently increases blood volume, cardiac output and capillary flow to supply the excess body mass with nutrients and oxygen (38-40). Despite this hemodynamic expansion, reduced tissue perfusion in the obese has been reported, which may affect drugs' V_d (38, 41, 42). Obese and lean subjects reported to have comparable serum albumin and total protein concentrations (43). However, an increased level of alpha-1-acid glycoprotein have been reported in obese populations, suggestion possible reduction in free fraction of basic drugs (43). Variations of V_d in the obese are considerable, with

their direction and magnitude difficult to predict. The most common practice to predict change of V_d is by extrapolating it from the total body weight (38). The change in clearance in the obese seems of lesser significance compared to V_d (38). Results from studies investigating the effect of obesity on CYP enzymes expression and function were inconclusive (44). Alterations of CYP2E1 and CYP3A activities have been reported to be increased and decreased, respectively (44).

The exact physiological alteration in the obese–pregnant women has not been fully studied. A report on zidovudine concentration, a water-soluble drug, showed that two obese pregnant patients had a lower maternal zidovudine plasma level than the average population (45).

1.1.2.2 Risk factors associated with obesity in pregnancy

Being obese in pregnancy is a risk factor for antenatal, intrapartum and postpartum complications. Table 1.3 lists some of these complications for both the mother and foetus (46). Obese pregnant women have an increased risk of CS, compared to non-obese pregnant women. The next sections will discuss CS prevalence, complications and measures taken to reduce post-CS infections.

Table 1.3: Summary of several complications associated with obesity in pregnancy

Condition	BMI 18 – 24.9		BMI ≥ 40		Adjusted OR (*p* value for trends)	95% CI	Reference
	Prevalence (%)	Prevalence (n/N) or (n)	Prevalence (%)	Prevalence (n/N) or (n)			
Miscarriage (≤20 weeks gestation)	7.8	257/3302	14.3	51/359	2.49	1.45–4.26	(47)
Intrauterine foetal death (>22 weeks gestation)	0.253	589/233160	0.559[a]	82/14669[1]	1.86	1.39–2.47	(48)
Gestational diabetes	0.937	2185/233160	9.292[a]	1363/14669[a]	11.01	0.25–11.82	(48)
Gestational hypertension disease	5.042	3351/66463	17.262	536/3105	3.55[b] (<0.001)	3.26–3.86	(49)
Preeclampsia	3.095	7217/233160	10.641[a]	1561/14669[a]	4.44	4.17–4.72	(48)
Eclampsia	0.037	131/353212	0.070	22/31 392	2.3	1.4–3.7	(50)
Birth weight >4500 g	2.4	5697/233160	6.4[a]	940/14669[a]	2.74	2.55–2.95	(48)
Preterm birth (22–27 weeks gestation)	0.17	1703/1014513	0.52	56/10855	2.91	2.21–3.81	(51)
Severe congenital malformations	3.40	25713	4.70	529	1.37	1.26–1.49	(52)
Malformation of the nervous system	0.09	695	0.18	20	1.88	1.20–2.94	(52)
Congenital heart defect	1.56	11807	2.26	257	1.44	1.27–1.63	(52)
Neonate sepsis	2.06	1367/66463	3.83	119/3105	1.55[b] (<0.001)	1.28–1.87	(49)
Neonate ICU	8.85	5880/66463	14.46	449/3105	1.38[b] (<0.001)	1.26–1.51	(49)
Caesarean section	**22.4**	**14872/66463**	**46.9**	**1457/3105**	**2.01[b]** (<0.001)	**1.93–2.10**	(49)

Table adapted from (46), with permission from Deutscher Aerzte-Verlag Gmbh.
BMI body mass index, CI confidence interval, ICU, intensive care unit, n number of cases per subgroup; N size of the subgroup ; OR, odds ratio. [a] for BMI ≥ 35 kg/m^2, [b] Adjusted risk ratio

1.1.3 Caesarean section complications in obese pregnant women

The rate of CS deliveries in the UK is 23.8% for singleton pregnancies (53). The exact rate of CS among obese–pregnant population in UK is not available, the rate is expected higher than of that in lean–pregnant. In 2001, a study of pregnancy within London hospitals indicated increased CS delivers (elective or emergency) in overweight– and obese-pregnant compared to lean cohort (54). A report by the NHS East Midlands Maternity Network stated that obese–pregnant are more likely to be delivered by CS than pregnant women with normal BMI (55). In US, the rate of CS in obese–pregnant women increases to 33.8%. In class II or III obesity cases, the CS rate can be as high as 47.4% compared to 20.7% in normal-body weight pregnant (6).

As is the case with all surgeries, the CS encompasses complications, including risk of bleeding, risk of blood clots, pain, side effects from anaesthesia, and risk of infections. Table 1.4 displays prevalence of different types of CS in non–obese and obese populations and associated prevalence of complications for each group (46). Although CS is accompanied by abdominal infections and endometritis, low grade evidence suggested CS as a favoured mode of delivery in morbidly obese pregnancies driven by the anaesthetic risks of an emergency CS (35).

To complicate matters further, epidural anaesthesia tends to have an effect on the PK of drugs, which alters the hemodynamic of the body, causing decreased systematic vascular resistance and pooling blood to tissue (56, 57). In practice, the use of IV isotonic fluid is recommended to avoid hypotension (57), but it also leads to further alterations in the drug PK by way of an increase in maternal V_d (56).

Table 1.4: Summary of the complications associated with caesarean section in obese pregnant

Condition	BMI 18 – 24.9		BMI ≥ 40		Adjusted OR (*p* value for trends)	95% CI	References
	Prevalence (%)	Prevalence (n/N)	Prevalence (%)	Prevalence (n/N)			
Caesarean section (total)	22.4	14 872/66463	46.9	1457/3105	2.01[b] (<0.001)	1.93–2.10	(49)
Primary Caesarean section	8.0	5323/66 463	19.3	600/3105	2.02[b] (<0.001)	1.88–2.18	(49)
Emergency Caesarean section	9.9	22974/233 160	17.2	2529/14669[a]	2.11	2.01–2.21	(48)
Premature placental abruption	1.4	937/66 463	1.6	51/3105	0.84[b] (0.04)	0.63–1.11	(49)
Puerperal fever	1.7	1132/66	2.7	83/3105	1.37[b]	1.10–1.71	(49)
Severe puerperal infection	0.4	260/66463	0.7	23/3105	1.75[b] (<0.001)	1.15–2.68	(49)
Postoperative wound healing complications	0.3	209/66463	0.6	19/3105	2.17[b] (<0.001)	1.34–3.51	(49)
Complications of anaesthesia and obstetric interventions	0.149	528/353212	0.245	77/31392	1.5	1.2–2.0	(50)
Severe postpartum haemorrhage with transfusion	0.422	1489/353212	0.312	98/31392	0.7	0.6–0.9	(50)
Sepsis	0.323	1140/353212	0.401	126/31392	1.4	1.2–1.7	(50)
Obstetric shock	0.016	56/353212	0.022	7/31392	1.5	0.7–3.3	(50)
ICU admission	0.073	259/353212	0.188	59/31392	2.4	1.8–3.3	(50)
Combined severe maternal morbidity or mortality	1.4	5057/353212	2.0	637/31392	1.4	1.3–1.5	(50)

Table adopted from (46), with permission from DEUTSCHER AERZTE-VERLAG GMBH.

BMI body mass index, CI confidence interval, ICU, intensive care unit, n number of cases per subgroup; N size of the subgroup; OR, odds ratio. [a] for BMI ≥ 35 kg/m^2, [b] Adjusted risk ratio

1.1.4 Post caesarean section infections in obese pregnant women

The rate of post-CS infection is almost 10% in the UK's general population, of which 0.6% will require hospital re-admission (7). A study evaluating the risk factors for post-CS infection showed that obesity was a major risk factors for post-CS infection (7). For every five unit increment of pregnant BMI, the incidence of post-CS wound infection was reported to double (58, 59). There are some possible reasons for the reported high rates of post-CS infections in obese pregnant women. Pregnancy alters the maternal immune system via different mechanisms, which are not fully understood, to allow development of the foetus (35). To further complicate the situation, it is evident that immunosuppression originates in adipose tissue (60). Additionally, a higher degree of hypoperfusion is associated with increased depth of subcutaneous adipose tissue (61). This may result in a low level of tissue oxygenation, a low level of neutrophils delivery to the tissue, and thus reduced capability to prevent infection within adipose tissue (10, 61). Compared to their non–obese counterparts, the obese population is threatened by a strong possibility of post-CS infections, arising from an increased volume of the distribution of antibiotics (leading to reduced levels in plasma and at the site of incision), prolonged operations, and compromised metabolic function (10, 13, 14).

There are different types of post-CS infections that include fever, wound, surgical site infections (SSI), endometritis (the inflammation of the lining of the uterus), and urinary tract infections (7, 8, 12). The most occurring type of SSI is Superficial incisional infection (Figure 1.2) (62). The most common pathogens causing post-CS infections are those that exist in skin and in the genital tract, and most notably when the

membrane is ruptured, allowing the pathogens from intrauterine cavity to invade, especially in premature labour cases (63). Post-CS infections are commonly polymicrobial (8). Isolated pathogens from endometrium and wound infections include *Ureaplasma urealyticum*, *Staphylococcus epidermidis,* group B streptococcus and other streptococcus species, *Staphylococcus aureus* and *coagulase negative staphylococci*, *Enterococcus faecalis*, *Escherichia coli* (*E. Coli*) and other aerobic gram-negative rods, anaerobes and genital mycoplasmas (63-68). Surgical sites can be contaminated by normal flora in the skin at the time of the CS surgery, and infected by *Staphylococcus aureus* and *coagulase negative staphylococci* (69, 70).

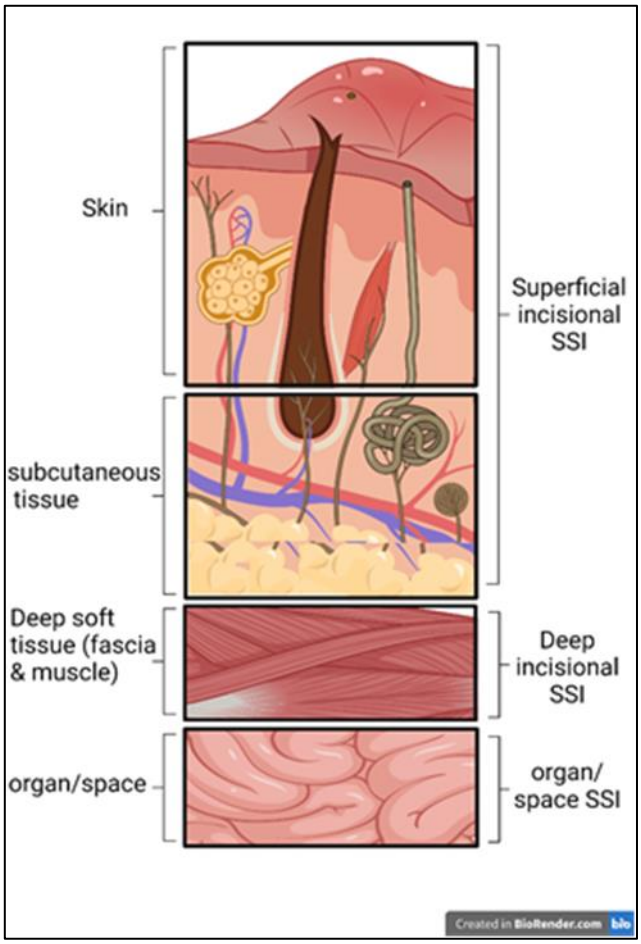


Figure 1.2: Cross-sectional schematic of abdominal wall and the classifications of surgical site infection according to the Centers for Disease Control and Prevention
Figure adopted from (71), with permission from Elsevier

1.1.5 Preventive measures to decrease post Caesarean section infection in obese pregnant women

Various measures are effective to decrease post-CS SSI include: skin preparation by chlorohexidine, hair removal by clippers, cleansing the vaginal area by povidone-iodine and, closing the subcutaneous tissue by suture (in cases where the wound thickness is >2cm) and using prophylactic antibiotics (66).

1.1.5.1 Use of prophylactic antibiotics

A Cochrane systematic review of the effect of prophylactic antibiotic showed a 60–70% decrease in the rate of post-CS wound infections, endometritis and other serious infections (8). Although there were conflicts suggestions regarding optimal antibiotic administration in CS, it is recommended to administer a single dose of IV antibiotic 15–60 min prior to the skin incision (72-74). The most common prophylactic antibiotics are cephalosporins; cefazolin (in the US), and cefuroxime (in the UK).

1.1.5.2 Potency of cefazolin and cefuroxime antibiotics

Both cefazolin and cefuroxime are bactericidal antibiotics, which inhibit bacterial cell wall synthesis (75, 76). Due to their broad-spectrum activities and minimal side effects, they are used to treat a proven (selected gram-positive and gram-negative microorganisms) or empirically suspected selection of microorganisms (76). Cefazolin and cefuroxime display time-dependent killing properties: bacterial killing is improved when the bacteria are exposed to concentrations above the minimum inhibitory concentration (MIC) for longer durations (77, 78).

The MIC (specifically MIC_{90}, which refers to the lowest concentration of cefazolin and cefuroxime at which 90% of bacteria isolates are inhibited) required to cover common

pathogens in CS surgeries has been widely discussed. A two-fold increase in the MIC of cefazolin, from 4 to 8 mg/L, to cover gram-negative organisms has been recommended after considering the emerging data on bacterial resistance in CS, whereas the recommended MIC for gram-positive bacteria is 1 mg/L (10, 79, 80). Recent guidelines have suggested an MIC breakpoint ≤4 mg/L (81). Several studies have explored the appropriateness of cefazolin concentration in plasma and/or tissue at both 4 and 8 mg/L or µg/g (13, 14). The effective MIC of cefuroxime against post-CS infection-causing common bacteria has been discussed elsewhere (Chapter 4: 4.1.3.4 Part A: Cefuroxime levels in plasma and adipose tissue, Table 4.1) (82). Although the suggested breakpoint of cefuroxime MIC of most common causative bacteria of post-CS infections is less than 1 mg/L, resistant strains may require a higher concentration. For example, 20% of *E. Coli* and 50% of *Klebsiella* require an MIC of 4–16mg/L and >3.1 mg/L, respectively (83-86). In this research, MIC of 2, 4 and 8 mg/L (plasma) or µg/g (adipose tissue) of cefazolin or cefuroxime were chosen to evaluate the appropriate antibacterial coverage for the intended population. As higher rates of SSI were associated with lower antibiotic plasma and adipose tissue concentrations (87). In a surgical setting, prophylactic antibiotic concentrations (total plasma, free plasma or targeted tissue concentrations) are anticipated to reach and be sustained >MIC for the duration of procedure. This is expressed by time of plasma concentration above MIC (T>MIC) and time of free plasma concentration above MIC (*f*T>MIC) (88, 89).

1.1.5.3 Intravenous cefazolin pharmacokinetics in different populations

Cefazolin has a range of reported lipophilicity values; the reported (measured or predicted) cefazolin logP values were −0.58, −0.4, 0.471 and 0.3 (16, 90-93). Notably,

cefazolin is highly bound to plasma proteins; moreover, the cefazolin–protein binding saturation was proven, following which the concentration-dependent fraction unbound (f_u) of cefazolin was reported (cefazolin f_u reported in healthy subjects were 0.11, 0.19 and 0.225) (94, 95).

Cefazolin is excreted (100%) unchanged through urine via the kidneys (96). In addition to the glomerular filtration of cefazolin by the kidneys, various transporters are involved in the tubular secretion of cefazolin (96-99). Cefazolin PK is affected by intra- and inter-population variability. The PK of cefazolin in non–pregnant healthy adults with a normal BMI in comparison to pregnant women with a normal BM, obese non–pregnant and pregnant with a higher BMI (BMI ≥30 Kg/m^2) is discussed below. Table 1.5 summaries the PK of cefazolin and cefuroxime in different populations of interest.

Philipson et al. examined cefazolin PK in pregnant women at 24 weeks of gestation and postpartum when the menstrual cycle returned and breastfeeding ceased (100). Postpartum women were considered to be healthy subjects with normal BMI in the population comparison. With regard to healthy non–pregnant adults with normal BMI vs. pregnant adults, f_u of cefazolin increased during pregnancy to 0.25 (101). This increase is due to the decreased plasma proteins in the pregnant population. Cefazolin V_d and CL were higher in the second trimester of pregnancy than in the same postpartum subjects. The overall changes in PK resulted in decreased plasma area under the curve (AUC) in pregnant women.

In pregnant women at the time of delivery by CS, the V_d reported was less than that reported by Philipson et al. in pregnant women in the second trimester (100, 102). It can be assumed that the apparent cefazolin V_d would increase due to the increased f_u

of cefazolin during the progression of pregnancy and increased total body weight. However, the observed decrease in cefazolin V_d can be related to alterations of hemodynamic in CS caused by anaesthesia and blood loss, which affects drugs' PK (103); it is less likely that the decrease was due to the analytical methods used in each study. Cefazolin CL values were nearly similar in both studies. In overweight patients with limb infections, the reported cefazolin V_d and CL were nearly similar to non–pregnant subjects with normal BMI (104).

The f_u of cefazolin was higher in the obese–pregnant populations (BMI ≥30 Kg/m^2) at the time of CS compared to the non–pregnant adults (12). Cefazolin V_d and CL were higher in obese–pregnant subjects (BMI range 37.3–46.2 Kg/m^2) at the time of delivery compared to non–pregnant adults (16). The effects of obesity during pregnancy compared to normal body weight during pregnancy remained inconclusive in these studies due to the different dosages used in each study (i.e. effects of cefazolin dose-dependent f_u on cefazolin apparent V_d). The reported cefazolin V_d values in different population indicate extracellular distribution of cefazolin, without intracellular distribution (105).

In CS, cefazolin is administered intravenously in varying doses at the preoperative stage. Some guidelines recommend a dose of 2 g for subjects weighing <120 kg, and 3 g for those weighing ≥120 kg; both doses of 3 g and 4 g have been evaluated in obese pregnant women (10, 13, 15, 106).

Table 1.5: Reported pharmacokinetic parameters of cefazolin and cefuroxime in different populations of interest

Population	f_u	V_d (L)	C_{max}-normalised dose (mg/L/mg)	V_d[a] (L/Kg)	V_{ss}[b](L/Kg)	CL (L/h)
	Cefazolin					
Non–pregnant	0.225 (94, 107)	11.6 (100)	0.15 (100)	0.186(100)	0.149	4.66 (100)
Pregnant (24 GW)	0.25 (101)	11.9 (100)	0.12 (100)	0.172(100)	0.137	7.30 (100)
Pregnant, at time of delivery (39.1 GW)		9.44 (102)	0.08 (102)	0.11(102)	0.09	7.18 (102)
Overweight	0.15 (104)		N/A	0.17(104)	0.136	3.72 (104)
Obese[c] and pregnant at time of CS	0.29 (12)	13.36 (16) 13.45 (16)	0.073 (16) 0.074 (16)		0.174 (16) 0.17 (16)	8.4 (16) 6.6 (16)
	Cefuroxime					
Non–pregnant	0.67 (108)	16.3 (109)	0.068 (109)	0.264	0.211	11.9 (109)
Pregnant (29 GW)	N/R	17.8 (109)	0.058 (109)	0.276	0.221	16.9 (109)
Pregnant, at time of CS (39.5 GW)	N/R		N/A	0.842 (9)[e]	0.673 0.351[d]	32.75 (9)[e]
Pregnant, at time of delivery (41 GW)	N/R	19.3 (109)	0.058 (109)	0.26	0.21	15.5 (109)
Obese[c]	N/R		N/A		0.192 (110)	8.39±3.07 (110)

CS caesarean section, CL clearance, C_{max} maximum concentration, f_u Fraction unbound, GW Gestational weeks, N/R not reported, N/A, not applicable, V_d Volume of distribution, V_{ss} volume of distribution at steady state.

[a] the V_d (L/Kg) is based on the participants mean weight reported in the study.

[b] The V_{ss} is considered as 80% of V_d (111) unless it has reference (stated in the study) or calculated.

[c] Body Mass Index ≥ 30 kg/m^2

[d] V_{ss} calculated using equation V_{ss} = Clearance * Mean residence time of drug in the body (9).

[e] The value could be incorrectly calculated/reported in the clinical study, possibly incorrect unit

1.1.5.4 Intravenous cefuroxime pharmacokinetics in different populations

Cefuroxime is a second-generation cephalosporin that was first used intramuscularly at the pre-CS stage; today, it is commonly administered intravenously (112). Unlike the inconclusive lipophilicity status reported for cefazolin, the overall reported lipophilicity values of cefuroxime were –0.9 and –0.167 (9, 93, 113-115). Its use in pregnancy is favoured due to its fewer side effects and low level of protein binding (9). The extent of cefuroxime bound to protein was only reported in healthy adults. The fraction of cefuroxime bound to plasma protein was 0.33 (108). The reduction in plasma protein during pregnancy may not considerably affect the fraction of free cefuroxime concentrations as compared to cefazolin. Similar to cefazolin, cefuroxime is excreted unchanged via the kidneys (9, 114, 115).

Philipson et al. reported the V_d of cefuroxime in pregnant women during and after delivery (109). These values helped understanding gestational-age changes impact on cefuroxime PK. In this study, cefuroxime V_d increased in pregnancy at 29 weeks (17.8 L) and then increased at the time of delivery (19.3 L). Cefuroxime CL increased in pregnant women (16.9 L/h). At delivery, the clearance was decreased (15.5 L/h). Lalic-Popovic et al. reported substantially increased V_d and CL in pregnant women at delivery compared to a similar population in Philipson et al.'s study (CL: 0.842 L/Kg, and V_d: 32.59 L/h) (9, 109).

In obese subjects undergoing abdominal surgery, the volume of distribution at steady state (V_{ss}) was higher, while the CL was lower compared to healthy subjects with normal BMI (109, 110). The V_d values in different population of interest indicates extracellular distribution of cefuroxime without intracellular distribution, as of cefazolin

case; nevertheless, reported V_d values in cefuroxime indicates extended distribution of cefuroxime compared to cefazolin (105)

Cefuroxime doses used at the pre-CS stage are either 750 or 1500 mg (56). It has been postulated that 750 mg dose may not achieve sufficient MIC for most common bacteria in the maternal blood of the population in question (56).

The paucity of data and the need for optimising doses to achieve therapeutic concentration, especially in the case of antibiotics, necessitate further studies to be carried out in order to predict optimal dosages. The next section will briefly discuss the concept of physiologically based pharmacokinetics (PBPK) and the tools needed for an effective prediction.

1.1.6 Physiologically based Pharmacokinetics

The concept of PBPK was first introduced by Teorell and colleagues in 1937, and further developed by other researchers (116, 117). In basic terms, PBPK divides the body organs and tissues into compartments, where each has its own specified volume and perfusion rate (116). Every species and population has their own modified models that represent their physiology, anatomy and biochemical compositions. Accordingly, mechanistic extrapolation is possible from one species or population to another, enabling prediction of PK and PD outcomes from pre-clinical data (118). This scaling accounts for physiological, anatomical and biochemical differences from one population to another to predict an outcome; which all of these factors are causative of variability of an outcome in different populations either healthy or diseased. Thus, the PBPK-based predictions ensure a much higher degree of certainty than other

extrapolations offered according to scaling approaches, involving compartmental or non-compartmental parameters, i.e. weight, age and/or sex (119).

1.1.6.1 Modelling using Physiologically-based Pharmacokinetics

Modelling relies on different numerical, mathematical and statistical analyses to simulate an outcome. PBPK modelling consists of two parts: (a) systematic data, which represent the chosen model with certain physiology, anatomy and biochemistry (e.g. transporters and enzymes abundance), and (b) drug-dependant data (including formulation properties), which represent physiochemical properties of drugs that affect their dispositions in the body (Figure 1.3) (116). There are different, robustly programmed PBPK simulators in the market, containing information of both systems and drugs. These simulators have their own advantages and disadvantages. Simcyp®, for instance, is a programmatic simulator with both a complex PBPK and PD models (120).

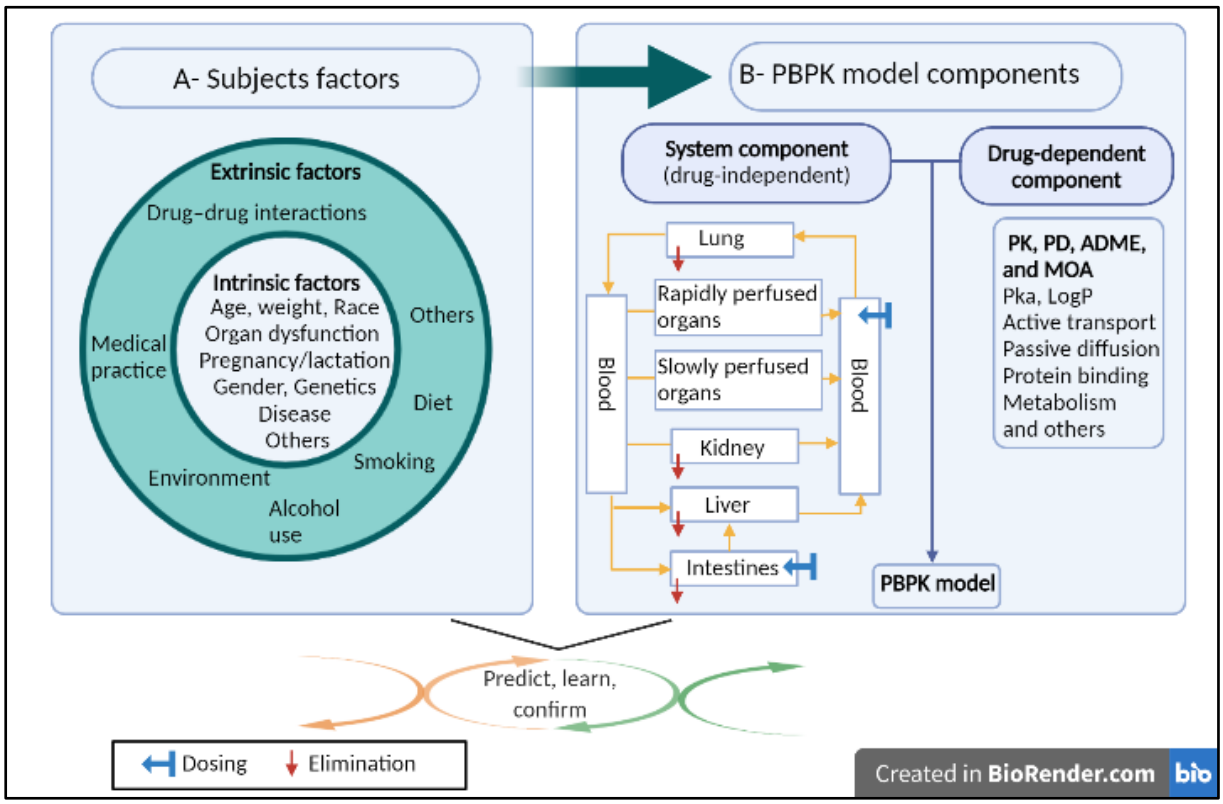


Figure 1.3: Building and applications of physiologically based pharmacokinetic (PBPK) modelling to predict the effects of numerous intrinsic and extrinsic elements on drug Pharmacokinetics and Pharmacodynamics
Figure adopted from elsewhere (121), with permission from JOHN WILEY AND SONS
ADME absorption, distribution, metabolism, and excretion; MOA mechanism of action; PBPK physiologically based pharmacokinetic; PD pharmacodynamics; PK pharmacokinetics.

1.1.6.2 Simcyp®

Simcyp® is a user-friendly platform, designed for Model-Based Drug Development (MBDD); it is annually updated and developed (120). The simulator consists of system and drug databases, incorporated into Simcyp® Data Management system (120). The simulator covers a diverse range of populations—healthy or unhealthy—children, adults, the elderly, the obese, and the pregnant population. In addition, it contains a variety of validated drugs, and users can create new drugs and new sample populations; can also adjust the physiochemical parameter of an existing drug, or change the physiological, anatomical or biochemical composition of an existing population. Additionally, the Simcyp® simulator contains permeability-limited organs

models. Functions within Simcyp® are substantial; user can use the Parameter Estimations function to estimate PK data, and/or use the Sensitively Analysis to optimise a PK data, predict Drug-Drug interactions and/or extrapolation an *in vitro* data to *in vivo* parameters (e.g. the Simcyp® In Vitro (Data) Analysis (SIVA) Toolkit). The following sections will describe PBPK models related to the current project — distribution and permeability-limited kidney models, pregnant populations (including feto-placenta), and obese and morbidly obese models — and their development within the Simcyp® simulator. Certain information and figures presented in the following sections are from the help file incorporated into Simcyp® with permission from the parent company (i.e., Certara).

1.1.6.3 Distribution Physiological-based pharmacokinetics models in Simcyp®

Users can select either the minimal or full PBPK distribution models. The minimal PBPK model, with its Single Adjustment Compartment, is a representation of the systematic compartment, and all tissues, except those of the portal vein and liver, are lumped into one compartment (Figure 1.4) (122). The volume of the systematic compartment in the minimal PBPK model within Simcyp® is described by equation **(1.3)** (122). If the minimal PBPK distribution model is selected, the V_{ss} value can be added manually or predicted using methods 1, 2 or 3 (discussed in 1.1.6.7); however, if the full PBPK distribution model is selected, the V_{ss} is required to be predicted using methods 1, 2 or 3 (122). Relevantly to this project, the full distribution PBPK model and available distribution algorithmic features are discussed. Certain prediction parameters described in the next section are not exclusively accessible to full PBPK models (e.g., V_{ss} predictions methods) (122).

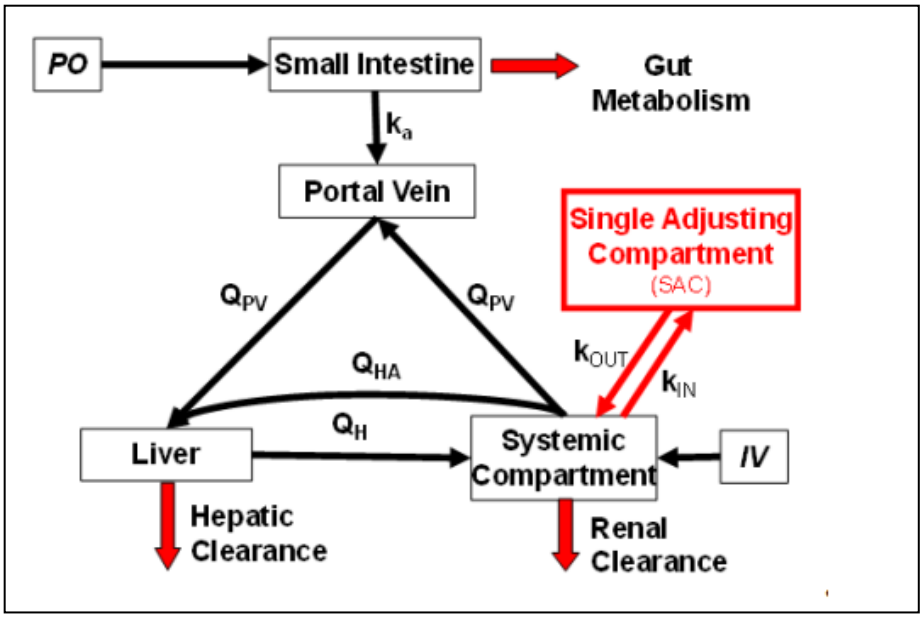


Figure 1.4: The Minimal physiologically based pharmacokinetic distribution model within simcyp

PO oral dose, IV intravenous, SAC Single Adjusting Compartment, Q_{PV} Blood flow in the portal vein, Q_H Hepatic blood flow, Q_{HA} Arterial hepatic blood flow, K_a , K_{OUT} Elimination rate constant, K_N Input rate constant. Images used with permission from Certara UK (123)

$$\text{Volume of systematic compartment } (\frac{\text{L}}{\text{Kg}}) = V_{ss} - V_H - V_{SAC} \quad \textbf{(1.3)}$$

Where the V_{ss} is volume of distribution at steady state for an average person, V_H is the apparent hepatic volume, and V_{SAC} apparent volume related with the Single Adjusting Compartment.

1.1.6.4 The Full Physiological-based pharmacokinetic distribution model in Simcyp® and functions (methods) to predict V_{ss}

The full PBPK model describes drug distribution using time-based numeral equations to predict drugs' concentrations in different compartmental body organs (blood and/or plasma, adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, pancreas, skin and spleen) (Figure 1.5) (124). Complex covariates' effects (e.g., age, sex, weight, height (BSA) and tissue volume), that cause inter-individual variability and thus affect the drug's PK/PD are accounted for in the simulator (124). Predictions of these

covariates in virtual populations are generated using correlated Monte Carlo sampling. Tissue volumes are essential in predicting V_{ss} and concentrations in each tissue (equations **(1.1)** and **(1.4)**) (125, 126).

In compartmental PBPK modelling, the virtual blood perfusion to a compartmental organ can be perfusion-limited or permeability-limited (discussed in sections 1.1.6.5 and 1.1.6.6). In the full distribution PBPK model within Simcyp®, the default distribution is set to perfusion-limited; users can switch to a permeability-limited distribution (124). Pre-built multi-compartment permeability-limited organs are available in the simulator (gut, liver, kidney, lung and brain) (124). As well, users can add an additional organ (perfusion- or permeability-limited); as in Figure 1.5, named “Generic”, users can define the volume and blood flow to the new organ (124). Tissues’ fundamental data (pH, membrane potential and binding protein) and the relative volume of wet tissues (comprised of extracellular water, intracellular water, neutral lipids, neutral phospholipids, fractions of subcellular organelle and acidic phospholipids) are addressed in the simulator (127, 128).

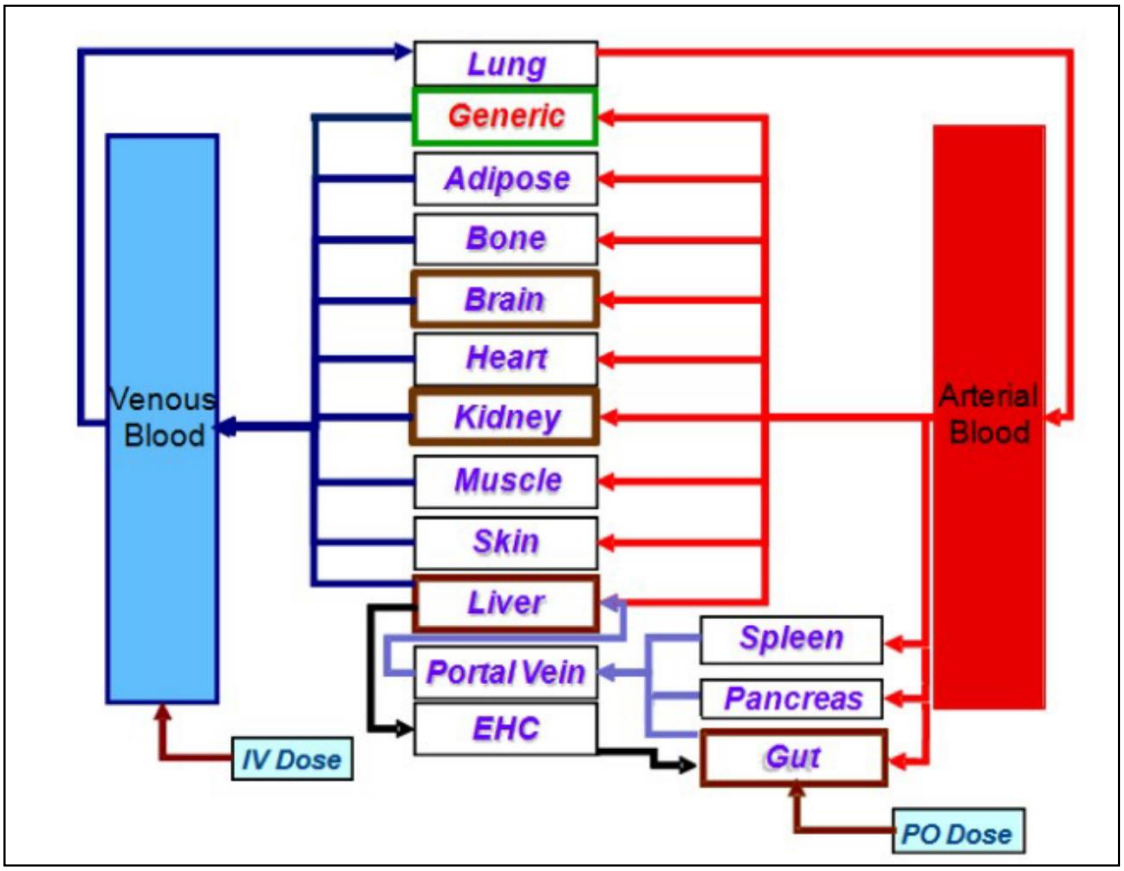


Figure 1.5: Full physiologically based pharmacokinetic distribution model within simcyp

IV intravenous dose; PO oral dose. Images used with permission from Certara UK (123)

$$V_{ss} = V_p + V_e * E{:}P \sum_t V_t * K_p \quad \textbf{(1.4)}$$

Where V_{ss} is volume of distribution at steady state; V_p is volume of plasma; V_e is volume of erythrocyte; E:P is the erythrocyte-plasma partition coefficient; V_t is tissue (t) volume; and K_p is the tissue-plasma partition coefficient for tissue t.

1.1.6.5 Perfusion-limited distribution

The assumption in perfusion-limited perfusion is that the capillary membrane (which separates blood from tissue) is particularly permeable to most drugs, especially small lipophilic molecules, and that diffusion into the interstitial fluid is very fast (124, 129). In this type of perfusion, the blood flow to the tissue is the rate-limiting factor that affects drug distribution into the tissue (Figure 1.6) (124, 129).

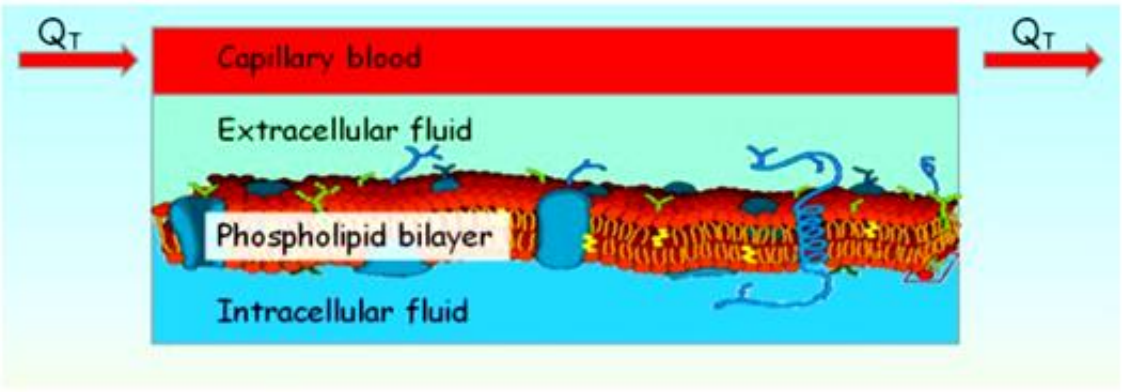


Figure 1.6: Perfusion-limited distribution of drug to tissue

Q_T Blood flow to unspecified tissue. Images used with permission from Certara UK (123, 124)

1.1.6.6 Permeability-limited distribution

When drugs are unable to diffuse quickly into and out of a tissue, the rate-limiting factor affecting their distribution is permeability (124). The permeability process depends on both the drug's properties and the structure of the capillary-tissue membrane (124). The drug's ability to permeate tissue may limit its distribution; consequently, redistribution may be constrained by the drug's release from the tissue (124).

Uptake and efflux transporters on both the intracellular and extracellular sides and passive clearance permeability parameters of a permeability-limited compartment can be defined by users (124). Thus, in addition to tissue concentration prediction, intracellular and extracellular concentrations can be simulated (124).

1.1.6.7 Methods to predict K_p and V_{ss} within Simcyp®

Knowledge of drug distribution to individual tissues enables prediction of the V_{ss}, equation **(1.4)** (126). Due to ethical restrictions and practical limitations, experimental data on tissue: plasma partition coefficients (K_p or $P_{t:p}$) are not frequently available, especially for human tissues (130). Within the simulator, users can add individual

values of K_p or estimate a universal K_p scaler and then predict a V_{ss} value using equation **(1.4)** (125, 130).

Methods within Simcyp® to predict K_p are as follows: a) using built-in mechanistic Methods (1, 2 or 3) from physicochemical and *in vitro* data, or b) estimating a K_p scalar from a V_{ss} predicted *in vitro* in animal species (131) . Additionally, users can both predict the K_p scaler using Methods 1, 2 or 3 and adjust/optimise an individual tissue: plasma partition coefficient if the user has clinical data on the relevant tissue (131). The estimated K_p scaler function accounts for inter-individual variability (132). The default value of the K_p scaler is 1, which represents no scaling factor for K_p (131). Notably, when an organ is set as a permeability-limited distribution to account for the transporter, the organ tissue: plasma partition coefficient is not used within the model; instead, the added permeability-limited data are used (132).

The mechanistic methods used to predict K_p and V_{ss} are the corrected Poulin and Theil methods (Method 1), the Rodgers and Rowland method (Method 2) and ion permeability incorporated into the Rodgers and Rowland method (Method 3) (90, 122, 133-138). In all methods, it is generally assumed that tissues are homogenised (i.e., well-stirred and perfusion-limited), molecules are passively permeated (i.e., no active transporter), the distribution is described by binding to natural lipids and natural phospholipids, and all bindings are considered non-saturating processes (130). A schematic representation of the concepts and assumptions of Methods 1, 2 and 3 is illustrated in Figure 1.7 A, B and C, respectively; Table 1.6 summarises the main and specific assumptions of each method and the data required to use a specific method (130, 138, 139).

Method 1 for predicting tissue-partition coefficients for adipose and non-adipose tissues, proposed by Poulin and Theil (2002) and then modified by Berezhkovskiy (2004), is described by equations **(1.5)** and **(1.6)**, respectively (130, 133, 134, 139). In addition to the general assumption of all methods previously discussed, this method does not account for the degree of molecules' ionisation within a tissue (i.e., not specific to a compound type: neutral, base or acid) (130, 133, 134, 139). The drug reversibly binds to proteins in plasma and tissue interstitial space; it is distributed homogenously within the tissue (130, 133, 134, 139).

Method 2, described by Rodgers and co-authors, presented alternate equations that included splitting the tissue water volume into intracellular and extracellular components as well as adding the tissue to acidic phospholipid, which accounts for its effects on drug distribution (90, 130, 135-137). At a steady state, Rodgers and co-authors assumed that only the unbound, unionised component of the drug can move across cell membranes passively and that unbound, unionised drug concentrations are in balance between intracellular and extracellular water (including plasma). These equations are anticipated to improve the prediction of tissue: plasma partition coefficients and thus V_{ss} for strong bases by explicitly accounting for the degree of ionisation of a molecule at the pH of the compartment in question (90, 130, 135-137). Rodgers et al. use the terms K_p and K_{pu} instead of $P_{t:p}$ as described in equation **(1.7)** (90, 130, 135-137). Two separate models are proposed in this method depending on the compound type: a) moderate to strong bases ($pK_a \geq 7$) and ampholytes (with at least one basic $pK_a \geq 7$) (equation **(1.8)**), and b) other compound types (neutrals, weak bases ($pK_a < 7$), acids and ampholytes without a basic $pK_a \geq 7$) (equation **(1.9)**) (90, 130, 135-137).

The unionised form of moderate to strong bases is assumed to dissolve in tissue water, where it can partition into neutral lipids and neutral phospholipids (130). Binding to acidic phospholipids is thought to control the distribution of moderate to strong bases and ampholytes (containing at least one basic pK_a 7) within the tissue. As described in equation **(1.8)** , $[AP^-]_t$ is the concentration of acidic phospholipids in tissue, and Ka_{AP} is the drug's affinity constant for acidic phospholipids. The application of a differential equation to red blood cells containing acidic phospholipids yields Ka_{AP} values (130). Notably, red blood cell Ka_{AP} values are thought to closely resemble Ka_{AP} values for other tissues (130).

Binding to extracellular proteins rather than acidic phospholipids is assumed to predominate for other compound types, i.e., those ampholytes without a basic pK_a 7, neutrals, weak bases (pK_a 7) and acids (130). This is described in equation **(1.9)**, where Ka_{PR} represents a drug's affinity constant for extracellular tissue protein (albumin or lipoprotein) and $[PR]_t$ represents the extracellular tissue protein concentration in tissue (90, 130, 135-137). Ka_{PR} can be estimated using a method proposed by Rodgers et al., or it can be calculated directly using an experimental equilibrium dissociation constant (K_D) (90, 130, 135-137).

Method 3, the Rodgers and Rowland method with ion membrane permeability, is similar to Method 2; in advance, it accounts for the permeation of ionised compounds (equation **(1.10)**) (90, 130, 135-139). Drug distribution into tissue is composed of unbound drug fractions in both intracellular and extracellular water as well as drugs bound to proteins in extracellular water and lipids in the intracellular segment, assembled with Method 2 (90, 130, 135-139). Contrasting with Method 2, Method 3 is enhanced to include the membrane potential as well as the passive permeation of

ionised molecules (the Nernst-Planck equation) through cell membranes, as in equation **(1.11)** (90, 130, 135-139). Method 3 is dependent on the knowledge of tissue membrane potential values, which is lacking in the literature; these values are required in the parametrisation of the system and affect the model's performance (138-140).

The selection of a proper method to predict K_p, and thus V_{ss} is summarised in Table 1.7 (138-140). The general selection depends on compound type (i.e., neutral compounds, all molecules are unionised), the proportion of passive permeability of ionised fractions vs. neutral fractions and tissue-specific membrane potential (138-140).

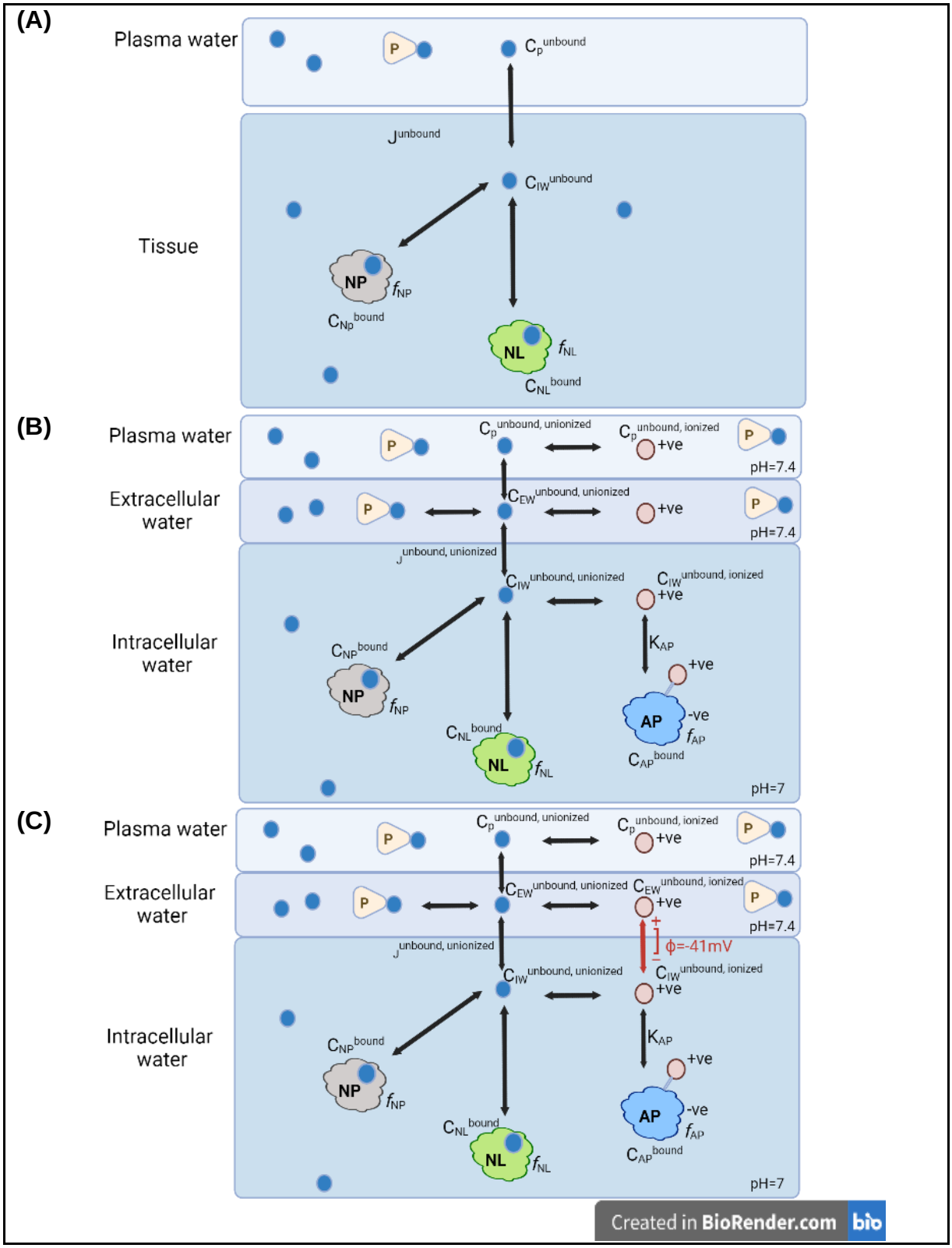


Figure 1.7: General schemes of Methods 1,2 and 3 used in Simcyp® to predict tissue : plasma partition coefficient

C_p, C_{EW} and C_{IW} plasma, Extracellular water and intracellular water concentrations (either unbound or bound, ionised or unionised);NP neutral phospholipids; NL Neutral Lipids; AP Acidic phospholipid; f_{NP}, f_{NL} and f_{AP} Volume fraction of neutral phospholipids, Neutral Lipids and Acidic phospholipids, J Flux, K_{AP} association constant with Acidic phospholipids. Adopted with permission from (138) and (139).

Table 1.6: Summery of Methods (1,2 and 3) used to predict tissue partition coefficient within Simcyp®

Method:		**Method 1**	**Method 2**	**Method 3**
General assumptions		• Homogenised tissue • Exclusive passive diffusion (no transporter involvement) • The distribution is described by binding to neutral lipids and neutral phospholipids		
Method-specific assumptions	Tissue	Adipose or non-adipose tissue	Differential tissue compositions (IW, EW, NP, NL, PR, pH_{IW}, pH_{EW})	
	Compound	Not specific for compound type	Compound type specific	
	Permeability		Ionization within IW and EW	
			Ionized fraction is not permeable	Ionized fraction is permeable
Requirement		Requires limited composition data	Requires composition and pH data	Requires composition, pH, ion permeability and membrane potential data

IW of Intracellular water, EW Extracellular water, pH_{IW} and pH_{EW} pH of Intracellular and Extracellular water, NP neutral phospholipids, NL Neutral Lipids, Adopted with permission from (139)

$$P_{t:p} = \frac{\left[P_{o:w} * \left(V_{nlt} + 0.3V_{pht}\right)\right] + \left(\frac{V_{wt}}{fu_t} + 0.7V_{pht}\right)}{\left[P_{o:w} * \left(V_{nlp} + 0.3V_{php}\right)\right] + \left(\frac{V_{wt}}{fu} + 0.7V_{pht}\right)} \quad \textbf{(1.5)}$$

$$P_{t:p} = \frac{\left[D_{vo:w} * \left(V_{nlt} + 0.3V_{pht}\right)\right] + \left(V_{wt} + 0.7V_{pht}\right)}{\left[D_{vo:w} * \left(V_{nlp} + 0.3V_{php}\right)\right] + \left(\frac{V_{wp}}{fu} + 0.7V_{pht}\right)} \quad \textbf{(1.6)}$$

$$P_{t:p} = K_p = K_{pu} * fu \quad \textbf{(1.7)}$$

$$K_{pu} = f_{EW} + \frac{X_{IW}}{X_{EW}} f_{IW} + \left(\frac{P * f_{NL} + (0.3P + 0.7) f_{NP}}{X_{EW}}\right) + \left(\frac{\boldsymbol{Ka_{AP}[AP^-]_t} * \boldsymbol{Y_{IW}}}{\boldsymbol{X_{EW}}}\right) \quad \textbf{(1.8)}$$

$$K_{pu} = f_{EW} + \frac{X_{IW}}{X_{EW}} f_{IW} + \left(\frac{P * f_{NL} + (0.3P + 0.7) f_{NP}}{X_{EW}}\right) + \boldsymbol{Ka_{PR}[PR]_t} \quad \textbf{(1.9)}$$

$$K_{pu} = f_{EW} + \boldsymbol{Ka_{PR}[PR]_t} + (f_{IW} * X_{IW} + P_{nl} f_{nl} + P_{np} f_{np} + (\boldsymbol{Ka_{AP}[AP^-]_t} * \boldsymbol{Y_{IW}}) * \frac{K_{p\ uu,uu}}{X_{EW}} \quad \textbf{(1.10)}$$

$$K_{p\ uu,uu} = \frac{C_{IW,unbound\ unionized}}{C_{EW,unbound\ unionized}} = \frac{1 + \sum_{i=1,2} \left(\frac{P^{unbound,ionised,i}}{P^{unbound,unionised,i}} \frac{N}{e^{N_i} - 1} Y^{i_{EW}}\right)}{1 + \sum_{i=1,2} \left(\frac{P^{unbound,ionised,i}}{P^{unbound,unionised}} \frac{N}{e^{N_i} - 1} e^{N_i}\ Y^{i_{IW}}\right)} \quad \textbf{(1.11)}$$

Where $P_{t:p}$ is tissue partition coefficients; $P_{o:w}$ is octanol:buffer partition coefficient; V_{nlp} is fractional volume of neutral lipids in plasma V_{nlt} fractional volume of neutral lipids in tissue; V_{pht} is fractional volume of phospholipids in tissue; V_{wt} is fractional volume of water in tissue; fu_t is fraction unbound in tissues; f_u is fraction unbound in plasma; $D_{v\ o:w}$ is olive oil:buffer partition coefficient; K_{pu} is unbound tissue plasma partition coefficient K_p is tissue plasma partition coefficient; $K_{p\ uu,\ uu}$ is the Ratio of unbound unionized concentration in tissue to that in plasma; f is the fractional tissue volume; EW is extracellular water; IW is intracellular water; P is a partition coefficient for unionised species; X and Y are drug ionisation; Ka_{AP} is the affinity constant of a drug for acidic phospholipids; $[AP^-]_t$ is the concentration of AP in tissue t, Ka_{PR} the affinity constant of a drug for extracellular tissue protein, $[PR]_t$ the concentration of extracellular tissue protein (can be albumin or lipoprotein) in tissue t; $P^{unbound,ionised}$ is passive permeability coefficient of unbound, ionised molecules; $P^{unbound,unionised}$ is passive permeability coefficient of unbound, unionised molecules where monoprotic (i = 1), and diprotic (i = 2); N the Nernst constant, for cations and for anions; Y^i is calculated according to the pH, compound pK_a and type.

Table 1.7: Comparison of Methods 1 vs. Method 2, and Method 2 vs. Method 3 performance in prediction K_p and V_{ss}

Compound type	Method 1 vs Method 2 (139, 140)	Method 2 vs Method 3 (138)
Neutral compounds	Both methods performance were similar. However, for highly lipophilic neutral compounds, Method 2 was over-predictive	Similar performance
Acidic compounds	Method 2 performed better than Method 1, and it is recommended for acidic drug	Not reported
Basic compounds	With the exception of highly lipophilic compounds, Method 2 was marginally better	For strong basic, Method 3 performed better than Method 2

.

1.1.6.8 Elimination Physiological-based pharmacokinetics models: a focus on the permeability-limited kidney model in Simcyp® (Mech KiM)

The mechanistic permeability-limited kidney model (Mech KiM) was added to the PBPK model within Simcyp® to describe drug disposition and evaluate nephrotoxicity (124, 141, 142). *In vitro* data, physicochemical characteristics of a drug and data on renal physiology are utilised in this model to simulate glomerular filtration, secretion (active and passive) and renal tubular reabsorption (active and passive) (141). The sophisticated structure, complex functions/equations and variations of elements that affect predictions (e.g., compartments' and/or segments' volume, pH and flow rate) of the Mech KiM have been published (141, 142). The main features and functions related to this project will be discussed.

The structure of the Mech KiM is illustrated in Figure 1.8. The Mech KiM consists of three main compartments: a renal blood compartment, renal mass compartment and urinal/tubules compartment (124, 141). The nephron compartment is subdivided into

eight segments (141). The first segment represents the glomerulus, followed by the proximal tubule (three segments) and then segments of the loop of Henle, the distal tubule, followed by collecting ducts (subdivided into cortex and medullary) (141). The renal compartment is then connected to the bladder (124, 141). Conventional equations that conserve drug mass balance are used to simulate drug movement between compartments and through segments (141). The model represents the kidney arterial blood flow rate (Q_{kidney}), vein blood flow rate (Q_{vein}) and urinal flow (Q_{urine}) (124, 141). The model also describes the passive diffusion clearance (CL_{PD}) values in the different segments, as illustrated in Figure 1.8 (red arrows) (124, 141). The effects of both uptake (dashed-lined blue arrows in Figure 1.8) and efflux (dotted-line blue arrows in Figure 1.8) transporters in the apical and basolateral membranes of the three proximal tubule segments are also addressed in the model (124, 141). Selectively, drug metabolism can be accounted for in these segments (green arrows in Figure 1.8) (124, 141). Thus, the following aspects are characteristic functions incorporated into the Mech KiM model:

- Renal blood bypass in two steps (red circles in Figure 1.8): before glomerulus (bypass 1), and before the Loop of Henle and succeeding segments (bypass 2) (124, 141).
- The glomerular filtration rate (GFR) is directly from the renal blood compartment to the urinal compartment, specifically the glomerulus (filtration) (124, 141).
- Fluid equilibrium within the kidney, which involves fluid flow into and out of the urinal compartments, renal blood compartments and renal mass cell compartments (reabsorption and secretion) (124, 141).

- Passive permeability across each renal cell compartment on the basolateral and apical membranes (passive representation of secretion and reabsorption) (124, 141).
- Each of the proximal tubular cell compartments (three segments) has transporters on the basolateral and apical membranes (representation of the active elements of reabsorption and secretion) (124, 141).
- Changes in the activity and regional distribution of transporters within the proximal tubular cell compartments can be addressed (124, 141).
- Representation of metabolic clearance (within each of the proximal tubular cell compartments) (124, 141).

The GFR value can be either added by the user (all males will have similar values and all females will have similar values that are different from the male values) or simulated based on creatinine clearance. In latter option, different subjects will have different GFR values (143). The GFR value is simulated by either the Cockcroft-Gault (CG) equation (referred to as Method 1 in Simcyp®) or the modification of diet in renal disease (MDRD) equation (referred to as Method 2 in Simcyp®); equations **(1.12)** and **(1.13)** represent these methods, respectively (141, 144-146).

The available transporters in the Mech KiM are illustrated in Figure 1.9; users can also add a defined renal transporter (i.e., on the apical or basolateral sides with uptake or efflux functions) (143). Allometric scaling of renal transporters from *in vitro* data (i.e., CHO and/or HEK293, etc.) is available via system scalars consisting of a) relative expression factor (REF) or relative activity factor (RAF), b) proximal tubule cells per gram of kidney (PTC-PGK) and c) kidney weight (141, 147).

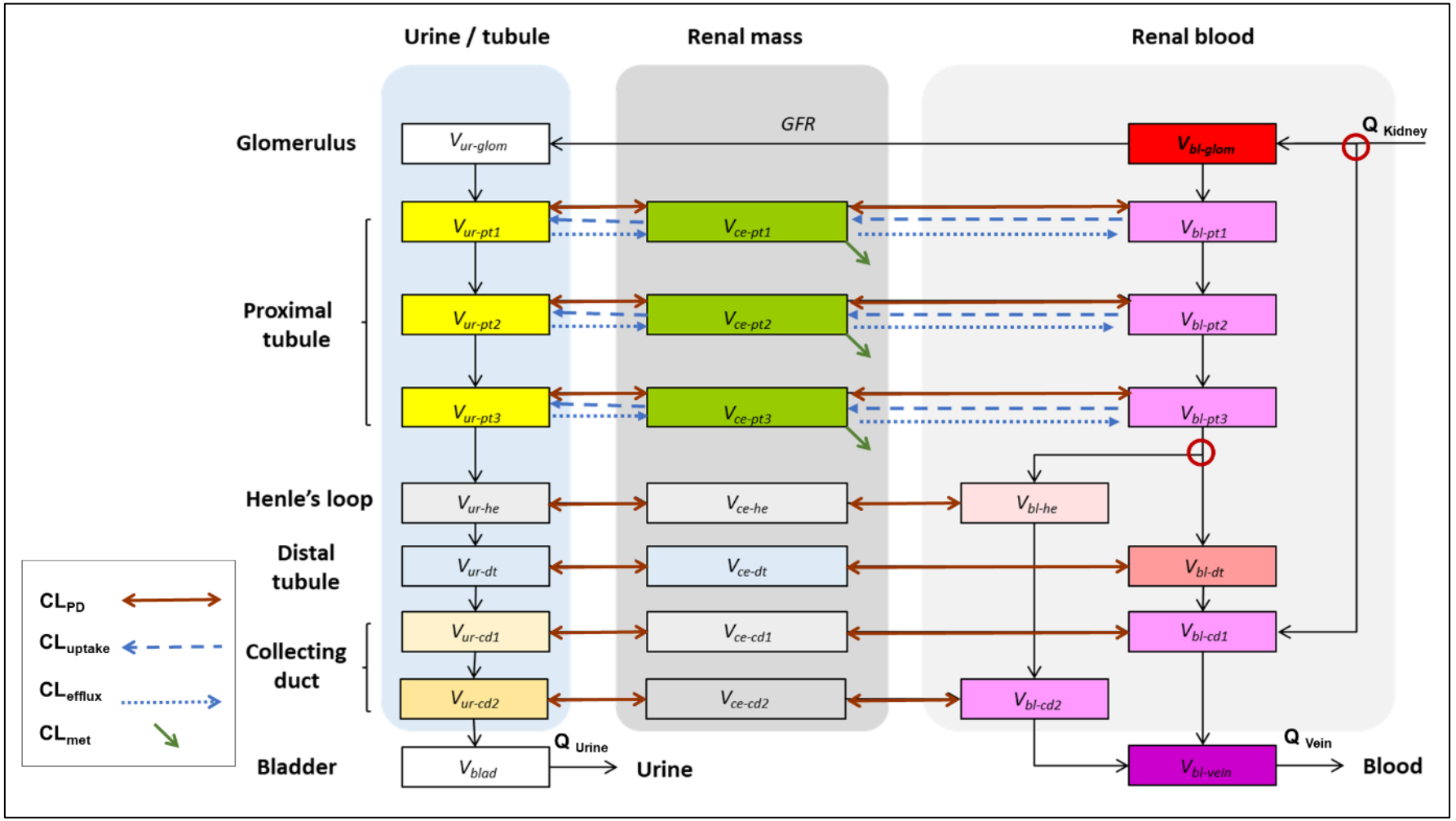


Figure 1.8: The permeability-limited kidney model (Mech Kim) incorporated into Simcyp® model

Q_{kidney} the arterial blood flow rate, Q_{vein} the vein blood flow rate, Q_{urine} urinal flow, V segment volume, bl blood compartment, ce cellular compartment, ur urine compartment, glom glomerulus, pt1, 2 and 3 proximal tubule segment 1, 2 and 3, he Loop of Henle, dt distal tubule, cd1 and 2 collecting duct segment 1 and 2, blad bladder, CL_{PD} passive diffusion clearance, CL_{uptake} uptake clearance, CL_{efflux} efflux clearance, Cl_{met} metabolic clearance. Adopted from (123); Image used with permission from Certara UK (123)

$$\text{GFR} = \frac{(140 - Age) * Weight}{0.814 * Serum\ Creatine} * \frac{1.73}{BSA} \ [* 0.85\ if\ Female] \quad \textbf{(1.12)}$$

$$\text{GFR} = 175 * (\frac{serum\ Creatine}{88.42})^{-1.154} * (age)^{-0.203} \ [* 0.742\ if\ Female] \quad \textbf{(1.13)}$$

Where GFR is glomerular filtration rate (mL/min/1.73m^2); BSA is body surface area.

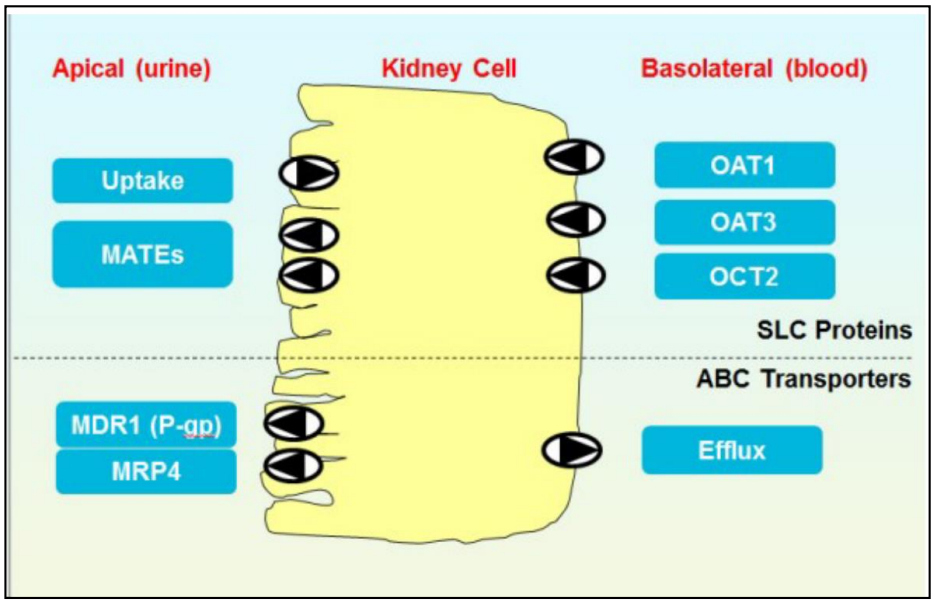


Figure 1.9: The renal transporters available in the Mech Kim model within simcyp

MATE multi-drug and toxin extrusion protein, MDR (P-gp) multi-drug resistance glycoprotein, MRP multi-drug resistant associated proteins, OAT organic anion transporter, OCT organic cation transporter, SLC solute carrier, ABC adenosine triphosphate-binding cassette. Images used with permission from Certara UK (123)

1.1.6.9 Sim–pregnancy Physiological-based pharmacokinetics model within Simcyp®

The Sim–pregnancy model is a representation of a healthy, pregnant Caucasian woman bearing a single foetus at an age between 18 and 45 years (148). The interface of the pregnancy tab contains additional data compared to the healthy population,

which contains the gestational changes of organs and the expansion of the new organ (i.e., foeto-placental) (148). To activate the Sim–pregnancy model, the Full PBPK distribution (section 1.1.6.4) must be selected (148). The user can select either one of the two comprehensive pregnancy models: A) the maternal model or B) the full feto-maternal model, which contains the multi-compartment placenta model (148). The maternal model represents age-dependent gestational changes with the addition of the foeto-placental unit (foetus, placenta, amniotic fluid and uterus) as one compartment; meanwhile, the full feto-maternal model characterises the components of the foeto-placental unit in more detail (i.e., it represents PK data for each feto-placental unit) (148). The maternal physiological data and their coding into the simulator have been published; Figure 1.10 (A) represents the pregnancy PBPK model developed within the Simcyp® simulator (149). Gestational changes in tissues' physiology (adipose, blood and glomerular filtration rate, blood flow to kidneys, skin and brain) are considered and allied to such tissues (148). Abduljalil et al. described average gestational changes incorporated into the generic Sim–pregnancy model using over 35 algorithmic formulas developed by collecting and analysing time variant physiological, anatomical and metabolic changes during pregnancy in the literature (149). The foeto-placental data have been published elsewhere; Figure 1.10 (B) represents the full feto-maternal model (permeability-limited placenta model) incorporated into Simcyp® (150-152).

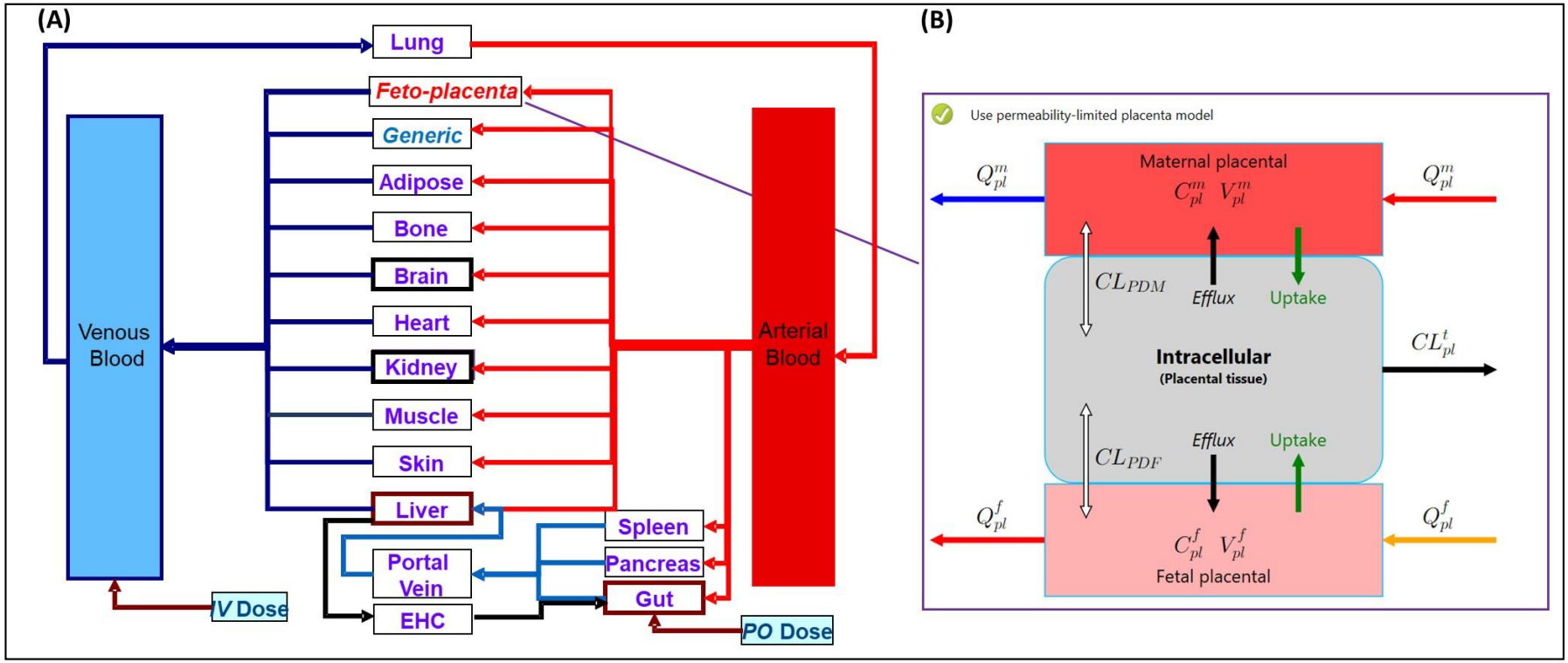


Figure 1.10: The Sim–pregnancy physiological-based pharmacokinetics model (A), coupled with the multi-compartment placenta model (B)

IV intravenous dose, PO oral dose, EHC enterohepatic recirculation, Q_{pl}^m, C_{pl}^m, and V_{pl}^m blood flow, concentration and Volume of maternal placenta, Q_{pl}^f, C_{p}^f, and V_{pl}^f blood flow, concentration and volume of feto-placenta, CL_{PDM} and CL_{PDF} passive diffusion in maternal placental side and fetal-placental side. Images used with permission from Certara UK (123)

1.1.6.10 Gestational changes in the Sim–pregnancy Physiological-based pharmacokinetics model compared to Sim–healthy volunteers

Basic equations utilised to develop the gestational changes of the generic Sim–pregnancy PBPK model and its graphical expression in the Simcyp® simulator in version 20 are discussed in the following sections. The Sim–pregnant PBPK model was not exclusively developed using these formulas, detailed development of such equations are discussed in Abduljalil et al. (149). The gestational changes scripted into Sim–pregnancy can be easily modified by the user.

1.1.6.11 Demographics

Data on pregnancy frequencies at each age range from the British Conception Statistics, England and Wales (2008) Office for National Statistics (http://www.statistics.gov.uk) was utilised to determine maternal age distribution incorporated into the Sim–pregnancy model (153). Accordingly, the following parameters (α = 4.5 and β = 30.35) were used in Equation **(1.14)** to describe age distribution within Sim–pregnancy (153, 154). The frequency of the phenotypes of enzymes and transporter abundance within the pregnancy population (i.e., intermediate metaboliser, extensive metaboliser, poor transporting phenotype or intermediate transporting phenotype) is updated within the simulator (153). Total body weight gain during pregnancy is scripted into the Sim–pregnancy model according to Equation **(1.15)** (32, 149, 155-167). Users can select a gestational age (0–40 weeks) from the trial design tab (168).

$$f(x) = \frac{\alpha}{\beta}\left(\frac{x}{\beta}\right)^{\alpha-1} e^{-(x/\beta)^{\alpha}} \quad \textbf{(1.14)}$$

Where α is shape factor of distribution and β is the scale factor of distribution

$$\text{TBW (kg)} = 61.1 + 0.2409\ \text{GA} + 0.0038\ \text{GA}^2 \quad \textbf{(1.15)}$$

Where TBW is Total body weight, GA is Gestational age in weeks

1.1.6.12 Organs, tissue and blood flow

1.1.6.13 Tissue volume

The addition of foetal volume and gestational changes in plasma volume, red blood cell volume and total fat mass (expressed as volume in simulator using specific gravity) are described in equations **(1.16)**, **(1.17)**, **(1.18)** and **(1.19)**, respectively (32, 149, 155-167, 169-189). The graphical expansion of these parameters over the gestational period and incorporation in Simcyp® are presented in Figure 1.11 (123).

$$\text{fetal volume (mL)} = 0.01\exp\left\{\left(\frac{0.955}{0.0702}\right)(1-\exp(-0.0702\ \text{GA}))\right\} \quad \textbf{(1.16)}$$

$$\text{Plasma volume (L)} = 2.50 - 0.0223\ \text{GA} + 0.0042\ \text{GA}^2 - 0.00007\ \text{GA}^3 \quad \textbf{(1.17)}$$

$$\text{Red Blood Cell volume (L)} = 1.49 + 0.0098\ \text{GA} \quad \textbf{(1.18)}$$

$$\text{Total fat mass (kg)} = 17.14 + 0.1305\ \text{GA} + 0.0008\ \text{GA}^2 \quad \textbf{(1.19)}$$

Where GA is Gestational age in weeks

1.1.6.14 Blood flows

The change in cardiac output and consequent alteration of blood flow percentages to the kidneys, brain, adipose tissues and the new organ (foeto-placenta) are incorporated into the Sim–pregnancy model. Equations **(1.20)**, **(1.21)** and **(1.22)** represent the gestational changes of cardiac output and blood flows to the uterine and

kidneys (effective renal blood flow), respectively (149, 155, 172, 190-214). Figure 1.12 represents the gestational increase of cardiac output (A) and the gestational percentages of cardiac blood flows to the foeto-placenta (B), kidney (C) and adipose (D) (123).

$$\text{Cardiac output (L/h)} = 301 + 5.916\ \text{GA} - 0.088\ \text{GA}^2 \quad \textbf{(1.20)}$$

$$\text{Uterine blood flow (L/h)} = 1.71 + 0.2068\ \text{GA} + 0.0841\ \text{GA}^2 - 0.0015\ \text{GA}^3 \quad \textbf{(1.21)}$$

$$\text{Effective renal blood flow (L/h)} = 53 + 2.6616\ \text{GA} - 0.0661\ \text{GA}^2 \quad \textbf{(1.22)}$$

Where GA is Gestational age in weeks

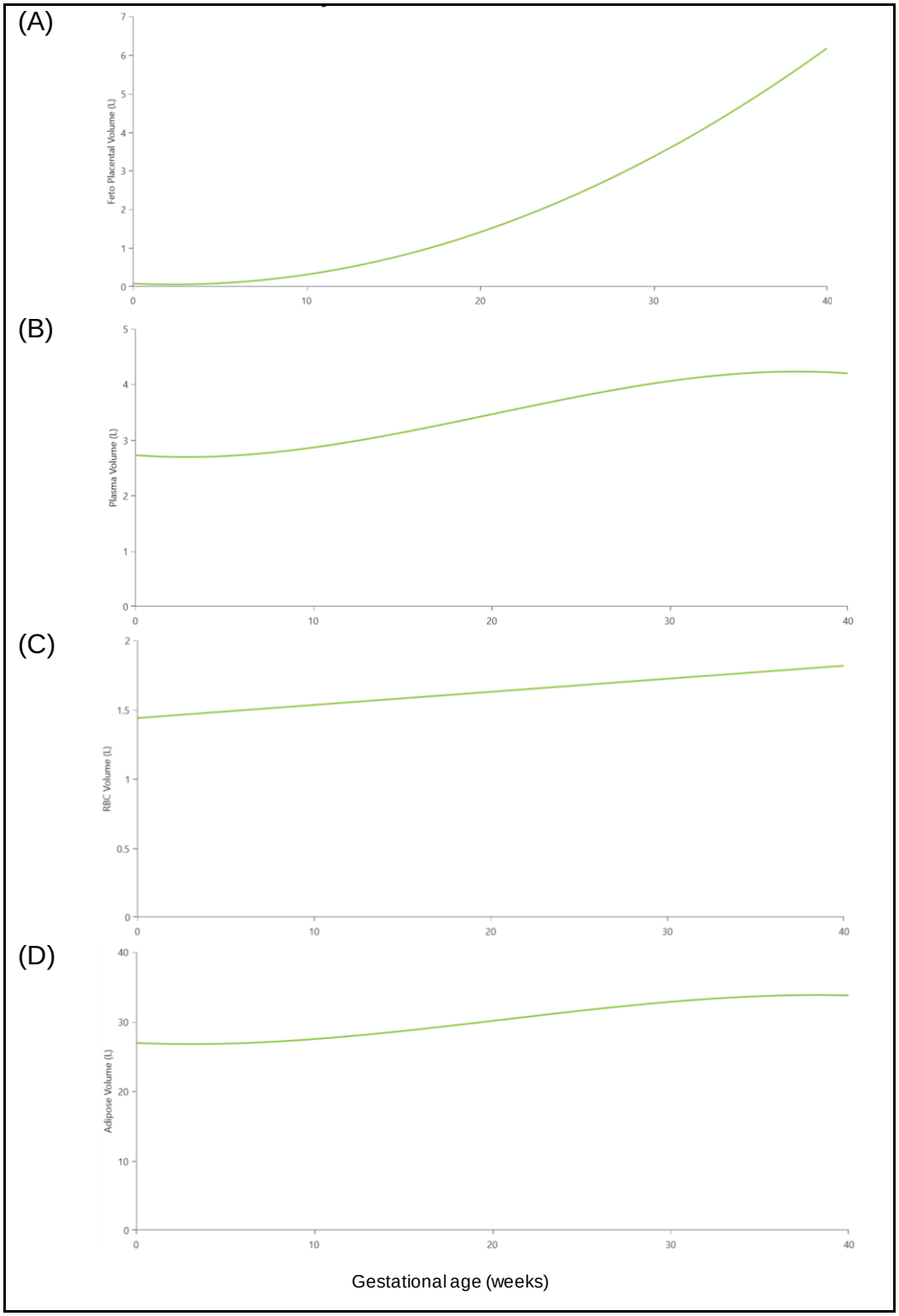


Figure 1.11: Graphical gestational changes of foeto placental volume (A), plasma volume (B), red blood cells volume (C) and adipose volume (D) in Sim–Pregnant physiological-based pharmacokinetics model

Images used with permission from Certara UK (123)

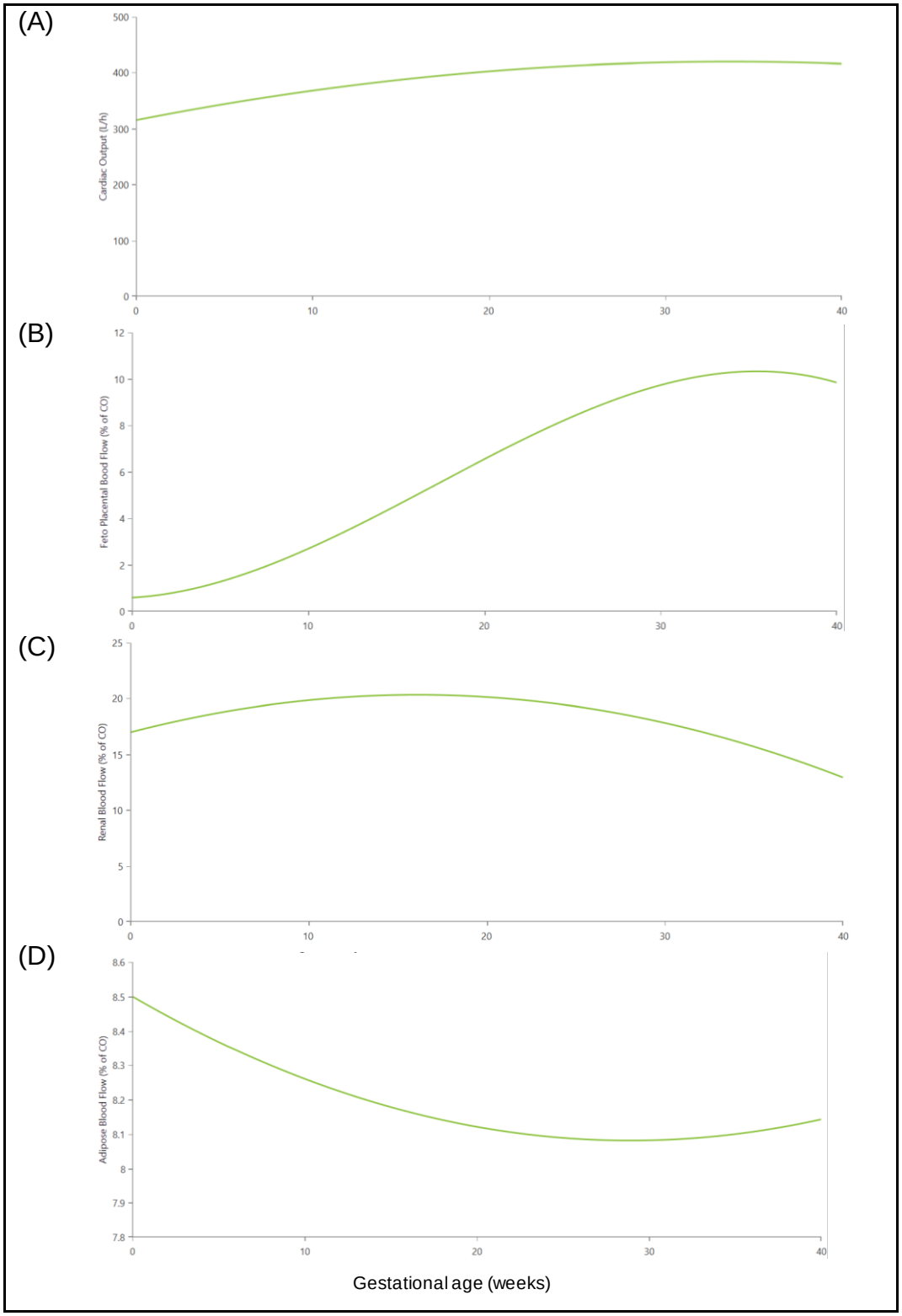


Figure 1.12: Graphical gestational changes of cardiac output (A), percentages of blood flows to foeto-placenta (B), kidney (C) and adipose (D) in Sim–Pregnant physiological-based pharmacokinetics model

Images used with permission from Certara UK (123)

1.1.6.15 Composition

The gestational expansion of total body water and change in extracellular and intracellular water at the time variant are described by equations **(1.23)**, **(1.24)** and **(1.25)**, respectively (149, 156, 165, 169, 172, 191-195, 215-219). The diagrammatic extracellular and intracellular water changes during the pregnancy period coded into the Sim–pregnancy model are shown in Figure 1.13 (123).

$$\text{Total body water (L)} = 31.67 + 0.275\ \text{GA} + 0.0024\ \text{GA}^2 \quad \textbf{(1.23)}$$

$$\text{Extracellular water (L)} = 11.86 + 0.0187\ \text{GA} + 0.0016\ \text{GA}^2 \quad \textbf{(1.24)}$$

$$\text{Intracellular water (L)} = 19.81 + 0.5941\ \text{GA} - 0.0007\ \text{GA}^2 \quad \textbf{(1.25)}$$

Where GA is Gestational age in weeks

1.1.6.16 Blood binding

The gestational reduction in blood protein levels, discussed in section 1.1.1.3 Distribution, are incorporated into the Sim–pregnancy model for albumin and alpha-1-acid glycoproteins. The collected data were utilised to describe the reduction of plasma protein levels in general and for albumin and alpha-1-acid glycoprotein specifically by equations **(1.26)**, **(1.27)** and **(1.28)**, respectively (149, 163, 176, 180, 193, 220-231). Figure 1.14 represents the reduction in albumin and alpha-1-acid glycoprotein plasma levels in the Sim–pregnancy model (123).

$$\text{Plasma protein (g/L)} = 69.7 + 0.2085\ \text{GA} - 0.0305\ \text{GA}^2 + 0.0006\ \text{GA}^3 \quad \textbf{(1.26)}$$

$$\text{Albumin (g/L)} = 45.8 - 0.1775\ \text{GA} - 0.0033\ \text{GA}^2 \quad \textbf{(1.27)}$$

$$\text{Alpha-1-acid glycoprotein (g/L)} = 0.74 - 0.0088\ \text{GA} + 0.0001\ \text{GA}^2 \quad \textbf{(1.28)}$$

Where GA is Gestational age in weeks

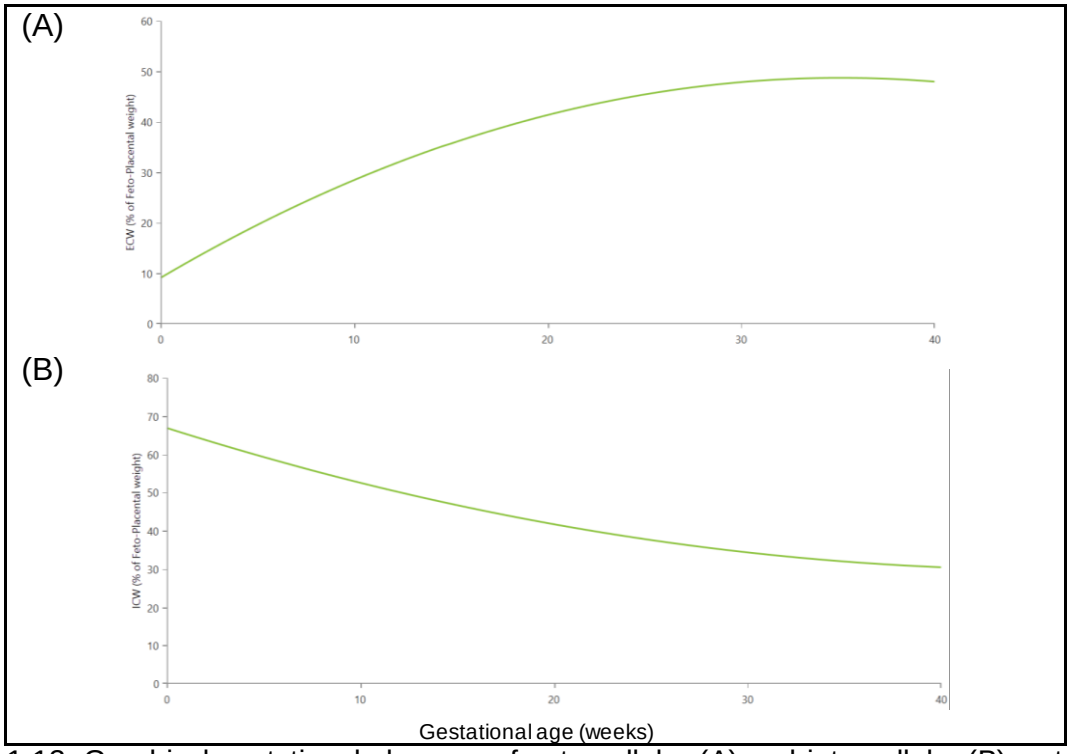


Figure 1.13: Graphical gestational changes of extracellular (A) and intracellular (B) water in Sim–Pregnant physiological-based pharmacokinetics model
Images used with permission from Certara UK (123)

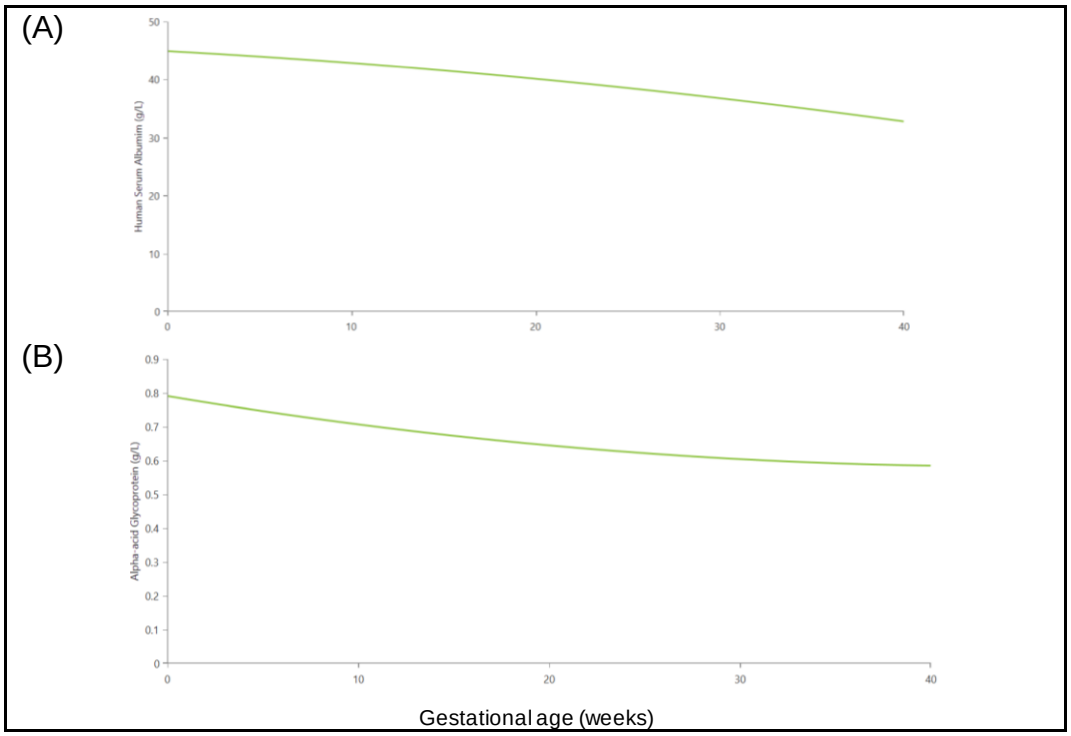


Figure 1.14: Graphical gestational changes of albumin (A) and alpha-1-acid glycoprotein (B) levels in Sim–Pregnant physiological-based pharmacokinetics model
Images used with permission from Certara UK (123)

1.1.6.17 Renal function

The complex gestational changes of renal function discussed in section 1.1.1.5 Elimination, have been described algorithmically by Abduljalil el al. (149, 155, 197-202, 232-234). The key physiological element of renal function is the GFR; the gestational alteration of GFR is described in equation **(1.29)**, and graphically displayed in Figure 1.15 (A) (123, 149).

$$\text{Glomerular Filtration Rate (mL/min)} = 114 + 3.2367\ \text{GA} - 0.0572\ \text{GA}^2 \quad \textbf{(1.29)}$$

Where GA is Gestational age in weeks

1.1.6.18 Enzymes and transporters

Gestational changes of certain enzymes are incorporated into Sim–pregnancy models (hepatic enzymes CYP1A2, CYP2D6 and CYP3A4; intestinal enzyme CYP2D6). Other transporters/enzyme activities during pregnancy can be easily adjusted by the user. The decreased activity of hepatic CYP1A2 and increased activity of hepatic CYP3A4 and CYP2D6 are described by equations **(1.30)**, **(1.31)** and **(1.32)**, respectively (149, 235). These equations were updated recently in the newer Simcyp® version, as in Figure 1.15 (B-D) (123).

$$\text{CYP1A2 activity (\%)} = 100 - 3.5814\ \text{GA} + 0.0495\ \text{GA}^2 \quad \textbf{(1.30)}$$

$$\text{CYP3A4 activity (\%)} = 100 + 2.9826\ \text{GA} - 0.0741\ \text{GA}^2 \quad \textbf{(1.31)}$$

$$\text{CYP2D6 activity (\%)} = 100 + 2.2695\ \text{GA} - 0.0348\ \text{GA}^2 \quad \textbf{(1.32)}$$

Where GA is Gestational age in weeks

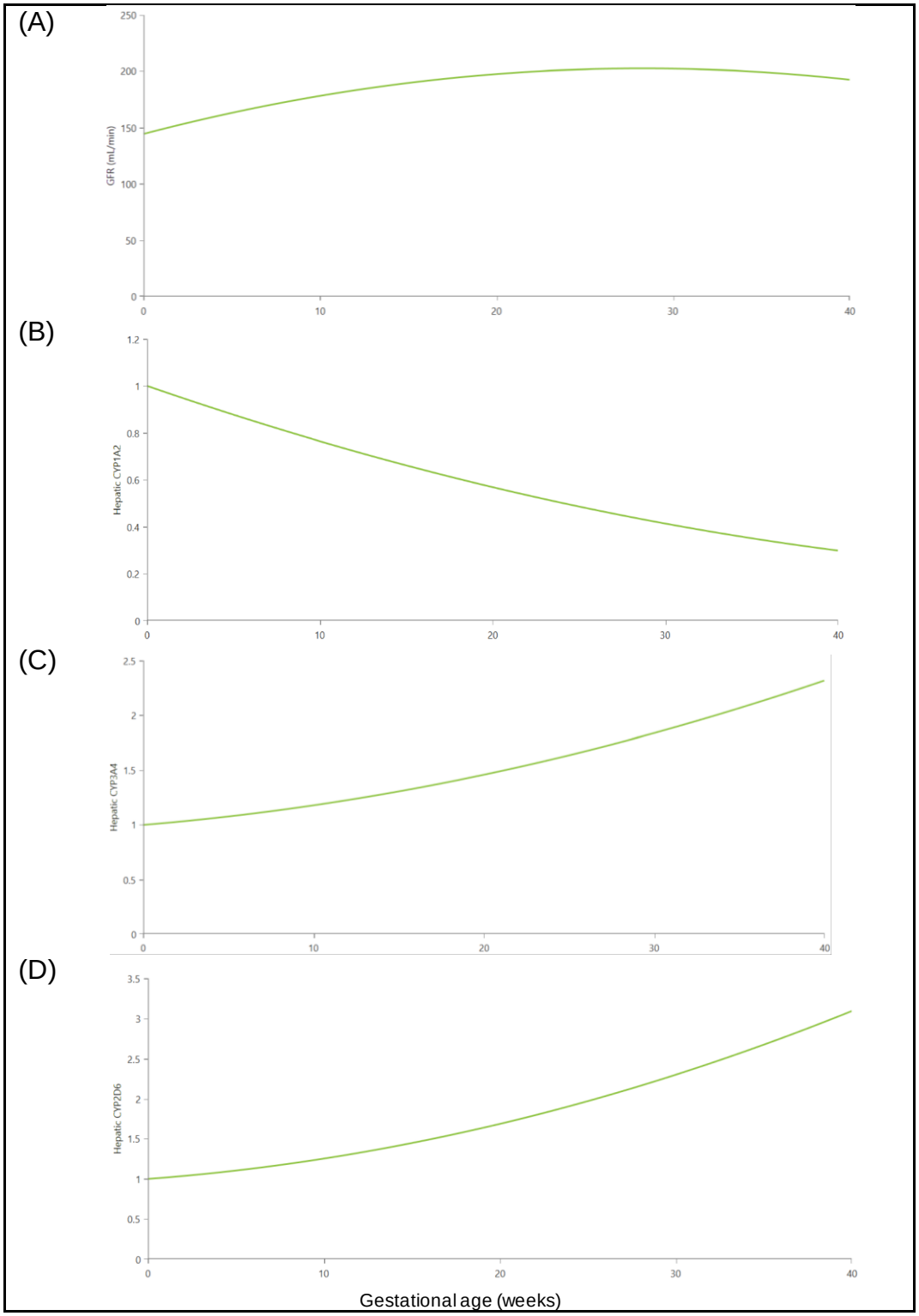


Figure 1.15: Graphical gestational changes of glomerular filtration rate (A) activates of hepatic CYP1A2 (B), CYP3A4 (C) and CYP2D6 (D) in Sim–Pregnant physiological-based pharmacokinetics model

Images used with permission from Certara UK (123)

1.1.6.19 Foeto-placental compartment

The foeto-placental full compartment coupled in Sim–pregnancy, selected electively, is briefly discussed (Figure 1.10, B). In the current project, the looped foeto-placental compartment was sufficient to serve the aim of this thesis. The generic foeto-placental model in Simcyp® has been published in detail elsewhere (150-152). The foeto-placental model accounts for the placenta, foetus and amniotic fluid changes during the pregnancy period between 15 and 40 weeks of gestation (236).

