# Prophylactic cefazolin concentrations in lumbar spine surgery: a comparison of tissue and plasma cefazolin concentrations to body composition

Dr Thomas B. Pett
MBChB

A thesis submitted in partial fulfilment of the requirements for
the degree of Master of Health Science
the University of Auckland, 2023

**Abstract**

Surgical site infections (SSI) are a leading complication in orthopaedic surgery. Weight- or BMI-based prophylactic antibiotics have been proven to reduce SSI. In orthopaedic surgery cefazolin is commonly used as the prophylactic antibiotic of choice however the scientific basis for the weight-based regimen often used is weak. The aim of this research was to report the relationship between the plasma and surgical site concentration of prophylactic cefazolin with measures of body composition.

Lean mass weight was demonstrated to be the strongest predictor for plasma cefazolin concentration. Weight was the other measurement of body composition to be found significant. Muscle tissue cefazolin concentrations correlated with plasma concentrations taken at the same time point. No significant correlation between measurements of body composition and muscle tissue cefazolin concentrations were found.

All plasma and tissue cefazolin concentrations were found to achieve the minimal inhibitory concentration required to act as a successful prophylactic agent in lumbar spine surgery.

This is the first study to demonstrate a linkage between lean body mass weight and plasma cefazolin concentration. Further research is warranted exploring cefazolin concentrations with regards to other tissue of the lumbar spine as well as extreme BMIs in lumbar spine surgery.

***Dedication***

*R. D. - my partner in adventures and disasters*

*My Family – the foundation I build from*

**Acknowledgements**

The following people and organisations I would like to thank for their support and patience with this thesis:

My supervisor and operating surgeon- Associate Professor Joe Baker

Operating surgeons- Mr Godwin Choy and Mr Hamish Deverall

My colleagues- Dr Tanushk Martyn and Dr Benjamin Petry

Laboratory Colleagues- Dr Megan Grainger and Karla Watson

Waikato Hospital and Braemar Hospital

Waikato Hospital Orthopaedic Department

# Contents


Abstract 2

Dedication 3

Acknowledgements 4

Contents 5

1. Introduction 10

1.1 Background 11

1.1.1 Defining Surgical Site Infection 11

1.1.2 Epidemiology of SSI in Orthopaedic Surgery 12

1.1.3 Surgical site infections and the cost to society 13

1.1.4 Risk factors for surgical site infections 14

1.2 Current methods to prevent surgical site infections 14

1.2.1 Pre-operative 14

1.2.1.i Decolonisation of *S. aureus* 15

1.2.1.ii Pre-operative nutrition 15

1.2.1.iii Glycaemic control 15

1.2.1.iv Pre-operative bathing 16

1.2.1.v Hair removal 16

1.2.2 Peri-operative 16

1.2.2.i Surgical site preparation 16

1.2.2.ii Theatre air flow 17

1.2.2.iii Oxygenation 17

1.2.2.iv Peri-operative Antibiotics 17

1.2.3 Post-operative 18

1.2.3.i Protein supplementation 18

1.2.3.ii Post-operative antibiotics 18

1.2.3.iii Blood transfusion 19

1.3 The use of antibiotics in preventing surgical site infections 19

1.3.1 Cephalosporins 19

1.3.1.1 Cefazolin Minimal Inhibitory Concentration 20

1.3.2 The timing of antibiotic administration 20

1.3.3 The dosage of antibiotics 22

1.3.3.1 Incidence of SSI based on weight and BMI dosing 23

1.3.3.2 Tissue penetration in relation to antibiotic dose 25

1.3.4 Redosing and post-operative cefazolin 27

1.3.5 Summary 29

1.4 Measurements of body composition 30

1.4.1 Body mass (weight) 30

1.4.2 Body Mass Index 31

1.4.3 Bioelectrical impedance analysis 32

1.5 Objectives of this research 33

2 Methods 35

2.1 Design of the study 36

2.2 Ethics 36

2.3 Consent 36

2.4 Participants 37

2.4.1 Inclusion Criteria 37

2.4.2 Exclusion Criteria 37

2.5 Data Collection 37

2.6 Assessment of body composition 37

2.7 Dosage of Cefazolin 40

2.8 Sample collection 40

2.8.1 Plasma sample collection 40

2.8.2 Tissue sample collection 40

2.8.3 Storage and transport 41

2.9 Sample analysis 41

2.9.1 Plasma cefazolin level analysis 41

2.9.2 Tissue cefazolin levels analysis 41

2.9.2.i Standards and Reagents 41

2.9.2.ii Sample Preparation 42

2.9.2.iii High Performance Liquid Chromatography 42

2.9.2.iv Specificity and Selectivity 43

2.9.2.v Accuracy assessed by recovery 44

2.9.2.vi Cefazolin degradation 45

2.9.2.vii Cefaclor 46

2.9.3 Quality Control 47

2.10 Data Storage 47

2.11 Statistical analysis 47

3 Results 48

3.1 Basic demographic data 49

3.2 Body mass, BMI and BIA basic statistical results 49

3.2.1 Weight 49

3.2.2 Height 49

3.2.3 BMI 50

3.2.4 Fat mass 50

3.2.5 Lean mass 50

3.3 Individual cefazolin dosing received by participants 52

3.4 Tissue and plasma sample cefazolin levels basic statistics 52

3.4.1 Tissue and plasma samples grouped statistics 52

3.4.2 Tissue and plasma samples individual participants' response 56

3.4.3 Achievement of minimal inhibitory concentration for cefazolin 56

3.5 Removal of potential confounder for statistical analysis of predictors 67

3.6 Univariate Analysis 67

3.6.1 Plasma sample Pearson Correlation Matrix 67

3.6.2 Tissue sample Pearson Correlation Matrix 68

3.7 Linear Regression analysis 76

3.7.1 Plasma sample Linear Regression Analysis 76

3.7.2 Tissue sample Linear Regression Analysis 76

3.7.2.i 30-minute tissue concentration regression analysis 76

3.7.2.ii 60-minute tissue concentration regression analysis 77

3.8 Summary of results 77

4 Discussion 85

4.1 Introduction 86

4.2 Aims of study 87

4.3 Measurement of tissue and plasma cefazolin concentrations in lumbar spine surgery 87

4.4 Achievement of the MIC for tissue and plasma cefazolin concentration 88

4.5 Relationship between tissue and plasma cefazolin concentrations and measures of body composition 90

4.5.1 Relationship between plasma cefazolin concentrations and measures of body composition 90

4.5.2 Relationship between tissue cefazolin concentration and measures of body composition 91

4.6 Determine which measure of body composition is best associated with tissue and plasma concentration of cefazolin 92

4.7 Limitations of study 93

4.7.1 Impact of COVID-19 94

4.8 Areas of future research 94

4.9 Conclusion 95

Appendix 1 – Participant Information Sheet 96

Appendix 2 – Participant Consent Form 104

Bibliography 106

# Chapter 1

# Introduction

## 1.1 Background

This thesis examines the use of cefazolin as a prophylactic antibiotic in lumbar spinal surgery. Specifically, it will look at the concentration of cefazolin in tissue and plasma samples from participants undergoing surgery. It will compare these findings to the participant's body habitus, including; weight, Body Mass Index (BMI) and measurements of Bioelectrical Impedance Analysis (BIA), to see if BIA measurements (including lean and fat mass) are a more accurate way of predicting cefazolin levels.

If this study shows a correlation between BIA and tissue antibiotic concentration it may allow for more precise antibiotic dosing prior to surgery thereby reducing surgical site infections (SSI).

### 1.1.1 Defining Surgical Site Infection

The Centre for Disease Control and Prevention (CDC) defines SSI by a set of criteria relating the infection to acuity and location (1). Depending on the type of operative procedure and type of SSI, these occur within a 30 to 90-day period after the operative procedure. SSI can occur at any wound level, often broken down into superficial (skin and subcutaneous tissue) and deep (fascia and below) level. Other standard definitions used in orthopaedic surgery include prosthetic joint infection and implant-related infection, where artificial constructs are commonly used.

Surgery can be categorised as either clean, clean-contaminated, contaminated or dirty (figure 1.1) (2, 1). Clean surgery involves surgery outside of contaminated areas, such as the gut or lungs. Clean-contaminated involves surgery through an area of the body such as the respiratory tract or gastrointestinal tract under controlled conditions. Contaminated surgery involves surgery where there is gross spillage from internal organs, which was not planned. Dirty surgery involves purulent material or traumatic (contaminated) wounds. Within orthopaedic surgery, the majority of surgery involving artificial implants comes under the 'clean' category.

A SSI occurs when the surgical wound and/or site is contaminated by a bacterial organism that has purulent activity (1). Most often the organisms are from an endogenous source, being that of skin flora (3, 4). It is also possible for infection to occur from exogenous sources, such as the surgical wound being exposed to bacteria from the outside. Causes of exogenous infections can include surgeon and instrument sources, as well as from early exposure of the wound to outside sources prior to the skin fully healing and closing. In the case of SSI when a prosthesis is involved, such as arthroplasty surgery, other sources of infection can also occur. For example, haematogenous spread where organisms spread from other sites of contamination (5, 6). This can occur in patients who have sepsis from other sources, for example pneumonia and urinary tract infections.

### 1.1.2 Epidemiology of SSI in Orthopaedic Surgery

Estimated SSI rates in patients are variable, with estimates ranging from 2% to up to 20% of all operative procedures (7). An overall rate of orthopaedic SSI is difficult as most data is separated based on the type of surgery being performed, which leads to variable reports. Part of the issue with regards to the wide incidence rates are due to the fact that surveillance of SSI is difficult once patients are discharged from hospital back to the care of their primary provider (7). A large majority of SSI are likely treated in the community, moreover, several presumed SSIs are likely treated when they do not exist.(7) There are also wide differences in the incidence of SSIs, including within orthopaedics. This depends on several factors, including the site of incision and the procedure being performed (7). The overall infection rate in orthopaedics is 2.8%, although this varies depending on the surgical site and operating centre being analysed (8, 9)

In spine surgery, there is a variable incidence of SSI dependent on the procedure. An incidence rate of pooled spine SSI is 3.1% from an international meta-analysis, which is high compared to the combined SSI rate of hip and knee arthroplasty in New Zealand of 0.91% (10, 11). This variability in incidence is due to several factors; the surgical approach used, the surgical procedure being undertaken, whether

metalware is used and patient comorbidities, such as obesity. There is also evidence to suggest that the SSI rate is affected by the spinal level that surgery occurs at and whether the approach is either anterior or posterior (10, 12). In spine surgery, a posterior approach has been demonstrated to have an increased rate of SSI compared to an anterior approach (5.0% in posterior, 2.3% anterior) (10, 12).

### 1.1.3 Surgical site infections and the cost to society

SSIs are important due to the burden that they place upon both the patient and the healthcare system. For patients who suffer a SSI this can lead to increased time spent in hospital, further surgical procedures, increased pain, side effects from medication required to treat the SSI and an increase in the possibility of mortality (7, 13). For the healthcare system, several estimates exist to quantify the cost of treating SSI: estimates from reviews by the CDC range from USD$10,443 to $25,546 for SSIs in the United States of America. Other studies have shown that patients with a SSI cost 2 to 2.9 times as much compared to patients without a SSI (2, 14)

In orthopaedic surgery, SSIs are further complicated by the use of implants, such as those in joint arthroplasty surgery and internal fixation of fractures. The outcomes from infected implants or prosthesis are difficult to manage, often with multiple revision surgeries required. This is due to the formation of biofilms by infective bacteria on artificial surfaces, leading to more resistance and difficulty in treatment (15).

Worldwide, it is estimated that over 310 million major surgeries are performed each year (16). In New Zealand alone, there are over 20,000 orthopaedic joint procedures registered each year (17). There are no available figures on the total amount of orthopaedic surgery or spinal surgery that occur each year in New Zealand, nor on the amount of SSIs that occur and the cost to the healthcare system.

From the New Zealand Joint Registry data, which has collected data on arthroplasty surgery from 1999, 18.9% of all total hip revision surgery was performed for deep infection in 2022 (106 cases). This figure being an increase from 8.7% in 2012 (46 cases) (17).

### 1.1.4 Risk factors for surgical site infections

There are various known risk factors that can contribute to the development of surgical site infections. For ease of consideration these risk factors can be divided into the categories: patient, surgical and physiological factors. Patient related factors include older age, obesity, diabetes mellitus, ischaemia to surgical area (poor blood supply), and smoking amongst other lesser factors (18). Surgical risk factors include; errors or inadequacies in the surgical preparation of the site and field, underdosing of prophylactic antibiotics, inadequate hand hygiene and equipment. Physiological factors include; hypoxia, hyperglycaemia, hypothermia and trauma/shock (18).