In the placental tab, the placental weight, composition, volume, maternal blood flow to the placenta and transporter abundance gestational changes are described in the model; this can be edited by the user (237). Amniotic fluid volume changes during the pregnancy period are defined in the model (238).

The male and female foetus parameters ontogeny in regards to demographics (height, weight and body surface area) (152, 239), foetal tissue growth and change of blood flow to these tissues (adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, pancreas, blood and skin) (151, 240, 241), tissue compositions (extracellular and intracellular water and fat) (242) and foetal blood binding (haematocrit, Albumin and Alpha-1 acid glycoprotein) are incorporated in the model (243).

If the permeability-limited placenta (full feto-placenta) model is activated, the mechanistic placenta and foetal models will be applied (147). As illustrated in Figure 1.10 (B), passive clearance of drugs to and from the placenta is added in the model (147). Additionally, placenta transporters (both foetal and maternal sides) are available in the model for users to define (153).

1.1.6.20 Obesity Physiological-based pharmacokinetics models within Simcyp®

The Simcyp® simulator contains obese and morbidly obese PBPK models (244). Data regarding key demographical, physical and biochemical parameters of obese and morbidly populations were collected, analysed and utilised to develop these models (244, 245). Major population-specific parameters incorporated into the Sim–obese and Sim–morbidly obese models are briefly discussed in the next section

1.1.6.21 Physiological changes incorporated into the Sim–obese and morbidly obese Physiological-based pharmacokinetics models

In line with the WHO's definition of obesity, the obese population in Simcyp® were the subjects of a BMI between 30–40 Kg/m^2 (obese class I and obese class II), while the morbidly obese population were subjects of a BMI >40 kg/m^2 (obese class III) (244, 245). Information regarding obesity and morbid obesity frequency age distribution for males and females from the dataset of the Health Survey for England (2003) was used to describe the age distribution for each gender in obesity models within the simulator (245). Height and body surface area were parametrised in Sim-obese and Sim–morbidly obese models (245).

Hemodynamic changes in obese populations discussed in section 1.1.2.1 were incorporated into the obese Simcyp® populations. The increased cardiac output in obese and morbidly obese populations (by around 15%) compared to lean subjects was scripted in models; this was achievable through the incorporation of an algorithmic relationship with BSA (244, 245). The change in cardiac output is directly applied to percentage changes in blood perfusion to tissues in these PBPK populations (244-248). However, the percentages of blood flows to certain organs (e.g., the brain and

kidney) were not increased according to the increased cardiac output; as evident in the literature, the blood flows to these tissue are similar to those of the lean cohort (43, 244, 245, 249, 250). To account for this similarity, the *in silico* blood flows to these organs decreased after increasing cardiac output in the obese population. The absolute blood flows to the remaining tissues were increased according to the updated cardiac output (244, 245). The reported increase in plasma alpha-1 acid glycoprotein by 103% and 123% is scripted into the obese and morbidly obese PBPK models, respectively (43, 244, 245, 251, 252).

The liver volume enlargement in obese and morbidly obese populations is updated in these models; the models are also parametrised for enzyme expression changes (244, 245). The increased expression of CYP2E1 (60%) and decreased expression of CYP3A4 (36%) and CYP3A5 (41%) are applied to the models (244, 245, 250, 253-264). Additionally, the observed increase in GFR in the obese and morbidly obese are coded in the virtual models (244, 245).

1.1.6.22 Developing a combined obese–pregnant Physiological-based pharmacokinetics model within Simcyp®

As previously addressed, the knowledge of physiological changes and their influence on drugs' PK is limited for the obese–pregnant population (section 1.1.2.1). It is possible to explore the PK of a drug of interest in an obese–pregnant virtual population without incorporating data on physiological changes in such a population. As the Sim–pregnancy algorithmic gestational changes are applied to a healthy population (pre-pregnant or baseline), a combined obese and pregnant model within the Simcyp® simulator is achievable by adjusting the basic physiological and biochemical

parameters of these healthy data (pre-pregnant or baseline) to obese subjects. This will allow bypassing the development of PK and physiological equations describing the PK exclusively in obese–pregnant PBPK models in such time-restricted projects.

The rationale of this PhD project is to investigate the appropriateness of cefuroxime doses used in clinical practice for obese–pregnant women requiring CS. It aims to determine whether cefuroxime plasma and adipose tissue concentrations are above MIC in the obese–pregnant population at the time of CS. The exact hypothesis, aims and objectives are listed in the following sections.

1.2 Hypothesis

Obese–pregnant women with a BMI ≥30 kg/m^2 and requiring CS will have decreased cefuroxime levels, possibly sub-therapeutic, in plasma and adipose tissue at the time of skin incision or skin closure compared to pregnant adults with a BMI of 18.5–29.99 kg/m^2. Accordingly, they may require additional or higher initial doses of prophylactic cefuroxime. Table 1.8 represents the facts and assumptions used, which the hypothesis was generated.

Table 1.8: Facts and assumptions lead to the generation of current project hypothesis

Facts	Assumption
Obesity causes diluent effects to drugs' concentrations (38)	Cefuroxime plasma and tissue concentrations in obese–pregnant women are decreased compared to normal body weight pregnant adults
Obese subjects have tissue hypo-perfusion (38, 41, 42)	Cefuroxime concentrations in obese–pregnant adults at the site of CS (adipose tissue) is decreased compared to pregnant adults with normal body weight.
A higher rate of post-CS infection is reported in obese–pregnant compared to non–obese pregnant women (58, 59)	Although obese–pregnant adults' immune systems are compromised due to different factors, increased rate of post-CS infection can be explained by prophylactic antibiotic sub-therapeutic concentrations.
Cefazolin, a comparable antibiotic used in CS, has a range of LogP values, while cefuroxime is hydrophilic (9, 16, 90-93, 113-115).	The reported cefuroxime and cefazolin logP ranges suggested lowered logP of cefuroxime compared to cefazolin. Postulating that cefuroxime penetration to adipose tissue is lower than that of cefazolin in CS.

1.3 Aims

The general aim of this project is to investigate the appropriateness of cefuroxime dose given prophylactically to obese– pregnant women at the time of CS. The detailed aims are:

- To undertake a thorough literature search to identify and report cefuroxime concentration in maternal plasma and/or adipose tissue at the time of skin

incision and skin closure in pregnant women (obese and non–obese) requiring CS.

- To undertake a thorough literature search to report and compare the rate of post CS infections in pregnant women (obese and non–obese) who have received a single dose of IV cefuroxime at the pre-CS stage without co-administration of antibiotics compared to placebo (or no antibiotic) or other antibiotics.
- To undertake a thorough literature search to identify and report cefazolin concentration in maternal plasma and/or adipose tissue at the time of skin incision and skin closure in obese–pregnant women requiring CS.
- To undertake a thorough literature search to report the rate of post CS infections in obese–pregnant women who have received a single dose of IV cefazolin at the pre-CS stage without co-administration of antibiotics compared to placebo (or no antibiotic) or other antibiotics.
- To conduct a partial validation of previously described method quantifying cefuroxime in human blood and subcutaneous adipose tissue using high-performance liquid chromatography (HPLC).
- To measure, clinically, cefuroxime concentrations in plasma and adipose tissue in obese and non–obese pregnant women at time of CS.
- To report rate of post-CS infections in obese and non–obese pregnant women when given cefuroxime at time of CS.
- To develop PBPK models of obese–pregnant women and predict cefuroxime and cefazolin plasma and adipose tissue concentration in the obese and non–obese pregnant population when different doses are administered at 39.5 weeks' gestation.

- In case of sub-therapeutic cefuroxime plasma/adipose tissue concentrations is proven, to recommend suitable dose for cefuroxime in obese–pregnant women requiring CS.

1.4 Objectives

- To systematically review the literature: to assess whether the administration of different doses of IV cefuroxime at the pre-CS stage will provide sufficient coverage against common microbial pathogens in pregnant women requiring CS by attaining a sufficient MIC (i.e. 2, 4 or 8 mg/L or 2, 4 or 8 μg/g) in both plasma and adipose tissue. (Chapter 2)
- To systematically review the literature: to assess whether the administration of different doses of a single IV cefuroxime at the pre-CS stage will provide sufficient coverage against common microbial pathogens in this population by reducing the rate and possibility of infection at the post-CS stage compared to placebo (or no antibiotic) or other prophylactic antibiotics given prior to CS. (Chapter 2)
- To systematically review the literature: to assess whether the administration of different doses of IV cefazolin at the pre-CS stage will provide sufficient coverage against common microbial pathogens in the obese–pregnant women requiring CS by attaining a sufficient MIC (i.e. 2, 4 or 8 mg/L or 2, 4 or 8 μg/g) in both plasma and adipose tissue. (Chapter 2)
- To systematically review the literature: to assess whether the administration of different doses of a single IV cefazolin at the pre-CS stage will provide sufficient coverage against common microbial pathogens in the obese–pregnant women requiring CS by reducing the rate and possibility of infection at the post-CS

stage compared to placebo (or no antibiotic) or other prophylactic antibiotics given prior to CS. (Chapter 2)

- Test the linearity of cefuroxime, carry-over effect, lower limit of detection (LLOD) and lower limit of quantification (LLOQ) and matrix effect (blood and adipose tissue) on cefuroxime chromatograms. (Chapter 3)
- To develop a protocol for a pilot study to measure cefuroxime concentrations in plasma and adipose tissue at time of CS (Chapter 4).
- To conduct a clinical pilot study at a maternity hospital to collect samples (plasma and adipose tissues) from obese and non–obese pregnant women, and report post-CS infections after administration cefuroxime at time of CS.
- Using Simcyp® simulator, cefazolin and cefuroxime compounds disposition were predicted and compared to clinical PK data in different populations of interest. (Chapter 5).
- Pregnant population within Simcyp® were adjusted to predict cefazolin and cefuroxime in obese–pregnant. (Chapter 5).

Chapter 2: Systematic Review to Evaluate Pharmacokinetics of Cefuroxime and Cefazolin in Obese–Pregnant Women Undergoing Caesarean Section and Rate of Surgical Site Infection

The aim of this chapter was to systematically review literature to collect data for the development of PBPK models and the design of a pilot clinical study to measure cefuroxime levels in obese-pregnant women. Due to variations in the data available for PK and the SSI post-CS, it was decided that separate systematic reviews be conducted for each antibiotic and for each domain. These systematic reviews are referred to as: (a) systematic review of studies on the outcomes of cefuroxime PK (CFX-PK) in pregnant women; (b) systematic review of studies on postsurgical cefuroxime infection (CFX-INF); (c) systematic review of studies of cefazolin PK (CFZ-PK) outcomes in pregnant women; and (d) systematic review of postsurgical cefazolin infection (CFZ-INF).

Publication from this chapter:

- The systematic review of CFX-PK and CFX-INF was published in European Journal of Obstetrics & Gynecology and Reproductive Biology, the manuscript was incorporated to this thesis upon permission statement by publisher: Elsevier (https://www.elsevier.com/about/policies/copyright/permissions) "Authors can include their articles in full or in part in a thesis or dissertation for non-commercial purposes.". Numbering of heading, tables and figures were only adjusted to be consistence with the current report. The Full article reference is: Alrammaal HH, Batchelor HK, Morris RK, Chong HP. Efficacy of perioperative cefuroxime as a prophylactic antibiotic in women requiring caesarean section: A systematic review. Eur J Obstet Gynecol Reprod Biol.

2019 Nov;242:71-78. doi: 10.1016/j.ejogrb.2019.08.022. Epub 2019 Aug 31. PMID: 31569027. Which can be found at: https://pubmed.ncbi.nlm.nih.gov/31569027/. Contributions of authors were mentioned within the paper.

- The CFX-PK and CFX-INF systematic review was presented also at the Annual Academic Meeting held at the Royal College of Obstetricians and Gynaecologists in London on 17–18 January 2019 as a **poster presentation** and published at the An International Journal of Obstetrics & Gynaecology. The poster is included in the appendix section, Figure 7.1. The Full reference is: (2019), Blair Bell Research Society: Annual Academic Meeting Abstracts. BJOG: Int J Obstet Gy, 126: e125-e141. https://doi.org/10.1111/1471-0528.15677 .
- A part of CFZ-PK meta-analysis was **presented as a poster** abstract at the Annual Academic Meeting of Royal College of Obstetricians and Gynaecologists held in London on the 27th and 28th of February 2020; The poster is included in the appendix section, Figure 7.2.
- Note: In the published journal article, one value regarding cefuroxime concentration was incorrectly typed only in–text (underlined in current thesis version). This value was correctly added in figures and tables. It was advised to correct this value in any publishable updates of the systematic review.

Manuscript number: (1)

Status: published

Publication title: "Efficacy of perioperative cefuroxime as a prophylactic antibiotic in women requiring caesarean section: a systematic review"

The page number of the thesis: 66

2.1 Efficacy of perioperative cefuroxime as a prophylactic antibiotic in women requiring caesarean section: a systematic review

Short running title: Cefuroxime pharmacokinetics and infection rates after CS

Authors

[1,2]Hanadi H. Alrammaal BSc MSc

[2]Dr Hannah K. Batchelor, BSc PhD

[3,4]Dr Katie Morris MBChB, PhD

[3,4]Dr Hsu P. Chong

[1]Clinical Pharmacy department, Collage of Pharmacy, Umm Al-Qura University, Mecca, Mecca Province, Saudi Arabia

[2] School of Pharmacy, Institute of Clinical Sciences, Robert Aitken Building, University of Birmingham, Edgbaston, Birmingham, United Kingdom, B15 2TT

[3]Department of Fetal and Maternal Medicine, Birmingham Women's and Children's NHS Foundation Trust, Edgbaston, Birmingham B15 2TG, UK

[4]Institute for Metabolic and Systems Research, University of Birmingham, Birmingham, United Kingdom

Corresponding author

Dr Hsu Phern Chong

Academic Department

3rd Floor

Birmingham Women's Hospital

Telephone:

Email address:

Tweetable abstract (110 characters)

Inconclusive data reported on cefuroxime levels in women requiring C-Section, with no data in obese women.

2.1.1 Abstract

Intravenous (IV) Cefuroxime (CFX) is widely used in Caesarean Section (CS) as a prophylactic antibiotic. The objective of this systematic review to compare CFX concentration in maternal blood and adipose tissue with the incidence of surgical site infection (SSI) following IV CFX in non-obese and obese women undergoing CS. A search in Medline, EMBASE, Cochrane, Web of Science, CINHAL Plus, Scopus and Google Scholar was conducted without language or date restrictions. Published articles or abstracts reporting CFX concentration or rates of SSI following CFX IV administration in adult women requiring CS were included. Studies were screened by title and abstract. Quality of studies was assessed via the ClinPK Statement checklist (Pharmacokinetics studies), or Joanna Briggs Institute Critical Appraisal Tools (SSI studies). The Cochrane Effective Practice and Organisation of Care checklist evaluated the risk of bias (SSI studies). There were no studies evaluating CFX concentrations in obese women undergoing CS. For non-obese women, CFX plasma concentrations ranged from 9.85 to 95.25mg/L within 30-60min of administration (1500mg dose; 4 articles, n=108 women). Plasma CFX concentrations were above the minimum inhibitory concentration (8mg/L) for up to 3 hours post-dose. No studies reported on CFX concentration in adipose tissue. Reported rates of SSI were 4.7% and 6.8% after administration of a single 1500mg dose of CFX administrated after cord

clamping (n=144 women). There is limited data on pharmacokinetics of CFX for CS. There were no studies that reported CFX concentrations or SSI in obese women.

Funding: Not applicable.

Keywords: Cefuroxime; Caesarean Section; Pharmacokinetics; Surgical Site Infection; Pregnant women; obese.

2.1.2 Introduction

The use of perioperative antibiotics has transformed the surgical landscape and it is standard practice for intravenous (IV) administration of a broad-spectrum antibiotic to minimise the incidence of surgical site infection (SSI). A single dose of IV prophylactic antibiotics is recommended at the time of caesarean section (CS) before skin incision (265). However, it is unclear if there is any advantage of one antibiotic over another, both in terms of choice of drug and dosage, in obese women undergoing CS. (12, 14, 265, 266)

The prevalence of maternal obesity varies across the UK; in the East of England, the rate is 6.23%, whilst in London 3.46% are obese (36). The rate of CS in the obese population is 33.8% rising to 47.4% in class II or III obesity (6). The rate of post-CS infection is higher among obese pregnant women compared to those who are not obese (7). Women who were overweight, obese and morbidly obese had an adjusted odds ratio for infection of 1.64 (95% CI 1.22-2.1), 2.41 (95%CI 1.73-3.37) and 3.67 (95%CI 2.62-5.16) respectively (7).

The optimal doses of peri-CS antibiotics in the obese pregnant population is unclear. Several studies have investigated an increased dose of cefazolin (CFZ) (3 g) for obese

pregnant women to achieve adequate antibiotic levels compared to the usual dose (2g) (10, 11, 13). Cefuroxime (CFX) is a second-generation cephalosporin, used in pregnancy due to a low incidence of side effects and a low level of protein binding (9). As with CFZ, CFX is excreted in an unaltered form by the kidneys. However, the lipophilicity of CFX is much lower than CFZ (logP values -0.167 vs. 0.3) (9, 90, 114, 115). Therefore, higher doses of CFX may be required to achieve adequate adipose tissue levels and thus prevent SSI. The minimum inhibitory concentration (MIC) is the lowest concentration of an antibiotic that inhibits the growth of a certain strain of bacteria. The MIC of CFX for the most common causative bacteria of post-CS infections is <1mg/L (56). Certain strains may require a higher concentration of 4mg/L or 8mg/L (81, 83, 267). In this review, MIC of 4 and 8 mg/L were chosen to evaluate appropriate CFX coverage for the intended population to ensure proper antibiotic coverage against these strains.

We sought to systematically review the literature to compare CFX concentrations in plasma and subcutaneous adipose tissue in non-obese and obese pregnant women requiring CS who were administered IV CFX peri-operatively; and to compare incidence of SSI in both groups.

Due to variations in reported data for pharmacokinetics (PK) and the infection rates, we conducted separate systematic reviews for each output parameter: (a) the CFX PK systematic review (CFX-PK) of studies on CFX concentrations in plasma and adipose tissue in pregnant women and (b) the systematic review of CFX and postsurgical infection (CFX-INF).

2.1.2.1 Scope of the Research

In March 2018, a search was conducted using the Cochrane Library's Cochrane Database of Systematic Reviews (CDSR) to identify any systematic reviews or meta-analyses addressing the search questions. There were no systematic reviews or meta-analyses evaluating the PK or rate of infection of CFX in pregnant women undergoing CS.

2.1.2.2 Reporting Strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist was used as guidance thorough this systematic review (Appendixes: Table 7.7) (268).

2.1.3 Methods

2.1.3.1 Search Strategy and data bases

Relevant electronic databases were searched using a research strategy that was constructed using Medical Subject Headings (MeSH) terms and keywords from MEDLINE, using the term harvesting template suggested by the National Institute of Health (Appendixes: S1). The strategy was then adapted to the other databases to establish homogenous search terms in all the databases selected (Appendixes: Table 7.7).

The following databases were searched including all available years, with no restrictions on the language or study setting: MEDLINE/Ovid (1948 to March 2018), Embase (1974 to March 2018), the Cochrane Library's CDSR, CINAHL Plus, Scopus and Web of Science (search until March 2018 for CFX-PK, until April for CFX-INF). The search was updated in May 2019.

An additional specific search was conducted using Google Scholar, with the following phrases for each systematic review with no time restrictions. The first two-hundred titles were reviewed.

- CFX-PK “pharmacokinetics of cefuroxime in pregnancy”. Last searched on 31 May 2019
- CFX-INF “Cefuroxime and infection in caesarean section”. Last searched on 31 May 2019

The references of the included articles, were screened to identify additional articles of interest; the articles identified were then added and screened for eligibility.

2.1.3.2 Protocol and registration

The systematic reviews’ protocols were registered on the International prospective register of systematic reviews (CRD42018106945 and CRD42018107192).

2.1.3.3 Relevant Articles

References yielded from the databases were exported into EndNote X8, and the duplicates were removed. The articles were then screened by titles and abstracts (Appendices Table 7.1). Full text of each article was examined to further assess eligibility.

2.1.3.4 Quality Assessment and Risk of Bias

The quality and risk of bias assessment were done by two reviewers (HA, HB), in case of conflicts, a discussion was made with a third reviewer for a final decision (HC).

2.1.3.5 Pharmacokinetics studies:

For the CFX-PK, the ClinPK Statement checklist was used to evaluate the quality of the methodology in each of the PK studies and risk of bias (269). This checklist was

comprehensively formulated specifically for PK studies; it was used previously in a similar study (21). The checklist contained 24 items; however, 4 items (11, 12, 20 and 21) were excluded from this study because they were irrelevant to the inclusion criteria (Appendices: Table 7.2). If the item was applicable and existed in the study, it was scored as 2. If the item was applicable and did not exist in the study, it was scored as a 0 for that item. If the item was not applicable or there was insufficient data, the item was scored as 1. Therefore, the total score for the modified ClinPK Statement checklist used in this study was 40.

2.1.3.6 Infection studies:

Study quality for CFX-INF was assessed by the Joanna Briggs Institute (JBI) Critical Appraisal Tools (270). This tool was selected as it comprehensively assesses different types of study design. For the risk of bias, the Cochrane Effective Practice and Organisation of Care (EPOC) checklist was used (271).

2.1.3.7 Data Analysis

Whenever applicable, the mean and standard deviation (SD) was calculated for the concentration or rate of infection. Meta-analysis and statistical analysis was performed where applicable.

2.1.4 Results

2.1.4.1 Cefuroxime pharmacokinetics systematic review

2.1.4.2 Literature Retrieval and Study Selection

Sixty-six titles were identified from the search strategy. After removing duplicates, 48 records were screened for inclusion based on the titles and abstracts, resulting in 37

studies being excluded (Figure 2.1). The full text of 11 studies were reviewed to assess eligibility. Four studies fulfilled the inclusion criteria, and were evaluated for the risk of bias and quality. The PK data was then extracted for further analysis. None of those studies reported PK in obese pregnant women. No additional studies were identified that were eligible for inclusion in the updated search conducted in May 2019.

2.1.4.3 Data Extraction

The PK data was extracted from the studies directly as reported or calculated based on figures presented (e.g. extraction from the time-concentration curve using an online website (272)) (Table 2.1). A time-concentration curve was generated for each of the included studies (Figure 2.2). In the study by Bousfield et al.(273), the data provided for the maternal blood was presented as scattered data in a graph with no differentiation of each time-point for each patient, nor mean plasma concentration for each time point, based on 10 patients.

2.1.4.4 Comparison of PK findings from Cefuroxime dose in women prior to CS

The studies identified in non-obese pregnant women will be discussed in chronological order in order to evaluate the growing body of knowledge of CFX PK in CS. It should be noted that the quality of three of the studies was low. The ClinPK Statement checklist scores were 24, 25 and 31 out of 40 for the Bousfield et al.(273), Roumen et al. (274) and Holt et al. (56) studies, respectively. However, Lalic-Popovic et al. (9) scored higher, with a ClinPK Statement checklist score of 38/40 (Appendixes: Table 7.3). All studies were included, despite low ClinPK score, due to the paucity of data identified. This difference in quality was primarily due to the date of publication and the greater emphasis on methodology and reporting in recent times. All of the studies measured CFX concentrations using high-performance liquid chromatography, with

the exception of the study by Bousfield et al.(273), in which the agar plate diffusion method was used. None of the included studies investigated CFX adipose tissue concentrations.

At time points closest to one hour, there was great variation in reported mean CFX concentrations: 9.47 mg/L, 32.55 mg/L and 74.76 mg/L in Lalic-Popovic et al. (9), Roumen et al. (274) and Bousfield et al. (273) studies, respectively (average CFX concentration was calculated from four points closer to one hour in Bousfield et al. (273)). These differences may relate to the interpatient variability or the assay methods used.

Bousfield et al.(273) investigated an IV bolus administration of 1500 mg of CFX pre-delivery in 10 pregnant women in labour who delivered vaginally and 10 pregnant women scheduled for elective CS. In the CS deliveries, the CFX injection time varied from 48 min to 337 min prior to delivery. The maternal blood was analysed for CFX quantification before delivery and at 30 min intervals until delivery.

Roumen et al.(274) evaluated the PK of CFX in 6 pregnant patients with preterm premature membrane ruptures, 4 of whom underwent CS. The authors measured CFX concentrations in the maternal plasma, amniotic fluid, umbilical cord blood and placental blood after three IV doses of 1500 mg of CFX (8 hours apart) (274). The mean± SD CFX plasma levels and were 32.55±5.20 mg/L 1 hour after the injection (for 4 patients) and 1.50±0.43 mg/L after 8 hours (for 3 patients). This study did not report the body weights of the pregnant women.

Holt et al.(56) compared two doses of CFX (750 vs. 1500 mg) in patients that had similar median body weights (71.5 vs. 74 kg, respectively). The authors reported that the CFX placental transfer during delivery was unaffected by the mother's body weight,

with no information reported regarding the effect of mothers' body weight on maternal CFX concentration. Additionally, in the low dose group (750 mg of CFX), the mean sampling time was shorter than in the higher dose group (1500 mg of CFX), although this was not statistically significant: 65 minutes [95% confidence interval (CI)=48.8–96.5] vs. 37 minutes (95% CI=22.8–58.3), respectively. In a subset analysis with comparable sampling time of the low dose group (47 minutes, 95% CI=32.1–67.5), maternal blood CFX concentrations were dose dependent. The group administered the higher CFX dose (1500 mg) had significantly higher CFX concentrations in the maternal blood when compared to the subset of those administered the lower dose (750 mg) (CFX concentration 51.9 vs. 19.4, P<0.001) (56). Holt et al.(56) concluded that 31% of women administered the lower dose (750 mg) would have insufficient CFX concentrations against some strains of *Escherichia coli* (*E. coli*) and *Klebsiella* at 75 min post administration.

In a recent study, a dose of 1500 mg CFX pre-CS was investigated in healthy, hypertensive and diabetic pregnant women (9). Initial means of CFX plasma concentration were comparable among the three groups. The mean CFX concentration in plasma at delivery in the diabetic group was significantly higher than those in the other groups (18.54±7.30 mg/L in diabetic group, 9.47±6.28 mg/L in control group and 11.53±8.54 mg/L in hypertensive group; P≤ 0.05). The diabetic group had shorter sampling time (t=56.14±31.12 min) compared to the control group (t=99.28±47.76 min) and hypertensive group (t=79.57±54.04 min). It should be noted that the minimum CFX plasma concentration reported at time of delivery were 0.9 mg/L, 2.04 mg/L and 9.85 mg/L in the control, hypertensive and diabetic group, respectively. Lean body weight-normalised volume of distribution, a hypothetical volume expressing the extent of drug

distribution in plasma and body, of CFX was significantly lower in the diabetic group than in the control and hypertensive groups (537.78±91.73ml/kg in diabetic group; 1364.58±621.98 m/Kg in control group and 1120.92±515.24 ml/Kg in hypertensive group; P≤ 0.05). This difference in volume of distribution may relate to the low logP value of CFX and its poor penetration into lipophilic tissues.

2.1.4.5 Cefuroxime and Caesarean Section surgical site infection

2.1.4.6 Literature Retrieval, Study Selection and Data Extraction

Fifty-seven records were screened for inclusion based on the titles and abstracts (Figure 2.1). The full text of 13 studies were reviewed to assess their eligibility. Three studies were eligible, and they were evaluated for the risk of bias and quality. Data were extracted (Table 2.2). Included studies evaluated single dose CFX administered after umbilical cord clamping; of those, 2 studies evaluated 1500 mg dosages and 1 study evaluated a 750 mg dose. Further data interpretation was not manageable as the 2 studies had different comparators; one study compared CFX to no antibiotic and the other to ampicillin/sulbactam antibiotic (275-277). No additional studies were identified that were eligible for inclusion in the updated search conducted in May 2019

2.1.4.7 Comparison of rate of infection reported in selected studies

All three studies were randomised controlled trials (275-277). Two studies had acceptable quality assessment and risk of bias evaluation, except for Kristensen et al (Appendixes: Table 7.4 and Table 7.5) (275). Kirstensen et al. (275) compared CFX (750mg) vs. no antibiotic, Rizk et al. (276) compared CFX (1500mg) vs. no antibiotic and Ziogos et al. (277) compared CFX (1500mg) vs. ampicillin/Sulbactam (3g). Patients were monitored for infection by medical team while patients were hospitalised for delivery until discharged in all studies (275-277); in addition to a weekly clinical and

laboratory monitoring for 30-days in Ziogos et al.(277) and at a 6 weeks post-OP visit in Rizk et al.(276). Reported rates of infection are listed in Table 2.2. Kristensen et al. (275) demonstrated a clear advantage of IV CFX, where 1.96% of patients had a defined infection in the treatment group compared to 19.2% in the group that did not receive antibiotics. Although this was not replicated in the Rizk et al. (276) study, as higher dose of CFX (1500mg) was given to 59 women and no antibiotic to 61 women. The rate of SSI were 6.8% in CFX group compared to 4.9% in the "no antibiotic" groups. Finally, in the Ziogos et al. (277) study the incidence of SSI was 4.7% in 85 women who received 1500 mg CFX compared to 6.6% in 91 women who received ampicillin/Sulbactam.

The mean± SD of body weights of the women in Rizk et al. (276) study were 87±23 in CFX group vs. 81±17 in control group; while in Kristensen et al. (275) it were, at operation, 74.9± 11.7 in CFX group vs. 75.6 ± 11.5 in control group. In Ziogos et al. (277), 44.7% of CFX group and 41.8% of the ampicillin/sulbactam group had a BMI of ≥ 30 kg/m^2; the authors did not report infection rate when groups are classified by antibiotic administered and BMI. In this study, the univariate analysis showed no association between the overall post-op infection or post-op SSI with BMI (regardless to the antibiotic assigned). Our robust search strategy did not identify studies evaluating the SSI after CS in obese pregnant women who were given CFX perioperatively.

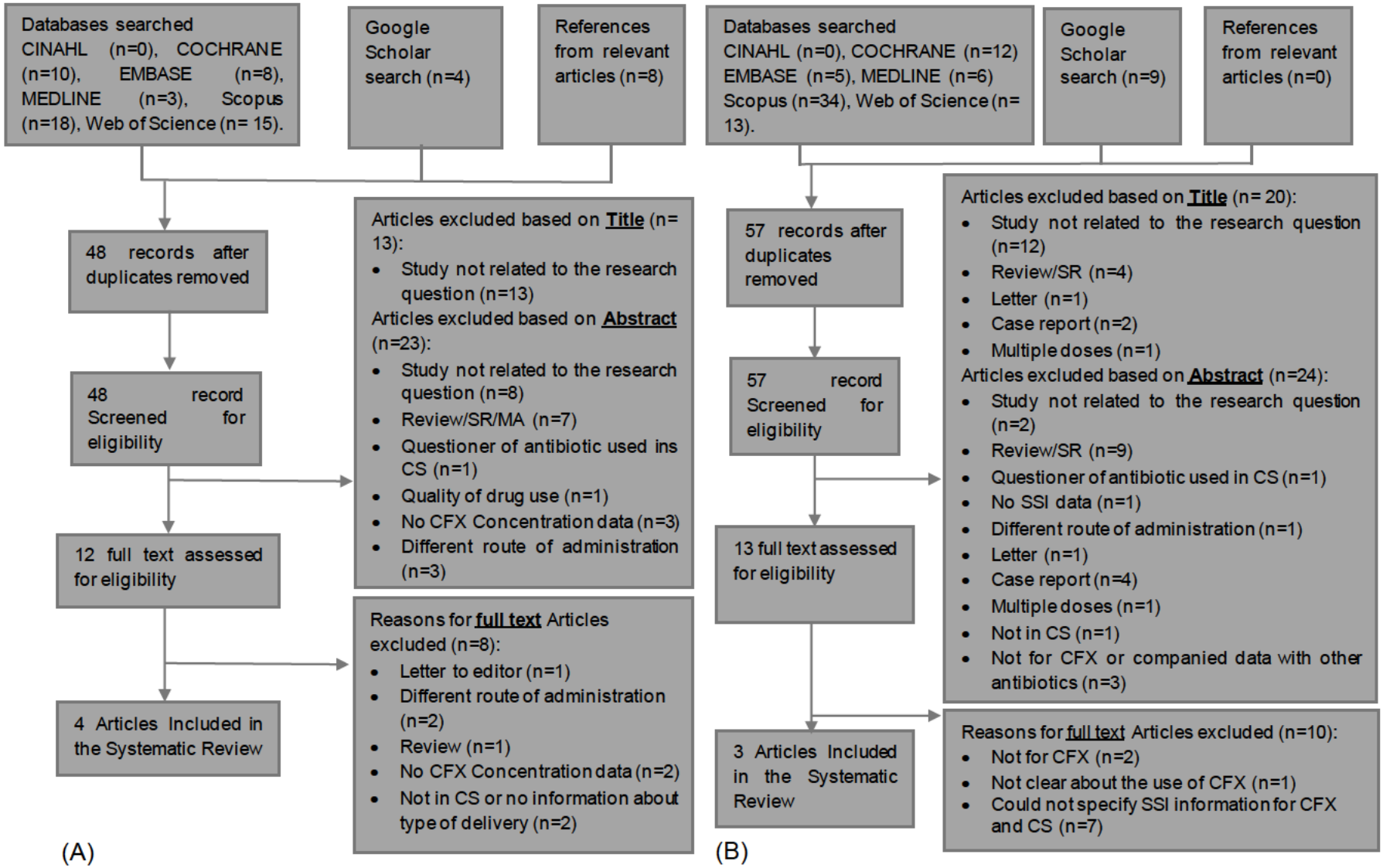


Figure 2.1: Flow diagram of full text (A) Cefuroxime Pharmacokinetics studies and (B) that were screened, assessed for eligibility SR: systematic review, MA: meta-analysis, CS: caesarean section, CFX: Cefuroxime.

Table 2.1: Summary of the data extracted from the eligible studies of Cefuroxime pharmacokinetics systematic review.

Study	Aims and Subjects	Sample size	Intervention	Type of sample	Cefuroxime Concentration mg/L at time point	ClinPK score /40
Bousfield et al. (273)	Aim: To investigate the placental transfer of IV cefuroxime Subjects: Pregnant women delivered by elective CS	10	IV cefuroxime (1500 mg), pre-CS	Maternal blood	C_t 48 min = 74.76[a] C_t 88 min = 32.95[a]	24
Roumen et al. (274)	Aim: To investigate cefuroxime PK in maternal blood and amniotic fluid and in foetal placenta, plasma cefuroxime concentration in women with preterm premature rupture of the membrane. Subjects: Pregnant women with preterm premature membrane rupture	4	IV cefuroxime (1500 mg TID), first dose was pre-CS	Maternal blood	Data for the 1st dose: C_t 1 hour = 32.55[b] C_t 8 hours = 1.5[b]	25
Holt et al. (56)	Aim: To compare concentrations of different cefuroxime doses in pregnant women at time of delivery. Subjects: Pregnant women at risk of infection (CS).	39	IV cefuroxime (750 or 1500 mg), pre-CS	Maternal blood	Group A (750 mg) (n = 26): C_t 65 min = 14.9[c] Subset of Group A (750 mg) (n = 20): C_t 47 min = 19.4[c] Group B (1500 mg) (n = 13): C_t 37 min = 51.9[c]	31
Lalic-Popovic et al. (9)	Aim: To report transplacental transfer of cefuroxime in pregnant women vs. pregnant with GD or HT. Subjects: Healthy pregnant women, pregnant women with GD or pregnant women with HT	53	IV cefuroxime (1500 mg), pre-CS	Maternal plasma	C_t 61.67 min in control = 41.14[c] C_t 99.28 min in control = 9.47[c] C_t 46.28 min in HT = 40.03[c] C_t 79.57 min in HT = 11.53[c] C_t 28.50 min in GD = 42.95[c] C_t 56.14 min in GD = 18.54[c]	38

CS: caesarean section, IV: intravenous, TID: 3 times per day, GE: gestational diabetes, HT: hypertension, C_t: concentration at time point

[a] Time and cefuroxime concentration are means of individual patients' data reported.

[b] Cefuroxime concentrations are means of individual patients' data reported.

[c] Time and cefuroxime concentration are reported as mean

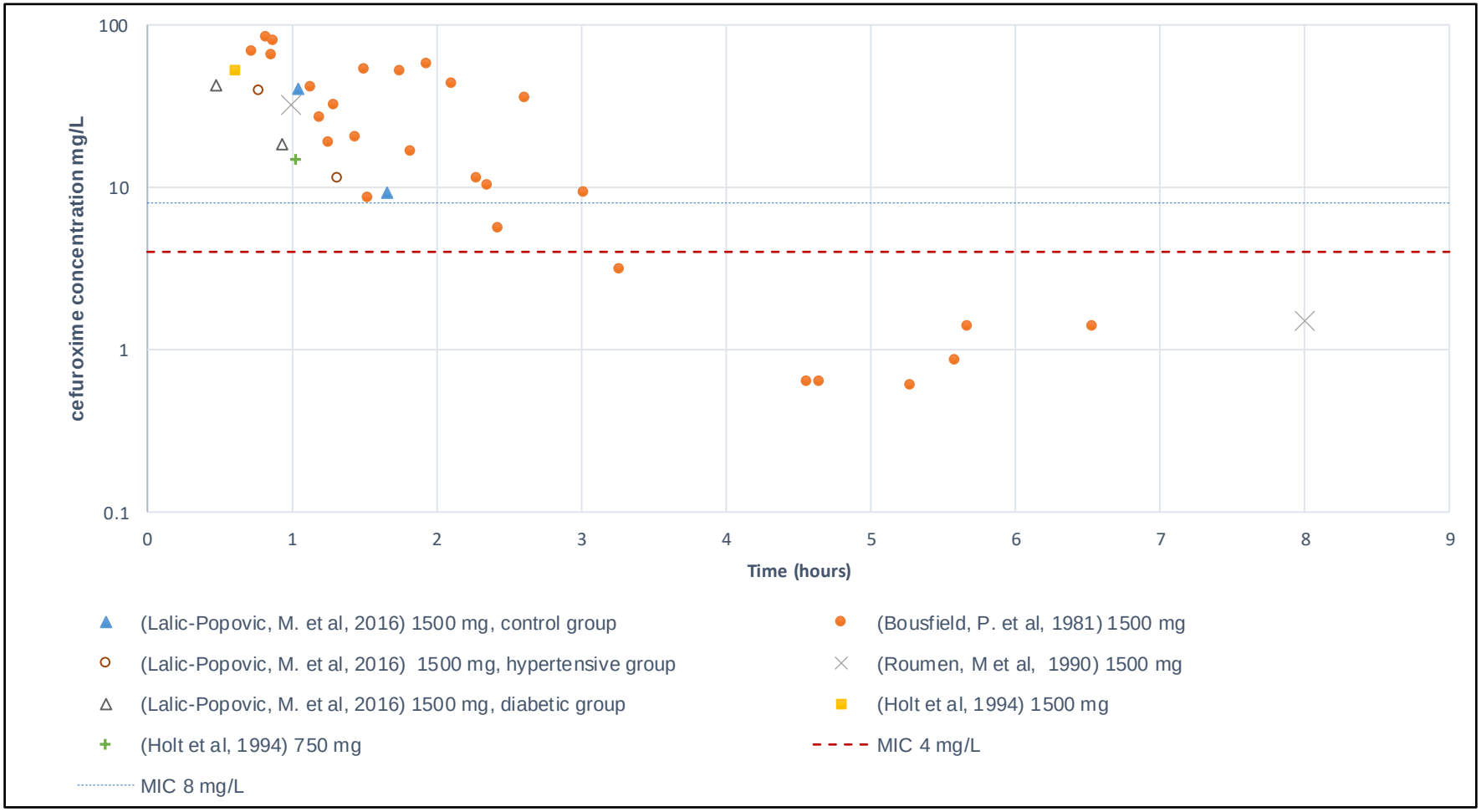


Figure 2.2: Reported Cefuroxime concentration in maternal plasma after an intravenous dose of 1500 mg or 750 mg in pregnant women underwent caesarean section.

Table 2.2: Summary of extracted data from Cefuroxime post Caesarean section infection systematic review.

Study	Type of infection	Definition by Author	Time of administration	Cefuroxime			Comparator		
				dose (mg)	n(N)	%	Name (dose)	n (N)	%
Kristensen et al. (275)	all post CS INF		after cord clamping	750	2(102)	1.96	no antibiotic	19(99)	19.19
	post CS endometritis	"FM, uterine tenderness and abnormal lochia."			1(102)	0.96		6(99)	6.06
	wound infection	"FM. cellulitis, and/or purulent discharge from the wound."			0(102)	0		1(99)	1.01
	UTI	"FM. and a positive urine culture (> 105 colonies per millilitre)."			0(102)	0		2(99)	2.02
	pneumonia	"FM and abnormal clinical or radiological finding"			0(102)	0		1(99)	1.01
	FUO	"FM occurring in the absence of any objective finding"			1(102)	0.96		9(99)	9.09
Rizk et al. (276)	febrile morbidity	"temperature of >38°c (excluding the first 2 postoperative days)"	after cord clamping	1500	6(59)	10.2	no antibiotic	6(61)	9.8
	endometritis	"uterine tenderness and offensive lochia accompanying fever in the absence of other obvious factors indicating infection elsewhere with or without positive endometrial cultures"			3(59)	5.1		2(61)	3.3
	wound infections	"presence of wound induration, erythema or purulent discharge with or without positive cultures from the incision site, with or without fever"			1(59)	1.7		1(61)	1.6
	UTI	"Presence of more than 10^5 colonies of a single bacterial species per milliliter of the postoperative urine specimen"			3(59)	5.1		3(61)	4.9
Ziogos et al. (277)	All post CS INF	As by (278)	after umbilical cord clamping	1500	5(85)	5.9	AMP/SAM (3 g)	8(91)	8.8
	Post CS Endometritis	Fever ≥38°C with one or more of the following: foul smelling lochia, uterine tenderness and "leucocytosis of >12.000 after exclusion other site of infection that developed within the first 5 days after the delivery"			2(85)	2.4		2(91)	2.2
	Post CS SSI (including Endometritis)	As by (278)			4(85)	4.7		6(91)	6.6

AMP/SAM: ampicillin/Sulbactam; CS: caesarean section; FUO: fever of unknown origin; FM: Fever morbidity, defined by author as oral temperature of 28°C, two readings 6 h apart, excluding the first 24 h following CS; INF: infection; n number of patients developed infection; N total number of patients

2.1.5 Discussion

2.1.5.1 Cefuroxime plasma and adipose tissue concentration in obese pregnant women

There was no information regarding CFX concentrations in obese pregnant women to allow comparison to non-obese women. Therefore, we evaluated plasma CFX concentrations in the non-obese population from literature identified through our search (9.85 to 95.25 mg/L within 30-60 min of administration 1500 mg CFX) with reported median CFX plasma concertation in the same population (57.2 mg/L, 82 min after administration of 2g CFZ) (10). The lipophilicity of CFX is lower than of CFZ, logP values are -0.167 and 0.3, respectively (9, 90, 114, 115). This fact, suggests that CFX is less able to penetrate adipose tissue compared to CFZ; therefore, the concentrations of CFX theoretically suspected to be lower than the reported median CFZ adipose tissue concentrations. The median adipose concentration values for CFZ in an obese population at skin incision ranged from 4.70 to 10.7 µg/g following a 2 g dose of CFZ and from 6.35 to 22.4 µg/g following a 3 g dose of CFZ (10, 11, 13, 14).

One study reported plasma and adipose tissue concentration of CFX in six morbidly obese non-pregnant patients (110). The mean± SD maximum concentration (C_{max}) of CFX in plasma was 66.8±18.9mg/L at 0.60±0.22 hour post dose, while CFX C_{max} in adipose tissue was 39.2±26.4 µg/g at 1.00±0.28 hour post dose (110). Although these results show reasonable penetration of CFX into adipose tissue, the authors reported insufficient MIC against *E. Coli* at times of prolonged surgeries (110). However, due to the unique physiological changes associated with pregnancy, specifically, with plasma volume expansion, it is inaccurate practice to extrapolate these results to the pregnant cohort of women undergoing CS (21).

2.1.5.2 Rate of post caesarean section infection

The lowest rate of infection in the CFX group was reported by Kristensen et al. (275) which is interesting because patients received lower doses of CFX (750 mg) at non-elective CS. The relatively short period of follow-up (till discharge) could explain the low rate of infection (7). That this study was undertaken in 1990 could be a reflection both of the bacterial sensitivity to antibiotics and the population at the time. As previously indicated, maternal BMI has increased with time, as has antimicrobial resistance in recent times (279).

The highest rate of infection in the CFX groups was found in the study by Rizk et al. (276). Mean± SD weight reported in this study was 87±23 kg for CFX group; this study had the fewest number of patients (n=59) for CFX group compared to same group in other studies. However, in this study only patients requiring elective CS were included, in contrast to the other studies (non-elective in Kristensen et al. (275) and both elective and non-elective in Ziogos et al. (277)).

A recent systematic review highlighted the need for evaluating infection control bundle, infection control practice and intrinsic risk factors to assess their impact on post CS SSI (280). These would not have been in existence at the time the studies on perioperative CFX and infection were undertaken, and thus, none of the studies reported on the delivery of infection prevention bundles of care. We are therefore unable to comment if bundles of care impacted on the SSI rate.

To the best of our knowledge there were no studies specifically investigating the rate of infection when CFX was administrated 30-60 min before skin incision; in this review, all included studies administrated CFX after cord clamping. This is not the current

practice, since it will not allow enough time for the drug to reach the adipose tissue and provide sufficient coverage at the time of surgery (14).

A published protocol of a Danish randomised control study evaluating the rate of infection post CS was found (281); the study aimed to compare a single dose of 1500 mg CFX administered 15-60 minutes before skin incision to the same dose after cord clamping. However, the study status was withdrawn in 2013, with no patients enrolled. The exact reasons for withdrawal of the study are unknown (281).

2.1.6 Strengths of the study

This research is the first systematic view that addressed CFX concentrations and rate of SSI in pregnant women requiring CS. The novelty of this search will enable further research in this field to determine appropriate dosing strategies for CFX.

2.1.7 Study limitations

Our search strategy could not identify one related study in CFX-INF; nevertheless, the study was not eligible (282). In CFX-PK, a total of 108 pregnant women were included in the CFX group, of whom 5 delivered spontaneously. It was considered that the type of anaesthesia and fluid administered in CS could affect the PK of drugs, specifically volume of distribution. Nevertheless, the effect of these convergences could be minor.

2.1.8 Conclusions

To the best of our knowledge, there is no study evaluating the effectiveness of CFX (in terms of CFX concentration or rate of infection post-CS) as a single IV dose peri-CS in obese pregnant women. Additionally, there were no studies assessing CFX concentrations in adipose tissue of non-obese pregnant women requiring CS. Evidence regarding the use of CFX in non-obese pregnant women requiring CS is very

sparse. There were no studies evaluating the rate of infection of single dose CFX administered as the current guidelines (30-60 min before skin incision). Owing to the importance of antibiotic resistance, future research should prioritise evaluating CFX concentration in adipose tissue for both non-obese and obese pregnant women to ensure therapeutic effectiveness from a pharmacological and clinical perspective.

2.1.9 Funding

Not applicable

2.1.10 Contribution to Authorship

HAR was involved in search planning, screening, data extraction, quality assessment, interpretation of outcome data and writing of manuscript. HB and HC were involved in search conceptualization, data extraction, quality assessment, interpretation of outcome data and editing of manuscript. KM was involved in search conceptualization, interpretation of outcome data and final editing of manuscript.

2.1.11 Declaration of Competing Interest

The Authors have no conflicts to declare.

2.1.12 Acknowledgment

We would like to thank Dr.Victoria Hodgetts Morton for her guidance in writing the manuscript.

2.1.13 Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejogrb.2019.08.022

2.2 Cefazolin pharmacokinetics and rate of infection post caesarean section: Systematic review and Meta-analysis

2.2.1.1 Scope of the research

The CDSR was searched in March 2018 to identify systematic review and meta-analysis of identical objectives. There was one meta-analysis abstract evaluating cefazolin pharmacokinetics in obese–pregnant women, this analysis was limited to include only randomised controlled trials (RCT) (283). The current research includes RCT, observational and other types of study design (Appendixes: Table 7.8).

2.2.1.2 Reporting strategy

The PRISMA statement was used while conducting this systematic review as guidance (268).

2.2.2 Methods

2.2.2.1 Search strategy and databases

The search strategy and databases described in section 2.1.3.1 were used in this systematic review. The databases were searched on April 2018; for CFZ-PK, a search update was conducted in September 2019. The MeSH terms and keywords used for the search are listed in Appendixes: section 7.1.1.1. Phrases used for Google Scholar search were as following:

- CFZ-PK “pharmacokinetics of cefazolin in obese pregnant”
- CFZ INF: “cefazolin and cesarean wound complications and infection in obese* pregnant women”

2.2.2.2 Protocol and registration

The registration number of CFZ-PK and CFZ-INF systematic review protocols' on the International prospective register of systematic reviews are CRD42018108904 and CRD42018108919, respectively.

2.2.2.3 Relevant articles

Using EndNote X8, duplicate of references from databases were removed. The references were then screened in sequence by tittles, abstract and full text.

2.2.2.4 Eligibility criteria

Studies that reported cefazolin concentrations in adult pregnant women requiring CS when cefazolin was given perioperatively were included in CFZ-PK review. For CFZ-INF, studies reported incidence of SSI after CS when cefazolin was given perioperatively to obese–pregnant women were considered eligible. Detailed eligibility criteria are listed in Appendixes Table 7.8.

2.2.2.5 Data extraction

The data of interest were extracted from the included studies to an Excel sheet, with calculations or data extraction from figures, whenever appropriate. The data extracted included:

- Details about the demographics of the population: age, BMI, co-morbidities (in addition to pregnancy) and ethnicity.
- Cefazolin concentrations in plasma or adipose tissue any anytime point.
- Rate of infection in a population following cefazolin administered as a prophylactic antibiotic in CS.

- Details about the overall infection control procedure to note other measures to reduce infection which may include: skin preparation with chlorohexidine, hair removal by clippers, cleansing the vaginal area by povidone-iodine, if the wound thickness is >2 cm and closure of subcutaneous tissue by suture.
- Details about how the post-surgical infection was measured (self-report, report by healthcare team, confirmation by healthcare team, use of additional antibiotics etc)
- Where possible details about the infection to include the location (SSI, UTI and endometritis) and timing of infection report and duration of infection.

2.2.2.6 Quality assessment and risk of bias

2.2.2.7 Pharmacokinetics studies

The ClinPK Statement checklist was used to assess the quality of the methodology and risk of bias of the included studies in the CFZ-PK systematic review (269). The checklist was utilised as described in section 2.1.3.5.

2.2.2.8 Infection studies

The studies' quality and risk of bias of eligible studies of CFX-INF systematic review were evaluated using the JBI Critical Appraisal Tools and the EPOC checklist.

2.2.2.9 Data Analysis

The means and SD were calculated for cefazolin concentrations; rate of infection was calculated. Meta-analysis was performed, whenever applicable. In case of conducting a meta-analysis, a random effect would be use to reflect differences of data distribution between studies. A sub group-analysis and sensitivity analysis were performed if necessary.

2.2.3 Results

2.2.3.1 Cefazolin pharmacokinetics systematic review and meta-analysis

2.2.3.2 Literature retrieval and study selection

Forty-one titles were assessed for eligibility after removing duplicates. Eight full texts were thoroughly reviewed, one study was excluded and seven studies were included in the CFZ-PK systematic review (Figure 2.3).

The quality assessments and risks of bias of the studies were acceptable. The ClinPK score of the eligible studies ranged from 34 to 39 out of 40 (Appendix: Table 7.10). Data of interest were extracted, and Table 2.3 shows the means and median of cefazolin concentrations in the plasma and total adipose tissue. All such means and medians were above the MIC of 4 mg/L or µg/g, but the MIC of 8 µg/g was not achieved in all the included studies.

An empirical analysis was performed that included all seven studies, in which non–obese (BMI 20–29.9 kg/m^2) subjects who were administered 2 g of cefazolin were compared with obese (BMI ≥30 kg/m^2) cohort who received different doses (2, 3, and 4 g) of cefazolin. Table 2.4 represents the means and the median of the empirical analysis of the cefazolin concentration reported in the included studies. The means and the median were above the MIC of 8 mg/L or 8 µg/g, except one study where the mean adipose cefazolin concentration at the time of skin closure in the non–obese pregnant group (BMI 20–29.9 kg/m^2) that received 2 g of cefazolin was 7.5 µg/g.

A subgroup analysis was conducted to report specific cefazolin concentrations stratified by obese group. The non–obese subjects (BMI 20–29.9 kg/m^2) who received 2 g of cefazolin were compared with the combined classes I and II obese subjects (BMI

30–39.9 kg/m^2, to whom were administered either 2 or 3 g of cefazolin) and the class III obese subjects (BMI ≥40 kg/m^2, to whom were administered either 2 or 3 g of cefazolin) (Figure 2.4). In this subgroup analysis, the studies of Young et al. (16), Groff et al. (12) and Stitely et al. (15) were excluded because the groups they studies had BMIs >30 kg/m^2 (Young et al. and Groff et al.) or >35 kg/m^2 (Stitely et al.) with no subgrouping differentiation according to BMI (12, 15, 16). Figure 2.4 illustrates the averages of the reported medians of the cefazolin concentration in both the plasma and total adipose tissue at time of skin incision and the skin closure. The number of patients pooled for each concentration from the four included studies are shown within the bars (for the plasma) or above the bars (for the adipose tissue). The exact average concentrations are presented below the bars. The average cefazolin concentrations in the maternal plasma were above the MIC of 8 mg/L. All the obese–pregnant (BMI ≥30 kg/m^2) who received 2 g of cefazolin had a cefazolin concentration above the MIC of 4 µg/g but under the MIC of 8 µg/g in adipose tissue at both times of the skin incision and the skin closure. Similarly, the class III obese pregnant women (BMI ≥40 kg/m^2) who received a higher dose (3 g) of cefazolin had an adipose concentration under the MIC of 8 µg/g at time of the skin incision and closure. Table 2.5 represent the percentages of the reported cefazolin concentration samples under the MIC of 4 or 8 mg/ml or µg/g in each study.

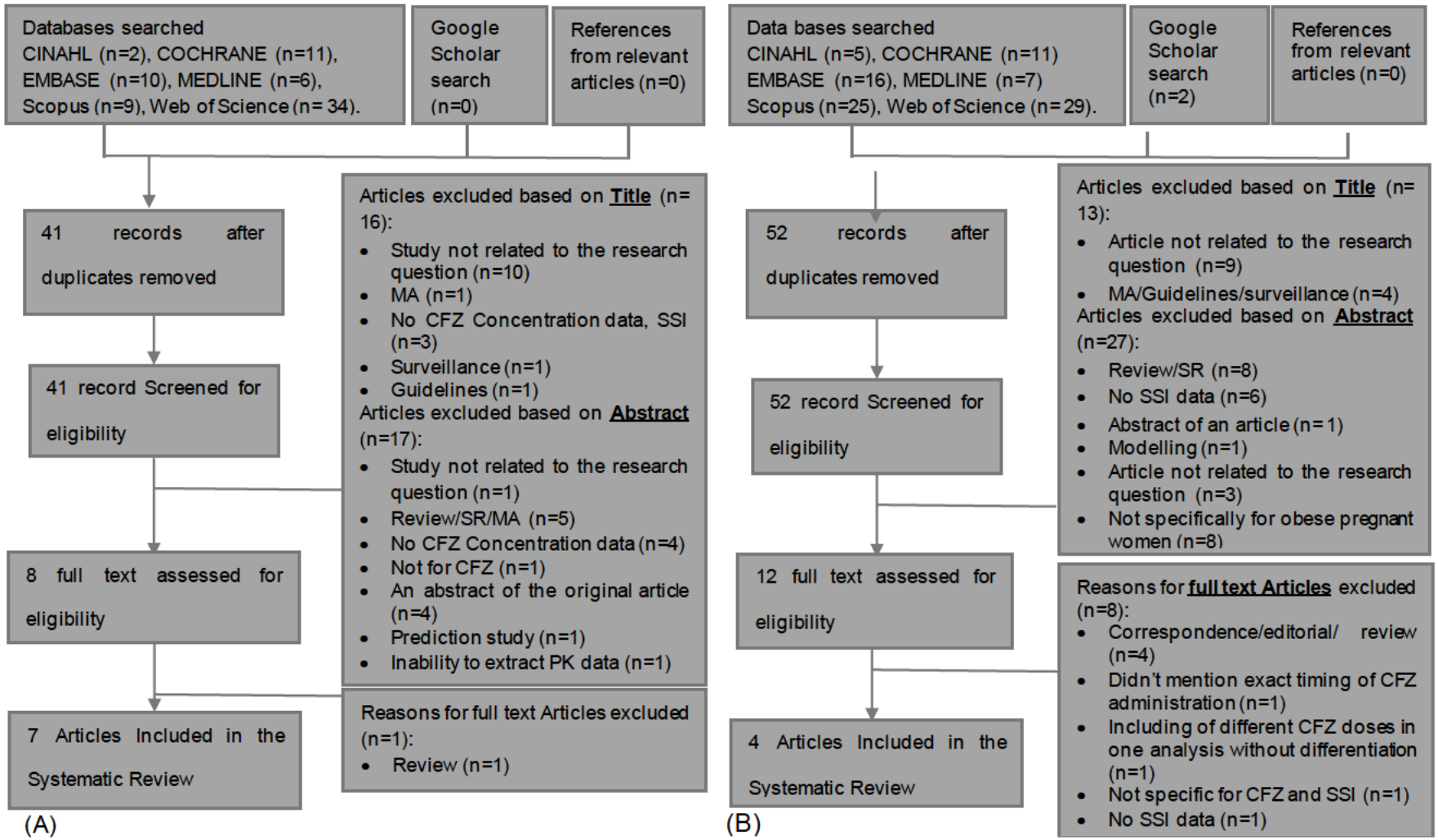


Figure 2.3: Flow diagram of cefazolin (A) Pharmacokinetics and (B) surgical site infections studies that were screened, assessed for eligibility.

SR: systematic review, SSI: surgical site infection, MA: meta-analysis, CS: caesarean section, CFZ: Cefazolin

Table 2.3: Summary of the data extracted from the eligible studies of cefazolin pharmacokinetics systematic review.

Study	BMI Kg/m^2	Average serum concentration mg/L[a]				Average adipose tissue concentration µg/g[a]				Clin pk score
		2 g Group		3 g or more Group[b]		2 g Group		3 g or more Group[b]		
		Incision	Closure	Incision	Closure	Incision	Closure	Incision	Closure	
Pevzner et al.	<30	65.20±30	NM			9.37±2.7	9.07±6.4			35
	30–39.9	54.9±41.3	NM			6.37±2.3	6.61±3.3			
	≥40	49.94±20.7	NM			4.35±1.2	4.70±1.5			
Stitely et al.	>35	155.45±50.97	83.49±34.97	346.16±166.01	150.12±55.54	18.36±6.68	21.73±16.02	40.11±24.10	34.89±17.42	39
Young et al.	>30	94.52	59.38	175.65	100	7.40 (5.4–9.4)	11.80 (9.2–16.2)	12.00(5.4–16.0)	14.6 (10.8–20.8)	38
Swank et al.	<30	57.2 (48.6–66.3)[c]	NM			8.7 (8.4–11.2)[c]	7.5 (5.0–10.1)			35
	30–40	42.60(27.3–64.8)[c]	NM	166.7(143.8–185.7)	NM	6.50(4.2–7.2)[c]	5.10 (4.0–9.3)	22.40(20.3–34,4)	24.80(18.9–34.1)	
	>40	39.7(35.5–71.5)[c]	NM	176.5(127.9–192.6)	NM	4.70(3.1–5.0)[c]	4.30(3.5–6.2)	9.60(7.6–15.8)	7.1(6.2–9.8)	
Maggio et al.	All >30	100.7(90.4–134.2)	75.1(68.9–78.4)	153.7(123.7–166)	83.8(61.7–115.3)	9.4(5.1–13.4)	8.4(4.4–13.2)	11.7(7.0–18.3)	8.7(6.7–18.8)	36
	30–39.9					10.7(7.2–21.2)	11.4(7.6–18.5)	13.0(10.1–24.2)	15.2(8.5–21.2)	
	≥40					8.9(5.0–10.6)	5.6(4.1–9.0)	7.0(6.2–13.1)	7.5(5.4–8.5)	
Kram et al.	30–39.9	NM	41.6(26.3–57.0)			5.30(3.0–9.6)	4.45(2.78–7.25)			37
	≥40			NM	45.3(36.7–68.3)			6.35(3.90–8.40)	6.90(2.60–10.6)	
Groff et al.	<25	135.4[d]	84[d]							34
	≥30	144[d]	91.2[d]							

[a] all data reported were median and IQR except that reported by Pevzner et al. and Stitely et al., were means and SD

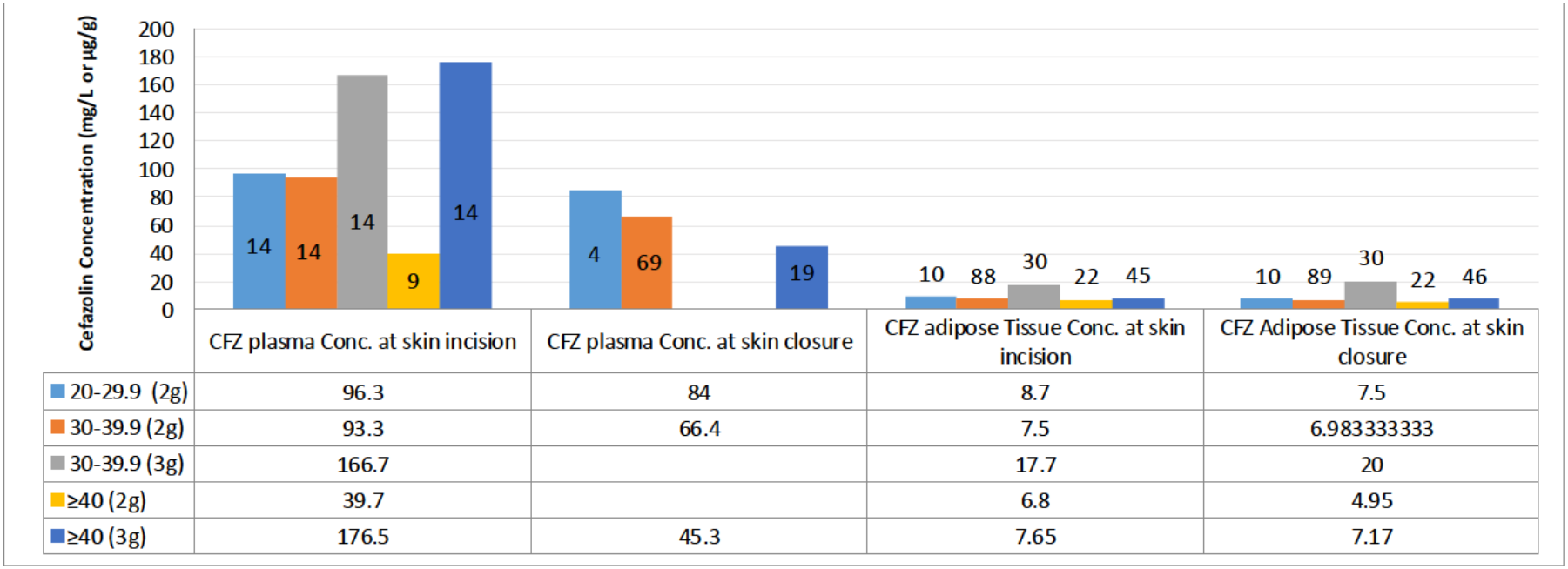
[b] all studies in 3 g or more group evaluated the use of 3 g except Sistelly et al. compared 2 g with 4 g;

[c] data of the 2 g group in Swank et al. were median of the reported means in Pevzner et al.

[d] The timing of sampling was ≈7 min after administration (selected as initial) and ≈6 min after delivery (selected as Closure). CFZ: cefazolin, NM not measured, AT adipose Tissue, P Plasma,

Table 2.4: Summery of cefazolin pharmacokinetics systematic review empirical analysis of lean–pregnant women (BMI 20–29.9 kg/m²) vs. obese–pregnant (BMI ≥30kg/m²) when given diffrent doses of cefazolin (2, 3 or 4 g)

	Average of Serum Concentration mg/L						Average of Adipose Tissue Concentration µg/g					
Dose:	2g Group		3 g Group		4g Group		2g Group		3 g Group		4g Group	
Time at skin:	Incision	Closure	Incision	Closure	Incision	Closure	Incision	Closure	Incision	Closure	Incision	Closure
BMI: 20–29.9 kg/m²	96.3	84					8.7	7.5				
BMI: ≥30 kg/m²	96.16	70.17	168.14	76.36	346.16	150.12	8.61	9.29	12.41	12.42	40.11	34.98



	CFZ plasma Conc. at skin incision	CFZ plasma Conc. at skin closure	CFZ adipose Tissue Conc. at skin incision	CFZ Adipose Tissue Conc. at skin closure
20-29.9 (2g)	96.3	84	8.7	7.5
30-39.9 (2g)	93.3	66.4	7.5	6.983333333
30-39.9 (3g)	166.7		17.7	20
≥40 (2g)	39.7		6.8	4.95
≥40 (3g)	176.5	45.3	7.65	7.17

Figure 2.4: Illustration of sub grouping analysis of cefazolin pharmacokinetics systematic review.
The numbers within or above columns are numbers of participants in each group in the sub-analysis. CFZ cefazolin; Conc. concentrations.

Table 2.5: Reported percentages of cefazolin samples under the minimum inhibitory concentration of 4 or 8 mg/L or µg/g in the eligible studies of CFZ-PK systematic review.

Study	Definition of inadequate cefazolin concentration by author	sample	Dose (g)	BMI kg/m²	% of samples under MIC	
					MIC of 4 mg/L or µg/g	MIC of 8 mg/L or µg/g
Pevzner et al. (284)	"Patients with initial or closing adipose concentration less than 4 µg/g"	adipose tissue	2	30–39.9	0%	
				≥ 40	40%	
Kram et al. (13)	"Inadequate concentrations in one or more tissue types"	adipose tissue	2 or 3	≥ 37.3	59.5%	86.9%
	"Within one or both blood sample types"	blood	2 or 3	≥37.3		8.33%
Maggio et al. (14)	"Adipose tissue concentration- opening"	adipose tissue	2	30–39.9		33.3%
			3	30–39.9		6.3%
			2	≥ 40		46.2%
			3	≥40		53.9%
	"Adipose tissue concentration- closing"	adipose tissue	2	30–39.9		33.3%
			3	30–39.9		18.8%
			2	≥40		69.2%
			3	≥40		66.7%
Swank et al. (10)	Percentage of samples with MIC < 8 µg/g, %- initial	adipose tissue	3	30–40		0%
			3	>40		28.6%
	Percentage of samples with MIC < 8 µg/g, %- closure	adipose tissue	3	30–40		7.1%
			3	>40		57.1%
Young et al. (16)	Adipose samples< 4 µg/g prior to fascial entry	adipose tissue	2	>30	7.7%	
			3	>30	16.7%	

2.2.3.3 Meta-analysis of the cefazolin concentrations in the adipose tissue

Of the seven studies that reported cefazolin concentrations in obese–pregnant women, six studies reported cefazolin total adipose tissue concentrations (10, 13-16, 284). A meta-analysis of the cefazolin initial adipose tissue concentrations was conducted to compare the 2 g cefazolin dose with the >2 g cefazolin dose in the obese–pregnant women (BMI ≥30 kg/m^2). A total of four studies were included in the meta-analysis which a comparison of 2 g vs. >2 g cefazolin were investigated in obese–pregnant

women (BMI ≥30 kg/m^2) in included studies (13-16). One study had reported the mean and SD of the initial cefazolin adipose tissue concentration (15), and the other studies had reported the median and interquartile range (13, 14, 16). The means and SDs were estimated using the Wan et al. and Cochrane Handbook equations, respectively (285, 286). Equation **(2.1)** is the Wan et al. equation for estimating the mean, and equation **(2.2)** is the Cochrane Handbook equation for estimating the SD from the interquartile range (285, 286). The meta-analysis of the four studies revealed significant lower cefazolin concentrations at time of skin incision *(p* = 0.03*)*, thus favouring the 2 g dose (as a function of the lower value had an improved outcome) over the >2 g dose in the obese–pregnant women (BMI ≥30 kg/m^2) (I^2 = 31%) (Figure 2.5, A). A sensitivity analysis was conducted for this meta-analysis, and the results are reported in the next section (2.2.3.3.1.1Sensitivity-analysis).

Another meta-analysis was conducted of the initial cefazolin concentrations in the adipose tissue after the administration of 2 g of cefazolin to the obese–pregnant women (BMI 30–39.9 kg/m^2) versus the morbidly obese–pregnant women (BMI ≥40 kg/m^2). Two studies were eligible for inclusion in the analysis. In one study, the mean and SD were estimated using equations 2.1 and 2.2, respectively (14). Giving a 2 g dose of cefazolin to obese– vs. morbidly obese–pregnant women showed significantly lower initial cefazolin adipose tissue concentrations in the morbidly obese–pregnant women compared than the obese cohort *(p* = 0.005*; I^2* = 0%) (Figure 2.6, A).

In another subset of the meta-analysis, the administration of 3 g of cefazolin to obese–pregnant women (BMI 30–39.9 kg/m^2) and to morbidly obese–pregnant women (BMI ≥40 kg/m^2) was investigated. The mean and SD of the two included studies were estimated using equations 2.1 and 2.2, respectively (10, 14). The 3 g dose of cefazolin

led to a significant lower initial adipose tissue concentration in the morbidly obese–pregnant women than in the obese pregnant women (*p* = 0.006; I^2 = 70%) (Figure 2.6, B).

2.2.3.3.1.1 Sensitivity-analysis

A sensitivity analysis of the 2 g vs. >2 g cefazolin dose to obese-pregnant women was performed. Omitting the study of Stitely et al., which investigated the 2 g dose vs. the 4 g dose in obese–pregnant women, revealed an insignificant difference in the initial cefazolin adipose tissue concentration when 2 g vs. 3 g of cefazolin was given to the obese–pregnant women (*p* = 0.07; I^2 = 0%) (Figure 2.5, B). When the study of Maggio et al. was further deleted, the initial cefazolin adipose tissue concentration insignificantly differed in the obese–pregnant women after the administration of 2 g vs. 3 g of cefazolin (*p*=0.21; I^2=0%) (Figure 2.5, C). All in all, the clinical significant dependent on the target MIC and whether sufficient number of samples reached the cut-off MIC, which will be discussed in other sections (section 2.2.4.2).

$$X \approx \frac{q_1 + m + q_3}{3} \quad \textbf{(2.1)}$$

$$S \approx \frac{q_3 - q_1}{1.35} \quad \textbf{(2.2)}$$

Where X is mean, q_1 is the first quartile, q_3 is the third quartile, m is the median and S is the standard deviation

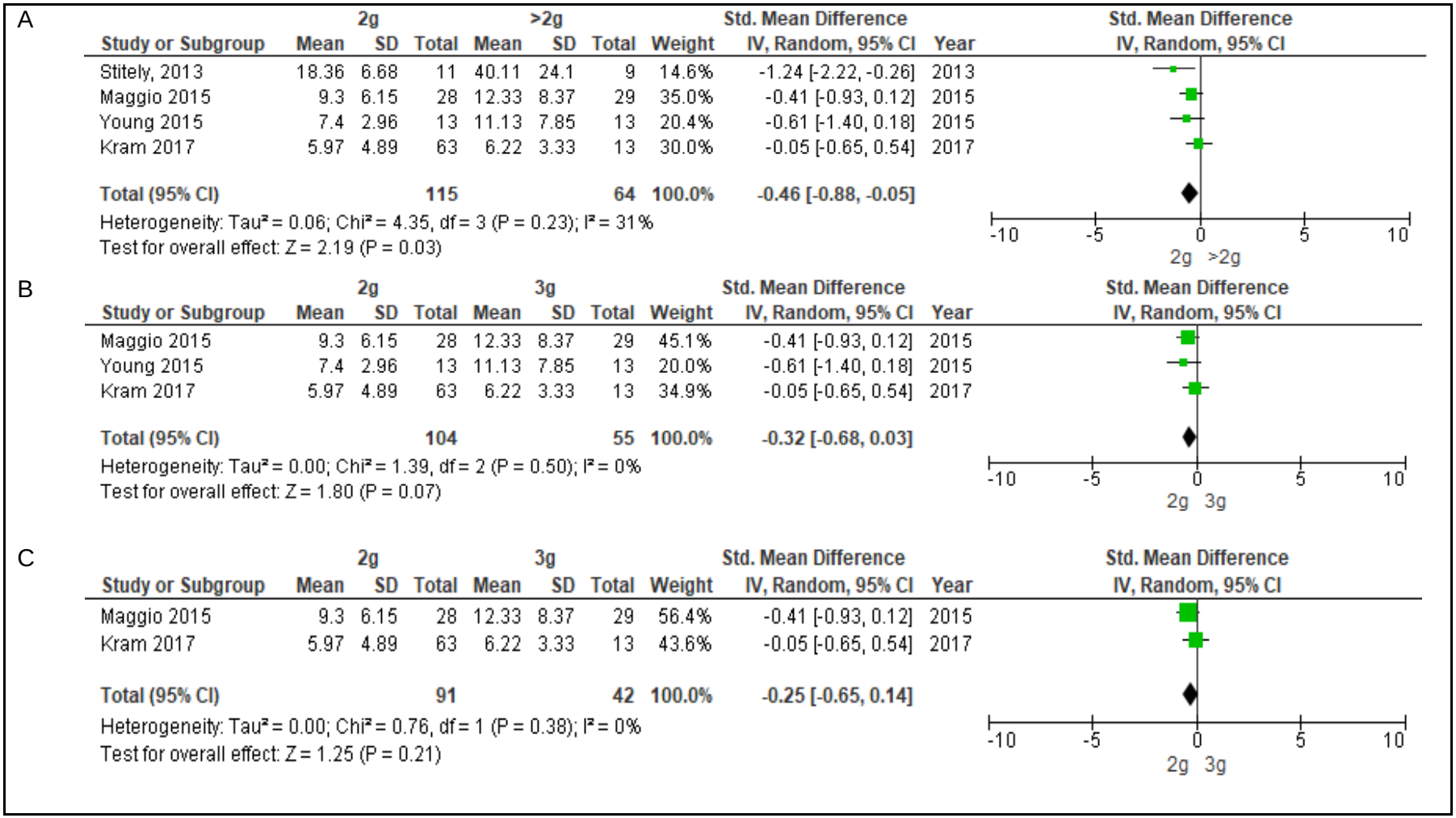


Figure 2.5: Meta-analysis of cefazolin initial total adipose tissue concentrations in obese–pregnant women (BMI ≥30 kg/m^2)
(A) Represent meta-analysis of obese–pregnant when given 2 g vs. >2g; (B) and (C) sensitivity-analysis of meta-analysis whereas different studies were omitted due to increased heterogeneity

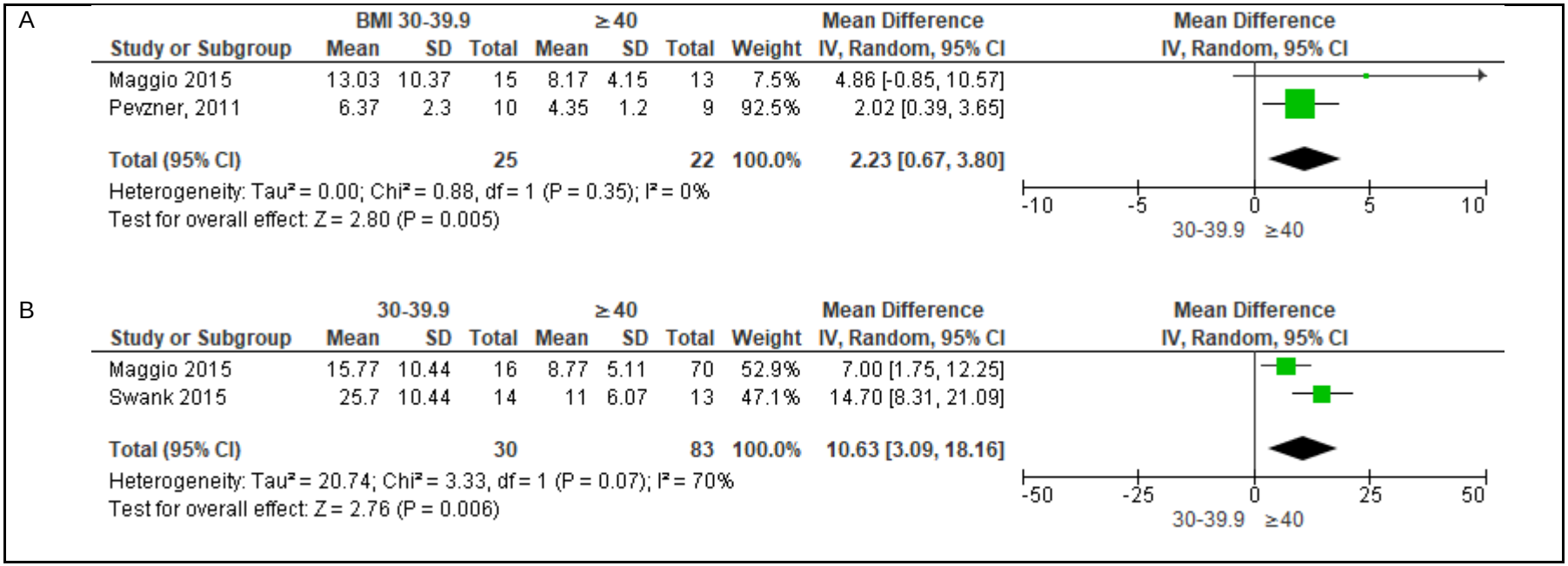


Figure 2.6: Meta-analysis of cefazolin initial total adipose concentrations in obese–pregnant women with a BMI of 30–30.9 kg/m^2 vs. ≥40 kg/m^2 when given (A) 2 g or (B) 3 g dose of cefazolin

2.2.3.4 Cefazolin post Caesarean section infection systematic review

After removing duplicates, fifty-two titles were assessed for eligibility. Twelve full texts were thoroughly reviewed, and four articles were included in the CFZ-INF systematic review (Figure 2.3). The studies' quality assessments and risks of bias were considered acceptable (Appendix: Table 7.11 and Table 7.12). Table 2.6 summarises the data extracted from the eligible studies in the CFZ-INF systematic review. Only one study was originally conducted to report SSI post-CS (287); the other studies aimed to measure cefazolin levels in obese–pregnant women, but their insufficient sample sizes affect their SSI detection. The study conducted focusing on CFZ-INF was a retrospective study (287); one study was a double-blind randomised controlled trial (14); one study was randomised (15); and one study was an observational study (284). In terms of the scope of the studies, one study reported the SSI when a dose of 2 g of cefazolin was given to pregnant women with an increasing BMI (<30 kg/m^2 vs. 30–39.9 kg/m^2 vs. ≥40 kg/m^2); two studies compared 2 g vs. 3 g of cefazolin given to obese–pregnant women and one study compared 2 g vs. 4 g of cefazolin given to obese–pregnant women (BMI ≥35 kg/m^2).

The highest SSI rate was 40% in the obese–pregnant women (BMI ≥40 kg/m^2) who were given 2 g of cefazolin (n= 5 women) and there was no SSI incidence in the obese–pregnant women with a lower BMI (<30 kg/m^2 or 30–39.9 kg/m^2) who were given a similar dose of cefazolin (n = 10 women in each group arm) (284). The next highest SSI rate was 13.1% in the morbidly obese–pregnant women who were given 2 g or 3 g of cefazolin (175 and 160 women, respectively) (287). One study did not report any SSI incidence in the obese–pregnant women given 2 g or 4 g of cefazolin (15). The

last study reported higher SSI rates of 6.89% vs. 3.57% in the obese group that was given 3 g vs. 2 g of cefazolin, respectively (14).

2.2.3.5 Cefazolin post Caesarean section infection meta-analysis

A meta-analysis of three studies that reported >2 g vs. 2 g of cefazolin given pre-CS to obese–pregnant women were conducted. Figure 2.7 (A) shows a forest plot of the non-significant differences in the SSI rates in the obese–pregnant women when given 2 g vs. >2 g of cefazolin pre-CS (P = 0.78, I^2=0%). A sub-group analysis of studies reported 2 g vs. 3 g was conducted (Figure 2.7, B). The SSI incidence insignificantly differed when 2 g vs. 3 g of cefazolin was given to obese–pregnant women.

Table 2.6: Summary of extracted data from cefazolin post caesarean section infection systematic review.

	Type of follow up	Duration of follow up	Definition of SSI by authors	Time of cefazolin administration	Dose (g)	BMI kg/m²(range)	Participants developing SSI (Total Number of Participants)	%
Pevzner et al., 2011	Chart review	6 weeks postpartum	Not defined	Preoperative 30–60 min before skin incision	2	<30	0(10)	0
					2	30–39.9	0(10)	0
					2	≥40	2(5)	40
Stitely et al., 2013	Review of medical record at 6–8 weeks postpartum; if participants had no medical documentation of postpartum visit, telephone contact was made to obtain information	6–8 weeks postpartum	As defined by National Healthcare Safety Network criteria (288)	Preoperative, a mean of 12 min before skin incision	2	46.02	0(11)	0
					4	43.23	0(9)	0
Ahmadzia et al., 2015	Retrospective review of chart and medical record	30 days postpartum	as defined by CDC[a]	Preoperative	2	49.9 (46.8–54.2)	23(175)	13.1
					3	53 (49.6–56.9)	21(160)	13.1
Maggio et al., 2015	review of medical record and participants contact at 6–8 weeks post CS	6–8 weeks postpartum [b]	Not defined	Preoperative, 22 min before skin incision	2	38.9 (35.4–45.6)	1(28)	3.57
					3	39.3 (36.7–44.8)	2(29)	6.89

CS: caesarean section; CDC: Centers for Disease Control and Prevention; SSI: surgical site infection.

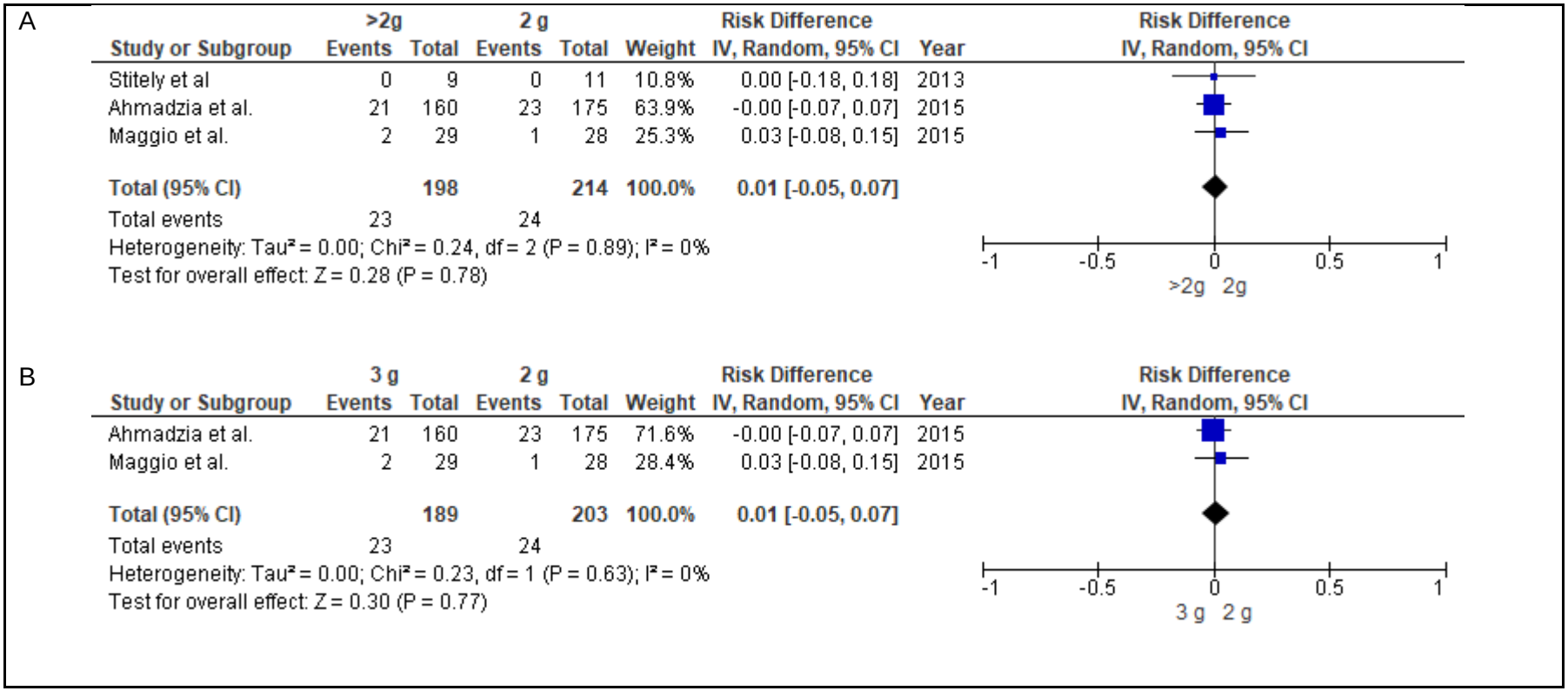


Figure 2.7: Meta-analysis of studies reported 2 g vs. higher doses of cefazolin initial total adipose concentrations in obese–pregnant women

2.2.4 Discussion

2.2.4.1 Cefazolin pharmacokinetics systematic review

In contrast to the dearth of published research on cefuroxime, there is considerable evidence to evaluate the use of cefazolin in pregnant women requiring CS. Nevertheless, the optimal doses of cefazolin that can be administered to obese–pregnant women is still a subject of controversy. This is due to the targeted MIC of most pathogens that cause infection post-CS. MICs of 2, 4, and 8 mg/L or µg/g have been reported (13, 14, 81).

One of the excluded studies on CFZ-PK fulfilled the eligibility criteria, but it was excluded due to the inability to extract the cefazolin levels from the abstract's Figure (289). The authors of this study investigated only the cefazolin levels in the maternal blood and the umbilical cord blood; cefazolin adipose tissue was not reported in the abstract.

A relatively higher total adipose cefazolin concentration at time of skin closure than time of skin incision was observed in certain obese–pregnant women (BMI ≥ 30 kg/m^2) groups who were given either 2 g or >2 g of cefazolin (Table 2.3). This can be explained by the differences in the time of cefazolin administration to the initial skin incision reported; i.e., the time to the maximum adipose tissue concentration was less than the time from cefazolin administration to the skin incision. All the studies reported administration of cefazolin 30–60 min pre-CS, except in Young et al. (within 30 min, with the average time from the administration to fascial entry, 3–4 min) and Stitely et al. (the average time from the cefazolin administration to the skin incision was 12 min) (15, 16).

Due to probable skewed data of cefazolin concentrations, most of the cefazolin concentrations data were reported as medians and interquartile ranges in the eligible studies for the CFZ-PK systematic review. In the current CFZ-PK analysis and subgroup analysis, the average medians were calculated and are presented in Table 2.4 and Figure 2.4. Although this is not an optimal statistical practice, these average medians give an empirical idea of combined limited information of interest. This was not the case in the CFZ-PK meta-analysis, as means were predicted from the medians (equation 2.1).

2.2.4.2 Meta-analysis of cefazolin concentrations in adipose tissue and dealing with missing data

The current meta-analysis of four studies showed a significantly higher cefazolin adipose tissue concentration at the time of the adipose opening when a dose of >2 g vs. 2 g of cefazolin was given to obese pregnant women (Figure 2.5, A). The between-study heterogeneity is considerably unimportant to moderate heterogeneity (I^2 = 31%)(286). In general, reasons for detected heterogeneity can be clinical, methodological and/or statistical differences between the studies (286). In the current meta-analysis (Figure 2.5, A), all the studies compared 2 g to 3 g of cefazolin, expect for one study that compared 2 g to 4 g (15). Another variance between the studies is the differences in the time from the cefazolin administration to the time of the adipose sample collection (initial), which was discussed in section 2.2.4.1. Finally, in Kram et al., obese class I and II pregnant women (BMI 30–39.9 kg/m^2) were given 2g of cefazolin, whereas obese class III pregnant women (BMI ≥40 kg/m^2) were given 3 g of cefazolin.

After a sensitivity analysis was conducted (Figure 2.5) by sequentially deleting the study of Stitely et al. (B) and then the study of Young et al. (C), the heterogeneity became insignificant (I^2 = 0%). However cefazolin adipose tissue concentrations at time of skin incision were statistically insignificant when 2 g of cefazolin was given to obese–pregnant women compared to 3 g. Nevertheless, Kram et al. was included in both sensitivity analysis studies, where the discussed dosing strategy (2 g for obese classes I and II and 3 g for obese class III) possibly affected the insignificant outcome observed in the sensitivity analysis.

When the meta-analysis was stratified according to the BMI (Figure 2.6), where the obese–pregnant women with a BMI of 30–39.9 kg/m^2 vs. a BMI ≥40 kg/m^2 were given (A) a 2 g or (B) 3 g dose of cefazolin; the initial cefazolin adipose tissue concentrations significantly differ between obese groups (2 g: P = 0.005; and 3 g: P = 0.006). The heterogeneity was unimportant in the 2 g case (I^2 = 0%), but it was high in the 3 g case (I^2 = 70%) which represent possible substantial heterogeneity. By reviewing the studies' methodology, they had relatively similar sampling time; interestingly, the studies included in the lower heterogeneity meta-analysis (i.e. 2 g) used different analytical method to analyse cefazolin concentrations, whereas the studies that were included in the meta-analysis with higher heterogeneity (i.e., 3 g) used similar analytical methods. In this case, the between-studies heterogeneity can't be explained; often, a high heterogeneity indicates differences in the methods used (286).

Most of the studies included in the meta-analysis reported the median and the interquartile range rather than the means and SDs, which suggests that skewed data of cefazolin plasma and adipose tissue concentrations were observed in these studies. According to the Cochrane Handbook regarding dealing with missing data in

conducting a meta-analysis, (A) studies with missing data can be omitted from the analysis; (B) missing data can be imputed from related data in the study, or (C) missing data may be statistically calculated. Obviously, options A and B are unfeasible in the current CFZ-PK meta-analysis to retrieve the missing data, i.e., the means and SDs of cefazolin adipose tissue concentrations. Weir et al. had systematically reviewed available approaches to estimating the means and SDs (290). They performed different scenarios, from deleting a study or studies with missing data to estimating missing data. The overall conclusion was that estimating SDs from ranges preserves the precision and performs best compared to deleting the study with missing data. While estimating the means prevents loss of precision and shows the best performance compared to omitting a study or studies by using the median and interquartile range formula, superior results were observed when studies were omitted in some cases. In a practical session hosted by Cochrane Training and presented by the author above, the author had suggested the use of the Cochrane equation to estimate the means (equation 2.2) and the use of the Wan et al. equation to estimate the SD (equation 2.1) whenever medians and interquartile ranges are reported (285, 286, 290, 291). Thus, these methods were selected to estimate the means and SDs in the meta-analysis. Furthermore, Shi et al. developed a statistical method of reporting the extent of the skewedness of data when reporting medians and interquartile ranges, which are incorporated into a user-friendly online calculator (292, 293). The skewedness of datasets used for meta-analysis were investigated using the website; out of 10 datasets used to calculate the means and SDs, Shi et al. considered two datasets significantly skewed (10, 14).

A previous meta-analysis abstract by Maggio et al. showed sufficient coverage of 2 g cefazolin across classes I, II, and III obese–pregnant patients, which presented results according to a target MIC of 2 µg/g. All in all, the optimal measure to investigate the appropriateness of cefazolin (a time-dependent antibiotic, section 1.1.5.2) dosage in obese–pregnant women is not statistical investigation of the different cefazolin concentrations of 2 g and >2 g, but measuring the time of the cefazolin adipose concentration above the target MIC for the desired time (i.e., T>MIC). None of the included studies had investigated the T>MIC. However, in the 2 g obese–pregnant group, out of the nine data points of cefazolin adipose tissue concentrations at the time of skin closure (in means or medians) reported, 66.7% of the reported cefazolin concentrations were under the MIC of 8 µg/g, whereas in the >2 g dose group, out of the seven cefazolin time-concentration data reported at the time of skin closure, 42.9% were under the MIC of 8 µg/g (Table 2.3). All the reported means and medians of the plasma and adipose tissue concentrations were above the MIC of 4 mg/L and 4 µg/g. Nevertheless, the reported medians of the adipose concentrations in the included CFZ-PK studies suggest skewed data and variations of the reported adipose concentrations. Five of the CFZ-PK studies had reported percentages of cefazolin adipose tissue or blood samples with concentrations under a desired MIC (Table 2.5). In one study, the cefazolin adipose tissue samples (initial or closure) of the obese–pregnant women (BMI ≥40 kg/m^2) who received 2 g of cefazolin reportedly had concentrations that were 40% less than the MIC of 4 µg/g (284). In another study, 33.3% of the samples were under the MIC of 8 µg/g of obese group with a BMI 30–39.9 kg/m^2 who were given 2 g dose of cefazolin (14). In Young et al., 7.7% of the cefazolin adipose tissue samples were under the MIC of 4 µg/g in the obese–pregnant

women (BMI >30 kg/m^2, reported median 39.3 kg/m^2) who received 2 g of cefazolin; whereas it were 16.7% in the obese–pregnant (BMI >30 kg/m^2, reported median 39 kg/m^2) who received 3 g of cefazolin (16). In obese–pregnant with higher BMI (≥40 kg/m^2) who were given a 2 g dose of cefazolin, the samples of cefazolin adipose concentrations at time of skin incision and closure were under the MIC of 8 µg/g by 46.2% and 69.2%, respectively; whereas those of same obese group who received 3 g dose, 53.9% and 66.7% of the cefazolin adipose tissue samples were under the MIC of 8 µg/g at time of the skin incision and closure, respectively (14).

Current guidelines for cefazolin susceptibility testing identified a MIC breakpoint of ≤2 mg/L for *Enterobacteriaceae* and a MIC of ≤4 mg/L for *Staphylococcus spp.* as sensitive for cefazolin (81). Table 2.7 represent summary of the conclusion driven by reported cefazolin mean (or medians) and individual values reported under a targeted MIC adipose concentrations in studies included in CFZ-PK systematic review. Aiming for a MIC of 4 µg/g, a 2 g dose of cefazolin seems sufficient against common pathogens that cause SSI post-CS in obese–pregnant women with a BMI of 30–39.9 kg/m^2. However, data are insufficient to support the administration of a similar dose to obese–pregnant women with a higher BMI (≥ 40 kg/m^2) when a MIC of 4 µg/g is desired; whereas when aiming for the upper limit of MIC (8 µg/g), the dose of 3 g appears inadequate for obese–pregnant women with a BMI of ≥ 40 kg/m^2 to achieve an appropriate MIC at the site of the skin incision.

In cases where it is desired that the cefazolin adipose concentrations achieve a target MIC, it is crucial to investigate T>MIC. If a target MIC is achieved and not maintained for the duration of the CS, the optimal practice is to re-administer cefazolin than

increase the dose, due to the time-killing properties discussed in section 1.1.5.2. If a target MIC is not reached in the adipose tissue, a higher cefazolin dose can be suggested; but in this case, studies investigating cefazolin placental transfer are warranted.

Table 2.7: Dose efficiency in the highlight of reported means (or medians) and individual values of cefazolin adipose tissue concntrations

BMI	**30–39.9 kg/m²**		**>40 kg/m²**	
MIC	2	3	2	3
2 µg/g	Data Support this dose	Data Support this dose	Data Support this dose	Data Support this dose
4 µg/g	Data Support this dose	Data Support this dose	Inconclusive (Reported Means and medians were above the MIC; the reported individual of cefazolin adipose tissue concentrations under MIC was 40%)	Inconclusive
8 µg/g	Insufficient dose, may require higher or repeatable dose	Data Support this	Insufficient, may require higher or repeatable dose	Insufficient, may require higher or repeatable dose

2.2.4.3 Cefazolin post Caesarean section infection systematic review

To the best of our knowledge, the current CFZ-INF systematic review is the first systematic review to report studies that investigated SSI after the administration of cefazolin pre-CS to obese–pregnant women. Although four studies were included in the CFZ-INF systematic review, only one retrospective study was directed originally to

report incidence of SSI in obese–pregnant women (287). This study had the highest sample size among all the studies. The SSI rates reported in the former study in which obese–pregnant women were given 2 g vs. 3 g were comparable (13.1%); however, the 3 g dose group had a higher BMI than the 2 g dose group. From this study, it can be postulated that giving a decreased dose of 2 g of cefazolin to both groups might have resulted in non-comparable SSIs; i.e., a higher SSI rate in the obese–pregnant women with a higher BMI. All in all, the author considered the reported SSI incidence "too high" in terms of the consequent mortality and cost of management of the SSI (287). The study reported several limitations that included the possibility of a missing SSI due to a merely retrospective medical review with no participant contact; possibly different CS protocols of different centres that might have affected the SSI rate and might not have been reported in the study; and a sample size that was insufficient to report the intended 67% SSI reduction (287). Interestingly, in one study that compared the 2g and 3g cefazolin, the 3g-dose group had a higher SSI than the 2g-dose group; but this study reported higher inadequate cefazolin adipose concentration samples (samples of concentrations less than the MIC of 8 µg/g) in the 3g group compared to the 2g group (Table 2.5) (14). However, in another study, the levels of cefazolin adipose concentration of two participants who developed post-CS SSI (out of five recruited) of obese pregnant women (BMI ≥40 kg/m^2) were 2.7 µg/g and 2.9 µg/g at the initial skin incision and 3.8 µg/g and 2.8 µg/g at the time of skin closure when a 2g dose of cefazolin was given pre-CS (284). These data could support the augmented SSI preventive effect of selecting a target cut-point MIC of 4 µg/g rather than 2 µg/g; while the current data cannot support the same for a MIC of 8 µg/g.

2.2.4.4 Cefazolin post Caesarean section infection meta-analysis

The current meta-analysis and sub-group analysis showed no significant difference in SSI incidence post-CS when given 2g vs. 3 g or > 2g of cefazolin to obese-pregnant women. However, the studies' limitations discussed in section 2.2.4.3 possibly affected the observed insignificant results.

In 2021, two systematic reviews and meta-analyses were published regarding SSI. One focused on SSI generally following CS in obese–pregnant women (regardless of the type or dose of the antibiotic given) (294), while the other focused on SSI when cefazolin was given to pregnant women in general (obese and non–obese) (295). None of the reviews had a scope similar to that of the current meta-analysis of CFZ-INF studies.

From the viewpoint of the SSI incidence and the recruited CFZ-INF studies, the 2 g dose seems insufficient for obese–pregnant women with a BMI ≥40 kg/m^2.

2.2.4.5 Study limitations

In the CFZ-PK systematic review, it was desired that the obese groups be divided into those with a BMI of 30–39.9 and those with a BMI ≥40 kg/m^2. There was a slight risk of the two groups overlapping with the obese groups in the study of Swank et al. (24 patients with a BMI of 30–40 kg/m^2 and 23 patients with a BMI >40 kg/m^2). Additionally, in the results synthesis presented in Table 2.4, , one means reported by Stitely et al. was added to the median of the cefazolin concentrations in the 2g group. This is not an optimal practice; however, this would not significantly affect the results where the conclusions were mainly driven by other results synthesis.

2.2.5 Conclusion

Different studies have investigated cefazolin levels in obese–pregnant women at the time of CS. Due to the diverse MIC target cut-offs, and small sample size led to discrepancy of recommendation in these studies. Targeting a higher cefazolin adipose tissue MIC will require either a 3 g dose of cefazolin or re-dosing when a longer CS is expected or both, especially with obese–pregnant women with a BMI of ≥40 kg/m^2.

2.3 Narrative review of eligible studies published since last update of systematic review

In July 2021, three databases (Cochrane, MEDLINE, and Embase) were updated to explore eligible studies recently published on CFX-PK, CFX-INF, CFZ-PK, and CFZ-INF. Only three databases were updated due to Ph.D. time limitations, and these databases were selected due to their robust retrieving results from saved searches compared to other databases.

2.3.1 Cefuroxime pharmacokinetics and post caesarean section infection

For CFX-PK, no study has evaluated the cefuroxime levels in obese–pregnant women, and neither there was no update to of CFX-PK in non–obese pregnant women.

For CFX-INF, a recent study had investigated the infection rate post-CS when the patients were given 2 g of cefuroxime at different timing of surgery; one of the group had a 2 g cefuroxime 30–45 min before CS (296). The full article was not accessible. All in all, the study reported the superior effect of single dose cefuroxime given 30 min before CS compared to other tested dosing intervals in terms of infection prevention and reduction of the length of the hospital stay (296).

2.3.2 Cefazolin pharmacokinetics and post caesarean section infection

A recent study was considered eligible for inclusion in the CFZ-PK systematic review (297). The study evaluated the use of 2 g (only one patient received 3 g) of cefazolin in 12 obese–pregnant women (BMI >35 kg/m^2) at the time of elective CS. Furthermore, the data were utilised to simulate cefazolin disposition in obese–pregnant women given different doses of cefazolin using Pmetrics. The author reported the probability of target attainment at a *f*T>MIC of 2 µg/g for a 3 h. The total cefazolin plasma concentrations were above the MIC of 8 mg/L for the 180 min sampling duration, and the minimum and maximum free cefazolin plasma concentrations were 7.7 mg/L and 73.1 mg/L, respectively. The free median adipose tissue interstitial fluid (ISF) remained above the MIC of 2 µg/g at each sampling point; however, two participants did not maintain cefazolin adipose concentrations above 2 µg/g for the 150 min sampling duration. Eley et al. suggested re-administration of cefazolin at 2 hours when the duration of the CS might exceed 3 hours for patients weighing 90–150 kg to maintain a free ISF adipose tissue concentration >2 µg/g. The authors accredited that higher MIC targets would require different doses. There was no comparison in the study; and thus the current meta-analysis of CFZ-PK would be unchanged; as would the pooled descriptive analysis because eleven participants received 2 g of cefazolin and one received 3 g; and the reported adipose cefazolin concentrations were of unbound ISF concentrations (i.e. cannot be added to the comparison of total cefazolin adipose concentration meta-analysis) (297). The cefazolin re-dosing suggestion of Eley et al. supports the conclusion in section 2.2.5.

Regarding the update search of CFZ-INF, no study was considered eligible; however, two recent studies have investigated SSI when giving cefazolin. A retrospective study

demonstrated no superior effect of a 3 g dose of cefazolin compared to 2 g in decreasing post-CS SSI rates (both non–obese and obese–pregnant) at time of CS (298). However, the study reported significant differences in the BMI (and other demographic characteristics) in the 2 g vs. 3 g doses; the participants who received the 3 g dose were less obese, had lower estimated blood loss, and were less diabetic. Generally, the study did not report SSI rates specifically for obese–pregnant women, thus, illegible. Another recent randomised study compared the SSI rate when using staples vs. suture in obese–pregnant women (BMI ≥40 kg/m^2) (299). However, 93% of the women received cefazolin pre-CS, 17% co-administered azithromycin and 12% had vaginal antisepsis with iodine. Thus, the study was illegible (299).

Chapter 3: Partial Validation to Quantify Cefuroxime in Blood and Adipose Tissue using High Performance Liquid Chromatography

The aim of this chapter was to provide an analytical method to measure cefuroxime in plasma and adipose tissue samples from non obese and obese–pregnant women undergoing CS.

3.1 Introduction

Cefuroxime levels in plasma and subcutaneous adipose tissue have not previously been investigated in obese–pregnant women requiring caesarean sections (300). A protocol for a pilot study to investigate cefuroxime concentrations in plasma and subcutaneous adipose tissue of obese–pregnant women requiring caesarean sections was formulated and submitted for review (IRAS244803 - RG_18-237), later it was approved (chapter 4). This pilot study will permit transfer of plasma and adipose samples from women undergoing CS to be provided to the University of Birmingham for analysis to support this research project. A validated analytical method was required to measure cefuroxime in plasma and adipose tissue. Plasma and adipose tissue can be analysed, to quantify cefuroxime concentration through different methods: reverse phase high-performance liquid chromatography (RP-HPLC) is a commonly used method (110). Different sample preparations have been described, to analyse cefuroxime and similar compounds (cefazolin) in biological samples (13, 110, 301). The development and validation of a method to analyse compounds in biological samples, needs to be fully validated (new compound or new method), partially validated (previously described protocol in the literature with minor changes in method

or sample processing procedure) or cross-validated (validation of similar methods across different laboratories) (302).

Previously described methods reported as validated method to measure cefuroxime concentrations in human blood and subcutaneous adipose tissue was selected and minor alterations were applied (due to different HPLC machine available at Pharmacy laboratory at University of Birmingham and different column); thus at least a partial validation was required (Table 3.1) (13, 110). According to Food and Drug Administration (FDA) guidelines, "one intra-assay accuracy and precision determination to a nearly full validation" can be investigated through partial validation (302). In the context of this PhD timeframe, linearity, carry-over, detection and quantification limits, stability, accuracy and selectivity were investigated. The acceptance criteria for these tests were similar to both the FDA's and the European Medicine Agency's (EMA) guidelines; however, EMA guidelines were more precise in describing the practical handling of this data (303, 304).

Table 3.1: Previously described method and sample preparation to quantify cefuroxime in human plasma and adipose tissue and changes made to the current method

	Previously described	Changes made
HPLC conditions (110)		
HPLC machine	Agilent 1100	Agilent 1260
Column	C_{18} Discovery®; Supelco, Bellefonte, PA	C_{18} InfinityLab Poroshell
Mobile phase	0.05M potassium dihydrogen phosphate buffer and acetonitrile (90:10)	0.05M potassium dihydrogen phosphate buffer and acetonitrile (85:15)
Flow rate	2mL/min	1.2 ml/min
Injection volume	20µL	20 µL
Sample preparation of adipose tissue (13, 305)		
Sample treatment	Fat were finely sliced	fat tissue was homogenised
Addition of liquid	PBS added at a mass of double the fat weight, extracted at 4°C for 5 h	PBS added at a mass of double the fat weight
Sonication	samples sonicated for 10 min at 35% power (5 seconds pulse) followed by cooling (10 seconds)	samples sonicated for 10 min at 35% power (5 seconds pulse) followed by cooling (10 seconds)
Centrifugation	centrifuged at 5000g	centrifuged for 30 min at 14000 speed

PBS Phosphate buffer saline

3.2 Aims and objectives

3.2.1 Aims

To investigate and conduct a partial validation of previously described methods reported as validated methods quantifying cefuroxime in human blood and subcutaneous adipose tissue with minor changes (Table 3.1). (13, 110).

3.2.2 Objectives

1- Test the **linearity** of cefuroxime concentrations using AUC.

2- Test the **carry-over** effect to investigate contamination of blank samples by the analytes (re-appearance of analyte peak).
3- Investigate the **lower limit of detection** (LLOD) and **lower limit of quantification** (LLOQ) in an aqueous solution, blood and adipose tissue.
4- Examine the **matrix effect** (blood and adipose tissue) on cefuroxime chromatograms.
5- Test the **stability** of cefuroxime (in terms of peak shape by means of diode-array detector system peak purity investigated, retention time (RT) and quantity) in aqueous solution, blood and adipose tissue, after sample processing and the extraction of cefuroxime from animal blood and adipose tissue under relevant storage conditions.
6- Develop a sample preparation protocol, to obtain an accepted **recovery** percentage of cefuroxime when spiked to blood and fat tissue.
7- Select an **internal standard** (IS), to optimise the quantification of cefuroxime in blood and adipose tissue.
8- Investigate the cefuroxime octanol/water partition coefficient (**LogP**).
9- Test the **selectivity** of this method, by using different compounds (caffeine, paracetamol, omeprazole, ibuprofen).

3.3 Methods

3.3.1 Optimising HPLC conditions

3.3.1.1 Chemicals

Cefuroxime (Cefuroxime sodium: SIGMA), vancomycin (Vancomycin HCL: TOKU-E), cefazolin (Cefazolin Sodium: Acros Organics 98%), ceftriaxone (Ceftriaxone Sodium: Sigma-Aldrich) indapamide (Alfa Aesar), phenacetine (MedChemExpress), potassium

dihydrogen phosphate (Alfa Aesar) phosphate buffer saline (SIGMA), acetonitrile (Sigma-Aldrich), 1-Octanol (Merck).

3.3.1.2 Apparatus

An Agilent 1260 series high-performance liquid chromatography with diode-array detector system (HPLC-DAD) was used. Data obtained was analysed using Chromatography Data System (OpenLab Software, Agilent).

3.3.1.3 Chromatographic conditions

A C18 Column (InfinityLab Poroshell 120 EC-C18, 3.0 x 150 mm, 2.7 µm) connected to a SecurityGuard column (SecurityGuard™ cartridges for C18 HPLC column 4.0 x 3 mm) were used. Different detection wavelengths reported in literature were tested at 274 nm (301), 275 nm (9, 306) and 280 nm (110) to record the most effective response. The flow rate was set at 1.2 ml/min (110), injection volume was 20 µL (110). All samples were filtered before injection using a 13 mm polytetrafluoroethylene (0.45 µm) syringe filter (Fisher brand). The sample run time was 30 min.

3.3.1.4 Mobile phase selection

Different mobile phase solutions, proportions and pH adjustment were tested, to produce an efficient cefuroxime peak shape and a clear chromatogram that is clearly differentiated from the blank and tissue noise peaks. Cefuroxime solutions (100 µg/ml) were analysed using different mobile phase solutions in which all were isocratic:

1- A mixture of **0.05 M potassium dihydrogen phosphate buffer (**pH=4.7**): acetonitrile**. Different proportions of acetonitrile were used at 30%, 20%, 15% and 10%.

2- A mixture of **0.05 M potassium dihydrogen phosphate buffer (pH adjusted to 2.5): acetonitrile**. Different proportions of acetonitrile were used at 30%, 20%, 15% and 10%.

3- A mixture of methanol: water. Different proportions of methanol were used at 25% and 60%.

3.3.2 Linearity (Calibration curve)

Linearity was tested in triplicate and a calibration curve was established using different cefuroxime concentrations in a solution (water: Acetonitrile 50:50) (0.5, 1, 10, 25, 50, 100 and 150 µg/ml). Excel 2016 was used, to fit a linear least square regression to the generated data. Cefuroxime sodium is freely soluble in water (100 mg/ml) (307).

3.3.3 Carry-over

A high concentration (150 µg/ml) of cefuroxime solution (acetonitrile: water 40:60), was injected in HPLC, followed by a blank sample (acetonitrile: water 50:50), to test the effect of carry-over.

3.3.4 Lower limit of detection and lower limit of quantification in solution

A series of low cefuroxime concentration solutions (0.0001–0.4 µg/ml of water: Acetonitrile 60:40) were analysed, to investigate the LLOD and LLOQ of cefuroxime in the solutions using the Visual Evaluation Method (308). In this method, low concentrations of analyte were injected into HPLC starting with the lowest concentration. The first analyte peak that appears in the chromatogram was the LLOD concentration, while the first quantification of the analyte peak (i.e., AUC) was the LLOQ concentration. (308). Additionally, LLOD and LLOQ were calculated from a

calibration curve using equations 3.1 and 3.2, to compare the measured and calculated results (308).

$$\text{Detection limit} = 3.3\left(\frac{\text{standard deviation of the response (SD)}}{\text{slope of the calibration curve (S)}}\right) \quad \textbf{(3.1)}$$

$$\text{Quantification limit} = 10\left(\frac{\text{SD}}{\text{S}}\right) \quad \textbf{(3.2)}$$

Where SD is the standard deviation of the AUC of the lowest concentration tested.

3.3.5 Matrix effect

Subcutaneous adipose tissue (pig adipose tissue purchased from Birmingham Indoor Market, B5 4RW) was spiked (a known concentration of cefuroxime solution was added to a known weight of adipose tissue using a pipette) with a cefuroxime solution (50 µg/ml of water: Acetonitrile 60:40 solution), to test the matrix effect on cefuroxime chromatograms.

3.3.6 Accuracy

3.3.6.1 Using phosphate buffer saline

Recovery percentages of high, medium and low cefuroxime concentrations (200 µg/ml, 50 µg/ml and 10 µg/ml of water: Acetonitrile 60:40 solution) after spiking subcutaneous adipose tissue, were tested and compared to quality control samples (samples of the same concentration of cefuroxime solution used to spike the tissue). A known adipose tissue weight was spiked directly with cefuroxime solution. Then, phosphate buffer saline (PBS) was added (at a mass of double the fat weight) (305). The fat tissue was homogenised (Homogeniser 150 Fisherbrand with stainless steel probes 7 mm). The samples were then sonicated (Sonicator with Probe, Fisherbrand) for 10 min at 35% power (5 sec pulse), followed by cooling (10 sec) (13). The samples were then

centrifuged (VWR Mega Star 600R) for 30 min at 14000 speed using a graduated filter Eppendorf (0.22 µm cellulose acetate). Supernatants were injected into HPLC. The remaining adipose tissue in the Eppendorf filter was spiked with PBS (0.5 ml) and centrifuged again, to extract all cefuroxime and supernatant injected into HPLC. The extraction process was not controlled, analyte extraction was dependent on the collection of supernatants. The sum of all AUC represented the concentration of cefuroxime added. The acceptance criteria for an accurate method was selected as a recovery percentage within ± 15% of the quality control sample (a sample of a known concentration of cefuroxime solution, not in the matrix) (308).

3.3.6.2 Using Methanol as extraction solvent

A trial using methanol as a liquid extractor was carried out, to improve the partitioning of cefuroxime (200 µg/ml) from the adipose tissue to the liquid phase. The method is the same as described above (Using phosphate buffer saline), although methanol was used instead of PBS.

3.3.6.3 Testing the effect of sample preparation (adipose tissue accuracy) on cefuroxime chromatogram

As the extraction of cefuroxime analyte had various steps, the analyte could be lost and/or degraded. A trial to explore the effect of sample preparation on cefuroxime quantification and chromatograms was carried out in the solution (not the matrix), to compare the results with those of the quality control sample (the sample was not processed). The cefuroxime solution and PBS were homogenised for 3, 5 and 10 min, and then these samples were analysed. Other samples of the cefuroxime solution and PBS were homogenised for 3, 5 and 10 min, and then sonicated as described; these

samples were then analysed. Lastly, to test the loss of cefuroxime during centrifugation, the cefuroxime solution and PBS were homogenised for 3, 5 and 10 min, and then sonicated and centrifuged once as described above; these samples were then analysed. PBS was added to the Eppendorf filter and centrifuged again; these samples were subsequently analysed. This process was repeated for a last time (third run) and the samples were analysed.

3.3.7 Stability

3.3.7.1 Stability of cefuroxime solution

A quality control sample of the cefuroxime solution was prepared (stock solution) and analysed on day 1; the stock solution was stored in amber glass at 4°C. Samples from the stock solution were analysed on day 7 and day 14.

3.3.7.2 Stability of cefuroxime in frozen animal adipose tissue (Freeze-thaw stability)

The aim of this experiment was to test the effect of adipose tissue and its constituents on the cefuroxime chromatogram. Different subcutaneous adipose tissue sections were spiked with low, medium and high cefuroxime concentrations (10, 50 and 200 µg/ml); the spiked adipose tissues (low, medium and high cefuroxime concentrations) were processed and analysed on day 1, as described in Accuracy and Using phosphate buffer saline section 3.3.6.1. Other spiked adipose tissues were stored in a −80°C freezer. On day 7 of storage, 3 different spiked adipose tissues (low, medium and high cefuroxime concentrations) were processed and analysed, while another 3 different spiked adipose tissues (low, medium and high cefuroxime concentrations) were processed and analysed on day 14 of storage in a −80°C freezer.

3.3.7.3 Stability of cefuroxime samples after extraction from animal adipose tissue (Extracted or processed sample stability)

The spiked adipose tissues with cefuroxime (low, medium and high concentrations) were processed and analysed on day 1, as described in the Accuracy and Using phosphate buffer saline sections (3.3.6.1). The extracted samples were stored in amber HPLC vials at 4°C. These samples were then analysed again on day 7 and day 14.

3.3.8 Octanol/water partition coefficient (LogP) of cefuroxime: Shake-flask method

Cefuroxime was dissolved in 100 ml of water (200 µg/ml); 3 ml of this solution was added to 3 ml of n-octanol and that solution was shaken for 24 hours at 4°C. Then, the two phases were allowed to separate for 24 hours and after separation the cefuroxime content was measured by HPLC in both the water and the n-octanol. The partition coefficient (LogP) of the cefuroxime was calculated using equation 3.3 (309).

$$\mathrm{LogP_{oct/wat}} = \mathrm{Log}\left(\frac{[\text{Solute in octanol}]}{[\text{Solute in water}]}\right) \quad \textbf{(3.3)}$$

3.3.9 Selection of internal standard

The cefuroxime solution samples were analysed in the presence of different compounds (cefazolin, ceftriaxone, indapamide, vancomycin and phenacetine), to select is the most appropriate internal standard.

3.4 Results

3.4.1 Optimising HPLC conditions

An efficient separation in terms of peak shape (symmetrical, no tailing) and convenient retention time (RT ≤20 min) was achieved when the mixture of 0.05 M potassium dihydrogen phosphate buffer (pH adjusted to 2.5): acetonitrile (85:15) was used as a mobile phase (Figure 3.1, G). This was selected as a mobile phase for all further experiments.

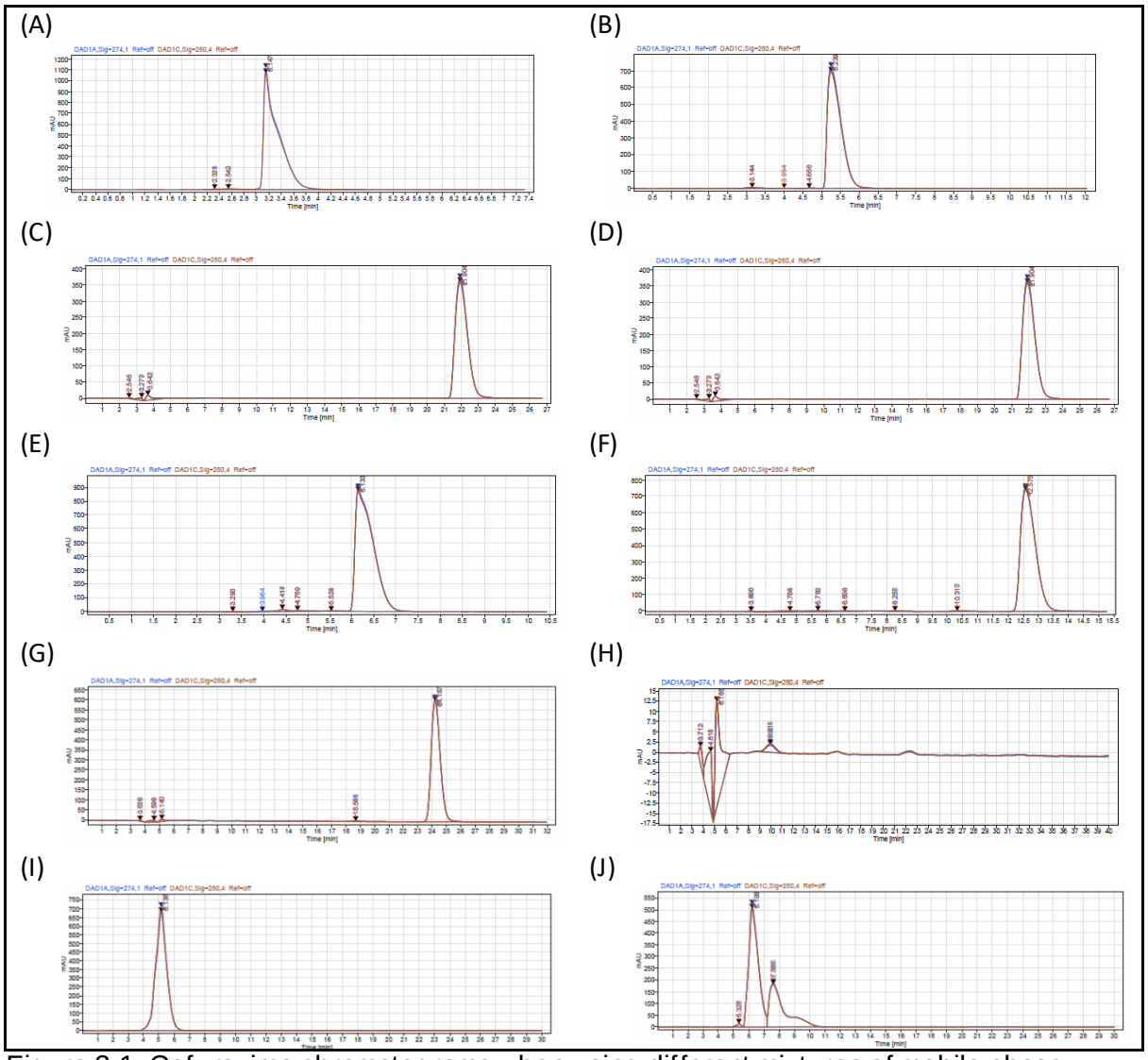


Figure 3.1: Cefuroxime chromatograms when using different mixtures of mobile phase
A mixture of 0.05 M potassium dihydrogen phosphate buffer: acetonitrile with different proportion of acetonitrile were used (A) 30%, (B) 20%, (C) 15%, (D) 10%; and same mixture

with adjusted pH (2.5) when different proportion of acetonitrile were used (E) 30%, (F) 20%, (G) 15% and (H) 10%; and when a mixture of methanol: water were investigated with different proportion of methanol were used (I) 60% and (J) 25%.

3.4.2 Linearity (Calibration curve)

The generated AUC showed linearity over the tested cefuroxime concentrations from 0.5 to 150 µg/ml, Y = 239.62x - 290.19 and R^2 = 0.9995, where X is cefuroxime concentration, Y is AUC and R is the correlation coefficient (Figure 3.2).

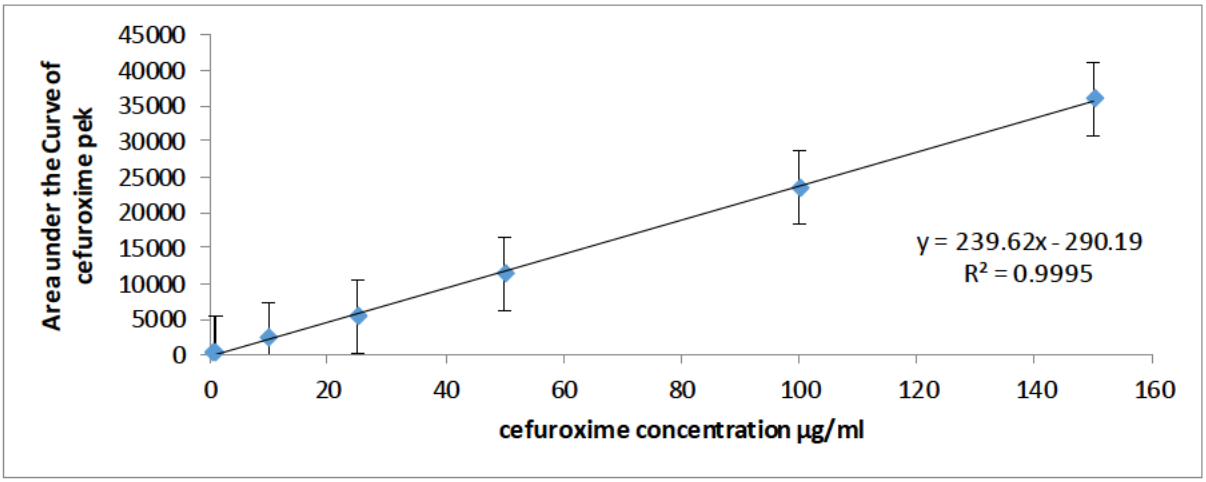


Figure 3.2: Calibration curve of cefuroxime solution using the mobile phase 0.05 M potassium dihydrogen phosphate buffer (adjusted to pH 2.5): acetonitrile (85:15) at a detection wavelength of 274 nm.

3.4.3 Carry-over

The analysis of a blank sample after an analysis of high cefuroxime concentration showed no interference.

3.4.4 Lower limit of detection and lower limit of quantification in solution

The LLOD and LLOQ of cefuroxime in solutions when analysed by HPLC (Visual Evaluation Method) were 0.1 µg/ml and 0.3 µg/ml, respectively. However, the calculated LLOD and LLOQ were 0.18 µg/ml and 0.55 µg/ml, respectively. Notably, the LLOQ calculated using the Standard Deviation of the Response and the Slope method

is comparable to the LLOQ reported in the validated HPLC method (0.5 µg/ml), the study did not report the LLOD (110).

3.4.5 Matrix effect

There was no matrix interference on cefuroxime peak (Figure 3.3).

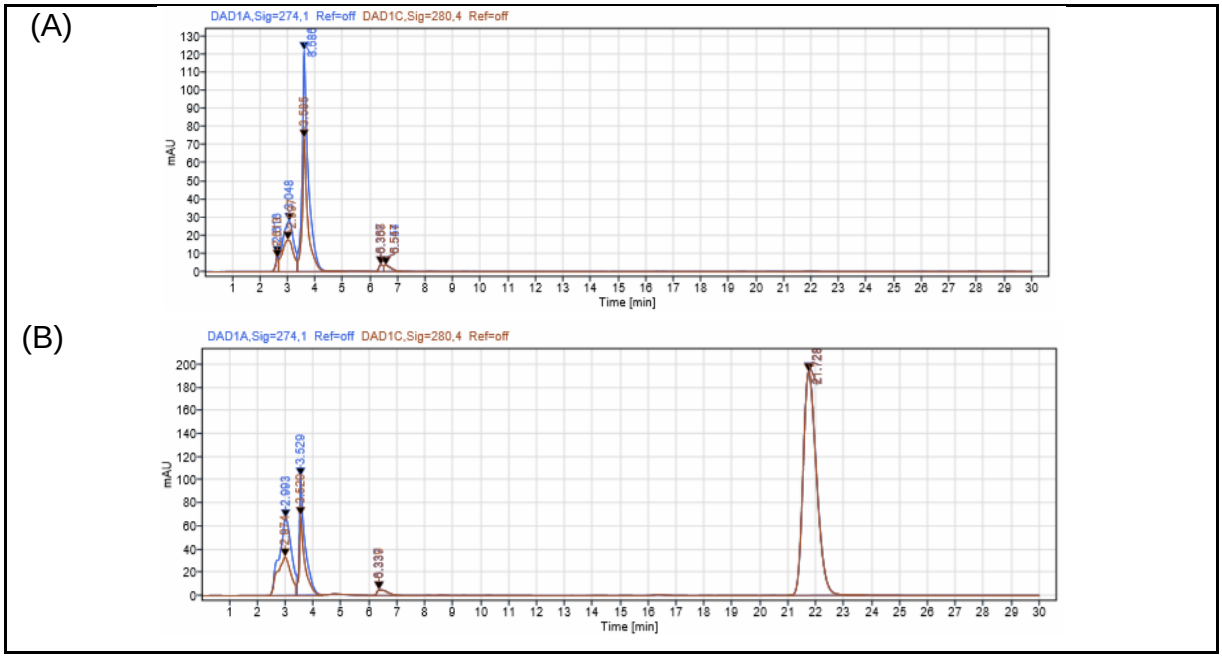


Figure 3.3: Adipose tissue effect on Cefuroxime chromatogram
(A) Analysed sample of adipose tissue without cefuroxime, (B) analysed sample of adipose tissue with cefuroxime

3.4.6 Accuracy

3.4.6.1 Using phosphate buffer saline

The recovery percentages of cefuroxime were 122.80%, 107.13% and 95.02% for low, medium and high concentrations, respectively, compared to the quality control samples.

3.4.6.2 Using Methanol

The recovery percentage of cefuroxime was 86.84%. However, methanol had an effect on cefuroxime peak (fronting and shoulder peaks) (Figure 3.4).

3.4.6.3 Testing the effect of sample preparation (adipose tissue accuracy) on cefuroxime chromatogram

The sample preparation and cefuroxime extraction from adipose tissue, did not affect cefuroxime peak shape. A cefuroxime chromatogram showed a related substance peak, as explained in the Discussion section 3.5.

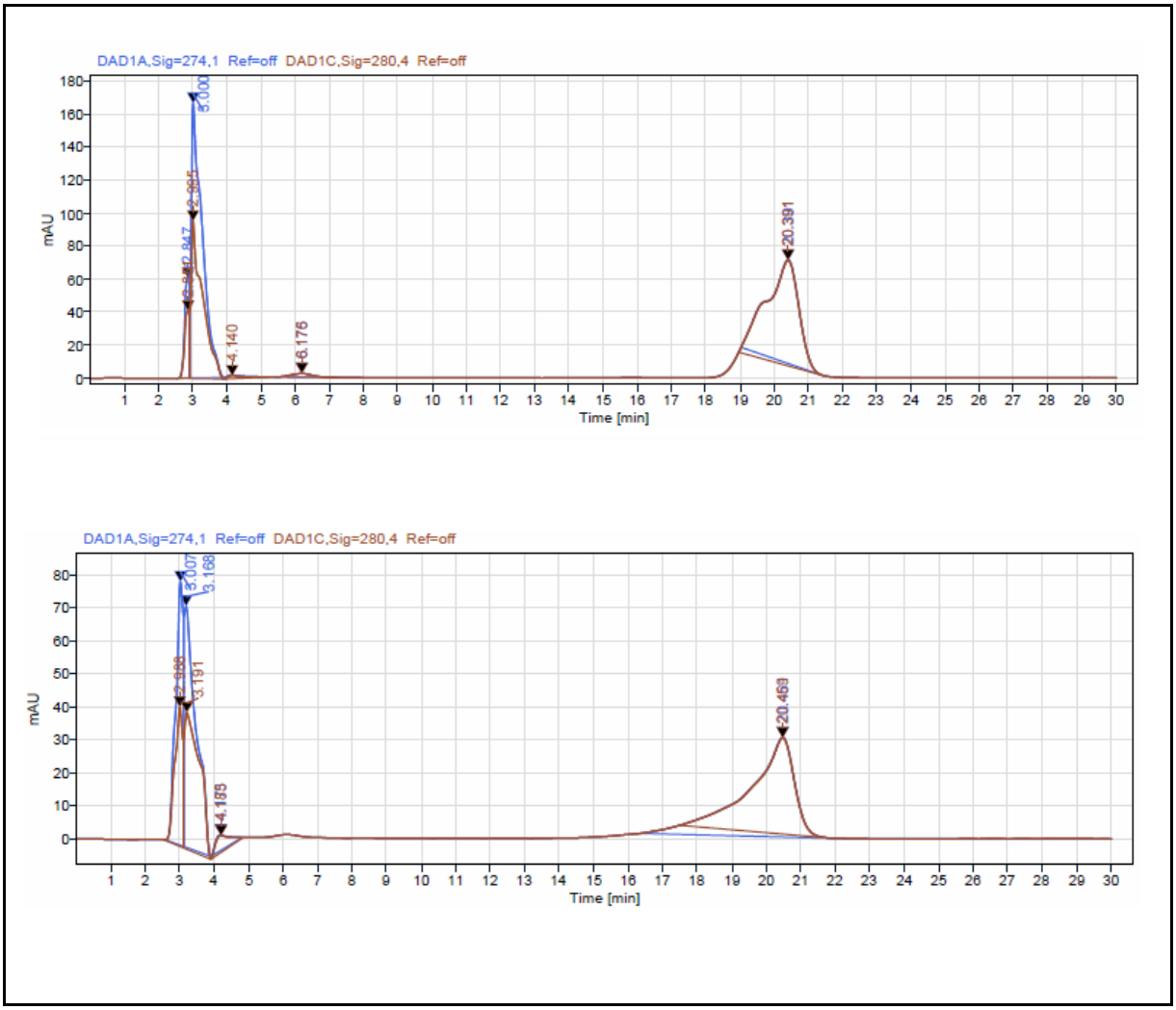


Figure 3.4: Effect of methanol on cefuroxime peak when used as liquid extraction from adipose tissue

3.4.7 Stability

The stability of the cefuroxime solutions and the stability of the cefuroxime in frozen animal adipose tissue were within the acceptance criteria; however, the extracted cefuroxime sample stability was above the accepted range for high and medium cefuroxime concentrations (129.84% and 118.94%, respectively). The cefuroxime chromatograms showed related substance peaks (degradation or present in cefuroxime isoforms) in all stability studies (Figure 3.5).

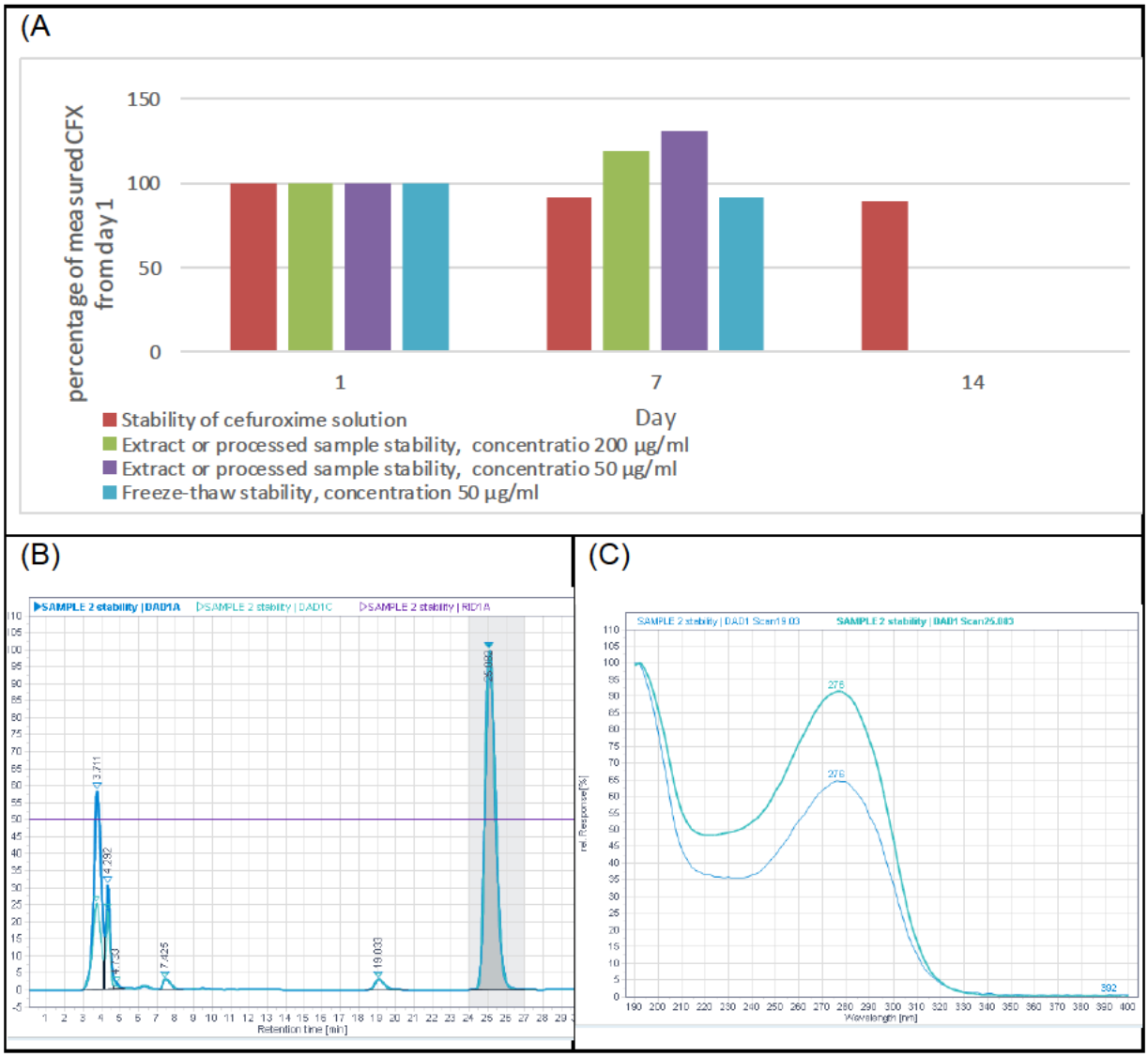


Figure 3.5: Stability of Cefuroxime in solution, frozen adipose tissue and after extraction (A), cefuroxime chromatogram in extraction stability studies (B), and extracted UV spectrum at a retention time of 19 and 25 minutes (C).

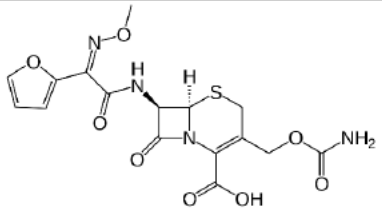
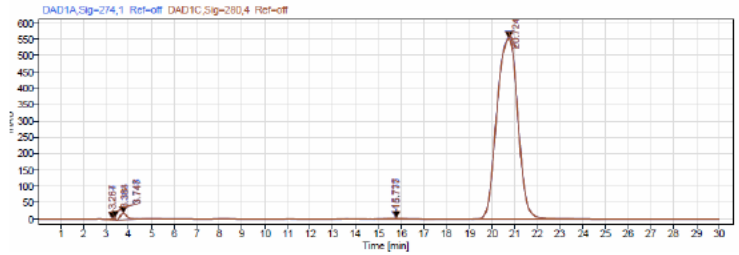
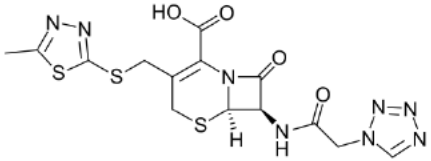
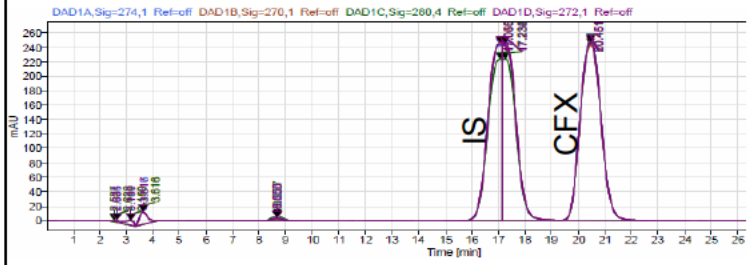
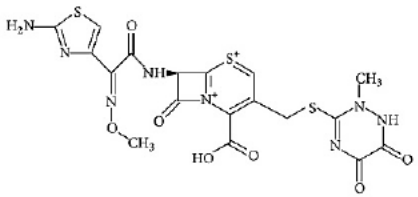
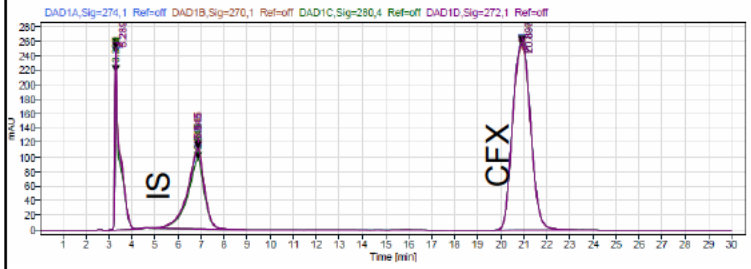
3.4.8 Octanol/water partition coefficient (LogP) of cefuroxime: Shake-flask method

The measured cefuroxime $LogP_{oct/wat}$ was −1.5. This represented the higher affinity of cefuroxime in the aqueous phase compared to the lipid phase. Reported cefuroxime logP in literature are listed in Table 3.2.

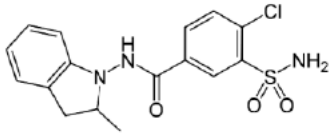
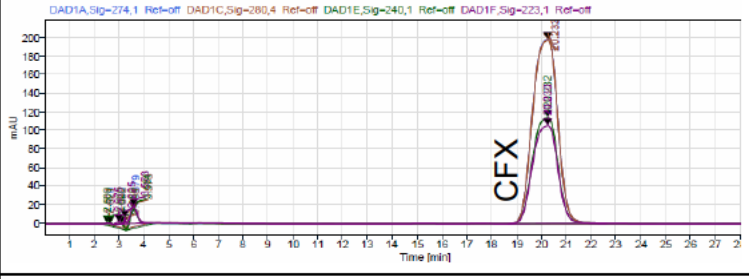
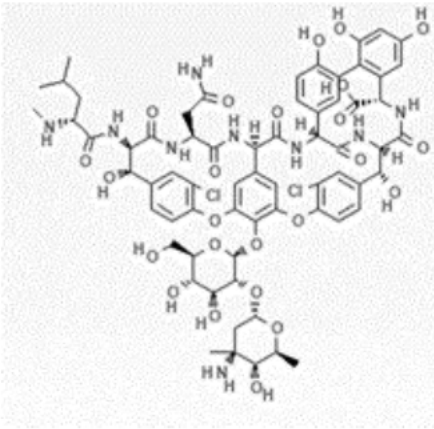
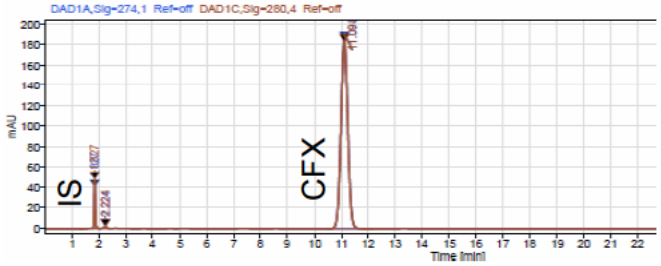
3.4.9 Selection of internal standard

Table 3.2 represents the IS investigated with their chemical structure, LogP and chromatogram of the IS with cefuroxime. Four compounds were not tested due to time restrictions; yet, they were mentioned in the table.

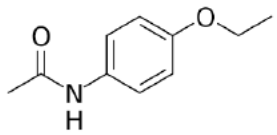
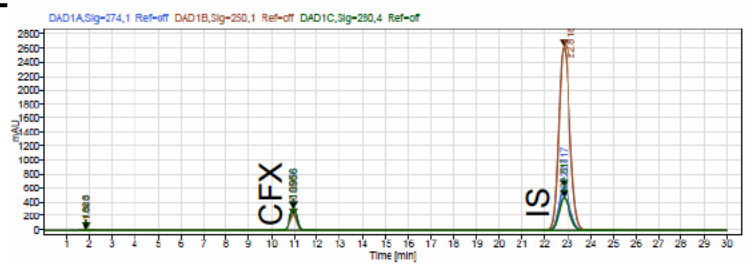
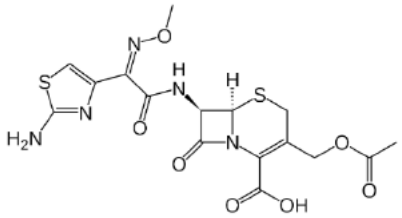
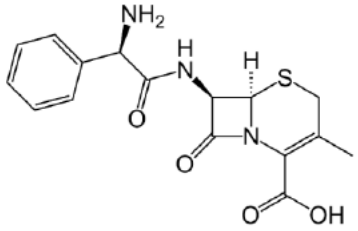
Table 3.2: Chemical structure, LogP and chromatogram of proposed internal standard and cefuroxime in solution*

Compound	Structure	LogP reported or predicted	Separation	Reference for usage as IS
Cefuroxime (analyte)	 (310)	−1.5 (result from shake-flask method) 0.034 fitted in a PBPK model (96) 0.4 (115) −0.9 (113)		NA
Cefazolin	 (311)	−0.58 (312) (313)		(301) (314)
Ceftriaxone	 (315)	−2.1 ± 0.19 (316) −0.01 (317) −1.9 (317) −1.7 (318)		

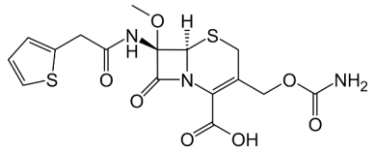
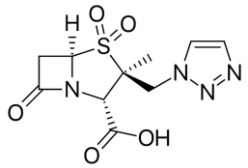
Continue Table 3.2: Chemical structure, LogP and chromatogram of proposed internal standard and cefuroxime in solution

Compound	Structure	LogP reported or predicted	Separation	Reference for usage as IS
Indapamide	 (319)	2.2 (320)		(321)
Vancomycin*	 (322)	−3.1 (318)		

Continue Table 3.2: Chemical structure, LogP and chromatogram of proposed internal standard and cefuroxime in solution

Compound	Structure	LogP reported or predicted	Separation	Reference for usage as IS
Phenacetin*	 (323)	1.58 (324, 325)		(326)
cefotaxime	 (327)	−0.5 (328) 0.14 (328) −1.4 (328)	Not Tested	(329)
cefalexin	 (330)	0.65 (331) 0.55 (332) −2.1 (332)	Not Tested	(333) (334)

Continue Table 3.2: Chemical structure, LogP and chromatogram of proposed internal standard and cefuroxime in solution

Compound	Structure	LogP reported or predicted	Separation	Reference for usage as IS
cefoxitin	 (335)	−0.02 (336) 0.22 (336) 0.29 (336)	Not Tested	(337)
tazobactam	 (338)	−1.8 (339) −1.4 (339)	Not Tested	(340)

* all tested IS were tested using C18 column with as described in method; except for Vancomycin and phenacetine, a C18 column with a particle size of 5 µm was used

IS internal standard; CFX cefuroxime

3.5 Discussion

Cefuroxime is an acidic compound that at a high pH value is (ionised) soluble in polar solvents, while at a lower pH value (unionised) is more soluble in organic solvents (341). The adjustment of mobile phase pH had a crucial role on cefuroxime peak symmetry. This can be explained by the pK_a of cefuroxime (pK_a 3.15). By adjusting the pH to 2.5, unionised cefuroxime had higher affinity for the stationery phase (apolar) than the mobile phase. It is recommended that the pH of the mobile phase should be ± 1 unit different from the pK_a of the analyte, as it can improve selectivity, peak shape and RT; however, extremely low and high pH can affect column lifetime (342). The RT shifting of the cefuroxime peak was observed. This is related to adjusting the mobile phase pH closer to the pK_a of the analyte (pH sensitivity); another reason could be the uncontrolled temperature of the column. The peak (RT 16 min) on the cefuroxime chromatogram observed in the stability studies, is a "related substance" to cefuroxime, as shown on the extracted UV spectra (Figure 3.5: B and C). This is not caused by the matrix effect, as this peak was observed in the stock solution stability.

It is worth noting the effect of methanol on cefuroxime separation with or without mobile phase pH adjustment (when used either as a mobile phase or as a solvent in adipose tissue recovery); although methanol is widely used in analytical chemistry, various reports in the literature highlighted that separation of compound and peak shape can be different when methanol is used as a mobile phase compared to acetonitrile (343). Methanol had an accepted elution when used as 60% of the mobile phase; however, the matrix interfered with the cefuroxime peak at a decreased RT (4 min).

The recoveries of medium and high cefuroxime concentrations were within the acceptance criteria, with the low cefuroxime concentration 7% above the acceptance

criteria; however, the quality control sample was 8.76 µg/ml and recovery was 10.75 µg/ml. The accuracy and extraction of cefuroxime from the adipose tissue can be optimised, by using an internal standard and optimisation extraction of cefuroxime from adipose tissue (using a solid-phase extraction and/or tissue lysis) (344).

The researchers questioned the requirement of using an IS, as the FDA and EMA used the word “should” (345). However, in the trial to use IS, phenacetine represented a desirable RT and efficient peak shape, compared to other compounds. Nevertheless, due to differences between phenacetine and cefuroxime LogP, a trial to prove similar extractions of cefuroxime and phenacetine from adipose tissue is required. Other compounds with a similar LogP to cefuroxime were intended to investigate; however, compounds with a similar LogP and structure would elute at relatively similar times (e.g. cefazolin Table 3.2).

The extraction of cefuroxime from adipose tissue using a solid-phase extraction is a promising alternative option. However, solid-phase extraction is not available at the current Pharmacy research laboratory; additionally, this can be time-consuming. Different tissue lysis compounds/kits have been used to digest adipose tissue. This can be beneficial in extraction cefuroxime; however, this could also increase the small lipid content in samples injected in HPLC (344). The efficiency of the current cefuroxime adipose tissue samples homogenisation can be investigated further by spiking homogenised adipose tissue with cefuroxime and then analysing samples to ensure optimal extraction and recovery of cefuroxime.

Regarding cefuroxime related peak appeared in stability testing, it is recommended to further investigate it through force degradation tests to understand degradation pathways (346). It is postulated that the increased recoveries observed in stability testing can be related to a cefuroxime degradation products, DAD investigation of cefuroxime recovery peak should be directed to ensure that the increased in recovery is not from impurity or degradation under the main cefuroxime peak.

Quantifying free drug concentration in ISF via Microdialysis has been widely used for clinical solid tissues and whenever IV infusion is used. Indeed, the use of Microdialysis has significant advantages; however, it is a minimal invasive technique which requires validation and calibration while in the operation room, with further analysis by HPLC or other analytical method (301). In the forthcoming PK pilot study at Birmingham Women's Hospital, the collection of solid samples are acceptable, while discussion regarding the optimal analytical method to measure cefuroxime was questioned.

3.6 Limitations and optimisations

1- Column pressure was a constant issue; this can have a great impact on analyte and IS RT. The increased column pressure could be related to the use of the relatively small particle size of the C18 column, and the wrong installation of the guard column. The validation of this method can be optimised using a C18 column with a particle size of 5 µm connected to a C18 guard.

2- Due to high deviations observed in established calibration curve (Figure 3.2), it was suggested to conduct a precision test to ensure appropriateness of cefuroxime weighing. If this was still an issue, it is recommended to use a single robust calibration curve instead of averages of triplicate measures. Further, it

was recommended to use broader linearity (maximum and minimum linear concentrations).

3- The recovery of cefuroxime from adipose tissue can be optimised, by using a representative IS. The appropriateness of using an IS should be investigated in adipose tissue, to evidence the descriptive recovery of IS, compared to the analyte.
4- The reported validated method in the literature to quantify cefuroxime in blood and adipose tissue, is a part of a PK study. This study had no appendices to review the exact results of the validation. In a PhD timeframe, with a focus on PK study, conducting a full validation can be challenging.
5- The column temperature and the samples temperature (preparation and injection) were not controlled, though the column temperature and sample preparation and injection temperature were maintained at room temperature of around 25°C within the laboratory. The validation of this method can be improved by controlling the column temperature, the autosampler tray temperature and sample preparations at low temperature (to ensure minimum penetration of Fatty compound in tested solution) by liquid nitrogen,
6- Filter adsorption studies can be performed to assess analyte loss caused by membrane filter adsorption.

The initial selection of HPLC method was based on the availability of HPLC in pharmacy research laboratory, where this research was being conducted, and the wide reported use of HPLC in similar studies as the current study to measure cefuroxime/cefazolin in plasma and adipose tissue (9, 78, 102). Due to the discussed limitations of the current HPLC method and time-frame in a PhD, It was decided to use

mass spectrometry (MS) in quantifying cefuroxime in another laboratory within the University of Birmingham with an MS expert assistance. The use of MS to quantify cefuroxime and cefazolin in plasma and adipose tissue has been largely reported in the literature (16, 297, 329, 347, 348). Initial discussion was made with other colleagues to prepare a protocol to quantify cefuroxime in plasma and adipose tissue using cefuroxime isotope as an IS. In addition to the high separation capability and sensitivity of MS; the added advantage of using MS with cefuroxime isotope will enable compensation of any technical variations and losing of analyte during sample preparations, storage and testing (349). Unfortunately, due to COVID-19 pandemic, the analytical method development using MS was suspended.

3.7 Conclusion

Partial validation, and indeed validation, to quantify cefuroxime in plasma and subcutaneous adipose tissue is a broad area. The reported method showed a cefuroxime recovery from adipose tissue slightly outside of the acceptance range; further optimisation needs to be conducted. With consideration of the PhD timeframe, the use of MS and assistance in analysing cefuroxime via MS is considered acceptable.

Chapter 4: Clinical Study to Assess the Pharmacokinetics of Cefuroxime and its Prophylactic Efficacy in Lean and Obese Pregnant Women after Caesarean Section (C-LACE)

The aim of this chapter was to develop a protocol to conduct a pilot study to collect blood and adipose tissue samples from pregnant women (obese and normal body wright) undergoing elective CS at Birmingham women's hospital. Further, to follow participants postpartum and review participants' file to record incidences of post-CS infections.

Publication from this chapter:

The protocol to conduct the pilot study at Birmingham women's hospital was published in Pilot and Feasibility Studies Journal, the manuscript was incorporated to this thesis theses upon permission statement by publisher BioMed Central (BMC) (https://www.biomedcentral.com/about/policies/license-agreement) "You are free to: •Share — copy and redistribute the material in any medium or format. •Adapt — remix, transform, and build upon the material for any purpose, even commercially. Under the following terms: •Attribution— you must give appropriate credit, provide a link to the license, and indicate if changes were made. You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use. •No additional restrictions—you may not apply legal terms or technological measures that legally restrict others from doing anything the license permits".; and by Journal (https://pilotfeasibilitystudies.biomedcentral.com/about) "As an author of an article published in Pilot and Feasibility Studies you retain the copyright of your article and you are free to reproduce and disseminate your work (for further details, see the BMC license agreement)". Numbering of heading, tables and figures were only adjusted to be consistence with the current report. The Full article reference is:

Alrammaal HH, Batchelor HK, Chong HP, Hodgetts Morton V, Morris RK. Prophylactic perioperative cefuroxime levels in plasma and adipose tissue at the time of caesarean section (C-LACE): a protocol for a pilot experimental, prospective study with non-probability sampling to determine interpatient variability. Pilot Feasibility Stud. 2021;7(1):54. doi:10.1186/s40814-021-00794-3. Which can be found at: https://pilotfeasibilitystudies.biomedcentral.com/articles/10.1186/s40814-021-00794-3 . Contributions of authors were mentioned within the paper.

This chapter is branched to:

- The published protocol of C-LACE study (section 4.1);
- Pathway of C-LACE study and logistics at Birmingham Women's Hospital (section 4.2);
- Summary of anticipated data cleaning and management plan (section 4.3);
- COVID-19 pandemic disruption on conducting C-LACE study (section 4.4).

Considerations regarding this chapter:

- The current publication represent the C-LACE study protocol where approved by the Health Research Authority (HRA) and Health and Care Research Wales (HCRW) ethics. The study protocol submitted and approved by HRA is included in appendixes (section 7.2.2.1).
- Amendment: One minor amendment was submitted, where only the Chief Investigator role/ship was shifted from Dr.Hannah Batchelor to Dr.Victoria Hodgetts-Morton. This was approved by HRA (appendixes section7.2.4.1).

Manuscript number: (2)

Status: published

Publication title: "Prophylactic Perioperative Cefuroxime Levels in Plasma and Adipose Tissue at the Time of Caesarean Section (C-LACE): A Protocol for a Pilot experimental, prospective study with non-probability sampling to determine interpatient variability."

The page number of the thesis: **126**

4.1 Prophylactic Perioperative Cefuroxime Levels in Plasma and Adipose Tissue at the Time of Caesarean Section (C-LACE): A Protocol for a Pilot experimental, prospective study with non-probability sampling to determine interpatient variability.

[1,2]Hanadi H. Alrammaal BSc MSc

[1,3]Dr Hannah K. Batchelor, BSc PhD

[4,5]Dr Hsu P. Chong

[5,6]Victoria Hodgetts Morton

[5,6]Dr R. Katie Morris MBChB, PhD

[1] School of Pharmacy, Institute of Clinical Sciences, Robert Aitken Building, University of Birmingham, Edgbaston, Birmingham, United Kingdom, B15 2TT

[2] Clinical Pharmacy department, Collage of Pharmacy, Umm Al-Qura University, Makkah, Makkah Province, Saudi Arabia

[3] Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, United Kingdom

[4] Rosie Maternity Hospital, Robinson Way, Cambridge CB2 0SQ, United Kingdom.

[5] Department of Fetal and Maternal Medicine, Birmingham Women's and Children's NHS Foundation Trust, Edgbaston, Birmingham B15 2TG, UK

[6] Institute for Metabolic and Systems Research, University of Birmingham, Birmingham, United Kingdom

Corresponding author

Hanadi Hassan Alrammaal

School of Pharmacy, Institute of Clinical Sciences, Robert Aitken Building, University of Birmingham, Edgbaston, Birmingham, United Kingdom, B15 2TT

Email: [redacted]

Telephone: [redacted]

4.1.1 Abstract

Background: The aim of the C-LACE study is to measure cefuroxime concentration in plasma and adipose tissue of non-obese and obese pregnant women undergoing caesarean section.

Methods: This study plans to compare maternal cefuroxime concentrations (plasma and adipose tissue), at time of skin incision and time of skin closure during a caesarean section from non-obese (Body Mass Index BMI<30 kg/m^2) and obese (BMI≥30 kg/m^2) pregnant women. The incidence of post-surgical site infection will also be measured. At least 15 participants are required for each arm (non-obese vs obese) with a total of 30 participants. The study participants will be followed up between 30 and 40 days post caesarean section to record details of any post-caesarean surgical infection to explore correlations between BMI; measured cefuroxime concentrations and post caesarean infection rates.

Discussion: This pilot study will allow the development of a model testing the inter-patient variability in plasma and adipose tissue concentrations of cefuroxime. The results will facilitate the development of a larger study to determine whether differences in cefuroxime plasma and tissue concentration in obese and non-obese women can support development of a physiologically-based pharmacokinetic model. This model

can then be used to propose dosing adjustments that can be used in a further trial to optimise cefuroxime dosing for women undergoing Caesarean section.

Trial Registration: https://www.isrctn.com/; ISRCTN17527512 (Registered on 26/10/2020).

Keywords

Caesarean section; Cefuroxime; Pharmacokinetics; Obese; Pregnant women; Surgical site infection.

Administrative information

This protocol follows the SPIRIT checklist (items of the checklist are numbered in a curly brackets).

Title {1}	Prophylactic Perioperative Cefuroxime Levels in plasma and adipose tissue at the Time of Caesarean Section (C-LACE): A Protocol for a Pilot experimental, prospective study with non-probability sampling to determine interpatient variability to define the sample size for a full trial
Trial registration {2a}	https://www.isrctn.com/; ISRCTN17527512 (Registered on 26/10/2020).
Protocol version {3}	C-LACE protocol v0.4, 02 January 2020
Funding {4}	Royal Embassy of Saudi Arabia, Cultural Bureau in London; from funds associated with HHA's PhD.
Roles and responsibilities {5a}	[1,2]Hanadi H. Alrammaal BSc MSc [1,3]Dr Hannah K. Batchelor, BSc PhD [4,5]Dr Hsu P. Chong [5,6]Victoria Hodgetts Morton [5,6]Dr R. Katie Morris MBChB, PhD [1] School of Pharmacy, Institute of Clinical Sciences, Robert Aitken Building, University of Birmingham, Edgbaston, Birmingham, United Kingdom, B15 2TT [2] Clinical Pharmacy department, Collage of Pharmacy, Umm Al-Qura University, Makkah, Makkah Province, Saudi Arabia [3] Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, United Kingdom [4] Rosie Maternity Hospital, Robinson Way, Cambridge CB2 0SQ, United Kingdom. [5] Department of Fetal and Maternal Medicine, Birmingham Women's and Children's NHS Foundation Trust, Edgbaston, Birmingham B15 2TG, UK [6] Institute for Metabolic and Systems Research, University of Birmingham, Birmingham, United Kingdom

	Chief investigator: Hannah Batchelor is a senior lecturer in pharmaceutics, formulation and drug delivery within the School of Pharmacy at the University of Birmingham. Her research focuses on the design and manipulation of medicines to create age-appropriate drug formulations to maximise clinical efficacy in paediatric patients. Hannah is an expert in prediction of medicines performance and has experience in the design and development of novel physiologically based pharmacokinetic models. She has experience in working on trials that evaluate human tissue samples (eg PaedGIFT #IRAS 251909). An integral part of her department's work is the development of drug delivery systems, including construction of new formulations, stability testing and development to market together with social and behavioural research into the perceptions, attitudes and values of patients towards medicines use. Hannah managed the ACCEPT study, a successful multi-centre, portfolio status clinical trial which involved 14 NHS sites across the West Midlands and recruited in excess of 600 participants. This study involved interacting with both paediatric patients and their parents to identify their views towards children's medicines. http://www.hra.nhs.uk/news/research-summaries/accept-methodology-to-assess-acceptability-of-paediatric-medicines/ Local Principal Investigator: Dr Katie Morris, Reader in Maternal and Fetal Health, University of Birmingham. Her research aims to improve the care for women with multiple pregnancies, fetal anomalies, growth restriction, reducing infection in maternity and prevention of preterm birth, employing prognostic and diagnostic research, modelling, systematic reviews and clinical trials. She has received research funding from the HTA, RfPB, MRC and charities. She sits on the RCOG Research Committee, Scientific Advisory Committee, RfPB West Midlands Panel, British Maternal and Fetal Medicine Society Executive Committee as scientific abstract co-ordinator and is Clinical Speciality Lead for Reproductive Health and Childbirth for the West Midlands. She was CI of the NIHR RfPB funded PREPS trial which was a randomised controlled trial recruiting women undergoing CS. Victoria Hodgetts-Morton is a clinical research fellow working on large clinical trials at Birmingham Women's Hospital including C-Stitch and PREPS. Researcher: Hanadi Alrammaal is a PhD student at the School of Pharmacy, University of Birmingham under the supervision of Hannah Batchelor. Hanadi is an experience researcher and has been trained in both GCP and handling of tissues. This study forms the basis of her PhD.
Trial sponsor {5b}	University of Birmingham Dr Birgit Whitman [redacted] Head of Research Governance and Integrity
Role the trial sponsor and funder {5c}	Study funder and sponsor had no role in the study design; collection, management, analysis, and interpretation of data; nor in writing of the report or the decision to submit the report for publication.

4.1.2 Background {6a}

The prevalence of obesity in pregnant women in England at the time of booking a Caesarean section (CS) has been reported to be 16% (350, 351). One complication of obesity in pregnancy relates to the requirement for operative delivery, i.e. CS. The rate of CS in an obese (Body Mass Index BMI≥30 kg/m^2) pregnant population is 33.8% (6).

The rate of post-CS infection is higher amongst obese pregnant women compared to those who are not obese; the adjusted odds ratio of surgical site infection in an obese population was reported to be 2.41 (95% CI 1.73-3.37) (7). Prophylactic single dose perioperative intravenous (IV) antibiotic is recommended for CS; and several studies have examined the use of alternative antibiotics, with cefazolin being the most commonly used in the US and cefuroxime in the UK. Numerous studies have focussed on the use of cefazolin in obese patients undergoing CS to gain an understanding of optimal dosing and clinical outcomes (12). Several studies proposed an increased dose of cefazolin (3g) compared to the standard dose (2g) for obese pregnant women to achieve sufficient antibiotic levels during CS (10, 11, 13). It was postulated by Holt et al. (in 1994) that doses of cefuroxime up to 1500mg may not achieve sufficient minimum inhibitory concentration (MIC) for most of the common bacteria in maternal blood during CS (56). Sub-therapeutic antibiotic concentration is a crucial factor in global bacterial resistance. It is essential that the therapeutic concentration of antibiotic is achieved in both maternal blood and at the site of incision during CS for effective therapy. No study to date has measured cefuroxime concentrations in blood and adipose tissue in obese pregnant women undergoing CS (300). Thus, a study to explore the drug concentration in this population at the time of CS is essential. This study will analyse cefuroxime concentration in plasma and adipose tissue samples to accurately measure levels achieved in non-obese and obese pregnant women undergoing CS to confirm if therapeutical cefuroxime levels are reached in plasma and adipose tissue. Further, to use the measured cefuroxime concentrations to verify a physiologically-based pharmacokinetic (PBPK) model of cefuroxime in CS to explore the impact of (i) dose and (ii) BMI post administration of cefuroxime on both blood and

tissue cefuroxime concentrations in an obese population and suggest a dose adjustment if required and applicable.

4.1.2.1 Objectives {7}

The main objective of conducting this pilot and feasibility study is to assess the process and protocol of recruiting patients, sample collection, follow-up and procedure of C-LACE. Also to gain an understanding of cefuroxime concentrations in normal-body weight pregnant vs. obese pregnant women. The primary objective is to measure the concentration of cefuroxime in the collected blood and tissue samples using an appropriate analytical method and to assess whether cefuroxime administered provides concentrations above the MIC in both the blood and adipose tissue for all patients regardless of their BMI. Additionally, to assess whether there are differences in cefuroxime concentrations from obese vs non-obese women.

4.1.2.2 Trial design {8}

A single centre experimental, prospective pilot study with non-probability sampling.

4.1.3 Methods/Design

4.1.3.1 Aims and methodologies

To measure cefuroxime concentration in plasma and adipose tissue (within the incision) at time of skin incision and skin closure in obese and non-obese pregnant patients requiring CS. Moreover, to explore the relationship between dose of cefuroxime administered and concentration measured in both blood and adipose tissue with respect to BMI of the patient. To measure the incidence of post CS infections in obese and non-obese pregnant women at 30-40 days post CS; and to assess if the

rate of infection is correlated with BMI, dose administered and cefuroxime blood or adipose tissue concentrations.

Cefuroxime is administrated to pregnant women undergoing CS as per Birmingham Women's Hospital protocol; i.e. IV infusion 30-60 minutes before skin incision.

4.1.3.2 Outcomes {12}

4.1.3.3 Outcomes of C-LACE pilot and feasibility study are:

- Patients' recruitment rate.
- Collecting samples strategies'.
- Analytical method for Sample analysis.
- Data collection and forms completion.
- Follow-up and participants' retention.

4.1.3.4 Part A: Cefuroxime levels in plasma and adipose tissue

The minimum inhibitory concentration is not harmonised between guidelines, thus the time above the minimum inhibitory concentration (T>MIC) will be reported for MIC values of 1, 4 and 8 mg/L(12, 301) as these values correlate to the most common bacteria involved in post CS surgical infections (Table 4.1).

Table 4.1: Most common bacteria causative of infection post Caesarean Section and the minimum inhibitory concentration range/Breakpoint

	Microorganism	MIC range/Breakpoint (mg/L) *(12, 81, 301, 352)*
Gram Negative Bacteria	*Escherichia coli*	0.001-8
	Haemophilus influenzae	2
	Klebsiella oxytoca	8
	Proteus mirabilis	0.001-8
	Ureaplasma urealyticum	ND
Gram Positive Bacteria	*Enterococcus faecalis*	ND
	Staphylococcus aureus	4
	Staphylococcus epidermidis	0.25-4
	Streptococcus pneumoniae	1
Other Organisms	*Raoultella ornithinolytica*	0.001-8
	Morganella morganii	ND

4.1.3.5 Part B: Surgical site Infection

The rate of surgical site infection will be reported in non-obese and obese pregnant women undergoing CS. This study will follow the definition of surgical infection as per the definitions set out by the US Centre's for Disease Control and Prevention (Centre's for Disease Control and Prevention 2019) (353).

4.1.3.6 Target population

The target population are non-obese and obese pregnant women undergoing elective CS at Birmingham Women's Hospital.

4.1.3.7 Eligibility criteria {10}

4.1.3.7.1.1 Inclusion Criteria

- Pregnant women 18 years to 50 years old, with a singleton pregnancy undergoing elective CS at 37 weeks or over.
- Participant is able to give consent and agree to sample storage.
- Participant agrees to be contacted for follow-up

- Participant contributing to the study needs to be able to read and/or understand English.

4.1.3.7.1.2 Exclusion criteria

- Multiple pregnancy
- Previous CS that resulted in a surgical site infection
- Emergency CS.
- Any non-elective CS including those requiring early delivery without threat to maternal or foetal health that were not previously planned.
- Body Mass Index (BMI) less than18 kg/m^2 or greater than or equal to 45kg/m^2 (At time of first pregnancy appointment and at time of delivery).
- Currently enrolled in a randomized controlled trial for an intervention to reduce post-operative surgical site infection
- Diabetes (type 1, type 2 or gestational)
- Hypertension
- Renal disease
- Cardiovascular disease (e.g. Maternal structural cardiac disease)
- Liver disease
- Inflammatory bowel disease (e.g. Crohn's disease or ulcerative colitis)
- Cephalosporin or penicillin allergy
- Administration of antibiotic within 1 week prior to delivery
- Suspected pre-existing infection (including Chorioamnionitis)
- Autoimmune disease (e.g. Systemic lupus erythematosus, Rheumatoid arthritis)
- Chronic use of corticosteroid

- History of wound breakdown in an abdominal surgery
- Prior laparotomy for any indication (e.g. Previous ovarian cystectomy or previous bowel surgery)

4.1.3.8 Setting {9}

This single centre study will take place at Birmingham Women's Hospital which is a large teaching hospital that is part of Birmingham Women's and Children's Hospital NHS Foundation Trust.

4.1.3.9 Participant selection and enrolment

Any patient (18-50 years) who requires a CS as part of their clinical care will be screened for inclusion. The potential participants will be identified using hospital clinical lists, where lists relate to a pre-booked CS a Good Clinical Practice (GCP) trained research midwife who is a member of the clinical care team will assess eligibility of patients to participate in the study according to inclusion/exclusion criteria using Screening Form. The woman will then be provided with the participant information sheet at this meeting by the research midwife. In addition, if a pregnant woman is booked for CS at a meeting they may be informed about the study by a research midwife or their clinical care team and provided with a participant information sheet. The participants will receive information about the study well in advance (at prior clinic appointments) and at least 24 hours prior to CS so that all participants have sufficient time to consider taking part.

This study plans to compare data from obese (BMI≥30 kg/m^2) and non-obese women (BMI<30 kg/m^2) at time of booking (10-14 weeks of pregnancy). At least 15 participants

are required for each arm with a total of 30 participants. The study sequence is summarised in Table 4.2.

Table 4.2: Summary of the C-LACE study sequence and methodology

Timing	Activity
Caesarean Section Booking clinic	Clinic list will be reviewed for eligible participants using the Screening Form
On day of Caesarean Section	A venflon cannula will be inserted into the woman as part of the research process to enable withdrawal of the required blood samples without the need for additional needles. This second access point will ensure that the blood sampling is minimally disruptive to the CS procedure.
At 30-40 days post Caesarean Section	Participant's medical file and notes will be reviewed by the research midwife to identify any post-surgical site infection which will be recorded on the 6 week outcome form Research midwife will attempt to contact the participant 3 times between 30 and 40 days post CS to complete the follow up interview form.
Samples analysis	Blood and adipose tissue will be analysed by LC-MS/MS (340)

GCP: Good Clinical Practice, CS: Caesarean Section, LC-MS/MS: Liquid Chromatography with tandem mass spectrometry

4.1.3.10 Screening for eligibility and recruitment {15}

A GCP trained research midwife will assess eligibility of patients to participate in the study according to inclusion/exclusion criteria using Screening Form.

4.1.3.11 Ineligible and non-recruited participants

Certain ineligible are stated in exclusion criteria. Research midwives/clinician will review and reassess each potential participant and confirm eligibility of patients to participate in the study at the day of CS.

4.1.3.12 Patient consent {26a}

A suitably qualified research midwife or doctor who is also part of the clinical care team will introduce the study to the woman at their pre-operative assessment. A Participant

Information Sheet (PIS) will be provided to facilitate this process. Investigators will ensure that they adequately explain the aim, anticipated benefits and potential hazards of taking part in the trial to the woman. They will also stress that participation is voluntary and that the woman is free to refuse to take part and may withdraw from the trial at any time. The woman will be given adequate time to read the PIS and to discuss their potential participation and be given the opportunity to ask questions. If the woman expresses an interest in participating in the trial, they will be asked to sign and date the latest version of the Informed Consent Form at least 24 hours prior to CS. The woman will be asked for her consent to withdraw 3 blood samples (with a maximum volume of 10mL blood per sample) and 2 fat tissue and for collection of outcome data from their medical records and a telephone interview at 30-40 days postnatal that will take approximately 5-15 minutes.

4.1.3.13 Withdrawal of study participants

Each participant has the right to withdraw from the study at any time and this will not affect their clinical care. In addition, the Investigator may discontinue to participate from the study at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Withdrawal of Consent

Withdrawal from the study will result in exclusion of the data for that participant from analysis; provided that the withdrawal occurs within 3 months of data collection.

4.1.3.14 Study assessments {13}

The study assessment and paperwork associated are summarised in Table 4.2 (forms are in the online supplementary file).

4.1.3.15 Data collection methods {18a}

Background information will be collected from each participant using the Baseline Form. This information is required for interpretation of the data and dissemination.

Following collection of the data from all forms (Screening, Consent, Baseline, Eligibility Reassessment, Operation, Discharge, follow up interview, 6 week Record Check) will be filled, signed and shared via secure system; this system can be accessed at the University and the Hospital and ensures security of data, any data entered will be anonymised.

The study has two parts: part A, which is blood and adipose tissue sampling for cefuroxime concentrations; part B, is a follow up of patients postpartum at between 30-40 days to record the incidence of post-surgical infection.

Table 4.3: Schedule of assessments and associated paperwork

Visit	Screening form	Consent form	Baseline form	Eligibility Reassessment form	CS Procedure form	Collection and labelling of samples	Discharge form	Follow up interview form	Medical Record check form
Eligibility Check	×								
Consent at least 24 h pre CS		×	×	×					
Day of CS until discharge					×	×	×		
Follow-up								×	×

4.1.3.16 Part A: Sample collection at Caesarean Section

The clinical care team will record the time of cefuroxime administration, dose administered, type of anaesthesia used, blood loss and other relevant clinical information. Five samples will be collected: 1 blood sample at time of skin incision, 1 blood sample at time of skin closure, 1 blood sample at recovery room (<3 hours following CS), 1 adipose tissue sample (approximately 1 cm from the skin in the middle of the Pfannesnstiel or vertical midline incision) at skin incision and one adipose tissue sample (from a similar location to sample 1) just prior to skin closure.

4.1.3.16.1.1 Blood sample and adipose tissue analysis

The concentration of cefuroxime in blood and adipose tissue will be quantified using standard laboratory methods (340).

4.1.3.17 Part B: Follow up for post-surgical infection

At between 30-40 days post CS, the study participants will be contacted by telephone by a research nurse/midwife from the research team and a review will be made to record any type of post-surgical infection. The Follow-up Interview Form will be used to record the details of any infection reported. If the participant attended to the hospital for a postnatal appointment at 30-40 days, the interview can be conducted at that time to minimise the burden to the participant.

4.1.3.17.1.1 Plans to promote participant retention and complete follow-up {18b}

Patient medical notes will be reviewed by a member of the clinical care team using the 30-40 days Outcome Form to identify whether there has been a reported post-surgical

infection. Post-surgical infection is defined as per the definitions set out by the US Centre's for Disease Control and Prevention (353).