## 1.2 Current methods to prevent surgical site infections

Numerous methods exist to assist in preventing the development of SSIs. These can be classified into three categories; pre-operative, peri-operative and post-operative.

### 1.2.1 Pre-operative

There are many processes which are carried out in the pre-operative phase. Some of these are done the day before or day of surgery, whilst others are in the weeks or months prior to elective cases. Most of these processes are commonly used around the world in an attempt to prevent SSI, but there are variable levels of evidence to support them.

#### 1.2.1.i Decolonisation of *S. aureus*

The majority of SSI are due to *Staphylococcus aureus* infections (48.6% in hip prostheses SSI) (3). MRSA (Methicillin-Resistant *S. aureus*) has become a more prevalent issue over the years with difficulties in its treatment due to antibiotic resistance. Several studies have looked at screening patients who may have nasal colonisation of *S. aureus* and then subsequent decolonisation treatment to see if this helps reduce the SSI rate. These studies have variable strengths and limitations but seem to suggest a trend towards SSI reduction with MRSA decolonisation (19, 20, 21, 22).

#### 1.2.1.ii Pre-operative nutrition

Serum albumin levels are used as a simple sign of nutrition in patients with regards to protein levels and intake. Low levels of albumin in orthopaedic surgical patients has been shown to increase the risk of developing SSI (23). Pre-operative albumin levels can be performed to see if patients are malnourished and at increased risk of SSI. No evidence currently exists showing successful methods that elevate albumin and in turn reduce SSI rate post-surgery. Further research in this area is required.

#### 1.2.1.iii Glycaemic control

Glycaemic control has been shown to be of importance as hyperglycaemia in the pre-, peri- and post-operative phases carry an increased risk of infection (2, 24, 25). This is most important in patients with known Diabetes Mellitus, both type 1 and type 2, although it is also important in those without it (2, 24). Studies have shown variable odds ratios (OR) for this in surgical fields. Caputo et al. demonstrated a statistically significant OR of 2.11 for SSI in those with peri-operative hyperglycaemia in spinal surgery (25). Elevated glycated haemoglobin (HbA1c) levels, a way of measuring glycaemic levels over a longer period of time, have been shown to correlate with increased risk of developing SSI (26). Zhuang et al. showed elevated HbA1c levels pre-operatively gave an increased OR 1.52 for developing

SSI in lumbar fusion patients (27). Reducing the HbA1c to levels of 50 mmol/mol or below with diabetes treatment optimisation are recommended to reduce the risk of SSI (27, 28).

#### 1.2.1.iv Pre-operative bathing

Pre-operative bathing is where the patient uses an antiseptic agent the day of, or prior to surgery. This is to wash the entire body in an attempt to reduce the skin colony count of possible infective organisms. Several studies performed looking at different antiseptic solutions have shown that it assists in reducing the skin colony count in the patients. However, this does not have a conclusive effect upon the SSI incidence compared to those who do not perform pre-operative bathing. Thus there is weak evidence to support its use (29, 30).

#### 1.2.1.v Hair removal

Hair removal in several orthopaedic surgeries is commonly done in an attempt to prevent SSIs alongside assisting adequate exposure, suturing and the adequate use of dressings. Randomised control trials and a meta-analysis have shown that hair removal does not have a conclusive preventative effect on SSI rates (31, 32, 33)

### 1.2.2 Peri-operative

#### 1.2.2.i Surgical site preparation

Surgical site preparation has been shown to be beneficial in reducing SSI occurrence (34). This is when the skin that is either being incised or draped is prepped with an alcohol or aqueous based solution, with chlorahexadine and iodine being common additives. A meta-analysis of multiple randomised

control trials found that alcohol-based solutions are more successful at reducing SSI rates than aqueous solutions, with strong evidence to back this (34).

#### 1.2.2.ii Theatre air flow

Laminar flow ventilation systems in theatre is where the air is made unidirectional. The idea is to create positive air pressure that passes through filtration units to direct air streams away from the surgical field. This includes directing airborne pathogens away from the surgical site. Initial studies from the 1970s and 1980s provided supporting evidence for this theory (35, 36). More recent studies, however, have not been able to show any benefit from the use of laminar flow and analysis of joint registries around the world have led to no conclusive evidence to support its use in reducing SSI (37, 38).

#### 1.2.2.iii Oxygenation

An important physiological intervention that has been shown to assist the reduction of SSI is that of peri-operative oxygenation. This involves giving patients a higher percentage fraction of oxygen compared to usual (80% vs 30%). A meta-analysis of several RCTs that have been performed in this area demonstrates that it is beneficial towards reducing the SSI rate for intubated patients whilst having surgery (34).

#### 1.2.2.iv Peri-operative Antibiotics

Peri-operative antibiotics given prophylactically prior to first incision have been demonstrated to assist in preventing SSIs (39, 40). To be effective they must be given within a 2 hour time frame prior

to first incision (41). Doses given after the incision has been made have not been shown to have an effect on reducing SSI (42, 43, 44, 45). Further detail is considered in this thesis below.

### 1.2.3 Post-operative

Many of the pre- and peri- surgical interventions mentioned continue into the pre-operative phase. This is particularly those that have an impact on the overall wellbeing of the patient, such as glycaemic control and nutritional status. The post-operative phase continues until the wound has fully closed and healed, although this has variable lengths of time depending on the surgical wound and factors relating to the patient.

#### 1.2.3.i Protein supplementation

The use of protein supplementation post-operatively was shown to assist in reducing SSI in a randomised control trial by Khalooeifard et al., conducted on 80 posterior spine fusion patients (46). It involved giving 1.2 g of protein via whey supplement for one month post-surgery. The protein group had a reduction in SSI compared to the placebo group amongst improved wound healing, higher rates of vertebral fusion and increased albumin levels (44). More research is required to further investigate its applicability.

#### 1.2.3.ii Post-operative antibiotics

It is common practice internationally for prophylactic antibiotics to be continued post operatively for a 24 hour period after prosthetic joint arthroplasty and also for some orthopaedic trauma surgery. Despite this being a routine practice in many parts of the world, there is strong evidence from several studies and meta-analyses to support the use of only single dose antibiotics (44, 45, 47).

#### 1.2.3.iii Blood transfusion

Blood transfusions are often given to patients who have a significant drop in their haemoglobin levels when checked post-operatively. Despite the benefit blood transfusions can bring in the recovery phase, it does contain an increased risk of SSI (2, 48, 49, 50). Whilst there is strong evidence behind this there is no evidence to support the risks of SSI outweighing the risks and effects from anaemia. It is, therefore, recommended to be assessed on a case-by-case basis.

## 1.3 The use of antibiotics in preventing surgical site infections

The use of prophylactic antibiotics has proven to be an important step in reducing SSI.

Whilst all antibiotics are effective in assisting in the treatment of certain infections, not all antibiotics are able to work as a prophylactic agent. This is due to their limitations in coverage or bioavailability. One of the most successfully used antibiotic groups for prophylactic agents is cephalosporins because of its coverage of common SSI bacteria (*S. aureus)*.

### 1.3.1 Cephalosporins

Cephalosporins belong to the beta lactam class of antibiotics. They are a bactericidal drug that assist in disrupting the bacterial wall formation and are perhaps the most commonly used agents for surgical prophylaxis globally. Focus shall be on the most commonly used cephalosporin for antibiotic prophylaxis in New Zealand, Cefazolin.

Cefazolin is a first generation cephalosporin that has a wide range of cover against Gram positive bacteria, such as *S. aureus* (51). It can also cover certain Gram negative bacteria, such as *Escherichia coli (E. coli)*. It is a water-soluble drug with a half-life of 1.8 hours and its effectiveness is reliant on the

free concentration of the drug (52). Cefazolin is excreted via the renal system without any metabolism changes to its structure.

#### 1.3.1.i Cefazolin Minimal Inhibitory Concentration

The minimal inhibitory concentration (MIC) is the level at which an antibiotic becomes inhibitory for bacteria growth. The MIC level above which cefazolin provides a bactericidal environment, and therefore protection against infection, varies depending on the bacteria being targeted. The accepted MIC is 2 mg/L for *S. aureus* (53, 54). Cefazolin is also a time-dependant antibiotic, meaning that the total time its concentration is above the MIC is when it is bactericidal. Additionally, higher concentrations above the MIC does not change the effectiveness of cefazolin (52).

### 1.3.2 The timing of antibiotic administration

Appropriate timing of antibiotic administration is essential to ensure the antibiotics is effective when it needs to be. Early studies, including those by McKittrick and Wheelock, and Blower et al. found that post-operative antibiotics did not assist in preventing surgical site infections in elective abdominal surgery as compared to patients who received no antibiotics post-surgery(42, 43). The major flaw of these studies was that the antibiotic was given post-surgery. This meant they were ineffective as a prophylactic agent, which is required prior to the event of surgery.

It was not until the 1960s that prophylactic surgical antibiotics were noted to be effective in preventing SSI. In a landmark study, Burke et al. demonstrated that guinea pigs who received an antibiotic dose 4 hours prior to a contaminated incision being made had reduced rates of infection compared to guinea pigs who received no prior antibiotics (55). This key discovery was the first to prove that the timing of antibiotic dosage was important to SSI prevention.

The findings by Burke et al. were soon after trialled on a human cohort. In a controlled study by Bernard and Cole, patients undergoing abdominal surgery either did or did not receive pre-operative antibiotics. The findings showed a greatly reduced SSI rate in the pre-operative antibiotic group (39). Further studies by Feltis et al. demonstrated similar results in a cohort of General Surgical patients who either received pre-surgical antibiotics or none at all (40). It quickly became common practice to give pre-operative antibiotics as a preventive measure against surgical site infections across surgical specialties.

Research over the following decades further defined when prophylactic antibiotics should be given. In a prospective study on multi-speciality elective patients, Classen et al. found an increase in wound infections when patients had an antibiotic dose 2 or more hours prior to wound incision, as compared to the within 2 hours group (56).

In a meta-analysis by de Jonge et al. (14 studies) on the timing of surgical prophylactic antibiotics and surgical site infections, it was demonstrated that no difference occurred between antibiotics given within the 120 minute window (41). This study included patients from many specialities including; arthroplasty, abdominal/general, cardiothoracic, vascular and gynaecology. They concluded by supporting Classen et al. findings that giving antibiotics more than 120 minutes before incision increased SSI rates.

Whilst no evidence supports the need for dosing closer than 120 minutes prior to first incision, the World Health Organisation does recommend taking into account the half-life of the antibiotic being used (57). For the example of Cefazolin, which has a shorter half-life of 1.8 hours, it is recommended that it is given 60 or less minutes prior to surgery to allow the MIC be maintained for as long as possible without further dosing (57).

### 1.3.3 The dosage of antibiotics

Many organisations follow a simple weight-based system for cefazolin dosing, recommending a dose of between 1 and 3 grams based on weight. The theory being the greater the mass, the higher the dose required to achieve the MIC. The current Waikato Hospital policy for cefazolin as a surgical prophylactic agent in orthopaedics is below.

Surgical Prophylaxis – Orthopaedic – Waikato Hospital

Cefazolin

- Cefazolin 2 g IV (3 g if patient >120 kg), within the 60 minutes prior to knife to skin
- Re-dose cefazolin 2 g IV (3 g if patient >120 kg) every 4 hours after the first dose until skin closure
    - If eGFR <30 ml/min – re-dose 6 hourly
    - For dialysis patients – re-dose 8 hourly
- Also consider re-dosing if there had been >1500 ml blood loss to ensure adequate antimicrobial levels up until wound closure

Figure 1.1- Reproduction of prophylactic guidelines for Orthopaedic surgery at Waikato Hospital. Available via the Microguide App (58).

The initial dosage of cefazolin given to a patient prophylactically was 1 g, irrespective of weight. It was not until a study by Forse et al. that dosage started to change. In their 1984 study, they found that morbidly obese patients undergoing gastroplasty had a much higher rate of SSI compared to the leaner population (16.5% vs 2.5% respectively), with all patients having received 1 g of cefazolin prophylactically (59). They also performed adipose tissue sampling on these groups to show that the MIC required for prophylactic cover was not reached in the obese population with only a 1 g dosage. They increased the dose in an obese cohort to 2 g and found that this reduced the rate of SSI drastically, nearly comparable to the normal weight population.