4.1.3.18 Data management {19} and confidentiality {27}

All participants will be given a study ID upon recruitment to the study. A spreadsheet that links study ID to patient ID will be held at Birmingham Women's and Children's Hospital NHS Foundation Trust under the control of the local PI. This information will be password protected and stored on an encrypted computer that conforms to the standard NHS security requirements.

The study ID will be used on all forms; this information provides anonymised data to the University of Birmingham that no longer has any personal identifiable data. Following collection the data from all forms will be entered on a secure system at the NHS site (undertaken by the research midwife); this system can be accessed at the University and ensures anonymity for the participants.

All processes will be carried out with respect to participant confidentiality. All participant data will anonymised by being replaced with a study ID number prior to data sharing with the University of Birmingham. The anonymity code will only be known to the local site PI and associated research nurse/midwife team. The data will be controlled by usernames and encrypted passwords.

4.1.3.19 Statistics {20a}, sample size {14} and study assessment

There is no predefined sample size yet sufficient numbers are required to provide an estimate of average values as well as the level of inter-patient variability. Previous work to measure the concentration of antibiotics in plasma and in tissue in obese and non-obese pregnant women following CS has used sample sizes of 14 in each arm to detect

differences in adipose antibiotic concentration (10, 16). Therefore in this study we plan to recruit 15 women in each arm (BMI <30 kg/m^2 and BMI ≥30 kg/m^2). The BMI measurements will be taken from the women at their time of booking appointment which occurs between 10-14 weeks of pregnancy. The BMI of participants immediately prior to CS will be determined to review any major changes during their pregnancy which may affect the results, for example a change from <30 kg/m^2 to an extremely obese participant at CS.

The differences in mean concentration values in plasma and adipose tissue between the obese and non-obese populations will be compared using ANOVA analysis to determine whether there are significant differences. SPSS software will be used for all statistical analysis. The rate and type of infection will be analysed. The rate of infections will be analysed in correlation with (1) dose administered, (2) BMI, (3) cefuroxime concentrations at both sites at time of skin incision and skin closure.

4.1.3.20 Success criteria and assessment of C-LACE pilot and feasibility study

These criteria applied to participants consented and did not withdraw from the study at any time-point of C-LACE study

- Patients' recruitment rate: As per Birmingham Women's Hospital Caesarean Section rate, recruitment of 30 patients would be achievable easily within 3 months. If this was not achieved, the protocol (specific inclusion and exclusion criteria) would be reviewed and reassessed.

- Collecting samples: This pilot and feasibility study would be considered successful if plasma and adipose tissue samples at the time of incision and time of closure and

plasma sample at time of recovery were successfully collected for ≥75% of participant in each arm. If this was not achievable, the sampling strategies will be revised and reassessed.

- Sample analysis: This pilot and feasibility study would be considered successful if ≥80% of plasma and adipose tissue samples collected were successfully analysed via the analytical method described; If not, sampling handling, transferring and sample analysis protocol will be revised and reassessed.

- Data collecting: This pilot and feasibility study would be considered successful if ≥ 80% of data collecting forms was fulfilled for each participant; if not, logistics and study procedure of C-LACE study will be revised at Birmingham Women's Hospital.

- Follow-up: The described follow-up protocol will be considered successful if participants' retention were ≥ 80%. The participant will be considered as lost to follow up, if the participant couldn't contacted and the patients file and notes were not reviewed.

Analysis and sub-analysis of outcomes and success criteria of C-LACE pilot and feasibility study will be made to explore the reasons of unsuccessful.

4.1.3.21 Missing data and lost to follow-up {20C}

Every attempt will be made to collect full follow-up data for all study participants; it is thus anticipated that missing data will be minimal. The main analysis will use available data only, however, the amount of missing data will be assessed, and if necessary, sensitivity analyses will be undertaken. If the participant couldn't be contacted, but the patients' medical records and notes were reviewed, the participant will be considered

enrolled in the follow up. The patient will be considered as lost to follow up, if the participant couldn't contacted and the patients file and notes were not reviewed.

4.1.3.22 Data monitoring {21a}

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. This study does not include blinding and patients are following the standard clinical pathway where the only intervention is the taking of adipose tissue and plasma samples therefore a data monitoring committee is not required.

4.1.3.23 Access to data {29}

All data will be collected only after the participant freely gave written consent to process their data in accordance with GDPR (this consent in included within the consent form for the study). Direct access to shared secure system will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

4.1.3.24 Harms {22}

The research protocol does not expose the participants to any risks as the study does not affect their clinical treatment. The collection of additional blood samples and adipose tissue samples during CS are the only additional study specific procedures. These additional blood samples will be taken by a clinical member of the research team trained in phlebotomy. There is a consent request for these study specific procedures to minimise the changes to routine clinical care and reduce the burden to participants. The risks associated with this research are minimal to participants.

All participants will be contacted at 30-40 days following CS to ask about post-surgical infections; this will not affect their clinical care in any way and does not pose any risks to participants.

4.1.3.25 Provisions for post-trial care {30}

Participants within this trial will follow standard clinical procedures with the intervention being taking plasma and adipose tissues samples thus there is no specific post-trial care. As the participants are NHS patients, indemnity is provided through the NHS schemes. The University of Birmingham provides clinical research insurance for studies that it sponsors. This insurance is provided via membership of UMAL.

4.1.3.26 Auditing {23}

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

4.1.3.27 Protocol amendments {25}

The Investigator will submit and, where necessary, obtain approval from the Health Research Authority (HRA) and Health and Care Research Wales (HCRW) for all substantial amendments to the original approved documents.

4.1.4 Discussion

4.1.4.1 Interpatient Pharmacokinetic variability of cefuroxime

Due to the great variability associated with pharmacokinetic measurements, the use of PBPK modelling is a common tool to understand the distribution of drugs. Specialist software packages are available that include details on pregnant population characteristic's. SimCYP software will be used to model the blood and adipose concentrations of cefuroxime and to compare this modelled data to the experimental

results from the C-LACE study. It is anticipated that this clinical data coupled with PBPK models will inform any future changes to doses of cefuroxime used for CS, particularly in relation to obese women.

4.1.4.2 Definition of the End of Study

The end of study is the date at which the follow-up interview is conducted at 30-40 days following CS. This is where the follow-up ends for all participants.

4.1.4.3 Patient and public involvement

A similar study conducted by Victoria Hodgetts Morton and Katie Morris (PREPS trial) involved the public and patients; those involved were supportive of any research undertaken to minimise the risks of post-surgical infections following CS(354). Informal discussions about this study with women who have had CS have all been positive as the burden of participation is minimised where possible.

4.1.4.4 Ethics and dissemination {31a-b-c}

Results of this trial will be submitted for publication in a peer-reviewed journal. The manuscript will be prepared by HHA and HKB with contributions from the local PI (RKM) and the local researcher (VHM) and authorship will be determined by mutual agreement. Authors must acknowledge that the trial was performed with the support of Saudi Arabian Government. Currently, there is no Plans for granting public access to the full protocol, participant-level dataset, and statistical code

4.1.4.5 Provenance and peer review

The protocol has been peer reviewed within the research team and by the research and governance team at the University of Birmingham as part of the agreement to sponsor the study and by the University of Birmingham's Clinical Research

Compliance Team (CRCT). Further, this protocol have been reviewed and approved by HRA and HCRW.

4.1.5 Trial Status

Ethical approval is granted (C-LACE protocol v0.4, 02 January 2020); the anticipated date of recruitment was 1st of September 2020; requirement expected to be completed on 1st of December 2020). This study was delayed due to COVID-19 epidemic.

4.1.6 List of Abbreviations

BMI Body Mass Index

CS Caesarean Section

GCP Good Clinical Practice

IV Intravenous

LC-MS/MS Liquid Chromatography with tandem mass spectrometry

MIC Minimum Inhibitory Concentration

PBPK physiologically based pharmacokinetic

PIS Participant Information Sheet

T>MIC Time above the Minimum Inhibitory Concentration

4.1.7 Declaration

4.1.7.1 Ethics approval {24} and consent to participate

The study has been approved by the Health Research Authority (HRA) and Health and Care Research Wales (HCRW)(19/EM/0356). Written, informed consent to participate will be obtained from all participants.

4.1.7.2 Consent for publication

Not applicable

4.1.7.3 Availability of data and materials

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations. Access to the data retrieved from participants will be restricted. The clinical research team are those based at the Hospital and the paperwork will not be transferred to the University of Birmingham. Following collection the data from all forms will be entered on a secure system at the NHS site (undertaken by the research midwife); this system can be accessed at the University and ensures anonymity for the participants. Researcher from the research team from the University of Birmingham will used the anonymous data to perform the data analysis.

4.1.7.4 Competing interests {28}

The authors declare that they have no competing interests

4.1.7.5 Funding

This work was supported by Saudi Arabian Government (Royal Embassy of Saudi Arabia, Cultural Bureau in London) from funds associated with HHA's PhD.

Authors' contributions

HHA, HKB and HPC led the proposal. HHA, HKB, HPC and RKM developed the protocol and methodology. HHA, HKB, HPC drafted the protocol. All authors reviewed and commented on the protocol.

Acknowledgements

Not applicable

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40814-021-00794-3.

Additional file 1.

Additional file 2. C-LACE Screening Form.

Additional file 3. SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*.

4.2 Pathway of C-LACE study and logistics at Birmingham Women's Hospital

4.2.1 Before requirement of participant into C-LACE study

4.2.2 Acronym and logo of C-LACE study

Identification parts were included in the pilot study to ensure easy communication while conducting research with the hospital staff. Specifically, an acronym and a logo were developed. The acronym, C-LACE, denoted “Cefuroxime level at CS time”; it was determined that “LACE” may have a calming effect on participants. The logo can be seen in Figure 4.1; it used the letter C and a lace-like fabric pattern.



Figure 4.1: Logo of C-LACE study

4.2.3 The anticipated preparations and results of C-LACE

The C-LACE forms that were developed, which are discussed in section 4.1.3.15, were adopted with respect to format and flow from a previous feasibility study conducted at clinical setting including Birmingham Women's Hospital (PREPS) (354, 355). That study aimed to investigate the effectiveness of using 0.05% chlorhexidine for vaginal cleansing to reduce post-CS infections; its control group used no cleansing (354, 355).

The same follow-up interview and six-week outcome forms were chosen to be used in the present study as in PREPS (with some minor alterations) (354, 355). Where applicable, the flow and strategy used in PREPS was chosen for the C-LACE study. This was done to provide the hospital staff with a familiar routine to follow rather than introducing a new strategy; as hospital staff were familiar with PREPS study. Consequently, it would encourage the staff to participate in the study. It was planned to conduct an introductory lecture of C-LACE study to the hospital staff interested in conducting the pilot study and this would be followed by a workshop.

It was intended to prepare and distributed packages which contains several printed C-LACE forms: screening, consent, patient contact details, baseline, reassessment of eligibility, operative, discharge, 6 weeks outcome and interview forms. They would also include trial identity wristbands, consent stickers, and sample collection label stickers. The first page would contain a study steps checklist. One pack and checklist was to be used for each participant

Subsequently, two papers would be posted in the outpatient clinic as the leading strategy for recruiting eligible women. The sheets suggested introduction of C-LACE study to possible eligible women (suggestions for consenting), shared the steps involved in the study, and mentioned completing paperwork. The aforementioned checklist and both these papers are discussed in the following pages. An account was created on twitter aiming to provide more information on C-LACE study (found at https://mobile.twitter.com/CLACE28360041).

The anticipated enrolment rate and follow up (as per defined) would be expected to be adequate. The discussed PREPS study at Birmingham Women's Hospital had a

recruitment rate of 128% of targeted sample size, 93% of them received the intervention (355). Follow up rates using medical records and telephone were 96% and 68%, respectively (355). The study authors' perspective of the C-LACE enrolment rate of 30 obese–pregnant and 30 lean pregnant women would be completed within around 4 weeks.

The anticipated PK results from the C-LACE study are discussed in the highlight of PBPK modelling results in section 6.1 (Anticipated cefuroxime concentrations results from C-LACE study).

C-LACE Pathway and checklist

Please tick bullet point to confirm action

- Discuss C-LACE at earliest opportunity, i.e. when booking CS
- Provide the Patient information sheet

Consent-Complete: -

- Use the envelope in pack to record participant trial number
- Consent form at least 24 before CS (1 pack, 1 main notes, 1 patient)
- Inform Dr.Victoria Hodgetts-Morton of patients' enrolment
- Consent sticker (Stick on main notes)
- Baseline form (pack)
- Patient Contact Information Form (pack)

Day of CS

- Complete the Eligibility Reassessment Form within 24 h prior to CS (Pack)
- Place C-LACE wristband on
- Insert venflon cannula to withdraw blood samples
- **Contact Researcher to collect samples** (see below)

- Complete Operative form (pack)
- Complete Samples sticker (pack) stick it to sample box
- 1 **blood** sample at time of **skin incision**
- 1 **adipose tissue** sample at time of **skin incision**
- 1 **blood sample** at time of **skin closure**
- 1 blood sample in recovery
- 1 **adipose tissue** sample at time of **skin closure**
- All Samples inside a box with ice
- Handle samples to researcher to transfer samples

- Complete Discharge Form (pack)

At 6 weeks post CS

- Review patient notes and complete the 6 Weeks Outcome Form (Pack)
- Interview patient by phone and complete interview form (pack)

- After completing interview form, Discard contact Details form and hand pack to **researcher**

Dr.Victoria Hodgetts-Morton Contact Details: [redacted]

Researcher contact details:

Name : Hanadi AlRammaal Email: [redacted]

Phone (mobile): (Deleted in thesis for privacy)

C-LACE completing the paperwork steps

Step	Paperwork	Where to find	Where to leave
1	Screening form	Pack	Pack
2	Patient information sheet	Pack	Patient
3	Consent form	Pack	Top copy – Pack Middle copy – in main notes Bottom copy – to patient
4	Consent sticker	Pack	Stick to main notes (medical file)
5	Patient contact details form	Pack	Pack *
6	Baseline form	Pack	Pack
7	Reassessment of eligibility form **PLEASE complete this within 24H pre CS**	pack	Leave the form in pack, and **contact researcher (see below)** to prepare for sample transfer to UoB
8	Trial identity band	pack	patients
9	Insert cannula		patients
10	Operative form	pack	Pack
11	Samples Sticker	pack	Stick to samples box, for researcher to pick up ASAP
12	Discharge form	pack	Pack
13	6 weeks outcome form	Pack	Pack
14	Interview form	pack	pack
15	*** After finishing the interview form, discard the contact details form and handle the pack to researcher**		

Researcher contact details:

Hanadi AlRammaal

Email: [redacted].

Phone (mobile): (Deleted in thesis for privacy)

Please confirm **Date** and estimated **Time** for pick up

Suggestions for consenting

Introduce yourself

Explain that you would like to talk to her about some research to see if she would like to take part.
That she does not have to take part if she doesn't want to and that if she does consent, she is free to withdraw at any time

Explain the study

As with all operations there is a small risk of developing an infection afterwards, C-LACE is looking at whether the usual antibiotic given before CS is sufficient to reduce this risk. And wither the drug concentration is affected by patients' weight and height.

NO change in the usual medical care plan

The care both you and baby receive will stay exactly the same, except we will have samples from your blood and small amount from belly fat tissue to measure the antibiotic concentration.

Explain about the samples

The samples will then be taking after you are numb from the anaesthetic. We will then contact you again, 30–40 days after you've had your baby for a 10 minute phone conversation to see how you're recovery went.

4.3 Summary of anticipated data cleaning and management plan

Once data collection sheets are shared, data will be coded and entered in Excel. The data cleaning process would be done as described by Royal Papworth Hospital (356). Data distribution (i.e., normal or skewed distribution) will be statistically evaluated, and an appropriate statistical method (statistical analysis test, central tendency and variability) will be used accordingly.

4.4 COVID-19 pandemic disruption on conducting C-LACE study

4.4.1 Suspension of C-LACE study and current status

The researcher and supervisors had been planning and developing the protocol that would be used in the study since 2018. The C-LACE study was a top priority as it was the backbone of the research, alongside the systematic review and laboratory work. As conducting and completing the C-LACE study would provide valuable information that would be used to directly assess current cefuroxime dosing in clinical setting and to assist with PBPK modelling.

The ethical approval of C-LACE study was granted in late January 2020—but just two months later, the WHO declared COVID-19 as a pandemic (357). This affected the current research. Healthcare resources, including staff, were burdened by the crisis, and research within hospitals that was unrelated to COVID-19 was suspended. Further, the laboratories at the University of Birmingham closed for several months. This affected the validation process with respect to developing an analytical method for measuring cefuroxime in the blood and adipose samples that would be collected during the C-LACE study. Several attempts were made to begin the C-LACE study despite these challenges, but none were successful—mainly due to staff shortages.

The C-LACE study is at a steady status, where a Confirmation of Capacity & Capability was granted from Birmingham Women's and Children's Hospital NHS Foundation Trust R&D in January 2021. Nonetheless, the researcher's supervisors decided to submit the PhD thesis in the middle of 2021 without the results of the C-LACE study. The decision was made because it remained difficult to predict the start of the C-LACE study.

Chapter 5: Application of Physiologically-Based Pharmacokinetics Cefazolin and Cefuroxime Models in Healthy, Pregnancy and Obese Models and Development of a Combined Pregnancy and Obese Physiologically-Based Pharmacokinetics Model

The aim of this chapter was to use available PBPK models within Simcyp® simulator to predict cefazolin and cefuroxime concentrations (plasma and adipose) in lean non–pregnant, lean–pregnant, obese and obese–pregnant populations

Manuscript number: (3)

Status: Published

Publication title: "Application of a Physiologically Based Pharmacokinetic Model to Predict Cefazolin and Cefuroxime Disposition in Obese Pregnant Women Undergoing Caesarean Section"

The page number of the thesis: 137

5.1 Application of a Physiologically Based Pharmacokinetic Model to Predict Cefazolin and Cefuroxime Disposition in Obese Pregnant Women Undergoing Caesarean Section.

Hanadi H. Alrammaal[1, 2*], Khaled Abduljalil[3], Victoria Hodgetts Morton[4, 5], R. Katie Morris[4, 5], John F Marriott[1], Hsu P. Chong[6], Hannah K. Batchelor[7].

[1]School of Pharmacy, Institute of Clinical Sciences, Robert Aitken Building, University of Birmingham, Birmingham, United Kingdom.

[2]Clinical Pharmacy department, Collage of Pharmacy, Umm Al-Qura University, Makkah, Makkah Province, Saudi Arabia

[3]Certara UK Limited, Simcyp Division, Sheffield, United Kingdom.

[4]Department of Fetal and Maternal Medicine, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom.

[5]Institute for Metabolic and Systems Research, University of Birmingham, Birmingham, United Kingdom

[6]Rosie Maternity Hospital, Robinson Way, Cambridge, United Kingdom.

[7]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom.

*** Correspondence:**

Hanadi Hassan Alrammaal

Keywords: Cefuroxime, Cefazolin, Physiologically-based pharmacokinetic model, Caesarean section, Obese–pregnant, Adipose exposure.

This manuscript is written in British English

5.1.1 Abstract

Intravenous (IV) cefuroxime and cefazolin are used prophylactically in caesarean section (CS). Currently, there are concerns regarding sub-optimal dosing in obese–pregnant women compared to lean–pregnant prior to CS. The current study used a physiologically-based pharmacokinetic (PBPK) model to predict cefazolin and cefuroxime pharmacokinetics in obese–pregnant women at the time of CS and the duration that the drug concentration remains above a target concentration (2, 4 or 8 µg/ml or µg/g). Cefazolin and cefuroxime PBPK models were first built using clinical data in lean non–pregnant and in obese non–pregnant populations. Models were then used to predict cefazolin and cefuroxime pharmacokinetics data in lean– and obese–pregnant. Cefazolin and cefuroxime models sufficiently described plasma, unbound plasma and unbound adipose interstitial fluid (ISF) concentrations in non–pregnant and pregnant populations. Predictions indicated that an IV 1500 mg cefuroxime dose can achieve unbound plasma and unbound ISF cefuroxime concentration of ≥8 µg/g up to 2 hours post dose in obese–pregnant. Re-dosing should be considered if CS was not completed within 2-hours post cefuroxime administration for both lean– or obese–pregnant, if cefuroxime concentrations of ≥8 µg/mL is required. A time of 15–30 minutes between cefuroxime administration and the start of skin incision in obese–pregnant women is preferable to provide sufficient protection. A clinical study to measure cefuroxime adipose concentration in pregnant and obese–pregnant women is warranted.

5.1.2 Introduction

Cefuroxime and cefazolin are used widely as intravenous prophylactic antibiotics in surgical settings (9, 10). Although their efficacy has been compared in clinical practice, their pharmacokinetics differ (Table 5.1) (76, 358). The plasma unbound fraction (f_u) of cefazolin and cefuroxime are different in lean non pregnant; 0.225 and 0.67, respectively (94, 107, 108). While the reported lipophilicity of cefuroxime is lower than cefazolin (Log P values of -0.90 vs. -0.58, respectively) (91, 359). Cefuroxime's apparent volume of distribution (V_d) is approximately 1.4–fold higher than that of cefazolin in non–obese non–pregnant subjects (100, 109). Both drugs are cleared entirely by the kidneys (97-99, 113). Transporter medicated clearance is believed to be responsible for 50–80% of cefazolin total clearance (360). While for cefuroxime, approximately 45–50% is reported to be cleared by tubular secretion (108).

Physiological and biochemical alterations, observed in pregnancy, can greatly impact drug kinetics (361, 362). The increased incidence of obesity within the pregnant population further complicates predictions of drug kinetics as obesity can also affect drugs kinetics (363). The extent and direction of clinical impacts of both obesity and gestation on pharmacokinetics and pharmacodynamics (PK/PD) has not been clearly established for the majority of drugs. While cefazolin PK/PD in obese–pregnant women, body mass index (BMI) ≥30 kg/m^2, has been investigated due to concerns around sub-optimal dosing in this population compared with those of normal body weight (BMI 18.50–24.99 kg/m^2); to date, there is no information regarding disposition of cefuroxime in an obese–pregnant population (10-16, 300).

The physiologically-based pharmacokinetics (PBPK) approach has the potential to predict drug exposure in pregnant women based on the drug's physiochemical

properties and knowledge of physiological changes during pregnancy (364). The motivation to conduct this study is to predict cefazolin and cefuroxime concentrations in plasma, total adipose tissue and adipose interstitial fluid (ISF) in non–obese and obese–pregnant women using a PBPK approach to ensure optimised dosing of these drugs at caesarean section (CS). This study aims were: to develop PBPK models for cefuroxime and cefazolin in non–pregnant, non–obese subjects as well as in obese non–pregnant subjects using available clinical data of serum and adipose tissue (total adipose concentrations or free antibiotic adipose ISF concentration) for cefazolin and cefuroxime concentrations; subsequently to use the developed model to predict the exposure in non–obese pregnant and in obese–pregnant populations. Although the minimum inhibitory concentrations required to inhibit the growth of 90% of organisms (MIC_{90}) of cefuroxime and cefazolin for most of the common causative bacteria of post CS infections is 1–2 µg/mL, various bacterial strains may require higher concentrations. Simulated plasma and adipose tissue concentrations of different doses were compared to a range of MIC_{90} (i.e. 2, 4 and 8 µg/mL) (12, 301). The timing of administration relative to skin incision was also investigated to ensure that concentrations are >MIC_{90} at the time of incision and during CS. This report follows the checklist suggested for pharmacometric studies (365).

Table 5.1: Reported pharmacokinetic parameters of cefazolin and cefuroxime in different populations

Population	f_u	V_d (L)	V_d[a] (L/Kg)	V_{ss}[b](L/Kg)	CL (L/h)
Cefazolin					
Non–pregnant	0.225 (94, 107)	11.6 (100)	0.186(100)	0.149	4.66 (100)
Pregnant (24 GW)	0.25 (101)	11.9 (100)	0.172(100)	0.137	7.30 (100)
Pregnant, at time of delivery (39.1 GW)		9.44 (102)	0.11(102)	0.09	7.18 (102)
Overweight	0.15 (104)		0.17(104)	0.136	3.72 (104)
Obese[c] and pregnant at time of CS	0.29 (12)	13.36 (16) 13.45 (16)		0.174 (16) 0.17 (16)	8.4 (16) 6.6 (16)
Cefuroxime					
Non–pregnant	0.67 (108)	16.3 (109)	0.264	0.211	11.9 (109)
Pregnant (29 GW)	N/R	17.8 (109)	0.276	0.221	16.9 (109)
Pregnant, at time of CS (39.5 GW)	N/R		0.842 (9)	0.673 0.351[d]	32.75 (9)
Pregnant, at time of delivery (41 GW)	N/R	19.3 (109)	0.26	0.21	15.5 (109)
Obese[c]	N/R			0.192 (110)	8.39±3.07 (110)

CS caesarean section, CL clearance, f_u Fraction unbound, GW Gestational weeks, N/R not reported, V_d Volume of distribution, V_{ss} volume of distribution at steady state.

[a] the V_d (L/Kg) is based on the participants mean weight reported in the study.

[b] The V_{ss} is considered as 80% of V_d (111) unless it has reference (stated in the study) or calculated.

[c] Body Mass Index ≥ 30 kg/m^2

[d] V_{ss} calculated using equation V_{ss} = Clearance * Mean residence time of drug in the body (9).

5.1.3 Materials and Methods

5.1.3.1 Model building

Both cefazolin and cefuroxime disposition were investigated in: a) non–obese (lean), non–pregnant (BMI 18.50–24.99 kg/m^2), b) obese non–pregnant (BMI ≥30 kg/m^2), c) pregnant non–obese (lean–pregnant) (BMI 18.50–24.99 kg/m^2) and d) obese–pregnant (BMI ≥30 kg/m^2) populations. As *in vivo* cefuroxime disposition data was unavailable for obese–pregnant (BMI ≥30 kg/m^2) populations, cefazolin was selected to build a new obese–pregnant model; then, cefuroxime concentration in obese–pregnant women was simulated using the developed obese–pregnant population model.

The Simcyp Simulator (Simcyp Version 20 Release 1) was used. The compound files were developed using a full PBPK (multi-compartment) distribution model and the volume of distribution at steady state (V_{ss}) was predicted using Rodgers and Rowland method within the Simcyp simulator (90, 135). To predict the free cefazolin and cefuroxime ISF adipose concentrations, the adipose tissue extracellular fraction unbound (f_{ut}) was assumed to be equal to plasma f_u. The glomerular filtration rate is predicted using the modification of diet in renal disease (MDRD) equation (146). The mechanistic kidney permeability model (Mech KiM) was used to account for transporters kinetics (Table 5.2) (141, 142). The pregnancy model was coupled with the Mech KiM model as illustrated in supplementary document, Figure 7.3. For predicting the exposure in the obese non–pregnant population, the obese population model within the simulator was used. For predicting the exposure during pregnancy, the pregnancy population model within the simulator was selected. Physiological changes during pregnancy relevant to this model and the way they have been

incorporation in the simulator during pregnancy have been published (149, 366). The maternal PBPK model was used without incorporation of the multi-compartmental feto-placental model; this was considered sufficient to serve the aim of this study. Key equations used to predict cefazolin and cefuroxime disposition in lean–pregnant, obese– and morbidly obese–pregnant are illustrated in supplementary document (Equations **(7.2)** to **(7.16)**).

For obese–pregnant, the default non–pregnant physiology of obese subjects or morbidly obese (in terms of tissue blood flows) in simulator was used as a pre-pregnancy baseline; for which the physiological changes during pregnancy were applied (supplementary document Table 7.14). Any change to the tissue blood flow during pregnancy were kept as pre-defined in the simulator for the pregnant subjects. The pre-pregnant weight was changed in the population demographic to allow prediction of pregnancy weight (at 39.5 gestational weeks) of either 97.8 Kg (obese–pregnant) or 128 kg (morbidly obese–pregnant) to reflect weight in obese–pregnant subjects.

5.1.3.2 Physiologically Based Pharmacokinetic simulation design

Covariates (age, sex, and gestational age) were set to the mean as reported in the clinical studies. If these covariates were not reported, the default populations' predefined characteristics within the simulator were selected (Table 5.3 and Table 5.4). If the predicted mean weight was different from the reported mean weight in clinical studies by more than ±5 kg, the weight within the simulator was adjusted to the mean reported weight ±5 kg. This was done by adjusting the non–pregnant bodyweight code within the Lua script in the population demographic. Simulations were executed after matching the doses to those used in the clinical studies. All virtual studies were set to

20 trials per run. Empowering the simulations of all studies using 20 virtual trials for the reported sample size was selected to increase the confidence around the obtained PK parameters.

Table 5.2: Physiochemical properties and pharmacokinetics values of cefazolin and cefuroxime for healthy subjects used in compounds building using simcyp 20.

Compound	**Cefazolin**		**Cefuroxime**	
Parameter	Value	Reference	Value	Reference
Molecular weight (g/mol)	454.5	(367)	424.39	(359)
Log P	-0.58	(368)	-1.5[1]	
Compound Type	Monoprotic Acid		Monoprotic Acid	(359)
Acid PKa	3.6	(368)	3.15	(359)
B/P	0.55	(369)	0.56	(113)
f_u	0.225	(94, 107)	0.67	(108)
V_{ss} (L/Kg)	0.1[2]		0.226[2]	
K_p Scalar	1		1	
Adipose tissue: plasma partition coefficient	Default (0.06)		Default (0.12)	
Lung tissue: plasma partition coefficient	0.2[3]	(370)	Default (0.43)	
Muscle tissue: plasma partition coefficient	0.03[3]	(370)	Default (0.25)	
Skin tissue: plasma partition coefficient	0.09	(371)	Default (0.53)	
Kidney tissue: plasma partition coefficient	Default (0.21)		Default (0.36)	
$Cl_{PD, basel}$ (mL/min/10^6 cells)	2.07E-08	(369)	N/A	
$Cl_{PD, apical}$ (mL/min/10^6 cells)	2.07E-08	(369)	N/A	
$fu_{Kidney\ cell}$	1		N/A	
fu_{Urine}	1		N/A	
$CL_{int,T}$ (µL/min/10^6 cells) by $T_{up,b}$ (OAT1)	0.208	(369)	9.62[4]	(113)
$CL_{int,T}$ (µL/min/10^6 cells) by $T_{up,b}$ (OAT3)	7.28	(369)	N/A	
$CL_{int,T}$ (µL/min/10^6 cells) by $T_{eff,a}$ (MRP4)	41.43	(99)	10^5	(113)

B/P blood to plasma partition ratio, Log P partition coefficient, f_u fraction unbound, V_{ss} volume of distribution at steady state, Kp tissue-to-plasma partition coefficient, $CL_{int,T}$ *in vitro* transporter-mediated intrinsic clearance Cl_{PD} passive diffusion clearance, Clr renal clearance, $T_{eff,a}$ efflux transporter on apical membrane, $T_{up,b}$ uptake transporter on basolateral membrane, N/A not applicable.

[1] Measured using shake-flask method

[2] Predicted using Rodgers and Rowland method within the Simcyp simulator (90, 135).

[3] Mean value from rats and rabbits (370)

[4] Optimised by Hsu et al. (113) using serum concentration time profile

[5] Optimised by Hsu et al. (113) based on urine data

Table 5.3: Summary of study designs used for cefazolin Physiologically Based Pharmacokinetic model building.

Population	Study code and reference	Population model used	Dose (mg)	Infusion time (min)	Number of subjects (proportion of female)	Age years (minimum-maximum)	Weight (kg)	Mean GA (weeks)
Lean non–pregnant	001 (100)	Sim-Healthy Volunteers	500	2	6 (1)	N/S[1]	62.3	N/A
	002 (372)	Sim-Healthy Volunteers	1000	N/S (used 2 min)	17 (0)	21–42	N/S[1]	N/A
Pregnant	003 (100)	Sim-Pregnancy	500	2	6 (1)	N/S[1]	67.9[2]	24.5
	004 (10, 284)	Sim-Pregnancy	2000	4	10 (1)	28 (23–32)	65.7[2]	39.1
	005 (102)	Sim-Pregnancy	1000	IV bolus (N/S used 1 min)	20 (1)	32.5(23–43)	79.3[2]	39
Obese	006 (78)	Sim-Obese	2000	IV bolus (N/S used 1 min)	37 (0.7)	18–60	127[2]	N/A
Obese–pregnant	007 (16)	Sim-Pregnancy[3]	2000	3	13 (1)	29 (23.5–34)	113.6[2, 4]	39.3
	008 (16)	Sim-Pregnancy[3]	3000	3	13 (1)	31(30–35)	108[2, 5]	39
	009 (14)	Sim-Pregnancy[3]	2000	IV bolus (N/S used 1 min)	28(1)	30 (25.5–34)	103 [2, 6]	39.2
Morbidly obese–pregnant	0091 (15)	Sim-Pregnancy[7]	2000	IV push (0.75 min)	11 (1)	31.09[1]	129.14[2]	38.75

GA gestational age, N/A not applicable, N/S not stated in the clinical study,
[1] Used the default value of the selected population in Simcyp simulator
[2] Weight was adjusted within simulator to report the mean observed weight ± 5 Kg
[3] The pre-pregnancy tissue flow rate was modified as obese
[4] Reported BMI at time of delivery as 42.9 Kg/m^2, suggested weight as 113.6 if height is around 163cm
[5] Reported BMI at time of delivery as 41.8 Kg/m^2, suggested weight as 108 if height is around 163cm
[6] Reported BMI at time of delivery as 38.9 Kg/m^2, suggested weight as 103 if height is around 163cm
[7] The pre-pregnancy tissue flow rate was modified as morbidly obese

Table 5.4: Summary of study designs used for cefuroxime Physiologically Based Pharmacokinetic model building.

Population	Study code and reference	Population model used	Dose (mg)	Infusion (min)	Number of subjects (proportion of female)	Age (minimum–maximum) years	Weight (kg)	Mean GA (weeks)
Lean non–pregnant	01 (305)	Sim-Healthy Volunteers	1500	N/S (used 2 min)	12 (N/S[1])	N/S[1]	N/S[1]	N/A
	02 (109)	Sim-Healthy Volunteers	750	1	7 (1)	N/S[1]	61.7[2]	N/A
	03 (373)	Sim-Healthy Volunteers	1500	30	23 (0.26)	(19–31)	76[2]	N/A
	04 Garton A (374)	Sim-Healthy Volunteers	750	20	10 (0.5)	32 (18–48)	72	N/A
	05 Garton B (374)	Sim-Healthy Volunteers	1500	20	10 (0.5)	32 (18–48)	72[2]	N/A
Pregnant	06 Philipson PRG (109)	Sim-Pregnancy	750	1	7 (1)	N/S[1]	64.4	29
	07 Philipson DLR (109)	Sim-Pregnancy	750	1	7 (1)	N/S[1]	74	41(used 40)
	08 (9)	Sim-Pregnancy	1500	1	18 (1)	23–37	78.94	39.5
	09 (273)	Sim-Pregnancy	1500	IV bolus (N/S used 1 min)	10 (1)	16–32[3]	74.4[2]	At term (used 39)
	091 (273)	Sim-Pregnancy	1500	IV bolus (N/S used 1 min)	10 (1)	17–36[3]	70.3[2]	At term (used 39)
Obese	092 (110)	Sim-Morbidly Obese	1500	15[4]	6 (1)	19–76	131.66[2]	N/A
Obese–pregnant	1	Sim-Pregnancy[5]	750	1	50 (1)	23–37	97.8[6]	39.5
	2	Sim-Pregnancy[5]	1500	1	50 (1)	23–37	97.8[6]	39.5
Morbidly obese–pregnant	3	Sim-Pregnancy[7]	750	1	50 (1)	23–37	128[8]	39.5
	4	Sim-Pregnancy[7]	1500	1	50 (1)	23–37	128[8]	39.5

GA gestational age, DLR at time of delivery, N/A not applicable, N/S not stated in the clinical study, PRG pregnant. [1] Used the default value of the selected population in simcyp simulator; [2] Weight was adjusted within simulator to report the mean observed weight ± 5 Kg; [3] The minimal age for pregnant in simcyp is 20 years; [4] Didn't mention the exact infusion time, stated as "short term infusion" suggested as 15 min; [5] The pre-pregnancy tissue flow rate was modified as obese; [6] A pre-pregnancy weight of 80 Kg was selected; [7] The pre-pregnancy tissue flow rate was modified as morbidly obese; [8] A pre-pregnancy weight of 105 Kg was selected.

5.1.3.3 Evaluation and refinement of cefazolin PBPK model in lean non–pregnant, obese non–pregnant, lean–pregnant and obese–pregnant subjects.

The physiochemical and pharmacokinetic inputs for the PBPK model of cefazolin are shown in Table 5.2 (375). Cefazolin is excreted (approximately 100%) as unchanged drug in the urine (376). In addition to the glomerular filtration of cefazolin, different transporters are involved in its tubular secretion (97-99). Recently, the activity of renal OAT3 has been shown to increase by approximately 2.2 during the 1st trimester, 1.7 during the 2nd trimester and 1.3–fold during the 3rd trimester (377). The permeability limited kidney model was used to represent the transporters involved in cefazolin clearance. The gestational age-dependent activity of OAT3 was updated in maternal model according to a recent publication as shown in Equation **(5.1)** (375).

The **PBPK cefazolin** model was evaluated using a) two independent pharmacokinetics studies on lean non–pregnant subjects (100, 372), b) three independent pharmacokinetics studies conducted in pregnant populations (10, 100, 102, 284), c) one study in an obese population (78) and d) four independent pharmacokinetics datasets from obese– and morbidly obese–pregnant populations (14-16) (Figure 5.1).

$$\text{Renal OAT3 pregnancy (fold change)} = 1 * (1 + 0.195 * \text{GW} - 0.0093 * \text{GW}^2 + 0.0001154 * \text{GW}^3) \quad \textbf{(5.1)}$$

Where GW is Gestational Week.

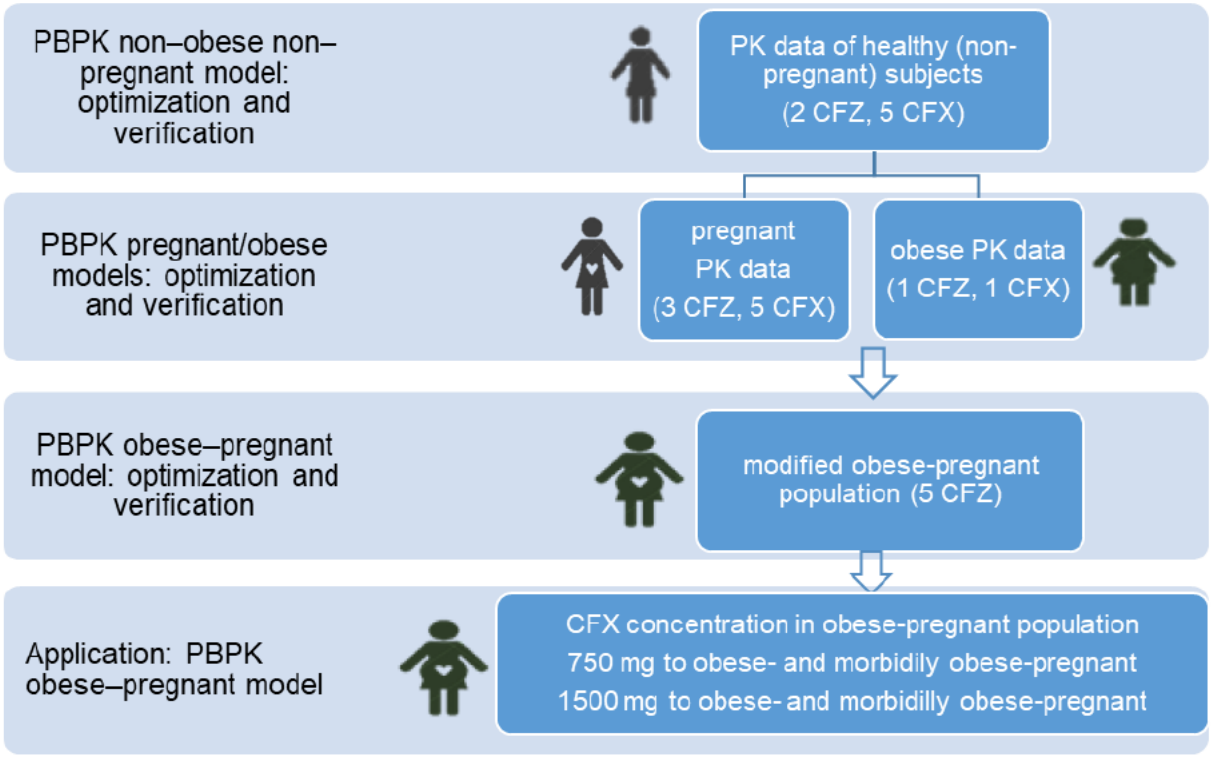


Figure 5.1: Summary of study optimization, evaluation and application of physiologically-based pharmacokinetics models in population of interest
Number in practice represent number of clinical pharmacokinetics data sets used for validation of each drug physiologically-based pharmacokinetics model in an exact population; CFX cefuroxime, CFZ cefazolin, PBPK Physiologically-based pharmacokinetics, PK pharmacokinetics.

5.1.3.4 Evaluation and refinement of cefuroxime PBPK model in lean non–pregnant, obese non–pregnant, lean–pregnant subjects.

An existing compound file for cefuroxime has been developed previously by Hsu et al. using the Simcyp simulator version 12.1 in lean non–pregnant subjects (113). The model was reproducible in version 20. The physiochemical properties and pharmacokinetic parameters of the PBPK cefuroxime model are summarised in in Table 5.2. The predicted Log P used in Hsu et al. was -0.9, this was replaced by an experimental value of -1.5 measured in-house using the shake-flask method (supplementary document, 7.3.1.1 Method7.3.1.1). Despite the fact that the model was

not sensitive to the Log P change, the measured value was kept in the model for documentation purposes. A global tissues-plasma partition coefficient (Kp) scalar of 1 was used. The Mech KiM model was used to describe the uptake and efflux of active secretion of cefuroxime in the kidney as described by Hsu et al. (113).

The PBPK cefuroxime model was assessed using a) five pharmacokinetics datasets from lean non–pregnant subjects (109, 305, 373, 374), b) five observed pharmacokinetics datasets from pregnant populations (9, 109, 273) and c) a single set of observed pharmacokinetics data in obese patients who underwent abdominal surgery (110) (Figure 5.1).

5.1.3.5 Acceptance Criteria

The PBPK models were considered successful if the simulated plasma area under the curve (AUC) and/or plasma maximum concentration (C_{max}) (or first reported time concentration point) were within 2–fold of the observed AUC and/or C_{max}. Additionally, visual checks of the observed and simulated concentration-time profiles were performed and considered for accepting the model prediction. Study quality and sample size of studies selected for optimisation of the PBPK models were considered; and any limitation in the study methodology that may affect results of observed cefuroxime and/or cefazolin concentration was countered (378, 379). For all model executions, the reported sample size in each clinical study was replicated in 20 trials of virtual populations.

5.1.3.6 Application of obese–pregnant model to predict cefuroxime disposition

Four clinical scenarios were explored, where 750 mg (study 1) and 1500 mg (study 2) doses of cefuroxime were tested in the obese–pregnant population (BMI 30–34.9

kg/m^2); further, 750 mg (study 3) and 1500 mg (study 4) doses of cefuroxime were tested in the morbidly obese–pregnant population (BMI ≥35 kg/m^2). In each scenario, the plasma concentrations were presented for cefuroxime administered at 15, 30 or 60 min before the start of skin incision in CS.

Sources and approaches to analyse constants (or assumptions), covariates and scripted algorithm of selected PBPK models in different populations of interest have been discussed in literature (90, 135, 141, 142, 146, 149, 245, 366). The scope of the current study is utilisation of these PBPK models to predict cefazolin and cefuroxime in different populations including obese–pregnant population without the necessity to develop algorithmic equations exclusively for this population; rather using scripted obese population's physiological data and pregnancy gestational changes algorithm within simulator.

Due to unavailability of adipose tissue concentrations in healthy volunteer, the selected models were first "piloted" using observed data of plasma, free plasma and unbound adipose tissue concentrations of cefazolin and cefuroxime in subjects undergoing surgical procedures.

5.1.4 Results

5.1.4.1 Cefazolin PBPK model

The observed cefazolin total, free plasma and adipose tissue ISF concentrations utilised to pilot cefazolin PBPK predictions and simulated concentrations are presented in Figure 5.2. Simulated cefazolin AUC and C_{max} (or the first reported time concentration point) were within 2–fold of the observed data; the exception was the total adipose concentration in Stitely et al. (Table 5.5 and Table 5.6). The observed

and mean simulated plasma concentrations time profiles for cefazolin are shown in Figure 5.3; and cefazolin total adipose concentrations time profiles are shown in Figure 5.4.

All simulated cefazolin plasma concentrations (for the examined doses of 500, 1000, 2000 and 3000 mg) were above the MIC_{90}, of 8 µg/mL for a mean time of 6.11 hours, 4 µg/mL for a mean time of 7.92 hours and 2 µg/mL for a mean time of 9.8 hours following infusion. In an obese–pregnant population (BMI ≥30 kg/m^2), the simulated time of free cefazolin plasma concentrations were above the MIC_{90} (*f*T>MIC) of 8 µg/mL was a mean of 3.52 hours, 4 µg/mL up to a mean of 5.28 hours and 2 µg/mL up to a mean of 7.16 hours following infusion of a 2000 mg dose (supplementary document, Results section Table 7.15).

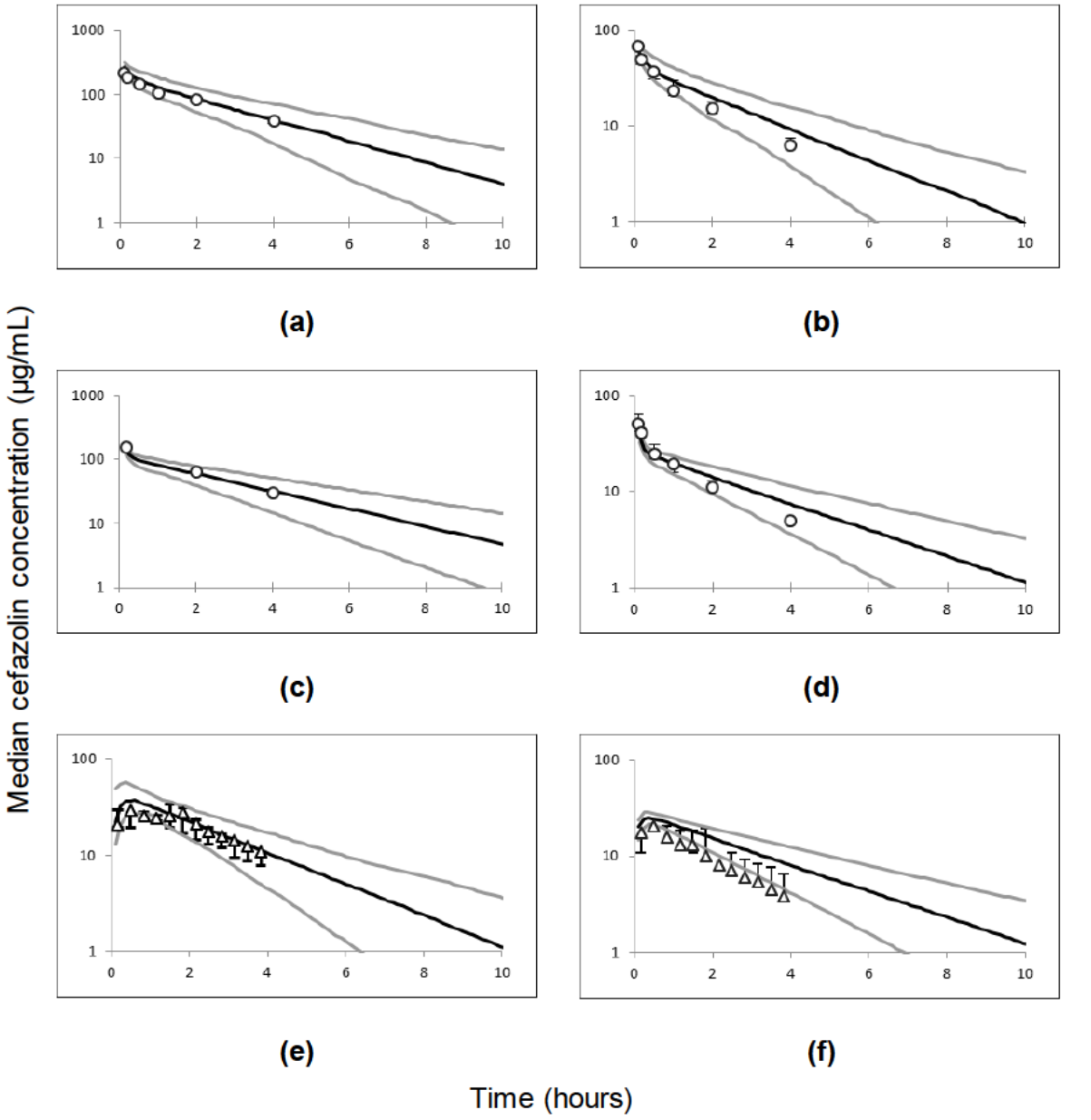


Figure 5.2: Simulated and observed cefazolin time concentrations curves in non–obese and obese subjects required surgical procedures

Time-concentrations curves represent observed and simulated cefazolin **(a)** total plasma, **(b)** unbound plasma, and **(e)** unbound adipose tissue cefazolin concentrations in non–obese subjects; and simulated and observed **(c)** total plasma, **(d)** unbound plasma, and **(f)** unbound adipose tissue cefazolin concentrations in obese subjects (380).

Table 5.5: Observed vs simulated area under the curve, serum concentration and clearance of cefazolin.

Study code and reference	AUC (mg/L.h)			Cefazolin serum concentration (µg/mL) at time point				Clearance (L/h)		
	Obs.	Sim.	Ratio[1]	Time point (min)	Obs.	Sim. (5^{th}, 95^{th} percentile)	Ratio[1]	Obs.	Sim.	Ratio[1]
001 (100)	110	130.27	1.18	$C_{10.8}$	73.24	66.26 (51.82, 82.85)	0.90	4.04	3.91	0.97
002 (372)	236.15[2]	262.60	1.11	$C_{4.4}$	188.6	152.72 (128.60, 179.57)	0.81	3.8	4.01	1.06
003 (100)	75.7	84.37	1.11	C_{9}	60.07	57.38 (45.56, 73.06)	0.96	7.3	6.22	0.85
004 (10, 284)	N/R[3]	362.97	N/A	C_{82}	57.2	95.74 (60.07, 135.05)	1.67	N/R	5.76	N/A
005 (102)	139.27[2]	172.24	1.24	C_{max}	82.53	105.60 (86.73, 126.67)	1.28	7.18	5.99	0.83
006 (78)	N/R[3]	415.86	N/A	C_{16}	159.96	131.55 (110.07, 155.41)	0.82	N/R	5.04	N/A
007 (16)	234.3	307.48	1.31	C_{max}	146.15	163.31 (133.85, 203.67)	1.12	8.4	6.17	0.73
008 (16)	453.4	446.82	0.99	C_{max}	223.74	249.68 (199.69, 301.13)	1.12	6.6	6.35	0.96
009 (14)	N/R[3]	316.33	N/A	$C_{24.5}$	100.7	116.01 (84.16, 161.83)	1.15	N/R	6.53	N/A
0091 (15)	N/R[3]	294.52	N/A	C_{12}	155.45	115.28 (85.84, 153.94)	0.74	N/R	7.04	N/A

AUC area under the curve, C_{max} maximum concentration, N/A not applicable, N/R not reported, Obs. Observed, Sim. Simulated, numbers in brackets represent the 5^{th} and 95^{th} percentile.

[1] Ratio of predicted/observed

[2] calculated using $\text{Area under the curve} = \frac{\text{Dose}}{\text{Clearance}}$

[3] The AUC could not be estimated, insufficient concentration-time points

Table 5.6: Observed and simulated adipose tissue concentrations of cefazolin and simulated time above MIC_{90}

Study code and reference	Adipose tissue concentration (µg/g) at time point				Simulated time (hour) of total adipose tissue concentration above MIC_{90}			Simulated time (hour) of free ISF adipose tissue concentration above MIC_{90}		
	Time point (min)	Obs.	Pred. (5^{th}, 95^{th} percentile)	Ratio[1]	T>MIC of 2 µg/g	T>MIC of 4 µg/g	T>MIC of 8 µg/g	T>MIC of 2 µg/g	T>MIC of 4 µg/g	T>MIC of 8 µg/g
001 (100)	C_{max}	N/R	4.52 (3.12, 6.29)	N/A	2.04	0.6	NA	4.08	2.52	1.08
002 (372)	C_{max}	N/R	7.62 (5.17, 10.84)	N/A	3.6	2.04	NA	6	4.2	2.52
003 (100)	C_{max}	N/R	4.01 (2.56, 5.72)	N/A	1.32	0.24	NA	3	1.8	0.72
004 (10, 284)	$C_{31.5}$	8.7	14.21 (10.90,18.55)	1.63	3.72	2.52	1.44	5.76	4.56	3.24
005 (102)	C_{max}	N/R	6.88 (4.47, 10.11)	N/A	2.52	1.32	NA	4.8	3.36	2.04
006 (78)	C_{16}	8.78	9.18 (7.33, 10.91)	1.05	5.52	3.24	0.96	9	6.48	4.08
007 (16)	C_{25}	12.4	9.85 (7.30, 13.86)	0.79	4.08	2.4	0.84	7.08	5.28	3.48
008 (16)	C_{28}	16.8	14.39 (10.78, 19.59)	0.86	5.04	3.36	1.8	8.04	6.24	4.44
009 (14)	$C_{24.5}$	9.4	10.08 (7.67, 13.79)	1.07	4.08	2.4	0.96	6.96	5.16	3.48
0091 (15)	C_{12}	18.36± 6.68	8.89 (6.16, 12.05)	0.48[2]	4.08	2.28	0.6	7.44	5.4	3.48
	C_{60}	21.73±16.02	6.85 (4.95, 9.01)	0.32[2]						

C_{max} maximum concentration, ISF interstitial fluid, N/A not applicable, N/R not reported, T>MIC Time above the minimum inhibitory concentration.

[1] Ratio of predicted/observed

[2] Outside the 2–fold criteria.

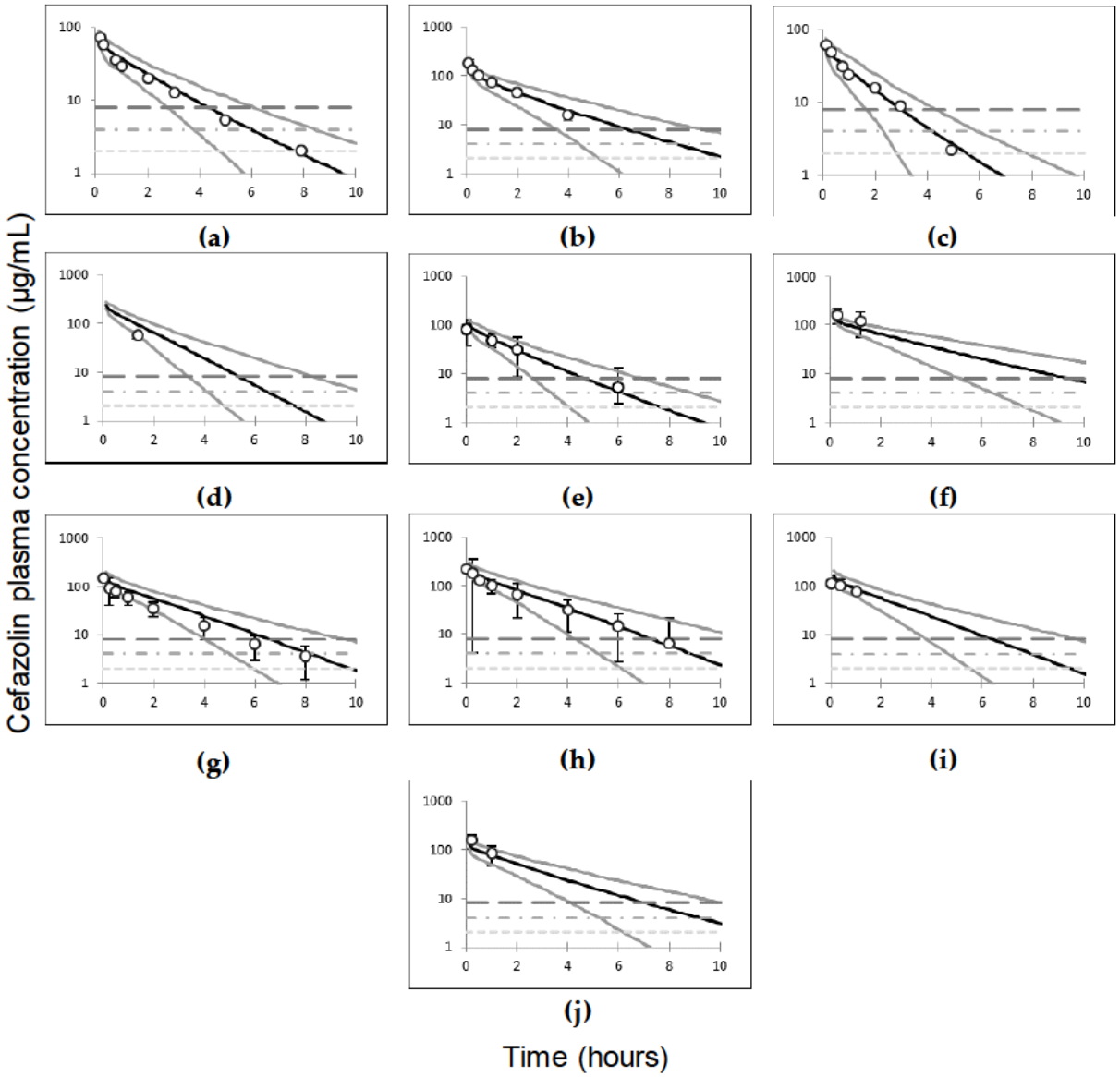


Figure 5.3: Simulated and observed plasma time concentrations curves of cefazolin in different population.
(a) and **(b)** represent simulated and observed plasma time concentrations curves of cefazolin in lean non–pregnant subjects (100, 372); **(c)**, **(d)** and **(e)** in pregnant subjects (10, 100, 102, 284); **(f)** in obese subjects (78); **(g)**, **(h)**, **(i)** and **(j)** in obese–pregnant subjects (14-16). Errors bars represent standard deviation in **(b)**, **(f)** and **(j)**; interquartile range in **(d)** and **(i)**; 95th and 5th percentile in **(e)**; and standard error of mean in **(g) and (h).**

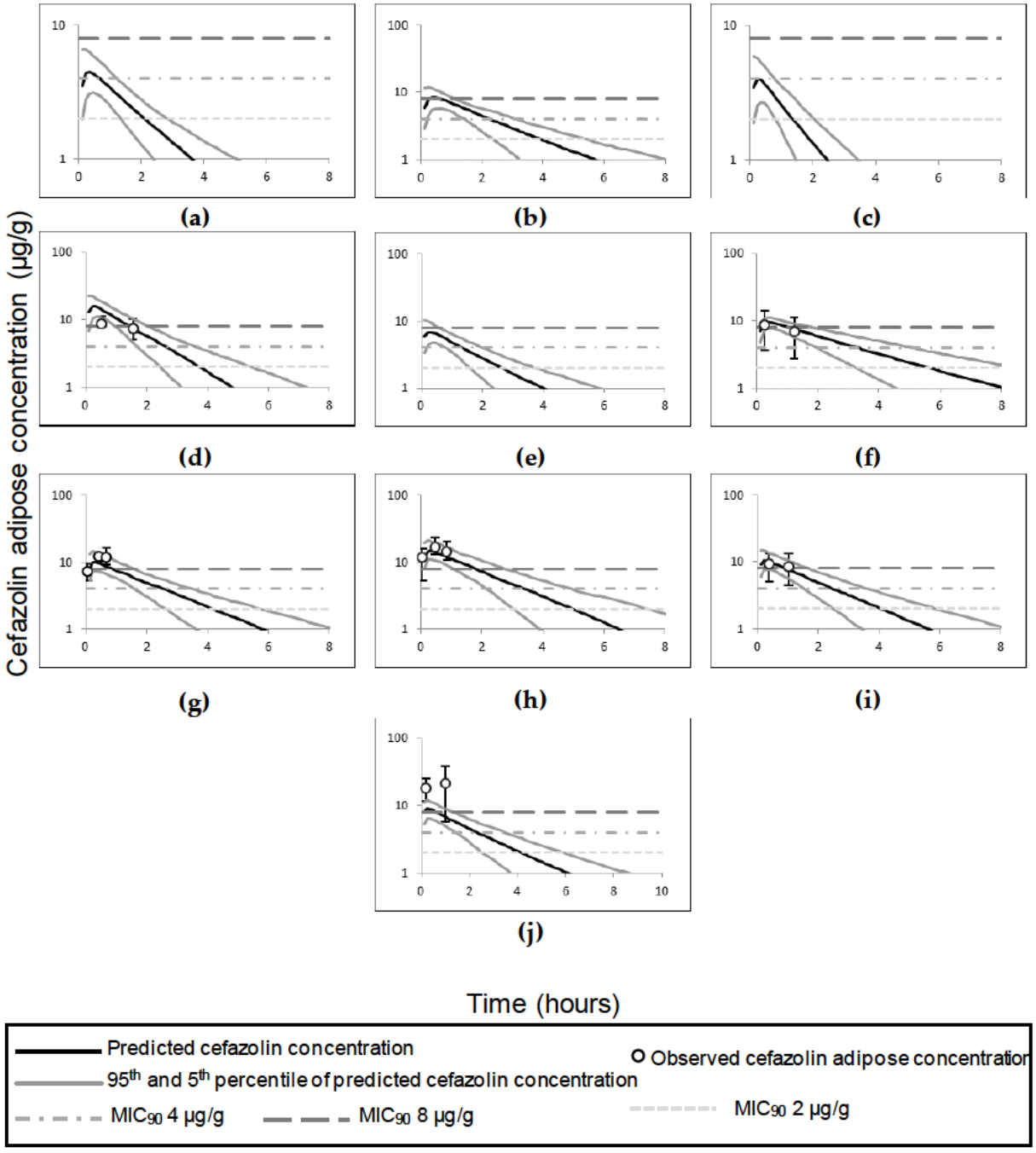


Figure 5.4: Simulated and observed adipose tissue time concentrations curves of cefazolin in different population.

(a) and **(b)** represent simulated and observed adipose time concentrations curves of cefazolin in lean non–pregnant subjects (100, 372); **(c)**, **(d)** and **(e)** in pregnant subjects (10, 100, 102, 284); **(f)** in obese subjects (78); **(g)**, **(h), (i)** and **(j)** in obese–pregnant subjects (14-16). Errors bars represent interquartile range in **(d), (g), (h) and (i)**; and standard deviations in **(f)** and **(j).**

5.1.4.2 Cefuroxime PBPK model

The observed and simulated cefuroxime total, free plasma and adipose tissue ISF concentrations utilised to preliminary predict cefuroxime concentrations are presented in Figure 5.5.

All simulated data of AUC and C_{max} (or first reported time concentration point) cefuroxime concentrations (serum and adipose tissue) were within 2–fold of the observed data; the exceptions were the AUC in Lalic-Popovic et al. and the adipose tissue concentrations in Lovering et al. (Table 5.7 and Table 5.8). The observed and mean simulated plasma concentrations time profiles for cefuroxime are shown in Figure 5.6; and cefuroxime adipose concentrations time profiles are shown in Figure 5.7. The predicted cefuroxime plasma 5th percentile was superior in describing cefuroxime clearance phase compared to the predicted mean in lean pregnant (Figure 5.66 f-j). The predicted 5th percentile of cefuroxime plasma concentration was utilised to evaluate cefuroxime T>MIC in both lean– and obese–pregnant at time of CS. The predicted 5th percentile of cefuroxime plasma concentration in lean–pregnant at term, was ≥MIC_{90} of 8 µg/mL up to 1.6 hours post 750 mg cefuroxime dose; and 2.3 hours post 1500 mg cefuroxime dose (supplementary document, Table 7.16.

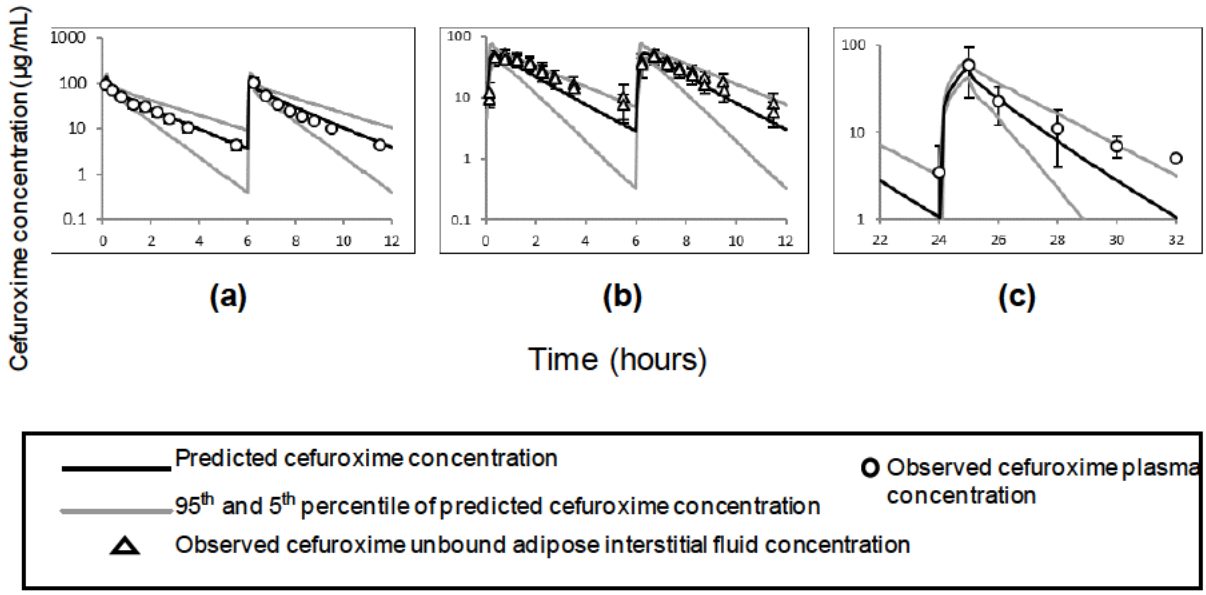


Figure 5.5: Simulated and observed cefuroxime time concentrations curves in overweight subjects
(a) Total plasma and **(b)** adipose tissue cefuroxime concentrations in overweight subjects required surgery (381), and **(c)** unbound plasma cefuroxime concentrations in overweight subjects required cerebral Microdialysis for neurochemical monitoring (306)

5.1.4.3 Cefuroxime PBPK model application in obese–pregnant population

Simulated 5th percentile cefuroxime plasma concentrations were above the MIC_{90} of 8 µg/mL for a duration of 1.63 hours after 750 mg dose and 2.45 hours after 1500 mg dose; and above 4 µg/mL for a duration of 2.45 hours post 750 mg dose, and 3.45 hours post 1500 mg dose and for both obese–pregnant and morbidly obese–pregnant populations (Supplementary document Table 7.16). The simulated 5th percentile of cefuroxime *f*T>MIC of 8 µg/mL was 3.9 hours, 4 µg/mL was 3 hours, and 2 µg/mL was 2 hours following infusion of a 1500 mg dose in in both obese–pregnant categories (studies code 2 and 4) (Supplementary document Table 7.16). In all *in silico* scenarios, cefuroxime mean total adipose tissue concentrations were above the MIC_{90} of 2 µg/g, only higher doses of cefuroxime (i.e., 1500 mg) achieved an MIC_{90} ≥4 µg/g, the MIC_{90} of 8 µg/g was not reached in both obese–pregnant categories. Cefuroxime free ISF concentrations were ≥8 µg/g for up to around 1.86 hours (5th percentile: 1.25 hours) after 750 mg dose and up to around 3.3 hours (5th percentile: 2.2 hours) after 1500 mg

dose both obese–pregnant and morbidly obese–pregnant populations (Supplementary document Table 7.17.

Table 5.7: Observed vs simulated area under the curve, serum concentration and clearance of cefuroxime

Study code and reference	AUC (mg/L.h)			Cefuroxime serum concentration (µg/mL) at time point				Clearance (L/h)		
	Obs.	Sim.	Ratio[1]	Time point (min)	Obs.	Sim. (5th, 95th percentile)	Ratio[1]	Obs.	Sim.	Ratio[1]
01 (305)	N/R[2]	150.99	N/A	C_{10}	130.8	91.04 (69.23,114.79)	0.70	N/R	11.36	N/A
02 (109)	60.8	75.79	1.25	$C_{9.6}$	51.48	52.19 (41.15, 63.31)	1.01	11.9	12.14	1.02
03 (373)	124	153.89	1.24	$C_{16.8}$	56.35	65.21 (53.63, 78.74)	1.16	12.09[3]	10.99	0.91
04 (374)	77	78.03	1.01	C_{20}	75.29	58.61 (45.73, 73.63)	0.78	9.74[3]	11.48	1.18
05 (374)	137	157.20	1.15	C_{18}	82.08	110.83 (86.74,138.67)	1.35	10.9[3]	11.48	1.05
06 (109)	42.0	61.09	1.45	$C_{7.8}$	43.56	45.29 (37.65, 53.94)	1.04	16.9	11.98	0.71
07 (109)	46.7	63.33	1.36	C_{9}	43.83	39.73 (33.94, 46.55)	0.91	15.5	11.57	0.75
08 (9)	50.79	123.15[4]	2.42[4]	$C_{62.67}$	41.14	38.30 (26.21, 51.46)	0.93	32.75	11.76[4]	0.36[4]
09 (273)	N/R	121.51	N/A	C_{30}	55.2	56.02 (42.21, 71.86)	1.01	N/R	11.92	N/A
091(273)	N/R	123.86	N/A	C_{48}	74.8	45.51 (31.75, 57.04)	0.61	N/R	11.57	N/A
092 (110)	158.7	130.76	0.82	C_{30}	64.25	48.13 (39.02, 57.18)	0.75	8.39	12.33	1.47
1	N/A	60.98	N/A	C_{max}	N/A	37.75 (30.74, 45.99)	N/A	N/A	11.98	N/A
2	N/A	121.97	N/A	C_{max}	N/A	75.51(61.48, 91.97)	N/A	N/A	11.98	N/A
3	N/A	57.40	N/A	C_{max}	N/A	31.76 (25.84, 38.35)	N/A	N/A	12.85	N/A
4	N/A	114.79	N/A	C_{max}	N/A	63.52 (51.67, 76.70)	N/A	N/A	12.85	N/A

AUC area under the curve, C_{max} maximum concentration, N/A not applicable, N/R not reported, Sim. Simulated, numbers in brackets represent the 5th and 95th percentile.

[1] Ratio of predicted/observed

[2] The AUC could not be estimated, insufficient concentration-time points

[3] Calculated using $\text{Clearance} = \frac{\text{Dose}}{\text{Area under the curve}}$

[4] Outside the 2–fold criteria

Table 5.8: Observed and simulated adipose tissue concentrations of cefuroxime and simulated time above MIC_{90}

Study code and reference	Adipose tissue concentration (µg/g) at time point				Simulated time (hour) of total adipose tissue concentration above MIC_{90}			Simulated time (hour) of free ISF adipose tissue concentration above MIC_{90}		
	Time point (min)	Obs.	Pred. (5^{th}, 95^{th} percentile)	Ratio[1]	T>MIC of 2 µg/g	T>MIC of 4 µg/g	T>MIC of 8 µg/g	T>MIC of 2 µg/g	T>MIC of 4 µg/g	T>MIC of 8 µg/g
01 (305)	C_{10}	16.5	6.55 (3.38, 10.48)	0.40[2]	2.35	1.09	N/A	6.32	4.88	3.49
02 (109)	C_{max}	N/R	4.24 (2.44, 6.83)	N/A	1.14	0.18	N/A	4.32	3.18	2.10
03 (373)	C_{max}	N/R	5.53 (3.51, 7.93)	N/A	2.52	1.26	N/A	6.60	5.10	3.72
04 (374)	C_{max}	N/R	3.19 (1.87, 4.60)	N/A	1.215	N/A	N/A	4.95	3.60	2.30
05 (374)	C_{max}	N/R	6.36 (3.84, 9.10)	N/A	2.46	1.20	N/A	6.36	4.92	3.60
06 (109)	C_{max}	N/R	3.15 (2.25, 4.49)	N/A	0.9	N/A	N/A	4.14	3.00	1.86
07 (109)	C_{max}	N/R	2.88 (2.16, 3.80)	N/A	0.84	NA	N/A	4.50	3.24	2.04
08 (9)	C_{max}	N/R	5.72 (4.35, 7.32)	N/A	2.04	0.84	N/A	5.76	4.44	3.24
09 (273)	C_{max}	N/R	6.14 (3.75, 10.81)	N/A	1.92	0.84	N/A	5.52	4.32	3.12
091 (273)	C_{max}	N/R	6.88 (3.86, 10.45)	N/A	1.92	0.84	NA	5.40	4.20	3.12
092 (110)	C_{max}	36.06[3]	33.74[3] (29.62, 37.84)	0.94	2.1	0.66	N/A	6.72	5.10	3.48
Scenario 1 [b]	C_{max}	N/A	2.72 (1.82, 4.05)	N/A	0.6	N/A	N/A	4.68	3.24	1.92
Scenario 2 [b]	C_{max}	N/A	5.43 (3.64, 8.10)	N/A	1.92	0.6	N/A	6.12	4.68	3.24
Scenario 3 [b]	C_{max}	N/A	2.22 (1.68, 2.88)	N/A	0.36	N/A	N/A	4.92	3.36	1.8
Scenario 4 [b]	C_{max}	N/A	4.44 (3.36, 5.75)	N/A	1.8	0.36	N/A	6.48	4.92	3.36

C_{max} maximum concentration, N/A not applicable, N/R not reported, T>MIC Time above the minimum inhibitory concentration, numbers in brackets represent the 5^{th} and 95^{th} percentile.

[1] Ratio of predicted/observed; [2] outside the 2-fold criteria; [3] unbound adipose interstitial fluid

[4] Assessed scenarios: 750 mg (scenario 1) and 1500 mg (scenario 2) in the obese–pregnant population and 750 mg (scenario 3) and 1500 mg (scenario 4) in the morbidly obese–pregnant population

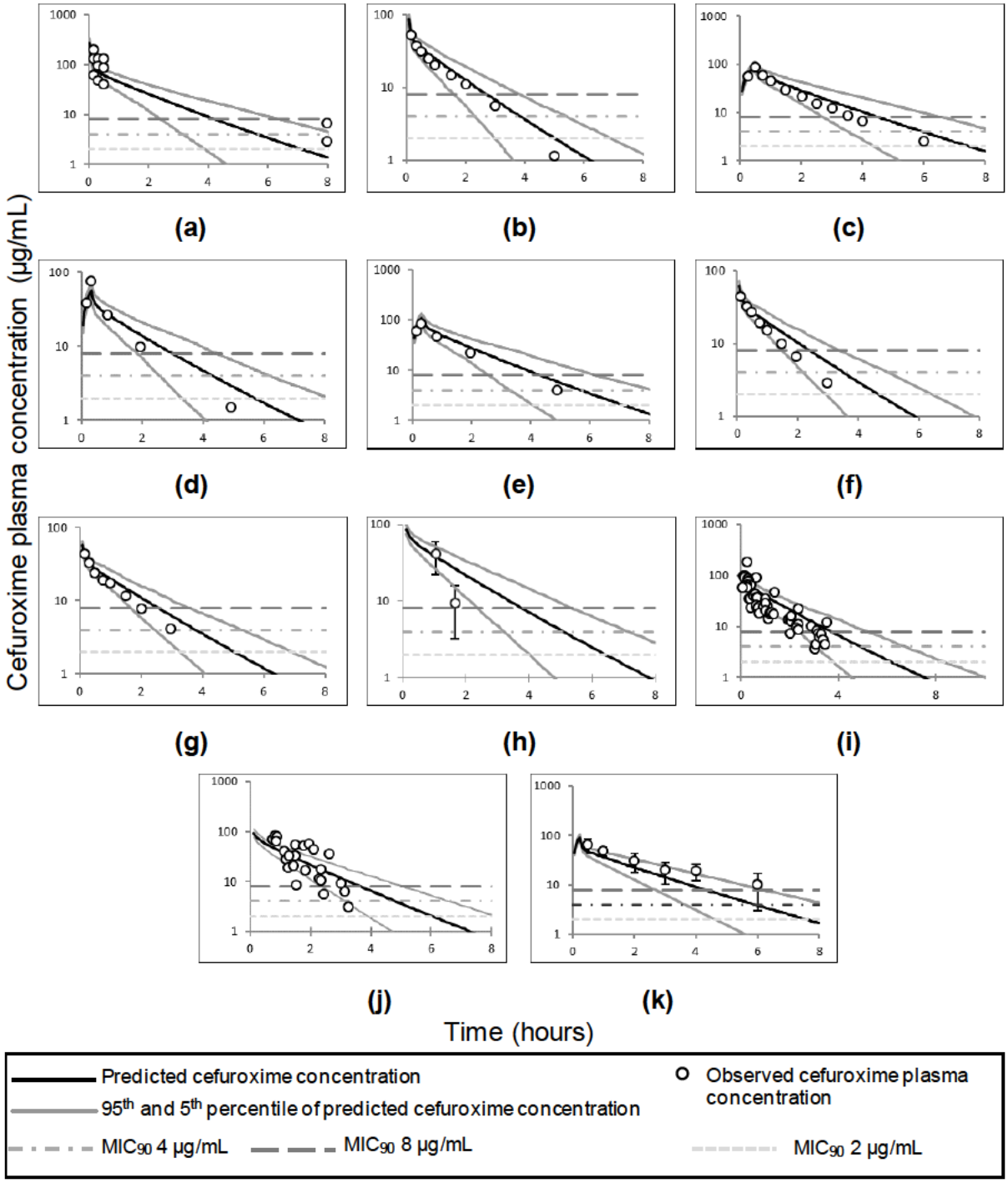


Figure 5.6: Simulated and observed plasma time concentrations curves of cefuroxime in different population

(a), **(b)**, **(c)**, **(d)** and **(e)** represent simulated and observed plasma-time concentrations curves of cefuroxime in lean non–pregnant subjects (109, 273, 305, 373, 374); **(f)**, **(g)**, **(h)**, **(i) and (j)** in lean pregnant subjects (9, 109); and **(k)** in obese subjects (110). Errors bars standard deviation of the observed concentrations in **(h) and (k)**.

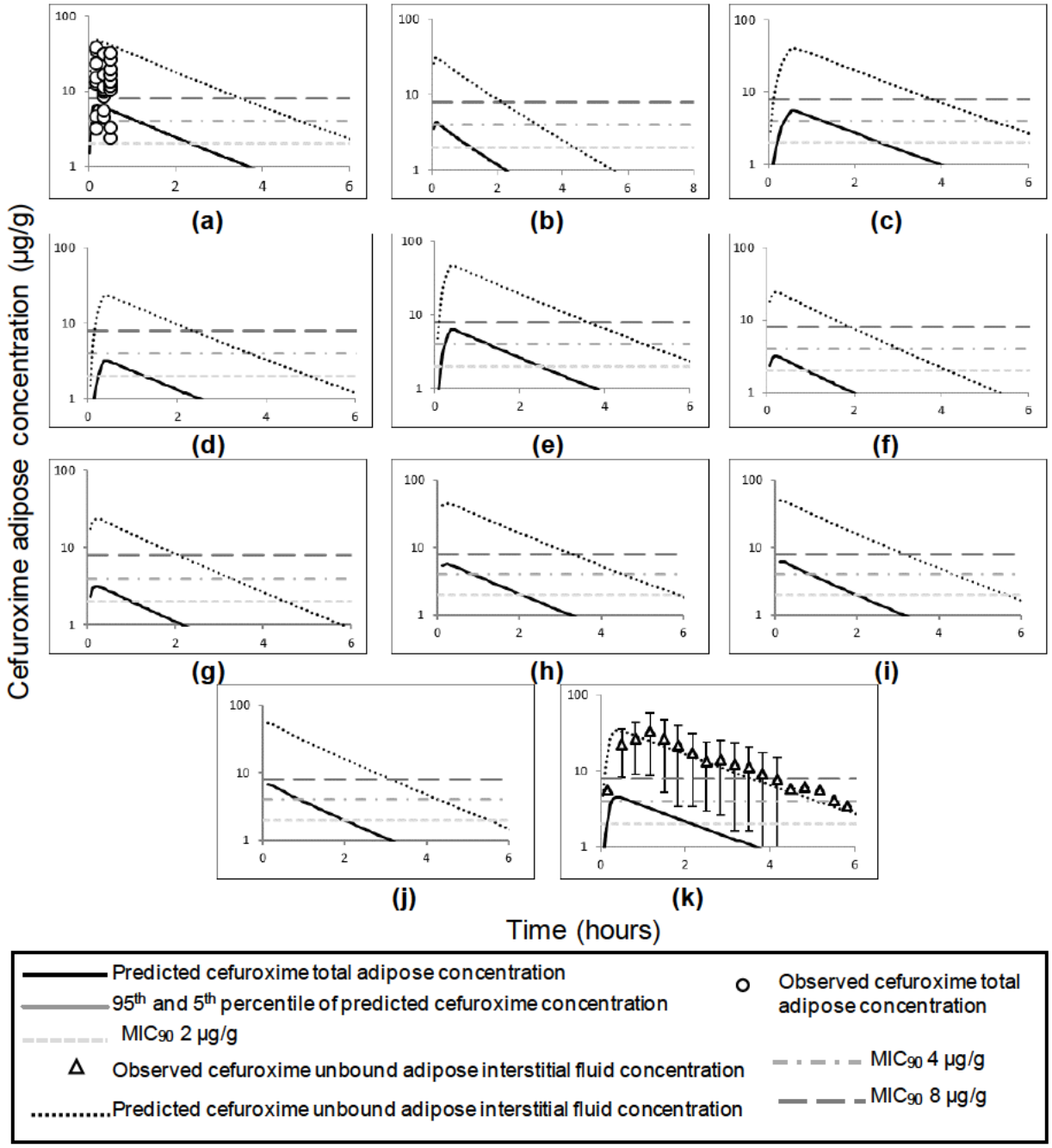


Figure 5.7: Simulated and observed adipose tissue time concentrations curves of cefuroxime in different population.
(a), **(b)**, **(c)**, **(d)** and **(e)** represent simulated and observed adipose-time concentrations curves of cefuroxime in lean non–pregnant subjects (109, 273, 305, 373, 374); **(f)**, **(g)**, **(h)**, **(i) and (j)** in pregnant subjects (9, 109); **(k)** in obese subjects (110). Errors bars represent standard deviation of the observed concentrations in **(k)**.

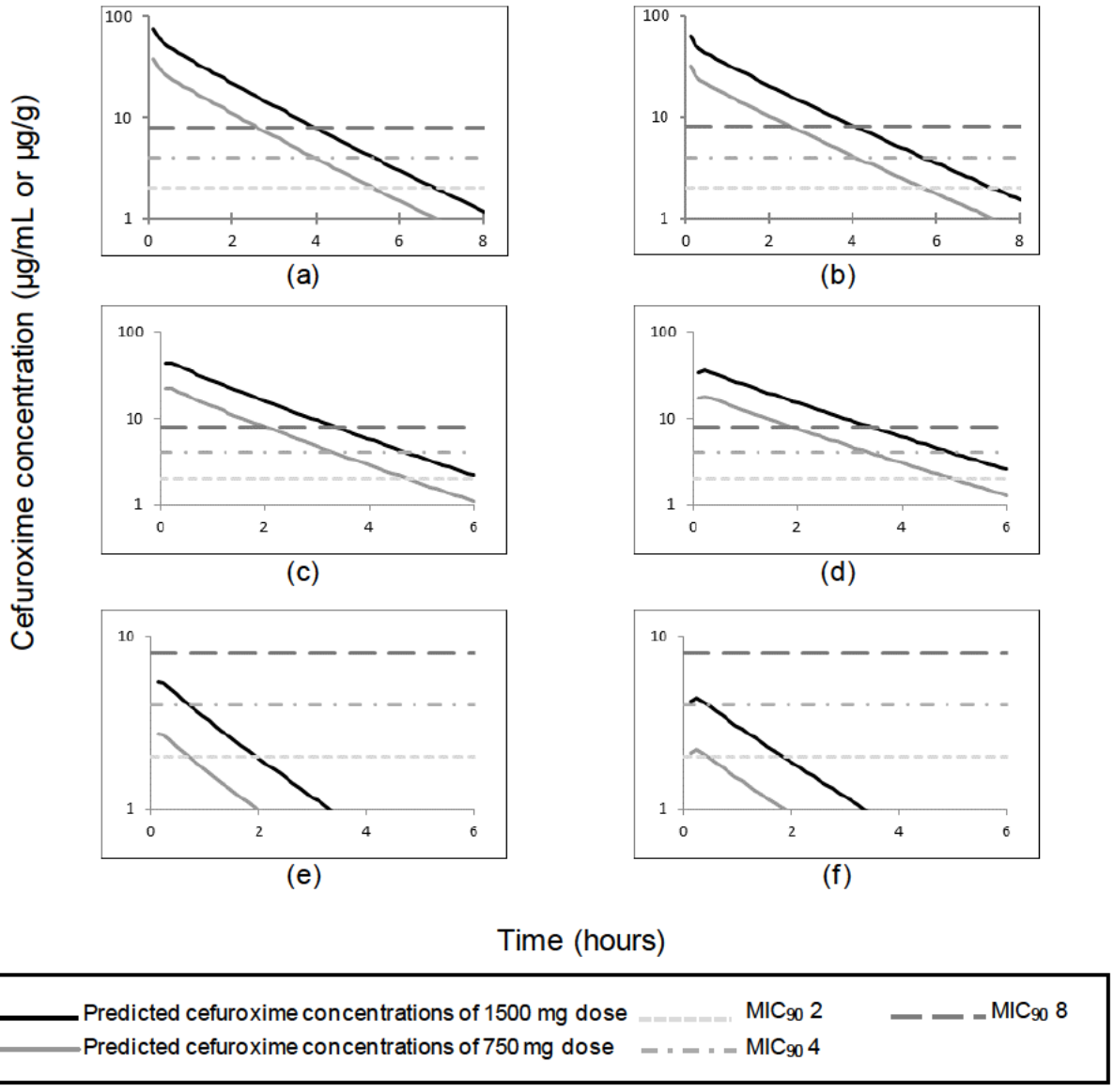


Figure 5.8: Simulated plasma, unbound adipose interstitial fluid and total adipose concentrations-time curves of cefuroxime (750 and 1500 mg doses) in obese–pregnant population.

Predicted mean cefuroxime plasma **(a)**, unbound adipose interstitial fluid **(c)**, and total adipose **(e)** concentrations in obese–pregnant of 750 and 1500 mg doses; and predicted mean cefuroxime plasma **(b)**, unbound adipose interstitial fluid **(d)**, and total adipose **(f)** concentrations in morbidly obese–pregnant of 750 and 1500 mg doses.

5.1.5 Discussion

The current study developed an obese–pregnant PBPK model to describe plasma and adipose tissue concentrations of cefazolin. This model was utilized to predict cefuroxime plasma and adipose tissue concentrations in non–obese pregnant (BMI 18.50–24.99 kg/m^2) and obese–pregnant women (BMI ≥30 kg/m^2).

5.1.5.1 Cefazolin

The PBPK model of cefazolin was considered adequate in predicting both plasma and adipose concentrations of cefazolin; however, the simulated cefazolin total adipose concentrations in morbidly obese–pregnant women (study code 0091), were not within two-fold of the clinically reported mean adipose tissue concentration of 18.36±6.68 µg/g at time of skin incision and 21.73±16.02 µg/g at time of skin closure following 2000 mg cefazolin administration (15). The simulated adipose concentration in lean–pregnant at 31 min was 14.21 µg/g compared to an observed median value of 8.7 µg/g (interquartile range 5.7–11.2 µg/g); and at 82 min, the simulated adipose concentration was 8.3 µg/g compared to an observed value of 7.5 µg/g (interquartile range 5–10.1 µg/g) (10, 284). The simulated total adipose tissue cefazolin concentrations in obese–pregnant at 4, 24 25, 41 and 60 min were 5.29, 10.08, 9.85, 8.68 and 7.62 µg/g, respectively; compared to observed values of 7.4, 9.4, 12.4, 11.8 and 8.4 µg/g, respectively following 2000 mg cefazolin dose administrations (studies codes 007 and 009) (14, 16). Additionally, higher dose of cefazolin (3000 mg) was adequately described via the PBPK obese–pregnant model (study code 008) (16). The under predicted values compared to observed values in Stitely et al. suggests higher distribution *in vivo* compared to the simulation. The current under-simulated result of cefazolin adipose tissue concentrations in morbidly obese–pregnant were accepted,

as the PBPK lean– and obese–pregnant models sufficiently described cefazolin observed adipose tissue concentration in lean– and obese–pregnant women.

The model indicates that cefazolin doses of ≥1000 mg can achieve a mean total cefazolin adipose tissue concentration above the MIC_{90} of 4 µg/g in all populations; while a dose of 500 can achieve similar MIC_{90} (i.e., 4 µg/g) in only lean non–pregnant subjects or lean–pregnant subjects. A cefazolin dose of ≥2000 mg can achieve higher MIC_{90} (i.e., 8 µg/g) in all included populations. In all tested cefazolin scenarios, the free cefazolin adipose ISF concentrations were >8 µg/g. Increased body weight in pregnant women decreases concentrations (plasma and adipose) at a time point compared to lean–pregnant, while the T>MIC are relatively similar or higher in obese compared to non–obese cohorts.

5.1.5.2 Cefuroxime

The PBPK cefuroxime model successfully predicted cefuroxime plasma, free plasma and ISF adipose concentrations. Limited data are available for cefuroxime exposure in homogenised total adipose tissue (both intracellular and extracellular) or intracellular solely. The model simulated comparable plasma concentration to the observed value at 60 min in pregnant women (9). The simulated concentrations of cefuroxime in pregnant women suggested higher *in vivo* clearance than of that predicted *in silico* (9). In a study of obese participants, where cefuroxime adipose tissue was sampled via clinical microdialysis (free extracellular cefuroxime), the simulated plasma and adipose concentrations were comparable to the observed values (110). The differences of simulated and observed cefuroxime adipose tissue concentrations in Lovering et al. study possibly suggest higher *in vivo* intracellular cefuroxime penetration than of that predicted (305). Cefuroxime doses of 750 mg and 1500 mg provided an unbound

cefuroxime adipose ISF concentrations above the MIC_{90} of 8 µg/g for means of 2.07 hours and 3.50 hours, respectively in lean non–pregnant, obese non–pregnant, and lean–pregnant populations. The PBPK cefuroxime model was used to explore cefuroxime disposition in obese–pregnant women. Similar doses within different obese–pregnant categorises had relatively comparable T>MIC and *f*T>MIC of plasma cefuroxime concentrations (Table 5.9).

In pregnant women at same gestational age with different body weight, although a comparable dose (1500 mg) achieved higher cefuroxime C_{max} (plasma or adipose) in lean-pregnant than obese cohorts, the simulated T>MIC were similar or shorter in lean–pregnant compared to of those obese– or morbidly obese–pregnant (studies code 08–091, 2 and 4). This was also noticed in predicted T>MIC of obese (non–pregnant) subjects compared to lean (non–pregnant) subjects (studies code 092 and 01). The elongated T>MIC of plasma, free plasma and ISF cefuroxime concentration with an increased weight can be explained by an increased cefuroxime half-life due to increased V_d in obese subjects compared to lean cohort, the effect of CL on drug disposition in obese subjects seems minor compared to V_d (38).

A prime factor affecting T>MIC after CS is timing of administration cefuroxime; longitudinal timing from cefuroxime administration to start of CS decreases T>MIC after surgery. It is recommended to administer prophylactic antibiotics 15–60 min before skin incision to allow drug distribution to adipose tissue and ensure sufficient T>MIC after completion of surgery (382). This study emphasised timing from prophylactic antibiotic administration to skin incision; administration of 1500 mg cefuroxime 15, 30 or 60 min before skin incision simulated a plasma T>MIC of 8 µg/mL after skin incision in obese–pregnant categories for the 5^{th} percentile of 2.2, 1.95 and 1.45 hours,

respectively. And *f*T>MIC of 1.75, 1.5 and 1 hour/s after virtual administration of 1500 mg at 15, 30 and 60 min before skin incision (Table 5.9). The current PBPK adequately predicted the mean time to maximum cefuroxime adipose tissue concentration (t_{max}) in Hosmann et al. (ISF) and Lovering et al. (total adipose), while the predicted unbound cefuroxime ISF adipose tissue concentrations t_{max} was shorter compared to observed time in Barbour et al..The simulated cefuroxime total adipose concentration t_{max} (1500 mg) in obese–pregnant was 7.2 min, and in morbidly obese–pregnant was 14.4 min. If a 750 mg cefuroxime dose was given 60 min pre-CS, at the end of 1 hour duration CS (i.e. 2 hours post cefuroxime dose), only around 45.2% of obese–pregnant subjects will achieve a cefuroxime free plasma concentration ≥8 µg/mL; and 44.2% of unbound cefuroxime adipose tissue ISF. For the same scenarios (750 mg cefuroxime dose given virtually 60 min pre-CS), 37.5%of morbidly obese–pregnant subjects will attain a cefuroxime free plasma concentration ≥8 µg/mL, and 36.2% will achieve a free cefuroxime ISF adipose tissue concentration of 8 µg/g or above at the end of 1 hour duration CS. These data support cefuroxime administration of no longer than 30 min before skin incision in different obese–pregnant categories to allow sufficient cefuroxime adipose T>MIC during and after surgery.

Table 5.9: Time above the minimum inhibitory concentration of plasma and free plasma (2, 4 and 8 µg/mL) cefuroxime concentrations of different cefuroxime doses given virtually to obese–pregnant and morbidly obese–pregnant

Dose (mg)	Time from administration to skin incision	MIC_{90} (µg/mL)	Obese–pregnant women		Morbidly obese–pregnant	
			T>MIC hours	*f*T>MIC hours	T>MIC hours	*f*T>MIC hours
750	15 min	2	5.03(3.15, 7.55)	4.43(2.75, 6.75)	5.39(3.25, 8.05)	4.67(2.75, 7.05)
		4	3.71(2.15, 5.63)	3.11(1.75, 4.67)	3.83(2.25, 5.75)	3.11(1.75, 4.95)
		8	2.27(1.45, 3.71)	1.67(0.95, 2.75)	2.27(1.31, 3.55)	1.55(0.85, 2.65)
	30 min	2	4.78(2.9, 7.3)	4.18(2.5, 6.5)	5.14(3, 7.8)	4.42(2.5, 6.8)
		4	3.46(1.9, 5.38)	2.86(1.5, 4.42)	3.58(2, 5.5)	2.86(1.5, 4.7)
		8	2.02(1.2, 3.46)	1.42(0.7, 2.5)	2.02(1.06, 3.3)	1.3(0.6, 2.4)
	1 h	2	4.28(2.4, 6.8)	3.68(2, 6)	4.64(2.5, 7.3)	3.92(2, 6.3)
		4	2.96(1.4, 4.88)	2.36(1, 3.92)	3.08(1.5, 5)	2.36(1, 4.2)
		8	1.52(0.7, 2.96)	0.92(0.2, 2)	1.52(0.56, 2.8)	0.8(0.1, 1.9)
1500	15 min	2	6.59(3.95, 9.35)	5.87(3.55, 8.65)	7.07(4.15, 10.35)	6.35(3.75, 9.25)
		4	5.03(3.15, 7.55)	4.43(2.75, 6.75)	5.39(3.25, 8.05)	4.67(2.75, 7.05)
		8	3.71(2.15, 5.65)	3.11(1.75, 4.65)	3.83(2.25, 5.75)	3.11(1.75, 4.95)
	30 min	2	6.34(3.7, 9.1)	5.62(3.3, 8.4)	6.82(3.9, 10.1)	6.1(3.5, 9)
		4	4.78(2.9, 7.3)	4.18(2.5, 6.5)	5.14(3, 7.8)	4.42(2.5, 6.8)
		8	3.46(1.9, 5.4)	2.86(1.5, 4.4)	3.58(2, 5.5)	2.86(1.5, 4.7)
	1 h	2	5.84(3.2, 8.6)	5.12(2.8, 7.9)	6.32(3.4, 9.6)	5.6(3, 8.5)
		4	4.28(2.4, 6.8)	3.68(2, 6)	4.64(2.5, 7.3)	3.92(2, 6.3)
		8	2.96(1.4, 4.9)	2.36(1, 3.9)	3.08(1.5, 5)	2.36(1, 4.2)

MIC_{90} minimum inhibitory concentration required to inhibit the growth of 90% of organisms, *f*T>MIC time of free concentration above the minimum inhibitory concentration, T>MIC Time above the minimum inhibitory concentration. Numbers in brackets represent T>MIC and, *f*T>MIC of 5^{th}, and 95^{th} percentile.

The reported mean cefuroxime total adipose tissue (intracellular and extracellular) penetration percentages compared to systematic plasma cefuroxime concentration was 16% post 1500 mg dose (19% at 30 min)(305). The reported mean plasma concentrations of lean–pregnant women undergoing CS at around 1 hour post cefuroxime dosing were 14.9 µg/mL (750 mg dose) and 41.14 µg/mL (1500 mg dose) (9, 56). Direct extrapolation (of 19% cefuroxime penetrations to total adipose tissue compared to the predicted mean cefuroxime plasma concentrations) would suggest a total adipose concentration at around 1 hour of 2.8 µg/g (750 mg) and 7.8 µg/g (1500 mg) in lean–pregnant. While such extrapolation in obese– or morbidly obese– pregnant would propose total cefuroxime concentrations of around 4.5 µg/g (750 mg dose) and 8.9 µg/g (1500 mg dose) at 30 minutes post dosing according to the mean predicted plasma concentrations in such populations.

The current guidelines recommend re-dosing prophylactic antibiotic when the surgery duration exceeds the antibiotic half-life; the mean reported half-life of cefuroxime in pregnant women is 75.63±22.10 min (9, 383). Other guidelines recommend re-dosing cefuroxime if surgery exceed 2 half-lives of prophylactic antibiotic (106). According to observed clinical cefuroxime plasma concentration in lean–pregnant and predicted mean and 5th percentile of cefuroxime plasma, free plasma and ISF adipose concentrations (irrespective to total cefuroxime adipose tissue concentration), cefuroxime 750 mg dose is sufficient if: (1) a MIC_{90} of 4 µg/g or µg/mL are desired for at least 95% of women at 1 hour post dose (redoes if the CS is delayed more than 2 hours to maintain MIC_{90} ≥4 µg/g or µg/mL), or (2) a MIC_{90} of 8 µg/g or µg/mL are desired for at least 75% of women at 1 hour post dose (consider redosing if the CS is delayed more than around 1.5 hours post dose, to maintain cefuroxime plasma above target

MIC_{90} of 4 µg/mL) (Table 7.17 and Table 7.18). In other situations, cefuroxime 1500 mg dose is recommended if MIC_{90} of ≥8 µg/g are desired for at least 95% of women at 2 hour post dose; If the CS is delayed >2 hours post cefuroxime administration of 1500 mg in lean–, obese– or morbidly obese–pregnant, a second dose might be required to maintain cefuroxime concentrations ≥8 µg/g or µg/mL (Table 7.17 and Table 7.18). Balancing the benefit vs. the maternal/foetal safety should be considered (384).

In the last decade, vast number of studies have reported antibiotic concentrations when given prophylactically at the time of clean–clean-contaminated surgeries with inconsistent recommendations. Since 2011, 8 trials were conducted to measure cefazolin concentrations in obese-pregnant women undergoing CS, where all studies measured cefazolin plasma concentrations (10, 12-16, 284, 297), 6 studies measured cefazolin total adipose tissue concentrations (10, 13-16, 284, 297), and 1 study measured free cefazolin ISF adipose concentration (297). Number of studies reported percentages of adipose tissue samples did not reach a target MIC_{90}.

The dilemma in prophylactic antibiotic dosing in different population undergoing a procedure arises from a lack of MIC_{90} cut-off value standardisation and small sample size of the clinical studies. Another reason for disagreement within literature regarding antibiotic posology in a surgical procedure is the imprecise guidelines of the targeted site or cellular targeted segment of antibiotic concentration; i.e., plasma, free plasma, total adipose (extracellular and intracellular) or/and adipose ISF (free extracellular) concentration.

According to a report by the Antibiotic Stewardship Program regarding the ‘Dosing of antimicrobials for Obese Patients’, limited evidence for beta-lactam antibiotics, such

as penicillins and cephalosporins, regarding dose adjustment in obese patients was acknowledged. The reported statement, 'These are hydrophilic drugs and are not usually dosed according to weight', is accurate; however, other factors may control hydrophilic antibiotic penetration into adipose tissue, such as its pK_a and its ionization in biological tissue (385). Nevertheless, the report mentioned the recommended increased prophylactic doses of hydrophilic cefazolin and vancomycin for subjects weighing > 120 kg and 80 kg, respectively, by The South Australian Expert Advisory Group on Antimicrobial Resistance (385, 386). The main factors directed such dose adjustment are the alterations of V_d and CL in obese population (387).

The predictions of cefuroxime intracellular concentrations require further optimisation. The selected Rodgers and Rowland method to predict cefuroxime plasma: tissue K_p and V_{ss} do not account for transporters involvement in drug tissue cellular penetrations neither penetration of ionized drug molecules, while it accounts for the concentrations of ionized molecules formed within the segment of a tissue (90, 135-137). Cefuroxime is an acidic compound (pK_a 3.15), it is expected that increased of ionized cefuroxime molecules are formed at a biological tissue (pH $\simeq$ 7–7.4) compared to the unionized molecules (388). Further techniques can be incorporated into the cefuroxime PBPK model to improve adipose intracellular cefuroxime concentration prediction. The selection of the Rodgers and Rowland method with ion membrane permeability, named Method 3 in Simcyp, may enhance the prediction of intracellular adipose cefuroxime penetration, as it will allow ionized forms to permeate the intracellular segment of a tissue (90, 135-138). However, this method requires parametrisation of tissue pH, tissue composition, ion permeability, and membrane potential data, which might not be available in literature (138, 140). Both Methods 2 and 3 neglect transport involvement

in compound disposition through a tissue. To account for transporter involvement, if proven *in vitro*, the selected permeability-limited adipose tissue can be further optimised to improve the prediction of cefuroxime disposition to intracellular adipose tissue. This will require extensive data gathering and analysis of the physiological and chemical structure of adipose tissue and *in vitro* analysis of transporters involved in cefuroxime adipose penetration, if applicable (389). Then, the development of an *in vitro* to *in vivo* correlation; and allocate a proper scaling factor/s. A permeability-limited model of adipose tissue can be parametrised according to the observed *in vitro* data (390, 391).

Although this PBPK model adequately described cefuroxime unbound ISF adipose tissue concentrations obese subjects, a clinical study is required to measure cefuroxime adipose concentration at the site of skin incision in CS. If sub-therapeutic adipose tissue cefuroxime concentration is proven, timing and dosage of cefuroxime should be reviewed. If a target MIC_{90} is reached and sustained for sufficient time before the surgery interval, timing from cefuroxime administration to skin incision should be decreased (not less than 7 min) or the dose regimen augmented with a second dose rather than increasing a single dose of cefuroxime due to the time-killing properties of cefuroxime (77). If the clinical dose of 1500 mg fails to attain the desired T>MIC or does not reach a desired MIC_{90} value at any point, a study is warranted to investigate the efficacy, safety and transplacental transfer of higher cefuroxime doses or consider an alternative prophylactic antibiotic. A protocol has been developed by our group to investigate cefuroxime pharmacokinetics in obese–pregnant women during CS, but the clinical study was postponed due to the COVID–19 pandemic (82).

Cefazolin was selected as a reference drug to guide building knowledge of cefuroxime disposition in obese–pregnant women based on several similarities between cefazolin and cefuroxime (i.e., used in the same setting as CS, non-metabolised by liver, and relatively have similar pK_a and molecular weight) (97-99). However, it is acknowledged that there are several physiochemical and PK differences. For example, the chemical structure, f_u, LogP, and dose-dependent f_u characteristics of cefazolin and the involvement of transporters OAT3 in cefazolin clearance are dissimilarities between the two compounds (91, 93-95, 97, 108).

The current study had a few limitations. Several studies used for the verification of pregnant and/or obese–pregnant PBPK model were of women undergoing CS. In the current study, the models were not parametrised to count for the effect of CS on pharmacokinetics. It may be considered a limitation because fluid administered and the type of anaesthesia given at time of CS can alter the pharmacokinetics of drugs, mainly V_d. Another possible limitation of the current prediction of adipose tissue antibiotic concentration that was based on the total subcutaneous adipose tissue in the body (i.e. not specifically in abdominal tissue). This study assumed that cefuroxime and cefazolin distributes evenly to different types of adipose tissues in the body. Additionally, changes in the activity of renal transporters during pregnancy, if modified by obesity, were not considered in the model for OAT1 and MRP4. The current PBPK model did not account for the reported cefazolin f_u dose-dependent kinetics; nevertheless, the model sufficiently described cefazolin concentrations post different escalating doses. Finally, no dynamic PD model was incorporated in the developed models.

5.1.6 Conclusion

The PBPK model developed for obese–pregnant women is adequate for predicting plasma cefuroxime concentrations in that population. While this study presented the simulated data for adipose tissue cefuroxime concentrations in pregnant and obese–pregnant women, a clinical study to quantify cefuroxime concentration in adipose tissue is necessary to validate the obese–pregnant model and further investigate cefuroxime posology in the population of interest.

Author Contributions: Conceptualization, H.H.A, H.K.B, V.H.M, K.M, J.F.M, K.A and HPC; methodology, H.H.A, H.K.B. and K.A; software, H.H.A, H.K.B. and K.A; validation, H.H.A, H.K.B. and K.A; investigation V.H.M, K.M and J.F.M; writing—original draft preparation, H.H.A, H.K.B. and K.A; writing—review and editing, H.H.A, H.K.B, V.H.M, K.M, J.F.M, K.A and HPC; supervision, H.K.B, V.H.M, K.M, J.F.M, K.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Royal Embassy of Saudi Arabia, Cultural Bureau in London in association with HHA's PhD funding.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable.

Acknowledgments: Certara UK Limited (Simcyp Division) granted free access to the Simcyp Simulator through an academic licence (subject to conditions). The authors acknowledge the support and guidance provided by Dr.Gopal Pawar during model optimisation.

Conflicts of Interest: KA is a principal scientist at Certara UK Limited. All authors, including KA, declare that the research was conducted in the absence of any

commercial or financial relationships that could be construed as a potential conflict of interest. Certara had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Chapter 6: Anticipated Results from C-LACE Study, General Discussion, Conclusion and Application

6.1 Anticipated cefuroxime concentrations results from C-LACE study

The expected cefuroxime adipose tissue concentrations in non–obese pregnant women, and anticipated cefuroxime plasma and adipose tissue concentrations in obese–pregnant women, will be discussed with the help of information gathered from a systematic review and PBPK modelling.

Cefuroxime mean penetration to total adipose tissue (total extracellular and intracellular) relative to systematic concentrations was reported as 16% in subjects undergoing hip replacement surgery (19% at 30 min). In other studies, the calculated average penetration percentage to ISF (unbound cefuroxime) at the distribution phase was around 50–66% (Table 6.1) (110, 305). As illustrated by Barbour et al. (obese subjects) and Hanberg et al.'s studies, the different percentages of cefuroxime plasm: adipose tissue partitioning at different time points and high penetration rates at the distribution phase (up to 66% at 0.37 hours post administration of cefuroxime) may indicate that cefuroxime penetration to ISF adipose tissue is dependent on cefuroxime systematic concentration (i.e., representation of equilibrium distribution) and is not entirely restricted by its logP properties. While the reported total cefuroxime adipose penetration percentages in Lovering et al. indicates limited penetration of cefuroxime to adipose intracellular segment.

Table 6.1: Percentages of cefuroxime penetration to adipose tissue compared to cefuroxime systematic plasma concentrations

Study and reference	**Reported cefuroxime plasma concentrations at time point**		**Reported cefuroxime adipose concentrations**			**% of Cefuroxime adipose tissue penetration compared to plasma concentrations**
	Time (h)	Concentration (mg/L)	Subcellular concentration	Time (h)	Concentration (µg/g)	
Lovering et al. (305)	0.17	130.8	Both intracellular and extracellular concentrations	0.17	16.5	13.2[a]
	0.33	94.3		0.33	13.5	15.4[a]
	0.50	83.3		0.50	15	19.1[a]
Barbour et al. (110)	0.50	64.25	Extracellular unbound concentration	0.50	21.93	34.12[b]
	0.99	48.60		0.83	26.22	53.96[b]
	2	30.37		2.17	17.20	56.62[b]
	3	19.63		3.17	12.25	62.43[b]
	4	19.16		4.17	7.74	40.39[b]
	6	10.28		5.83	3.44	33.45[b]
Hanberg et al., (381)	0.12	96.74	Extracellular unbound concentration	0.12	8.75	9.05[b]
	0.37	71.80		0.37	44.02	61.31[b]
	0.75	51.12		0.75	46.65	91.24[b]
	1.25	34.64		1.25	40.52	116.98[b]
	1.74	29.60		1.74	36.08	121.92[b]
	2.25	22.33		2.25	26.12	116.98[b]
	2.74	16.44		2.74	20.21	122.94[b]
	3.50	10.69		3.50	15.37	143.89[b]
	5.50	4.44		5.50	9.43	212.37[b]

[a] Reported in the study
[b] Calculated from the observed plasma and adipose tissue concentrations at a relevant similar time points

A selected average cefuroxime penetration to adipose tissue of 16–100% of systematic cefuroxime concentrations would predict adipose tissue concentrations, as illustrated in Table 6.2. The 16% could represent both total intracellular and extracellular concentrations, while ~40–100% could represent the unbound extracellular (i.e., ISF) concentration. As described in Table 6.2, the dose intensity greatly impacted cefuroxime adipose concentration compared to increased body weight. According to current PBPK obese–pregnant cefuroxime model, an increase in body weight by around 20% and 30% would cause a reduction in cefuroxime unbound ISF adipose tissue concentrations by around 2.11 µg/g (7.3%) and 5 µg/g (17.2%) compared to lean pregnant women. Statistical differences are not relevant to the cefuroxime PK/PD correlation; clinical significance depends on reaching a targeted MIC. The application and assessment of cefuroxime posology in obese–pregnant women, according to the above findings (i.e., body weight and cefuroxime unbound ISF adipose tissue concentration linkage), is dependent on the observed adipose tissue concentration in lean–pregnant women, which is not currently available.

Table 6.2: Predicted cefuroxime adipose concentrations via direct extrapolation from reported plasma concentrations in lean–pregnant undergoing caesarean section (16% and 100%), and via PBPK modelling and predicted effect of body weight on cefuroxime adipose tissue concentrations

Study and reference	**Dose (mg)**	**Time (min)**	**Reported plasma conc. (mg/L)**	**Expected cefuroxime adipose concentrations (µg/g) if penetration percentages of systematic concentrations to adipose tissue were 16%–100%**	**Predicted cefuroxime total adipose concentrations using PBPK model in different virtual population**			**Predicted cefuroxime unbound ISF adipose concentrations using PBPK model in different virtual population**		
					non–obese pregnant	Obese–pregnant	Morbidly obese–pregnant	non–obese pregnant	Obese–pregnant	Morbidly obese–pregnant
Holt et al. (56)	750	65	14.9	2.38–14.9	1.77	1.62	1.45	14.17	13.15	11.803
Holt et al. (56)	1500	37	51.9	8.30–51.9	4.69	4.23	3.67	37.64	34.41	29.87
Bousfield et al. (273)	1500	48	74.76	11.96–74.76	4.19	3.79	3.34	33.61	30.84	27.16
Bousfield et al. (273)	1500	60	32.55	5.21–32.55	3.71	3.39	3.02	29.79	27.54	24.58
Lalic–popovic et al. (9)	1500	61.67	41.14	6.58–41.14	3.67	3.35	2.99	29.43	27.23	24.34
Bousfield et al. (273)	1500	88	32.95	5.27–32.95	2.82	2.63	2.41	22.60	21.35	19.62
Lalic–popovic et al. (9)	1500	99.28	9.47	1.52–9.47	2.53	2.38	2.21	20.29	19.35	17.98
							Predicted weight (Kg):	80.19	97	127.21
							Average reduction of predicted adipose concentrations in obese pregnant group compared to lean pregnant (of 1500 mg dose):	Reference value	2.11	5
							% reduction of predicted adipose concentrations in obese pregnant group compared to lean pregnant (of 1500 mg dose):	Reference value	7.3	17.2

A key element in assessing the suggested timing of cefuroxime administration relative to CS initiation is cefuroxime adipose tissue t_{max}. These were discussed previously in the highlight of PBPK prediction chapter, note that this was based on predictions alone as there was no clinical data available (5.1.5.2 Cefuroxime).

In the Lovering study, the last sampled total adipose tissue concentration was 30 min post administration. In the coherent adipose tissue sampling studies of Barbour et al. (obese subjects) and Hanberg et al., cefuroxime unbound ISF adipose t_{max} were 1.00±0.28 hours and 45 min (range: 23–75 min), respectively (110, 305). The current PBPK models adequately predicted the mean cefuroxime adipose tissue t_{max} concentration in Hosmann et al. (ISF) and Lovering et al. (total adipose), while the predicted cefuroxime ISF adipose tissue concentrations t_{max} was under predicted compared to observed time in Barbour et al. The PBPK predicted cefuroxime adipose (total) t_{max} was 14 min in lean–pregnant, 7 min obese–pregnant, and 14 min in the morbidly obese–pregnant women.

The current predicted cefuroxime adipose tissue concentrations (either via direct extrapolation of cefuroxime adipose tissue penetrations or PBPK modelling) and predicted adipose tissue t_{max} support giving cefuroxime 15–30 min before skin incision at time of CS, instead of 60 min. The targeted MIC determines whether cefuroxime current doses (750 mg and 1500 mg) are sufficient or if an additional dose is required (Discussed in 5.1.5.2 Cefuroxime).

6.2 General discussion

Due to discussed similarities of cefazolin and cefuroxime compounds (5.1.5 Discussion), cefazolin was chosen as a reference drug to aid in the development of

knowledge about cefuroxime disposition in obese—pregnant (96-99). However, there are a number of physiochemical and PK variations of cefazolin and cefuroxime to be cautiously considered (9, 16, 90-99, 108, 113-115). Identification of these differences controlled the extent of utilisation of cefazolin as a reference drug and thus, limited interconnection of the knowledge of PK from cefazolin to cefuroxime in the obese–pregnant population. To elaborate, for example, the correlation between cefazolin doses, subjects' weight, and reported cefazolin adipose concentrations from the cefazolin systematic review was not investigated in the context of this research. Due to the dose-dependent f_u effect, which is not considered a characteristic PK parameter of cefuroxime and other PK dissimilarities, this correlation may not be necessary applied to the case of cefuroxime.

Literature review and systematic review provided knowledge of cefazolin and cefuroxime PK in populations of interest; additionally, identified gaps in knowledge and elements causing disagreements in dosage recommendations between studies reporting concentrations of an antibiotic given prophylactically in a population. The research gap for cefuroxime is considered empirical and population gap; while for cefazolin is empirical and theoretical gap. The question of whether a prophylactic antibiotic should be given to pregnant women at the time of CS has been addressed in a meta-analysis with a clear advantage of giving antibiotics compared to no antibiotic in CS; however, the consequences of administration of an antibiotic at the time of CS on neonate was uncertain (392).

Although several studies had investigated cefazolin deposition in obese–pregnant women when given different doses and several attempts were conducted to combine evidence to draw conclusions, it was challenging to reach a conclusion. This relates to

the discussed studies' heterogeneity, small sample size and different targeted MIC values. Despite between studies similarities/differences of antibiotics' plasma or tissue concentrations, the disagreement of dose recommendation arises mainly in the targeted MIC values.

Another reason for disagreement within literature regarding antibiotic posology in a surgical procedure is the unclear cellular targeted segment. Many studies had reported the total adipose concentrations via conventional adipose tissue collection and analytical sampling, while other reported the unbound antibiotic adipose ISF concentrations via Microdialysis. The extracellular (i.e. ISF), in cefuroxime and cefazolin cases, is higher compared to intracellular. Figure 6.1 (A), represent theoretical cefazolin and cefuroxime penetration to extracellular and intracellular segments of adipose tissue, the yellow tab represent the blood flow and blue colour shade represent the concentrations intensity (darker shade represent higher concentration). When reporting the total adipose tissue concentrations in clinical setting (via conventional method), the concentration is not representing the actual concentration in each cellular segment (Figure 6.1, B). For certain medication, the intracellular concentrations correlate to drug PK/PD rather than extracellular or total tissue concentrations. It is unusual to measure intracellular drug concentrations *in vivo*; however, it is possible *in vitro (389).* Overall, to best of our knowledge, there is no information regarding the optimal cellular targeting segment in cefazolin or cefuroxime. The PBPK modelling enable investigating such penetrations with acceptable validity.

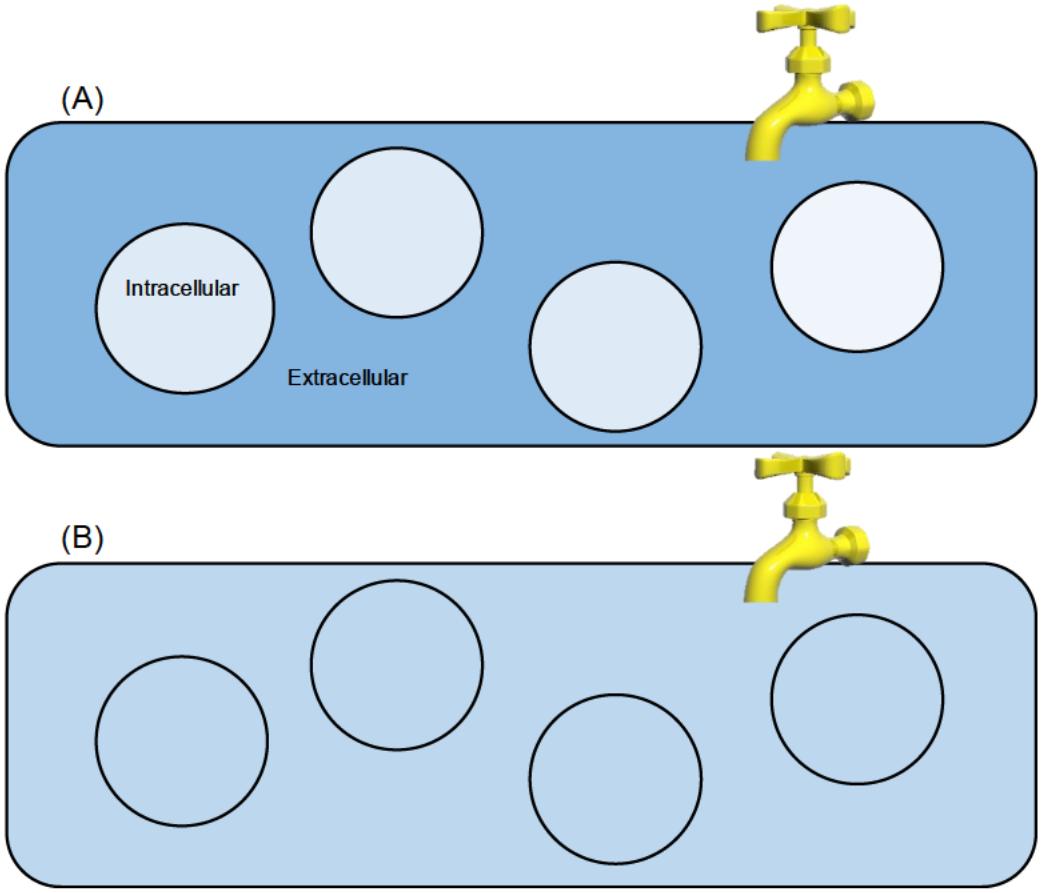


Figure 6.1: Cefazolin and cefuroxime penetration to adipose extracellular and intracellular segments

(A) Represent the actual penetration (no penetration or limited penetration), and (B) Represent the false impression from conventional adipose tissue sampling and analysing

The hypothesis that decreased cefuroxime adipose tissue concentrations in obese–pregnant compared to lean cohort can be supported by the observed significant differences of cefazolin initial concentration in different obesity groups when given similar doses (2.2.3.3 Meta-analysis of the cefazolin concentrations in the adipose tissue: Figure 2.6). Decreased dug tissue concentrations in the obese–pregnant population can be explained by increased V_d and CL due to both gestation and obesity effects and decreased tissue vascularity due to increased adiposity (10). Some studies included in the CFZ-PK systematic review investigated correlations of pregnant body-based weight (BMI, actual or lean body weight) and cefazolin dose and/or

concentrations with divergent results (12-14, 284). It is anticipated to update and further investigate cefazolin systematic review in terms of correlating body weight, cefazolin dose, cefazolin plasma and adipose concentrations. Additionally, it was planned to clinically investigate the correlations of cefuroxime in the C-LACE study. The correlation between body weight and cefazolin adipose tissue can be extrapolated to cefuroxime to some extent, while a correlation between an increased dose and an observed cefazolin adipose tissue concentration cannot be applied to cefuroxime, as discussed previously. Thoughtful consideration of pathophysiological effects of obesity on the rate of SSI (for example, decreased immunity) before correlation of an obese class, antibiotic (dose, concentrations) and reported SSI rate.

The current project emphasises the impractical linear extrapolation of PK from one population to another and the value of using a compound as a reference drug to guide knowledge of another drug's PK.

In 2013, US-based organisations, the American Society of Health-System Pharmacists and the Infectious Diseases Society of America suggested a 2 g dose of cefazolin when used prophylactically in surgery and an increasing dose of 3 g to those patients weighing ≥ 120 kg (106). In April 2019, the Australian Therapeutic Guidelines updated the previous recommendation of 2 g of cefazolin given within 60 min of skin incision in CS, regardless of mothers' weight, to an increased dose of 3 g of cefazolin for women weighing > 120 kg (297). The National Institute for Health & Care Excellence guidelines in the UK recommend cefuroxime in CS without dose adjustment (74). Until around 2018, cefuroxime prophylactic doses in CS settings were either 750 mg or 1500 mg at Birmingham Women's Hospital, regardless of the mother's weight. This was changed to a standardised dose of 1500 mg.

The selection of adipose tissue as a target tissue to evaluate the effectiveness of intraoperative cefazolin and whether an increased dose should be suggested in obese patients (non–pregnant) undergoing clean–clean-contaminated surgery has been recently discussed by Blum et al. (393). The author claimed that increasing the cefazolin dose: 1) will not increase adipose concentrations due to its hydrophilicity, and 2) will not improve SSI prevention due to cefazolin time-dependent kinetics (393). In regards to the second claim, it is accepted for the obese–pregnant population if a targeted adipose MIC is achieved and maintained for sufficient time (i.e., duration of CS); scenarios of failing to achieve a targeted MIC or T>MIC were discussed (5.1.5.2 Cefuroxime).

Regarding the first claim, the reported increased cefazolin adipose tissue concentrations with an increased dose cannot support or reject the author's statements due to the effect of cefazolin dose-dependent f_u. For cefuroxime, however, in the study by Lalic-Popovic et al., the diabetic group with a higher mean body weight compared to the control group (statistically insignificant), the diabetic group had a significantly higher dose per lean body compared to the control group (9). This observation, if proven that the diabetic condition does not affect drug distribution to fat tissue, may support the author's claims. According to Barbour et al., cefuroxime adipose tissue ISF: plasma penetration percentages; the cefuroxime penetration to and from the ISF adipose tissue is in equilibrium with plasma cefuroxime concentrations (Table 6.1) (110). The *in silico* sensitivity analysis of LogP and cefuroxime adipose tissue concentration showed no effect of logP on cefuroxime adipose tissue concentrations — although this could be a false effect related to the use of Method 2 rather than Method 3 (explained further in paragraph of PBPK model optimisation). The reported

high cefuroxime adipose penetration rates and sensitivity analysis of cefuroxime LogP counteract the claims by Blum et al. (increased antibiotics dose will not increase adipose tissue concentrations) and the current project assumption of limited cefuroxime adipose tissue penetration due to its hydrophilicity characteristics (393). However, reported cefuroxime V_d values and total adipose tissue suggest limited intracellular penetration, the limited penetration can be driven by numerus physiochemical properties not only LogP. Overall, a final conclusion can be drawn from a large clinical study assessing cefuroxime concentrations in plasma and adipose tissue (or other targeted tissue/s) at the time of delivery for both obese and non–obese pregnant women.

If dose adjustment is required, it is recommended in the guidelines that weight-dose adjustment should be based on 'time of delivery weight' rather than pre-pregnancy weight, first antenatal booking, or time of booking. The weight based adjustment element and cut-off value to guide dose adjustment are suggested for further investigation; i.e. dose adjustment based on body weight or BMI, and cute-value of body weight to increase cefuroxime dose.

The best phrase can be used to coherently describe the ratio of model predictions' benefits to their uncertainty, as said by George Box: 'All models are wrong, some models are useful' (394). PBPK modelling is greatly implemented and required by drug authorities to assess drugs' PD/PK and posology (395-397). Simcyp®, specifically, has been utilised widely to inform PD/PK of new drug applications in the FDA (398). The Simcyp® simulator was selected to conduct the current study due to its sophisticated–user friendlily characteristic to build the obese–pregnant population in a reasonable time scale. As discussed previously (section 1.1.6.22), developing new

equations/algorithms exclusively for the obese–pregnant population was not the scope of this study.

There was no further investigation of cefuroxime dosage regimens adjustment (if required) via Simcyp® modelling; as models needs further validation of cefuroxime intracellular adipose tissue penetration. To the best of our knowledge, there is no clinical information regarding cefuroxime penetration into biological adipose tissue in non-diseased subjects and does not require surgery (305, 399, 400).

The approach used to predict cefazolin and cefuroxime concentrations in obese–pregnant women was the PBPK 'Bottom-Up' approach. Additionally, tissue: plasma partitioning and CL was optimised using observed data (i.e. population pharmacokinetics); thus, the 'Top-Down' approach was partially involved in such work (401, 402). Although the 'Top-Down' approach can be utilised to optimise prediction of cefuroxime adipose tissue, optimisation of observed cefuroxime adipose tissue from subjects undergoing hip replacement to optimise of healthy PBPK model can be a limitation due to discussed possible hemodynamic alterations from the use of aesthesia and isotonic solutions within surgery, which may affect the drug's PK.

Information on cefuroxime concentrations in adipose tissue in healthy subjects can be utilised to optimise the PBPK model at the initial PBPK model development stage, which is ethically restricted. Coherent gathering and analysis of clinically reported cefuroxime tissue concentrations can be utilised to further optimise the PBPK cefuroxime model (i.e. population pharmacokinetics).

In the context of the current project, once data are available of either approach — *in vitro in vivo* cefuroxime adipose tissue partition or clinical measurements of cefuroxime

adipose tissue concentrations in healthy or populations of interest— the PBPK cefuroxime model can be optimised accordingly. The validity of the PBPK model can be assessed; the model predictions would be further 'trusted' after such validation.

General strengths, limitations and possible optimisations have been previously discussed; the strengths and limitations of the whole project will be discussed. The strength of this project relied on collaborative work between clinical and academic sectors; in which a concern regarding optimal cefuroxime dose has been addressed by a physician in a clinical setting due to observed increased SSI rate post CS, conformation of lacking information in literature has led to the current project. The development of the C-LACE protocol was a result of extensive meetings between academics and clinicians at Birmingham Women's Hospital. The C-LACE protocol was patient-oriented. To exemplify, the addition of using a venflon cannula was to ensure preserving a precious moment of mothers holding their babies for the first time (crossed hands) and minimising distribution of the CS procedure, while ensuring withdrawal of blood samples at the time of skin closure. Although deviations in routine healthcare practice are expected to be minimised in observational studies, such an addition is of ethical necessity. The systematic review search was coherent; several databases were screened, for English and non-English publications. The coherent systematic review and literature review of this project identified the element of controversies between studies related to the current project; i.e. dosing antibiotic in obese population compared to lean cohort, which had been discussed previously. Systematic review and protocol of C-LACE were peer-reviewed and were adjusted/optimised at the time of review. This project supports the mission of the 'Health Equity' approach, where time, effort and resources should be directed to ensure equal treatment within and across

population/s (403). The 'one-dose-fits-all' approach is disclaimed intensely in the current project.

The major constraint of the current project is that it was not conducted as planned due to the COVID-19 pandemic. This was discussed in section 4.4.1: Suspension of C-LACE study and current status. It was initially aimed to utilise the results of cefuroxime plasma and adipose tissue concentrations from the C-LACE study in the development of a PBPK obese–pregnant model using Simcyp®. Information collected from 30 participants would be utilised to improve the validity of the PBPK models and predictions in obese–pregnant women. Then, cefuroxime PK can be explored in the PBPK models within Simcyp® to predict cefuroxime concentrations in: 1) larger sample size of population, 2) extreme obesity, and 3) predict other dose strategies, if required. Having no data on cefuroxime adipose tissue concentrations in pregnant women, as well as plasma and adipose tissue concentrations in obese–pregnant women, it was agreed to use cefazolin as a reference drug to apprise regarding PBPK obese–pregnant models' efficiency in predicting plasma and adipose tissue concentrations. However, the differences between cefazolin and cefuroxime were considered. All in all, researchers of this project are aiming to conduct the C-LACE study and continue the above mentioned prediction.

6.2.1 Key findings and fulfilment of the research aims and objectives

- There is no clinical information on cefuroxime plasma and adipose tissue concentrations or the rate of SSI (when given cefuroxime) in obese–pregnant women undergoing CS.

- There is no clinical information on cefuroxime adipose tissue concentrations in pregnant women with lean body weight at the time of CS.
- Studies have reported a rate of SSI when cefuroxime was prophylactically given to pregnant women at the time of CS; cefuroxime was given after cord clamping—which is not the current clinical practice.
- Both cefuroxime doses (750 mg and 1500 mg) achieved sufficient plasma concentrations; in one study, plasma T>MIC was up to around 3 hours (Figure 2.2) post 1500 mg cefuroxime dose in lean–pregnant women at the time of CS.
- A dose of 750 mg cefuroxime had a clinical plasma concentration of 14.9 mg/L at around 1 hour; cefuroxime adipose concentrations predict range was 2.38–14.9 μg/g (directly extrapolated from observed cefuroxime adipose tissue penetration in subjects undergoing surgeries 16–100%; where lower concentrations represent total adipose concentrations, and higher represents unbound adipose ISF concentrations).
- A dose of 1500 mg in lean–pregnant reported as 41.14 mg/L post 1 hour of cefuroxime dosing and predicted values of adipose tissue was 6.58–41.14 μg/g in lean–pregnant women (directly extrapolated from observed cefuroxime adipose tissue penetration in subjects undergoing surgeries 16–100%; where lower percentages represent total adipose concentrations, and higher represents unbound adipose ISF concentrations).
- A dose of 1500 mg in obese–pregnant predicted (PBPK) a plasma concentration of around 33.18 mg/L post 1 hour of cefuroxime dosing; and via directly extrapolation from observed cefuroxime total adipose tissue penetration in subjects undergoing surgeries (16–100%; as lower represent total adipose

and higher represent unbound adipose ISF concentrations), cefuroxime adipose tissue concentrations would be around 5.3–33.18 µg/g post 1 hour of dosing in obese–pregnant.

- Identification of knowledge gaps and elements of recommendation disagreements (MIC value, targeted tissue site to measure antibiotic efficacy, BMI vs. actual body weight for dose adjustment).
- Cefazolin and cefuroxime dose recommendations depend on the targeted MIC value, which is a point of argument in the published literature.
- Given similar doses to different obesity classes in obese–pregnant women yielded significantly different initial cefazolin adipose tissue concentrations.
- PBPK modelling of cefuroxime adipose tissue concentrations was independent of its logP value, this can be validated by the use of Method 3 to account for ionised molecules penetration.
- To optimally quantify cefuroxime in adipose tissue: 1) sensitivity of MS is superior to HPLC, and 2) use Microdialysis for targeted cefuroxime unbound ISF or conventional sampling technique for total adipose concentration (intracellular and extracellular), the Microdialysis requires calibrations at the time of surgery.

6.3 Applications

The implications of this research may direct nation and international guidelines regarding the dosing of prophylactic antibiotics in CS or other procedures requiring prophylactic antibiotics.

Extensive informative meetings between physicians and researchers were conducted to discuss gaps in knowledge in assessing cefuroxime clinical doses and to develop a constructive, patient-oriented protocol to evaluate cefuroxime doses in the population of interest.

In the last decade, different studies have reported antibiotic concentrations when given prophylactically at the time of clean–clean-contaminated surgeries with inconsistent recommendations. The dilemma in prophylactic antibiotic dosing in different population undergoing a procedure arises from a lack of MIC value standardisation. Knowing such gaps may direct further investigation of prophylactic antibiotic concentrations research, limit unnecessary research, and preserve time, effort and resources.

The current project aims were parallel with those of the Antibiotic Stewardship Programme, where antibiotic prescribing should be optimised to decrease: (1) Infection rate, (2) Treatment failures, (3) *Clostridioides difficile* infections, (4) Adverse effects, (5) Antibiotic resistance, and (6) Hospital costs and lengths of stay(404).

The current project findings would inform the suggested project between authorities (including decision making and guiding of program as such of Antibiotic Stewardship Program), clinicians and researchers to further investigate dosing of prophylactic cefuroxime in obese–pregnant women.

The focus of clinical research, specifically PK and dosing-wise, in the past few decades has been on special populations, and their deviation from the typically recruited healthy population in the drug development process. The current combined obese–pregnant PBPK modelling would support posology in such a population and the anticipated individualised treatment in the future of subjects within this population via model-

informed software (405). Notably, cefuroxime and cefazolin are not metabolized by liver enzymes; other applications of the developed obese–pregnant PBPK population will required updating the bassline of the pre-pregnancy metabolizer enzymes to those of obese or morbidly obese PBPK models (244). Overall, assessing model performance is a critical step that should be guided by authorities before introducing dosing-informed models in a clinical setting.

6.4 Conclusion

The current report suggests cefazolin dose recommendations according to several targeted adipose MIC values. Using PBPK modelling, when targeting for an MIC value of 8 µg/g, a dose of 750 mg seems insufficient for obese–pregnant women at the time of CS. *In silico* prediction suggests the administration of cefuroxime 15–30 min before a skin incision in CS. PBPK continually supports knowledge of drugs' PK, specifically if there are ethical or pandemic restrictions as of the current project. A constructive collaborative project between clinicians, researchers and authorities is essential to standardise antibiotics PK/PD correlations and MIC values. A clinical study to measure cefuroxime adipose tissue concentration in obese–pregnant women is required. If cefuroxime targeted T>MIC is insufficient, re-dosing should be considered rather than an increased dose.

6.5 Future work and recommendations

The suggested future work, with directed references, are listed in chronological order.

- **What is the target MIC cut-off value? Should the target MIC value be standardised or should it be per clinical setting? Does targeting for higher MIC values (i.e. 8 and 16 mg/L or µg/g) would considered as prophylactic**

management? A major reason for disagreements regarding the published studies recommendation about posology of antibiotics in a special population lies within the target MIC value (10, 79-81, 83-86, 393).

- **Should antibiotic plasma concentrations be related solely to antibiotic efficacy? Alternatively, should it include a site of incision PK/PD correlation (as of current clinical practice)? Is adipose tissue targeting considered the optimal tissue to assess concentration at the site of incision of CS? Is extracellular, intracellular or both prophylactic antibiotic concentrations relates to its efficacy?(393)**
- **What are the correlations between cefuroxime doses, plasma and adipose concentrations in obese–pregnant women?**
- **What is the correlation between increased body weight and clinical reduction in cefazolin/cefuroxime plasma and adipose tissue concentrations in obese–pregnant women?**
- Optimisation of C-LACE study: **Analytical method used to measure cefuroxime in adipose tissue.** It is acceptable to collect samples of adipose tissue via the conventional method (i.e. collecting physical adipose tissue) in the pilot study and analyse it using MS; if the pilot study then proceeds to a larger study, use the Microdialysis technique for unbound adipose ISF concentrations (which needs calibrations at the time of CS) and the conventional method for total adipose antibiotic concentrations. In both adipose collections technique, concentrations data will be utilised to validate the PBPK model and then predict the other cellular segment/s concentrations.

- C-LACE study: **Is there a need for a larger clinical study? First, validate the PBPK model.** If data from the C-LACE study proven to: 1) cefuroxime concentrations (at targeted site, plasma, adipose, both or others) under an targeted MIC, and or 2) T>MIC were under-therapeutic, it is suggested to first validate the PBPK models and then conduct an extended virtual studies to investigate cefuroxime levels in obese–pregnant women.
- Optimisation of the PBPK model: if the model was optimised and validated. **What is the consequence (PK/PD) of an increased or added cefuroxime dose to the foetal?** Validate the model using the multi–compartment placenta model to predict cefuroxime foetal transfer (406)**. When to re-dose (according to surgery duration or post-delivery, regardless of surgery duration to bypass foetal excessive transfer of cefuroxime (384))? Should cefuroxime dose be adjusted based on BMI or weight (lean, total or adjusted body weight)? Does a subject with normal body weight but high waist circumference (Abdominal or truncal obesity) need dose adjustment?** According to literature, adjusted body weight and establishing a dose factor, such as that used in aminoglycosides, seems promising for cefuroxime dose adjustment if required (385, 407). **Consider re-dosing with topical cefuroxime formula** (408).

If the predicted results were sub-therapeutics (under MIC, or insufficient T>MIC of a targeted tissue), a larger clinical study is warranted. The *in silico* prediction will allow understanding of the possible effects of an increased or repeated dose of cefuroxime and the clinical implications of this on foetal and guide posology in clinical study.

- Clinical study to investigate PK/PD of cefuroxime and rate of SSI in lean– and obese–pregnant women and cefuroxime placental transfer at time of CS (**multi-centres? What is the sample size?**). As discussed, a thoughtful consideration of the targeted MIC value and targeted site of efficacy should be made before conducting such research. Using MS is essential in such a study.
- **If cefuroxime was therapeutically inadequate in CS setting for either lean– or obese–pregnant women, what other antibiotics could be used prophylactically in such a setting?** (392)**.**
- **Guidelines update.** Whenever the dose adjustment of cefuroxime is advisory, the dose adjustments (either actual, lean or adjusted body weight) should be stated at the time of CS rather than at the 'time of booking'.
- **What is the algorithmic correlation of V_d and V_{ss}** (409, 410)

Chapter 7: Appendix

7.1 Appendix of Chapter 2 "Systematic Review to Evaluate Pharmacokinetics of Cefuroxime and Cefazolin in Obese–Pregnant Women Undergoing Caesarean Section and Rate of Surgical Site Infection"

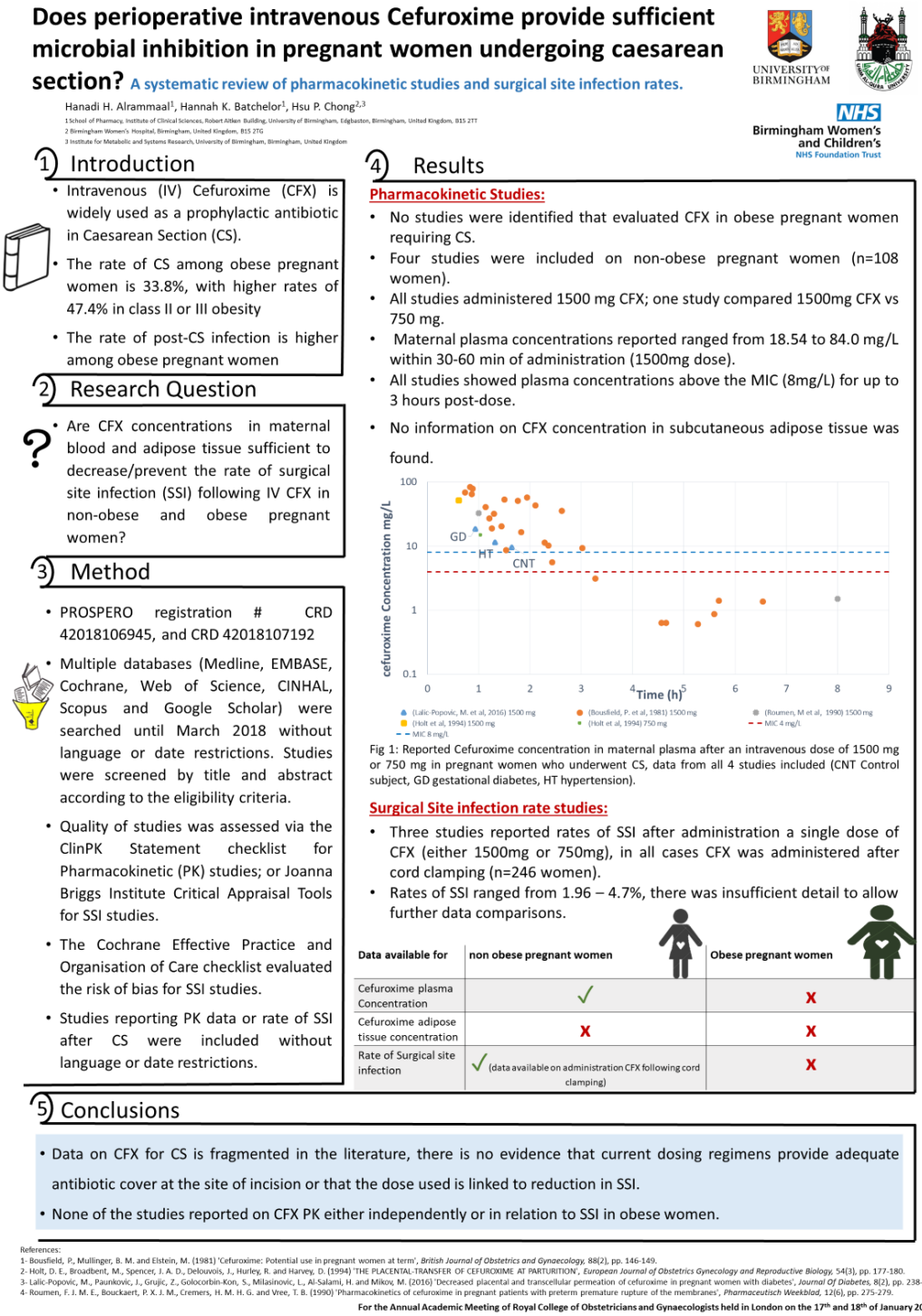


Figure 7.1: Poster presentation of cefuroxime systematic review at the Annual Academic Meeting held at the Royal College of Obstetricians and Gynaecologists

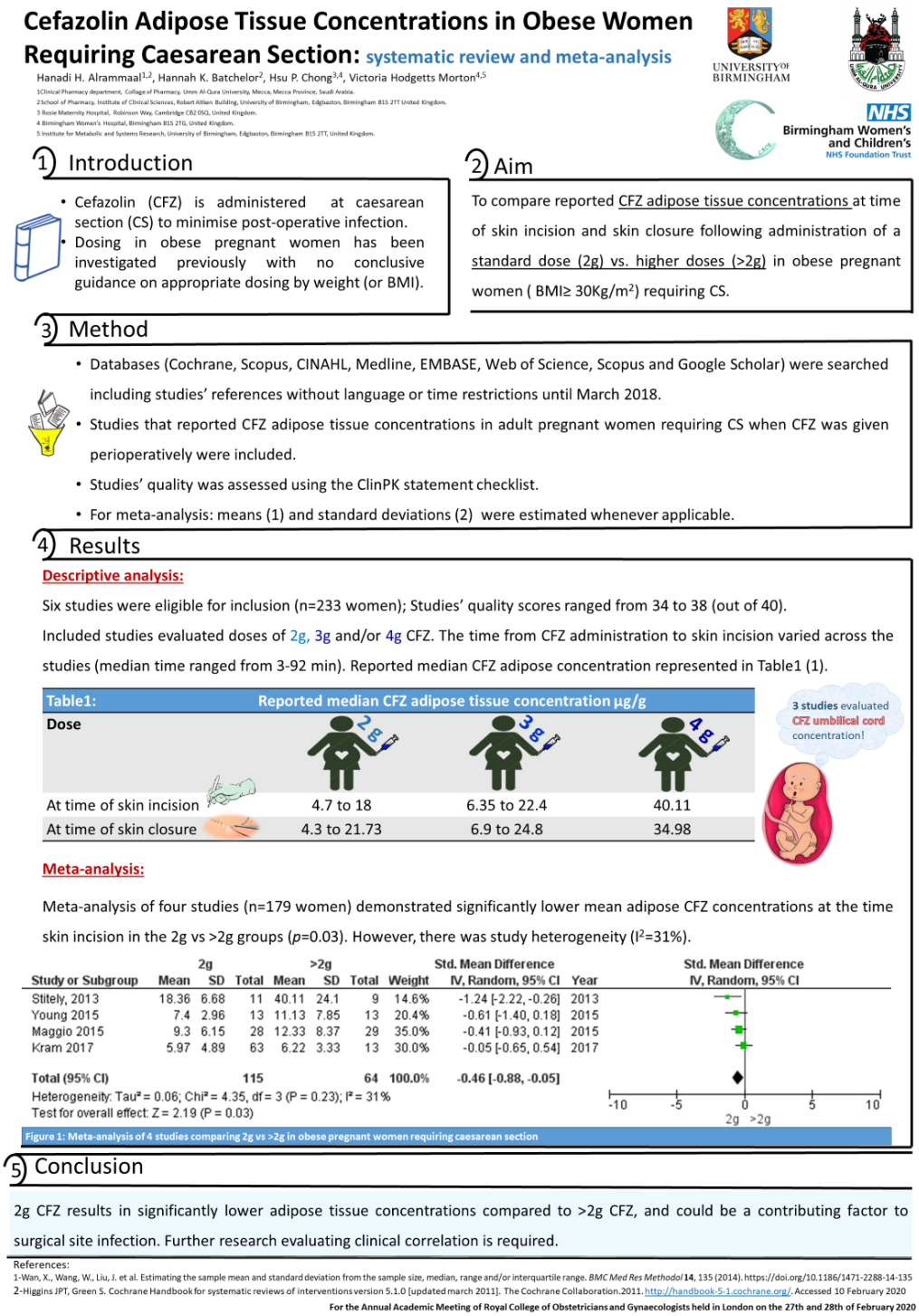


Figure 7.2: part of CFZ-PK meta-analysis presented as a poster abstract at the Annual Academic Meeting of Royal College of Obstetricians and Gynaecologists

7.1.1 Appendix of the published study "Efficacy of perioperative cefuroxime as a prophylactic antibiotic in women requiring caesarean section: A systematic review:

7.1.1.1 Appendix S1: Search Strategy (example from Medline)

1- exp Pregnancy/ or exp PREGNANT WOMEN/ or exp Pregnancy Complications/
2- (Gravid or child bearing or childbearing or gravidity or intrauterine pregnancy or labor presentation or labour presentation or pregnancy maintenance or pregnancy trimesters or diseases complicating pregnancy or gestational complication or pregnancy complications, cardiovascular or pregnancy complications, haematologic or pregnancy complications, hematologic or pregnancy complications, infectious or pregnancy complications, neoplastic or pregnancy complications, parasitic).mp.
3- #1 or #2
4- exp Cesarean Section/
5- (birth,caesarean or caesarean birth or caesarian birth or caesarian section or cesarean delivery or cesarian section or elective repeat cesarean section or fetectomy or repeated cesarotomy or sectio caesarea).mp.
6- #4 or #5
7- exp CEFUROXIME/
8- (alporin or altacef or anaptivan or aprok or aprokam or biocefal or cefoxurime or cefumax or (cefuroxime and dextrose in duplex container) or cefuroxime sodium or ceplus or ceroxime or curocef or curoxim or curoxima or curoxime or eroxmit or froxal or fucerox or furoxime or intracef or kefazol or kefurim or kefurox or kesint or laxinat or maxil or polixima or prokam or sn 107 or supacef or ucefaxim or ultroxim or uroxime or vekfazolin).mp.
9- (anikef or axetil e47 or axetine or axoril or axurocef or bearcef or cci 15641 or cci15641 or cefamar or ceflour or cefogen or ceftil or ceftin or cefudura or cefuhexal or cefuracet or cefurax or cefuril or cefuro-puren or cefurox-wolff or cefuroxime 1 acetoxyethyl ester or cefuroxime axetil ester or cefutil or cekonin or celocid or cepazine or cervin or cethixim or cetoxil or deltrox or elobact or froxime or kalcef or magnaspor or mufexine or novador or oraxim or romicef or roxbi or sharox-500 or sn 407 or sn407 or ximaken or zinat or zinnat or zonef or zoref).mp.
10- #7 or #8 or #9
11- exp PHARMACOKINETICS/
12- (body drug relation or drug body relation or kinetic profile or pharmaceutical kinetic or pharmaco-kinetic or pharmacological kinetic or tissue concentration or blood concentration or Plasma concentration or clearance or CL or distribution or volume of distribution or VD or absorption or metabolism or excretion or maximum concentration or Cmax or time to maximum concentration or Tmax or Ctrough or area under the curve or AUC or half-life or half life or "t1/2" or trough concentration or protein binding).mp
13- #11 or #12
14- exp Surgical wound infection/
15- (SSI or (surgical infection) or (surgical infections) or (infection, surgical) or (post-operative wound infection) or (post-operative wound infections)).mp.
16- #14 or #15
17- #3 and #6 and #10 and #13 (for CFX-PK)
18- #3 and #6 and #10 and #16 (for CFX-INF)

Table 7.1: Cefuroxime pharmacokinetics and infection systematic reviews' inclusion and exclusion criteria.

	Cefuroxime PK		**Cefuroxime SSI**	
	Inclusion	Exclusion	Inclusion	Exclusion
Study	• Quantitative study (randomised controlled trial, controlled trial, observational study, single case study) • Published research	• Qualitative study (audit, systematic review, literature review, meta-analysis) • Simulation/predicted study with no observed/clinical data	• Quantitative or qualitative study (randomised controlled trial, controlled trial, observational study, audit) • Published research	• Single case study, systematic review, literature review, meta-analysis • Simulation/predicted study with no observed/clinical data
Population	• Pregnant women • 18 years old or older • Patients undergoing caesarean sections (scheduled or emergency)	• Patients undergoing some other surgery	• Pregnant women • 18 years old or older • Patients undergoing caesarean sections (scheduled or emergency)	• Patients undergoing some other surgery
Intervention	• Administered cefuroxime intravenously perioperative caesarean section, with or without other prophylactic antibiotics	• Administered other prophylactic antibiotics than cefuroxime	• Administered single dose of cefuroxime intravenously perioperative caesarean section, without other prophylactic antibiotics	• Administration of cefuroxime with other prophylactic antibiotic • Administered other prophylactic antibiotics • Multiple doses of Cefuroxime • Other formula (example intramuscular)
Outcomes	• At least one observed pharmacokinetics parameter including Maternal plasma or adipose tissue Cefuroxime concentration (i.e., clearance, area under the curve, maximum concentration)	• Other outcomes (e.g., surgical site infection rate, hospital stay rate) • Predicted pharmacokinetics data	• Any post-surgical infection outcome (including SSI)	• Other outcomes for example hospital stay.

Table 7.2: ClinPK Statement checklist scoring system for the studies assessed in the cefuroxime pharmacokinetics systematic review. Adopted from (269)

section	item number	checklist item
Title/ abstract	1	The title identifies the drug(s) and patient population(s) studied
	2	The abstract minimally includes the name of the drug(s) studied, route of administration, population in whom it was studied, and results of the primary objective and major clinical pharmacokinetic findings
Background	3	Pharmacokinetic data (i.e. absorption, distribution, metabolism, excretion) that are known and relevant to the drugs being studied are described
	4	An explanation of the study rationale is provided
	5	Specific objectives or hypotheses are provided
Method	6	Eligibility criteria of study participants are described
	7	Co-administration (or lack thereof) of study drug(s) with other potentially interacting drugs or food within this study is described
	8	Drug preparation and administration characteristics, including dose, route, formulation, infusion duration (if applicable), and frequency are described
	9	Body fluid or tissue sampling (timing, frequency, and storage) for quantitative drug measurement are described
	10	Validation of quantitative bioanalytical methods used in the study are referenced or described if applicable
	11	Pharmacokinetic modeling methods and software used are described, including assumptions made regarding the number of compartments and order of kinetics (zero, first, or mixed order)
	12	For population pharmacokinetic studies, covariates incorporated into pharmacokinetic models are identified and described
	13	Formulas for calculated variables (such as creatinine clearance, body surface area, AUC, and adjusted body weight) are provided or referenced
	14	The specific body weight used in drug dosing and pharmacokinetic calculations are reported (i.e. ideal body weight vs. actual body weight vs. adjusted body weight)
	15	Statistical methods, including software used, are described
Results	16	Study withdrawals or subjects lost to follow-up (or lack thereof) are reported
	17	Quantification of missing or excluded data is provided if applicable
	18	All relevant variables that may explain inter- and intrapatient pharmacokinetic variability (including age, sex, end-organ function, ethnicity, weight or BMI, health status or severity of illness, and pertinent co-morbidities) are provided with appropriate measures of variance
	19	Results of pharmacokinetic analyses are reported with appropriate measures of precision (such as range or 95 % confidence intervals)
	20	Studies in patients receiving extracorporeal drug removal (i.e. dialysis) should report the mode of drug removal, type of filters used, duration of therapy, and relevant flow rates
	21	In studies of drug bioavailability comparing two formulations of the same drug, F, AUC, Cmax, and tmax should be reported
discussion/ conclusion	22	Study limitations describing potential sources of bias and imprecision, where relevant, should be described
	23	The relevance of study findings (applicability, external validity) is described
other information	24	Funding sources and conflicts of interest for the authors are disclosed

Table 7.3: ClinPK quality assessment result of cefuroxime pharmacokinetics systematic review

Section	Checklist Item Number	(Bousfield et al. 1981)	(Roumen et al. 1990)	(Holt et al, 1994)	(Lalic-Popovic et al., 2016)
		Score out of 40			
Title/abstract	1	2	2	2	2
	2	2	1	2	2
Background	3	2	1	2	2
	4	2	2	2	2
	5	0	0	1	2
Methods	6	2	2	2	2
	7	1	2	1	2
	8	2	2	2	2
	9	2	2	2	2
	10	2	2	2	2
	11	0	2	2	1
	12	1	1	1	2
	13	0	0	2	2
Results	14	2	0	1	2
	15	1	1	1	2
	16	1	1	2	2
	17	0	1	2	2
Discussion/conclusion	18	0	1	0	1
	19	2	2	2	2
Other information	20	0	0	0	2
Total mark 40		24	25	31	38

Table 7.4: Risk of bias assessment of cefuroxime post caesarean section systematic review by the Cochrane Effective Practice and Organisation of Care checklist

Item	Study		
	Kristensen et al., 1990	Rizk et al., 1998	Ziogos et al., 2010
	Judgment (Low risk, High risk, Unclear)		
Adequate sequence generation?	High risk	Low risk	Low risk
Allocation concealment?	High risk	Low risk	Low risk
Blinding?	High risk	Low risk	High risk
Incomplete outcome data addressed?	Unclear	unclear	Unclear risk
Free of selective reporting?	Unclear	Low risk	Low risk
Free of other bias?	Low risk	Low risk	Low risk
Baseline outcomes similar?	Low risk	Low risk	Low risk
Free of contamination?	Low risk	Low risk	Low risk
Baseline characteristics similar?	Low risk	Low risk	Low risk

Table 7.5: Quality assessment of studies included in cefuroxime post caesarean section infection systematic review by Joanna Briggs Institute Critical Appraisal Checklist for Randomized Controlled Trials

Item	Study		
	Kristensen et al., 1990	Rizk et al., 1998	Ziogos et al., 2010
	Judgment (Yes, No, Unclear, not applicable)		
1. Was true randomization used for assignment of participants to treatment groups?	No	Yes	Yes
2. Was allocation to treatment groups concealed?	Unclear	Yes	Yes
3. Were treatment groups similar at the baseline?	Yes	Yes	Yes
4. Were participants blind to treatment assignment?	Yes	Yes	Yes
5. Were those delivering treatment blind to treatment assignment?	Yes	Yes	No
6. Were outcomes assessors blind to treatment assignment?	Yes	Yes	No
7. Were treatment groups treated identically other than the intervention of interest?	Unclear	Yes	Yes
8. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	Unclear	Yes	Yes
9. Were participants analyzed in the groups to which they were randomized?	Unclear	Yes	Yes
10. Were outcomes measured in the same way for treatment groups?	Yes	Yes	Yes
11. Were outcomes measured in a reliable way?	No	Yes	Yes
12. Was appropriate statistical analysis used?	Yes	Yes	Yes
13. Was the trial design appropriate, and any deviations from the standard rct design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?	Yes	Unclear	Unclear

Table 7.6: Summary of the terms used for the cefuroxime pharmacokinetic and infection systematic review in MEDLINE database.

Term	Selected terms	MeSH terms	Keywords	Boolean term
Pregnant	Pregnancy	gestation pregnancies pregnancy	child bearing childbearing gravidity intrauterine pregnancy labor presentation labour presentation pregnancy maintenance pregnancy trimesters	OR
	Pregnant woman	pregnant woman pregnant women woman, pregnant women, pregnant	Gravid	OR
	Pregnancy Complications	complication, pregnancy complications, pregnancy pregnancy complication pregnancy complications	diseases complicating pregnancy gestational complication pregnancy complications, cardiovascular pregnancy complications, haematologic pregnancy complications, hematologic pregnancy complications, infectious pregnancy complications, neoplastic pregnancy complications, parasitic	OR
C-section	Cesarean Section	abdominal deliveries abdominal delivery c section (ob) c-section (ob) c-sections (ob) caesarean section caesarean sections cesarean section cesarean sections deliveries, abdominal delivery, abdominal postcesarean section	birth,caesarean caesarean birth caesarian birth caesarian section cesarean delivery cesarian section elective repeat cesarean section fetectomy repeated cesarotomy sectio caesarea	And

Term	Selected terms	MeSH terms	Keywords	Boolean term
Cefuroxime	Cefuroxime	cefuroxime cephuroxime ketocef zinacef	alporin or altacef or anaptivan or aprok or aprokam or biocefal or cefoxurime or cefumax or (cefuroxime and dextrose in duplex container) or cefuroxime sodium or ceplus or ceroxime or curocef or curoxim or curoxima or curoxime or eroxmit or froxal or fucerox or furoxime or intracef or kefazol or kefurim or kefurox or kesint or laxinat or maxil or polixima or prokam or sn 107 or supacef or ucefaxim or ultroxim or uroxime or vekfazolin or zinacef	OR
	cefuroxime axetil		anikef or axetil e47 or axetine or axoril or axurocef or bearcef or cci 15641 or cci15641 or cefamar or ceflour or cefogen or ceftil or ceftin or cefudura or cefuhexal or cefuracet or cefurax or cefuril or cefuro-puren or cefurox-wolff or cefuroxime 1 acetoxyethyl ester or cefuroxime axetil ester or cefutil or cekonin or celocid or cepazine or cervin or cethixim or cetoxil or deltrox or elobact or froxime or kalcef or magnaspor or mufexine or novador or oraxim or romicef or roxbi or sharox-500 or sn 407 or sn407 or ximaken or zinat or zinnat or zonef or zoref	And
Pharmacokinetics	Pharmacokinetics	drug kinetics kinetics, drug pharmacokinetics	body drug relation or drug body relation or kinetic profile (drug) or pharmaceutical kinetic or pharmaco-kinetic or pharmacological kinetic or Tissue concentration or Blood concentration or Plasma concentration or clearance or CL or distribution or volume of distribution or VD or absorption or metabolism or excretion or maximum concentration or Cmax or time to maximum concentration or Tmax or Ctrough or area under the curve or AUC or half-life or half life or "t1/2" or trough concentration or protein binding	
infection	Surgical wound infection	infection, postoperative wound infection, surgical site infection, surgical wound infections, postoperative wound infections, surgical site infections, surgical wound postoperative wound infection	SSI surgical infection surgical infections infection, surgical post-operative wound infection post-operative wound infections	

Term	Selected terms	MeSH terms	Keywords	Boolean term
		postoperative wound infections surgical site infection surgical site infections surgical wound infection surgical wound infections wound infection, postoperative wound infection, surgical wound infections, postoperative wound infections, surgical		

Table 7.7: the PRISMA Statement checklist for current study

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Cover
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1,2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5,6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	appendix
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5,6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	appendix
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency ($e.g., I^2$) for each meta-analysis.	8
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	NA
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	appendix
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table1,2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	appendix
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table1,2 and Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	NA
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	13-15
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	16

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097
For more information, visit: www.prisma-statement.org.

7.1.2 Appendix of Cefazolin pharmacokinetics systematic review and Meta-analysis and rate of infection post Caesarean section.

7.1.2.1 Appendix S2: Search Strategy (example from Medline)

1- exp Pregnancy/ or exp PREGNANT WOMEN/ or exp Pregnancy Complications/
2- (Gravid or child bearing or childbearing or gravidity or intrauterine pregnancy or labor presentation or labour presentation or pregnancy maintenance or pregnancy trimesters or diseases complicating pregnancy or gestational complication or pregnancy complications, cardiovascular or pregnancy complications, haematologic or pregnancy complications, hematologic or pregnancy complications, infectious or pregnancy complications, neoplastic or pregnancy complications, parasitic).mp.
3- #1 or #2
4- exp Cesarean Section/
5- (birth,caesarean or caesarean birth or caesarian birth or caesarian section or cesarean delivery or cesarian section or elective repeat cesarean section or fetectomy or repeated cesarotomy or sectio caesarea).mp.
6- #4 or #5
7- exp PHARMACOKINETICS/
8- (body drug relation or drug body relation or kinetic profile or pharmaceutical kinetic or pharmaco-kinetic or pharmacological kinetic or tissue concentration or blood concentration or Plasma concentration or clearance or CL or distribution or volume of distribution or VD or absorption or metabolism or excretion or maximum concentration or Cmax or time to maximum concentration or Tmax or Ctrough or area under the curve or AUC or half-life or half life or "t1/2" or trough concentration or protein binding).mp
9- #7 or #8
10- exp Surgical wound infection/
11- (SSI or (surgical infection) or (surgical infections) or (infection, surgical) or (post-operative wound infection) or (post-operative wound infections)).mp.
12- #10 or #11
13- exp Obesity/ or exp overweight/
14- (Obese or High BMI or High body mass index or adipose tissue hyperplasia or adipositas or adiposity or alimentary obesity or (body weight, excess) or fat overload syndrome or nutritional obesity or obesitas or (BMI 30 kg/m2) or (BMI 30 kg m2) or (body mass index 30 kg/m2) (body mass index 30 kg m2)).map
15- #13 or #14
16- exp CEFAZOLIN/
17- ((ancef in dextrose 5%) or Ansef or anzolin or basocef or biozolin or cefacidal or cefamezine or cefarad or cefazin or cefazol or (cefazolin and dextrose) or cefazolina or cefazoline or (cefazoline panpharma) or celmetin or cezolin or elzogram or faxilen or fazolin or fonvicol or izacef or kefarin or kefazin or kefazolin or kezolin or kofatol or kurgan or lupex or oricef or orizolin or reflin or sanzol or stancef or stazolin or surzolin or uzolin or vulmizolin or zaconil or zolecef or zolicef or zolidina).mp.
18- #16 or #17

19- #3 and #6 and #9 and #15 and #18 **(for CFZ-PK)**
20- #3 and #6 and #12 and #15 and #18 **(for CFZ-INF)**

Table 7.8: Cefazolin pharmacokinetics and infection systematic reviews' inclusion and exclusion criteria.

	Cefazolin PK		Cefazolin INF	
	Inclusion	**Exclusion**	**Inclusion**	**Exclusion**
Study	• Quantitative study (randomised controlled trial, controlled trial, observational study, single case study) • Published research	• Qualitative study (audit, systematic review, literature review, meta-analysis) • Unpublished research • Simulation/predicted study with no observed/clinical data	• Quantitative or qualitative study (randomised controlled trial, controlled trial, observational study, audit) • Published research	• Single case study, systematic review, literature review, meta-analysis • Simulation/predicted study with no observed/clinical data
Population	• Pregnant women • 18 years old or older • BMI is 30 or above • undergoing caesarean sections (scheduled or emergency)	• Patients undergoing some other surgery	• Pregnant women • 18 years old or older • BMI is 30 or above • undergoing caesarean sections (scheduled or emergency)	• Patients undergoing some other surgery
Intervention	• Administered Cefazolin intravenously pre-caesarean section, with or without other prophylactic antibiotics	• Administered other prophylactic antibiotics	• Administered single dose of Cefazolin intravenously pre-caesarean section, without other prophylactic antibiotics	• Administration of cefazolin with other prophylactic antibiotic • Administered other prophylactic antibiotics • Multiple doses of cefazolin • Other formula (example intramuscular)
Outcomes	• At least one observed pharmacokinetics parameter including Maternal plasma or adipose tissue cefazolin concentration (i.e., clearance, area under the curve, maximum concentration)	• Other outcomes (e.g., surgical site infection rate, hospital stay rate) • Predicted pharmacokinetics data	• Any post-surgical infection outcome (including SSI)	• Other outcomes for example hospital stay.

Table 7.9: Summary of the terms used for the cefazolin pharmacokinetic and surgical site infection systematic review in the MEDLINE database.

Term	Selected terms	MeSH terms	Keywords	Boolean term
Pregnant	Pregnancy	gestation pregnancies pregnancy	child bearing childbearing gravidity intrauterine pregnancy labor presentation labour presentation pregnancy maintenance pregnancy trimesters	OR
	Pregnant woman	pregnant woman pregnant women woman, pregnant women, pregnant	Gravid	OR
	Pregnancy Complications	complication, pregnancy complications, pregnancy pregnancy complication pregnancy complications	diseases complicating pregnancy gestational complication pregnancy complications, cardiovascular pregnancy complications, haematologic pregnancy complications, hematologic pregnancy complications, infectious pregnancy complications, neoplastic pregnancy complications, parasitic	AND
Obese	Obesity	appetite depressants body weight diet, reducing skinfold thickness lipectomy anti-obesity agents bariatrics	Obese High BMI High body mass index	
	overweight		adipose tissue hyperplasia adipositas adiposity [MeSH Descriptor] alimentary obesity body weight,excess fat overload syndrome nutritional obesity obesitas BMI 30 kg/m^2 bmi 30 kg m2	

Term	Selected terms	MeSH terms	Keywords	Boolean term
			body mass index 30 kg/m2 body mass index 30 kg m2	
C-section	Cesarean Section	abdominal deliveries abdominal delivery c section (ob) c-section (ob) c-sections (ob) caesarean section caesarean sections cesarean section cesarean sections deliveries, abdominal delivery, abdominal postcesarean section	birth,caesarean caesarean birth caesarian birth caesarian section cesarean delivery cesarian section elective repeat cesarean section fetectomy repeated cesarotomy sectio caesarea	And
Cefazolin	Cefazolin	ancef cefamedin cefamezine cefazolin cefazolin sodium cephamezine cephazolin cephazolin, sodium gramaxin kefzol sodium, cefazolin sodium cephazolin totacef	(ancef in dextrose 5%) or Ansef or anzolin or basocef or biozolin or cefacidal or cefamezine or cefarad or cefazin or cefazol or (cefazolin and dextrose) or cefazolina or cefazoline or (cefazoline panpharma) or celmetin or cezolin or elzogram or faxilen or fazolin or fonvicol or izacef or kefarin or kefazin or kefazolin or kezolin or kofatol or kurgan or lupex or oricef or orizolin or reflin or sanzol or stancef or stazolin or surzolin or uzolin or vulmizolin or zaconil or zolecef or zolicef or zolidina	OR
Pharmacokinetics	Pharmacokinetics	drug kinetics kinetics, drug pharmacokinetics	body drug relation or drug body relation or kinetic profile (drug) or pharmaceutical kinetic or pharmaco-kinetic or pharmacological kinetic or Tissue concentration or Blood concentration or Plasma concentration or clearance or CL or distribution or volume of distribution or VD or absorption or metabolism or excretion or maximum concentration or Cmax or time to maximum concentration or Tmax or Ctrough or area under the curve or AUC or half-life or half life or "t1/2" or trough concentration or protein binding	
infection	Surgical wound infection	infection, postoperative wound infection, surgical site	SSI surgical infection	

Term	Selected terms	MeSH terms	Keywords	Boolean term
		infection, surgical wound infections, postoperative wound infections, surgical site infections, surgical wound postoperative wound infection postoperative wound infections surgical site infection surgical site infections surgical wound infection surgical wound infections wound infection, postoperative wound infection, surgical wound infections, postoperative wound infections, surgical	surgical infections infection, surgical post-operative wound infection post-operative wound infections	

Table 7.10: ClinPK quality assessment result of cefazolin pharmacokinetics systematic review

Section	Checklist item number	Pevzner et al., 2011	Stitely et al., 2013	Maggio et al., 2015)	Swank et al. 2015	Young et al., 2015)	Kram et al., 2017)	Groff et al., 2017)
		Score out of 40						
Title/Abstract	1	2	2	2	2	2	2	2
	2	2	2	2	2	2	2	2
Background	3	0	1	1	1	1	0	1
	4	2	2	2	2	2	2	2
	5	2	2	2	1	1	2	2
Methods	6	2	2	2	2	2	2	2
	7	1	2	2	1	2	2	2
	8	2	2	2	2	2	2	2
	9	2	2	2	2	2	2	2
	10	2	2	2	2	2	2	2
	11	1	2	1	2	2	1	1
	12	2	2	2	2	2	2	2
	13	2	2	2	2	2	2	2
Results	14	2	2	2	2	2	2	1
	15	2	2	2	1	2	2	1
	16	2	2	1	2	2	2	1
	17	2	2	1	2	2	2	1
Discussion/ Conclusion	18	1	2	2	2	2	2	2
	19	2	2	2	2	2	2	2
Other Information	20	2	2	2	0	2	2	2
Total Mark 40		35	39	36	35	38	37	34

Table 7.11: Risk of bias assessment of cefazolin post caesarean section infection systematic review by the Cochrane Effective Practice and Organisation of Care checklist

Item	Study			
	Pevzner et al., 2011	Stitely et al., 2013	Ahmadzia et al., 2015	Maggio et al., 2015
	Judgment (Low risk, High risk, Unclear)			
Adequate sequence generation?	High risk	Low risk	High risk	Low risk
Allocation concealment?	High risk	High risk	High risk	Unclear
Blinding?	High risk	High risk	High risk	Low risk
Incomplete outcome data addressed?	High risk	Unclear	Low risk	Low risk
Free of selective reporting?	Low risk	Unclear	Low risk	Low risk
Free of other bias?	Low risk	Unclear	Low risk	Low risk
Baseline outcomes similar?	Low risk	Low risk	Low risk	Low risk
Free of contamination?	High risk	High risk	High risk	High risk
Baseline characteristics similar?	Low risk	Low risk	High risk	Low risk

Table 7.12: Quality assessment of studies included in cefazolin post caesarean section infection systematic review by Joanna Briggs Institute Critical Appraisal Checklist for Randomized Controlled Trials

Item	Study			
	Pevzner et al., 2011	Stitely et al., 2013	Ahmadzia et al., 2015	Maggio et al., 2015
	Judgment (Yes, No, Unclear, not applicable)			
1. Was true randomization used for assignment of participants to treatment groups?	NA	Yes	NA	Yes
2. Was allocation to treatment groups concealed?	NA	No	NA	Yes
3. Were treatment groups similar at the baseline?	Yes	Yes	No	Yes
4. Were participants blind to treatment assignment?	NA	No	NA	Yes
5. Were those delivering treatment blind to treatment assignment?	NA	No	NA	Yes
6. Were outcomes assessors blind to treatment assignment?	NA	No	NA	Yes
7. Were treatment groups treated identically other than the intervention of interest?	NA	Unclear	No	Unclear
8. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	No	Unclear	Yes	Yes
9. Were participants analyzed in the groups to which they were randomized?	NA	Yes	NA	Yes
10. Were outcomes measured in the same way for treatment groups?	Yes	Yes	Yes	Yes
11. Were outcomes measured in a reliable way?	Yes	Yes	Yes	Yes
12. Was appropriate statistical analysis used?	Yes	Yes	Yes	Yes
13. Was the trial design appropriate, and any deviations from the standard rct design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?	NA	Yes	NA	Yes

Table 7.13: the PRISMA Statement checklist for cefazolin systematic review and meta-analysis

Section/topic	#	Checklist item	Reported on section #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Section 2.2
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	NA
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	NA
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	1.4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Section 2.2.2.2
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	appendix
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Section 2.2.2.1
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	appendix
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Section 2.2.2.3
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	NA
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Section 2.2.2.5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Section 2.2.2.6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Section 2.2.2.9

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency ($e.g., I^2$) for each meta-analysis.	Section 2.2.2.9
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	NA
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Section 2.2.2.9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	appendix
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 2.6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	appendix
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 2.6
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Sections 2.2.3.3 and 2.2.3.5
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	NA
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Sections 2.2.3.3 and 2.2.3.3.1.1
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Section 2.2.4
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Section 2.2.4.5
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Section 2.2.5
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

7.2 Appendix of Chapter 4 "Observational Study to Assess the Pharmacokinetics of Cefuroxime and its Prophylactic Efficacy in Obese Pregnant women after Caesarean Section (C-LACE)"

7.2.1 Appendix of the published Protocol "Prophylactic Perioperative Cefuroxime Levels in Plasma and Adipose Tissue at the Time of Caesarean Section (C-LACE): A Protocol for a Pilot experimental, prospective study with non-probability sampling to determine interpatient variability."

7.2.1.1 Additional file 1

CONSENT FORM for the participant {32}

C-LACE: Cefuroxime Levels After Caesarean Section

Participant Study ID Number: ☐☐☐☐☐

Please initial box

1	I confirm that I have read and understood the Participant information sheet (v0.2 16-09-2019) for the above study.	
2	I have had the opportunity to ask questions and all my questions have been answered to my satisfaction.	
3	I understand my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
4	I understand that sections of my medical notes may be reviewed by responsible individuals from Birmingham Women's Hospital, University of Birmingham as Sponsor or regulatory authorities, where it is relevant to my taking part in research. I give permission for these individuals to have access to these records.	
5	I Agree to my medical records being accessed to share the following information about me (in an anonymised format) with the University of Birmingham (ethnicity; height; weight; site from where sample(s) removed; Caesarean Section operative information; follow up information).	
6	I agree to the collection of blood samples and tissue samples taken during Caesarean Section and for these samples to be transported and stored at the University of Birmingham for use in this research project.	
7	I agree to being contacted by a research midwife from Birmingham Women's Hospital by telephone 30-40 days after my Caesarean Section and being asked questions about whether I have had an infection as a result of my C-section.	
8	The procedures regarding confidentiality have been clearly explained to me (e.g. anonymization of data, etc.).	
9	I acknowledge that the data will be stored at the University of Birmingham for period of 10 years and Birmingham Women's Hospital for 25 years in line with local Hospital policy, with the access restricted only to the research team.	
10	The use of the data in research, publications has been explained to me. I will not be mentioned by name in any publications as the data will be coded and non-identifiable.	
11	I agree to my personal information being collected and processed for the purpose of the study in accordance with General Data Protection Regulation (GDPR).	
12	I agree to participate in this study.	
13	OPTIONAL: I agree to any remaining tissue to be used in other research at the University of Birmingham with the relevant ethical approval. I understand that this will be a donation from me to the University of Birmingham.	

Participant name:_____

Signature:_____ Date: _____

Name of person taking consent:_____

Signature:_____ Date: _____

Original copy for the study site file, one copy for the medical records and one for the participant

Sample collection at C-Section {33}

The clinical care team will use the following forms: Operation Form and Discharge Form to record the time of cefuroxime administration, dose administered, type of anaesthesia used, blood loss and other information (see documents/appendixes). Five samples will be collected: 1 blood sample at time of skin incision, 1 blood sample at time of skin closure, 1 blood sample at recovery room, 1 adipose tissue sample (approximately 1 cm from the skin in the middle of the Pfannesnstiel or vertical midline incision) at skin incision and one adipose tissue sample just prior to skin closure.

The first sample of blood will be taken at time of skin incision. After the operation, take another blood sample will be collected as close to the time of delivery as possible and the final blood sample will be taken in the recovery room. The samples will either be taken from a cannula inserted in the participant's hand via a cannula, or taken by a very small 'butterfly' needle from the participant's hand or foot. If the samples are taken from their foot this will be numb from the anaesthetic and will not hurt. The research team will discuss with the participant how best to take these samples in line with their personal preferences.

Just before your baby is delivered, we will ask your doctor to remove a small amount of the fat from under the skin on your tummy. This will be a very small amount (about the size of a 50p coin). During your C-section, you will have an anaesthetic so you should not feel any pain. Your doctor will deliver the baby and placenta then stich the womb. Just before your skin is stitched, your doctor will remove a second 50p sized sample of fat. Taking these small fat samples will take less than a minute each so the overall procedure is only a maximum of two minutes longer than without the fat samples.

The securely sealed specimen sample tube will be labelled with the participant identification number which will correspond to the details held on the background information (Sample Label). These samples will be stored refrigerated as soon after collection as possible by the laboratories at Birmingham Women's and Children's Hospital NHS Foundation Trust and all samples will be couriered within a cool box (on ice) to the University of Birmingham (using an approved courier for transport of human samples). Prior to any human tissue transport, all human tissues will be packed into a sealed container, and labelled with the following information: Human Tissue Sample, Sample Reference Number, Tissue type, details of medium/ preservative, date of packing.

Upon arrival at the University the samples will be handled according to the University of Birmingham Health and Safety Guidance (GUIDANCE/26/WHBFT/14) and in accordance with the HTA and local procedures. Samples will be stored at the University of Birmingham in dedicated locked areas of -80°C freezers that are suitable for storage of human samples.

At the University of Birmingham, the process of characterisation will be performed within a laboratory settings on batches of samples. SOPs are in place for subsequent processing of samples. The work at the University of Birmingham will be conducted within the Pharmaceutics Laboratory in the Sir Robert Aitken building. The facilities are HTA compliant.

Sample Storage and Destruction

Local storage of samples at Birmingham Women's Hospital will be managed by the Laboratory R&D team where samples will be refrigerated upon receipt and then packaged appropriately for transfer to the University of Birmingham using an approved courier.

All human tissue will be packed into a sealed container prior to transport and labelled with the following information:

Human Tissue Sample
Sample Reference Number:
Tissue Type:
Details of medium/preservative if applicable: Note that no preservative will be used for these samples.
Date of packing:
UN 3373 warning label

The container will then be packed into a box and labelled with an approved human tissue sample hazard warning label – UN 3373.

Samples will be stored at the University of Birmingham in appropriate freezer boxes within a -80°C locked freezer in compliance with the HTA legislation. Each freezer box will be labelled with: (i) the name of the researcher; (ii) the PI's name; (iii) the study name; (iv) the study ethics reference number; (v) the date the trial began; (vi) the sample type in the box; (vii) the latest date by which the contents of the box must be destroyed/disposed of. Each sample will be labelled with the study identification number (which will not include any personal details of the participant).

The research team have requested consent to store any remaining tissue that is left over after this study to use for other research at the University of Birmingham. This further research will require approval from a research ethics committee. If no further research is planned and the samples need to be discarded this will be undertaken by placing samples into double autoclave bags and sent for autoclaving. Autoclaved waste will then be placed into yellow clinical waste bags and marked for incineration.

Sample Handling in the laboratory

Human samples will be handled according to the University of Birmingham Health and Safety Guidance (GUIDANCE/26/WHBFT/14) and in accordance with the HTA and local procedures. Samples will be stored at the University of Birmingham in dedicated locked areas of -80°C freezers that are suitable for storage of human samples.

University of Birmingham Health and Safety Policy (UHSP/9/BS/14) requires that risk assessment of all work with biological materials must be carried out in advance of work commencing. This work was submitted to the University of Birmingham Advisory Group for the Control of Biological Hazards "Measuring cefuroxime concentration in human plasma and adipose tissue" - Ref: AG 16_03 (approved in May 2018).

All work will be conducted within ADCP level 2 containment. As per standard laboratory practice the researcher will wear laboratory coat and nitrile gloves when handling human samples.

Chemgene HLD4L for disinfection validated to BS EN 14476:2005 will be used to clean all apparatus both prior to and following contact with the human samples used within this study. This will be diluted to 1:20 (with deionised water) to ensure that there is no risk of contamination due to contact with human samples.

Human blood and tissue will be kept in-80°C freezers, which will be located in a room with limited access to authorised staff. These freezers are specified for human tissue storage only (not animal origin).

Methodology for characterisation of samples collected

The concentration of cefuroxime in blood and adipose tissue will be quantified using standard laboratory methods. Full methodology is available as SOPs within the pharmacy research laboratory.