#### 1.3.3.i Incidence of SSI based on weight and BMI dosing

After Forse et al. proved a link between weight, cefazolin dosing and the rate of SSI, research turned towards trying to reduce the SSI rate further. This was done by increasing the cefazolin dose to a higher level in more obese populations. The theory was antibiotics coverage would share a linear relationship to SSI rates.

Rondon et al. retrospectively analysed dosing in primary joint arthroplasty to see if under-dosing of cefazolin led to an increase in SSI in a cohort of 17,393 patients. They found that of the >120kg group, 95.9% of them were under-dosed, with the appropriate dose by the study's standard being 3g for those >120kg. They reported that under-dosing of cefazolin prior to surgery had an increased risk of prosthetic joint infection (PJI) (1.51% vs 0.86% p=0.02), although this description was not stratified by weight nor the actual dose received. Patients who weighed >120kg had an increased rate of SSI compared to those <120kg (3.25% vs 0.83% respectively) (60). While this does demonstrate an increased rate of infection in patient's greater than 120kg, there was no demonstration of a decreased risk in preventing PJI for the specific patients greater than 120kg that received 3 g of cefazolin. As the results were not stratified by weight nor the actual dose received, it was not possible to conclude that patients over 120kg who received 2 g had an increased rate of PJI or SSI compared to a cohort receiving 3 g (60).

Morris at el. looked at under-dosing in total hip and knee arthroplasty using the New Zealand Joint Registry (55). They defined under-dosing as a cefazolin dose less than 2 g in patients with a weight of 80-120kg and less than 3 g for those >120kg. They demonstrated that under-dosing, when compared to recommended dosing, caused an increase in SSI (2.52% vs 0.98% p<0.0001) (61). However, when this was broken down into weight categories, there was no statistical difference between those who received 3 g and those who received 2 g or less in the >120kg group. They concluded that the increased rate of SSI in higher weight patients was due to under-dosing.

A retrospective case-control study was performed by Hussain et al. to see if there was an increase in SSI in patients >120kg who had received 2 g cefazolin prophylactically, as compared to those <120kg who also received 2 g cefazolin (304 patients). The patients for this study came from several specialities and were all elective cases. They found that there was a near double rate of SSI in those > 120kg, however, this evidence was not statistically significant (8.6% vs 4.6% p=0.25), which could be attributed to the small sample size used (62). They concluded that larger studies are required to see if this trend does have more evidence to support it. An issue discovered, as they discuss, with retrospective studies on SSI is that many patients who may develop wound issues see their primary physician for care and treatment. This potentially means many cases are not recorded by researchers who only analyse hospital/inpatient records.

Peppard et al., retrospectively reported on the SSI rate of surgical patients >100kg who had either received 2 g or 3 g cefazolin prophylactically (436 patients, multiple speciality cases). They found that there was no significant difference between the two groups with SSI rates (7.2% and 7.4% for the 2 g and 3 g groups respectively, p=0.95) (63).

Unger and Stein also performed a respective study looking at SSI rates for surgical patients who had received 2 g cefazolin divided by BMI (either under or above a BMI of 30 $kg/m^2$). They found that there was no significant difference between the two groups with regards to SSI (7 vs 5 cases, 185 patients total, p=0.56) (64).

In summary, both Rondon et al. and Morris et al. have very similar findings, but fail to prove that 3g of cefazolin in patients weighing over 120kg reduces the rate of SSI (60, 61). Both propose that obese patients have a higher rate of SSI and that giving less than 2 g cefazolin at any weight increases the risk of SSI. Hussain et al. did demonstrate an increase in SSI in patients weighing over 120kg compared to those weighing under 120kg when 2 g was given to patients irrespective of weight. However, this was not statistically significant and could be attributed to obese patients having a higher rate of SSI in

the first place (56). Peppard et al. and Unger and Stein also demonstrated that a 2 g dose in obese patients did not increase the rate of SSI, though both had smaller patient numbers (63, 64).

#### 1.3.3.ii Tissue penetration in relation to antibiotic dose

Another area of research has been whether the pharmacokinetic properties of cefazolin are upheld in an obese population. This is done by comparing blood and tissue samples to see if the required MIC is found.

In a study by Swank et al., 57 patients undergoing caesarean delivery who had BMIs of 30 kg/m² or greater had adipose tissue samples taken. These samples were analysed to see if those who received 3 g of cefazolin had increased concentrations compared to those who received a 2 g dose. The authors found that in the 2 g group no patients with a BMI of 40 kg/m² or greater reached the specified MIC of 8 mg/L, as compared to 71% of the same BMI group who received 3 g. The authors concluded this as evidence for 3 g to be used in obese patients (65). An obvious difference with this study is that the MIC was set at 8 mg/L, which at this time was the breakpoint for *Enterobacteriaceae* (this has subsequently been lowered to 2 mg/L, but remains 8 mg/L for resistant strains). However, the main bacteria that is targeted with prophylactic cefazolin is *S. aureus*, which has an MIC of 2 mg/L (53). Taking this into account, all patients in this study when using the 2 mg/L MIC reached a satisfactory level in both the 2 g and 3 g group. This suggests that unless resistant *Enterobacteriaceae* strains are specifically being targeted prophylactically (of which there are more suitable antibiotic options) that there was no evidence found to support increased dosage of cefazolin above 2 g. With regards to orthopaedic surgery, the majority of infections are caused by gram positive bacteria such as *S. aureus*, so the findings do not support any increase in dosage for patients over 120kg.

Sharareh et al. analysed trabecular bone concentrations of cefazolin and vancomycin in 34 patients undergoing knee or hip arthroplasty. They found that when a dosage of 2 g was used in those weighing

over 70kg, 92.6% (25 patients) achieved the MIC, with a further breakdown showing that all patients with a BMI greater than 35 kg/m² achieved the MIC with only a 2 g dose (3 patients) (66). Interestingly, they also found that of the 4 patients whose weight was less than 70kg and who received 1g cefazolin, only 75% reached the MIC. Whilst this study does demonstrate that 2 g cefazolin reaches the MIC for patients of a greater BMI, it is limited in its application as bone is not a common site of SSI and also due to the small numbers that made up the study. It is, however, reassuring knowledge for orthopaedic surgeons that there is penetration into bone tissue.

In a randomised control trial (RCT) by Young et al. obese patients undergoing caesarean section were randomised to be given either 2 g or 3 g cefazolin prophylactically. Plasma and adipose tissue samples were then taken and analysed, using liquid chromatography-mass spectrometry, for cefazolin levels. They found that whilst there was a difference between cefazolin levels in the two groups, both groups were well above the cefazolin MIC in regards to the plasma levels (67). They also demonstrated that in the adipose tissue samples both groups reached above the MIC required. This study had limited participants, with only 26 patients in total. The evidence it provided, however, is significant in demonstrating that there is no clinical requirement for increased dosing above 2 g in patients with an increased BMI, with regards to adipose tissue alone.

Another RCT was performed by Maggio et al. on obese women undergoing a caesarean section. In this study, 58 patients with BMIs over 30 kg/m² were either given 2 g or 3 g cefazolin pre-operatively, with adipose tissue samples taken during surgery. They found that all patients' tissue samples were above the required MIC 2 mg/L (68). They did mention that they were using a cut-off of 8 mg/L for *S. aureus*, which at the time was the recommended MIC from Clinical and Laboratory Standards Institute (53), and there was no significant difference between the groups when using this higher MIC. Despite the changes in MIC targets, no evidence was found to support an increase in dosage based on BMI.

Ho et al. performed a study where patients with a BMI of 40 kg/m² or greater either received 2 g or 3 g of cefazolin pre-operatively via either IV bolus or IV infusion, over 30 minutes. They then took serum

samples up until 360 minutes. They found that serum levels of cefazolin remained above 8 mg/L for the full 360 minutes (69). Only 5 patients received 3 g during the trial. Due to these low numbers the study was not statistically significant. The 3 g doses were also given via a 30 minute infusion, rather than an IV bolus which is standard in most areas.

Stitely et al. performed another RCT looking at cefazolin dosing and obese patients undergoing caesarean sections. They had 20 patients with a BMI over 35 kg/m² randomised to receive either 2 g or 4 g cefazolin prophylactically before first incision, prior to collection of plasma and subcutaneous tissue samples. Whilst there was a statistically significant difference between the two doses and the concentrations achieved, it was also noted that all plasma and tissue concentrations of both groups were above the set MIC of 4 mg/L (70). The authors concluded that there was no obvious benefit to the increased dose.

In summary, whilst several studies have reported that 3 g dosing is required in obese patients due to their findings, most of these were using elevated MIC for cefazolin. When a more appropriate MIC is used (2 mg/L) there is no difference between a 2 g and 3 g dose. The studies mentioned have also been of small numbers, leading to very few statistically significant results.

### 1.3.4 Redosing and post-operative cefazolin

Orthopaedic surgery has a variable length of time depending on the procedure being undertaken. This is important for a wide variety of decision making instances, including whether cefazolin requires further intra-operative dosing.

The normal time between cefazolin dosage is 8 hours. Effective bactericidal protection is obtained if the cefazolin levels remain above the MIC for 60% of this time (4.8hrs). This does not have to be for the entire 8 hours, due to the post-antibiotic effect, where continued inhibition remains even below the MIC (71).

Most guidelines recommend intra-operative re-dosing after 2 lengths of the half-life (1.8hrs). Thus, it is recommended identifying procedures that approach or are over 4 hours in length to receive a further dose of cefazolin (72, 73, 74). It is difficult to assess from the current literature how a figure of two half-lives has been chosen as a reference point for further dosing, especially since various studies have differing time lengths for re-dosing, and in fact sometimes do not use cefazolin half-life as an actual reason. Some studies do not exclusively use cefazolin as the only antibiotic, and some even under-dose compared to what is currently the standard dose (75, 76, 77).

One such study was by Scher, who assessed the influence of redosing on SSI. It was found that those who received a single dose of cefazolin had an increased rate of SSI, compared to those who received the same pre-operative dose as well as a re-dose of cefazolin after 3 hours for procedures that lasted longer than 3 hours (6.1 vs 1.3 respectively $p<0.01$) (75). A large issue with this study is that patients received only 1 g of cefazolin prophylactically irrespective of weight or BMI.

Chen et al. looked at tissue concentrations in patient's whose operation time went over 4 hours. They studied patients undergoing gastric bypass, finding that adipose tissue samples remained above the MIC required for 4.8 hours post cefazolin dosage (71). Whilst this study does not answer whether re-dosing is required or not, it does suggest that 4 hours for re-dosing may not have a strong pharmacokinetic basis behind it.

Another consideration is that of extended prophylaxis beyond the surgery itself. The standard practice for arthroplasty surgery is that of a prophylactic dose prior to surgery, and a further two doses after surgery to complete a 24 hour coverage. There is no clinical trial that specifically studies cefazolin and the difference between a single dose and a 24 hour duration with regards to arthroplasty. There have been two meta-analyses conducted in recent years studying post-surgical duration of a group of collective antibiotics. They have found no evidence to support extended dosing beyond that of a pre-operative dose (44, 45). This has also been shown in spinal surgery with extended antibiotics beyond surgery not aiding in reducing SSI rates (47).

The first of these meta-analyses was by Siddiqi et al., which included 14 studies comparing single dose antibiotics to a 24 hour course. The results demonstrated no statistically significant associations (OR 0.96, CI 0.73-1.26) (44). The other meta-analysis was by Ryan et al. which also included 14 randomised control trials (RCT), several of them overlapping with those in Siddiqi et al. Similarly, they found no statistically significant associations between the two groups (OR 0.92 CI 0.56-1.51) (45). Some flaws in both of these meta-analyses is that cefazolin was not the exclusive antibiotic used and several of the RCTs did not use appropriate modern dosing standards.

In summary, little evidence exists to support redosing in the majority of patients, due to much of the evidence now being outdated and inapplicable. There is also little evidence to support extended duration of antibiotics, including cefazolin, beyond the time of surgery. Further research is warranted.

### 1.3.5 Summary

Several robust studies have demonstrated that there is a clear benefit in the timing of cefazolin being given in a 2 hour period prior to first incision. Whilst cefazolin has a shorter half-life than other antibiotics, no smaller time period has been shown to be beneficial in reducing SSI.

The is clear evidence from studies demonstrating that an increased cefazolin dose is required in patients of a larger body mass, with there being the options of 1 or 2 g to be given. Despite several studies looking at both cefazolin tissue levels and the SSI rate with regards to the use of 3 g of cefazolin, none of these have demonstrated any evidence to support this higher dose, regardless of the patient's body mass.