7.2.1.2 Additional file 2





UNIVERSITY OF BIRMINGHAM

NHS
Birmingham Women's and Children's
NHS Foundation Trust

C-LACE Screening Form

Section 1 - Details of the Participant

Initials of the woman: *First, Middle, Last*		Date of Birth: *e.g. 31-JAN-1997* D D - M M M - Y Y Y Y

Section 2 - Screenings

Inclusion Checklist		
Age of woman is between 18 years to 50 years? *Tick one*	No	Yes
With a singleton pregnancy? *Tick one*	No	Yes
Having an elective Caesarean Section? *Tick one*	No	Yes
Caesarean scheduled for at 37 weeks or over (≥37 weeks)? *Tick one*	No	Yes
With no known allergy to cefuroxime, cephalosporin or penicillin? *Tick one*	No	Yes
Exclusion Checklist		
Previously had an infection following a Caesarean Section? *Tick one*	No	Yes
Body Mass Index (BMI) less than 18 kg/m^2 at first pregnancy appointment? *Tick one*	No	Yes
Body Mass Index (BMI) greater than or equal to 45kg/m^2 at first pregnancy appointment? *Tick one*	No	Yes
Currently enrolled in an RCT for an intervention to reduce post-operative surgical site infection? *Tick one*	No	Yes
Has Diabetes (type 1, Type 2 or gestation)? *Tick one*	No	Yes
Diagnosed with Hypertension? *Tick one*	No	Yes
Diagnosed with renal disease? *Tick one*	No	Yes
Has a Cardiovascular Disease (eg. Maternal structural cardiac disease)? *Tick one*	No	Yes
Has liver disease? *Tick one*	No	Yes
Has inflammatory bowel disease (eg Crohn's disease or ulcerative colitis)? *Tick one*	No	Yes
Prior laparotomy for any indication (eg. Previous ovarian cystectomy or bowel surgery)? *Tick one*	No	Yes
Suspected pre-existing infection (including Chorioamnionitis)? *Tick one*	No	Yes
Has autoimmune disease (eg SLE, Rheumatoid arthritis)? *Tick one*	No	Yes
Chronic use of corticosteroid? *Tick one*	No	Yes
History of wound breakdown in an abdominal surgery? *Tick one*	No	Yes
If this form was completed more than 1 week pre CS please do not answer this question: Administration of antibiotic within 1 week prior to delivery?	No	Yes
Please note: if any of the **shaded boxes** above are ticked, then the woman is **ineligible** to take part in this study. Please sign and date the form. If the women is **eligible** then please provide the participant information sheet to the woman.		
Does the woman wish to take part in C-LACE study?	**No**	Yes

Section 3 - Form Completion Details

Full name of person completing form:	Signature of person completing form:	Date: D D - M M M - Y Y Y Y

IRAS#244803

C-LACE BaselineForm

Section 1 - Participant Details

Participant Trial No: ☐☐☐☐☐

Section 2 - Baseline Demographics

Booking BMI: _____.___kg/m^2 *Booking BMI: BMI at time of booking CS*	Booking Weight: _____.___kg *Booking weight: BMI at time of booking CS*	Height: _____cm

Ethnicity: *Tick one*

◯ British European ◯ Irish European ◯ East European ◯ North European ◯ South European ◯ West European
◯ North African ◯ Sub-Saharan African ◯ Middle Eastern ◯ Indian ◯ Pakistani ◯ Bangladeshi ◯ Chinese
◯ Other Far East ◯ South East Asia ◯ Caribbean ◯ Other ◯ Declined to give information

Section 3 - Pre-pregnancy Medical Conditions and Pregnancy History

Please tick NO if the conditions do not apply.

What is the woman's parity? *e.g. 01, 02, etc.* ☐☐ | Number of previous caesarean sections? ◯ 0 ◯ 1 ◯ 2 ◯ 3 ◯ ≥4

Has the woman ever had any other open abdominal surgery (excluding caesarean section)? *Tick one* ◯ No ◯ Yes

If **YES**, please specify:

Section 4 - This Pregnancy

Estimated Date of Delivery: *e.g. 31-JAN-2019* D D - M M M - Y Y Y Y

Section 5 - Smoking, Drugs & Alcohol Use

Was the woman an **ongoing** smoker at **booking** in this pregnancy? *Tick one* ◯ No ◯ Yes

Has the woman used non prescribed recreational drugs in this pregnancy (e.g. cocaine)? *Tick one* ◯ No ◯ Yes

If **YES**, please specify _____

Has the woman consumed alcohol in this pregnancy? *Tick one* ◯ No ◯ Yes | If **YES**, how many units per week? _____units

Section 6 - Form Completion Details

Full name of person completing form:	Signature of person completing form:	Date: *e.g. 31-JAN-2017* D D - M M M - Y Y Y Y

C-LACE eligibility reassessment form

PLEASE complete this within 24H pre CS

Section 1 - Participant Details
Participant Study ID number:

Section 2 – Current BMI		
Current BMI: _____.___ kg/m^2	Current Weight: _____.___ kg	Height: _____ cm

Section 3 – Eligibility re-assessment		
Inclusion Checklist		
Having an **elective** Caesarean Section? *Tick one*	No	Yes
Caesarean scheduled for at 37 weeks or over (≥37weeks)?? *Tick one*	No	Yes
With no known allergy to cefuroxime, cephalosporin or penicillin? *Tick one*	No	Yes
Exclusion Checklist		
Currently enrolled in an RCT for an intervention to reduce post-operative surgical site infection? *Tick one*	No	Yes
Current Body Mass Index (BMI) less than or equal to 18kg/m^2? *Tick one*	No	Yes
Current Body Mass Index (BMI) greater than or equal to 45kg/m^2? *Tick one*	No	Yes
Has Diabetes (type 1, Type 2 or gestation)? *Tick one*	No	Yes
Has pregnancy induced hypertension? *Tick one*	No	Yes
Has a Cardiovascular Disease (eg. Maternal structural cardiac disease)? *Tick one*	No	Yes
Has Renal disease? *Tick one*	No	Yes
Has Liver disease? *Tick one*	No	Yes
Has suspected pre-existing infection (including Chorioamnionitis)? *Tick one*	No	Yes
Has received an antibiotic in the past 1 week prior to delivery?	No	Yes
Please note: if any of the **shaded boxes** above are ticked, then the woman is **ineligible** to take part in this study. Please sign and date the form.		

Section 4 - Form Completion Details		
Full name of person completing form:	Signature of person completing form:	Date: D D - M M M - Y Y Y Y

C-LACE Operation Form

Section 1 - Participant Details

Participant Study ID Number:

Section 2 – Current weight and height

Current Weight:_____.___kg	Height:_____cm	Gestational age in weeks at time of CS _____Weeks

Section 3: Current medication within the last 48 hours prior to CS:

	Drug Name/ Strength e.g:	Dose	Frequency	Route	Starting Date	Discontinuation date	Comments
01					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	
02					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	
03					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	
04					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	If more than 4 medicines, complete this table and tick this box ◯

Section 4 - Details of the Caesarean Section

Was the Caesaran section performed in labour? *Tick one* ◯ No ◯ Yes	If **YES**, how many cm dilated? _____cm
Did the woman have rupture of membranes before the caesarean section? *Tick one* ◯ No ◯ Yes	
If **YES**, date of rupture of membranes: D D - M M M - Y Y Y Y	Time of rupture of membranes: *24hr* H H. M M
Type of anaesthesia *Tick one* ◯ General ◯ Regional	Time of administration of anesthesia *24hr* H H. M M
Any additional fluid administered *e.g. IV isotonic fluid*? ◯ No ◯ Yes	Type of the fluid :*g. IV isotonic fluid*
Estimated fluid administrated:_____mls	Estimated blood loss:_____mls

Section 5: Prophylactic antibiotic administration:

	Drug Name: *e.g Cefuroxime*	Dose : *e.g 1500 mg*	Frequency *e.g once*	Route *e.g IV*	Exact time of antibiotic given *24 h*	Timing from administration to knife to skin incision *e.g 30 min prior Skin incision or 60 min post Skin closure*	Comments
01					H H - M M		
02					H H - M M		
03					H H - M M		
04					H H - M M		If more than 4 antibiotics, complete this table and tick this box ◯

Section 6 - Operation Details

Date of CS decision: *e.g.31-JAN-2019* D D - M M M - Y Y Y Y	Time of CS decision: *24 h* H H - M M
Date of knife to skin: *e.g.31-JAN-2019* D D - M M M - Y Y Y Y	Time knife to skin: *24 h* H H - M M
Date of knife to uterus: *e.g.31-JAN-2019* D D - M M M - Y Y Y Y	Time of knife to uterus: *24 h* H H - M M
Date baby delivered: *e.g.31-JAN-2019* D D - M M M - Y Y Y Y	Time baby delivered: *24 h* H H - M M
Date placenta delivered: *e.g.31-JAN-2019* D D - M M M - Y Y Y Y	Time placenta delivered: *24 h* H H - M M
Date Skin Closure: *e.g.31-JAN-2019* D D - M M M - Y Y Y Y	Time skin closure skin: *24 h* H H - M M
Date woman out of theatre: *e.g.31-JAN-2019* D D - M M M - Y Y Y Y	Time out pf theatre: *24 h* H H - M M
Abdominal incision *Tick one* ◯ Midline ◯ Transverse	Thickness of adipose tissue ____cm ◯ Estimate ◯ Measured
Uterine incision: *Tick one* ◯ Lower segment ◯ Classical ◯ T shaped ◯ Extension into upper segment	

Section 7 - Details of samples collected

Sample number	Type of sample	Site of fat tissue collected	Date taken *e.g.31-JAN-2019*	Time taken *24 h*	Time within CS *Tick one*
1	◯ Blood sample		D D - M M M - Y Y Y Y	H H - M M	◯ at time of skin incision ◯ at time of skin closure
2	◯ Blood sample		D D - M M M - Y Y Y Y	H H - M M	◯ at time of skin incision ◯ at time of skin closure
2	◯ Blood sample		D D - M M M - Y Y Y Y	H H - M M	◯ during recovery
3	◯ adipose tissue sample	◯ upper segment of fat layer ◯ middle segment of fat layer ◯ lower segment of fat layer	D D - M M M - Y Y Y Y	H H - M M	◯ at time of skin incision ◯ at time of skin closure
4	◯ adipose tissue sample	◯ upper segment of fat layer ◯ middle segment of fat layer ◯ lower segment of fat layer	D D - M M M - Y Y Y Y	H H - M M	◯ at time of skin incision ◯ at time of skin closure

Section 10 - Form Completion Details

Full name of person completing form:	Signature of person completing form:	Date: *e.g. 31-JAN-2019* D D - M M M - Y Y Y Y





UNIVERSITY OF BIRMINGHAM

NHS
Birmingham Women's and Children's
NHS Foundation Trust

C-LACE Trial Discharge

Section 1 - Participant Details

Patient trial No:

Section 2 - Discharge Details

Discharge date: *e.g. 31-JAN-2017* D D - M M M - Y Y Y Y

Discharge time: *24hr* H H . M M

Section 3 - Antibiotics

Was the woman prescribed a course of antibiotics postnatally? *Tick one* ◯ No ◯ Yes

If **No**, please go to Section 4, If **YES**, complete the following regarding antibiotics given postnatally

	Drug Name/ Strength e.g: *e.g Cefuroxime*	**Dose** *e.g 1500 mg*	**Frequency** *e.g twice*	**Route** *e.g. IV*	**Starting Date** *e.g. 31-JAN-2019*	**Discontinuation date** *e.g. 31-JAN-2019*	**Comments**
1					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	
2					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	If more than 2 antibiotic, complete this table and tick this box ◯

Was the woman discharged on antibiotics? *Tick one* ◯ No ◯ Yes

Were the antibiotics used for a suspected/confirmed surgical site infection (e.g. uterine, pelvic, abdominal wound, perineal)? *Tick one* ◯ No ◯ Yes

If the discharge antibiotic are as reported in table above, please go to section 4; if the discharge antibiotic different from reported antibiotic in table above, then complete this table regarding discharge antibiotic

	Drug Name/ Strength e.g: *e.g Cefuroxime*	**Dose** *e.g 1500 mg*	**Frequency** *e.g twice*	**Route** *e.g. IV*	**Starting Date** *e.g. 31-JAN-2019*	**Discontinuation date** *e.g. 31-JAN-2019*	**Comments**
1					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	
2					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	If more than 5 antibiotic, complete this table and tick this box ◯

Section 4 - Suspected or Confirmed Infection

Did the woman develop a fever **(≥38°C)** during her inpatient stay? *Tick one* ◯ No; Temp <38°C ◯ Yes; Temp≥38°C

Was an infection suspected and/or confirmed in the uterus/pelvis? *Tick one* ◯ No ◯ Yes

If **YES**, was infection prompted by maternal and/or clinical concern? *Tick one*
◯ Maternal concern
◯ Clinical concern
◯ Both

Was an infection suspected and/or confirmed in the abdominal wound? *Tick one* ◯ No ◯ Yes

If **YES**, was infection prompted by maternal and/or clinical concern? *Tick one*
◯ Maternal concern
◯ Clinical concern
◯ Both

Was an infection suspected and/or confirmed in the perineum? *Tick one* ◯ No ◯ Yes

If **YES**, was infection prompted by maternal and/or clinical concern? *Tick one*
◯ Maternal concern
◯ Clinical concern
◯ Both

If **any shaded box is ticked in section 4**, please complete rest of form. If not, please sign and date the form

Section 5 - Signs & Symptoms

Did the woman have any of the following? *Answer all*

Abnormal uterine tenderness	◯ No	◯ Yes	Abdominal wound tenderness	◯ No	◯ Yes
Abnormal uterine involution	◯ No	◯ Yes	Abnormal lochia	◯ No	◯ Yes
Abnormal temperature	◯ No	◯ Yes			

Did the woman have a clinical diagnosis of endometritis requiring treatment? *Tick one* ◯ No ◯ Yes

If **YES**, date of clinical diagnosis of endometritis: *e.g. 31-JAN-2017* D D - M M M - Y Y Y Y

Did the woman meet the **CDC definition of endometritis** below? (see CDC definition) *Tick one* ◯ No ◯ Yes

The definition as per the CDC are: Patient has organisms cultured from endometrial fluid or tissue (including amniotic fluid) OR Patient has at least two of the following: fever (≥38°C), pain or tenderness (uterine or abdominal), or purulent drainage from uterus (day 0 - 30)

Section 6 - Microbiology

Did the woman have any microbiology samples taken post operatively? *Tick one* ◯ No ◯ Yes

If **No**, go to Section 7

Was a **blood culture** sample taken? *Tick one* ◯ No ◯ Yes

If **YES**, did the **blood cultures** grow any micro-organisms? *Tick one*
◯ No
◯ Yes

If **YES**, please specify the micro-organisms:

Was a **skin wound swab** sample taken? *Tick one* ◯ No ◯ Yes

If **YES**, did the **skin wound swab** grow any micro-organisms? *Tick one*
◯ No
◯ Yes

If **YES**, please specify the micro-organisms:

Was a **vaginal swab** sample taken? *Tick one* ◯ No ◯ Yes

If **YES**, did the **vaginal swab** grow any micro-organisms? *Tick one*
◯ No
◯ Yes

If **YES**, please specify the micro-organisms:

Was an **MSU** sample taken? *Tick one* ◯ No ◯ Yes

If **YES**, did the **MSU** grow any micro-organisms? *Tick one*
◯ No
◯ Yes

If **YES**, please specify the micro-organisms:

If **any other** samples were taken, please specify site and micro-organism if positive culture:

Section 7 - Severity of Infection

Was the woman admitted to level 2 or level 3 care (e.g. HDU) as a result of a suspected/confirmed infection? *Tick one* ◯ No ◯ Yes

Did the woman develop sepsis prior to discharge from hospital? *Tick one* ◯ No ◯ Yes

If **YES**, date of diagnosis of sepsis: *e.g. 31-JAN-2017* D D - M M M - Y Y Y Y

Did the infection delay the woman's discharge from hospital? *Tick one* ◯ No ◯ Yes

7.2.1.3 Additional file 3



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	√
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	√
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	√
Funding	4	Sources and types of financial, material, and other support	√
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	√
	5b	Name and contact information for the trial sponsor	√
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	√
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA single centre trial

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	√
	6b	Explanation for choice of comparators	NA no comparator
Objectives	7	Specific objectives or hypotheses	√
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	√
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	√
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	√
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	NA Not RCT
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA Not RCT
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA Not RCT
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA Not RCT
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each	√

		outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	√
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	√
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	√
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA not RCT
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA not RCT
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA not RCT
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA not RCT
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA not RCT
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	√
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	√

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	√
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	√
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA not necessary in the pilot study
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	√
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	√
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA pilot study with no intervention
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	√
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	√
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	√
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	√
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	√
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA no ancillary studies

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	√
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	√
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	√
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	√
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	√
	31b	Authorship eligibility guidelines and any intended use of professional writers	√
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	√
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	√
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	√

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

7.2.2 Study Protocol and additional forms related to C-LACE study

7.2.2.1 Study protocol



C-LACE: Cefuroxime Levels After Caesarean Section

UNDERSTANDING PHARMACOKINETIC DISTRIBUTION OF CEFUROXIME WHEN GIVEN PRIOR TO CAESAREAN SECTION IN BODY FAT AND BLOOD

Chief Investigator:
Hannah Batchelor

Local Principal Investigator:
Katie Morris

Researchers:
Victoria Hodgetts-Morton
Hanadi Alrammaal



Summary

This protocol describes the study "Understanding pharmacokinetic distribution of cefuroxime when given prior to Caesarean section in body fat and blood". This study will be conducted at Birmingham Women's Hospital. This study will adhere to the University of Birmingham's Clinical Trials Quality Management System (QMS).. The study will be conducted in compliance with the protocol, the Data Protection Act (2018) and other regulatory requirements as appropriate. The research will be conducted according to the legal framework for use of human tissues in research embodied in the Human Tissue Act.

Version number and date: Version 0.4 2nd January 2020

Sponsor: University of Birmingham

Funder: Funding is available for this project as part of the researcher's (PhD student Hanadi Alrammaal) funding from the Saudi Arabian Government.

IRAS number: 244803

Sponsor Reference Number: RG_18-237 (ERN_18-1539)

REC Reference Number:

Chief Investigator: Dr Hannah Batchelor

Protocol Amendments				
The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version.				
Amendment number	**Date of amendment**	**Protocol version number**	**Type of amendment**	**Summary of amendment**

Contact list

Chief investigator:

Dr Hannah Batchelor
School of Pharmacy, Institute of Clinical Sciences, Robert Aitken Building, University of Birmingham, Edgbaston, B15 2TT
Tel: [redacted]
Email: [redacted]

Local Principal investigator

Dr Katie Morris
Birmingham Women's Hospital, Birmingham Women's and Children's NHS Foundation Trust, Edgbaston, Birmingham, B15 2TG
Institute of Metabolism and Systems Research, University of Birmingham, Edgbaston, B15 2TT
Email [redacted]

Researchers

Dr Victoria Hodgetts Morton
Birmingham Women's Hospital, Birmingham Women's and Children's NHS Foundation Trust, Edgbaston, Birmingham, B15 2TG
Institute of Applied Health Research, University of Birmingham, Edgbaston, B15 2TT
Email: [redacted]

Hanadi Alrammaal
School of Pharmacy, Institute of Clinical Sciences,
Robert Aitken Building, University of Birmingham, Edgbaston, B15 2TT
Email address: [redacted]

UNIVERSITY OF BIRMINGHAM
NHS Birmingham Women's and Children's NHS Foundation Trust

Signature Page

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to adhere to the signed University of Birmingham's Sponsorship CI declaration.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Trial Name: C-LACE: Cefuroxime Levels After Caesarean Section

Protocol Version Number: Version: __ __

Protocol Version Date: __ __/__ __/__ __ __ __

Chief Investigator:

Signature: ..... Date: ...../...../.....

Name: (please print):....Hannah Batchelor.....

Sponsor statement:

Where the University of Birmingham takes on the sponsor role for protocol development oversight, the signing of the IRAS form by the sponsor will serve as confirmation of approval of this protocol.

Table of Contents

Summary 1
Contact list 2
Chief investigator: 2
Local Principal investigator 2
Researchers 2
1. Summary and Scientific Justifications 6
1.1. Summary of the study 6
2. Synopsis 6
2.1. Protocol contributors 7
2.2. Study Flow Chart 7
2.3. Background and Scientific Justification 8
3. Aims and Objectives 8
3.1. Primary Aims 8
3.2. Secondary Aims 9
3.3. Objectives 9
4. Study Design 9
4.1. Patient and public involvement 9
4.2. Study setting 9
4.3. Research team 9
5. Study sequence 10
5.1. Participant identification 10
5.2. Inclusion criteria 11
5.3. Exclusion criteria 11
5.4. Recruitment for prospective study 11
5.5. Informed Consent 12
5.6. Discontinuation/Withdrawal of Participants from Study 13
5.7. Definition of the End of Study 13
5.1. Schedule of Assessments and associated paperwork 13
6. Study Procedures 14
6.1. Part A: Sample collection at C-Section 14
6.1.1. Sample Storage and Destruction 15
6.1.2. Sample Handling in the laboratory 16

UNIVERSITY OF BIRMINGHAM
NHS Birmingham Women's and Children's NHS Foundation Trust

6.1.3. Methodology for characterisation of samples collected 16
6.2. Part B: Follow up for post-surgical infection 16
6.2.1. Safety reporting 17
7. Statistics and Analysis 17
7.1. Description of Statistical Methods 17
7.2. The Number of Participants 17
7.3. Analysis of Outcome Measures 18
7.4. Specific secondary outcome 18
7.1. Missing data and lost to follow-up 18
8. Data Management 18
8.1. Access to Data 18
8.2. Data Recording and Record Keeping 20
8.2.1. Data Recording 20
8.2.2. Record Keeping 20
9. Dissemination of Study Results 20
10. Quality Assurance Procedures 20
11. Ethical and Regulatory Considerations 21
11.1. Peer review 21
11.2. Declaration of Helsinki 21
11.3. Guidelines for Good Clinical Practice 21
11.4. Approvals 21
11.5. Reporting 21
11.6. Participant Confidentiality 21
11.7. Expenses and Benefits 21
11.8. Assessment and management of risk 22
12. Finance and Insurance 22
12.1. Funding 22
12.2. Insurance 22
13. Publication Policy 22
14. References ..... **Error! Bookmark not defined.**

1. Summary and Scientific Justifications

1.1. Summary of the study

This study will evaluate the concentration of Cefuroxime in stomach adipose tissue and blood from pregnant women undergoing scheduled Caesarean section (standard care). Analysis will explore the impact of BMI (body mass index) vs dose of cefuroxime on the concentrations of drug determined. The study participants will be followed up between 30 and 40 days post C-section to record details of any post-caesarean infection to explore correlations in BMI:measured cefuroxime concentrations and post caesarean infection rates.

2. Synopsis

Study Title	C-LACE: Cefuroxime Levels After Caesarean Section Understanding pharmacokinetic distribution of cefuroxime when given prior to caesarean section in body fat and blood	
Internal ref.	RG_18-237 ERN_18-1539	
Study Design	Experimental, prospective study with non-probability sampling	
Study Participants	Women undergoing Caesarean-Section where cefuroxime is used as the prophylactic antibiotic	
Planned Sample Size	This study plans to compare data from obese (BMI>30 kg/m^2) and non-obese women (BMI<30 kg/m^2) at time of booking (10-14 weeks of pregnancy). At least 15 participants are required for each arm with a total of 30 participants	
Planned Study Period	18 months starting in November 2019	
	Objectives	**Outcome Measures**
Primary	To measure the concentration of cefuroxime in maternal blood and adipose tissue at defined periods post IV administration when used as a prophylactic antibiotic for Caesarean-section.	Measurement of cefuroxime concentration in blood and adipose tissue and comparison of values in obese and non-obese patients
Secondary	To measure the incidence of post-surgical site infections from the same patients at 30-40 days post C-section	Incidence of post-surgical site infections recorded and comparison of values in obese and non-obese patients

2.1. Protocol contributors

This protocol was written jointly by the researcher (Hanadi Alrammaal); chief investigator (Hannah Batchelor); local principal investigator (Katie Morris) and clinical researcher (Victoria Hodgetts-Morton).

2.2. Study Flow Chart

Timing	Activity
C-Section Booking clinic	Clinic list will be reviewed by the clinic team in conjunction with a research midwife employed at Birmingham Women's Hospital for eligible participants using the Screening form Eligible women will be provided with a participant information sheet by a research midwife
24 hours prior to CS	Woman reminded of the study by a research midwife using a second copy of the participant information sheet Eligibility of women rechecked by a research midwife using the reassessment of eligibility form, prior to obtaining consent.
Recruitment to study	GCP trained research midwife will talk to participant about the study and answer any questions; if participant willing the informed consent form will be completed (by research midwife) Research midwife will complete the baseline form. The consent sticker will be completed and placed in the notes by the research midwife The Trial ID band will be completed by the research midwife and placed on the participants wrist The research midwife will complete the participant contact details for the follow-up
On day of CS	Sample pots will be prepared by the research midwife and provided to the obstetrician A venflon cannula will be inserted into the woman as part of the research process to enable withdrawal of the required blood samples without the need for additional needles. This second access point will ensure that the blood sampling is minimally disruptive to the CS procedure. Research midwife will inform the researcher that samples will be available later that day Blood and adipose tissue samples will be collected at time of CS by the obstetrician These samples will be placed into the pre-prepared pots The pots will be labelled using the sample sticker by the research midwife and taken to laboratory services for storage and packaging Laboratory services will inform the pharmacy research lab at UoB that the samples are ready for collection. Operation form will be completed by the research midwife.
At 30-40 days post CS	Research midwife will complete the Discharge form using patient notes Research midwife will attempt to contact the participant 3 times between 30 and 40 days post CS to complete the follow up interview form.

	Participant's medical file and notes will be reviewed by the research midwife to identify any post-surgical site infection which will be recorded on the 6 week outcome form

CS = Caesarean Section

2.3. Background and Scientific Justification

It was estimated in the 2000s that the prevalence of obesity in pregnant women in England was 16-20% [1, 2]. Being obese and pregnant may have an effect on the mother and foetus. One complication of obesity in pregnancy relates to the requirement for operative delivery, i.e. Caesarean section (CS).

The rate of CS among a pregnant, obese population is 33.8% [3]. The rate of post-CS infection is higher among obese pregnant women compared to those who are not obese. Wloch et al found that the adjusted odds ratio of surgical site infection in the obese population was 2.41 (95% CI 1.73-3.37) [4]. There are various ways to decrease and/or prevent post-CS infections. Single dose pre-CS intravenous (IV) antibiotic is recommended and several studies have examined the use of different antibiotics in CS, with cefazolin in the US and cefuroxime in the UK being the most commonly used drugs. Numerous studies have focussed on the use of cefazolin in obese patients to gain an understanding of optimal dosing and clinical outcomes [5]. Several studies proposed an increased dose of cefazolin (3 g) compared to the usual dose (2g) for obese pregnant women to achieve sufficient antibiotic levels [6-8]. It was postulated by Holt et al. (in 1994) that doses of cefuroxime up to 1500mg may not achieve sufficient MIC for most of the common bacteria in the maternal blood during CS [9]. Sub-therapeutic antibiotic concentration is a crucial factor in global bacterial resistance. It is essential that the therapeutic concentration of cefuroxime is achieved in both maternal blood and at the site of incision during CS for effective therapy. This study will take blood and adipose tissue samples to accurately measure levels achieved. Due to the great variability associated with pharmacokinetic measurements the use of physiologically based pharmacokinetic (PBPK) modelling is a common tool to understand the distribution of drugs. Specialist software packages are available that include details on pregnant populations. In this study we will use SimCYP software to model the blood and adipose concentrations of cefuroxime and we will compare this modelled data to the experimental results. It is anticipated that this clinical data coupled with PBPK models will inform any future changes to doses of cefuroxime used for CS, particularly in relation to obese women.

To the best of our knowledge, no study to date has measured cefuroxime concentrations in blood and adipose tissue as a function of body mass index (BMI) in pregnant women who have had CS. Thus, a study to explore the drug concentration in this population at the time of CS is essential.

3. Aims and Objectives

3.1. Primary Aims

1- To measure cefuroxime concentration in maternal blood at time of skin incision and skin closure in obese and non-obese pregnant patients requiring CS.
2- To measure cefuroxime concentration in adipose tissue (within the incision) at time of skin incision and skin closure in obese and non-obese pregnant
3- To explore the relationship between dose of cefuroxime administered and concentration measured in both blood and adipose tissue with respect to the Body Mass Index (BMI) of the patient

UNIVERSITY OF BIRMINGHAM
NHS Birmingham Women's and Children's NHS Foundation Trust

3.2. Secondary Aims

1- To use the measured cefuroxime concentrations to verify a Physiologically based pharmacokinetic modelling (PBPK) model of cefuroxime in CS to explore the impact of (i) dose and (ii) time of incision post administration on both blood and tissue concentrations in an obese population
2- To measure the rate of infections in obese and non-obese pregnant women at 30-40 days post CS.
3- To assess if the rate of infection is correlated with BMI, dose administered and cefuroxime blood or adipose tissue concentrations.

3.3. Objectives

1- To collect maternal blood samples and samples of adipose tissue from the site of incision at specific times post cefuroxime administration (skin incision and skin closure) when used as a prophylactic antibiotic in CS
2- To measure the concentration of cefuroxime in the collected blood and tissue samples using an appropriate analytical method.
3- To use the measured concentrations of cefuroxime to verify a PBPK model of cefuroxime in pregnant women
4- To assess whether cefuroxime administered provides concentrations above the minimum inhibitory concentration (MIC) in both the blood and adipose tissue for all patients regardless of their BMI.

4. Study Design

4.1. Patient and public involvement

A similar study conducted by Victoria Hodgetts Morton and Katie Morris (PREPS trial) involved the public and patients; those involved were supportive of any research undertaken to minimise the risks of post-surgical infections following CS [10].

Informal discussions about this study with women who have had CS have all been positive as the burden of participation is minimised where possible.

4.2. Study setting

This single centre study will take place at Birmingham Women's Hospital which is a large teaching hospital that is part of Birmingham Women's and Children's Hospital NHS Foundation Trust.

In a typical month (based on June 2018) 15 women with a BMI>30 kg/m^2 had an elective CS; there are plenty of matched controls therefore there are sufficient patients that can be recruited to this study.

The clinical members of the research team; Victoria Hodgetts-Morton and Katie Morris are involved in several research studies and are experienced in clinical research specifically studies that reduce the risk of post surgical infection following CS.

4.3. Research team

The research team brings together clinical and academic expertise.

Chief investigator: Hannah Batchelor is a senior lecturer in pharmaceutics, formulation and drug delivery within the School of Pharmacy at the University of Birmingham. Her research focuses on the

design and manipulation of medicines to create age-appropriate drug formulations to maximise clinical efficacy in paediatric patients. Hannah is an expert in prediction of medicines performance and has experience in the design and development of novel physiologically based pharmacokinetic models. She has experience in working on trials that evaluate human tissue samples (eg PaedGIFT #IRAS 251909). An integral part of her department's work is the development of drug delivery systems, including construction of new formulations, stability testing and development to market together with social and behavioural research into the perceptions, attitudes and values of patients towards medicines use. Hannah managed the ACCEPT study, a successful multi-centre, portfolio status clinical trial which involved 14 NHS sites across the West Midlands and recruited in excess of 600 participants. This study involved interacting with both paediatric patients and their parents to identify their views towards children's medicines. http://www.hra.nhs.uk/news/research-summaries/accept-methodology-to-assess-acceptability-of-paediatric-medicines/

Local Principal Investigator: Dr Katie Morris, Reader in Maternal and Fetal Health, University of Birmingham. Her research aims to improve the care for women with multiple pregnancies, fetal anomalies, growth restriction, reducing infection in maternity and prevention of preterm birth, employing prognostic and diagnostic research, modelling, systematic reviews and clinical trials. She has received research funding from the HTA, RfPB, MRC and charities. She sits on the RCOG Research Committee, Scientific Advisory Committee, RfPB West Midlands Panel, British Maternal and Fetal Medicine Society Executive Committee as scientific abstract co-ordinator and is Clinical Speciality Lead for Reproductive Health and Childbirth for the West Midlands. She was CI of the NIHR RfPB funded PREPS trial which was a randomised controlled trial recruiting women undergoing CS.

Victoria Hodgetts-Morton is a clinical research fellow working on large clinical trials at Birmingham Women's Hospital including C-Stitch and PREPS.

Researcher: Hanadi Alrammaal is a PhD student at the School of Pharmacy, University of Birmingham under the supervision of Hannah Batchelor. Hanadi is an experience researcher and has been trained in both GCP and handling of tissues. This study forms the basis of her PhD.

5. Study sequence

5.1. Participant identification

Any patient (over 18 years) who requires a CS as part of their clinical care will be screened for inclusion.

The potential participants will be identified using hospital clinical lists, where lists relate to a pre-booked CS a GCP trained research midwife who is a member of the clinical care team will assess eligibility of patients to participate in the study according to inclusion/exclusion criteria (Screening Form). The woman will then be provided with the participant information sheet at this meeting by the research midwife.

In addition, if a pregnant woman is booked for CS at a meeting they may be informed about the study by a research midwife or their clinical care team and provided with a participant information sheet (Participant Information Sheet). A GCP trained research midwife will assess eligibility of patients to participate in the study according to inclusion/exclusion criteria (Screening Form).

The participants will receive information about the study well in advance (at prior clinic appointments) and at least 24 hours prior to CS so that all participants have sufficient time to consider taking part.

The study team agreed that patients' General Practitioner will not be notified of patients' participation in the study as it will not affect their care/treatment plan.

This study plans to compare data from obese (BMI>30 kg/m^2) and non-obese women (BMI<30 kg/m^2) at time of booking (10-14 weeks of pregnancy).

At least 15 participants are required for each arm with a total of 30 participants.

5.2. Inclusion criteria

- Pregnant women 18 years to 50 years old, with a singleton pregnancy undergoing elective Caesarean Section at 37 weeks or over (Category 4 CS); with no known allergy to cefuroxime, cephalosporin or penicillin.
- Participant is able to give consent and agree to sample storage.
- Participant agrees to be contacted for follow-up
- Participant contributing to the study needs to be able to read and/or understand English.

5.3. Exclusion criteria

- Multiple pregnancy
- Previous Caesarean Section that resulted in a surgical site infection
- Emergency Caesarean Section (Caesarean categories 1 & 2)
- Any non-elective Caesarean Section including those requiring early delivery without threat to maternal or foetal health that were not previously planned (Category 3 CS)
- Body Mass Index (BMI) less than18 kg/m^2 or greater than or equal to 45kg/m^2 (At time of first pregnancy appointment and at time of delivery).
- Participant contributing to the study is unable to read and/or understand English.
- Currently enrolled in an RCT for an intervention to reduce post-operative surgical site infection
- Diabetes (type 1, Type 2 or gestation)
- Hypertension
- Renal disease
- Cardiovascular disease (eg. Maternal structural cardiac disease)
- Liver disease
- Inflammatory bowel disease (eg Crohn's disease or ulcerative colitis)
- Cephalosporin or penicillin allergy
- Administration of antibiotic within 1 week prior to delivery
- Suspected pre-existing infection (including Chorioamnionitis)
- Autoimmune disease (eg SLE, Rheumatoid arthritis)
- Chronic use of corticosteroid
- History of wound breakdown in an abdominal surgery
- Prior laparotomy for any indication (eg. Previous ovarian cystectomy or previous bowel surgery)

5.4. Recruitment for prospective study

At least 24 hours before CS, the participant will be greeted by a GCP trained research nurse/midwife and asked whether they received the participant information sheet (Participant Information Sheet). They will then be asked if they have any questions about the information provided and whether they are interested in participating in the study. The study will be briefly verbally described to the potential participant. Again they will be provided with an opportunity to ask any questions. Research

midwives/clinician will review and reassess each potential participant and confirm eligibility of patients to participate in the study at the day of CS (Eligibility Reassessment Form).

5.5. Informed Consent

A suitably qualified research midwife or doctor who is also part of the clinical care team will introduce the study to the woman at their pre- operative assessment. A Participant Information Sheet (PIS) (Participant Information Sheet) will be provided to facilitate this process. Investigators will ensure that they adequately explain the aim, anticipated benefits and potential hazards of taking part in the trial to the woman. They will also stress that participation is voluntary and that the woman is free to refuse to take part and may withdraw from the trial at any time. The woman will be given adequate time to read the PIS and to discuss their potential participation and be given the opportunity to ask questions. If the woman expresses an interest in participating in the trial, they will be asked to sign and date the latest version of the Informed Consent Form (ICF) at least 24 hours prior to CS. The woman will be asked for her consent to withdraw 2 blood samples (with a maximum volume of 10mL blood per sample) and 2 fat tissue and for collection of outcome data from their medical records and a telephone interview at 30-40 days postnatal that will take approximately 5-15 minutes. It will be the responsibility of the investigator to obtain written informed consent for each participant prior to performing any trial related procedure. Research midwives and doctors can both take informed consent for the trial as long as they are GCP trained and this responsibility has been delegated to them by the Principal Investigator (PI) as captured on the Site Delegation Log. The consent form will be provided in triplicate (as a carbon copy version) to facilitate the process on a busy labour ward. Each box on the consent form should be initialled by the woman and the form signed by the woman and the delegated research staff. A copy of the consent form will be given to the woman, a copy filed in the medical records with the PIS and the original signed form will be retained at the study site file (Birmingham Women's Hospital). Once the woman is entered into the trial, the woman's trial number will be entered on the ICF maintained in the Investigator Site file only. Details of the informed consent discussions will be recorded in the woman's medical notes. This will include date of consent, the name of the trial, summary of discussion, version number of the PIS, the Study ID given to the participant and version number of the ICF. Where consent is obtained on the same day that the trial related assessments are due to start, a note should be made in the medical notes as to what time the consent was obtained and what time the procedures started. At the telephone interview the woman's continued willingness to participate in the trial will be ascertained and documented (C-LACE Follow Up Interview). Throughout the trial the woman will have the opportunity to ask questions about the trial. At the time of taking consent the woman will provide contact details to facilitate the telephone interview using Contact details form which will be managed by the research midwife.

Upon signing consent the woman will be allocated a participant study ID number; this will be a random sequence of 5 numbers. Each participant will be allocated a pack of study documents in an envelope labelled with the participant study ID number; all documents within the envelope will have the participant study ID number pre-filled. Blank copies of all paperwork will also be available to the research team should additional copies be required for any participant.

UNIVERSITY OF BIRMINGHAM
NHS Birmingham Women's and Children's NHS Foundation Trust

5.6. Discontinuation/Withdrawal of Participants from Study

Each participant has the right to withdraw from the study at any time and this will not affect their clinical care. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Withdrawal of Consent

Withdrawal from the study will result in exclusion of the data for that participant from analysis; provided that the withdrawal occurs within 3 months of data collection.

Withdrawal of consent will require the keeper of the database to inform the research team of the study ID so that the associated data can be deleted and excluded from further analysis. Where analysis has already taken place (within the 3 months) the data will be deleted and not included in the final analysis and dissemination. Samples already collected will be destroyed in accordance with the Human Tissue Act 2004. Withdrawal beyond 3 months following dissemination will not be possible (this is detailed in the patient information sheet), however, any remaining samples will be destroyed in accordance with the Human Tissue Act 2004.

5.7. Definition of the End of Study

The end of study is the date at which the follow-up interview is conducted at 30-40 days following CS. This is where the follow-up ends for all participants.

The study will run for a maximum of 18 months and research data generated by the study will be kept for a period of 10 years at university of Birmingham and for 25 years at the NHS trust. Standard procedures for archiving data will be followed at Birmingham Women's Hospital. Research data will be held electronically on the University password protected computers, only the Chief Investigator and the immediate research team will have access to the study data. Given the time to collate the data, we feel that the study outcomes may not be of particular interest to participants. The study outcomes will primarily be of interest to the scientific community and clinicians and will be published in peer-reviewed journals and disseminated at scientific conferences. A poster will be made available to the public for the participants to be informed in case they are interested to see the results of the study. This will be hosted on Dr Hannah Batchelor's personal web pages, as stated in the participant information sheet (www.hannahbatchelor.com).

5.1. Schedule of Assessments and associated paperwork

Visit	Screening form	Consent form	Baseline form	Eligibility Reassessment form	CS Procedure form	Collection of samples, samples labels	Discharge form	Follow up interview form	Medical Record check form
Eligibility Check	×								
Consent at least 24 h pre CS		×	×	×					

Day of CS until discharge					×		×		
Follow-up								×	×

6. Study Procedures

Upon signing consent for the study each participant will be allocated a study ID number to manage anonymity in data analysis and interpretation. A spreadsheet that links study ID to patient ID will be held at Birmingham Women's and Children's Hospital NHS Foundation Trust under the control of the local principal investigator. This information will be password protected and stored on an encrypted computer that conforms to the standard NHS security requirements.

Background information will be collected from each participant using the Baseline Form. This information is required for interpretation of the data and dissemination.

Following collection of the data from all forms (Screening, Consent, Baseline, Eligibility Reassessment, Operation, Discharge, follow up interview, 6 week Record Check) will be entered on a shared secure system (Bear Share: https://intranet.birmingham.ac.uk/it/teams/infrastructure/research/bear/BEARDataShare/BEARDataShare.aspx) which is provided by the University of Birmingham; this system can be accessed at the University and the Hospital and ensures security of data, any data entered will be anonymised.

The study has two parts: part A, which is blood and adipose tissue sampling for cefuroxime concentrations, part B, is a follow up of patients postpartum at between 30-40 days to record the incidence of post-surgical infection.

6.1. Part A: Sample collection at C-Section

The duration of the participant participation in part A will last as long as the CS procedure lasts. No additional visits for the collection of the additional human blood or tissue are required. However, a follow up interview will be conducted at 30-40 days postpartum (see 6.2.Part B: Follow up for post-surgical infection)

The clinical care team will use the following forms: Operation Form and Discharge Form to record the time of cefuroxime administration, dose administered, type of anaesthesia used, blood loss and other information (see documents/appendixes). Five samples will be collected: 1 blood sample at time of skin incision, 1 blood sample at time of skin closure, 1 blood sample at recovery room, 1 adipose tissue sample (approximately 1 cm from the skin in the middle of the Pfannesnstiel or vertical midline incision) at skin incision and one adipose tissue sample just prior to skin closure.

The first sample of blood will be taken at time of skin incision. After the operation, take another blood sample will be collected as close to the time of delivery as possible and the final blood sample will be taken in the recovery room. The samples will either be taken from a cannula inserted in the participant's hand via a cannula, or taken by a very small 'butterfly' needle from the participant's hand or foot. If the samples are taken from their foot this will be numb from the anaesthetic and will not hurt. The research team will discuss with the participant how best to take these samples in line with their personal preferences.

Just before your baby is delivered, we will ask your doctor to remove a small amount of the fat from under the skin on your tummy. This will be a very small amount (about the size of a 50p coin). During your C-section, you will have an anaesthetic so you should not feel any pain. Your doctor will deliver the baby and placenta then stich the womb. Just before your skin is stitched, your doctor will remove a second 50p sized sample of fat. Taking these small fat samples will take less than a minute each so the overall procedure is only a maximum of two minutes longer than without the fat samples.

The securely sealed specimen sample tube will be labelled with the participant identification number which will correspond to the details held on the background information (Sample Label). These samples will be stored refrigerated as soon after collection as possible by the laboratories at Birmingham Women's and Children's Hospital NHS Foundation Trust and all samples will be couriered within a cool box (on ice) to the University of Birmingham (using an approved courier for transport of human samples). Prior to any human tissue transport, all human tissues will be packed into a sealed container, and labelled with the following information: Human Tissue Sample, Sample Reference Number, Tissue type, details of medium/ preservative, date of packing.

Upon arrival at the University the samples will be handled according to the University of Birmingham Health and Safety Guidance (GUIDANCE/26/WHBFT/14) and in accordance with the HTA and local procedures. Samples will be stored at the University of Birmingham in dedicated locked areas of -80°C freezers that are suitable for storage of human samples.

At the University of Birmingham, the process of characterisation will be performed within a laboratory settings on batches of samples. SOPs are in place for subsequent processing of samples. The work at the University of Birmingham will be conducted within the Pharmaceutics Laboratory in the Sir Robert Aitken building. The facilities are HTA compliant.

6.1.1. Sample Storage and Destruction

Local storage of samples at Birmingham Women's Hospital will be managed by the Laboratory R&D team where samples will be refrigerated upon receipt and then packaged appropriately for transfer to the University of Birmingham using an approved courier.

All human tissue will be packed into a sealed container prior to transport and labelled with the following information:

Human Tissue Sample
Sample Reference Number:
Tissue Type:
Details of medium/preservative if applicable: Note that no preservative will be used for these samples.
Date of packing:
UN 3373 warning label

The container will then be packed into a box and labelled with an approved human tissue sample hazard warning label – UN 3373.

Samples will be stored at the University of Birmingham in appropriate freezer boxes within a -80°C locked freezer in compliance with the HTA legislation. Each freezer box will be labelled with: (i) the name of the researcher; (ii) the PI's name; (iii) the study name; (iv) the study ethics reference number; (v) the date the trial began; (vi) the sample type in the box; (vii) the latest date by which the contents of the box must be destroyed/disposed of. Each sample will be labelled with the study identification number (which will not include any personal details of the participant).

The research team have requested consent to store any remaining tissue that is left over after this study to use for other research at the University of Birmingham. This further research will require approval from a research ethics committee. If no further research is planned and the samples need to be discarded this will be undertaken by placing samples into double autoclave bags and sent for autoclaving. Autoclaved waste will then be placed into yellow clinical waste bags and marked for incineration.

6.1.2. Sample Handling in the laboratory

Human samples will be handled according to the University of Birmingham Health and Safety Guidance (GUIDANCE/26/WHBFT/14) and in accordance with the HTA and local procedures. Samples will be stored at the University of Birmingham in dedicated locked areas of -80°C freezers that are suitable for storage of human samples.

University of Birmingham Health and Safety Policy (UHSP/9/BS/14) requires that risk assessment of all work with biological materials must be carried out in advance of work commencing. This work was submitted to the University of Birmingham Advisory Group for the Control of Biological Hazards "Measuring cefuroxime concentration in human plasma and adipose tissue" - Ref: AG 16_03 (approved in May 2018).

All work will be conducted within ADCP level 2 containment. As per standard laboratory practice the researcher will wear laboratory coat and nitrile gloves when handling human samples.

Chemgene HLD4L for disinfection validated to BS EN 14476:2005 will be used to clean all apparatus both prior to and following contact with the human samples used within this study. This will be diluted to 1:20 (with deionised water) to ensure that there is no risk of contamination due to contact with human samples.

Human blood and tissue will be kept in-80°C freezers, which will be located in a room with limited access to authorised staff. These freezers are specified for human tissue storage only (not animal origin).

6.1.3. Methodology for characterisation of samples collected

The concentration of cefuroxime in blood and adipose tissue will be quantified using standard laboratory methods. Full methodology is available as SOPs within the pharmacy research laboratory.

6.2. Part B: Follow up for post-surgical infection

At between 30-40 days post CS, the study participants will be contacted by telephone by a research nurse/midwife from the research team and a review will be made to record any type of post surgical infection. The Follow-up Interview Form will be used to record the details of any infection reported. If the participant attended to the hospital for a postnatal appointment at 30-40 days, the interview can be conducted at that time to minimise the burden to the participant.

The participant will be contacted by the researcher/research nurse/midwife and reminded of the study. In addition to the signed informed consent, a verbal consent to participate in the follow-up aspect will be obtained and recorded on the follow up sheet. Additionally, patient medical notes will be reviewed by a member of the clinical care team using the 30-40 days Outcome Form to identify whether there has been a reported post-surgical infection. Post-surgical infection is defined as per the

definitions set out by the US Centre's for Disease Control and Prevention (Centre's for Disease Control and Prevention 2019) [11].

6.2.1. Safety reporting

The principles of safety reporting for non-CTIMP studies as outlined by the Health Research Authority will be used for this project.

In brief, only reports of Adverse events (AEs) Serious Adverse Events (SAEs) that are:

- related to the study (ie they resulted from any of the research procedures); defined as from taking the first blood sample to completion of the CS

will be reviewed by the research team to determine whether a change in protocol is required.

We plan to undertake simple reviews of participants following the first 5 procedures to ensure that our risk assessment is correct; for example if more than two of the first five participants experience an untoward event we will halt the study and investigate alternative methods of blood and adipose tissue collection.

Definition of AE and SAE (taken from NRES):

Adverse Events (AEs) are any unfavourable and unintended signs, including abnormal laboratory results, symptoms or a disease associated with treatment.

Serious Adverse Events (SAEs) are defined as any untoward medical occurrence(s) that at any dose results in death, hospitalisation or prolongation of existing hospitalisation, persistent or significant disability/incapacity or a congenital anomaly or birth defect.

7. Statistics and Analysis

7.1. Description of Statistical Methods

The differences in mean concentration values in blood and adipose tissue between the obese and non-obese populations will be compared using ANOVA analysis to determine whether there are significant differences. SPSS software will be used for all statistical analysis. As a PhD student at the University of Birmingham the researcher has access to an expert statistician (Sayeed Haque) who will assist with analysis.

7.2. The Number of Participants

There is no predefined sample size yet sufficient numbers are required to provide an estimate of average values as well as the level of variability. Previous work to measure the concentration of antibiotics in plasma and in tissue in obese and non-obese pregnant women following CS has used sample sizes of 14 in each arm to detect differences in adipose antibiotic concentration [12, 13]. Therefore in this study we plan to recruit 15 women in each arm (BMI <30 kg/m^2 and BMI >30 kg/m^2). The BMI measurements will be taken from the women at their time of booking appointment which occurs between 10-14 weeks of pregnancy. The BMI of participants immediately prior to CS will be

determined to review any major changes during their pregnancy which may affect the results, for example a change from <30 kg/m² to an extremely obese participant at CS.

7.3. Analysis of Outcome Measures

To compare the concentration of cefuroxime in maternal blood and adipose tissue in obese vs non-obese women following a C-section.

To determine how many of the concentrations are above the MIC for cefuroxime (8mg/L).

7.4. Specific secondary outcome

The patients will be contacted at between 30-40 days post CS to record any incidence of surgical site infections postpartum. The rate and type of infection will be recorded and analysed. The rate of infections will be analysed in correlation with (1) dose administered, (2) BMI, (3) cefuroxime concentrations at both sites at time of skin incision and skin closure.

7.1. Missing data and lost to follow-up

Every attempt will be made to collect full follow-up data for all study participants; it is thus anticipated that missing data will be minimal. The main analysis will use available data only, however, the amount of missing data will be assessed, and if necessary, sensitivity analyses will be undertaken. If the participant couldn't be contacted, but the patients' medical records and notes were reviewed, the participant will be considered enrolled in the follow up. The patient will be considered as lost to follow up, if the participant couldn't contacted and the patients file and notes were not reviewed.

8. Data Management.

In compliance with the Data Protection Act 2018, Good Clinical Practice standards (GCP) and General Data Protection Regulation 2018 (GDPR)

8.1. Access to Data

All data will be collected only after the participant freely gave written consent to process their data in accordance with GDPR (this consent in included within the consent form for the study).

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

Access to the data retrieved from participants will be restricted; the table below lists the data collected and who will have access to this information. The clinical research team are those based at the Hospital and the paperwork will not be transferred to the University of Birmingham.

Step	Paperwork	Who retains access
1	Screening form	Clinical research team in study site file
2	Patient information sheet	Patient retains a copy Copy retained in the study site file
3	Consent form	Top copy – Clinical research team in study site file Middle copy – in patients medical notes Bottom copy – to patient
4	Consent sticker	Stick to main notes (medical file)
5	Patient contact details form	Clinical research team in study site file
6	Baseline form	Clinical research team in study site file; anonymised on completion so data can be shared with University of Birmingham
7	Reassessment of eligibility form	Clinical research team in study site file; anonymised on completion so data can be shared with University of Birmingham
8	Trial identity band	Patient
9	Operative form	Clinical research team in study site file; anonymised on completion so data can be shared with University of Birmingham
10	Samples Sticker	Stick to samples box, for researcher to pick up ASAP
11	Discharge form	Clinical research team in study site file; anonymised on completion so data can be shared with University of Birmingham
12	6 weeks outcome form	Clinical research team in study site file; anonymised on completion so data can be shared with University of Birmingham
13	Follow-up interview form	Clinical research team in study site file; anonymised on completion so data can be shared with University of Birmingham

The personal identifiable data collected will include names on consent forms and contact details for the follow up telephone interview. This information will be stored at the local site (Birmingham Women's and Children's Hospital NHS Foundation Trust) and not transferred to the University of Birmingham. Paper transcripts of the background information will be securely stored at Birmingham

Women's Hospital prior to transcription into the shared secure system (Bear Share). All information transferred on to the designated study encrypted computer will have restricted password protected access to only those directly involved in the research.

All participants will be given a study ID upon recruitment to the study. A spreadsheet that links study ID to patient ID will be held at Birmingham Women's and Children's Hospital NHS Foundation Trust under the control of the local PI. This information will be password protected and stored on an encrypted computer that conforms to the standard NHS security requirements.

The study ID will be used on all forms; this information provides anonymised data to the University of Birmingham that no longer has any personal identifiable data. Following collection the data from all forms will be entered on a secure system at the NHS site (undertaken by the research midwife); this system can be accessed at the University and ensures anonymity for the participants. (Bear Share: https://intranet.birmingham.ac.uk/it/teams/infrastructure/research/bear/BEARDataShare/BEARDataShare.aspx).

8.2. Data Recording and Record Keeping

8.2.1. Data Recording

All processes will be carried out with respect to participant confidentiality. All participant data will anonymised by being replaced with a study ID number prior to data sharing with the University of Birmingham. The anonymity code will only be known to the local site PI and associated research nurse/midwife team. The data will be controlled by usernames and encrypted passwords.

8.2.2. Record Keeping

It is the responsibility of the PI to ensure all essential trial documentation and source documents (e.g. signed ICFs, Investigator Site Files, Pharmacy Files, participants' hospital notes etc.) at their site are securely retained for 25 years as per hospital policy. Original consent forms and relevant documentation will be retained at the study site (University of Birmingham) under the supervision of the CI for 10 years.

9. Dissemination of Study Results

The study will run for 18 months and research data generated by the study will be kept for a period of 10 years in accordance with the University of Birmingham policy [14]. Research data will be held electronically on the University password protected computers, only the Chief Investigator and the immediate research team will have access to the study data.

The study outcomes will primarily be of interest to the scientific community and clinicians and the findings from the study will also be reported in a non-identifiable format via peer-reviewed journals and disseminated at scientific conferences. A poster will be made available to the public for the participants to be informed in case they are interested to see the results of the study.

Medical practitioners especially paediatricians, nurses, pharmacists and psychologists may also find the results of this study relevant to their field of work. Findings of the study will be distributed to healthcare professionals and the public via the following website www.hannahbatchelor.com.

10. Quality Assurance Procedures

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

UNIVERSITY OF BIRMINGHAM
NHS Birmingham Women's and Children's NHS Foundation Trust

11. Ethical and Regulatory Considerations

11.1. Peer review

The protocol has been peer reviewed within the research team and by the research and governance team at the University of Birmingham as part of the agreement to sponsor the study and by the University of Birmingham's Clinical Research Compliance Team (CRCT).

11.2. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the UK Policy Framework for Health and Social Care Research, Good Clinical Practice (GCP) standards, the General Data Protection Regulation (GDPR 2018) and Declaration of Helsinki.

11.3. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice and in adherence with Sponsor policies and procedures. According to the MHRA's algorithm Is it a clinical trial of a medicinal product? This is not a CTIMP study as there is no difference to the participant's clinical pathway as a result of participating in this study.

11.4. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and HRA for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents. The CI and local PI will be responsible for ensuring that all the appropriate approvals from participating organisations are in place prior to patients being enrolled into the study.

11.5. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

Any breaches in protocol and/or GCP would be reported to the relevant ethics committee within 7 days. The Chief Investigator will notify the Sponsor within 24 hours of becoming aware of a violation.

11.6. Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant study ID number on all study documents and any electronic database, with the exception of the consent form, where participant name and initials may be added and the patient contact details that will be used for the follow up interview. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

The participant's confidentiality will be ensured via the processes described in section 8.2 (Data recording and record keeping).

11.7. Expenses and Benefits

No expenses or benefits will be paid as part of this study.

11.8. Assessment and management of risk

The research protocol does not expose the participants to any risks as the study does not affect their clinical treatment. The collection of additional blood samples and adipose tissue samples during CS are the only additional study specific procedures. These additional blood samples will be taken by a clinical member of the research team trained in phlebotomy. There is a consent request for these study specific procedures to minimise the changes to routine clinical care and reduce the burden to participants. The risks associated with this research are minimal to participants.

All participants will be contacted at 30-40 days following CS to ask about post-surgical infections; this will not affect their clinical care in any way and does not pose any risks to participants.

The researcher will be exposed to human tissue samples. The researcher will be trained in the safe handling of biological materials and will have received the necessary inoculations. The researcher will use appropriate personal protective equipment at all times when handling human materials and will ensure that these are disposed of in accordance with the HTA legislation.

12. Finance and Insurance

12.1. Funding

The trial will be financed from funds associated with Hanadi Alrammaal's PhD which is funded by the Saudi Government. The details of the funding have been drawn up in a separate agreement.

12.2. Insurance

As the participants are NHS patients, indemnity is provided through the NHS schemes. The University of Birmingham provides clinical research insurance for studies that it sponsors. This insurance is provided via membership of UMAL.

13. Publication Policy

Results of this trial will be submitted for publication in a peer-reviewed journal. The manuscript will be prepared by Hanadi Alrammaal and Dr.Hannah Batchelor with contributions from the local PI (Katie Morris) and the local researcher (Victoria Hodgetts-Morton) and authorship will be determined by mutual agreement.

Authors must acknowledge that the trial was performed with the support of Saudi Arabian Government.

UNIVERSITY OF BIRMINGHAM
NHS Birmingham Women's and Children's NHS Foundation Trust

References

1. Kanagalingam, M.G., et al., *Changes in booking body mass index over a decade: retrospective analysis from a Glasgow Maternity Hospital.* Bjog, 2005. **112**(10): p. 1431-3.
2. Heslehurst, N., et al., *Trends in maternal obesity incidence rates, demographic predictors, and health inequalities in 36,821 women over a 15-year period.* Bjog, 2007. **114**(2): p. 187-94.
3. Weiss, J.L., et al., *Obesity, obstetric complications and cesarean delivery rate--a population-based screening study.* Am J Obstet Gynecol, 2004. **190**(4): p. 1091-7.
4. Wloch, C., et al., *Risk factors for surgical site infection following caesarean section in England: results from a multicentre cohort study.* Bjog, 2012. **119**(11): p. 1324-33.
5. Groff, S.M., et al., *Effect of maternal obesity on Maternal-Fetal transfer of preoperative cefazolin at cesarean section.* Journal of Pediatric Pharmacology and Therapeutics, 2017. **22**(3): p. 227-232.
6. Swank, M.L., et al., *Increased 3-gram cefazolin dosing for cesarean delivery prophylaxis in obese women.* American Journal of Obstetrics and Gynecology, 2015. **213**(3).
7. Kram, J.J.F., et al., *Does current cefazolin dosing achieve adequate tissue and blood concentrations in obese women undergoing cesarean section?* European Journal of Obstetrics, Gynecology, & Reproductive Biology, 2017. **210**: p. 334-341.
8. Chan, K., et al., *Effects of maternal obesity on tissue concentrations of prophylactic cefazolin during cesarean delivery.* American Journal of Obstetrics and Gynecology, 2011. **204 (1 SUPPL.)**: p. S24.
9. Holt, D.E., et al., *THE PLACENTAL-TRANSFER OF CEFUROXIME AT PARTURITION.* European Journal of Obstetrics Gynecology and Reproductive Biology, 1994. **54**(3): p. 177-180.
10. Weckesser, A., et al., *Women's perspectives on caesarean section recovery, infection and the PREPS trial: a qualitative pilot study.* BMC Pregnancy and Childbirth, 2019. **19**(1): p. 245.
11. Prevention, C.f.D.C.a., *Surgical Site Infection (SSI) Event.* 2019: p. 34.
12. Swank, M.L., et al., *Increased 3-gram cefazolin dosing for cesarean delivery prophylaxis in obese women.* American Journal of Obstetrics and Gynecology, 2015. **213**(3): p. 415e1-415e8.
13. Young, O.M., et al., *Pharmacokinetics of cefazolin prophylaxis in obese gravidae at time of cesarean delivery.* American Journal of Obstetrics and Gynecology, 2015. **213**(4): p. 541.e1-541.e7.
14. Birmingham, U.o., *Research Data Management Policy.* https://intranet.birmingham.ac.uk/as/libraryservices/library/research/rdm/Policies/Research-Data-Management-Policy.aspxhttps://intranet.birmingham.ac.uk/as/libraryservices/library/research/rdm/Policies/Research-Data-Management-Policy.aspx, Updated January 2018.

7.2.3 Additional forms related to C-LACE study

7.2.3.1 Participant Information Sheet

Participant Information Sheet
C-LACE: Cefuroxime Levels After Caesarean Section

Full title of research study: understanding pharmacokinetic distribution of cefuroxime when given prior to caesarean section in body fat and blood.

Summary of the Participant Information Sheet:

Doctors and Pharmacists at Birmingham Women's Hospital and the University of Birmingham, School of Pharmacy are investigating how the body mass index of the woman affects the concentration of the standard antibiotic (Cefuroxime) given to women prior to having a Caesarean section. They are particularly interested in the concentration of antibiotic in the blood and at the site of incision (tummy).

You have received this information sheet as you are scheduled to have a Caesarean Section at Birmingham Women's Hospital and are eligible to take part in this study.

In this research study we will use information from you and your medical records. We will only use information that we need for the research study. We will let very few people know your name or contact details, and only if they really need it for this study. Everyone involved in this study will keep your data safe and secure. We will also follow all privacy rules.

At the end of the study we will save some of the data in case we need to check it and for future research.

We will make sure no-one can work out who you are from the reports we write.

The information sheet tells you more about this study and what it involves.

This study is sponsored by University of Birmingham.

Please read the following information and if you have any queries, please discuss them with your friends, relatives or your hospital doctor or midwife. Please ask if there is anything that is not clear or if you would like more information.

Why we are doing this research?

Antibiotics are given prior to a Caesarean section (C-section) to reduce the chance of an infection following the procedure. At Birmingham Women's Hospital, Cefuroxime is the antibiotic drug that is usually used (if a woman does not have a severe penicillin allergy, note that if you have a penicillin allergy then you are NOT eligible to participate in this trial). Every patient receives the same dose of antibiotic regardless of their height, weight and body mass index (BMI). Currently, approximately 1 in 10 women develop an infection following a C-section which can affect a woman's recovery.

The likelihood of getting an infection increases with the woman's body mass index and we want to know if this is related to the concentration of cefuroxime that is present in the blood and site of

incision at the time of the C-section.

This research will assess whether the same dose of the antibiotic (cefuroxime) provides an adequate antibiotic coverage/concentration in all women; or whether women with a higher body mass index (BMI) would benefit from a higher dose of the drug. We will look at the concentration of antibiotic in blood and fat samples (within the incision) to see what level of antibiotic cover is reached in all women who participate.

The results from this study will be used to develop computer based models that better predict what dose would be required for a woman based on her height and weight. This may influence a change in dosing for the future, and in so doing reduce the rate of infection after a C-Section.

This research study is being undertaken as part of an educational qualification (a PhD) by Hanadi Alrammaal, at the School of Pharmacy at the University of Birmingham under the supervision of Dr Victoria Hodgetts-Morton. The local Principal Investigator is Dr Katie Morris, who is an obstetrician at Birmingham Women's Hospital and a researcher at the University of Birmingham. The research project has been funded by the PhD grant for Hanadi Alrammaal which is paid by the Saudi government. The University of Birmingham are responsible for sponsoring the research.

As a participant what do I have to do?

We will need to use information from you and from your medical records for this research project.

This information will include your:

- Initials
- Name
- Contact details
- Body mass index (related to your height and weight)
- Details of the drugs used during your Caesarean Section
- General details about your health at the time of your Caesarean Section

People at Birmingham Women's Hospital will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead. Some of this information and the blood and fat samples will be transferred to the University of Birmingham, this information and the samples will use a coded number and there will be no identifiable information that is transferred to the University of Birmingham.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

We would also like your permission to take **three blood samples (each one of less than 10mL (two teaspoons)** and **two small samples of fat** from your tummy during your Caesarean section.

The first sample of blood will be taken at time of skin incision. After your operation, we will need to take another blood sample as close to the time of delivery as possible and the final blood sample will be taken in the recovery room. The samples will either be taken from a cannula inserted in your hand, the insertion of the cannula will be uncomfortable, or the samples taken by a very small 'butterfly' needle from your hand or foot. If the samples are taken from your foot this will be numb from the anaesthetic and will not hurt. We will discuss with you how best to take these samples in line with your personal preferences.

Just before your baby is delivered, we will ask your doctor to remove a small amount of the fat from under the skin on your tummy. This will be a very small amount (about the size of a 50p coin). During your C-section, you will have an anaesthetic so you should not feel any pain. Your doctor will deliver the baby and placenta then stich the womb. Just before your skin is stitched, your doctor will remove a second 50p sized sample of fat. Taking these small fat samples will take less than a minute each so the overall procedure is only a maximum of two minutes longer than without the fat samples.

By taking **these three samples of blood and two samples of fat**, we will be able to see how the Cefuroxime antibiotic concentration in your body changes during your operation. These samples will be collected into sample tubes and transported to the University of Birmingham. The analysis to determine the drug concentrations will be done at the University of Birmingham.

You will also be asked if you consent to any remaining tissue that is left over after this study is completed being used for other research at the University of Birmingham. This further research will require approval from a research ethics committee.

We would also like your permission to contact you by telephone between 30-40 days after your C-section to ask about whether you have had an infection as a result of the C-section. This telephone call will take a maximum of 15 minutes.

How long do I need to take part for?

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.

As well as the samples taken on the day of your C-Section we would like your permission to contact you by telephone once between 30-40 days after your C-section to ask a few questions and to find out if you have had an infection.

If we cannot contact you or you choose to stop taking part in the study, we would like to continue collecting information about your health from your medical notes. If you do not want this to happen, tell us and we will stop. We would like to look at your medical notes to see if there is any record of an infection as a result of your C-section. There are no additional Hospital visits involved in this study.

We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

Will people know that the samples belong to me?

The samples will be anonymised at the Hospital so the researchers at the University will not know who provided the sample. However, some details from your Hospital notes will be linked to the sample, using a study number. These include your: ethnicity; height; weight; location from where the fat sample was collected; times that samples were taken and additional details about the C-section procedure (anaesthetic used; dose of antibiotic used). This information will not include any details that can identify you.

Am I eligible to participate?

We will be seeking to recruit healthy women who are experiencing a normal pregnancy. We are interested in women with a range of body mass index values so that we know as much as possible about cefuroxime concentrations.
As this is a pilot study a limited budget prevents us from providing translation services for those participants unable to read and understand English. Participation has no direct benefits to participants thus exclusion on this basis does not affect the standard of care provided.

Are there any risks or benefits?

There are no direct health benefits to participants. The findings from this study will help us determine the appropriate antibiotic dosing strategy used in future for patients with a high BMI that need a C-section.

We do not believe that there are any major disadvantages or risks to taking part in this study. The additional procedures that you will receive are (i) insertion of an additional cannula for the taking of three additional blood samples and (ii) the removal of two samples of fat. The blood will be taken by a trained healthcare professional using standard methods to take blood; the risks associated with taking blood samples are minimal. The samples of fat will be taken from you whilst the skin is open for the C-section; you will be anaesthetized so will not feel any pain from this process. The amount of fat taken is small (about the size of a 50p coin) and this will not lead to visible differences to your body following your C-section.

Your doctor will explain all the risks that are associated with a C-section.

What happens when the research project is completed?

We will publish our results. This may take up to 3 years as it takes time to collect and analyse all the data that we need. We will not name any of the participants and all the data will be anonymised so no one will be able to identify you

We will also create a poster that describes this study that you (and other participants) can access once the work is complete. A link will be available on Dr Hannah Batchelor's personal web pages: www.hannahbatchelor.com

If you withdraw your consent to participate within 3 months of your C-section all data associated with you will be destroyed and the samples previously collected will be destroyed in accordance with the Human Tissue Act 2004 (HTA). A decision to withdraw after 3 months means that the data has already been analysed and anonymised it will not be possible to delete your data. All data will be anonymised in any publication and it will not be possible to identify you from this output.

What if something goes wrong?

If you wish to complain about any aspect of the way you have been approached or treated during the course of this study you should contact the following:

Birgit Whitman, the University of Birmingham's Head of Research Integrity and Governance, who is an independent point of contact for research participants:

E-mail: researchgovernance@contacts.bham.ac.uk Phone: tel. 0121 415 8011

Alternatively, please contact the hospital's Patient Advice and Liaison Service (PALS) Office on 0121 335 8226 or email BWC.formalcomplaints@nhs.net.

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against the University of Birmingham or Birmingham Women's Hospital but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).**Will my information be kept in private?**

Definitely, it is our main duty and priority to ensure that all your details will remain strictly confidential. Your name and any other personal details will be kept at the hospital. You will be given a study number, which will be recorded against your name and this record will be kept at the hospital, any documents or tissue leaving the hospital will only be identified with this study number.

The final page of this document provides full details of how your data will be managed.

Will I be offered reimbursements?

There will be no reimbursements offered as part of this study.

Who has reviewed the research?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect participants' interests. This study has been reviewed and given favourable opinion by the East Midlands (Leicester Central) Research Ethics Committee.

Thank you…for your time and for considering participation in this study!

If you have any questions at all, please contact:

Chief investigator: Dr Victoria Hodgetts Morton(v.a.h.morton@bham.ac.uk)

Local investigator: Dr Katie Morris (r.k.morris@bham.ac.uk)

Head of Research Governance and Integrity at the University of Birmingham: Dr Birgit Whitman researchgovernance@contacts.bham.ac.uk or on the phone: 0121 415 8011

If you would like independent advice about this study or have any complaints about this research, please contact the hospital's Patient Advice and Liaison Service (PALS) Office on 0121 335 8226 or email BWC.formalcomplaints@nhs.net.

Or you can note them here and bring this on the day of your appointment.

Appendix: Data Management Information

In order to carry out the research project described above, we will need to collect information about you, and some of this information will be your personal data. Under data protection law, we have to provide you with very specific information about what we do with your data and about your rights. We have set out below the key information you need to know about how we will use your personal data.

The University of Birmingham Edgbaston, Birmingham B15 2TT is the data controller for the personal data that we process in relation to you.

The legal justification we have under data protection law for processing your personal data is that it is necessary for our research, which is a task we carry out in the public interest. We will not share your personal data with any third party.

The University takes great care to ensure that personal data is handled, stored and disposed of confidentially and securely. Our staff receive regular data protection training, and the University has put in place organisational and technical measures so that personal data is processed in accordance with the data protection principles set out in data protection law.

The University has an Information Security Management System based on ISO27001 with a range of controls covering the protection of personal information. Annual security awareness training is mandatory for staff and the University is accredited under the NHS Information Governance Toolkit, the Payment Card Industry Data Security Standard and is in the process of gaining Cyber Essentials Plus for defined services.

In relation to this project, data collected will be kept strictly confidential in a secure locked cupboard at the University of Birmingham where access is restricted to the research team.

How long will my personal data be kept?

Your data will be retained at the University of Birmingham for 10 years after the publication of the research outcomes and for 25 years at Birmingham Women's Hospital. If you withdraw from the project, we will keep the information we have already obtained but, to safeguard your rights, we will use the minimum personally-identifiable information possible.

Your rights in relation to your data

- You may have the following rights in respect of your personal data:
- The right to access to your data (often referred to as a Subject Access Request).
- The right to rectification of inaccuracies in your data.
- The right to erasure of your data (in certain circumstances).
- The right to restrict processing of your data (in certain circumstances).
- The right to object to the processing of your data (in certain circumstances).
- The right to ask for your personal data to be transferred electronically to a third party.

However, your rights to access, change or move your information are limited, as we need to manage

your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the project, we will keep the information we have already obtained but, to safeguard your rights, we will use the minimum personally-identifiable information possible.

If you would like more information on your rights, would like to exercise any right or have any queries relating to our processing of your personal data, please contact:

The Information Compliance Manager, Legal Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT

Email: dataprotection@contacts.bham.ac.uk Telephone: +44 (0)121 414 3916

If you wish to make a complaint about how your data is being or has been processed, please contact our Data Protection Officer.

Mrs Carolyn Pike, OBE, The Data Protection Officer, Legal Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT

Email: dataprotection@contacts.bham.ac.uk Telephone: +44 (0)121 414 3916

You also have a right to complain to the Information Commissioner's Office (ICO) about the way in which we process your personal data. You can make a complaint using the ICO's website.

7.2.3.2 Contact Details Form

C-LACE Contact details Form

Section 1 - Participant Details

Initials: *First, Middle, Last*	Date of Birth: *e.g. 31-JAN-1997* D D - M M M - Y Y Y Y
Participant Study ID number:	

Section 2 – Participant Contact Details

Please complete **ALL** details to ensure we can contact the participant for the telephone interview

Last Name:	First Name:	
Contact phone numbers	Telephone Number (Home):	Telephone Number (Mobile):
Any comments on preferred day/time to call or preferred number		

Section 3 - Form Completion Details

Full name of person completing form:	Signature of person completing form:	Date: *e.g. 31-JAN-2017* D D - M M M - Y Y Y Y

7.2.3.3 Consent Sticker

Trial Consent Documentation
Sticker to be placed in main notes

PIS given: ◯ No ◯ Yes /Date D D - M M M - Y Y Y Y
Happy to participate: ◯ No ◯ Yes
Consented: ◯ No ◯ Yes / Date D D - M M M - Y Y Y Y
Consented by: Name Sig:
Consent forms filed
Top copy in pack ◯ Yes
Middle copy to patient ◯ Yes
Bottom copy in main notes ◯ Yes
Patient aware that she can withdraw ◯ Yes



IRAS# 244803, Consent Sticker v0.4 18th December 2019

7.2.3.4 Sample label

C-LACE Sample Sticker

Section 1 – participant Details

Participant Study ID number:

Section 2 – Box Content

This box contains (please tick box)	**Sample Reference Number: is patient Trial number/sample number** *e.g. 23722/1*
1 human blood sample, *taken at time of skin incision at CS procedure* ◯	/
1 human blood sample, *taken at time of skin closure at CS procedure* ◯	/
1 human blood sample, *taken during recovery from CS procedure* ◯	/
1 human adipose tissue sample, *taken at time of skin incision at CS procedure* ◯	/
1human adipose tissue sample, *taken at time of skin incision at CS procedure* ◯	/

Section 3 – addresses

This box intended to be transferred from Birmingham Women's Hospital

To: Pharmacy Research Lab, Sir Robert Aitken Institute for Medical Research, University of Birmingham, Edgbaston B15 2TT, Birmingham, UK

contact details: Mrs.**Hanadi** Alrammaal phone number **07477143102** or Email: **hha706@student.bham.ac.uk**

Section 4 - Form Completion Details

Full name of person completing form:	Signature of person completing form:	Date: *e.g. 31-JAN-2019* D D - M M M - Y Y Y Y

7.2.3.5 Follow up interview form

Postnatal Interview – Day 30-40
Cefuroxime levels after CS



(Please dispose of THIS SHEET CONFIDENTIALLY once interview is complete)

Participant Details

Surname:

Forename

Participant Study ID number

Home Phone Number

Mobile Phone Number

C-section Details

Date of C-section D D M M Y Y

Scheduled 30 day Interview Date D D M M Y Y

Pertinent information for contact:

(e.g. time of day to call; preferred day of week)

Please dispose of THIS SHEET CONFIDENTIALLY once interview is complete

Postnatal Interview – Day 30-40
Cefuroxime levels after CS

Day 30-40 Telephone Contact Log

30-40 Day contact attempts

This should be done between 30-40 days post C-Section – with a maximum of 3 attempts during this period where each attempt is separated by at least 2 days

Date Time	Days post CS	Notes
D D M M Y Y H H M M		Interview not conducted ☐ Interview conducted ☐ Notes:
D D M M Y Y H H M M		Interview not conducted ☐ Interview conducted ☐ Notes:
D D M M Y Y H H M M		Interview not conducted ☐ Interview conducted ☐ Notes:

Unable to Contact

If you are unable to contact the participant for an interview, please give details:

IRAS# 244803
Follow-up Questionnaire v0.2 *Page **1** of **6*** *16th September 2019*

30-40 day interview

Participant Details

Participant Study ID number ☐☐☐☐☐

Date of Interview D D M M Y Y

Telephone Interview ☐ In Hospital Interview ☐

Script

'Make Introductions….'

Thank you for agreeing to receive a further telephone interview following your C-section. We really appreciate your time.

You previously gave consent for us to call you; please can you confirm that you are willing to proceed with this interview: **Yes/No**

The interviews will not be recorded so please bear with me while I write information down.

General Questions:	Response categories
1. Do you feel you have recovered well from your caesarean section?	a) Not at all ☐ Notes: b) A little ☐ c) Quite a bit ☐ d) A lot ☐
2. Do you feel you can care for yourself as you would expect?	a) Not at all ☐ Notes: b) A little ☐ c) Quite a bit ☐ d) A lot ☐
3. Have you sought advice from a healthcare professional because of a problem with your CS? This does not include routine planned follow-up appointments	Yes ☐ No ☐ Notes:

Infection Questions:	Response categories
4. Have you been diagnosed with an infection since leaving hospital?	Yes ☐ No ☐
5. About your infection:	**If YES please continue; if NO please go to question 6**
>5a. Who diagnosed your infection?	a) GP Yes ☐ No ☐ b) Dr at the hospital where you had your baby Yes ☐ No ☐ c) Dr at a different hospital Yes ☐ No ☐ d) Other Yes ☐ No ☐ Please specify: ……
>5b. Where was/is your infection?	a) Womb/pelvis Yes ☐ No ☐ b) CS wound Yes ☐ No ☐ c) Vaginal/perineal wound Yes ☐ No ☐ d) Other Yes ☐ No ☐ Please specify: ……
>5c. Were you prescribed antibiotics for this infection?	Yes ☐ No ☐
>5d. Do you remember the name of the antibiotic?	Yes ☐ name of antibiotic_____ No ☐
>5e. Were you readmitted into hospital for this infection?	Yes ☐ No ☐
>5f. If **YES (to Q5e)**, was this the hospital where you had your baby?	**If NO please go to question 6** Yes ☐ No ☐ Date admitted D D M M Y Y Date discharged: D D M M Y Y

6. Have you had, or felt like you have had, a high temperature or fever since leaving hospital after your CS? >6a. If **YES**, did you seek medical advice? Provide details of advice received	Yes ☐ No ☐ Yes ☐ No ☐
7. Have you had any of the following concerns with your CS wound?	a) Redness Yes ☐ No ☐ b) Pain Yes ☐ No ☐ c) Discharge/oozing Yes ☐ No ☐ d) Heat Yes ☐ No ☐ e) Wound opening up Yes ☐ No ☐
8. Have you had any abnormal period-like pain since your CS?	Yes ☐ No ☐

Trial feedback	Response categories
9. Do you have any feedback about the trial?	Yes ☐ No ☐ If YES please tell us:
10. Have you found the interview process difficult?	a) Not at all ☐ Notes: b) A little ☐ c) Quite a bit ☐ d) A lot ☐

Length of interview

Duration: H H M M

Once this interview is completed, Please discard only first page with the this logo 

Interview conducted by: Name:

Date: D D - M M M - Y Y Y Y

Signature:

7.2.3.6 Medical Record 6 weeks outcome form

C-LACE Trial 6 week outcome form

Section 1 - Participant Details

Participant Study ID number:

Section 2 – Hospital Attendance

Did the women attend hospital for a possible infection **AFTER** her discharge home since giving birth? *Tick one* No Yes

If **YES**, how many attendances? *e.g. 01, 02* ____

If **NO**,- this form is complete, please sign and date form. If **YES**- please complete the remainder of the form

Please complete a separate form for each attendance

Which attendance does this form refer to? *e.g. 01, 02* ____

What was the outcome of the attendance? *Tick one*

- Attended triage and returned home
- Attended triage and admitted to hospital

Date attended: D D - M M M - Y Y Y Y

Time attended: *24hr* H H . M M

If **ADMITTED** to hospital, date of discharge: D D - M M M - Y Y Y Y

If **ADMITTED**, time discharged: *24hr* H H . M M

Section 3 - Antibiotics

Was the woman prescribed a course of antibiotics postnatally? *Tick one* No Yes

If **No**, please go to Section 4, If **YES**, complete the following regarding antibiotics given postnatally

	Drug Name/ Strength e.g: *e.g Cefuroxime*	**Dose** *e.g 1500 mg*	**Frequency** *e.g twice*	**Route** *e.g. IV*	**Starting Date** *e.g. 31-JAN-2019*	**Discontinuation date** *e.g. 31-JAN-2019*	**Comments**
1					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	
2					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	
3					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	
4					D D - M M M - Y Y Y Y	D D - M M M - Y Y Y Y	If more than 4 antibiotic, complete this table and tick this box

Were the antibiotics used for a suspected/confirmed surgical site infection (e.g. uterine, pelvic, abdominal wound, perineal)? *Tick one* No Yes

Section 4 - Suspected or Confirmed Infection

Did the woman develop a fever **(≥38°C)** during her inpatient stay? *Tick one* No; Temp <38°C Yes; Temp ≥38°C

Was an infection suspected and/or confirmed in the uterus/pelvis? *Tick one* No Yes

If **YES**, was infection prompted by maternal and/or clinical concern? *Tick one*
- Maternal concern
- Clinical concern
- Both

Was an infection suspected and/or confirmed in the abdominal wound? *Tick one* No Yes

If **YES**, was infection prompted by maternal and/or clinical concern? *Tick one*
- Maternal concern
- Clinical concern
- Both

Was an infection suspected and/or confirmed in the perineum? *Tick one* No Yes

If **YES**, was infection prompted by maternal and/or clinical concern? *Tick one*
- Maternal concern
- Clinical concern
- Both

Section 5 - Signs & Symptoms

Did the woman have any of the following? *Answer all*

Abnormal uterine tenderness	No	Yes	Abdominal wound tenderness	No	Yes
Abnormal uterine involution	No	Yes	Abnormal lochia	No	Yes
Abnormal temperature	No	Yes			

Did the woman have a clinical diagnosis of endometritis requiring treatment? *Tick one* No Yes

If **YES**, date of clinical diagnosis of endometritis: *e.g. 31-JAN-2017* D D - M M M - Y Y Y Y

Did the woman meet the **CDC definition of endometritis** below? (see CDC definition) *Tick one* No Yes

The definition as per the CDC are: Patient has organisms cultured from endometrial fluid or tissue (including amniotic fluid) OR Patient has at least two of the following: fever (≥38°C), pain or tenderness (uterine or abdominal), or purulent drainage from uterus (day 0 - 30)

Section 6 - Microbiology

Did the woman have any microbiology samples taken post operatively? *Tick one* No Yes

If **No**, go to Section 7

Was a **blood culture** sample taken? *Tick one* No Yes

If **YES**, did the **blood cultures** grow any micro-organisms? *Tick one* No Yes

If **YES**, please specify the micro-organisms:

Was a **skin wound swab** sample taken? *Tick one* No Yes

If **YES**, did the **skin wound swab** grow any micro-organisms? *Tick one* No Yes

If **YES**, please specify the micro-organisms:

Was a **vaginal swab** sample taken? *Tick one* No Yes

If **YES**, did the **vaginal swab** grow any micro-organisms? *Tick one* No Yes

If **YES**, please specify the micro-organisms:

Was an **MSU** sample taken? *Tick one* No Yes

If **YES**, did the **MSU** grow any micro-organisms? *Tick one* No Yes

If **YES**, please specify the micro-organisms:

If **any other** samples were taken, please specify site and micro-organism if positive culture:

Section 7 - Severity of Infection

Was the woman admitted to level 2 or level 3 care (e.g. HDU) as a result of a suspected/confirmed infection? *Tick one* No Yes

If **YES**, date of admittance to level 2 or level 3 care (e.g. HDU): *e.g. 31-JAN-2017* D D - M M M - Y Y Y Y

Did the woman develop sepsis during her attendance? *Tick one* No Yes

If **YES**, date of diagnosis of sepsis: *e.g. 31-JAN-2017* D D - M M M - Y Y Y Y

Section 8 - Form Completion Details

Full name of person completing form:

Signature of person completing form:

Date: D D - M M M - Y Y Y Y

7.2.4 Ethical approval and paperwork related to C-LACE study

7.2.4.1 Health Research Authority and Health and Care Research Wales Approval Letter

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Dr Hannah Batchelor
Senior Lecturer in Pharmaceutics, Formulation and Drug Delivery
University of Birmingham
University of Birmingham
School of Pharmacy
Birmingham
B15 2TT

Email: hra.approval@nhs.net
HCRW.approvals@wales.nhs.uk

22 January 2020

Dear Dr Batchelor

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Understanding pharmacokinetic distribution of cefuroxime when given prior to Caesarean section in body fat and blood**
IRAS project ID:	**244803**
REC reference:	**19/EM/0356**
Sponsor	**Organization not set**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report

(including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **244803**. Please quote this on all correspondence.

Yours sincerely,
Barbara Cuddon

Approvals Specialist

Email: hra.approval@nhs.net

Copy to: *Dr Birgit Whitman*

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
Contract/Study Agreement template [Letter]		25 September 2019
Contract/Study Agreement template [Letter]		
Covering letter on headed paper [Cover letter]		19 September 2019
Covering letter on headed paper [Cover letter]		26 December 2019
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Letter]		08 November 2019
HRA Schedule of Events [HRA Assessed Version]	1.0	22 November 2019
IRAS Application Form [IRAS_Form_21112019]		21 November 2019
IRAS Application Form XML file [IRAS_Form_21112019]		21 November 2019
Letter from sponsor [Sponsor letter]		08 November 2019
Non-validated questionnaire [Baseline form]	0.2	16 September 2019
Non-validated questionnaire [Sample label]	0.3	30 October 2019
Non-validated questionnaire [Operation form]	0.3	30 October 2019
Non-validated questionnaire [Screening form]	0.3	30 October 2019
Non-validated questionnaire [Discharge form]	0.2	16 September 2019
Non-validated questionnaire [Follow up interview form]	0.2	16 September 2019
Non-validated questionnaire [6 weeks outcome form]	0.2	16 September 2019
Non-validated questionnaire [Eligibility reassessment form]	0.2	16 September 2019
Non-validated questionnaire [Contact details]	0.2	16 September 2019
Participant consent form [Consent sticker]	0.4	18 December 2019
Participant consent form [ICF]	0.5	21 January 2020
Participant information sheet (PIS) [PIS Tracked change]	0.4	26 December 2019
Participant information sheet (PIS) [PIS Clean Version]	0.4	26 December 2019
Research protocol or project proposal [Protocol]	0.4	02 January 2020
Summary CV for Chief Investigator (CI) [Summary CV]		16 September 2019
Summary CV for student [CV]		25 November 2019
Summary CV for supervisor (student research) [CV]		09 September 2019
Summary CV for supervisor (student research) [CV]		16 September 2019
Summary CV for supervisor (student research) [CV]		06 May 2019
Summary of any applicable exclusions to sponsor insurance (non-NHS sponsors only) [Letter]	0.1	08 November 2019

IRAS project ID	244803

Information to support study set up

The below provides all parties with information to support the arranging and confirming of capacity and capability with participating NHS organisations in England and Wales. This is intended to be an accurate reflection of the study at the time of issue of this letter.

Types of participating NHS organisation	Expectations related to confirmation of capacity and capability	Agreement to be used	Funding arrangements	Oversight expectations	HR Good Practice Resource Pack expectations
All sites will perform the same research activities therefore there is only one site type.	Research activities should not commence at participating NHS organisations in England or Wales prior to their formal confirmation of capacity and capability to deliver the study.	An Organisation Information Document has been submitted and the sponsor is not requesting and does not expect any other site agreement to be used.	Study funding will be provided to sites as per the Organisation Information Document	It is expected that a Principal Investigator would be appointed at study sites.	No Honorary Research Contracts, Letters of Access or pre-engagement checks are expected for local staff employed by the participating NHS organisations. Where arrangements are not already in place, research staff not employed by the NHS host organisation undertaking any of the research activities listed in the research application would be expected to obtain an honorary research contract from one NHS organisation (if university employed), followed by Letters of Access for subsequent organisations. This would be on the basis of a Research Passport (if university employed) or an NHS to NHS confirmation of pre-engagement checks letter (if NHS employed). These should confirm enhanced DBS checks, including appropriate barred list checks, and occupational health clearance.

Other information to aid study set-up and delivery

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales in study set-up.
The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.

7.2.4.2 Amendment ethical approval letter

NHS
Health Research Authority

East Midlands - Leicester Central Research Ethics Committee

The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Tel [redacted]

24 August 2020

Mrs Hanadi H A Alrammaal
School of Pharmacy
University of Birmingham
Birmingham, UK
B15 2TT

Dear Mrs Alrammaal

Study title:	**Understanding pharmacokinetic distribution of cefuroxime when given prior to Caesarean section in body fat and blood**
REC reference:	**19/EM/0356**
Amendment number:	**AM 01 244803**
Amendment date:	**04/08/2020**
IRAS project ID:	**244803**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Completed Amendment Tool [Amendment Tool]	1	06 July 2020
Other [IRAS_Form]		04 August 2020
Participant information sheet (PIS) [PIS C-LACE v1.4]	1.4	27 July 2020
Research protocol or project proposal [C-LACE protocol v1.4]	1.4	27 July 2020
Summary CV for Chief Investigator (CI) [CV chief investigator]	1	27 July 2020

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Amendments related to COVID-19

We will update your research summary for the above study on the research summaries section of our website. During this public health emergency, it is vital that everyone can promptly identify all relevant research related to COVID-19 that is taking place globally. If you have not already done so, please register your study on a public registry as soon as possible and provide the HRA with the registration detail, which will be posted alongside other information relating to your project.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

HRA Learning

We are pleased to welcome researchers and research staff to our HRA Learning Events and online learning opportunities– see details at: https://www.hra.nhs.uk/planning-and-improving-research/learning/

IRAS Project ID - 244803:	**Please quote this number on all correspondence**

Yours sincerely

[redacted]

On Behalf Of
Mrs Rita Patel
Chair

E-mail: leicestercentral.rec@hra.nhs.uk

East Midlands - Leicester Central Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 14 August 2020

Committee Members:

Name	*Profession*	*Present*	*Notes*
Mr Richard Hovey	Business Analyst	Yes	
Mrs Rita Patel	Contracts and Innovation Manager - retired	Yes	Chair

Also in attendance:

Name	*Position (or reason for attending)*
Mr Taimoor Hassan	Approval Administrator

7.2.4.3 The Integrated Research Application System approved form

Welcome to the Integrated Research Application System

IRAS Project Filter

The integrated dataset required for your project will be created from the answers you give to the following questions. The system will generate only those questions and sections which (a) apply to your study type and (b) are required by the bodies reviewing your study. Please ensure you answer all the questions before proceeding with your applications.

Please complete the questions in order. If you change the response to a question, please select 'Save' and review all the questions as your change may have affected subsequent questions.