For the use of increased dosage of cefazolin to beyond 2 g, further studies that have supporting evidence will be required. Other areas in which dosage could be further investigated include by disease, such as if diabetic patients require a different dosage to standard patients.

There are no studies that support the redosing of cefazolin when the initial dose is 2 g or more. There is also little evidence to support the ongoing use of cefazolin beyond the prophylactic dose in any surgical setting, including arthroplasty.

### 1.4 Measurements of body composition

Body composition varies from person to person depending on a range of factors. There are also several ways of managing body composition; ranging from simple to complex; cheap to expensive. Most commonly used in day to day activity and medical fields are that of height and weight and body mass index (BMI). In more recent decades there have been developments in ways to calculate body composition more accurately; including bioelectrical impedance analysis, among others.

This is important due to the differences that exist in patients who have an increased body habitus of fat with regards to SSI. Patients who undergo an operative procedure with an obese fat mass are more likely to develop SSI compared to those with an equivalent mass made up of muscle tissue. Part of this can be attributed to inflammatory response caused by obesity, as well as comorbidities that exist commonly with obesity, including diabetes mellitus and cardiovascular disease (78, 79).

#### 1.4.1 Body mass (weight)

Body mass is a simple way of looking at body composition. Obvious issues with the use of this alone is that it in no way calculates what the mass is made up of (i.e., muscle or fat tissue) nor if the person is of healthy proportions.

### 1.4.2 Body Mass Index

BMI takes into account a person's mass and height to give a value. The value is obtained by dividing the weight (kg) by the height (m) squared. The units are kg/m².

$$BMI = \frac{weight\ (kg)}{metres^2 (m)}$$

BMI is able to be used to compare people of a similar height using predetermined figures in an attempt to classify body composition. These index figures vary depending on the population used, with Asian populations having lower index values compared to European populations (80). The index classifies people into one of several groups, including; underweight, normal, overweight and obese (81). The obese group has been split into further subgroups depending on the BMI, with a BMI over 40 kg/m² considered 'severe' obesity (82).

An issue with a BMI system is that it does not take into account the tissue composition that makes up the patient's mass (83). Patients who have a large percentage of muscle, above the average composition, find themselves classified as overweight or obese by BMI, despite having less fat mass than the average person. Examples of this include professional athletes, where larger muscle mass is required, including rugby and weightlifting among others. This is an important point as the physiological effects from muscle and fat differ.

| Body Mass Index kg/m² | Weight status |
|---|---|
| <18.5 | Underweight |
| 18.5 – 24.9 | Normal weight |
| 25.0 – 29.9 | Overweight |
| 30.0 – 34.9 | Obese class 1 |
| 35.0 – 39.9 | Obese class 2 |
| >40.0 | Obese class 3 |

Table 1.1- World Health Organisation classification of weight status based on BMI (F80)

### 1.4.3 Bioelectrical impedance analysis

BIA is a simple method of calculating a person's lean and fat mass. It further quantifies body composition more accurately than body mass or BMI alone.

BIA works by passing an electrical current through two points in the body (typically the wrist and ankle) and measures the resistance provided by the body tissue to determine quantities of tissue types. Depending on the frequency of the electrical current, it travels through different compartments of the body, such as passing through extracellular fluid at low frequencies and through both extracellular and intracellular at frequencies of 50 kilohertz. With the use of equations that have been calculated previously on subjects, it is possible to calculate the fat-free mass and fat mass of patients, alongside other measures such as total body water and compartmental break down.

BIA has been validated as a method of calculating body composition in subjects by using an appropriate equation with regards to age and gender (84, 85). It has been shown to be comparable in validity to other methods of calculating body composition, including hydrostatic weighing and dual-

energy X-ray absorptiometry (86, 8). The key to its validation is in using an appropriate, pre-formed equation for the patient that is being analysed, taking into account the ethnicity, age and body habitus amongst other baseline data.

Limitations to BIA do exist in patient groups where the correct equation is not used. These are often the extremes of body habitus, such as low body fat athletes and morbidly obese people. Equations for most groups have been developed, including athletes and morbidly obese, and for those that do not exist they are able to be developed.

Whilst there are several methods to calculate body tissue composition BIA is the most appropriate in a clinical setting. It has the benefits of being portable, being a small hand held device; efficient with it taking less than five minutes to complete, including set up; cost efficient as, apart from an initial cost in purchasing the machine, only electrode pads are required for the machines continual use.

BIA has been studied previously in clinical settings with regards to nutritional status and patients with certain illnesses (87, 88). Yet, no previous studies have been found using BIA to see if tissue antibiotic concentrations can be correlated with more precise body composition in a surgical setting.

### 1.5 Objectives of this research

The objectives of this research include:

1. Measure tissue and plasma concentrations of prophylactic cefazolin in elective lumbar spine surgery
2. Determine the prevalence of cefazolin MIC in elective lumbar spine surgery, using the hospital prophylactic regimen
3. Define the relationship between tissue and plasma cefazolin concentrations and measures of body composition (weight, BMI, BIA)

4. Determine which measure of body composition is best associated with tissue and plasma concentration of cefazolin

The significance behind this research is that if a link between body composition and antibiotic tissue concentrations can be demonstrated it would allow further research into this area. This being to see if tailored antibiotic dosage, based on bioimpedance analysis, can reduce the rate of SSIs. If a correlation can be demonstrated, this would lead to potentially large cost saving for healthcare providers. Furthermore, it would reduce the burden of disease on patients who previously may have obtained a SSI.

# Chapter 2

# Methods

## 2.1 Design of the study

This study was an observational, cross-sectional study. Participants undergoing lumbar spinal surgery in a non-acute setting were selected due to the uniform incision and the same target muscle group to be used for samples.

As a pilot study to determine if there is an association between body composition and tissue antibiotic concentration, 20 patients were recruited based on previous similar studies (82).

The study was conducted at two hospitals within the city of Hamilton, New Zealand; Waikato Hospital, which is the public tertiary hospital for the region; and Braemar Hospital, a private hospital.

## 2.2 Ethics

Approval for this study was granted by the Auckland Health Research Ethics Committee from the University of Auckland (Reference number AH23409).

Locality approval was given from both Waikato Hospital and Braemar Hospital for the study to be performed on their grounds.

## 2.3 Consent

Prospective participants were contacted prior to the day of surgery via phone. This was to explain the study and assess if they would be interested in participating. Those who identified as such were offered information pamphlets to be sent out to them if they wished.

On the day of surgery, participants previously contacted were met to further discuss the study. Written consent was obtained after discussion of risks and benefits. No risks beyond those of the original surgery were identified, with no effect on wound healing or recovery expected. There were no benefits identified from being part of the study.

Appendix 1 and 2 are the patient information sheet and consent form respectively.

All participants were offered the return of tissue material post completion of the study, in keeping with Tikanga Maori principles.

## 2.4 Participants

Participants were selected from those waiting for non-acute lumbar spine surgery at either Waikato Hospital or Braemar Hospital in Hamilton, New Zealand. Participants were selected from the public and private operating lists of two orthopaedic spine surgeons who operated across both sites.

### 2.4.1 Inclusion criteria

Inclusion criteria for participants required:

- Being over the age of 18 years
- Being able to understand and give informed consent for the study
- Spinal surgery to be a primary procedure
- Posterior approach to the lumbar spine

### 2.4.2 Exclusion criteria

Exclusion criteria included any of the following:

- Participants who received any antibiotics within 10 days of surgery
- Any known allergy or adverse reaction to cephalosporin or other antibiotics that would not allow the use of cefazolin
- Undergoing secondary or revision lumbar spine surgery and any infections present in the participant at the time of surgery
- If the initial approach of the lumbar surgery was not direct posterior (i.e., lateral or anterior approach started with)

## 2.5 Data collection

From participants baseline demographic data was collected, including age, ethnicity and gender.

## 2.6 Assessment of body composition

Weight (kilograms, kg) and height (metres, m) were measured using the same calibrated scales and wall tape measure with Body Mass Index ($kg/m^2$) calculated from this. Uniform clothing of a hospital gown and socks were worn by the patient whilst assessed.

Height was measured using a wall tape measure. Participants had their feet flat next to each other, with arms by the side and the participant asked to look straight ahead. The height recorder was the highest point of contact with the wall tape measure.

BMI was calculated using the weight and height measured on the day. For this, the standard equation below was used:

$$BMI = \frac{weight\ (kg)}{metres^2(m)}$$

BIA was then performed using a BodyStat Quadscan 4000 obtained from BodyStat Australia.

The participant was required to lie supine for 5 minutes to allow for body fluid to stabilise and distribute around the body. After this, electrodes were connected to the upper and lower limbs on the right side; one overlying the ulna styloid, one at the base of the metacarpal heads, one at the level of the ankle joint and one over the metatarsal heads. A standard preprogramed equation for the Australasian population for calculations was used to give measurements.

Figure 2.1- Picture demonstrating electrode placement (Bodystat© 2023- reproduced with permission)

Data collected from this included; fat and lean percentage, fat and lean mass (KG), dry lean mass (KG), total body water percentage and litres, extracellular and intracellular percentage and litres, and body cell mass (KG). A list of all collected parameters (referred to as 'predictors') is available in table 2.1

| Predictor | Description | Unit(s) |
|---|---|---|
| Weight | Body weight | Kilograms (kg) |
| Height | Body height | Metres (m) |
| BMI | Body mass index | $kg/m^2$ |
| Waist-to-hip ratio | Ratio between waist and hip circumference | - |
| Fat mass weight | Body fat mass weight | Kilograms (kg) |
| Lean mass weight | Body lean mass weight | Kilograms (kg) |
| Fat mass percentage | The percentage of body weight from fat mass | - |
| Lean mass percentage | The percentage of body weight from lean mass | - |
| Dry lean mass weight | Body dry lean mass weight | Kilograms (kg) |
| Total body water | The total body water volume | Litre (L) |
| Total body water percentage | The percentage of body that is water | - |
| Extracellular water | Water volume found outside of body cells | Litre (L) |
| Extracellular water percentage | The total body water that is extracellular fluid | - |
| Intracellular water | Water volume found inside of body cells | Litre (L) |
| Body cell mass weight | Body weight contributed by cells | Kilograms (kg) |

Table 2.1- List and description of predictors obtained from BIA

## 2.7 Dosage of Cefazolin

At both hospitals used as sites for the study, cefazolin is the antibiotic of choice for prophylaxis at the time of surgery. A dosing policy was in place by Waikato Hospital that patients over 120kg should receive 3 g of cefazolin, those under 70kg should receive 1 g of cefazolin and the rest 2 g. Whilst this was the standard policy, both hospital sites had the final decision made by the surgeon. No change to the standardised dose was given to participants.

## 2.8 Sample collection

Four samples in total were aimed to be taken from each patient; 1 plasma sample and 3 muscle tissue samples. Once the patient was anaesthetised and positioned, with the surgical team ready to proceed with scrubbing in, the dose of cefazolin was given. The time the cefazolin was given was recorded, as all collection times were made referring to this.

### 2.8.1 Plasma sample collection

At 30 minutes post cefazolin dose, a single plasma specimen was collected by the anaesthetist into an EDTA tube (Ethylenediaminetetraacetic acid). The plasma sample was then immediately delivered to the Waikato Hospital Laboratory, for storage at negative 80 degrees Celsius, to await transfer for analysis.

### 2.8.2 Tissue sample collection

Muscle samples were collected by the operating surgeon. These were collected at 30, 60 and 90 minutes post cefazolin dosing. Samples were taken using a clean scalpel and forceps from an undisturbed area of the erector spinae muscles deep to exposed tissue and not affected by previously used cautery. Sample size was aimed to be approximately 0.125$cm^3$. Once collected, the sample was immediately placed into a sterile tissue pot and stored on dry ice in the operating theatre whilst awaiting transport to Waikato Hospital Laboratory for storage.

Surgery was not unnecessarily prolonged to ensure the 90-minute sample collection, and so patients whose wound was closed prior to 90 minutes after cefazolin dosage would have only two muscle specimens.

### 2.8.3 Storage and transport

Once surgery and the plasma and tissue sample collection was completed the samples were moved to secure storage fridges at Waikato Hospital Laboratory. Samples were kept at under negative 80 degrees Celsius to prevent any breakdown of tissue until analysis.

## 2.9 Sample analysis

### 2.9.1 Plasma cefazolin level analysis

Plasma samples were transported at negative 80 degrees Celsius to Christchurch Hospital Laboratory, in Christchurch, where they were analysed by liquid chromatographic assay using a pre-existing method.