Please enter a short title for this project (maximum 70 characters)
C-LACE: Cefuroxime Levels After Caesarean Section

1. Is your project research?

◉ Yes ○ No

2. Select one category from the list below:

○ Clinical trial of an investigational medicinal product
○ Clinical investigation or other study of a medical device
○ Combined trial of an investigational medicinal product and an investigational medical device
○ Other clinical trial to study a novel intervention or randomised clinical trial to compare interventions in clinical practice
◉ Basic science study involving procedures with human participants
○ Study administering questionnaires/interviews for quantitative analysis, or using mixed quantitative/qualitative methodology
○ Study involving qualitative methods only
○ Study limited to working with human tissue samples (or other human biological samples) and data (specific project only)
○ Study limited to working with data (specific project only)
○ Research tissue bank
○ Research database

If your work does not fit any of these categories, select the option below:

○ Other study

2a. Will the study involve the use of any medical device without a CE Mark, or a CE marked device which has been modified or will be used outside its intended purposes?

○ Yes ◉ No

2b. Please answer the following question(s):

a) Does the study involve the use of any ionising radiation?	○ Yes	◉ No
b) Will you be taking new human tissue samples (or other human biological samples)?	◉ Yes	○ No
c) Will you be using existing human tissue samples (or other human biological samples)?	○ Yes	◉ No

d) Will the study involve any other clinical procedures with participants (e.g. MRI, ultrasound, physical examination)?	○ Yes	◉ No

3. In which countries of the UK will the research sites be located? *(Tick all that apply)*

☑ England
☐ Scotland
☐ Wales
☐ Northern Ireland

3a. In which country of the UK will the lead NHS R&D office be located:

◉ England
○ Scotland
○ Wales
○ Northern Ireland
○ This study does not involve the NHS

4. Which applications do you require?

☑ IRAS Form
☐ Confidentiality Advisory Group (CAG)
☐ Her Majesty's Prison and Probation Service (HMPPS)

Most research projects require review by a REC within the UK Health Departments' Research Ethics Service. Is your study exempt from REC review?

○ Yes ◉ No

5. Will any research sites in this study be NHS organisations?

◉ Yes ○ No

5a. Are all the research costs and infrastructure costs (funding for the support and facilities needed to carry out research e.g. NHS Support costs) for this study provided by a NIHR Biomedical Research Centre, NIHR Collaboration for Leadership in Health Research and Care (CLAHRC), NIHR Patient Safety Translational Research Centre or Medtech and In Vitro Diagnostic Cooperative in all study sites?

Please see information button for further details.

○ Yes ◉ No

Please see information button for further details.

5b. Do you wish to make an application for the study to be considered for NIHR Clinical Research Network (CRN) Support and inclusion in the NIHR Clinical Research Network Portfolio?

Please see information button for further details.

○ Yes ◉ No

The NIHR Clinical Research Network provides researchers with the practical support they need to make clinical studies

DRAFT DRAFT

happen in the NHS e.g. by providing access to the people and facilities needed to carry out research "on the ground".

If you select yes to this question, you must complete a NIHR Clinical Research Network (CRN) Portfolio Application Form (PAF) immediately after completing this project filter question and before submitting other applications. Failing to complete the PAF ahead of other applications e.g. HRA Approval, may mean that you will be unable to access NIHR CRN Support for your study.

6. Do you plan to include any participants who are children?

○ Yes ◉ No

7. Do you plan at any stage of the project to undertake intrusive research involving adults lacking capacity to consent for themselves?

○ Yes ◉ No

Answer Yes if you plan to recruit living participants aged 16 or over who lack capacity, or to retain them in the study following loss of capacity. Intrusive research means any research with the living requiring consent in law. This includes use of identifiable tissue samples or personal information, except where application is being made to the Confidentiality Advisory Group to set aside the common law duty of confidentiality in England and Wales. Please consult the guidance notes for further information on the legal frameworks for research involving adults lacking capacity in the UK.

8. Do you plan to include any participants who are prisoners or young offenders in the custody of HM Prison Service or who are offenders supervised by the probation service in England or Wales?

○ Yes ◉ No

9. Is the study or any part of it being undertaken as an educational project?

◉ Yes ○ No

Please describe briefly the involvement of the student(s):
This project is undertaken as part of a PhD research, the researcher is the PhD student

9a. Is the project being undertaken in part fulfilment of a PhD or other doctorate?

◉ Yes ○ No

10. Will this research be financially supported by the United States Department of Health and Human Services or any of its divisions, agencies or programs?

○ Yes ◉ No

11. Will identifiable patient data be accessed outside the care team without prior consent at any stage of the project (including identification of potential participants)?

○ Yes ◉ No

Integrated Research Application System
Application Form for Basic science study involving procedures with human participants

IRAS Form (project information)

Please refer to the E-Submission and Checklist tabs for instructions on submitting this application.

The Chief Investigator should complete this form. Guidance on the questions is available wherever you see this symbol displayed. We recommend reading the guidance first. The complete guidance and a glossary are available by selecting Help.

Please define any terms or acronyms that might not be familar to lay reviewers of the application.

Short title and version number: (maximum 70 characters - this will be inserted as header on all forms)
C-LACE: Cefuroxime Levels After Caesarean Section

Please complete these details after you have booked the REC application for review.

REC Name:
Leicester Central

REC Reference Number:
19/EM/0356

Submission date:
15/11/2019

PART A: Core study information

1. ADMINISTRATIVE DETAILS

A1. Full title of the research:

Understanding pharmacokinetic distribution of cefuroxime when given prior to Caesarean section in body fat and blood

A2-1. Educational projects

Name and contact details of student(s):

Student 1

	Title	Forename/Initials	Surname
	Mrs	Hanadi H A	Alrammaal
Address	School of Pharmacy University of Birmingham Birmingham, UK		
Post Code	B15 2TT		
E-mail	Hha706@student.bham.ac.uk		
Telephone	07477143102		
Fax			

Give details of the educational course or degree for which this research is being undertaken:
Name and level of course/ degree:

PhD in Pharmacy

Name of educational establishment:
University of Birmingham

Name and contact details of academic supervisor(s):

Academic supervisor 1

	Title Forename/Initials Surname
	Dr Hannah Batchelor
Address	School of Pharmacy
	College of Medical and Dental Sciences
	University of Birmingham, Birmingham, UK
Post Code	B15 2TT
E-mail	[redacted]
Telephone	[redacted]
Fax	

Academic supervisor 2

	Title Forename/Initials Surname
	Dr Victoria Hodgetts Morton
Address	Academic Unit, 3rd Floor
	Birmingham Women's Hospital
Post Code	B15 2TG
E-mail	[redacted]
Telephone	[redacted]
Fax	

Please state which academic supervisor(s) has responsibility for which student(s):
Please click "Save now" before completing this table. This will ensure that all of the student and academic supervisor details are shown correctly.

Student(s)	Academic supervisor(s)
Student 1 Mrs Hanadi H A Alrammaal	☑ Dr Hannah Batchelor ☑ Dr Victoria Hodgetts Morton

A copy of a current CV for the student and the academic supervisor (maximum 2 pages of A4) must be submitted with the application.

A2-2. Who will act as Chief Investigator for this study?

- ○ Student
- ◉ Academic supervisor
- ○ Other

A3-1. Chief Investigator:

	Title Forename/Initials Surname
	Dr Hannah Batchelor
Post	Senior Lecturer in Pharmaceutics, Formulation and Drug Delivery
Qualifications	BSc, PhD
ORCID ID	0000 0002 8729 9951
Employer	University of Birmingham
Work Address	University of Birmingham
	School of Pharmacy
	Birmingham
Post Code	B15 2TT
Work E-mail	[redacted]
* Personal E-mail	
Work Telephone	
* Personal Telephone/Mobile	
Fax	

** This information is optional. It will not be placed in the public domain or disclosed to any other third party without prior consent.*
A copy of a current CV (maximum 2 pages of A4) for the Chief Investigator must be submitted with the application.

A4. Who is the contact on behalf of the sponsor for all correspondence relating to applications for this project?
This contact will receive copies of all correspondence from REC and HRA/R&D reviewers that is sent to the CI.

	Title Forename/Initials Surname
	Dr Birgit Whitman
Address	Head of Research Governance and Integrity
	Room 117, Aston Webb Building
	University of Birmingham
Post Code	B15 2TT
E-mail	researchgovernance@contacts.bham.ac.uk
Telephone	01214158011
Fax	

A5-1. Research reference numbers. *Please give any relevant references for your study:*

Applicant's/organisation's own reference number, e.g. R & D (if available):	RG_18-237
Sponsor's/protocol number:	
Protocol Version:	v0.3
Protocol Date:	30/10/2019
Funder's reference number (enter the reference number or state not applicable):	Not applicable
Project website:	

Registry reference number(s):
The UK Policy Framework for Health and Social Care Research sets out the principle of making information about research publicly available. Furthermore, Article 19 of the World Medical Association Declaration of Helsinki adopted in 2008 states that "every clinical trial must be registered on a publicly accessible database before recruitment of the first subject"; and the International Committee of Medical Journal Editors (ICMJE) will consider a clinical trial for publication only if it has been registered in an appropriate registry. Please see guidance for more information.

DRAFT

International Standard Randomised Controlled Trial Number (ISRCTN):

ClinicalTrials.gov Identifier (NCT number):

Additional reference number(s):

Ref.Number Description	Reference Number

A5-2. Is this application linked to a previous study or another current application?

○ Yes ◉ No

Please give brief details and reference numbers.

2. OVERVIEW OF THE RESEARCH

To provide all the information required by review bodies and research information systems, we ask a number of specific questions. This section invites you to give an overview using language comprehensible to lay reviewers and members of the public. Please read the guidance notes for advice on this section.

A6-1. Summary of the study. *Please provide a brief summary of the research (maximum 300 words) using language easily understood by lay reviewers and members of the public. Where the research is reviewed by a REC within the UK Health Departments' Research Ethics Service, this summary will be published on the Health Research Authority (HRA) website following the ethical review. Please refer to the question specific guidance for this question.*

This study will evaluate the concentration of Cefuroxime in stomach adipose tissue and blood from pregnant women undergoing a scheduled Caesarean section. Analysis will explore the impact of BMI (body mass index) vs dose of cefuroxime on the concentrations of drug determined. The study participants will be followed up between 30 and 40 days post C-section to record details of any post-caesarean infection to explore correlations in BMI:measured cefuroxime concentrations and post caesarean infection rates.

A6-2. Summary of main issues. *Please summarise the main ethical, legal, or management issues arising from your study and say how you have addressed them.*

Not all studies raise significant issues. Some studies may have straightforward ethical or other issues that can be identified and managed routinely. Others may present significant issues requiring further consideration by a REC, HRA, or other review body (as appropriate to the issue). Studies that present a minimal risk to participants may raise complex organisational or legal issues. You should try to consider all the types of issues that the different reviewers may need to consider.

This study was designed to minimise the burden to participants yet maximise access to samples for research.

The main issues for ethical consideration are the collection and analysis of blood samples and adipose tissue samples from the site of incision. Our study setting and population were selected due to the links to routine clinical practice. During C-section the abdomen is open therefore there is direct access to harvest samples from adipose tissue. A second venflon cannula will be used to withdraw the three blood samples required for this study to minimise the burden of needles to the participant and also disruption during the procedure. Where possible we have tried to minimise the disruption to the participant during their clinical procedure.

We want to follow-up with women at between 30-40 days post C-section and have tried to minimise the length of interview. We have also asked for access to medical notes in cases where we cannot contact the women.

The anonymisation of samples minimises the risk of identification of participants and the close working relationship between the University and Hospital provides a background of collaborative research.

3. PURPOSE AND DESIGN OF THE RESEARCH

A7. Select the appropriate methodology description for this research. *Please tick all that apply:*

- ☐ Case series/ case note review
- ☐ Case control
- ☑ Cohort observation
- ☐ Controlled trial without randomisation
- ☐ Cross-sectional study
- ☐ Database analysis
- ☐ Epidemiology
- ☐ Feasibility/ pilot study
- ☑ Laboratory study
- ☐ Metanalysis
- ☐ Qualitative research
- ☐ Questionnaire, interview or observation study
- ☐ Randomised controlled trial
- ☐ Other (please specify)

A10. What is the principal research question/objective? *Please put this in language comprehensible to a lay person.*

This study will evaluate whether the concentrations of cefuroxime (the drug used as a prophylactic antibiotic) reach the same levels in blood and at the site of incision during a C-section when the patient is obese compared to a non-obese patient.

A11. What are the secondary research questions/objectives if applicable? *Please put this in language comprehensible to a lay person.*

To see if the concentration of cefuroxime reached in blood and tissue are linked to the rate of post surgical infection following a C-section.

A12. What is the scientific justification for the research? *Please put this in language comprehensible to a lay person.*

It was estimated in the 2000s that the prevalence of obesity in pregnant women in England was 16-20% [1, 2]. Being obese and pregnant may have an effect on the mother and foetus. One complication of obesity in pregnancy relates to the requirement for operative delivery, i.e. Caesarean section (CS).
The rate of CS among a pregnant, obese population is 33.8% [3]. The rate of post-CS infection is higher among obese pregnant women compared to those who are not obese. Wloch et al found that the adjusted odds ratio of surgical site infection in the obese population was 2.41 (95% CI 1.73-3.37) [4]. There are various ways to decrease and/or prevent post-CS infections. Single dose pre-CS intravenous (IV) antibiotic is recommended and several studies have examined the use of different antibiotics in CS, with cefazolin in the US and cefuroxime in the UK being the most commonly used drugs. Numerous studies have focussed on the use of cefazolin in obese patients to gain an understanding of optimal dosing and clinical outcomes [5]. Several studies proposed an increased dose of cefazolin (3 g) compared to the usual dose (2g) for obese pregnant women to achieve sufficient antibiotic levels [6-8]. It was postulated by Holt et al. (in 1994) that doses of cefuroxime up to 1500mg may not achieve sufficient MIC for most of the common bacteria in the maternal blood during CS [9]. Sub-therapeutic antibiotic concentration is a crucial factor in global bacterial resistance. It is essential that the therapeutic concentration of cefuroxime is achieved in both maternal plasma and at the site of incision during CS for effective therapy. This study will take plasma and adipose tissue samples to accurately measure levels achieved. Due to the great variability associated with pharmacokinetic measurements the use of physiologically based pharmacokinetic (PBPK) modelling is a common tool to understand the distribution of drugs. Specialist software packages are available that include details on pregnant populations. In this study we will use SimCYP software to model the plasma and adipose concentrations of cefuroxime and we will compare this modelled data to the experimental results. It is anticipated that this clinical data coupled with PBPK models will inform any future changes to doses of cefuroxime used for CS, particularly in relation to obese women.
To the best of our knowledge, no study to date has measured cefuroxime concentrations in plasma and adipose tissue as a function of body mass index (BMI) in pregnant women who have had CS. Thus, a study to explore the drug concentration in this population at the time of CS is essential.

DRAFT

A13. Please summarise your design and methodology. *It should be clear exactly what will happen to the research participant, how many times and in what order. Please complete this section in language comprehensible to the lay person. Do not simply reproduce or refer to the protocol. Further guidance is available in the guidance notes.*

Study Procedures
Upon signing consent for the study each participant will be allocated a study ID number to manage anonymity in data analysis and interpretation. A spreadsheet that links study ID to patient ID will be held at Birmingham Women's and Children's Hospital NHS Foundation Trust under the control of the local principal investigator. This information will be password protected and stored on an encrypted computer that conforms to the standard NHS security requirements. Background information will be collected from each participant using the Baseline Form. This information is required for interpretation of the data and dissemination.
Following collection of the data from all forms (Screening, Consent, Baseline, Eligibility Reassessment, Operation, Discharge, follow up interview, 6 week Record Check) all data will be entered on a shared secure system (Bear Share: https://intranet.birmingham.ac.uk/it/teams/infrastructure/researc h/bear/BEARDataShare/BEARDataShare.aspx) which is provided by the University of Birmingham; this system can be accessed at the University and the Hospital and ensures security of data, any data entered will be anonymised.
The study has two parts: part A, which is blood and adipose tissue sampling for cefuroxime concentrations, part B, is a follow up of patients postpartum at between 30-40 days to record the incidence of post-surgical infection.
Part A: Sample collection at C-Section
The duration of the participant participation in part A will last as long as the CS procedure lasts plus an additional up to two minutes to allow for collection of adipose tissue. No additional visits for the collection of the human blood or tissue are required. However, a follow up interview will be conducted at 30-40 days postpartum (see 6.2.Part B: Follow up for post-surgical infection)
The clinical care team will use the following forms: Operation Form and Discharge Form to record the time of cefuroxime administration, dose administered, type of anaesthesia used, blood loss and other information (see documents/appendixes). Five samples will be collected: 1 maternal blood sample at time of skin incision, 1 blood sample at time of skin closure, 1 blood sample in the recovery room, 1 adipose tissue sample (approximately 1 cm from the skin in the middle of the Pfannesnstiel or vertical midline incision) at skin incision and one adipose tissue sample just prior to skin closure.

Part B: Follow up for post-surgical infection
At between 30-40 days post CS, the study participants will be contacted by telephone by a research nurse/midwife from the research team and a review will be made to record any type of post surgical infection. The Follow-up Interview Form will be used to record the details of any infection reported. If the participant attended to the hospital for a postnatal appointment at 30-40 days, the interview will be conducted at that time to minimise the burden to the participant.
The participant will be contacted by the researcher/research nurse/midwife and reminded of the study. In addition to the signed informed consent, a verbal consent to participate in the follow-up aspect will be obtained and recorded on the follow up sheet. Additionally, patient medical notes will be reviewed by a member of the clinical care team using the 30-40 days Outcome Form to identify whether there has been a reported post-surgical infection. Post-surgical infection is defined as per the definitions set out by the US Centre's for Disease Control and Prevention (Centre's for Disease Control and Prevention 2019) [12].

A14-1. In which aspects of the research process have you actively involved, or will you involve, patients, service users, and/or their carers, or members of the public?

- [x] Design of the research
- [ ] Management of the research
- [ ] Undertaking the research
- [ ] Analysis of results
- [ ] Dissemination of findings
- [ ] None of the above

Give details of involvement, or if none please justify the absence of involvement.
Informal discussions about this study with women who have had CS have all been positive as the burden of participation is minimised where possible.
A similar study conducted by Victoria Hodgetts Morton and Katie Morris (PREPS trial) involved the public and patients; those involved were supportive of any research undertaken to minimise the risks of post-surgical infections following CS [10].

4. RISKS AND ETHICAL ISSUES

RESEARCH PARTICIPANTS

A15. What is the sample group or cohort to be studied in this research?

Select all that apply:

- [ ] Blood
- [ ] Cancer
- [ ] Cardiovascular
- [ ] Congenital Disorders
- [ ] Dementias and Neurodegenerative Diseases
- [ ] Diabetes
- [ ] Ear
- [ ] Eye
- [ ] Generic Health Relevance
- [ ] Infection
- [ ] Inflammatory and Immune System
- [ ] Injuries and Accidents
- [ ] Mental Health
- [ ] Metabolic and Endocrine
- [ ] Musculoskeletal
- [ ] Neurological
- [ ] Oral and Gastrointestinal
- [ ] Paediatrics
- [ ] Renal and Urogenital
- [x] Reproductive Health and Childbirth
- [ ] Respiratory
- [ ] Skin
- [ ] Stroke

Gender: Female participants only
Lower age limit: 18 Years
Upper age limit: 50 Years

A17-1. Please list the principal inclusion criteria (list the most important, max 5000 characters).

• Pregnant women 18 years to 50 years old, with a singleton pregnancy undergoing elective Caesarean Section at 37 weeks or over (Category 4 CS); with no known allergy to cefuroxime, cephalosporin or penicillin.
• Participant is able to give consent and agree to sample storage.
• Participant agrees to be contacted for follow-up
• Participant contributing to the study needs to be able to read and/or understand English.

A17-2. Please list the principal exclusion criteria (list the most important, max 5000 characters).

• Multiple pregnancy

DRAFT

• Previous Caesarean Section that resulted in a surgical site infection
• Emergency Caesarean Section (Caesarean categories 1 & 2)
• Any non-elective Caesarean Section including those requiring early delivery without threat to maternal or foetal health that were not previously planned (Category 3 CS)
• Body Mass Index (BMI) less than18 kg/m2 or greater than or equal to 45kg/m2 (At time of first pregnancy appointment or at time of delivery).
• Participant contributing to the study is unable to read and/or understand English.
• Currently enrolled in an RCT for an intervention to reduce post-operative surgical site infection
• Diabetes (type 1, Type 2 or gestation)
• Hypertension
• Renal disease
• Cardiovascular disease (eg. Maternal structural cardiac disease)
• Liver disease
• Inflammatory bowel disease (eg Crohn's disease or ulcerative colitis)
• Cephalosporin or penicillin allergy
• Administration of antibiotic within 1 week prior to delivery
• Suspected pre-existing infection (including Chorioamnionitis)
• Autoimmune disease (eg SLE, Rheumatoid arthritis)
• Chronic use of corticosteroid
• History of wound breakdown in an abdominal surgery
• Prior laparotomy for any indication (eg. Previous ovarian cystectomy or previous bowel surgery)

RESEARCH PROCEDURES, RISKS AND BENEFITS

A18. Give details of all non-clinical intervention(s) or procedure(s) that will be received by participants as part of the research protocol. *These include seeking consent, interviews, non-clinical observations and use of questionnaires.*

Please complete the columns for each intervention/procedure as follows:

1. Total number of interventions/procedures to be received by each participant as part of the research protocol.
2. If this intervention/procedure would be routinely given to participants as part of their care outside the research, how many of the total would be routine?
3. Average time taken per intervention/procedure (minutes, hours or days)
4. Details of who will conduct the intervention/procedure, and where it will take place.

Intervention or procedure	1	2	3	4
Provide background information to study participant	1	0	10 minutes	Research team (CI or nominated research midwife)via distribution of patient information sheet at previous appointment and within 24 hours prior to the procedure
Review inclusion/exclusion criteria and obtain consent	1	0	10 minutes	Research team (CI or nominated research midwife) within 24 hours prior to the procedure
Follow-up telephone call to participant (or participant can attend the Hospital for the follow up interview)	1		10 minutes	Research team (CI or nominated research midwife) by telephone 30-40 days post C-Section

A19. Give details of any clinical intervention(s) or procedure(s) to be received by participants as part of the research protocol. *These include uses of medicinal products or devices, other medical treatments or assessments, mental health interventions, imaging investigations and taking samples of human biological material. Include procedures which might be received as routine clinical care outside of the research.*

Please complete the columns for each intervention/procedure as follows:

1. Total number of interventions/procedures to be received by each participant as part of the research protocol.
2. If this intervention/procedure would be routinely given to participants as part of their care outside the research, how many of the total would be routine?
3. Average time taken per intervention/procedure (minutes, hours or days).
4. Details of who will conduct the intervention/procedure, and where it will take place.

Intervention or procedure	1	2	3	4

Insert cannula to take blood samples	1	0	10 minutes	Clinical member of the research team, likely to be the anaesthetist
Sample collection adipose tissue at skin incision	1	0	1 minute	Obstetrician in the delivery room
Sample collection adipose tissue at skin closure	1	0	1 minute	Obstetrician in the delivery room
Sample collection Maternal blood at skin incision	1	0	5 minutes	Clinical member of the research team trained in phlebotomy in the delivery room
Sample collection blood at skin closure	1	0	5 minutes	Clinical member of the research team trained in phlebotomy in the delivery room
Sample collection blood at recovery room	1	0	5 minutes	Clinical member of the research team trained in phlebotomy in the recovery room

A21. How long do you expect each participant to be in the study in total?

The duration of the study will be up to 40 days; with the first part taking place within 24 hours prior to the C-section, the C-section and the follow up being at between 30-40 days post the C-section.

A22. What are the potential risks and burdens for research participants and how will you minimise them?

For all studies, describe any potential adverse effects, pain, discomfort, distress, intrusion, inconvenience or changes to lifestyle. Only describe risks or burdens that could occur as a result of participation in the research. Say what steps would be taken to minimise risks and burdens as far as possible.

There are no direct health benefits to participants. However, the findings from this study will help us determine the appropriate antibiotic dosing strategy used in future for patients with a high BMI that need a C-section.

We do not believe that there are any major disadvantages or risks to taking part in this study. The only additional procedures that the participants will receive are (i) insertion of a dedicated venflon cannula for the taking of additional blood samples and (ii) the removal of two samples of fat. The blood will be taken by a trained healthcare professional using standard methods to take blood; the risks associated with taking blood samples are minimal. The samples of fat will be taken whilst the skin is open for the C-section; the participant will be anaesthetized so will not feel any pain from this process. The amount of fat taken is small (about the size of a 50p coin) and this will not lead to visible differences to the participant's body following the C-section.

The use of small samples was selected to minimise the risks to participants.

A24. What is the potential for benefit to research participants?

There are no direct health benefits to participants. However, the findings from this study will help us determine the appropriate antibiotic dosing strategy used in future for patients with a high BMI that need a C-section to ensure optimal antibiotic concentration and decrease bacterial resistance.

RECRUITMENT AND INFORMED CONSENT

In this section we ask you to describe the recruitment procedures for the study. Please give separate details for different study groups where appropriate.

A27-1. How will potential participants, records or samples be identified? Who will carry this out and what resources will be used?*For example, identification may involve a disease register, computerised search of GP records, or review of medical records. Indicate whether this will be done by the direct healthcare team or by researchers acting under arrangements with the responsible care organisation(s).*

Any patient (between 18 -50 years) who requires a CS as part of their clinical care will be screened for inclusion. The potential participants will be identified using hospital notes and future clinic lists, this will be undertaken by the clinical care team within Birmingham Women's and Children's Hospital NHS Foundation Trust who will have access to the

DRAFT

clinical notes.
Pregnant women have appointments at Birmingham Women's Hospital during pregnancy and at any of these meetings they will be informed of the study and provided with a participant information sheet (Participant Information Sheet) if they are booked for CS. The participants will receive information about the study at least 24 hours prior to CS.

A27-2. Will the identification of potential participants involve reviewing or screening the identifiable personal information of patients, service users or any other person?

(•) Yes ( ) No

Please give details below:
Any patient (between 18 -50 years) who requires a CS as part of their clinical care will be screened for inclusion. The potential participants will be identified using hospital notes and future clinic lists, this will be undertaken by the clinical care team within Birmingham Women's and Children's Hospital NHS Foundation Trust who will have access to the clinical notes.

A27-3. Describe what measures will be taken to ensure there is no breach of any duty of confidentiality owed to patients, service users or any other person in the process of identifying potential participants. *Indicate what steps have been or will be taken to inform patients and service users of the potential use of their records for this purpose. Describe the arrangements to ensure that the wishes of patients and service users regarding access to their records are respected. Please consult the guidance notes on this topic.*

Identification of the potential participants will be undertaken using patient notes and clinic lists as is standard practice in research. This will be conducted by the clinical care teams and as a result there are no additional measures required to ensure that there is no breach of confidentiality

A27-4. Will researchers or individuals other than the direct care team have access to identifiable personal information of any potential participants?

( ) Yes (•) No

A28. Will any participants be recruited by publicity through posters, leaflets, adverts or websites?

( ) Yes (•) No

A29. How and by whom will potential participants first be approached?

At least 24 before CS, the participant will be greeted by a GCP trained research nurse/midwife and asked whether they received the participant information sheet (Participant Information Sheet). They will then be asked if they have any questions about the information provided and whether they are interested in participating in the study. The study will be briefly verbally described to the potential participant. Again they will be provided with an opportunity to ask any questions. Research midwives/clinician will review and reassess and confirm eligibility of patients to participate in the study at the day of CS (Eligibility Reassessment Form).

A30-1. Will you obtain informed consent from or on behalf of research participants?

(•) Yes ( ) No

If you will be obtaining consent from adult participants, please give details of who will take consent and how it will be done, with details of any steps to provide information (a written information sheet, videos, or interactive material). Arrangements for adults unable to consent for themselves should be described separately in Part B Section 6, and for children in Part B Section 7.

If you plan to seek informed consent from vulnerable groups, say how you will ensure that consent is voluntary and fully informed.

A suitably qualified research midwife or doctor will introduce the study to the woman at their pre- operative assessment. A Participant Information Sheet (PIS) will be provided to facilitate this process. Investigators will ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial

to the woman. They will also stress that participation is voluntary and that the woman is free to refuse to take part and may withdraw from the trial at any time. The woman will be given adequate time to read the PIS and to discuss their potential participation and be given the opportunity to ask questions. If the woman expresses an interest in participating in the trial, they will be asked to sign and date the latest version of the Informed Consent Form at least 24 hours prior to CS. The woman will be asked for her consent to withdraw 3 blood samples and 2 fat tissue and for collection of outcome data from their medical records and a telephone interview at 30-40 days postnatal that will take approximately 5-15 minutes. The woman will be asked to give explicit consent for the regulatory authorities, members of the research team and or representatives of the sponsor to be given direct access to their medical records. It will be the responsibility of the investigator to obtain written informed consent for each participant prior to performing any trial related procedure. Research midwives and doctors can both take informed consent for the trial as long as they are GCP trained and this responsibility has been delegated to them by the Principal Investigator (PI) as captured on the Site Delegation Log. The consent form will be provided in triplicate (as a carbon copy version) to facilitate the process on a busy labour ward. Each box on the consent form should be initialled by the woman and the form signed by the woman and the delegated research staff. A copy of the consent form will be given to the woman, a copy filed in the medical records with the PIS and the original signed form will be will be retained at the study site (Birmingham Women's Hospital). Once the woman is entered into the trial, the woman's trial number will be entered on the ICF maintained in the Investigator Site file. Details of the informed consent discussions will be recorded in the woman's medical notes. This will include date of consent, the name of the trial, summary of discussion, version number of the PIS, the Study ID given to the participant and version number of the ICF. Where consent is obtained on the same day that the trial related assessments are due to start, a note should be made in the medical notes as to what time the consent was obtained and what time the procedures started. At the telephone interview the woman's willingness to continue in the trial will be ascertained and documented (C-LACE Follow Up Interview). Throughout the trial the woman will have the opportunity to ask questions about the trial. At the time of taking consent the woman will provide contact details to facilitate the telephone interview using Contact details form which will be managed by the research midwife.
Upon signing consent the woman will be allocated a participant study ID number; this will be a random sequence of 5 numbers. Each participant will be allocated a pack of study documents in an envelope labelled with the participant study ID number; all documents within the envelope will have the participant study ID number pre-filled. Blank copies of all paperwork will also be available to the research team should additional copies be required for any participant.

If you are not obtaining consent, please explain why not.

Please enclose a copy of the information sheet(s) and consent form(s).

A30-2. Will you record informed consent (or advice from consultees) in writing?

(•) Yes ( ) No

A31. How long will you allow potential participants to decide whether or not to take part?

Pregnant women have appointments at Birmingham Women's Hospital during pregnancy and at any of these meetings they will be informed of the study and provided with a participant information sheet (Participant Information Sheet) if they are booked for CS. The participants will receive information about the study at least 24 hours prior to CS.

A32. Will you recruit any participants who are involved in current research or have recently been involved in any research prior to recruitment?

( ) Yes
( ) No
(•) Not Known

If Yes, please give details and justify their inclusion. If Not Known, what steps will you take to find out?
We will exclude any participants who are currently enrolled in an RCT for an intervention to reduce post-operative surgical site infection

However we will not check about there engagement in other clinical research as it is not relevant to this study.

A33-1. What arrangements have been made for persons who might not adequately understand verbal explanations or

written information given in English, or who have special communication needs? *(e.g. translation, use of interpreters)*

One of the inclusion criteria for the study is, "Participant contributing to the study needs to be able to read and/or understand English".
As there are no direct benefits to participants we have excluded those who cannot understand the study documentation as the study team are unable to be confident that they consent to the study.

A35. What steps would you take if a participant, who has given informed consent, loses capacity to consent during the study? *Tick one option only.*

- ○ The participant and all identifiable data or tissue collected would be withdrawn from the study. Data or tissue which is not identifiable to the research team may be retained.
- ○ The participant would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.
- ○ The participant would continue to be included in the study.
- ○ Not applicable – informed consent will not be sought from any participants in this research.
- ◉ Not applicable – it is not practicable for the research team to monitor capacity and continued capacity will be assumed.

Further details:

CONFIDENTIALITY

In this section, personal data means any data relating to a participant who could potentially be identified. It includes pseudonymised data capable of being linked to a participant through a unique code number.

Storage and use of personal data during the study

A36. Will you be undertaking any of the following activities at any stage (including in the identification of potential participants)? *(Tick as appropriate)*

- ☐ Access to medical records by those outside the direct healthcare team
- ☐ Access to social care records by those outside the direct social care team
- ☐ Electronic transfer by magnetic or optical media, email or computer networks
- ☐ Sharing of personal data with other organisations
- ☐ Export of personal data outside the EEA
- ☑ Use of personal addresses, postcodes, faxes, emails or telephone numbers
- ☐ Publication of direct quotations from respondents
- ☐ Publication of data that might allow identification of individuals
- ☐ Use of audio/visual recording devices
- ☑ Storage of personal data on any of the following:
  - ☑ Manual files (includes paper or film)
  - ☑ NHS computers
  - ☐ Social Care Service computers
  - ☐ Home or other personal computers
  - ☐ University computers
  - ☐ Private company computers
  - ☐ Laptop computers

Further details:
Personal data generated within this study will be retained at the Hospital and will not be transferred to the University of Birmingham
All personal data will be stored using standard NHS mechanisms to ensure that this is secure.

A37. Please describe the physical security arrangements for storage of personal data during the study?

The personal identifiable data collected will include names on consent forms and contact details for the follow up telephone interview. This information will be stored at the local site (Birmingham Women's and Children's Hospital NHS Foundation Trust) and not transferred to the University of Birmingham. Paper transcripts of the background information will be securely stored at Birmingham Women's Hospital prior to transcription into the shared secure system (Bear Share). All information transferred on to the designated study encrypted computer will have restricted password protected access to only those directly involved in the research.
All participants will be given a study ID upon recruitment to the study. A spreadsheet that links study ID to patient ID will be held at Birmingham Women's and Children's Hospital NHS Foundation Trust under the control of the local PI. This information will be password protected and stored on an encrypted computer that conforms to the standard NHS security requirements.
The study ID will be used on all forms; this information provides anonymised data to the University of Birmingham that no longer has any personal identifiable data. Following collection the data from all forms will be entered on a secure system at the NHS site (undertaken by the research midwife); this system can be accessed at the University and ensures anonymity for the participants. (Bear Share: https://intranet.birmingham.ac.uk/it/teams/infrastructure/research/bear/BEARDataShare/BEARDataShare.aspx).

A38. How will you ensure the confidentiality of personal data? *Please provide a general statement of the policy and procedures for ensuring confidentiality, e.g. anonymisation or pseudonymisation of data.*

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant study ID number on all study documents and any electronic database, that are shared with the University of Birmingham.
All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

The study ID will be used on all forms so that this information provides anonymised data to the research team at Birmingham. Those processing the data and responsible for the dissemination will only ever receive anonymised data.

A40. Who will have access to participants' personal data during the study? *Where access is by individuals outside the direct care team, please justify and say whether consent will be sought.*

Direct access to data will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations. This is clearly stated on the consent form. The clinical care team will review clinic notes to identify potential participants and a research nurse/midwife will complete the background information required to accompany each sample that is obtained. This is standard practice in research studies.

Storage and use of data after the end of the study

A41. Where will the data generated by the study be analysed and by whom?

The data will be analysed by Hanadi Alrammaal under the supervision of Dr Hannah Batchelor at the University of Birmingham. Analysis of the anonymised data will be undertaken in a secure office.

A42. Who will have control of and act as the custodian for the data generated by the study?

	Title Forename/Initials Surname
	Dr Hannah Batchelor
Post	Senior Lecturer in Pharmaceutics
Qualifications	BSc PhD
Work Address	School of Pharmacy
	College of Medical and Dental Sciences
	University of Birmingham, Birmingham, UK
Post Code	B15 2TT
Work Email	
Work Telephone	
Fax	

A43. How long will personal data be stored or accessed after the study has ended?

- ○ Less than 3 months
- ○ 3 – 6 months
- ○ 6 – 12 months
- ○ 12 months – 3 years
- ◉ Over 3 years

If longer than 12 months, please justify:
According to the University of Birmingham's Code of Practice all research data is stored for 10 years and at Birmingham Women's Hospital all research data is stored for 25 years according to their local policy.

A44. For how long will you store research data generated by the study?

Years: 10
Months:

A45. Please give details of the long term arrangements for storage of research data after the study has ended. *Say where data will be stored, who will have access and the arrangements to ensure security.*

The data will be securely stored within the office of Dr Hannah Batchelor in a locked cabinet to prevent any unauthorised access.
Access to the consent forms will be restricted to the immediate research team at Birmingham Women's Hospital.
Study data will be stored according to local policy at Birmingham Women's Hospital to ensure that this is securely archived.

INCENTIVES AND PAYMENTS

A46. Will research participants receive any payments, reimbursement of expenses or any other benefits or incentives for taking part in this research?

- ○ Yes
- ◉ No

A47. Will individual researchers receive any personal payment over and above normal salary, or any other benefits or incentives, for taking part in this research?

- ○ Yes
- ◉ No

A48. Does the Chief Investigator or any other investigator/collaborator have any direct personal involvement (e.g. financial, share holding, personal relationship etc.) in the organisations sponsoring or funding the research that may give rise to a possible conflict of interest?

- ○ Yes
- ◉ No

NOTIFICATION OF OTHER PROFESSIONALS

A49-1. Will you inform the participants' General Practitioners (and/or any other health or care professional responsible for their care) that they are taking part in the study?

- ○ Yes
- ◉ No

If Yes, please enclose a copy of the information sheet/letter for the GP/health professional with a version number and date.

PUBLICATION AND DISSEMINATION

A50. Will the research be registered on a public database?

The UK Policy Framework for Health and Social Care Research sets out the principle of making information about research publicly available. Furthermore, Article 19 of the World Medical Association Declaration of Helsinki adopted in 2008 states that "every clinical trial must be registered on a publicly accessible database before recruitment of the first subject"; and the International Committee of Medical Journal Editors (ICMJE) will consider a clinical trial for publication only if it has been registered in an appropriate registry. Please see guidance for more information.

- ◉ Yes
- ○ No

Please give details, or justify if not registering the research.
This study will be registered on clinicaltrials.gov

Please ensure that you have entered registry reference number(s) in question A5-1.

A51. How do you intend to report and disseminate the results of the study? *Tick as appropriate:*

- ☑ Peer reviewed scientific journals
- ☐ Internal report
- ☑ Conference presentation
- ☑ Publication on website
- ☐ Other publication
- ☐ Submission to regulatory authorities
- ☐ Access to raw data and right to publish freely by all investigators in study or by Independent Steering Committee on behalf of all investigators
- ☐ No plans to report or disseminate the results
- ☐ Other (please specify)

A52. If you will be using identifiable personal data, how will you ensure that anonymity will be maintained when publishing the results?

The study outcomes will primarily be of interest to the scientific community and clinicians and the findings from the study will also be reported in a non-identifiable format via peer-reviewed journals and disseminated at scientific conferences. A poster will be made available to the public for the participants to be informed in case they are interested to see the results of the study via the following website www.hannahbatchelor.com.

DRAFT

A53. Will you inform participants of the results?

(●) Yes ( ) No

Please give details of how you will inform participants or justify if not doing so.
Findings of the study will be distributed via the following website www.hannahbatchelor.com.
This information is detailed on the patient information sheets for this study.

5. Scientific and Statistical Review

A54. How has the scientific quality of the research been assessed? *Tick as appropriate:*

- [ ] Independent external review
- [ ] Review within a company
- [ ] Review within a multi-centre research group
- [x] Review within the Chief Investigator's institution or host organisation
- [x] Review within the research team
- [x] Review by educational supervisor
- [ ] Other

Justify and describe the review process and outcome. If the review has been undertaken but not seen by the researcher, give details of the body which has undertaken the review:
This has been reviewed by the research team and the research governance team at the University of Birmingham

For all studies except non-doctoral student research, please enclose a copy of any available scientific critique reports, together with any related correspondence.

For non-doctoral student research, please enclose a copy of the assessment from your educational supervisor/ institution.

A56. How have the statistical aspects of the research been reviewed? *Tick as appropriate:*

- [ ] Review by independent statistician commissioned by funder or sponsor
- [ ] Other review by independent statistician
- [ ] Review by company statistician
- [ ] Review by a statistician within the Chief Investigator's institution
- [x] Review by a statistician within the research team or multi-centre group
- [x] Review by educational supervisor
- [ ] Other review by individual with relevant statistical expertise
- [x] No review necessary as only frequencies and associations will be assessed – details of statistical input not required

In all cases please give details below of the individual responsible for reviewing the statistical aspects. If advice has been provided in confidence, give details of the department and institution concerned.

	Title	Forename/Initials	Surname
	Dr	Sayeed	Haque
Department	Institute of Applied Health Research		
Institution	University of Birmingham		
Work Address	University of Birmingham		
	College of Medical and Dental Sciences		
	Birmingham, UK		
Post Code	B15 2TT		
Telephone	[redacted]		
Fax			
Mobile			
E-mail	[redacted]		

Please enclose a copy of any available comments or reports from a statistician.

A57. What is the primary outcome measure for the study?

Comparison of cefuroxime concentration in blood and adipose tissue from obese and non-obese patients

A58. What are the secondary outcome measures? *(if any)*

Incidence of SSI recorded and comparison of values in obese and non-obese patients

A59. What is the sample size for the research? *How many participants/samples/data records do you plan to study in total? If there is more than one group, please give further details below.*

Total UK sample size: 30
Total international sample size (including UK): 30
Total in European Economic Area: 30

Further details:
There is no predefined sample size yet sufficient numbers are required to provide an estimate of average values as well as the level of variability. Previous work to measure the concentration of antibiotics in plasma and in tissue in obese and non-obese pregnant women following CS has used sample sizes of 14 in each arm to detect differences in adipose antibiotic concentration [2, 3]. Therefore in this study we plan to recruit 15 women in each arm (BMI <30 and BMI >30).

A60. How was the sample size decided upon? *If a formal sample size calculation was used, indicate how this was done, giving sufficient information to justify and reproduce the calculation.*

There is no predefined sample size yet sufficient numbers are required to provide an estimate of average values as well as the level of variability. Previous work to measure the concentration of antibiotics in plasma and in tissue in obese and non-obese pregnant women following CS has used sample sizes of 14 in each arm to detect differences in adipose antibiotic concentration [2, 3]. Therefore in this study we plan to recruit 15 women in each arm (BMI <30 and BMI >30).

A61. Will participants be allocated to groups at random?

( ) Yes (●) No

A62. Please describe the methods of analysis (statistical or other appropriate methods, e.g. for qualitative research) by which the data will be evaluated to meet the study objectives.

The differences in mean concentration values in plasma and adipose tissue between the obese and non-obese populations will be compared using ANOVA analysis to determine whether there are significant differences. SPSS software will be used for all statistical analysis.

6. MANAGEMENT OF THE RESEARCH

A63. Other key investigators/collaborators. *Please include all grant co-applicants, protocol co-authors and other key*

members of the Chief Investigator's team, including non-doctoral student researchers.

	Title	Forename/Initials	Surname
	Dr	Katie	Morris
Post	Reader in Maternal Fetal Medicine		
Qualifications	MBChB, PhD, MRCOG		
Employer	University of Birmingham		
Work Address	Institute of Applied Health Research College of Medical and Dental Sciences University of Birmingham, Birmingham, UK		
Post Code	B15 2TT		
Telephone			
Fax			
Mobile			
Work Email			

A64. Details of research sponsor(s)

A64-1. Sponsor

Lead Sponsor

Status:
- ○ NHS or HSC care organisation
- ◉ Academic
- ○ Pharmaceutical industry
- ○ Medical device industry
- ○ Local Authority
- ○ Other social care provider (including voluntary sector or private organisation)
- ○ Other

Commercial status: Non-Commercial

If Other, please specify:

Contact person

Name of organisation	University of Birmingham
Given name	Birgit
Family name	Whitman
Address	University of Birmingham
Town/city	Birmingham
Post code	B15 2TT
Country	UNITED KINGDOM
Telephone	
Fax	
E-mail	researchgovernance@contacts.bham.ac.uk

A65. Has external funding for the research been secured?

Please tick at least one check box.

- ☐ Funding secured from one or more funders
- ☐ External funding application to one or more funders in progress
- ☑ No application for external funding will be made

What type of research project is this?

- ○ Standalone project
- ○ Project that is part of a programme grant
- ○ Project that is part of a Centre grant
- ◉ Project that is part of a fellowship/ personal award/ research training award
- ○ Other

Other – please state:

A66. Has responsibility for any specific research activities or procedures been delegated to a subcontractor (other than a co-sponsor listed in A64-1) ? *Please give details of subcontractors if applicable.*

○ Yes ◉ No

A67. Has this or a similar application been previously rejected by a Research Ethics Committee in the UK or another country?

○ Yes ◉ No

Please provide a copy of the unfavourable opinion letter(s). You should explain in your answer to question A5-2 how the reasons for the unfavourable opinion have been addressed in this application.

A68-1. Give details of the lead NHS R&D contact for this research:

	Title	Forename/Initials	Surname
		Elizabeth	Adey
Organisation	Birmingham Women's and Children's NHS Foundation Trust		
Address	R&D Office Mindelsohn Way		
Post Code	B15 2TG		
Work Email	bwc.research@nhs.net		
Telephone			
Fax			
Mobile			

Details can be obtained from the NHS R&D Forum website: http://www.rdforum.nhs.uk

A69-1. How long do you expect the study to last in the UK?

DRAFT DRAFT

Planned start date: 06/01/2020
Planned end date: 30/04/2021
Total duration:
Years: 1 Months: 3 Days: 25

A70. Definition of the end of trial, and justification in the case where it is not the last visit of the last subject undergoing the trial (1)

The end of study is the date at which the follow-up interview is conducted at 30-40 days following CS. This is where the follow-up ends for all participants.

A71-1. Is this study?

- (•) Single centre
- ( ) Multicentre

A71-2. Where will the research take place? *(Tick as appropriate)*

- [x] England
- [ ] Scotland
- [ ] Wales
- [ ] Northern Ireland
- [ ] Other countries in European Economic Area

Total UK sites in study 1

Does this trial involve countries outside the EU?
( ) Yes ( ) No

A72. Which organisations in the UK will host the research? *Please indicate the type of organisation by ticking the box and give approximate numbers if known:*

- [x] NHS organisations in England 1
- [ ] NHS organisations in Wales
- [ ] NHS organisations in Scotland
- [ ] HSC organisations in Northern Ireland
- [ ] GP practices in England
- [ ] GP practices in Wales
- [ ] GP practices in Scotland
- [ ] GP practices in Northern Ireland
- [ ] Joint health and social care agencies (eg community mental health teams)
- [ ] Local authorities
- [ ] Phase 1 trial units
- [ ] Prison establishments
- [ ] Probation areas
- [ ] Independent (private or voluntary sector) organisations

- [ ] Educational establishments
- [ ] Independent research units
- [ ] Other (give details)

Total UK sites in study: 1

A73-1. Will potential participants be identified through any organisations other than the research sites listed above?

( ) Yes (•) No

A76. Insurance/ indemnity to meet potential legal liabilities

***Note:** in this question to NHS indemnity schemes include equivalent schemes provided by Health and Social Care (HSC) in Northern Ireland*

A76-1. What arrangements will be made for insurance and/or indemnity to meet the potential legal liability of the sponsor(s) for harm to participants arising from the management of the research? *Please tick box(es) as applicable.*

Note: Where a NHS organisation has agreed to act as sponsor or co-sponsor, indemnity is provided through NHS schemes. Indicate if this applies (there is no need to provide documentary evidence). For all other sponsors, please describe the arrangements and provide evidence.

- [ ] NHS indemnity scheme will apply (NHS sponsors only)
- [x] Other insurance or indemnity arrangements will apply (give details below)

The University of Birmingham has in force a Public Liability Policy and/or Clinical Trial policy which provides cover for claims for "negligent harm" and the activities here are included with that coverage

Please enclose a copy of relevant documents.

A76-2. What arrangements will be made for insurance and/ or indemnity to meet the potential legal liability of the sponsor(s) or employer(s) for harm to participants arising from the design of the research? *Please tick box(es) as applicable.*

Note: Where researchers with substantive NHS employment contracts have designed the research, indemnity is provided through NHS schemes. Indicate if this applies (there is no need to provide documentary evidence). For other protocol authors (e.g. company employees, university members), please describe the arrangements and provide evidence.

- [ ] NHS indemnity scheme will apply (protocol authors with NHS contracts only)
- [x] Other insurance or indemnity arrangements will apply (give details below)

The University of Birmingham has in force a Public Liability Policy and/or Clinical Trial policy which provides cover for claims for "negligent harm" and the activities here are included with that coverage

Please enclose a copy of relevant documents.

A76-3. What arrangements will be made for insurance and/ or indemnity to meet the potential legal liability of investigators/collaborators arising from harm to participants in the conduct of the research?

Note: Where the participants are NHS patients, indemnity is provided through the NHS schemes or through professional indemnity. Indicate if this applies to the whole study (there is no need to provide documentary evidence). Where non-NHS sites are to be included in the research, including private practices, please describe the arrangements which will be made at these sites and provide evidence.

- [x] NHS indemnity scheme or professional indemnity will apply (participants recruited at NHS sites only)

DRAFT

☐ Research includes non-NHS sites (give details of insurance/ indemnity arrangements for these sites below)

Please enclose a copy of relevant documents.

A78. Could the research lead to the development of a new product/process or the generation of intellectual property?

◯ Yes ◉ No ◯ Not sure

Part B: Section 5 – Use of newly obtained human tissue(or other human biological materials) for research purposes

1. What types of human tissue or other biological material will be included in the study?

Samples collected as part of this study will include:
Maternal blood samples collected at the time of incision and time of skin closure during a C-section and at time of waiting in recovery room
Adipose tissue collected from the abdomen of women at the time of incision and time of skin closure during a C-section

2. Who will collect the samples?

The samples will be collected by those undertaking the C-section and placed into appropriate sample containers. These samples will be stored refrigerated as soon after collection as possible by the laboratories at Birmingham Women's and Children's Hospital NHS Foundation Trust and all samples will be couriered within a cool box (on ice) to the University of Birmingham (using an approved courier for transport of human samples). Prior to any human tissue transport, all human tissues will be packed into a sealed container, and labelled with the following information: Human Tissue Sample, Sample Reference Number, Tissue type, details of medium/ preservative, date of packing.
Upon arrival at the University the samples will be handled according to the University of Birmingham Health and Safety Guidance (GUIDANCE/26/WHBFT/14) and in accordance with the HTA and local procedures. Samples will be stored at the University of Birmingham in dedicated locked areas of -80°C freezers that are suitable for storage of human samples.
At the University of Birmingham, the process of characterisation will be performed within a laboratory settings on batches of samples. SOPs are in place for subsequent processing of samples. The work at the University of Birmingham will be conducted within the Pharmaceutics Laboratory in the Sir Robert Aitken building. The facilities are HTA compliant.

3. Who will the samples be removed from?

☑ Living donors
☐ The deceased

4. Will informed consent be obtained from living donors for use of the samples? *Please tick as appropriate*

In this research?
◉ Yes ◯ No

In future research?
◉ Yes ◯ No ◯ Not applicable

6. Will any tissues or cells be used for human application or to carry out testing for human application in this research?

◯ Yes ◉ No

8. Will the samples be stored: *[Tick as appropriate]*

In fully anonymised form? *(link to donor broken)*
◯ Yes ◉ No

In linked anonymised form? *(linked to stored tissue but donor not identifiable to researchers)*
◉ Yes ◯ No

If Yes, say who will have access to the code and personal information about the donor.

All participants will be given a study ID upon recruitment to the study. A spreadsheet that links study ID to patient ID will be held at Birmingham Women's and Children's Hospital NHS Foundation Trust under the control of Katie Morris. This information will be password protected and stored on an encrypted computer that conforms to the standard NHS security requirements.
The study ID will be used on all paperwork to provide anonymised data to the research team at the University of Birmingham that no longer has any personal identifiable data. The relevant data will be entered on a shared secure system (Bear Share) (undertaken by the clinical research team under the supervision of Katie Morris); this system can be accessed at the University and ensures anonymity for the participants.

In a form in which the donor could be identifiable to researchers?
◯ Yes ◉ No

9. What types of test or analysis will be carried out on the samples?

The concentration of cefuroxime in plasma and adipose tissue will be quantified using high-performance liquid chromatography-ultraviolet (HPLC-UV). The methodology will replicate previous work conducted by Barbour et al (2009) [11]. Full methodology is available as SOPs within the pharmacy research laboratory.

10. Will the research involve the analysis or use of human DNA in the samples?

◯ Yes ◉ No

11. Is it possible that the research could produce findings of clinical significance for donors or their relatives?

◯ Yes ◉ No

12. If so, will arrangements be made to notify the individuals concerned?

◯ Yes ◯ No ◉ Not applicable

13. Give details of where the samples will be stored, who will have access and the custodial arrangements.

Human samples will be handled according to the University of Birmingham Health and Safety Guidance (GUIDANCE/26/WHBFT/14) and in accordance with the HTA and local procedures. Samples will be stored at the University of Birmingham in dedicated locked areas of -80°C freezers that are suitable for storage of human samples. The freezers will be located in a room with limited access to authorised staff. These freezers are specified for human tissue storage only (not animal origin).

14. What will happen to the samples at the end of the research? *Please tick all that apply and give further details.*

☐ Transfer to research tissue bank

DRAFT

(If the bank is in England, Wales or Northern Ireland the institution will require a licence from the Human Tissue Authority to store relevant material for possible further research.)

☑ Storage by research team pending ethical approval for use in another project

(Unless the researcher's institution holds a storage licence from the Human Tissue Authority, or the tissue is stored in Scotland, or it is not relevant material, a further application for ethical review should be submitted before the end of this project.)

☐ Storage by research team as part of a new research tissue bank

(The institution will require a licence from the Human Tissue Authority if the bank will be storing relevant material in England, Wales or Northern Ireland. A separate application for ethical review of the tissue bank may also be submitted.)

☐ Storage by research team of biological material which is not "relevant material" for the purposes of the Human Tissue Act

☑ Disposal in accordance with the Human Tissue Authority's Code of Practice

☐ Other

☐ Not yet known

Please give further details of the proposed arrangements:

The research team have requested consent to store any remaining tissue that is left over after this study to use for other research at the University of Birmingham. This further research will require approval from a research ethics committee. If no further approvals have been made prior to the end of the study the samples will be destroyed as per the HTA requirement.

PART C: Overview of research sites

Please enter details of the host organisations (Local Authority, NHS or other) in the UK that will be responsible for the research sites. *For further information please refer to guidance.*

Investigator identifier	Research site		Investigator Name	
IN1	◉ NHS/HSC Site ○ Non-NHS/HSC Site		Forename	Katie
			Middle name	
			Family name	Morris
	Organisation name	BIRMINGHAM WOMEN'S AND CHILDREN'S NHS FOUNDATION TRUST	Email	
	Address	STEELHOUSE LANE	Qualification (MD...)	MBChB, PhD, MRCOG
		BIRMINGHAM WEST MIDLANDS	Country	UNITED KINGDOM
	Post Code	B4 6NH		
	Country	ENGLAND		

DRAFT DRAFT

PART D: Declarations

D1. Declaration by Chief Investigator

1. The information in this form is accurate to the best of my knowledge and belief and I take full responsibility for it.
2. I undertake to fulfil the responsibilities of the chief investigator for this study as set out in the UK Policy Framework for Health and Social Care Research.
3. I undertake to abide by the ethical principles underlying the Declaration of Helsinki and good practice guidelines on the proper conduct of research.
4. If the research is approved I undertake to adhere to the study protocol, the terms of the full application as approved and any conditions set out by review bodies in giving approval.
5. I undertake to notify review bodies of substantial amendments to the protocol or the terms of the approved application, and to seek a favourable opinion from the main REC before implementing the amendment.
6. I undertake to submit annual progress reports setting out the progress of the research, as required by review bodies.
7. I am aware of my responsibility to be up to date and comply with the requirements of the law and relevant guidelines relating to security and confidentiality of patient or other personal data, including the need to register when necessary with the appropriate Data Protection Officer. I understand that I am not permitted to disclose identifiable data to third parties unless the disclosure has the consent of the data subject or, in the case of patient data in England and Wales, the disclosure is covered by the terms of an approval under Section 251 of the NHS Act 2006.
8. I understand that research records/data may be subject to inspection by review bodies for audit purposes if required.
9. I understand that any personal data in this application will be held by review bodies and their operational managers and that this will be managed according to the principles established in the Data Protection Act 2018.
10. I understand that the information contained in this application, any supporting documentation and all correspondence with review bodies or their operational managers relating to the application:
    - Will be held by the REC (where applicable) until at least 3 years after the end of the study; and by NHS R&D offices (where the research requires NHS management permission) in accordance with the NHS Code of Practice on Records Management.
    - May be disclosed to the operational managers of review bodies, or the appointing authority for the REC (where applicable), in order to check that the application has been processed correctly or to investigate any complaint.
    - May be seen by auditors appointed to undertake accreditation of RECs (where applicable).
    - Will be subject to the provisions of the Freedom of Information Acts and may be disclosed in response to requests made under the Acts except where statutory exemptions apply.
    - May be sent by email to REC members.
11. I understand that information relating to this research, including the contact details on this application, may be held on national research information systems, and that this will be managed according to the principles established in the Data Protection Act 2018.
12. Where the research is reviewed by a REC within the UK Health Departments Research Ethics Service, I understand that the summary of this study will be published on the website of the Health Research Authority (HRA) together with the contact point for enquiries named below. Publication will take place no earlier than 3 months after the issue of the ethics committee's final opinion or the withdrawal of the application.

Contact point for publication *(Not applicable for R&D Forms)*

HRA would like to include a contact point with the published summary of the study for those wishing to seek further information. We would be grateful if you would indicate one of the contact points below.

- (●) Chief Investigator
- ( ) Sponsor
- ( ) Study co-ordinator
- ( ) Student
- ( ) Other – please give details
- ( ) None

Access to application for training purposes *(Not applicable for R&D Forms)*

Optional – please tick as appropriate:

☑ I would be content for members of other RECs to have access to the information in the application in confidence for training purposes. All personal identifiers and references to sponsors, funders and research units would be removed.

Signature: .....

Print Name: Hannah Batchelor

Date: 08/11/2019 *(dd/mm/yyyy)*

IRAS Form Reference: 19/EM/0356 IRAS Version 5.13

DRAFT

D2. Declaration by the sponsor's representative

If there is more than one sponsor, this declaration should be signed on behalf of the co−sponsors by a representative of the lead sponsor named at A64-1.

I confirm that:

1. This research proposal has been discussed with the Chief Investigator and agreement in principle to sponsor the research is in place.
2. An appropriate process of scientific critique has demonstrated that this research proposal is worthwhile and of high scientific quality.
3. Any necessary indemnity or insurance arrangements, as described in question A76, will be in place before this research starts. Insurance or indemnity policies will be renewed for the duration of the study where necessary.
4. Arrangements will be in place before the study starts for the research team to access resources and support to deliver the research as proposed.
5. Arrangements to allocate responsibilities for the management, monitoring and reporting of the research will be in place before the research starts.
6. The responsibilities of sponsors set out in the UK Policy Framework for Health and Social Care Research will be fulfilled in relation to this research.

   Please note: *The declarations below do not form part of the application for approval above. They will not be considered by the Research Ethics Committee.*
7. Where the research is reviewed by a REC within the UK Health Departments Research Ethics Service, I understand that the summary of this study will be published on the website of the National Research Ethics Service (NRES), together with the contact point for enquiries named in this application. Publication will take place no earlier than 3 months after issue of the ethics committee's final opinion or the withdrawal of the application.
8. Specifically, for submissions to the Research Ethics Committees (RECs) I declare that any and all clinical trials approved by the HRA since 30th September 2013 (as defined on IRAS categories as clinical trials of medicines, devices, combination of medicines and devices or other clinical trials) have been registered on a publically accessible register in compliance with the HRA registration requirements for the UK, or that any deferral granted by the HRA still applies.

Signature: .....

Print Name:

Post:

Organisation:

Date: *(dd/mm/yyyy)*

D3. Declaration for student projects by academic supervisor(s)

1. I have read and approved both the research proposal and this application. I am satisfied that the scientific content of the research is satisfactory for an educational qualification at this level.

2. I undertake to fulfil the responsibilities of the supervisor for this study as set out in the UK Policy Framework for Health and Social Care Research.

3. I take responsibility for ensuring that this study is conducted in accordance with the ethical principles underlying the Declaration of Helsinki and good practice guidelines on the proper conduct of research, in conjunction with clinical supervisors as appropriate.

4. I take responsibility for ensuring that the applicant is up to date and complies with the requirements of the law and relevant guidelines relating to security and confidentiality of patient and other personal data, in conjunction with clinical supervisors as appropriate.

Academic supervisor 1

Signature: .....

Print Name: Hannah Batchelor

Post: Senior Lecturer in Pharmaceutics, Formulation and Drug Delivery

Organisation: University of Birmingham

Date: 08/11/2019 *(dd/mm/yyyy)*

Academic supervisor 2

Signature: .....

Print Name: Victoria Hodgetts Morton

Post: Clinical Research Fellow

Organisation: Birmingham Women's Hospital

Date: 08/11/2019 *(dd/mm/yyyy)*

7.2.4.4 University of Birmingham Trial sponsor and insurance letters

TO WHOM IT MAY CONCERN
Our Reference: **Q917**

9th October 2019

Dear Sir/Madam

THE UNIVERSITY OF BIRMINGHAM AND ALL ITS SUBSIDIARY COMPANIES

We confirm that the above Institution is a Member of U.M. Association Limited and Clinical Trials cover is in force in respect of the Trial below subject to the cover period, terms, conditions and exceptions:

CLINICAL TRIAL COVERAGE

Clinical Trial	**'C-LACE'** - Cefuroxime Levels After Caesarean Section
Certificate of Entry No.	UM034/95
Period of Indemnity	1st August 2019 to 31st July 2020
Limit Of Indemnity	£30,000,000 Limit any one event and in the aggregate for any one Period of Indemnity including claims costs and expenses for all Clinical Trials undertaken by the University
Basis of Cover	Legal Liability or No Fault Cover
Indemnity	To the University of Birmingham, its employees and its students
Cover provided by	U.M. Association Limited and Excess Cover Provider QBE Europe SA/NV

Yours faithfully

[redacted]

Paul Cusition
For U.M. Association Limited

5 St Helen's Place, London EC3A 6AB | T: 020 7847 8670 | www.umal.co.uk

U.M.Association Ltd (registered in England & Wales, no. 2731799) is the Appointed Representative (FCA firm reference no. 417806) of Hasilwood Management Services Ltd (registered in England & Wales, no. 9295343) which is authorised and regulated by the Financial Conduct Authority (FCA firm reference no. 665752). Hasilwood Management Services Ltd is a wholly owned subsidiary of U.M. Association Ltd. The registered address of both companies is 5 St Helen's Place, London, EC3A 6AB. Hasilwood Management Services Ltd VAT Registration Number: 212249835.

ISO 9001 REGISTERED FIRM

UNIVERSITY OF BIRMINGHAM

FINANCE OFFICE

To Whom It May Concern,

Friday, 8 November 2019

Dear Sir / Madam,

Project Title: Understanding pharmacokinetic distribution of cefuroxime when given prior to Caesarean section in body fat and blood

Sponsor Reference: RG_18-237

The University currently arranges Clinical Research Insurance through membership of UMAL, this cover is renewable annually on 1st August and the University ensures that there are no gaps in cover.

We confirm that the University will arrange insurance for the above research for the duration of the research project involving human participants including legal liability cover. This will be subject to the terms, conditions and exceptions of the relevant liability insurance policies as detailed in the current trial documentation.

The University will provide notification if there are any changes to the provider in a particular year or if the indemnity limits change. Copies of the trial specific annual confirmatory letters will be available upon request.

Yours faithfully,

[redacted]

Dr Birgit Whitman
Head of Research Governance and Integrity

UNIVERSITY OF BIRMINGHAM

FINANCE OFFICE

Dr Hannah Batchelor
School of Pharmacy
University of Birmingham

Friday, 8 November 2019

Dear Dr Hannah Batchelor

Project Title: **Understanding pharmacokinetic distribution of cefuroxime when given prior to Caesarean section in body fat and blood**
IRAS ID: **244803**
Sponsor Reference: **RG_18-237**
UoB Ethics Reference: **ERN_18-1539**

Under the requirements of UK Policy Framework for Health and Social Care Research, the University of Birmingham agrees to act as Sponsor for this project. Sponsorship is subject to you obtaining a favourable ethical opinion, HRA approval and NHS R&D management approval where appropriate.

As Chief Investigator, you must ensure that local study recruitment does not commence until all applicable approvals have been obtained. Where a study is or becomes multi-site you are responsible for ensuring that recruitment at external sites does not commence until local approvals have been obtained.

Following receipt of all relevant approvals, you should ensure that any subsequent amendments are notified to the Sponsor, REC, HRA and relevant NHS R&D Office(s), and that an annual progress report is submitted to the Sponsor, REC and NHS R&D departments where requested.

Please ensure you are familiar with the University of Birmingham Code of Practice for Research (http://www.birmingham.ac.uk/Documents/university/legal/research.pdf) and any appropriate College or School guidelines.

Finally please contact researchgovernance@contacts.bham.ac.uk should you have any queries.

You may show this letter to external organisations.

Yours sincerely,

[redacted]

Dr Birgit Whitman
Head of Research Governance and Integrity

cc: Hanadi Alrammaal (student researcher)

University of Birmingham Edgbaston Birmingham B15 2TT United Kingdom
w: www.finance.bham.ac.uk

7.2.4.5 Saudi Arabian financial insurance letter



ROYAL EMBASSY OF SAUDI ARABIA
CULTURAL BUREAU
LONDON

سفارة المملكة العربية السعودية
الملحقية الثقافية
لندن

ROYAL EMBASSY OF SAUDI ARABIA
CULTURAL BUREAU
LONDON

سفارة المملكة العربية السعودية
الملحقية الثقافية
لندن

Financial Sponsorship

University of Birmingham

The University of Birmingham , Edgbaston , Birmingham

Student Name	HANADI HASSAN A ALRAMMAAL
Our Ref	1054606262
Academic Stage	ESL / Phd
Course Degree Title	Pharmacy
Course Dates and Fees	09/01/2020-19/12/2020() @GBP 10000

The Saudi Arabian Cultural Bureau (SACB) hereby confirms that the above named is a Saudi Government Scholarship holder. SACB is therefore responsible for paying the students tuition fees of the above mentioned course and duration only. Payments will be made upon receipt of confirmation of registration. This financial sponsorship letter does not cover any On-Line or distance learning courses and students are NOT allowed to register for these courses without prior permission from SACB.
Given the above referenced student is part of the sponsorship scheme we require reports on his/her progress and attendance on biannual/semester based. Any unauthorised absence exceeding 2 days, fail to register, suspension or withdrawal from the course should be kindly reported to us without delay.
To ensure all payments are made a completed copy of this form in addition to all original invoices should be sent to the Bureau. Please note that payment will be made against original invoices only.
For further guidance please refer to the attached checklist.
To avoid delay in processing any documents please quote our reference on all correspondence relating to the student. Should you require any assistance do not hesitate to contact us on this email: communications@uk.moe.gov.sa

Yours sincerely,

Dr. Abdulaziz Saleh Alraddadi
Acting Saudi Cultural Attaché to the United Kingdom

[redacted]

630 Chiswick High Road, London W4 5RY Tel: [redacted] E-mail [redacted]

Please fill this form and return it to us to CONFIRM registration

Student Name	HANADI HASSAN A ALRAMMAAL
Our Ref	1054606262
Academic Stage	ESL / Phd
Course Degree Title	Pharmacy
Course Dates and Fees	09/01/2020-19/12/2020() @GBP 10000

To be completed by the School/College or university:
I confirm that the Mr. /Miss/Mrs. _____ has been registered for the program shown above and commenced his/her studies on __/__/____.

Name and contact details of Academic Supervisor/ Course Director / Tutor:
Name: _____ **Faculty:** _____
Department: _____ **Telephone:** _____
Email: _____

7.2.4.6 Birmingham Women's and Children's Hospital NHS Foundation Trust R&D Confirmation of Capacity & Capability letter

NHS
Birmingham Women's and Children's
NHS Foundation Trust

BIRMINGHAM WOMEN'S HOSPITAL DETAILS:
Research and Development
Birmingham Women's and Children's NHS Foundation Trust
Birmingham Women's Hospital
Mindelsohn Way
Edgbaston Birmingham B15 2TG
Tel: 0121 335 8046
BWC.research@ nhs.net

DATE: 12 Jan 2021

CI/PI Name: Katie Morris
Department: Fetal Medicine
Birmingham Women's and Children's NHS Foundation Trust

Dear PI

Re: Birmingham Women's and Children's Hospital NHS Foundation Trust R&D Confirmation of Capacity & Capability

Project Title: C-LACE: Cefuroxime Levels After Caesarean Section
IRAS Ref: 244803
R&D No: 20/BW/OGN/NO/478
Protocol: Version 1.4 dated 27 July 2020

Thank you for informing Birmingham Women's and Children's NHS Foundation Trust's R&D office of the above project.

I can confirm that Birmingham Women's & Children's NHS Foundation Trust has the capacity and capability to deliver the above referenced study and therefore is issuing R&D Confirmation of Capacity & Capability **for this project up to and including AM 01.**

Please find attached our signed agreements as confirmation.

R&D Confirmation of Capacity and Capability of the study is subject to the following conditions:

- If you have not already done so you must ensure that you and other members of the research team complete Good Clinical Practice ('GCP') training and a copy of your certificate is forwarded to the R&D Department before conducting your research.
- The research is conducted in line with the guidance given within the UK Policy Framework for Health and Social Care Research and where appropriate the Medicines for Human Use (Clinical Trial) Regulations 2004. You must also apply the principles of ICH Good Clinical Practice in conduct of your research.
- The research will be conducted in compliance with Trust Policies and carried out in accordance with the General Data Protection Regulation (2018), Human Tissue Act 2004, Health & Safety at Work Act and the Caldicott principles and NHS Code of Confidentiality.

- Any proposed changes or amendments to the protocol will be notified to the ethics committee, the research sponsor and the R&D Manager/R&D Facilitator.
- The UK Policy Framework for Health and Social Care Research published by the Health Research Authority (HRA) and the UK Health Departments make clear that appropriate allocation of responsibility, and clear understanding of responsibility is fundamental to good overall governance of research. You must ensure that all researchers not employed by the Trust follow the standardised procedures for issuing Honorary Research Contracts (HRC) or Letters of Access (LOA) as laid down in the Research in the HR Good Practice Resource Pack http://www.nihr.ac.uk/policy-and-standards/research-passports.htm
- Procedures are in place to ensure collection of high quality, accurate data and the integrity and conditionality of data during processing and storage.
- Upon completion of the research you must complete an End of Study report and submit this to Ethics and copy in the R&D Manager/R&D Facilitator at Birmingham Women's and Children's NHS Foundation Trust. http://www.hra.nhs.uk/research-community/end-of-study-and-beyond/notifying-the-end-of-study/
- If your project has been adopted by the National Institute for Health Research (NIHR) portfolio you will be required to provide monthly recruitment figures to the R&D Office (or manually update the R&D Database with this (EDGE) and address any issues affecting recruitment.
- That the Chief/Principal Investigator and the research team should be familiar with BWC trial Standard Operating Procedures. These SOPS can be found at the following location on the trust intranet. https://intranet.bwc.nhs.uk/departments/r/research/research-and-development/research-documents/
- The study will be run as outlined in roles and responsibilities section of the CI/PI Agreement which has been duly signed and returned to R & D

Please see attached Initial HRA approval and REC approval letter dated 22 Jan 2020 and REC approval for substantial amendments AM01 dated 24 Aug 2020 for documents reviewed and approved by R&D. All approved documents can be accessed via EDGE using the local project reference.

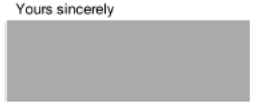
May I also draw your attention to the UK Policy Framework for Health and Social Care Research which can be found on the internet https://www.hra.nhs.uk/planning-and-improving-research/policies-standards-legislation/uk-policy-framework-health-social-care-research/ and remind you that all research within the Trust should be run to the standards as outlined in this document. Guidance and advice is always available from the Department of Research and Development should you require it at any stage of your project.

Your research activity is now covered by NHS indemnity as set out in HSG (96) 48, and your trial has been entered into the Trusts' database.

The Trust conducts regular audits to monitor 10% of all projects on an annual basis. You must comply with the requests as they have the authority to audit your site files at any time.

If you have any queries relating to R&D, please do not hesitate to contact me. The Trust wishes you success with your research.

Yours sincerely



Head of R & D

7.3 Appendix of Chapter 5 "Observational Study to Assess the Pharmacokinetics of Cefuroxime and its Prophylactic Efficacy in Obese Pregnant women after Caesarean Section (C-LACE)"

7.3.1 Application of Physiologically Based Pharmacokinetic Model to Predict Cefazolin and Cefuroxime Disposition in Obese Pregnant Women.

Supplementary Material

7.3.1.1 Method

7.3.1.2 Study selection for the Physiologically-based Pharmacokinetics models:

The selection of studies used in cefazolin or cefuroxime Physiologically-based Pharmacokinetics (PBPK) models was dependent on a) the availability of observed cefazolin and cefuroxime adipose tissue concentrations, b) the analytical techniques used in analysing drugs in blood and adipose tissue, c) ClinPK scores (269) and d) the overall availability of data.

7.3.1.3 Cefazolin

The Philipson study (healthy and pregnant populations) was selected because it represented the gestational differences in women. The study identified the PK of cefazolin of the subjects at the time of pregnancy and after delivery, on the return of the menstrual cycle and at the end of breastfeeding (100). Therefore, it will allow the comparison of cefazolin PK parameters at the time of pregnancy compared to a healthy population.

Ritta and Ravin used different dosing than Philipson et al. (100, 372). Elkomy et al. represented cefazolin PK at the time of caesarean section (CS) compared to other studies at the time of pregnancy (102). Pevzner et al. reported cefazolin concentrations in adipose tissue in normal-weight pregnant women at the time of CS (10, 284). Chen et al. reported cefazolin concentrations in adipose tissue in obese subjects undergoing bariatric surgery with no other conditions (78).

Many studies analysed cefazolin in the obese-pregnant population (10-16). Out of 40 score, the highest ClinPK scores were reported by Stitely et al. (39), Young et al. (38), Kram et al. (37) and Maggio et al. (36) (13-16). Kram et al. used a modified Kirby-Baure assay, which is not considered to be a robust technique for analysing cefazolin in blood and adipose tissue. As a result, Young et al. and Stitely et al. were selected to investigate the appropriately of the PBPK obese-pregnant model to predict cefazolin concentrations in plasma and adipose compared to observed data.

7.3.1.4 Cefuroxime

The study by Lovering et al. (healthy population) was selected because it reported cefuroxime adipose tissue concentrations in subjects underwent hip replacement, this was used to optimise cefuroxime adipose tissue: Plasma partition coefficient (305). Studies by Kegdel et al. and Garton et al. (healthy populations) were used previously in the study by Hsu et al. to predict cefuroxime concentrations in pregnancy PBPK model using Simcyp V12.1 (113, 373, 374). These studies were included in this work to capture the similarities and differences of in-silico prediction of Simcyp version 20 to the older version.

For the Philipson and Stiernstedt study, healthy and pregnant women were selected as representative of the gestational differences in women during pregnancy and at delivery (109). Additionally, it identified the PK of cefuroxime of these women after delivery, on the return of the menstrual cycle and cessation of breastfeeding. This data will allow the comparison of the cefuroxime PK parameter during pregnancy with the healthy population. Lalic et al. was selected because they reported the highest ClinPK score among other related studies (9, 300). Barbour et al. reported cefuroxime

concentrations in the blood and adipose tissue of obese women undergoing abdominal surgery (110).

7.3.1.5 Cefuroxime logP

The Log P used in Hsu et al. was -0.9; this was predicted using ADMET PredictorTM (113). In our study, Log P was measured using the shake-flask method. Cefuroxime was dissolved in 100 ml of water (200 µg/ml); 3 ml of this solution was added to 3 ml of n-octanol and that solution was shaken for 24 hours at 4°C. Then, the two phases were allowed to separate for 24 hours and after separation the cefuroxime content was measured by high-performance liquid chromatography in both the water and the n-octanol using a previously published validated method with minor modification (13, 110). The partition coefficient of the cefuroxime was calculated using equation **(7.1)** (309). The resulting Log P was -1.5. A sensitivity analysis of cefuroxime Log P range values from -3 to 3 was performed using cefuroxime-observed plasma concentrations in healthy subjects.

$$\mathrm{LogP_{oct/wat}} = \mathrm{Log}\left(\frac{[\text{Solute in octanol}]}{[\text{Solute in water}]}\right) \quad \textbf{(7.1)}$$

7.3.1.6 PBPK models of non–obese non–pregnant, obese, pregnant and obese–pregnant populations

Figure S1 represent the pregnancy PBPK model coupled with the kidney permeability model used within simcyp (141, 142, 149, 366). The GFR value is simulated the modification of diet in renal disease (MDRD) equation (referred to as Method 2 in Simcyp®); equation **(7.2)** represent this method (141, 146).

7.3.1.7 Organs, tissue and blood flow

7.3.1.8 Tissue volume

The addition of fetal volume and gestational changes in plasma volume, red blood cell volume and total fat mass (expressed as volume in simulator using specific gravity) are described in equations **(7.3)**, **(7.4)**, **(7.5)** and **(7.6)**, respectively (149).

7.3.1.9 Blood flows

The change in cardiac output and consequent alteration of blood flow percentages to the kidneys, brain, adipose tissues and the new organ (feto-placenta) are incorporated into the Sim–pregnancy model. Equations **(7.7)**, **(7.8)** and **(7.9)** represent the gestational changes of cardiac output and blood flows to the uterine and kidneys (effective renal blood flow), respectively (149).

7.3.1.10 Composition

The gestational expansion of total body water and change in extracellular and intracellular water at the time variant are described by equations **(7.10)**, **(7.11)** and **(7.12)**, respectively (149).

7.3.1.11 Blood binding

The gestational reduction in blood protein levels are incorporated into the Sim–pregnancy model for albumin and alpha-1-acid glycoproteins. The collected data were utilised to describe the reduction of plasma protein levels in general and for albumin and alpha-1-acid glycoprotein specifically by equations **(7.13)**, **(7.14)** and **(7.15)**, respectively (149).

7.3.1.12 Renal function

The complex gestational changes of renal function have been described algorithmically by Abduljalil el al. (149). The key physiological element of renal function is the GFR; the gestational alteration of GFR is described in equation **(7.16)** (123, 149).

7.3.1.13 Developing a combined obese–pregnant Physiological-based pharmacokinetics model within Simcyp®

The knowledge of physiological changes and their influence on drugs' PK is limited for the obese–pregnant population. It is possible to explore the PK of a drug of interest in an obese–pregnant virtual population without incorporating data on physiological changes in such a population. As the Sim–pregnancy algorithmic gestational changes are applied to a healthy population (pre-pregnant or baseline), a combined obese and pregnant model within the Simcyp® simulator is achievable by adjusting the basic physiological and biochemical parameters of these healthy (pre-pregnant or baseline) to obese subjects. This will allow bypassing the development of PK and physiological equations describing the PK exclusively in obese–pregnant PBPK models in such time-restricted projects. For obese–pregnant PBPK model, additional to the weight adjustment, the tissue blood flows of obese and morbidly obese subjects were used as the baseline (pre-pregnant) of obese– and morbidly obese–pregnant PBPK model (Table S 1).

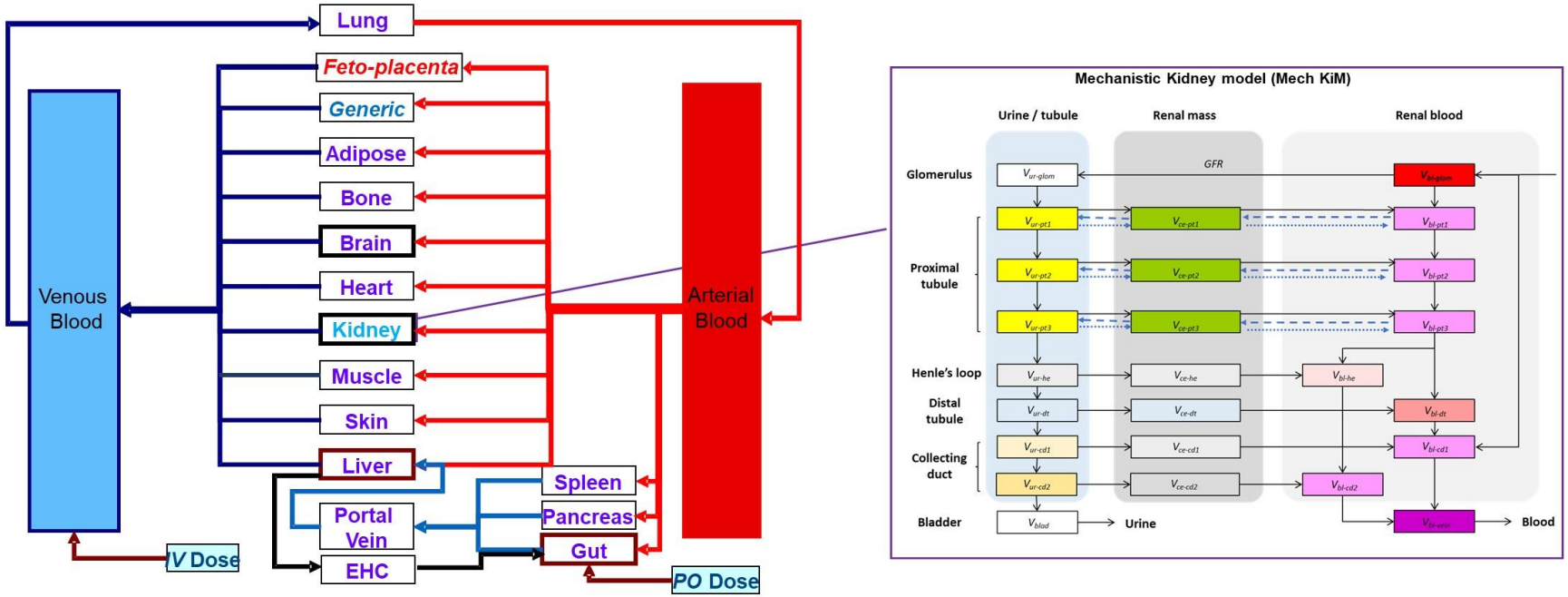


Figure 7.3: Pregnancy (Maternal) PBPK model coupled with MechKiM model

Note: For MechKiM model: both renal blood flow and GFR are gestational age dependent, hence the flows between kidney compartments are gestational age dependent (Solid black arrows). Broken arrows represent directions of efflux and uptake transporters.

$$\text{GFR} = 175 * (\frac{serum\ Creatine}{88.42})^{-1.154} * (age)^{-0.203}\ [*0.742\ if\ Female] \quad \textbf{(7.2)}$$

$$\text{fetal volume (mL)} = 0.01\exp\{\left(\frac{0.955}{0.0702}\right)(1-\exp(-0.0702\ \text{GA}))\} \quad \textbf{(7.3)}$$

$$\text{Plasma volume (L)} = 2.50 - 0.0223\ \text{GA} + 0.0042\ \text{GA}^2 - 0.00007\ \text{GA}^3 \quad \textbf{(7.4)}$$

$$\text{Red Blood Cell volume (L)} = 1.49 + 0.0098\ \text{GA} \quad \textbf{(7.5)}$$

$$\text{Total fat mass (kg)} = 17.14 + 0.1305\ \text{GA} + 0.0008\ \text{GA}^2 \quad \textbf{(7.6)}$$

$$\text{Cardiac output (L/h)} = 301 + 5.916\ \text{GA} - 0.088\ \text{GA}^2 \quad \textbf{(7.7)}$$

$$\text{Uterine blood flow (L/h)} = 1.71 + 0.2068\ \text{GA} + 0.0841\ \text{GA}^2 - 0.0015\ \text{GA}^3 \quad \textbf{(7.8)}$$

$$\text{Effective renal blood flow (L/h)} = 53 + 2.6616\ \text{GA} - 0.0661\ \text{GA}^2 \quad \textbf{(7.9)}$$

$$\text{Total body water (L)} = 31.67 + 0.275\ \text{GA} + 0.0024\ \text{GA}^2 \quad \textbf{(7.10)}$$

$$\text{Extracellular water (L)} = 11.86 + 0.0187\ \text{GA} + 0.0016\ \text{GA}^2 \quad \textbf{(7.11)}$$

$$\text{Intracellular water (L)} = 19.81 + 0.5941\ \text{GA} - 0.0007\ \text{GA}^2 \quad \textbf{(7.12)}$$

$$\text{Plasma protein (g/L)} = 69.7 + 0.2085\ \text{GA} - 0.0305\ \text{GA}^2 + 0.0006\ \text{GA}^3 \quad \textbf{(7.13)}$$

$$\text{Albumin (g/L)} = 45.8 - 0.1775\ \text{GA} - 0.0033\ \text{GA}^2 \quad \textbf{(7.14)}$$

$$\text{Alpha-1-acid glycoprotein (g/L)} = 0.74 - 0.0088\ \text{GA} + 0.0001\ \text{GA}^2 \quad \textbf{(7.15)}$$

$$\text{Glomerular Filtration Rate} = 114 + 3.2367\ \text{GA} - 0.0572\ \text{GA}^2 \quad \textbf{(7.16)}$$

Where GFR is glomerular filtration rate (mL/min/1.73m^2); GA is Gestational age in weeks

7.3.1.14 Results

Tables S2 and S3 represent simulated time above the minimum inhibitory concentrations required to inhibit the growth of 90% of most common organisms causing post CS infection (MIC_{90}) of cefazolin and cefuroxime, respectively.

Predicted time above MIC_{90} (2, 4 and 8 µg/mL or µg/g) of cefuroxime plasma, free plasma and adipose tissue concentration in lean non–pregnant, lean–pregnant, obese– and morbidly obese–pregnant after administration of either 750 mg or 1500 mg cefuroxime dose are presented in table S4. The percentages of subjects attaining

a target MIC_{90} at 1.5 hours and 2 hours are illustrated in table S5. The 1.5 hours and 2 hours were selected to investigate percentages of subjects achieving targeted MIC post cefuroxime administration of 30 min or 60 min pre CS of 1 hours.