### 2.9.2 Tissue cefazolin levels analysis

Muscle cefazolin concentration was determined using methodology developed based on methods previously described by Capoor et al. (87). The method was developed and performed under Physical Containment 1 and Physical Containment 2 laboratory conditions. The method was initially performed on porcine tissue to assess its validity.

#### 2.9.2.i Standards and Reagents

Potassium Phosphate (Merck, Ensure) was used to make a buffer solution (25 mM $KH_2PO_4$, pH 3.0-3.1).

Internal Standard stock (5 mg mL-1 Cefaclor) was made up in 25 mM $KH_2PO_4$ buffer. This was done to give a base level to compare cefazolin levels to.

Grinding buffer consisted of Cefaclor added to 25mM $KH_2PO_4$ buffer (0.28 mL of Cefaclor internal standard stock in 100 mL $KH_2PO_4$ buffer). This was used to extract cefazolin from the tissue samples.

All standards and reagents were stored in a freezer at – 80 °C until required.

#### 2.9.2.ii Sample Preparation

Tissue samples were accurately weighed to 200 mg (+/-11 mg), and 0.5ml grinding buffer was added. The sample was then homogenised using 5 x 2.5mm diameter beads (Biosepc Products) in a homogeniser (Bertin Technologies Precellys 24 Tissue Homogeniser) for 3 x 20 seconds at 5800rpm. The samples were vortexed to mix before sonicating on ice for 10 minutes and then centrifuging (5000-10000rpm for 30 minutes at 4 °C ). Samples were then filtered through either a 0.45 uM PTFE syringe filter prior to High Performance Liquid Chromatography.

After tissue preparation, tissue samples weighed ranged from 0.130g to 0.2004g (average 0.1907 +/- 0.013g).

The human tissue had various amounts of fat between patient samples. This caused a layer of fat to solidify on the top of the extracted sample even after filtering. To prevent this from blocking the High Performance Liquid Chromatography and interfering with the chromatogram, the extracted sample was left to settle. Then, the sample was taken out of the vial using a glass pasteur pipette by punching through the fat layer, and transferring to a new vial for analysis.

#### 2.9.2.iii High Performance Liquid Chromatography

A Waters Arc system with quaternary colvent manager-R (with inbuilt degasser), Sample Manager FTN-R and 2998 PDA detector was used for High Performance Liquid Chromatography analysis. This system was controlled using Empower™ 3 Chromatography Software. Separation was performed on a Kinetex C18 150x4.6mm, 2.6 µm, 100 Å column.

Mobile phase A (potassium phosphate, 25 mM, pH 3) and mobile phase B (acetonitrile) were used to create a gradient elution. The gradient conditions (with %B in parenthesis) were: 0 min (5%), 15 min (60%), 19 min (5%), with a total analysis time of 25 minutes. The flow rate was 0.8 mL min-1, with a 20 µL injection. Samples were held at 8 °C in the autosampler, the column was heated to 35 °C.

Cefaclor and cefazolin eluted at approximately 5.5 and 8.0 minutes respectively, and were both extracted at 270 nm.

Quantification of cefazolin was calculated using the ratio of cefazolin to the internal standard (Cefaclor) in the prepared samples.

### 2.9.2.iv Specificity and Selectivity

Specificity is the ability to ensure that the analytical procedures for sample preparation and HPLC measurement allows a reliable determination of cefazolin in the presence of components expected to be present in the solution (e.g. other compounds extracted from tissue, matrix, impurities in reagents).

The method was determined to be selective for cefazolin. Grinding buffer (blank matrix) was analysed with every batch of samples. This confirmed that there was no interfering peak arising from the sample preparation (Figure 2.2).

In the initial method performed on porcine tissue, no compounds were extracted from the tissue matrix, which co-eluted at the same time as cefazolin. Unspiked human tissue matrix was not used for analysis. However, analysis of the human tissue samples (containing cefazolin) did not show any co-elution with an unknown peak (Figure 2.3). This was confirmed by the UV plot and the Gaussian shape of the peak.

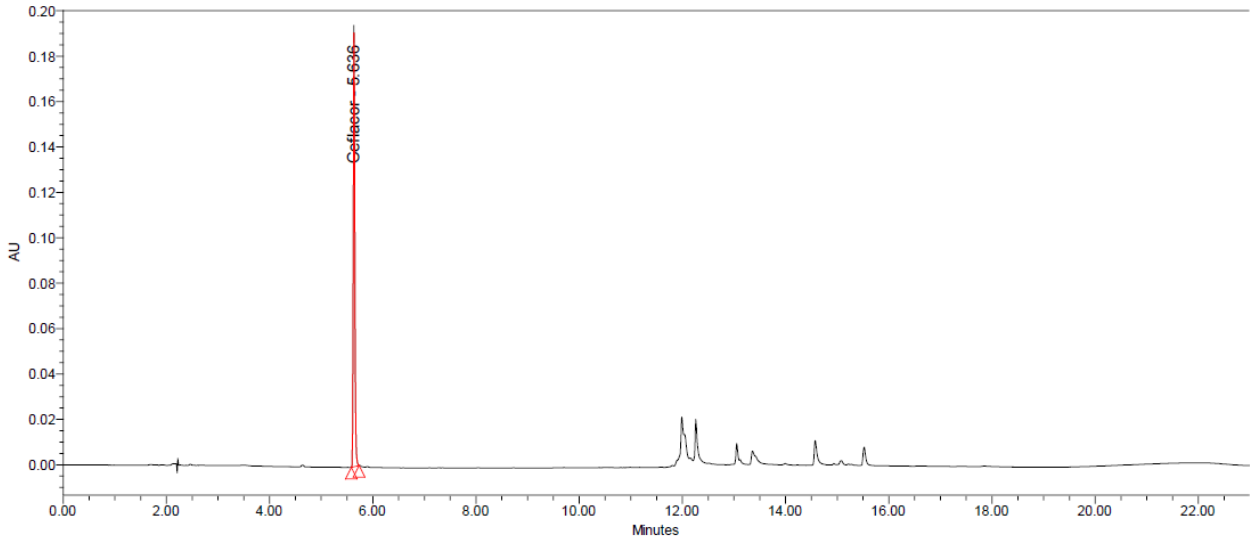


Figure 2.2- Grinding buffer (blank) demonstrating no interference at the elution time of cefazolin (approximately 8 minutes)

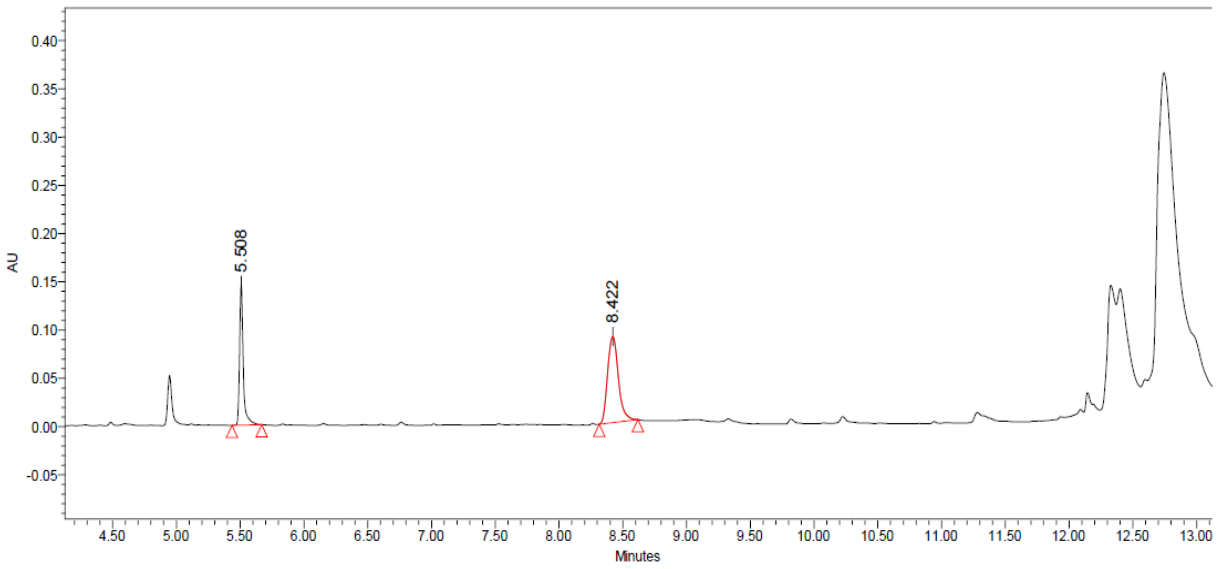


Figure 2.3- Patient sample demonstrating no co-elution of an unknown compound with cefazolin (8.422 minutes)

In some batches there was an interference that slowly eluted from the column. During a long batch analysis the unknown interference co-eluted with cefazolin. An example of an unknown interference moving through the chromatogram, and eventually co-eluting with the cefazolin peak, is shown in Figure 5 (appendix). It was not clear where this arose from but may have been caused due to old solvent or a compound being injected onto the column that elutes very slowly over time.

To mitigate this issue, Mobile phase A (buffer) was made fresh. Additionally, after each batch of samples the system was thoroughly flushed with water, then methanol, to prevent buildup on the column.

Any samples which contained the non-specific co-eluting peak were repeated.

#### 2.9.2.v Accuracy assessed by recovery

Accuracy could not be determined using a certified reference material (CRM) as there was not one commercially available. Instead, spikes of a known concentration of cefazolin were used. To determine the recovery of cefazolin, low and high spikes were analysed in both grinding buffer (blank matrix) and in tissue matrix. Batches of samples were prepared (triplicate, or quintuplet) and analysed on separate

days. This was to also take into consideration the inter-day repeatability and robustness of the method. A summary of the recovery is provided in Table 3.

A recovery of 90 – 110% is ideal, but 80 – 120% is acceptable when analysing low concentrations of the target analyte (which was carried out in this validation). The recoveries lie within 89 – 99 % of the expected value which is acceptable for the method.

### 2.9.2.vi Cefazolin degradation

Cefazolin is not stable at room temperature for an extended period of time. Degradation of cefazolin is observed in the chromatography; the observed cefazolin peak splits, with the split becoming more pronounced overtime (Figure 2.4)

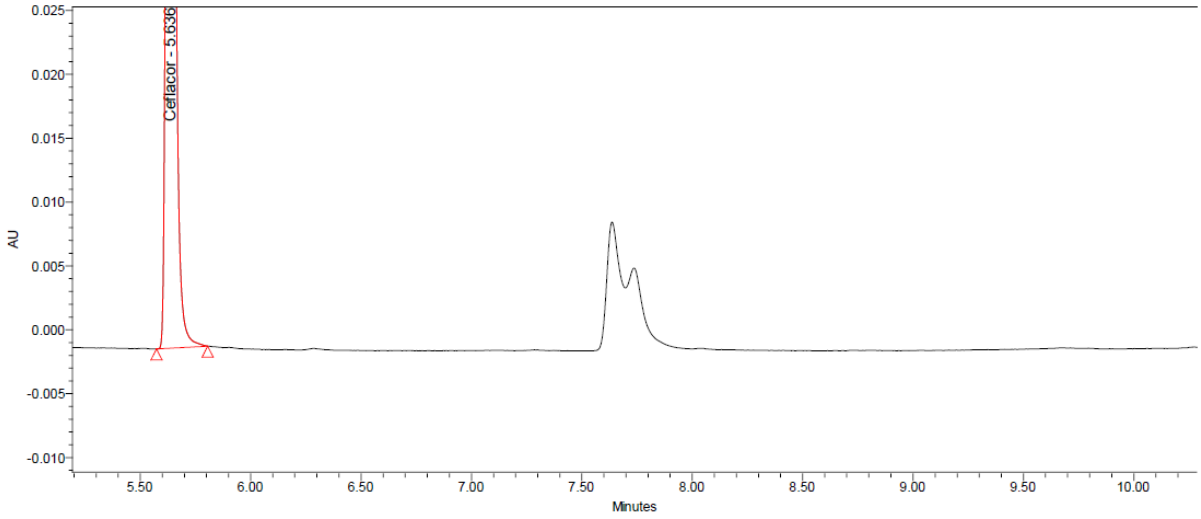


Figure 2.4 Splint cefazolin peak at approximately 7.75 minutes

To investigate whether or not the standards could still be used, a set of standards were left at room temperature for 48 hours, then analysed. The two cefazolin peaks were integrated separately (Figure 2.5).

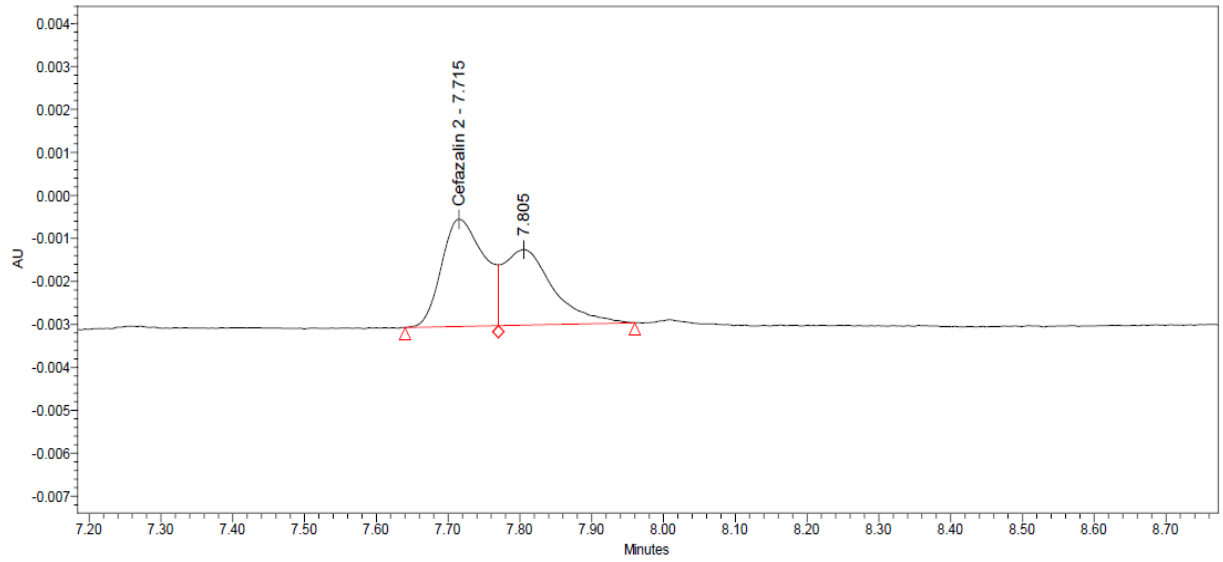


Figure 2.5 Integration of cefazolin peak when split

The sum of peak 1 and peak 2 were used to quantify cefazolin in a sample and this was compared to data calculated from a fresh set of standards (no splitting present). It was determined that the sum of peaks could be used when the peak shows a split at the top, as it does not affect the peak area. However, once the peak becomes two distinct peaks (observed in the lowest concentration standards), low level concentrations are unable to be accurately detected and the limit of detection is compromised. Fortunately, concentrations at this low level were not required for the samples that were currently being processed and these standards could be removed from the calibration curve.

To prevent the cefazolin peak degrading, standard and spike solutions were stored in the fridge (short term) or freezer (long term) when not in use. The autosampler was chilled to 8 °C during analysis. Working standards were remade weekly to prevent degradation.

#### 2.9.2.vii Cefaclor

The internal standard Cefaclor exhibited a reduced peak area over time. Therefore, if samples were required to be reinjected, analysis typically needed to occur within 72 hours. If reinjection was outside this timeframe, the internal standard peak area was monitored to determine if there was a reduced peak area. It is thought that this is from degradation since a colour change was observed in the grinding buffer solution that was old.

### 2.9.2 Quality Control

Calibration curves were analysed with every batch of samples and had R2 value of at least 0.998.

Each batch of samples was analysed with two recovery check samples. A low (0.71 µg L-1 cefazolin) and a high (1.77 µg L–1 cefazolin) were analysed. Acceptable recoveries are between 80 – 120 %.

## 2.10 Data Storage

All collected information was stored de-identified in an Excel document, on a secure cloud server at the University of Auckland.

## 2.11 Statistical analysis

Statistical analysis was performed using Microsoft Excel (version 2021).

Basic analysis including means, standard deviation and range were calculated using exploration equations available in Excel. T-tests were used to calculate p-values for measurements that had comparison between males and females.

Excel was also used to perform univariate analysis by Pearson Correlation Matrix for all the predictors that were collected and then compared to tissue and plasma sample cefazolin concentrations. Significant predictors were identified with their respective confidence interval and p-value.

Stepwise linear regression modelling was used to determine which significant variables from the univariate analysis best predicted final cefazolin concentration. The most significant predictor was then placed into an equation format by the software. This equation was then used in Excel to calculate the predicted concentrations for plasma and tissue samples.

# Chapter 3

# Results

### 3.1 Basic demographic data

All participants had basic demographic data recorded. This included age, gender and ethnicity. Participants are numbered by the order they were recruited from number 1 to 20.

12 participants were female (60%) and 8 patients were male (40%). The mean age was 61.5 years (standard deviation (SD) ±15.8, range 21 to 80 years).

Ethnicity was defined as New Zealand European, Maori, Asian and Other. 12 participants identified as New Zealand European (60%), 4 participants identified as Maori (20%), 1 participant identified as Asian (5%) and 3 participants identified as both New Zealand European and Maori (15%).

All procedures undertaken were all exclusively related to the lumbar spine.

### 3.2 Body mass, BMI and BIA basic statistical results

All participants' successfully underwent body habitus measurements including weight, BMI and BIA. Data that was collected from this analysis include; weight (mass), height, waist-to-hip ratio, fat mass percentage and weight, lean mass percentage and weight, dry lean mass weight, total body water (TBW)percentage and volume, extracellular water (ECW) percentage and volume, intracellular water (ICW) percentage and volume, and body cell mass weight. These will be referred to as predictors in the further analysis.

#### 3.2.1 Weight

The mean weight was 88.0 kg (SD ±25.4, range 58 to 151kg). The male mean weight was 98.3 kg (SD ±30.0, range 65 to 151 kg) and the female mean weight was 81.0 kg (SD ±20.3, range 58 to 129 kg) (male vs female, p=0.14).

#### 3.2.3 Height

The mean height was 169.7 cm (SD ±11.6, range 154.2 to 198 cm). The male mean height was 179.8 cm (SD ±10.3, range 166 to 198 cm) and the female mean height was 163.0 cm (SD ±6.5, range 154 to 175 cm) (male vs female, p=0.001).

### 3.2.3 BMI

The mean BMI was 30.2 kg/m² (SD ±6.52, range 21.7 to 44.8 kg/m²). Male mean BMI was 30.0 kg/m² (SD ±6.9, range 21.7 to 38.5 kg/m²) and female mean BMI was 30.3 kg/m² (SD ±6.6, range 22.5 to 44.8 kg/m²) (male vs female, p=0.9).

Five participants (25%) had a BMI under 25 kg/m², 6 participants (30%) had a BMI between 25 and 30 kg/m² and 9 participants (45%) had a BMI of 30 kg/m² or greater.

### 3.2.4 Fat mass

The mean fat mass percentage was 36.4% (SD ±9.46, range 19.4 to 52.1%). Male mean fat mass percentage was 27.8% (SD±5.80, range 19.4 to 34.9%) and female mean fat mass percentage was 42.2% (SD±6.50, range 32.1 to 52.1%) (male vs female, p=0.001).

The mean fat mass weight was 32.3kg (SD ±13.3, range 14.9 to 65.5 kg). Male mean fat mass weight was 28.2 kg (SD±12.6, range 14.9 to 45.7 kg) and female mean fat mass weight was 35.0 kg (SD±13.6, range 18.9 to 65.5 kg) (male vs female, p=0.273).

### 3.2.5 Lean mass

The mean lean mass percentage was 63.6% (SD ±9.5, range 48.8 to 80.5%). Male mean lean mass percentage was 72.2% (SD±5.80, range 65.1 to 80.6) and female mean lean mass percentage was 57.8% (SD±6.50, range 47.9 to 67.9) (male vs female, p=0.001).

The mean lean mass weight was 55.6 kg (SD ±17.7, range 35.4 to 105.3 kg). Male mean lean mass weight was 70.1 kg (SD±18.6, range 47.9 to 105.0 kg) and female mean lean mass weight was 45.9 kg (SD±7.94, range 35.4 to 62.5 kg) (male vs female, p=0.001).

| Participant Number | Age (years) | Gender | ABW (kg) | Height (cm) | BMI (kg/m²) | Waist/hip ratio | FMW (kg) | LMW (kg) | FMP (%) | LMP (%) | DLM (kg) | TBW (L) | TBW-P (%) | ECW (L) | ECW-P (%) | ICW (L) | ICW-P (%) | BCM (kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 80 | F | 72.3 | 167 | 25.9 | 1.05 | 28.1 | 44.2 | 38.8 | 61.2 | 8.1 | 36.2 | 50 | 16.9 | 23.4 | 19.5 | 26.9 | 27.8 |
| 2 | 58 | F | 103.6 | 162 | 39.5 | 0.94 | 54 | 49.6 | 52.1 | 47.9 | 11 | 38.6 | 37.3 | 18.8 | 18.1 | 23.3 | 22.5 | 33.4 |
| 3 | 59 | M | 105.3 | 169 | 36.9 | 0.97 | 36.8 | 68.5 | 34.9 | 65.1 | 17.4 | 51.1 | 48.6 | 21.4 | 20.4 | 30.4 | 28.7 | 43.2 |
| 4 | 75 | F | 67.5 | 157 | 27.4 | 1 | 27.8 | 39.7 | 41.1 | 58.9 | 6 | 33.7 | 49.9 | 15.8 | 23.4 | 17.9 | 26.5 | 25.6 |
| 5 | 78 | M | 76.7 | 183 | 22.9 | 0.86 | 14.9 | 61.8 | 19.4 | 80.6 | 11.8 | 50 | 65.2 | 20.5 | 26.7 | 27.7 | 36.1 | 39.6 |
| 6 | 61 | F | 65 | 157 | 26.4 | 0.9 | 25.5 | 39.5 | 39.2 | 60.8 | 7.6 | 31.9 | 49 | 14.9 | 23 | 17.2 | 26.5 | 24.6 |
| 7 | 43 | F | 92 | 175 | 30 | 0.88 | 37.4 | 54.6 | 40.7 | 59.3 | 16.4 | 38.2 | 41.5 | 17.9 | 19.5 | 22.8 | 24.8 | 32.5 |
| 8 | 58 | F | 59 | 162 | 22.5 | 0.83 | 18.9 | 40.1 | 32.1 | 67.9 | 8.2 | 31.9 | 54.1 | 14.7 | 25 | 16.6 | 28.2 | 23.8 |
| 9 | 44 | M | 151 | 198 | 38.5 | 0.99 | 45.7 | 105.3 | 30.3 | 69.7 | 25.8 | 79.5 | 52.6 | 32.8 | 21.7 | 45 | 29.8 | 64.4 |
| 10 | 66 | F | 87 | 159 | 34.4 | 0.93 | 42.9 | 44.1 | 49.3 | 50.7 | 8.9 | 35.3 | 40.5 | 16.7 | 19.2 | 20.9 | 24.1 | 29.9 |
| 11 | 69 | M | 81 | 181 | 24.7 | 0.92 | 15.8 | 65.2 | 19.5 | 80.5 | 14.2 | 51 | 63 | 21.6 | 26.6 | 27 | 33.3 | 38.5 |
| 12 | 61 | M | 65 | 173 | 21.7 | 0.98 | 17.1 | 47.9 | 26.3 | 73.7 | 10.8 | 37.1 | 57.1 | 15.8 | 24.3 | 21 | 32.4 | 30.1 |
| 13 | 75 | M | 104 | 182 | 31.4 | 1.04 | 30.2 | 73.8 | 29 | 71 | 16.8 | 57 | 54.8 | 24.3 | 23.4 | 30.9 | 29.7 | 44.1 |
| 14 | 66 | F | 78.2 | 168 | 27.7 | 0.94 | 29.6 | 48.6 | 37.9 | 62.1 | 10.9 | 37.7 | 48.2 | 18 | 23 | 20.1 | 25.7 | 28.7 |
| 15 | 73 | F | 83.2 | 169 | 29.1 | 0.93 | 30.1 | 53.1 | 36.2 | 63.8 | 10.8 | 42.3 | 50.8 | 19.7 | 23.7 | 22.7 | 27.3 | 32.4 |
| 16 | 31 | M | 129.8 | 186.5 | 37.3 | 0.94 | 43.6 | 86.2 | 33.6 | 66.4 | 26.8 | 59.4 | 45.8 | 24.7 | 19 | 36.2 | 27.9 | 51.7 |
| 17 | 67 | F | 58 | 157 | 23.5 | 0.91 | 22.6 | 35.4 | 39 | 61 | 5.6 | 29.8 | 51.3 | 14 | 24.1 | 15.5 | 26.8 | 22.2 |
| 18 | 75 | M | 74 | 166 | 26.9 | 0.98 | 21.6 | 52.4 | 29.2 | 70.8 | 10.3 | 42.1 | 56.9 | 18.4 | 24.9 | 22.6 | 30.5 | 32.2 |
| 19 | 70 | F | 77.5 | 154.2 | 32.7 | 0.85 | 37.9 | 39.6 | 48.9 | 51.1 | 6.6 | 32.9 | 42.5 | 15.9 | 20.5 | 18.5 | 23.9 | 26.4 |
| 20 | 21 | F | 128.9 | 169 | 44.8 | 0.95 | 65.5 | 62.5 | 51.2 | 48.8 | 18 | 44.5 | 34.8 | 21.6 | 16.9 | 28.5 | 22.3 | 40.8 |

Table 3.1- table demonstrating individual participants characteristics (age and gender (male=M, female=F) and body habitus recordings, including: Absolute body weight (ABW), height, body mass index (BMI), waist-to-hip ratio (waist/hip ratio), fat mass weight (FMW), lean mass weight (LMW), fat mass percentage (FMP), lean mass percentage (LMP), dry lean mass weight (DLM), Total body water volume (TBW), total body water percentage (TBW-P), extracellular water percentage (ECW), extracellular water volume (ECW-V), intracellular water volume (ICW), intracellular water percentage (ICW-P), body cell mass weight (BCM).