Table 7.14: Tissue blood flow rates in female subjects in Simcyp simulator

Population	**Predefined tissue blood flow as percentage of cardiac output (Mean predicted L/h)**			**Mean predicted tissue blood flow L/h using discussed equations at 40 weeks gestational**		
	Non–obese, non–pregnant (baseline of pregnant PBPK population)	Obese (baseline of obese–pregnant PBPK population)	Morbidly obese	Lean–pregnant	obese–pregnant	Morbidly obese–pregnant
Adipose	8.5 (20.62)	14.8 (37.34)	17.2 (58.42)	29.34	62.07	80.01
Bone	5 (17.42)	5 (17.36)	5 (18.63)	13.66	16.59	18.41
Brain	12 (41.81)	10.4 (37.36)	9.7 (36.50)	39.02	41.09	42.51
Stomach and esophagus	1 (3.48)	0.9 (3.12)	0.8 (2.98)	2.73	2.99	2.95
Small intestine	11 (35.75)	9.5 (31.98)	8.9 (32.42)	30.05	31.53	32.76
Villi	6 (20.90)	5.4 (18.56)	4.9 (18.38)	16.39	17.26	18.04
Large intestine	5 (14.85)	4.3 (13.17)	4 (14.04)	13.66	14.27	14.73
Heart	5 (14.85)	5 (14.85)	5 (17.40)	13.66	16.59	18.41
Kidney	17 (64.36)	14.7 (57.56)	13.8 (53.87)	46.68	49.05	51.07
Liver (arterial)	6.5 (22.64)	5.6 (20.20)	5.3 (19.87)	17.76	18.59	19.51
Liver (portal)	21.5 (68.48)	18.5 (61.22)	17.4 (62.85)	58.73	61.40	64.05
Lung	100 (348.38)	100 (347.21)	100 (372.52)	360.33	437.79	485.60
Muscle	12 (54.65)	12 (54.21)	12 (50.85)	32.78	39.83	44.18
Pancreas	1 (3.48)	1 (3.47)	1 (3.73)	2.73	3.32	3.68
Skin	5 (17.42)	6.6 (21.41)	7.2 (26.45)	29.19	46.81	56.64
Spleen	3 (7.88)	2.5 (7.02)	2.4 (8.08)	8.19	8.63	8.84
Feto-Placenta	0.6			35.49	43.12	47.82

Table 7.15: Simulated time of cefazolin plasma and free plasma above minimum inhibitory concentration required to inhibit the growth of 90% of organisms

Study code	**Dose (mg)**	**Time (hour) Simulated cefazolin plasma concentration above MIC90**			**Time (hour) Simulated free plasma cefazolin concentration above MIC90**		
		T>MIC of 2 µg/mL	T>MIC of 4 µg/mL	T>MIC of 8 µg/mL	*f*T>MIC of 2 µg/mL	*f*T>MIC of 4 µg/mL	*f*T>MIC of 8 µg/mL
001 ***(100)***	500	7.68	5.88	4.32	4.08	2.52	1.08
002 ***(372)***	1000	10.2	8.16	6.24	5.88	4.08	2.4
003 ***(100)***	500	5.4	4.2	2.88	2.88	1.8	0.72
004 ***(10, 284)***	2000	8.4	6.96	5.64	5.88	4.56	3.24
005 ***(102)***	1000	7.68	6.12	4.56	4.8	3.36	2.04
006 ***(78)***	2000	14.76	12	9.36	9.12	6.48	4.08
007 ***(16)***	2000	10.56	8.64	6.72	7.08	5.28	3.6
008 ***(16)***	3000	11.64	9.6	7.68	8.04	6.24	4.44
009 ***(14)***	2000	10.44	8.52	6.6	6.96	5.16	3.48
0091 ***(15)***	2000	11.28	9.12	7.08	7.44	5.4	3.48
Mean in all population (001-0091)	All doses	9.80	7.92	6.11	6.22	4.49	2.86
	Only 2000	11.09	9.05	7.08	7.30	5.38	3.58
Mean in Obese-pregnant (007, 009 and 0091)	2000	10.76	8.76	6.80	7.16	5.28	3.52

Table 7.16: Simulated time of cefuroxime plasma and free plasma above minimum inhibitory concentration required to inhibit the growth of 90% of organisms

Study code	**Dose (mg)**	**Time (hour) Simulated cefuroxime plasma concentration above MIC90**			**Time (hour) Simulated free plasma cefuroxime concentration above MIC90**		
		T>MIC of 2 µg/mL	T>MIC of 4 µg/mL	T>MIC of 8 µg/mL	*f*T>MIC of 2 µg/mL	*f*T>MIC of 4 µg/mL	*f*T>MIC of 8 µg/mL
01 *(305)*	1500	7.15	5.65	4.21	6.24	4.80	3.36
02 *(109)*	750	4.98	3.78	2.70	4.32	3.12	2.04
03 *(373)*	1500	7.44	5.88	4.44	6.54	5.04	3.66
04 *(374)*	750	5.67	4.28	2.97	4.86	3.51	2.25
05 *(374)*	1500	7.14	5.70	4.26	6.30	4.86	3.48
06 *(109)*	750	4.68	3.48	2.34	4.08	2.94	1.80
07 *(109)*	750	5.04 (3.2, 6.9)	3.72(2.4, 5.3)	2.52(1.6, 3.5)	4.44(2.9, 6.1)	3.18(2.1, 4.4)	1.98(1.3, 2.8)
08 *(9)*	1500	6.36 (4, 8.9)	5.04(3.1, 7.1)	3.72(2.3, 5.3)	5.76(3.6, 7.9)	4.44(2.8, 6.2)	3.24(1.9, 4.6)
09 *(273)*	1500	6.12(3.7,8.28)	4.80(3, 6.72)	3.60(2.28,5.2)	5.52(3.48,7.6)	4.32(2.76, 6)	3.12(1.92,4.4)
091 *(273)*	1500	6 (3.84, 8.04)	4.8 (3.12,6.6)	3.6(2.28,5.04)	5.4 (3.6, 7.44)	4.2(2.8, 5.88)	3.12(1.9,4.32)
092 *(110)*	1500	7.56	5.88	4.26	6.66	5.04	3.42
1	750	5.28(3.4, 7.8)	3.96(2.4, 6)	2.52 (1.7, 4)	4.68(3, 7)	3.36(2, 5)	1.92 (1.2, 3)
2	1500	6.84(4.2, 9.6)	5.28(3.4, 7.8)	3.96(2.4, 5.9)	6.12(3.8, 8.9)	4.68 (3, 7)	3.36 (2, 4.9)
3	750	5.64(3.5, 8.3)	4.08(2.5, 6)	2.52(1.56,3.8)	4.92(3, 7.3)	3.36(2, 5.2)	1.8(1.1, 2.9)
4	1500	7.32 (4.4, 10.6)	5.64 (3.5, 8.3)	4.08 (2.5, 6)	6.6 (4, 9.5)	4.92 (3, 7.3)	3.36 (2, 5.2)
Mean of studies 01-05 and 092	Both 750 and 1500	6.66	5.19	3.81	5.82	4.40	3.04
Mean of studies 01-091	Both 750 and 1500	6.19	4.82	3.51	5.47	4.13	2.86

Mean in all population (01-4)	Both 750 and 1500	6.21	4.80	3.45	5.50	4.12	2.79
Mean of obese-pregnant population BMI≥ 30 kg/m² (1-4)	Both 750 and 1500	6.27	4.74	3.27	5.58	4.08	2.61
Mean of obese-pregnant population BMI≥ 30 kg/m² (2 and 4)	1500	7.08	5.46	4.02	6.36	4.8	3.36
The 5th percentile of obese-pregnant population BMI≥ 30 kg/m² (1-4)	Both 750 and 1500	3.87	2.95	2.04	3.45	2.5	1.57
The 5th percentile of obese-pregnant population BMI≥ 30 kg/m² (1 and 3)	Only 750	3.45	2.45	1.63	3	2	1.15
The 5th percentile of obese-pregnant population BMI≥ 30 kg/m² (2 and 4)	Only 1500	4.3	3.45	2.45	3.9	3	2

*f*T>MIC time of free concentration above the minimum inhibitory concentration, T>MIC Time above the minimum inhibitory concentration

Table 7.17: Simulated time of cefuroxime plasma, free plasma and adipose tissue concentrations above minimum inhibitory concentration required to inhibit the growth of 90% of organisms (2, 4 and 8 µg/mL or µg/g) post dose of 750 mg and 1500 mg, and dose efficacy when given cefuroxime 30 min or 60 min pre CS of 1 hour.

population	MIC_{90}	Simulated mean T>MIC_{90} (hours) of 750 mg cefuroxime dose						Simulated mean T>MIC (hours) of 1500 mg cefuroxime dose					
		Plasma	Free plasma	Total adipose	ISF	Dose recommendation if cefuroxime was given 30 or 60 min before surgery (of 1 hour duration CS)		Plasma	Free plasma	Total adipose	ISF	Dose recommendation if cefuroxime was given 30 or 60 min before surgery (of 1 hour duration CS)	
						30 min or less	60 min					30 min or less	60 min
Lean Non–pregnant	2	4.98	4.32	1.14	4.32	Sufficient	Sufficient	7.15	6.24	2.35	6.32	Sufficient	Sufficient
	4	3.78	3.12	0.18	3.18	Sufficient	Sufficient	5.65	4.80	1.09	4.88	Sufficient	Sufficient
	8	2.70	2.04	N/A	2.10	Sufficient	Sufficient	4.21	3.36	N/A	3.49	Sufficient	Sufficient
Lean–Pregnant	2	5.04 (3.2, 6.9)	4.44 (2.9, 6.1)	0.84	4.5 (3, 6.1)	Sufficient	Sufficient	6.36 (4, 8.9)	5.76 (3.6, 7.9)	2.04	5.76 (3.7, 8)	Sufficient	Sufficient
	4	3.72 (2.4, 5.3)	3.18 (2.1, 4.4)	N/A	3.24 (2.2, 4.5)	Sufficient	Sufficient	5.04 (3.1, 7.1)	4.44 (2.8, 6.2)	0.84	4.44 (2.9, 6.2)	Sufficient	Sufficient
	8	2.52 (1.6, 3.5)	1.98 (1.3, 2.8)	N/A	2.04 (1.4, 2.9)	Sufficient	Sufficient	3.72 (2.3, 5.3)	3.24 (1.9, 4.6)	N/A	3.24 (2.2, 4.6)	Sufficient	Sufficient
Obese–pregnant	2	5.3 (3.4, 7.8)	4.7 (3, 7)	0.6	4.68 (3, 6.8)	Sufficient	Sufficient	6.84 (4.2, 9.6)	6.12 (3.8, 8.9)	1.92	6.12 (3.8, 8.8)	Sufficient	Sufficient
	4	3.96 (2.4, 6)	3.36 (2, 5)	N/A	3.24 (2.2, 4.9)	Sufficient	Sufficient	5.28 (3.4, 7.8)	4.68 (3, 7)	0.6	4.68 (3, 6.8)	Sufficient	Sufficient
	8	2.52 (1.7, 4)	**1.92 (1.2, 3)**	N/A	1.92 (1.3, 3)	Insufficient	Insufficient	3.96 (2.4, 5.9)	3.36 (2, 4.9)	N/A	3.24 (2.2, 4.9)	Sufficient	Sufficient
Morbidly Obese–pregnant	2	5.64 (3.5, 8.3)	4.92 (3, 7.3)	0.36	4.92 (3.1, 7.2)	Sufficient	Sufficient	7.32 (4.4, 10.6)	6.6 (4, 9.5)	1.8	6.48 (4.1, 9.4)	Sufficient	Sufficient

	4	4.08 (2.5, 6)	3.36 (2, 5.2)	N/A	3.36 (2.2, 5)	Sufficient	Sufficient	5.64 (3.5, 8.3)	4.92 (3, 7.3)	0.36	4.92 (3.1, 7.2)	Sufficient	Sufficient
	8	2.52 (1.56, 3.8)	**1.8 (1.1, 2.9)**	N/A	**1.8** (1.2, 2.8)	Insufficient	Insufficient	4.08 (2.5, 6)	3.36 (2, 5.2)	N/A	3.36 (2.2, 5)	Sufficient	Sufficient

Data in bracets represents 5th, 95th percentile

Table 7.18: Simulated percentages of Obese– and morbidly Obese–pregnant subjects achieving plasma, free plasma and interstitial fluid adipose tissue concentrations above minimum inhibitory concentration (2, 4 and 8 µg/mL or µg/g) at 1.5 hours and 2 hours post dose of 750 mg and1500 mg

population	**MIC_{90}**	**Simulated % of subjects achieved T>MIC_{90} (hours) post 750 mg cefuroxime dose at 1.5 hour (2 hours)**				**Dose current dose achieve a targeted % of subjects attaining a target MIC, if cefuroxime were given before surgery at 30 or 60 min before surgery**				**Simulated % of subjects achieved T>MIC_{90} (hours) post 1500 mg cefuroxime dose at 1.5 hour (2 hours)**				**Dose current dose achieve a targeted % of subjects attaining a target MIC, if cefuroxime were given before surgery at 30 or 60 min before surgery**			
		Plasma	Free plasma	Total adipose	ISF	30 min or less		60 min		Plasma	Free plasma	Total adipose	ISF	30 min or less		60 min	
						75 %	95 %	75 %	95 %					75 %	95 %	75 %	95 %
Obese–pregnant	2	100 (100)	100 (100)	0.4 (0)	100 (100)	Yes	Yes	Yes	Yes	100 (100)	100 (100)	84 (42)	100 (100)	Yes	Yes	Yes	Yes
	4	100 (99.6)	99.9 (97)	0 (0)	100 (97.2)	Yes	Yes	Yes	Yes	100 (100)	100 (100)	0.4 (0)	100 (100)	Yes	Yes	Yes	Yes
	8	98.6 (83.4)	84.6 (45.2)	0 (0)	85.7 (44.2)	Yes	No	No	No	100 (99.6)	99.9 (97)	0 (0)	100 (97.2)	Yes	Yes	Yes	Yes
Morbidly Obese–pregnant	2	100 (100)	100 (100)	0(0)	100 (100)	Yes	Yes	Yes	Yes	100 (100)	100 (100)	75.6 (34.2)	100 (100)	Yes	Yes	Yes	Yes
	4	100 (99.6)	99.9 (96.8)	0 (0)	100 (97.7)	Yes	Yes	Yes	Yes	100 (100)	100 (100)	0 (0)	100 (100)	Yes	Yes	Yes	Yes
	8	96.1 (79.5)	75.8 (37.5)	0 (0)	78.2 (36.2)	Yes	No	No	No	100 (99.6)	99.9 (96.8)	0 (0)	100 (97.7)	Yes	Yes	Yes	Yes

Chapter 8: List of References

1. McCormack S, Best B. Obstetric pharmacokinetic dosing studies are urgently needed. Front Pediatr. 2014;2(9).
2. Dawes M, Chowienczyk PJ. Pharmacokinetics in pregnancy. Best Pract Res Clin Obstet Gynaecol. 2001;15(6):819-26.
3. Anger GJ, Costantine MM, Piquette-Miller M. Chapter 4 - Pharmacokinetics in pregnancy Reproductive and Developmental Toxicology. San Diego: Academic Press; 2011. p. 39-45.
4. Team NP. National maternal and perinatal audit: Clinical report 2017. 2017.
5. Denison F, Aedla N, Keag O, Hor K, Reynolds R, Milne A, et al. Care of women with obesity in pregnancy: Green-top guideline No. 72. BJOG. 2019;126(3):e62-e106.
6. Weiss JL, Malone FD, Emig D, Ball RH, Nyberg DA, Comstock CH, et al. Obesity, obstetric complications and cesarean delivery rate--a population-based screening study. Am J Obstet Gynecol. 2004;190(4):1091-7.
7. Wloch C, Wilson J, Lamagni T, Harrington P, Charlett A, Sheridan E. Risk factors for surgical site infection following caesarean section in England: results from a multicentre cohort study. BJOG. 2012;119(11):1324-33.
8. Smaill FM, Gyte GM. Antibiotic prophylaxis versus no prophylaxis for preventing infection after cesarean section. Cochrane Database Syst Rev. 2010(1):CD007482.
9. Lalic-Popovic M, Paunkovic J, Grujic Z, Golocorbin-Kon S, Milasinovic L, Al-Salami H, et al. Decreased placental and transcellular permeation of cefuroxime in pregnant women with diabetes. J Diabetes. 2016;8(2):238-45.
10. Swank ML, Wing DA, Nicolau DP, McNulty JA. Increased 3-gram cefazolin dosing for cesarean delivery prophylaxis in obese women. Am J Obstet Gynecol. 2015;213(3).
11. Chan K, Krepel C, Edmiston Jr C, Pevzner L, Swank M, Wing DA. Effects of maternal obesity on tissue concentrations of prophylactic cefazolin during cesarean delivery. Am J Obstet Gynecol. 2011;204 (1 SUPPL.):S24.
12. Groff SM, Fallatah W, Yang S, Murphy J, Crutchfield C, Marzinke M, et al. Effect of maternal obesity on maternal-fetal transfer of preoperative cefazolin at cesarean section. J Pediatr Pharmacol Ther. 2017;22(3):227-32.
13. Kram JJF, Greer DM, Cabrera O, Burlage R, Forgie MM, Siddiqui DS. Does current cefazolin dosing achieve adequate tissue and blood concentrations in obese women undergoing cesarean section? Eur J Obstet Gynecol Reprod Biol. 2017;210:334-41.
14. Maggio L, Nicolau DP, DaCosta M, Rouse DJ, Hughes BL. Cefazolin prophylaxis in obese women undergoing cesarean delivery: A randomized controlled trial. Obstet Gynecol. 2015;125(5):1205-10.
15. Stitely M, Sweet M, Slain D, Alons L, Holls W, Hochberg C, et al. Plasma and tissue cefazolin concentrations in obese patients undergoing cesarean delivery and receiving differing pre-operative doses of drug. Surg Infect (Larchmt). 2013;14(5):455-9.
16. Young OM, Shaik IH, Twedt R, Binstock A, Althouse AD, Venkataramanan R, et al. Pharmacokinetics of cefazolin prophylaxis in obese gravidae at time of cesarean delivery. Am J Obstet Gynecol. 2015;213(4):541.e1-7.
17. Parry E, Shields R, Turnbull AC. Transit time in the small intestine in pregnancy. BJOG. 1970;77(10):900-1.

18. Anger GJ, Piquette-Miller M. Chapter 3 - Pharmacokinetics in pregnancy Reproductive and Developmental Toxicology (Second Edition): Academic Press; 2017. p. 39-49.
19. Feghali M, Venkataramanan R, Caritis S. Pharmacokinetics of drugs in pregnancy. Semin Perinatol. 2015;39(7):512-9.
20. Frederiksen MC. 79 - Consideration for the use of therapeutic drugs during pregnancy and the perinatal period A2 - Legato, Marianne J. Principles of Gender-Specific Medicine. San Diego: Academic Press; 2004. p. 869-73.
21. Pariente G, Leibson T, Carls A, Adams-Webber T, Ito S, Koren G. Pregnancy-associated changes in pharmacokinetics: A systematic review. PLoS Med. 2016;13(11):e1002160.
22. Aweeka FT, Hu C, Huang L, Best BM, Stek A, Lizak P, et al. Alteration in cytochrome P450 3A4 activity as measured by a urine cortisol assay in HIV-1-infected pregnant women and relationship to antiretroviral pharmacokinetics. HIV Med. 2015;16(3):176-83.
23. Wadelius M, Darj E, Frenne G, Rane A. Induction of CYP2D6 in pregnancy. Clin Pharmacol Ther. 1997;62(4):400-7.
24. Tomson T, Lindbom U, Ekqvist B, Sundqvist A. Disposition of carbamazepine and phenytoin in pregnancy. Epilepsia. 1994;35(1):131-5.
25. McGready R, Stepniewska K, Seaton E, Cho T, Cho D, Ginsberg A, et al. Pregnancy and use of oral contraceptives reduces the biotransformation of proguanil to cycloguanil. Eur J Clin Pharmacol. 2003;59(7):553-7.
26. Tsutsumi K, Kotegawa T, Matsuki S, Tanaka Y, Ishii Y, Kodama Y, et al. The effect of pregnancy on cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activities in humans. Clin Pharmacol Ther. 2001;70(2):121-5.
27. Pennell PB, Newport DJ, Stowe ZN, Helmers SL, Montgomery JQ, Henry TR. The impact of pregnancy and childbirth on the metabolism of lamotrigine. Neurology. 2004;62(2):292-5.
28. Brazier JL, Ritter J, Berland M, Khenfer D, Faucon G. Pharmacokinetics of caffeine during and after pregnancy. Dev Pharmacol Ther. 1983;6(5):315-22.
29. Conrad KP. Mechanisms of renal vasodilation and hyperfiltration during pregnancy. J Soc Gynecol Investig. 2004;11(7):438-48.
30. Anderson GD. Pregnancy-induced changes in pharmacokinetics: A mechanistic-based approach. Clin Pharmacokinet. 2005;44(10):989-1008.
31. De Sousa Mendes M, Hirt D, Urien S, Valade E, Bouazza N, Foissac F, et al. Physiologically-based pharmacokinetic modeling of renally excreted antiretroviral drugs in pregnant women. Br J Clin Pharmacol. 2015;80(5):1031-41.
32. Hebert MF, Easterling TR, Kirby B, Carr DB, Buchanan ML, Rutherford T, et al. Effects of pregnancy on CYP3A and P-glycoprotein activities as measured by disposition of midazolam and digoxin: a University of Washington specialized center of research study. Clin Pharmacol Ther. 2008;84(2):248-53.
33. CMACE R. Management of women with obesity in pregnancy. London RCOG. 2010.
34. WHO OE. Body mass index - BMI Euro.who.int: World Health Organization/Europe; [Available from: https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi.
35. Orr K, Chien P. Sepsis in obese pregnant women. Best Pract Res Clin Obstet Gynaecol. 2015;29(3):377-93.

36. Maternal obesity in the UK: Findings from a national project. Centre for Maternal and Child Enquiries CMACE London 2010. 2010.
37. Lewis G. Confidential enquiry into maternal and child health: Saving mother's lives: Reviewing maternal deaths to make motherhood safer-2003-2005: Executive summary and key recommendations: December 2007: CEMACH; 2007.
38. Knibbe CA, Brill MJ, van Rongen A, Diepstraten J, van der Graaf PH, Danhof M. Drug disposition in obesity: Toward evidence-based dosing. Annu Rev Pharmacol Toxicol. 2015;55:149-67.
39. Alexander JK, Dennis EW, Smith WG, Amad KH, Duncan WC, Austin RC. Blood volume, cardiac output, and distribution of systemic blood flow in extreme obesity. Cardiovasc Res Cent Bull. 1962;1:39-44.
40. Lemmens HJ, Bernstein DP, Brodsky JB. Estimating blood volume in obese and morbidly obese patients. Obes Surg. 2006;16(6):773-6.
41. Casati A, Putzu M. Anesthesia in the obese patient: Pharmacokinetic considerations. J Clin Anesth. 2005;17(2):134-45.
42. van Kralingen S, Taks M, Diepstraten J, van de Garde EM, van Dongen EP, Wiezer MJ, et al. Pharmacokinetics and protein binding of cefazolin in morbidly obese patients. Eur J Clin Pharmacol. 2011;67(10):985-92.
43. Blouin R, Kolpek J, Mann H. Influence of obesity on drug disposition. Clin Pharm. 1987;6(9):706-14.
44. Kotlyar M, Carson SW. Effects of obesity on the cytochrome P450 enzyme system. Int J Clin Pharmacol Ther. 1999;37(1):8-19.
45. Acosta EP, Schwebke K, Henry K, Fletcher CV. Disposition of zidovudine in obese pregnant women with human immunodeficiency virus type 1 infection. J Infect Dis. 1996;174(3):673.
46. Stubert J, Reister F, Hartmann S, Janni W. The Risks Associated With Obesity in Pregnancy. Dtsch Arztebl Int. 2018;115(16):276-83.
47. Felisbino-Mendes MS, Matozinhos FP, Miranda JJ, Villamor E, Velasquez-Melendez G. Maternal obesity and fetal deaths: results from the Brazilian cross-sectional Demographic Health Survey, 2006. BMC Pregnancy Childbirth. 2014;14(1):1-10.
48. Ovesen P, Rasmussen S, Kesmodel U. Effect of prepregnancy maternal overweight and obesity on pregnancy outcome. Obstet Gynecol. 2011;118(2 Part 1):305-12.
49. Kim SS, Zhu Y, Grantz KL, Hinkle SN, Chen Z, Wallace ME, et al. Obstetric and neonatal risks among obese women without chronic disease. Obstet Gynecol. 2016;128(1):104.
50. Lisonkova S, Muraca GM, Potts J, Liauw J, Chan W-S, Skoll A, et al. Association between prepregnancy body mass index and severe maternal morbidity. JAMA. 2017;318(18):1777-86.
51. Cnattingius S, Villamor E, Johansson S, Bonamy A-KE, Persson M, Wikström A-K, et al. Maternal obesity and risk of preterm delivery. JAMA. 2013;309(22):2362-70.
52. Persson M, Cnattingius S, Villamor E, Söderling J, Pasternak B, Stephansson O, et al. Risk of major congenital malformations in relation to maternal overweight and obesity severity: cohort study of 1.2 million singletons. BMJ. 2017;357.

53. Bragg F, Cromwell DA, Edozien LC, Gurol-Urganci I, Mahmood TA, Templeton A, et al. Variation in rates of caesarean section among English NHS trusts after accounting for maternal and clinical risk: Cross sectional study. BMJ. 2010;341:c5065.
54. Sebire NJ, Jolly M, Harris JP, Wadsworth J, Joffe M, Beard RW, et al. Maternal obesity and pregnancy outcome: A study of 287 213 pregnancies in London. Int J Obes. 2001;25(8):1175-82.
55. Network EMM. Pregnant women with a raised BMI, best practice standards of care 2015.
56. Holt DE, Broadbent M, Spencer JAD, Delouvois J, Hurley R, Harvey D. The placental-transfer of cefuroxime at parturition. Eur J Obstet Gynecol. 1994;54(3):177-80.
57. Langesæter E, Dyer RA. Maternal haemodynamic changes during spinal anaesthesia for caesarean section. Curr Opin Anaesthesiol. 2011;24(3):242-8.
58. Machado LS. Cesarean section in morbidly obese parturients: Practical implications and complications. N Am J Med Sci. 2012;4(1):13.
59. Perlow JH, Morgan MA. Massive maternal obesity and perioperative cesarean morbidity. Am J Obstet Gynecol. 1994;170(2):560-5.
60. Karlsson EA, Marcelin G, Webby RJ, Schultz-Cherry S. Review on the impact of pregnancy and obesity on influenza virus infection. Influenza Other Respir Viruses. 2012;6(6):449-60.
61. Anaya DA, Dellinger EP. The obese surgical patient: a susceptible host for infection. Surg Infect (Larchmt). 2006;7(5):473-80.
62. Lawson EH, Hall BL, Ko CY. Risk factors for superficial vs deep/organ-space surgical site onfections: Implications for quality improvement initiatives. JAMA Surgery. 2013;148(9):849-58.
63. Watts DH, Krohn MA, Hillier SL, Eschenbach DA. The association of occult amniotic fluid infection with gestational age and neonatal outcome among women in preterm labor. Obstet Gynecol. 1992;79(3):351-7.
64. Martens MG, Kolrud BL, Faro S, Maccato M, Hammill H. Development of wound infection or separation after cesarean delivery. Prospective evaluation of 2,431 cases. J Reprod Med. 1995;40(3):171-5.
65. Roberts S, Maccato M, Faro S, Pinell P. The microbiology of post-cesarean wound morbidity. Obstet Gynecol. 1993;81(3):383-6.
66. Kawakita T, Landy HJ. Surgical site infections after cesarean delivery: Epidemiology, prevention and treatment. Matern health neonatol perinatol. 2017;3(1):12.
67. Maccato M, Faro S, Summers KL. Wound infections after cesarean section with Mycoplasma hominis and Ureaplasma urealyticum a report of three cases. Diagn Microbiol Infect Dis. 1990;13(5):363-5.
68. Gibbs RS, Blanco JD, Bernstein S. Role of aerobic gram-negative bacilli in endometritis after cesarean section. Rev Infect Dis. 1985;7 Suppl 4:S690-5.
69. Emmons SL, Krohn M, Jackson M, Eschenbach DA. Development of wound infections among women undergoing cesarean section. Obstet Gynecol. 1988;72(4):559-64.
70. Zejnullahu VA, Isjanovska R, Sejfija Z, Zejnullahu VA. Surgical site infections after cesarean sections at the University Clinical Center of Kosovo: Rates, microbiological profile and risk factors. BMC Infect Dis. 2019;19(1):752.

71. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. Am J Infect Control. 1992;20(5):271-4.
72. Kamal SS, Hyldig N, Krych Ł, Greisen G, Krogfelt KA, Zachariassen G, et al. Impact of early exposure to cefuroxime on the composition of the gut microbiota in infants following cesarean delivery. J Pediatr. 2019;210:99-105. e2.
73. Heesen M, Klöhr S, Rossaint R, Allegeaert K, Deprest J, Van de Velde M, et al. Concerning the timing of antibiotic administration in women undergoing caesarean section: a systematic review and meta-analysis. BMJ Open. 2013;3(4):e002028.
74. Caesarean birth, NICE guideline [NG192] [Internet]. 2021 [cited 28/06/20121]. Available from: https://www.nice.org.uk/guidance/ng192.
75. Bergeron MG, Brusch JL, Barza M, Weinstein L. Bactericidal activity and pharmacology of cefazolin. Antimicrob Agents Chemother. 1973;4(4):396-401.
76. Gustaferro CA, Steckelberg JM, editors. Cephalosporin antimicrobial agents and related compounds. Mayo Clin Proc; 1991: Elsevier.
77. Viberg A, Lannergård A, Larsson A, Cars O, Karlsson MO, Sandström M. A population pharmacokinetic model for cefuroxime using cystatin C as a marker of renal function. Br J Clin Pharmacol. 2006;62(3):297-303.
78. Chen X, Brathwaite CE, Barkan A, Hall K, Chu G, Cherasard P, et al. Optimal cefazolin prophylactic dosing for bariatric surgery: No need for higher doses or intraoperative redosing. Obes Surg. 2017;27(3):626-9.
79. Turnidge JD, on behalf of the Subcommittee on Antimicrobial Susceptibility Testing of the C, Laboratory Standards I. Cefazolin and enterobacteriaceae: Rationale for revised susceptibility testing breakpoints. Clin Infect Dis. 2011;52(7):917-24.
80. Racusin DA, Vidaeff AC. Cesarean delivery in the obese parturient. Evid-based obstet gynecol. 2019:593-9.
81. Weinstein MP. M100-Performance standards for antimicrobial susceptibility testing, 28th edition: Clinical and Laboratory; 2018.
82. Alrammaal HH, Batchelor HK, Chong HP, Hodgetts Morton V, Morris RK. Prophylactic perioperative cefuroxime levels in plasma and adipose tissue at the time of caesarean section (C-LACE): a protocol for a pilot experimental, prospective study with non-probability sampling to determine interpatient variability. Pilot Feasibility Stud. 2021;7(1):54.
83. Andrews J. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001;48:5-16.
84. Washington JA, Jones RN, Gerlach EH, Murray PR, Allen SD, Knapp CC. Multicenter comparison of in vitro activities of FK-037, cefepime, ceftriaxone, ceftazidime, and cefuroxime. Antimicrob Agents Chemother. 1993;37(8):1696.
85. Shanahan PMA, Thomson CJ, Miles RS, Old D, Hood J, Gould IM, et al. Antibiotic resistance in nosocomial isolates in Scotland. Int J Antimicrob Agents. 1996;7(2):153-60.
86. ZINACEF® (cefuroxime for injection) [Internet]. FDA. 2001 [cited 04/07/2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2001/50643s11lbl.pdf.
87. Takayama Y, Komatsu T, Nakamura T, Tomoda Y, Toda M, Miura H, et al. Association of serum and fat tissue antibiotic concentrations with surgical site infections in lower gastrointestinal surgery. Surgery. 2021.

88. Nielsen EI, Cars O, Friberg LE. Pharmacokinetic/pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: A step toward model-based dose optimization. Antimicrob Agents Chemother. 2011;55(10):4619-30.
89. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis. 1998;26(1):1-10.
90. Rodgers T, Rowland M. Physiologically based pharmacokinetic modelling 2: Predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. J Pharm Sci. 2006;95(6):1238-57.
91. Zeilmaker-Roest GA, van Saet A, van Rosmalen J, Bahmany S, van Dijk A, Wildschut ED, et al. Potentially clinically relevant concentrations of Cefazolin, Midazolam, Propofol, and Sufentanil in auto-transfused blood in congenital cardiac surgery. J Cardiothorac Surg. 2018;13(1):64.
92. Zeilmaker-Roest GA, van Saet A, van Hoeven MP, Koch BC, van Rosmalen J, Kinzig M, et al. Recovery of cefazolin and clindamycin in in vitro pediatric CPB systems. Artif Organs. 2020;44(4):394-401.
93. Dąbrowska M, Komsta Ł, Krzek J, Kokoszka K. Lipophilicity study of eight cephalosporins by reversed-phase thin-layer chromatographic method. Biomed Chromatogr. 2015;29(11):1759-68.
94. Vella-Brincat JW, Begg EJ, Kirkpatrick CM, Zhang M, Chambers ST, Gallagher K. Protein binding of cefazolin is saturable in vivo both between and within patients. Br J Clin Pharmacol. 2007;63(6):753-7.
95. Karalis V, Tsantili-Kakoulidou A, Macheras P. Quantitative structure–pharmacokinetic relationships for disposition parameters of cephalosporins. Eur J Pharm Sci. 2003;20(1):115-23.
96. Dallmann A, Ince I, Solodenko J, Meyer M, Willmann S, Eissing T, et al. Physiologically based pharmacokinetic modeling of renally cleared drugs in pregnant women. Clin Pharmacokinet. 2017;56(12):1525-41.
97. Ueo H, Motohashi H, Katsura T, Inui K-i. Human organic anion transporter hOAT3 is a potent transporter of cephalosporin antibiotics, in comparison with hOAT1. Biochem Pharmacol. 2005;70(7):1104-13.
98. Uwai Y, Saito H, Inui K-i. Rat renal organic anion transporter rOAT1 mediates transport of urinary-excreted cephalosporins, but not of biliary-excreted cefoperazone. Drug Metab Pharmacokinet. 2002;17(2):125-9.
99. Ci L, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, Sugiyama Y. Involvement of MRP4 (ABCC4) in the luminal efflux of ceftizoxime and cefazolin in the kidney. Mol Pharmacol. 2007.
100. Philipson A, Stiernstedt G, Ehrnebo M. Comparison of the pharmacokinetics of cephradine and cefazolin in pregnant and non-pregnant women. Clin Pharmacokinet. 1987;12(2):136-44.
101. Allegaert K, Van Mieghem T, Verbesselt R, Vanhole C, Devlieger R, Cossey V, et al. Cefazolin plasma protein binding saturability during pregnancy. Methods Find Exp Clin Pharmacol. 2009;31(1):25-8.
102. Elkomy MH, Sultan P, Drover DR, Epshtein E, Galinkin JL, Carvalho B. Pharmacokinetics of prophylactic cefazolin in parturients undergoing cesarean delivery. Antimicrob Agents Chemother. 2014;58(6):3504-13.
103. Ram M, Lavie A, Lev S, Blecher Y, Amikam U, Shulman Y, et al. Cardiac hemodynamics before, during and after elective cesarean section under spinal anesthesia in low-risk women. J Perinatol. 2017;37(7):793-9.

104. Bhalodi AA, Housman ST, Shepard A, Nugent J, Nicolau DP. Tissue pharmacokinetics of cefazolin in patients with lower limb infections. Antimicrob Agents Chemother. 2013;57(11):5679-83.
105. Hall J, Guyton A. The body fluid compartments: extracellular and intracellular fluids; edema. Hall JE In: Guyton and Hall textbook of medical physiology 13th ed Philadelphia, Elsevier. 2016:305-21.
106. Bratzler DW, Dellinger EP, Olsen KM, Perl TM, Auwaerter PG, Bolon MK, et al. Clinical practice guidelines for antimicrobial prophylaxis in surgery. Am J Health Syst Pharm. 2013;70(3):195-283.
107. Decroix MO, Zini R, Chaumeil JC, Tillement JP. Cefazolin serum protein binding and its inhibition by bilirubin, fatty acids and other drugs. Biochem Pharmacol. 1988;37(14):2807-14.
108. Foord R. Cefuroxime: human pharmacokinetics. Antimicrob Agents Chemother. 1976;9(5):741-7.
109. Philipson A, Stiernstedt G. Pharmacokinetics of cefuroxime in pregnancy. Am J Obstet Gynecol. 1982;142(7):823-8.
110. Barbour A, Schmidt S, Rout WR, Ben-David K, Burkhardt O, Derendorf H. Soft tissue penetration of cefuroxime determined by clinical microdialysis in morbidly obese patients undergoing abdominal surgery. Int J Antimicrob Agents. 2009;34(3):231-5.
111. Vo M. C- Steady-state volume of distribution (Vss) Professional Education, Testing and Certification Organization International: Professional Education, Testing and Certification Organization International; 2003 [Available from: https://www.peoi.org/Courses/Coursesen/phkin/ch/ch3c.
112. Coppi G, Berti MA, Chehade A. A study of the transplacental transfer of cefuroxime in humans. Curr Ther Res Clin Exp. 1982;32:712-6.
113. Hsu V, de LTVM, Zhao P, Zhang L, Zheng JH, Nordmark A, et al. Towards quantitation of the effects of renal impairment and probenecid inhibition on kidney uptake and efflux transporters, using physiologically based pharmacokinetic modelling and simulations. Clin Pharmacokinet. 2014;53(3):283-93.
114. Ristuccia AM, Le Frock JL. Cerebrospinal fluid penetration of antimicrobials. Bacterial Meningitis. 45: Karger Publishers; 1992. p. 118-52.
115. Mrestani Y, Mrestani-Klaus C, Bretschneider B, Neubert RH. Improvement of lipophilicity and membrane transport of cefuroxime using in vitro models. Eur J Pharm Biopharm. 2004;58(3):653-7.
116. Badhan RKS. Physiologically based pharmacokinetic modelling in drug delivery. Computational pharmaceutics: Wiley; 2015. p. 263-91.
117. Teorell T. Kinetics of distribution of substances administered to the body, I: The extravascular modes of administration. Archives internationales de pharmacodynamie et de therapie. 1937;57:205-25.
118. Chan R, De Bruyn T, Wright M, Broccatelli F. Comparing mechanistic and preclinical predictions of volume of distribution on a large set of drugs. Pharm Res. 2018;35(4):87.
119. Buist H. Characterization and application of physiologically based pharmacokinetic models in risk assessment. 2010.
120. Jamei M, Marciniak S, Edwards D, Wragg K, Feng K, Barnett A, et al. The simcyp population based simulator: architecture, implementation, and quality assurance. In Silico Pharmacol. 2013;1(1):9.

121. Zhao P, Zhang L, Grillo J, Liu Q, Bullock J, Moon Y, et al. Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. Clin Pharmacol Ther. 2011;89(2):259-67.
122. Simcyp_Version.20. Help_File section: Compound screens > Distribution (PBPK) > Parameter definitions (Distribution) > Choose the model type 2021.
123. Simcyp_Version.20. Screenshot. 20 ed2021. p. image used with permission from Certara UK.
124. Simcyp_Version.20. Help_File section: Compound screens > Distribution (PBPK) > Distribution overview > Simcyp distribution models 2021.
125. Simcyp_Version.20. Help_File section: Compound screens > Distribution (PBPK) > Distribution overview > Volume of distribution 2021.
126. Sawada Y, Hanano M, Sugiyama Y, Harashima H, Iga T. Prediction of the volumes of distribution of basic drugs in humans based on data from animals. J Pharmacokinet Biopharm. 1984;12(6):587-96.
127. Simcyp_Version.20. Help_File section: Populations > Tissue composition screen > Parameter definitions (tissue composition) > Tissue composition and binding protein partition (tissue composition) 2021.
128. Simcyp_Version.20. Help_File section: Compound screens > Distribution (PBPK) > Distribution overview > Tissue composition data 2021.
129. Gibaldi M, Perrier D. Route of Administration and Drug Disposition. Drug Metab Rev. 1975;3(1):185-99.
130. Simcyp_Version.20. Help_File section: Compound screens > Distribution (PBPK) > Distribution overview > Calculation of tissue partition coefficients. 2021.
131. Simcyp_Version.20. Help_File section: Compound screens > Distribution (PBPK) > Parameter definitions (Distribution) > Kp scalar 2021.
132. Simcyp_Version.20. Help_File section: Compound Screens > Distribution (PBPK) > Parameter Definitions (Distribution) > Tissue: Plasma Partition Coefficient 2021.
133. Poulin P, Theil FP. Prediction of pharmacokinetics prior to in vivo studies. 1. Mechanism-based prediction of volume of distribution. J Pharm Sci. 2002;91(1):129-56.
134. Berezhkovskiy LM. Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination. J Pharm Sci. 2004;93(6):1628-40.
135. Rodgers T, Leahy D, Rowland M. Physiologically based pharmacokinetic modeling 1: Predicting the tissue distribution of moderate-to-strong bases. J Pharm Sci. 2005;94(6):1259-76.
136. Rodgers T, Leahy D, Rowland M. Tissue distribution of basic drugs: accounting for enantiomeric, compound and regional differences amongst beta-blocking drugs in rat. J Pharm Sci. 2005;94(6):1237-48.
137. Rodgers T, Rowland M. Mechanistic approaches to volume of distribution predictions: Understanding the processes. Pharm Res. 2007;24(5):918-33.
138. Gaohua L, Turner D, Fisher C, Riedmaier A, Musther H, Gardner L, et al. A novel mechanistic approach to predict the steady state volume of distribution (Vss) using the Fick-Nernst-Planck equation. Drug/Disease modeling - Absorption & PBPK: Drug/Disease modeling - Absorption & PBPK; 2016. p. 25.
139. CERTARA. Simcyp workshop : Hands-on experience with model-informed drug development: Incorporating population variability into mechanistic prediction of PK and modeling PK/PD. [Lecture book from Simcyp workshop]. In press 2018.

140. Obach RS, Lombardo F, Waters NJ. Trend analysis of a database of intravenous pharmacokinetic parameters in humans for 670 drug compounds. Drug Metab Dispos. 2008;36(7):1385-405.
141. Neuhoff S, Gaohua L, Burt H, Jamei M, Li L, Tucker GT, et al. Accounting for transporters in renal clearance: Towards a mechanistic kidney model (Mech KiM). In: Sugiyama Y, Steffansen B, editors. Transporters in Drug Development: Discovery, Optimization, Clinical Study and Regulation. New York, NY: Springer New York; 2013. p. 155-77.
142. Burt HJ, Neuhoff S, Almond L, Gaohua L, Harwood MD, Jamei M, et al. Metformin and cimetidine: Physiologically based pharmacokinetic modelling to investigate transporter mediated drug–drug interactions. Eur J Pharm Sci. 2016;88:70-82.
143. Simcyp_Version.20. Help_File section: Populations > Kidney data screen > Parameter definitions (kidney data) > Nephron parameters 2021.
144. Simcyp_Version.20. Help_File section: Compound screens > Elimination > Elimination overview > Non-metabolic renal clearance 2021.
145. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41.
146. Spruill WJ, Wade WE, Cobb HH, 3rd. Estimating glomerular filtration rate with a modification of diet in renal disease equation: implications for pharmacy. Am J Health Syst Pharm. 2007;64(6):652-60.
147. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy substrate and inhibitor > Transport 2021.
148. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy population data 2021.
149. Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H. Anatomical, physiological and metabolic changes with gestational age during normal pregnancy: a database for parameters required in physiologically based pharmacokinetic modelling. Clin Pharmacokinet. 2012;51(6):365-96.
150. Abduljalil K, Jamei M, Johnson TN. Fetal physiologically based pharmacokinetic models: Systems information on fetal blood components and binding proteins. Clin Pharmacokinet. 2020;59(5):629-42.
151. Abduljalil K, Jamei M, Johnson TN. Fetal physiologically based pharmacokinetic models: Systems information on the growth and composition of fetal organs. Clin Pharmacokinet. 2019;58(2):235-62.
152. Abduljalil K, Johnson TN, Rostami-Hodjegan A. Fetal physiologically-based pharmacokinetic models: Systems information on fetal biometry and gross composition. Clin Pharmacokinet. 2018;57(9):1149-71.
153. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Main population tabs 2021.
154. Simcyp_Version.20. Help_File section: Populations > Introduction to populations > Distribution functions. 2021.
155. Chapman AB, Abraham WT, Zamudio S, Coffin C, Merouani A, Young D, et al. Temporal relationships between hormonal and hemodynamic changes in early human pregnancy. Kidney Int. 1998;54(6):2056-63.
156. Butte NF, Ellis KJ, Wong WW, Hopkinson JM, Smith EO. Composition of gestational weight gain impacts maternal fat retention and infant birth weight. Am J Obstet Gynecol. 2003;189(5):1423-32.

157. Kopp-Hoolihan LE, van Loan MD, Wong WW, King JC. Longitudinal assessment of energy balance in well-nourished, pregnant women. Am J Clin Nutr. 1999;69(4):697-704.
158. Lof M, Olausson H, Bostrom K, Janerot-Sjöberg B, Sohlstrom A, Forsum E. Changes in basal metabolic rate during pregnancy in relation to changes in body weight and composition, cardiac output, insulin-like growth factor I, and thyroid hormones and in relation to fetal growth. Am J Clin Nutr. 2005;81(3):678-85.
159. Lof M, Hilakivi-Clarke L, Sandin SS, de Assis S, Yu W, Weiderpass E. Dietary fat intake and gestational weight gain in relation to estradiol and progesterone plasma levels during pregnancy: A longitudinal study in Swedish women. BMC Womens Health. 2009;9:10.
160. Abrams B, Selvin S. Maternal weight gain pattern and birth weight. Obstet Gynecol. 1995;86(2):163-9.
161. Catalano PM, Roman-Drago NM, Amini SB, Sims EA. Longitudinal changes in body composition and energy balance in lean women with normal and abnormal glucose tolerance during pregnancy. Am J Obstet Gynecol. 1998;179(1):156-65.
162. Groot L, Boekholt H, Spaaij C, van Raaij J, Drijvers JJM, Heijden L, et al. Energy balances of healthy Dutch women before and during pregnancy: Limited scope for metabolic adaptations in pregnancy. Am J Clin Nutr. 1994;59:827-32.
163. Edouard DA, Pannier BM, London GM, Cuche JL, Safar ME. Venous and arterial behavior during normal pregnancy. Am J Physiol. 1998;274(5):H1605-12.
164. Ghezzi F, Franchi M, Balestreri D, Lischetti B, Mele MC, Alberico S, et al. Bioelectrical impedance analysis during pregnancy and neonatal birth weight. Eur J Obstet Gynecol Reprod Biol. 2001;98(2):171-6.
165. Larciprete G, Valensise H, Vasapollo B, Altomare F, Sorge R, Casalino B, et al. Body composition during normal pregnancy: reference ranges. Acta Diabetol. 2003;40 Suppl 1:S225-32.
166. Wolfe WS, Sobal J, Olson CM, Frongillo EA, Jr., Williamson DF. Parity-associated weight gain and its modification by sociodemographic and behavioral factors: A prospective analysis in US women. Int J Obes Relat Metab Disord. 1997;21(9):802-10.
167. Hronek M, Klemera P, Tosner J, Hrnciarikova D, Zadak Z. Anthropometric measured fat-free mass as essential determinant of resting energy expenditure for pregnant and non-pregnant women. Nutrition. 2011;27(9):885-90.
168. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy population design. 2021.
169. Kopp-Hoolihan LE, van Loan MD, Wong WW, King JC. Fat mass deposition during pregnancy using a four-component model. J Appl Physiol (1985). 1999;87(1):196-202.
170. Goldberg GR, Prentice AM, Coward WA, Davies HL, Murgatroyd PR, Sawyer MB, et al. Longitudinal assessment of the components of energy balance in well-nourished lactating women. Am J Clin Nutr. 1991;54(5):788-98.
171. Lederman SA, Paxton A, Heymsfield SB, Wang J, Thornton J, Pierson RN, Jr. Body fat and water changes during pregnancy in women with different body weight and weight gain. Obstet Gynecol. 1997;90(4 Pt 1):483-8.
172. Van Loan MD, Kopp LE, King JC, Wong WW, Mayclin PL. Fluid changes during pregnancy: use of bioimpedance spectroscopy. J Appl Physiol (1985). 1995;78(3):1037-42.

173. Salas SP, Marshall G, Gutiérrez BL, Rosso P. Time course of maternal plasma volume and hormonal changes in women with preeclampsia or fetal growth restriction. Hypertension. 2006;47(2):203-8.
174. Bernstein IM, Ziegler W, Badger GJ. Plasma volume expansion in early pregnancy. Obstet Gynecol. 2001;97(5 Pt 1):669-72.
175. Lund CJ, Donovan JC. Blood volume during pregnancy. Significance of plasma and red cell volumes. Am J Obstet Gynecol. 1967;98(3):394-403.
176. Whittaker PG, Lind T. The intravascular mass of albumin during human pregnancy: a serial study in normal and diabetic women. Br J Obstet Gynaecol. 1993;100(6):587-92.
177. Campbell DM, MacGillivray I. Comparison of maternal response in first and second pregnancies in relation to baby weight. J Obstet Gynaecol Br Commonw. 1972;79(8):684-93.
178. Rovinsky JJ, Jaffin H. Cardiovascular hemodynamics in pregnancy. I. Blood and plasma volumes in multiple pregnancy. Am J Obstet Gynecol. 1965;93:1-15.
179. Caton WL, Roby CC, Reid DE, Caswell R, Maletskos CJ, Fluharty RG, et al. The circulating red cell volume and body hematocrit in normal pregnancy and the puerperium by direct measurement using radioactive red cells. Am J Obstet Gynecol. 1951;61(6):1207-17.
180. Honger PE. Intravascular mass of albumin in pre-eclampsia and normal pregnancy. Scand J Clin Lab Invest. 1967;19(3):283-7.
181. Verel D, Bury JD, Hope A. Blood volume changes in pregnancy and the puerperium. Clin Sci. 1956;15(1):1-7.
182. Whittaker PG, Macphail S, Lind T. Serial hematologic changes and pregnancy outcome. Obstet Gynecol. 1996;88(1):33-9.
183. Bruinse HW, van den Berg H, Haspels AA. Smoking and its effect on maternal plasma volume during and after normal pregnancy. Eur J Obstet Gynecol Reprod Biol. 1985;20(4):215-9.
184. Cope I. Plasma and blood volume changes in late and prolonged pregnancy. J Obstet Gynaecol Br Emp. 1958;65(6):877-94.
185. Hytten FE, Paintin DB. Increase in plasma volume during normal pregnancy. J Obstet Gynaecol Br Emp. 1963;70:402-7.
186. Pirani BB, Campbell DM, MacGillivray I. Plasma volume in normal first pregnancy. J Obstet Gynaecol Br Commonw. 1973;80(10):884-7.
187. Silver HM, Seebeck M, Carlson R. Comparison of total blood volume in normal, preeclamptic, and nonproteinuric gestational hypertensive pregnancy by simultaneous measurement of red blood cell and plasma volumes. Am J Obstet Gynecol. 1998;179(1):87-93.
188. Jirásek JE, Uher J, Uhrová M. Water and nitrogen content of the body of young human embryos. Am J Obstet Gynecol. 1966;96(6):868-71.
189. Wilcox M, Gardosi J, Mongelli M, Ray C, Johnson I. Birth weight from pregnancies dated by ultrasonography in a multicultural British population. BMJ. 1993;307(6904):588-91.
190. Clapp JF, 3rd, Capeless E. Cardiovascular function before, during, and after the first and subsequent pregnancies. Am J Cardiol. 1997;80(11):1469-73.
191. Lof M, Forsum E. Evaluation of bioimpedance spectroscopy for measurements of body water distribution in healthy women before, during, and after pregnancy. J Appl Physiol (1985). 2004;96(3):967-73.

192. P. D. Body composition techniques in health and disease. Cambridge: Cambridge University Press; 1995.
193. Frederiksen MC, Ruo TI, Chow MJ, Atkinson AJ, Jr. Theophylline pharmacokinetics in pregnancy. Clin Pharmacol Ther. 1986;40(3):321-8.
194. Valensise H, Andreoli A, Lello S, Magnani F, Romanini C, De Lorenzo A. Multifrequency bioelectrical impedance analysis in women with a normal and hypertensive pregnancy. Am J Clin Nutr. 2000;72(3):780-3.
195. van Marken Lichtenbelt WD, Snel YE, Brummer RJ, Koppeschaar HP. Deuterium and bromide dilution, and bioimpedance spectrometry independently show that growth hormone-deficient adults have an enlarged extracellular water compartment related to intracellular water. J Clin Endocrinol Metab. 1997;82(3):907-11.
196. Hale SA, Schonberg A, Badger GJ, Bernstein IM. Relationship between prepregnancy and early pregnancy uterine blood flow and resistance index. Reprod Sci. 2009;16(11):1091-6.
197. Dunlop W. Serial changes in renal haemodynamics during normal human pregnancy. Br J Obstet Gynaecol. 1981;88(1):1-9.
198. Assali NS, Dignam WJ, Dasgupta K. Renal function in human pregnancy. II. Effects of venous pooling on renal hemodynamics and water, electrolyte, and aldosterone excretion during gestation. J Lab Clin Med. 1959;54:394-408.
199. Irons DW, Baylis PH, Davison JM. Effect of atrial natriuretic peptide on renal hemodynamics and sodium excretion during human pregnancy. Am J Physiol. 1996;271(1 Pt 2):F239-42.
200. Sims EA, Krantz KE. Serial studies of renal function during pregnancy and the puerperium in normal women. J Clin Invest. 1958;37(12):1764-74.
201. Spaanderman M, Ekhart T, van Eyck J, de Leeuw P, Peeters L. Preeclampsia and maladaptation to pregnancy: A role for atrial natriuretic peptide? Kidney Int. 2001;60(4):1397-406.
202. Sturgiss SN, Wilkinson R, Davison JM. Renal reserve during human pregnancy. Am J Physiol. 1996;271(1 Pt 2):F16-20.
203. De Alvarez RR. Renal glomerulotubular mechanisms during normal pregnancy. I. Glomerular filtration rate, renal plasma flow, and creatinine clearance. Am J Obstet Gynecol. 1958;75(5):931-44.
204. Flo K, Wilsgaard T, Vårtun A, Acharya G. A longitudinal study of the relationship between maternal cardiac output measured by impedance cardiography and uterine artery blood flow in the second half of pregnancy. BJOG. 2010;117(7):837-44.
205. Thaler I, Manor D, Itskovitz J, Rottem S, Levit N, Timor-Tritsch I, et al. Changes in uterine blood flow during human pregnancy. Am J Obstet Gynecol. 1990;162(1):121-5.
206. Bernstein IM, Ziegler WF, Leavitt T, Badger GJ. Uterine artery hemodynamic adaptations through the menstrual cycle into early pregnancy. Obstet Gynecol. 2002;99(4):620-4.
207. Dickey RP, Hower JF. Ultrasonographic features of uterine blood flow during the first 16 weeks of pregnancy. Hum Reprod. 1995;10(9):2448-52.
208. Jeffreys RM, Stepanchak W, Lopez B, Hardis J, Clapp JF, 3rd. Uterine blood flow during supine rest and exercise after 28 weeks of gestation. BJOG. 2006;113(11):1239-47.

209. Konje JC, Howarth ES, Kaufmann P, Taylor DJ. Longitudinal quantification of uterine artery blood volume flow changes during gestation in pregnancies complicated by intrauterine growth restriction. BJOG. 2003;110(3):301-5.
210. Konje JC, Kaufmann P, Bell SC, Taylor DJ. A longitudinal study of quantitative uterine blood flow with the use of color power angiography in appropriate for gestational age pregnancies. Am J Obstet Gynecol. 2001;185(3):608-13.
211. Metcalfe J, Romney SL, Ramsey LH, Reid DE, Burwell CS. Estimation of uterine blood flow in normal human pregnancy at term. J Clin Investig. 1955;34(11):1632-8.
212. Palmer SK, Zamudio S, Coffin C, Parker S, Stamm E, Moore LG. Quantitative estimation of human uterine artery blood flow and pelvic blood flow redistribution in pregnancy. Obstet Gynecol. 1992;80(6):1000-6.
213. Rigano S, Ferrazzi E, Boito S, Pennati G, Padoan A, Galan H. Blood flow volume of uterine arteries in human pregnancies determined using 3D and bi-dimensional imaging, angio-Doppler, and fluid-dynamic modeling. Placenta. 2010;31(1):37-43.
214. Wilson MJ, Lopez M, Vargas M, Julian C, Tellez W, Rodriguez A, et al. Greater uterine artery blood flow during pregnancy in multigenerational (Andean) than shorter-term (European) high-altitude residents. Am J Physiol Regul Integr Comp Physiol. 2007;293(3):R1313-24.
215. Lukaski HC, Siders WA, Nielsen EJ, Hall CB. Total body water in pregnancy: assessment by using bioelectrical impedance. Am J Clin Nutr. 1994;59(3):578-85.
216. Pipe NG, Smith T, Halliday D, Edmonds CJ, Williams C, Coltart TM. Changes in fat, fat-free mass and body water in human normal pregnancy. Br J Obstet Gynaecol. 1979;86(12):929-40.
217. Seitchik J. Total body water and total body density of pregnant women. Obstet Gynecol. 1967;29(2):155-66.
218. Forsum E, Sadurskis A, Wager J. Resting metabolic rate and body composition of healthy Swedish women during pregnancy. Am J Clin Nutr. 1988;47(6):942-7.
219. Clark SL, Cotton DB, Lee W, Bishop C, Hill T, Southwick J, et al. Central hemodynamic assessment of normal term pregnancy. Am J Obstet Gynecol. 1989;161(6 Pt 1):1439-42.
220. Lundstrom P. Studies on erythroid elements and serum iron in normal pregnancy. Acta Soc Med Ups. 1950;55(1/2):1-83.
221. Sala C, Campise M, Ambroso G, Motta T, Zanchetti A, Morganti A. Atrial natriuretic peptide and hemodynamic changes during normal human pregnancy. Hypertension. 1995;25(4 Pt 1):631-6.
222. Duvekot JJ, Cheriex EC, Pieters FA, Menheere PP, Peeters LH. Early pregnancy changes in hemodynamics and volume homeostasis are consecutive adjustments triggered by a primary fall in systemic vascular tone. Am J Obstet Gynecol. 1993;169(6):1382-92.
223. Haram K, Augensen K, Elsayed S. Serum protein pattern in normal pregnancy with special reference to acute-phase reactants. Br J Obstet Gynaecol. 1983;90(2):139-45.
224. Pond SM, Kreek MJ, Tong TG, Raghunath J, Benowitz NL. Altered methadone pharmacokinetics in methadone-maintained pregnant women. J Pharmacol Exp Ther. 1985;233(1):1-6.

225. Connelly TJ, Ruo TI, Frederiksen MC, Atkinson AJ, Jr. Characterization of theophylline binding to serum proteins in pregnant and nonpregnant women. Clin Pharmacol Ther. 1990;47(1):68-72.
226. Mendenhall HW. Serum protein concentrations in pregnancy. I. Concentrations in maternal serum. Am J Obstet Gynecol. 1970;106(3):388-99.
227. Tsen LC, Tarshis J, Denson DD, Osathanondh R, Datta S, Bader AM. Measurements of maternal protein binding of bupivacaine throughout pregnancy. Anesth Analg. 1999;89(4):965-8.
228. Chu CY, Singla VP, Wang HP, Sweet B, Lai LT. Plasma alpha 1-acid glycoprotein levels in pregnancy. Clin Chim Acta. 1981;112(2):235-40.
229. Krauer B, Dayer P, Anner R. Changes in serum albumin and alpha 1-acid glycoprotein concentrations during pregnancy: An analysis of fetal-maternal pairs. Br J Obstet Gynaecol. 1984;91(9):875-81.
230. Tsen LC, Arthur GR, Datta S, Hornstein MD, Bader AM. Estrogen-induced changes in protein binding of bupivacaine during in vitro fertilization. Anesthesiology. 1997;87(4):879-83.
231. Wang HP, Chu CY. A solid-phase enzyme-linked immunosorbent assay for the quantitation of human plasma alpha 1-acid glycoprotein. Clin Chem. 1979;25(4):546-9.
232. Davison JM, Hytten FE. Glomerular filtration during and after pregnancy. J Obstet Gynaecol Br Commonw. 1974;81(8):588-95.
233. Lafayette RA, Druzin M, Sibley R, Derby G, Malik T, Huie P, et al. Nature of glomerular dysfunction in pre-eclampsia. Kidney Int. 1998;54(4):1240-9.
234. Moran P, Baylis PH, Lindheimer MD, Davison JM. Glomerular ultrafiltration in normal and preeclamptic pregnancy. J Am Soc Nephrol. 2003;14(3):648-52.
235. Tracy TS, Venkataramanan R, Glover DD, Caritis SN. Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. Am J Obstet Gynecol. 2005;192(2):633-9.
236. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy screen > Feto-placental tab 2021.
237. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy screen > Feto-placental tab > Placenta tab 2021.
238. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy screen > Feto-placental tab > Amniotic fluid tab 2021.
239. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy screen > Feto-placental tab > Fetal demographic tab 2021.
240. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy screen > Feto-placental tab > Fetal tissue volumes tab 2021.
241. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy screen > Feto-placental tab > Fetal blood flows tab. 2021.
242. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy screen > Feto-placental tab > Fetal composition tab. 2021.
243. Simcyp_Version.20. Help_File section: Simcyp pregnancy > Pregnancy screen > Feto-placental tab > Fetal blood binding tab. 2021.
244. Simcyp_Version.20. Help_File section: Populations > The built-in population libraries > Disease populations > Obesity populations 2021.

245. Ghobadi C, Johnson TN, Aarabi M, Almond LM, Allabi AC, Rowland-Yeo K, et al. Application of a systems approach to the bottom-up assessment of pharmacokinetics in obese patients. Clin Pharmacokinet. 2011;50(12):809-22.
246. Howgate EM, Rowland Yeo K, Proctor NJ, Tucker GT, Rostami-Hodjegan A. Prediction of in vivo drug clearance from in vitro data. I: Impact of inter-individual variability. Xenobiotica. 2006;36(6):473-97.
247. Colles SL, Dixon JB, Marks P, Strauss BJ, O'Brien PE. Preoperative weight loss with a very-low-energy diet: quantitation of changes in liver and abdominal fat by serial imaging. Am J Clin Nutr. 2006;84(2):304-11.
248. Vasan RS. Cardiac function and obesity. Heart. 2003;89(10):1127-9.
249. Messerli FH, Sundgaard-Riise K, Reisin E, Dreslinski G, Dunn FG, Frohlich E. Disparate cardiovascular effects of obesity and arterial hypertension. Am J Med. 1983;74(5):808-12.
250. Abernethy DR, Greenblatt DJ, Divoll M, Smith RB, Shader RI. The influence of obesity on the pharmacokinetics of oral alprazolam and triazolam. Clin Pharmacokinet. 1984;9(2):177-83.
251. Benedek IH, Blouin RA, McNamara PJ. Serum protein binding and the role of increased alpha 1-acid glycoprotein in moderately obese male subjects. Br J Clin Pharmacol. 1984;18(6):941-6.
252. Benedek IH, Fiske WD, 3rd, Griffen WO, Bell RM, Blouin RA, McNamara PJ. Serum alpha 1-acid glycoprotein and the binding of drugs in obesity. Br J Clin Pharmacol. 1983;16(6):751-4.
253. Caraco Y, Zylber-Katz E, Berry EM, Levy M. Significant weight reduction in obese subjects enhances carbamazepine elimination. Clin Pharmacol Ther. 1992;51(5):501-6.
254. Caraco Y, Zylber-Katz E, Berry EM, Levy M. Carbamazepine pharmacokinetics in obese and lean subjects. Ann Pharmacother. 1995;29(9):843-7.
255. Dunn TE, Ludwig EA, Slaughter RL, Camara DS, Jusko WJ. Pharmacokinetics and pharmacodynamics of methylprednisolone in obesity. Clin Pharmacol Ther. 1991;49(5):536-49.
256. Flechner SM, Kolbeinsson ME, Tam J, Lum B. The impact of body weight on cyclosporine pharmacokinetics in renal transplant recipients. Transplantation. 1989;47(5):806-10.
257. Greenblatt DJ, Abernethy DR, Locniskar A, Harmatz JS, Limjuco RA, Shader RI. Effect of age, gender, and obesity on midazolam kinetics. Anesthesiology. 1984;61(1):27-35.
258. Bentley JB, Vaughan RW, Gandolfi AJ, Cork RC. Halothane biotransformation in obese and nonobese patients. Anesthesiology. 1982;57(2):94-7.
259. Bentley JB, Vaughan RW, Miller MS, Calkins JM, Gandolfi AJ. Serum inorganic fluoride levels in obese patients during and after enflurane anesthesia. Anesth Analg. 1979;58(5):409-12.
260. Frink EJ, Jr., Malan TP, Jr., Brown EA, Morgan S, Brown BR, Jr. Plasma inorganic fluoride levels with sevoflurane anesthesia in morbidly obese and nonobese patients. Anesth Analg. 1993;76(6):1333-7.
261. Higuchi H, Satoh T, Arimura S, Kanno M, Endoh R. Serum inorganic fluoride levels in mildly obese patients during and after sevoflurane anesthesia. Anesth Analg. 1993;77(5):1018-21.

262. Miller MS, Gandolfi AJ, Vaughan RW, Bentley JB. Disposition of enflurane in obese patients. J Pharmacol Exp Ther. 1980.
263. O'Shea D, Davis SN, Kim RB, Wilkinson GR. Effect of fasting and obesity in humans on the 6-hydroxylation of chlorzoxazone: A putative probe of CYP2E1 activity. Clin Pharmacol Ther. 1994;56(4):359-67.
264. Young SR, Stoelting RK, Peterson C, Madura JA. Anesthetic biotransformation and renal function in obese patients during and after methoxyflurane or halothane anesthesia. Anesthesiology. 1975;42(4):451-7.
265. Women's NCCf, Health Cs. Caesarean section. 2011.
266. Lamont RF, Sobel J, Kusanovic JP, Vaisbuch E, Mazaki-Tovi S, Kim SK, et al. Current debate on the use of antibiotic prophylaxis for caesarean section. BJOG. 2011;118(2):193-201.
267. Testing TECoAS. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1. http://wwweucastorg. 2018.
268. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol. 2009;62(10):e1-e34.
269. Kanji S, Hayes M, Ling A, Shamseer L, Chant C, Edwards DJ, et al. Reporting guidelines for clinical pharmacokinetic studies: The ClinPK statement. Clin Pharmacokinet. 2015;54(7):783-95.
270. Critical appraisal tools - JBI: Joannabriggs.org; [Available from: http://joannabriggs.org/research/critical-appraisal-tools.html.
271. Practice CE, Group OoC. Suggested risk of bias criteria for EPOC reviews. 2015.
272. WebPlotDigitizer - Copyright 2010-2017 Ankit Rohatgi Arohatgi.info2018 [Available from: http://arohatgi.info/WebPlotDigitizer/app3_12/.
273. Bousfield P, Mullinger BM, Elstein M. Cefuroxime: Potential use in pregnant women at term. Br J Obstet Gynaecol. 1981;88(2):146-9.
274. Roumen FJME, Bouckaert PXJM, Cremers HMHG, Vree TB. Pharmacokinetics of cefuroxime in pregnant patients with preterm premature rupture of the membranes. Pharm Weekbl. 1990;12(6):275-9.
275. Kristensen GB, Beiter EC, Mather O. Single-dose cefuroxime prophylaxis in non-elective cesarean section. Acta Obstet Gynecol Scand. 1990;69(6):497-500.
276. Rizk DEE, Nsanze H, Mabrouk MH, Mustafa N, Thomas L, Kumar M. Systemic antibiotic prophylaxis in elective cesarean delivery. Int J Gynaecol Obstet. 1998;61(3):245-51.
277. Ziogos E, Tsiodras S, Matalliotakis I, Giamarellou H, Kanellakopoulou K. Ampicillin/Sulbactam versus Cefuroxime as antimicrobial prophylaxis for cesarean delivery: A randomized study. BMC Infect Dis. 2010;10.
278. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection, 1999. Am J Infect Control. 1999;27(2):97-134.
279. Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, et al. The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect. 2015;6:22-9.
280. Martin EK, Beckmann MM, Barnsbee LN, Halton KA, Merollini K, Graves N. Best practice perioperative strategies and surgical techniques for preventing

caesarean section surgical site infections: A systematic review of reviews and meta-analyses. BJOG. 2018;125(8):956-64.
281. RCT of Postoperative Infections Following Caesarean Section Infections Following Caesarean Section (APIPICS) [Internet]. clinicaltrials.gov. 2013. Available from: https://clinicaltrials.gov/ct2/show/NCT02009098?term=cefuroxime&draw=2&rank=13.
282. Tzingounis V, Makris N, Zolotas J, Michalas S, Aravantinos D. Cefuroxime prophylaxis in caesarean section. Pharmatherapeutica. 1982;3(2):140-2.
283. Maggio L, Miladinovic B, Slain D, Young OM, Dahlke JD, Stitely M, et al. Optimal dosing of pre-cesaren cefazolin prophylaxis in obese women: An individual patient data meta-analysis. Am J Obstet Gynecol. 2017;216(1):S188.
284. Pevzner L, Swank M, Krepel C, Wing DA, Chan K, Edmiston CEJ. Effects of maternal obesity on tissue concentrations of prophylactic cefazolin during cesarean delivery. Obstet Gynecol. 2011;117(4):877-82.
285. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol. 2014;14(1):135.
286. Higgins JP, Green S. Cochrane handbook for systematic reviews of interventions: Wiley Online Library; 2008.
287. Ahmadzia HK, Patel EM, Joshi D, Liao C, Witter F, Heine RP, et al. Obstetric surgical site infections: 2 grams compared with 3 grams of cefazolin in morbidly obese women. Obstet Gynecol. 2015;126(4):708-15.
288. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-32.
289. McKenney S, Farzin A, Yang S, Murphy J, Marzinke M, Lee C, et al. 230: Maternal-neonatal transfer of preoperative cefazolin at cesarean section (C/S). Am J Obstet Gynecol. 2015;212(1):S128.
290. Weir CJ, Butcher I, Assi V, Lewis SC, Murray GD, Langhorne P, et al. Dealing with missing standard deviation and mean values in meta-analysis of continuous outcomes: A systematic review. BMC Med Res Methodol. 2018;18(1):25.
291. Weir CM, Joanne Practical methods for handling missing summary statistics in meta-analysis of continuous outcomes. BMC Med Res Methodol2018. p. 25.
292. Shi J, Luo D, Wan X, Liu Y, Liu J, Bian Z, et al. Detecting the skewness of data from the sample size and the five-number summary. arXiv preprint arXiv:201005749. 2020.
293. Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range: Hong Kong Baptist University, DEPARTMENT OF MATHEMATICS; 2021 [Available from: https://www.math.hkbu.edu.hk/~tongt/papers/median2mean.html.
294. Słabuszewska-Jóźwiak A, Szymański JK, Jóźwiak Ł, Sarecka-Hujar B. A systematic review and meta-analysis of wound complications after a caesarean section in obese women. J Clin Med. 2021;10(4):675.
295. Li M, Shi B, Ma J, Peng X, Shi J. Comparing prophylactic use of cefazolin for SSI in cesarean section: A systematic review and meta-analysis. Arch Gynecol Obstet. 2021;303(2):313-20.

296. Tan X, Liu S, Song L, Sun A. Effects of antibiotics on prevention of infection, white blood cell counts, and C-reactive protein levels at different times in the perioperative period of cesarean section. Int J Clin Pharmacol Ther. 2020;58(6):310.
297. Eley VA, Christensen R, Ryan R, Jackson D, Parker SL, Smith M, et al. Prophylactic cefazolin dosing in women with body mass index >35 kg.m2 undergoing cesarean delivery: A pharmacokinetic study of plasma and interstitial fluid. Anesth Analg. 2020.
298. La Rosa M, Omere C, Redfern T, Abdelwahab M, Spencer N, Villarreal J, et al. The impact of low-dose versus high-dose antibiotic prophylaxis regimens on surgical site infection rates after cesarean delivery. Arch Gynecol Obstet. 2020.
299. Rodel RL, Gray KM, Quiner TE, Bodea Braescu A, Gerkin R, Perlow JH. Cesarean wound closure in body mass index 40 or greater comparing suture to staples: A randomized clinical trial. Am J Obstet Gynecol MFM. 2021.
300. Alrammaal HH, Batchelor HK, Morris K, Chong HP. Efficacy of perioperative cefuroxime as a prophylactic antibiotic in women requiring caesarean section: A systematic review. European Journal of Obstetrics and Gynecology and Reproductive Biology. 2019.
301. Skhirtladze-Dworschak K, Hutschala D, Reining G, Dittrich P, Bartunek A, Dworschak M, et al. Cefuroxime plasma and tissue concentrations in patients undergoing elective cardiac surgery: Continuous vs bolus application. A pilot study. Br J Clin Pharmacol. 2019.
302. FDA UDoHHS. Bioanalytical method validation, guidance for industry. http://www fda gov/cder/guidance/4252fnl htm. 2018.
303. Kaza M, Karaźniewicz-Łada M, Kosicka K, Siemiątkowska A, Rudzki PJ. Bioanalytical method validation: new FDA guidance vs. EMA guideline. Better or worse? J Pharm Biomed Anal. 2019;165:381-5.
304. Agency EM. Guideline on bioanalytical method validation. Committee for Medicinal Products for Human Use (EMEA/CHMP/EWP/192217/2009). 2011.
305. Lovering AM, Perez J, Bowker KE, Reeves DS, MacGowan AP, Bannister G. A comparison of the penetration of cefuroxime and cephamandole into bone, fat and haematoma fluid in patients undergoing total hip replacement. J Antimicrob Chemother. 1997;40(1):99-104.
306. Hosmann A, Ritscher LC, Burgmann H, Oesterreicher Z, Jager W, Poschner S, et al. Concentrations of cefuroxime in brain tissue of neurointensive care patients. Antimicrob Agents Chemother. 2018;62(2).
307. Wozniak TJ, Hicks JR. Analytical Profile of Cefuroxime Sodium. In: Florey K, editor. Analytical Profiles of Drug Substances. 20: Academic Press; 1991. p. 209-36.
308. Guideline IHT, editor Validation of analytical procedures: text and methodology Q2 (R1). International conference on harmonization, Geneva, Switzerland; 2005.
309. Comer J, Tam K. Lipophilicity profiles: theory and measurement. Wiley Online Library; 2001. p. 275-304.
310. Wikipedia. Cefuroxime. Wikipedia 15 September 2019 ed. Wikipedia, Free Encyclopedia: Wikipedia, Free Encyclopedia; 2019. p. 915884719.
311. TM P, Salgado H. Methods for qualitative analysis of cefazolin sodium raw material and pharmaceutical product. Physical Chemistry. 2013;3(2):29-38.
312. Wang L, Chen L, Lian G, Han L. Determination of partition and binding properties of solutes to stratum corneum. Int J Pharm. 2010;398(1-2):114-22.

313. Martin YC. Exploring QSAR: Hydrophobic, Electronic, and Steric Constants ACS Publications; 1996.
314. Piva G, Farin D, Gozlan I, Kitzes-Cohen R. HPLC method for determination of cefuroxime in plasma. Chromatographia. 2000;51(3):154-6.
315. Ethiraj R, Thiruvengadam E, Sampath VS, Vahid A, Raj J. Development and validation of stability indicating spectroscopic method for content analysis of Ceftriaxone sodium in Pharmaceuticals. Int Sch Res Notices. 2014;2014.
316. Lee S, Kim SK, Lee DY, Chae SY, Byun Y. Pharmacokinetics of a new, orally available ceftriaxone formulation in physical complexation with a cationic analogue of bile acid in rats. Antimicrob Agents Chemother. 2006;50(5):1869-71.
317. Ceftriaxone [Internet]. 2019 [cited 05/09/2019]. Available from: https://www.drugbank.ca/drugs/DB01212.
318. Shekar K, Roberts JA, Barnett AG, Diab S, Wallis SC, Fung YL, et al. Can physicochemical properties of antimicrobials be used to predict their pharmacokinetics during extracorporeal membrane oxygenation? Illustrative data from ovine models. Crit Care. 2015;19:437.
319. Wikipedia. Indapamide. Wikipedia Wikipedia, The Free Encyclopedia.: Wikipedia, The Free Encyclopedia.; 2019. p. 911496615.
320. Indapamide [Internet]. 2019 [cited 05/09/2019]. Available from: https://www.drugbank.ca/drugs/DB00808.
321. Can NO, Altiokka G, Aboul-Enein HY. Determination of cefuroxime axetil in tablets and biological fluids using liquid chromatography and flow injection analysis. Anal Chim Acta. 2006;576(2):246-52.
322. Wikipedia. Vancomycin. Wikipedia: Wikipedia, The Free Encyclopedia.; 2019. p. ID: 917393107.
323. Wikipedia. Phenacetin. Wikipedia. Wikipedia: Wikipedia, The Free Encyclopedia.; 2019. p. 917372182.
324. Nakagawa Y, Izumi K, Oikawa N, Sotomatsu T, Shigemura M, Fujita T. Analysis and prediction of hydrophobicity parameters of substituted acetanilides, benzamides and related aromatic compounds. Environ Toxicol Chem. 1992;11(7):901-16.
325. Phenacetine [Internet]. 2019 [cited 05/09/2019]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Phenacetin#section=Octanol-Water-Partition-Coefficient.
326. Zhao L, Zhao Y, Li Q, Chen X, Xiao F, He B, et al. A fast, sensitive, and high throughput method for the determination of cefuroxime lysine in dog plasma by UPLC–MS/MS. Talanta. 2012;89:84-90.
327. Wikipedia. Cefotaxime. Wikipedia. Wikipedia, The Free Encyclopedia.: Wikipedia, The Free Encyclopedia.; 2019. p. 915884634.
328. Cefotaxime [Internet]. 2019 [cited 09/09/2019]. Available from: https://www.drugbank.ca/drugs/DB00493.
329. Viberg A, Sandstrom M, Jansson B. Determination of cefuroxime in human serum or plasma by liquid chromatography with electrospray tandem mass spectrometry. Rapid Commun Mass Spectrom. 2004;18(6):707-10.
330. Wikipedia. Cefalexin. Wikipedia. Wikipedia, The Free Encyclopedia: Wikipedia, The Free Encyclopedia; 2019. p. 915947975.
331. Hansch C, Hoekman D, Leo A, Zhang L, Li P. The expanding role of quantitative structure-activity relationships (QSAR) in toxicology. Toxicol Lett. 1995;79(1-3):45-53.

332. Cephalexin [Internet]. 2019 [cited 09/09/2019]. Available from: https://www.drugbank.ca/drugs/DB00567.
333. Szlagowska A, Kaza M, Rudzki PJ. Validated HPLC method for determination of cefuroxime in human plasma. Acta Pol Pharm. 2010;67(6):677.
334. Al-Said MS, Al-Khamis KI, Niazy EM, El-Sayed YM, Al-Rashood KA, Al-Bella S, et al. Bioequivalence evaluation of two brands of cefuroxime 500 mg tablets (Cefuzime and Zinnat) in healthy human volunteers. Biopharm Drug Dispos. 2000;21(6):205-10.
335. Wikipedia. Cefoxitin. Wikipedia. Wikipedia, The Free Encyclopedia.: Wikipedia, The Free Encyclopedia.; 2019. p. 894713453.
336. Cefoxitin [Internet]. 2019 [cited 09/09/2019]. Available from: https://www.drugbank.ca/drugs/DB01331.
337. Pojar M, Mandak J, Malakova J, Jokesova I. Tissue and plasma concentrations of antibiotic during cardiac surgery with cardiopulmonary bypass--microdialysis study. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2008;152(1):139-45.
338. Wikipedia. Tazobactam. Wikipedia Wikipedia, The Free Encyclopedia.: Wikipedia, The Free Encyclopedia.; 2019. p. 849989952.
339. Tazobactam [Internet]. 2019 [cited 09/09/2019]. Available from: https://www.drugbank.ca/drugs/DB01606.
340. Hu X, Huang M, Liu J, Chen J, Shentu J. Simple and robust analysis of cefuroxime in human plasma by LC-MS/MS: Application to a bioequivalence study. Adv Pharmacol Sci. 2014;2014:981624-.
341. Cairns D. Essentials of pharmaceutical chemistry: Pharmaceutical Press; 2012.
342. Control pH during method development for better chromatography [Internet]. 2015 [cited 12/09/2019]. Available from: https://www.agilent.com/cs/library/technicaloverviews/public/5990-9984EN.pdf.
343. Shimadzu. Differences between using acetonitrile and methanol for reverse phase chromatography. https://www.shimadzu.com/an/hplc/support/lib/lctalk/35/35lab.html; 2019.
344. Marin RD, Crespo-Garcia S, Wilson AM, Sapieha P. RELi protocol: Optimization for protein extraction from white, brown and beige adipose tissues. MethodsX. 2019;6:918-28.
345. FDA. Evaluation of internal standard responses during chromatographic bioanalysis: Questions and answers. 2019 2019.
346. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. J Pharm Anal. 2014;4(3):159-65.
347. Partani P, Gurule S, Khuroo A, Monif T, Bhardwaj S. Liquid chromatography/electrospray tandem mass spectrometry method for the determination of cefuroxime in human plasma: Application to a pharmacokinetic study. J Chromatogr B. 2010;878(3):428-34.
348. Carlier M, Noë M, Roberts JA, Stove V, Verstraete AG, Lipman J, et al. Population pharmacokinetics and dosing simulations of cefuroxime in critically ill patients: non-standard dosing approaches are required to achieve therapeutic exposures. J Antimicrob Chemother. 2014;69(10):2797-803.
349. Bruheim P, Kvitvang HFN, Villas-Boas SG. Stable isotope coded derivatizing reagents as internal standards in metabolite profiling. J Chromatogr A. 2013;1296:196-203.

350. Kanagalingam MG, Forouhi NG, Greer IA, Sattar N. Changes in booking body mass index over a decade: Retrospective analysis from a Glasgow Maternity Hospital. BJOG. 2005;112(10):1431-3.
351. Heslehurst N, Ells LJ, Simpson H, Batterham A, Wilkinson J, Summerbell CD. Trends in maternal obesity incidence rates, demographic predictors, and health inequalities in 36,821 women over a 15-year period. BJOG. 2007;114(2):187-94.
352. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0 [Internet]. The European Committee on Antimicrobial Susceptibility Testing. 2020 [cited 09/03/2020]. Available from: http://www.eucast.org.
353. Prevention CfDCa. Surgical Site Infection (SSI) Event. 2019:34.
354. Weckesser A, Farmer N, Dam R, Wilson A, Morton VH, Morris RK. Women's perspectives on caesarean section recovery, infection and the PREPS trial: a qualitative pilot study. BMC Pregnancy Childbirth. 2019;19(1):245.
355. Hodgetts-Morton V, Hewitt CA, Wilson A, Farmer N, Weckesser A, Dixon E, et al. Vaginal preparation with chlorhexidine at cesarean section to reduce endometritis and prevent sepsis: A randomized pilot trial (PREPS). Acta Obstet Gynecol Scand. 2020;99(2):231-9.
356. Hospital RP. GD012_-_Data_Cleaning_v1.1_in_developement. Royal Papworth Hospital; 2021.
357. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. Acta Biomed. 2020;91(1):157-60.
358. Edwards Jr WH, Kaiser AB, Kernodle DS, Appleby TC, Edwards Sr WH, Martin III RS, et al. Cefuroxime versus cefazolin as prophylaxis in vascular surgery. J Vasc Surg. 1992;15(1):35-42.
359. Cefuroxime | C16H16N4O8S | ChemSpider [Internet]. 2020 [cited 26/08/2020]. Available from: http://www.chemspider.com/Chemical-Structure.4514699.html.
360. Brodwall EK, Bergan T, Orjavik O. Kidney transport of cefazolin in normal and impaired renal function. J Antimicrob Chemother. 1977;3(6):585-92.
361. Koren G, Pariente G. Pregnancy-associated changes in pharmacokinetics and their clinical implications. Pharm Res. 2018;35(3):61.
362. Abduljalil K, Pansari A, Jamei M. Prediction of maternal pharmacokinetics using physiologically based pharmacokinetic models: assessing the impact of the longitudinal changes in the activity of CYP1A2, CYP2D6 and CYP3A4 enzymes during pregnancy. J Pharmacokinet Pharmacodyn. 2020;47(4):361-83.
363. Smit C, De Hoogd S, Brüggemann RJM, Knibbe CAJ. Obesity and drug pharmacology: A review of the influence of obesity on pharmacokinetic and pharmacodynamic parameters. Expert Opin Drug Metab Toxicol. 2018;14(3):275-85.
364. Abduljalil K, Badhan RKS. Drug dosing during pregnancy—opportunities for physiologically based pharmacokinetic models. J Pharmacokinet Pharmacodyn. 2020;47(4):319-40.
365. Jamsen KM, McLeay SC, Barras MA, Green B. Reporting a population pharmacokinetic–pharmacodynamic study: A journal's perspective. Clin Pharmacokinet. 2014;53(2):111-22.
366. Gaohua L, Abduljalil K, Jamei M, Johnson TN, Rostami-Hodjegan A. A pregnancy physiologically based pharmacokinetic (p-PBPK) model for disposition of drugs metabolized by CYP1A2, CYP2D6 and CYP3A4. Br J Clin Pharmacol. 2012;74(5):873-85.

367. De Sarro A, Ammendola D, Zappala M, Grasso S, De Sarro G. Relationship between structure and convulsant properties of some beta-lactam antibiotics following intracerebroventricular microinjection in rats. Antimicrob Agents Chemother. 1995;39(1):232.
368. National center for biotechnology information. PubChem compound catabase; CID=33255, Cefazolin. [Internet]. Pubchem. 2021 [cited 22/10/2018]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/33255.
369. Mathialagan S, Piotrowski MA, Tess DA, Feng B, Litchfield J, Varma MV. Quantitative prediction of human renal clearance and drug-drug interactions of organic anion transporter substrates using in vitro transport data: A relative activity factor approach. Drug Metab Dispos. 2017;45(4):409-17.
370. Nishida M, Matsubara T, Murakawa T, Mine Y, Yokota Y, Goto S, et al. Cefazolin, a new semisynthetic cephalosporin antibiotic. III Absorption, excretion and tissue distribution in parenteral administration. The journal of antibiotics. 1970;23(4):184-94.
371. Tsuji A, Nishide K, Minami H, Nakashima E, Terasaki T, Yamana T. Physiologically based pharmacokinetic model for cefazolin in rabbits and its preliminary extrapolation to man. Drug Metab Disposition. 1985;13(6):729-39.
372. Rattie ES, Ravin LJ. Pharmacokinetic interpretation of blood levels and urinary excretion data for cefazolin and cephalothin after intravenous and intramuscular administration in humans. Antimicrob Agents Chemother. 1975;7(5):606-13.
373. Kagedal M, Nilsson D, Huledal G, Reinholdsson I, Cheng Y-F, Asenblad N, et al. A study of organic acid transporter-mediated pharmacokinetic interaction between NXY-059 and cefuroxime. J Clin Pharmacol. 2007;47(8):1043-9.
374. Garton A, Rennie R, Gilpin J, Marrelli M, Shafran S. Comparison of dose doubling with probenecid for sustaining serum cefuroxime levels. J Antimicrob Chemother. 1997;40(6):903-6.
375. Abduljalil K, Ning J, Pansari A, Pan X, Jamei M. Prediction of maternal and fetoplacental concentrations of cefazolin, cefuroxime and amoxicillin during pregnancy using bottom-up physiologically based pharmacokinetic models. Drug Metab Dispos. 2022.
376. Nightingale CH, Greene DS, Quintiliani R. Pharmacokinetics and clinical use of cephalosporin antibiotics. J Pharm Sci. 1975;64(12):1899-927.
377. Peng J, Ladumor MK, Unadkat JD. Prediction of pregnancy-induced changes in secretory and total renal clearance of drugs transported by organic anion transporters. Drug Metab Disposition. 2021.
378. Abduljalil K, Cain T, Humphries H, Rostami-Hodjegan A. Deciding on success criteria for predictability of pharmacokinetic parameters from in vitro studies: An analysis based on in vivo observations. Drug Metab Disposition. 2014;42(9):1478-84.
379. Shebley M, Sandhu P, Emami Riedmaier A, Jamei M, Narayanan R, Patel A, et al. Physiologically based pharmacokinetic model qualification and reporting procedures for regulatory submissions: A consortium perspective. Clin Pharmacol Ther. 2018;104(1):88-110.
380. Brill MJ, Houwink AP, Schmidt S, Van Dongen EP, Hazebroek EJ, van Ramshorst B, et al. Reduced subcutaneous tissue distribution of cefazolin in morbidly obese versus non-obese patients determined using clinical microdialysis. J Antimicrob Chemother. 2014;69(3):715-23.

381. Hanberg P, Bue M, Kabel J, Jørgensen AR, Jessen C, Søballe K, et al. Effects of tourniquet inflation on peri- and postoperative cefuroxime concentrations in bone and tissue. Acta Orthop. 2021;92(6):746-52.
382. Chopra T, Zhao JJ, Alangaden G, Wood MH, Kaye KS. Preventing surgical site infections after bariatric surgery: value of perioperative antibiotic regimens. Expert Rev Pharmacoecon Outcomes Res. 2010;10(3):317-28.
383. UK NGUT. Surgical site infections: prevention and treatment. 2019.
384. Bossung V, Lupatsii M, Dashdorj L, Tassiello O, Jonassen S, Pagel J, et al. Timing of antimicrobial prophylaxis for cesarean section is critical for gut microbiome development in term born infants. Gut microbes. 2022;14(1):2038855.
385. Committee TCALHNCAS. Updates on antimicrobial stewardship, dosing of antimicrobials for obese patients. 2018.
386. Resistance SAeAGoA. Surgical antimicrobial prophylaxis prescribing guideline. 2021.
387. Hanrahan TP, Lipman J, Roberts JA. Antibiotic dosing in obesity: a BIG challenge. Crit Care. 2016;20(1):240-.
388. Meisner H, Tenney K. pH as an indicator of free fatty acid release from adipocytes. J Lipid Res. 1977;18(6):774-6.
389. Tran CT, Huynh DT, Gargiulo C, Tran le BH, Huynh MH, Nguyen KH, et al. Adipose tissue can be generated in vitro by using adipocytes from human fat tissue mesenchymal stem cells seeded and cultured on fibrin gel sheet. Cell Tissue Bank. 2013;14(1):97-106.
390. Coppack SW, Chinkes DL, Miles JM, Patterson BW, Klein S. A multicompartmental model of in vivo adipose tissue glycerol kinetics and capillary permeability in lean and obese humans. Diabetes. 2005;54(7):1934-41.
391. Sherill BC, Dietschy JM. Permeability characteristics of the adipocyte cell membrane and partitioning characteristics of the adipocyte triglyceride core. J Membr Biol. 1975;23(3-4):367-83.
392. Smaill FM, Grivell RM. Antibiotic prophylaxis versus no prophylaxis for preventing infection after cesarean section. Cochrane Database Syst Rev. 2014;2014(10):Cd007482.
393. Blum S, Cunha CB, Cunha BA. Lack of pharmacokinetic basis of weight-based dosing and intra-operative re-dosing with cefazolin surgical prophylaxis in obese patients: Implications for antibiotic stewardship. Surg Infect (Larchmt). 2019;20(6):439-43.
394. Box GEP. Science and Statistics. J Am Stat Assoc. 1976;71(356):791-9.
395. Rowland M, Lesko L, Rostami-Hodjegan A. Physiologically based pharmacokinetics is impacting drug development and regulatory decision making. Wiley Online Library; 2015.
396. Wagner C, Zhao P, Pan Y, Hsu V, Grillo J, Huang S, et al. Application of physiologically based pharmacokinetic (PBPK) modeling to support dose selection: report of an FDA public workshop on PBPK. CPT Pharmacometrics Syst Pharmacol. 2015;4(4):226-30.
397. Shepard T, Scott G, Cole S, Nordmark A, Bouzom F. Physiologically based models in regulatory submissions: Output from the ABPI/MHRA forum on physiologically based modeling and simulation. CPT Pharmacometrics Syst Pharmacol. 2015;4(4):221-5.

398. Yoshida K, Budha N, Jin JY. Impact of physiologically based pharmacokinetic models on regulatory reviews and product labels: Frequent utilization in the field of oncology. Clin Pharmacol Ther. 2017;101(5):597-602.
399. Vree TB, Hekster YA. Pharmacokinetics and tissue concentrations of cefuroxime. Pharm Weekbl. 1990;12(6):262-7.
400. Andreasen JJ, Møller S, Hauch O, Jørgensen LN, Lester A, Mortensen I, et al. Peroperative concentrations of cefuroxime in serum and subcutaneous tissue following antimicrobial prophylaxis in gastrointestinal surgery. J Antimicrob Chemother. 1988;22(1):90-2.
401. Feng S, Shi J, Parrott N, Hu P, Weber C, Martin-Facklam M, et al. Combining 'Bottom-Up' and 'Top-Down' methods to assess ethnic difference in clearance: Bitopertin as an example. Clin Pharmacokinet. 2015;55.
402. Tsamandouras N, Rostami-Hodjegan A, Aarons L. Combining the 'bottom up'and 'top down'approaches in pharmacokinetic modelling: Fitting PBPK models to observed clinical data. Br J Clin Pharmacol. 2015;79(1):48-55.
403. Michael Marmot, Jessica Allen, Tammy Boyce, Peter Goldblatt, Morrison J. Health equity in england: The marmot review ten years on. London: Institute of health equity. 2020.
404. Centers for Disease Control and Prevention. Core elements of hospital antibiotic stewardship programs. 2021.
405. Abrahams E. Right drug-right patient-right time: Personalized medicine coalition. Clin Transl Sci. 2008;1(1):11-2.
406. Zachariassen G, Hyldig N, Joergensen JS, Nielsen DS, Greisen G. The half-life and exposure of cefuroxime varied in newborn infants after a caesarean section. Acta Paediatr. 2016;105(9):1074-8.
407. Polso AK, Lassiter JL, Nagel JL. Impact of hospital guideline for weight-based antimicrobial dosing in morbidly obese adults and comprehensive literature review. J Clin Pharm Ther. 2014;39(6):584-608.
408. Matushek KJ, Rosin E. Pharmacokinetics of cefazolin applied topically to the surgical wound. Arch Surg. 1991;126(7):890-3.
409. Berezhkovskiy LM. The connection between the steady state (Vss) and terminal (Vbeta) volumes of distribution in linear pharmacokinetics and the general proof that Vbeta >/= Vss. J Pharm Sci. 2007;96(6):1638-52.
410. Sobol E, Bialer M. Mathematical comparison between volume of distribution (V) and volume of distribution at steady-state (Vss) utilizing model-independent approach. Biopharm Drug Disposition. 2004;25(2):99-101.