### 3.3 Individual cefazolin dosing received by participants

18 of 20 participants (90%) received a cefazolin dose of 2 g. Two of those who received 2 g weighed over 120 kg in body mass. One participant received 1 gram of cefazolin, with this participant weighing 59kg. The final participant received 3 g of cefazolin with a body mass of 151kg.

### 3.4 Tissue and plasma sample cefazolin levels basic statistics

Basic statistical analysis was performed of the tissue and plasma samples with these analysed as a grouped and individual response to cefazolin.

#### 3.4.1 Tissue and plasma samples grouped statistics

Of the 20 participants, all had a 30- and 60-minute tissue sample obtained; 11 of the 20 participants had a 90 minute sample collected, with those that did not have a 90 minute sample collected, due to the surgery not being beyond the mark where collection could occur before wound closure. All participants had a 30 minute plasma sample successfully taken. The plasma cefazolin sample mean concentration was 34.1 mg/L (SD ±10.2, range 18.0 to 54.0 mg/L).

Figure 3.1 demonstrates the individual plasma cefazolin concentrations with wide variation, but suggestive of potentially two modes (one approximately25 mg/L and another approximately at 45 mg/L).

Mean tissue samples cefazolin concentrations were 44.4 mg/kg at 30-minutes (SD ±18.6, range 21.6 to 92.5 mg/kg), 43.8 mg/kg at 60-minutes (SD ±20.4, range 19.0 to 91.91 mg/kg), and 25.2 mg/kg at 90-minutes (SD ±25.2, range 5.7 to 102.0 mg/kg).

| Participant Number | Cefazolin dosage (g) | Plasma cefazolin concentration (mg/L) at 30 minutes | Tissue cefazolin concentration (mg/kg) at 30 minutes | Tissue cefazolin concentration (mg/kg) at 60 minutes | Tissue cefazolin concentration (mg/kg) at 90 minutes |
|---|---|---|---|---|---|
| 1 | 2 g | 45.5 | 45.2 | 40.1 | 32.9 |
| 2 | 2 g | 34 | 38.5 | 41.1 | 24.2 |
| 3 | 2 g | 25 | 48.8 | 76 | 34.6 |
| 4 | 2 g | 54 | 88.6 | 78.9 | 102 |
| 5 | 2 g | 24 | 25.3 | 20.7 | - |
| 6 | 2 g | 26 | 48.5 | 91.1 | 25.2 |
| 7 | 2 g | 29 | 32.7 | 28.3 | - |
| 8 | 1 g | 18 | 21.6 | 28.4 | - |
| 9 | 3 g | 33 | 36.7 | 30.7 | - |
| 10 | 2 g | 44 | 31.5 | 60.1 | 24.7 |
| 11 | 2 g | 26 | 45.6 | 33.2 | 18.1 |
| 12 | 2 g | 44 | 46.8 | 34.7 | 5.7 |
| 13 | 2 g | 24 | 33.9 | 27.3 | 18.3 |
| 14 | 2 g | 31 | 29 | 47.1 | - |
| 15 | 2 g | 37 | 92.5 | 57.8 | - |
| 16 | 2 g | 26 | 42.9 | 36.7 | - |
| 17 | 2 g | 45.8 | 59.3 | 59.2 | 38.9 |
| 18 | 2 g | 46.6 | 50.6 | 38.3 | 48.7 |
| 19 | 2 g | 42.9 | 45.6 | 26.6 | - |
| 20 | 2 g | 25.3 | 24.8 | 19 | - |

Table 3.2- Table demonstrating individual participants data with regards to cefazolin dosage received, 30-minute plasma cefazolin concentration recorded, 30-, 60- and 90-minute tissue cefazolin concentration recorded. Participants with no 90-minute tissue cefazolin concentration did not have sample obtained.

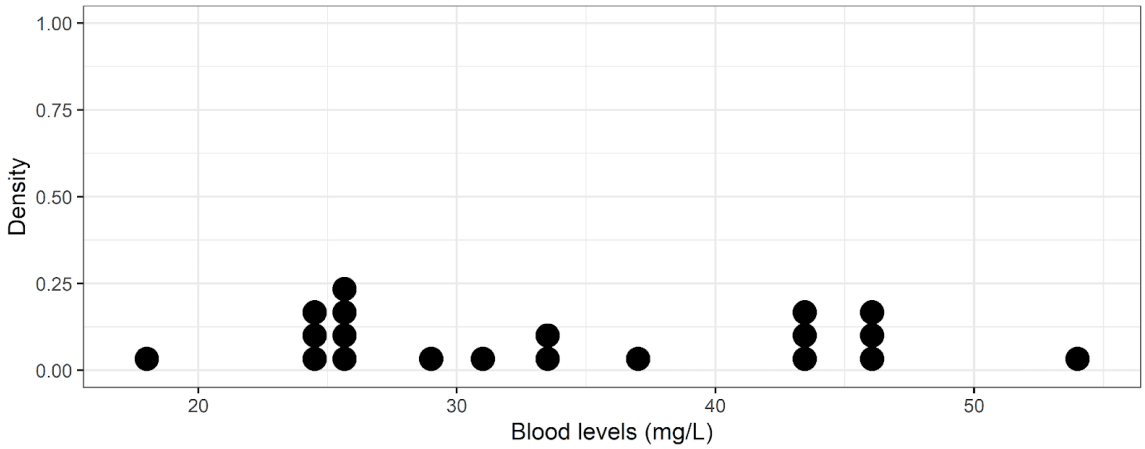


Figure 3.1- Column graph showing individual participants plasma cefazolin concentrations (blood levels on x-axis). Each dot represents a participant's plasma cefazolin concentration, with stacking occurring if results have the same integer recording.

| | 30-minute plasma sample concentration mg/L | 30-minute tissue sample concentration mg/Kg | 60-minute tissue sample concentration mg/Kg | 90-minute tissue sample concentration mg/Kg |
|---|---|---|---|---|
| Mean | 34.1 | 44.4 | 43.8 | 33.9 |
| Standard deviation | 10.2 | 18.6 | 20.4 | 25.3 |
| Minimum | 18.0 | 21.6 | 19.0 | 5.70 |
| Maximum | 54.0 | 92.5 | 91.1 | 102 |

Table 3.3- Plasma and tissue sample cefazolin concentrations mean (with standard deviation), minimum and maximum concentrations for participants as a group. N=20.

### 3.4.2 Tissue and plasma samples individual participants' response

The tissue cefazolin concentrations had varying trends in each participant, with some having concentration levels trending down from the initial dose, whilst others peaked in the second or third sample taken (observations 2, 3, 4, 6, 8, 10, 14, 17), as seen in figures 3.12 and 3.11. Of all participants, 65% (13 participants) had a peak in their concentration at the 30-minute sample, 30% (6 participants) had a peak in concentration at the 60-minute sample and 5% (1 participant) had a peak at the 90-minute sample.

When plasma and tissue cefazolin levels are compared to weight and BMI, wide variation is found in the cefazolin levels, as seen in scatterplot figures 3.2 to 3.9.

### 3.4.3 Achievement of minimal inhibitory concentration for cefazolin

The MIC for cefazolin as a prophylactic agent is 2 mg/kg for tissue and 2 mg/L for plasma.

All participants achieved both tissue and plasma MIC, with all samples obtained at all time points.

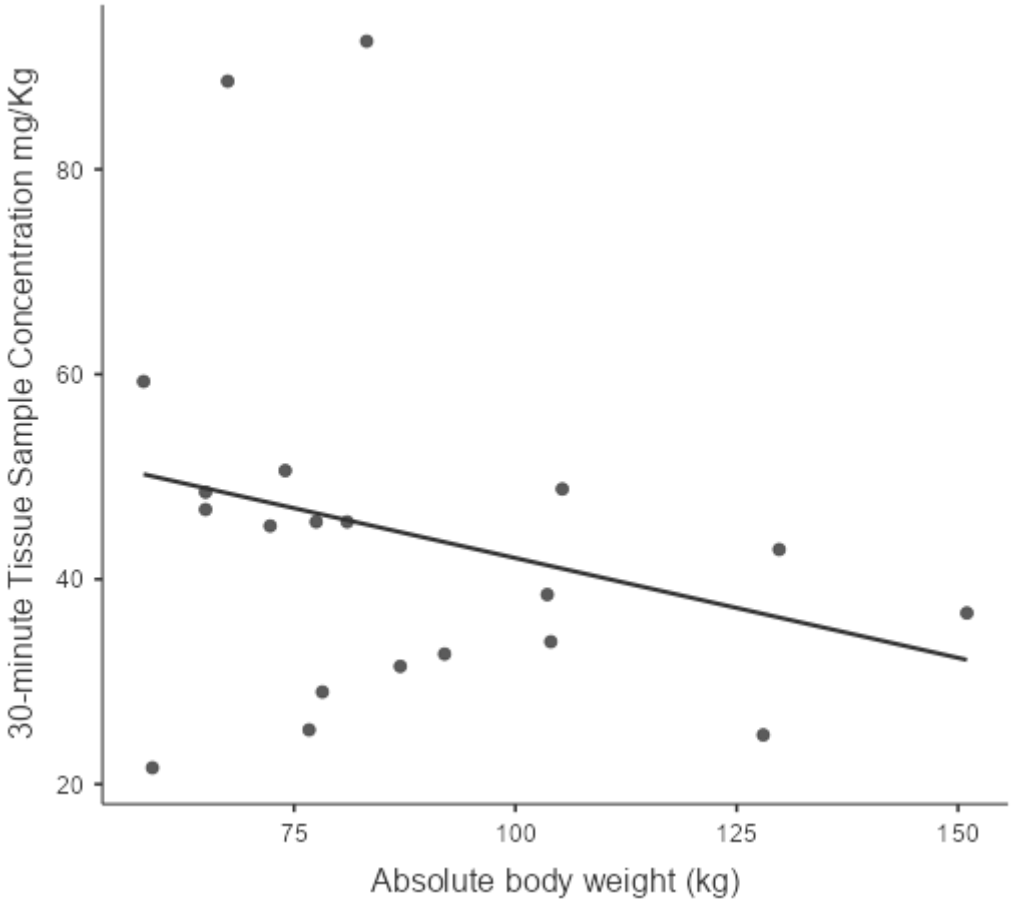


Figure 3.2- Scatterplot graph demonstrating participants' absolute body weight (x-axis) compared to the 30-minute tissue sample cefazolin concentration (y-axis). Each dot represents a participant's recording. Linear regression line demonstrated. N=20.

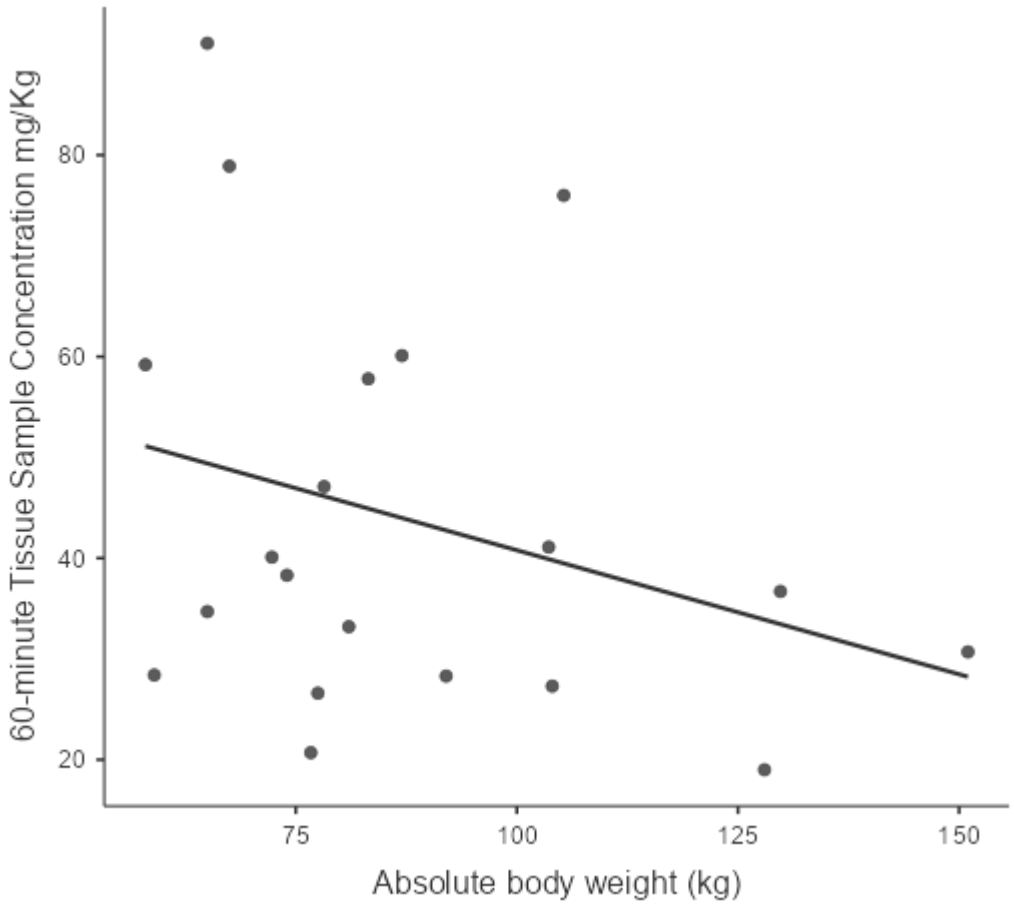


Figure 3.3- Scatterplot graph demonstrating participants' absolute body weight (x-axis) compared to the 60-minute tissue sample cefazolin concentration (y-axis). Each dot represents a participant's recording. Linear regression line demonstrated. N=20.

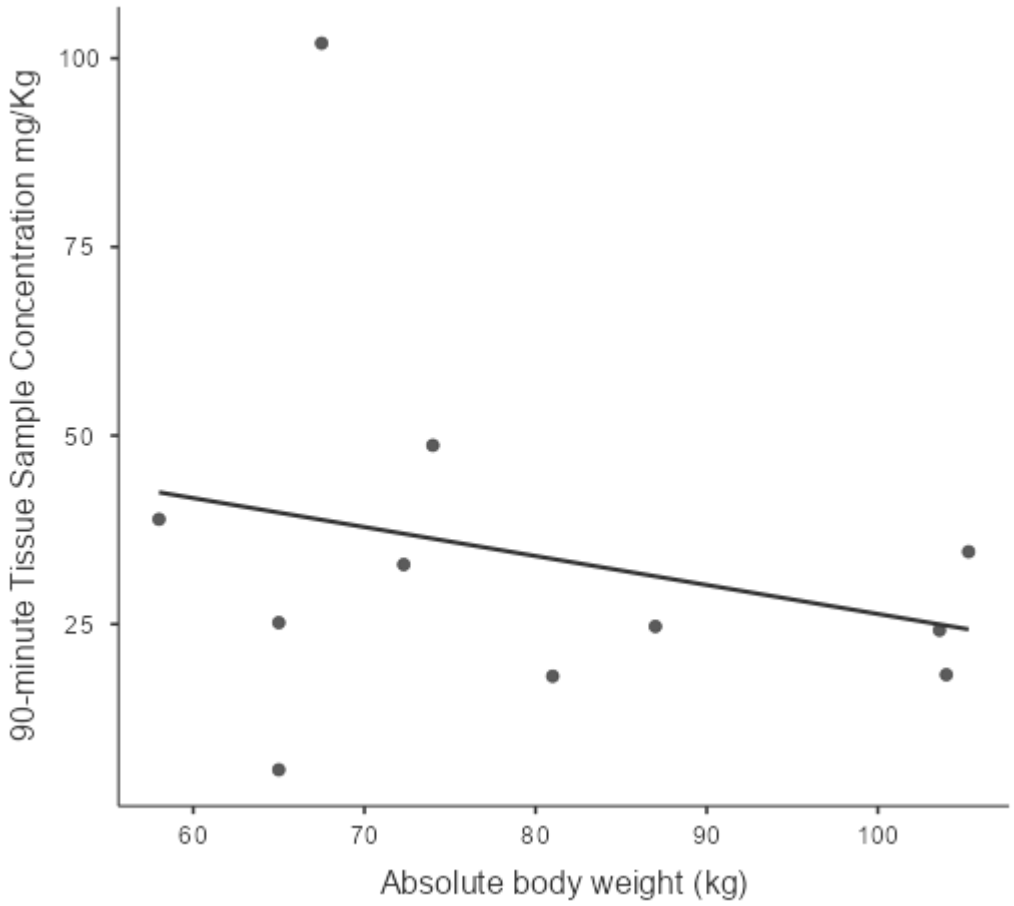


Figure 3.4- Scatterplot graph demonstrating participants' absolute body weight (x-axis) compared to the 90-minute tissue sample cefazolin concentration (y-axis). Each dot represents a participant's recording. Linear regression line demonstrated. N=11.

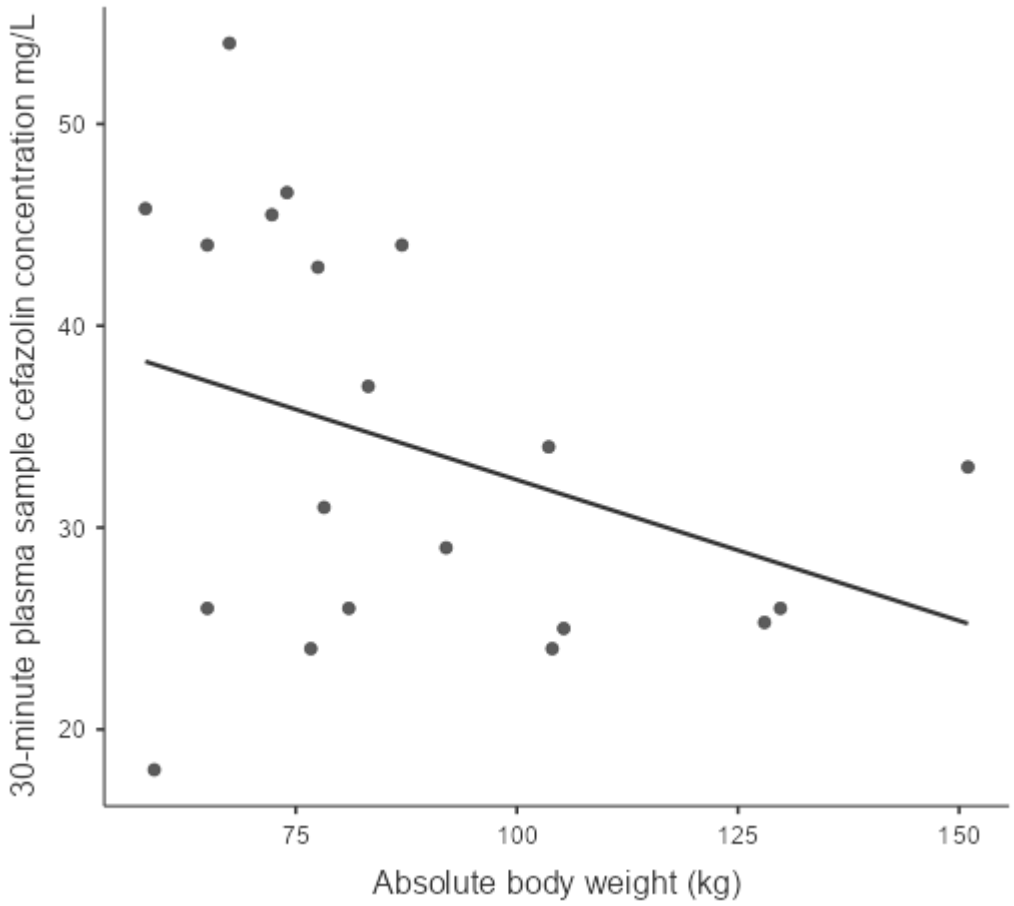


Figure 3.5- Scatterplot graph demonstrating participants' absolute body weight (x-axis) compared to the 30-minute plasma sample cefazolin concentration (y-axis). Each dot represents a participant's recording. Linear regression line demonstrated. N=20.

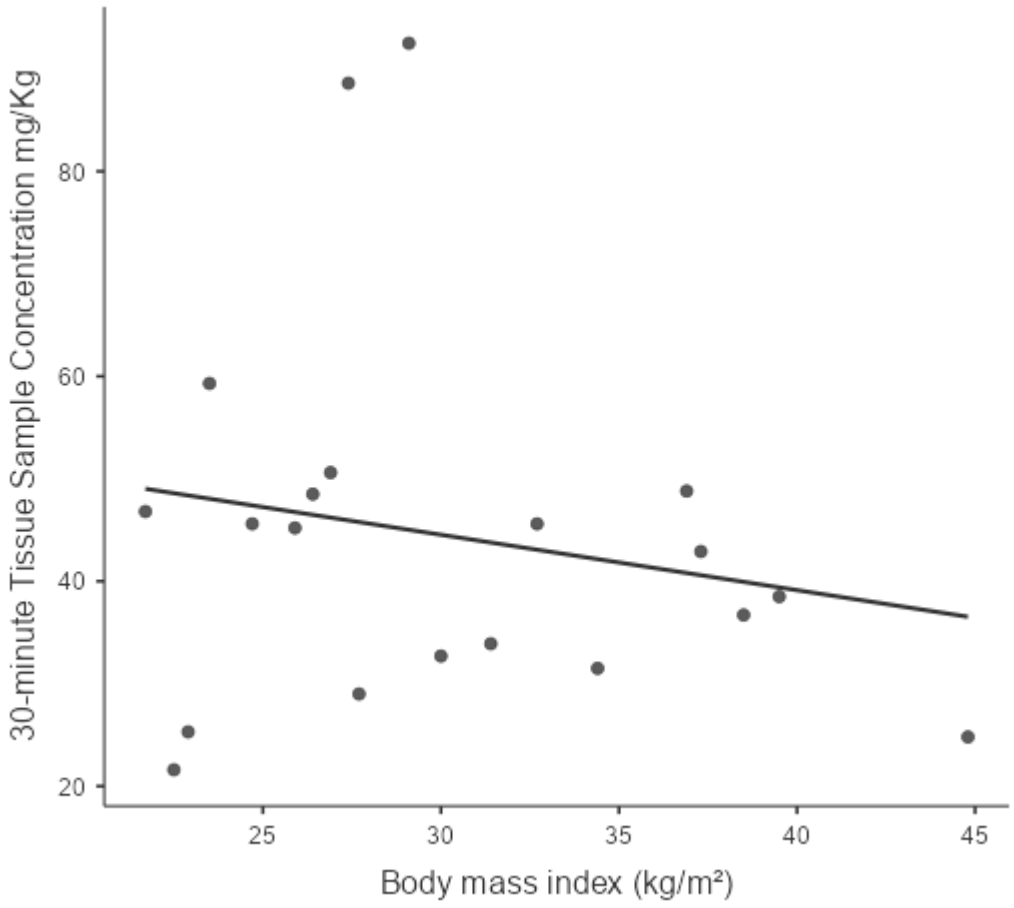


Figure 3.6- Scatterplot graph demonstrating participants' body mass index (x-axis) compared to the 30-minute tissue sample cefazolin concentration (y-axis). Each dot represents a participant's recording. Linear regression line demonstrated. N=20.

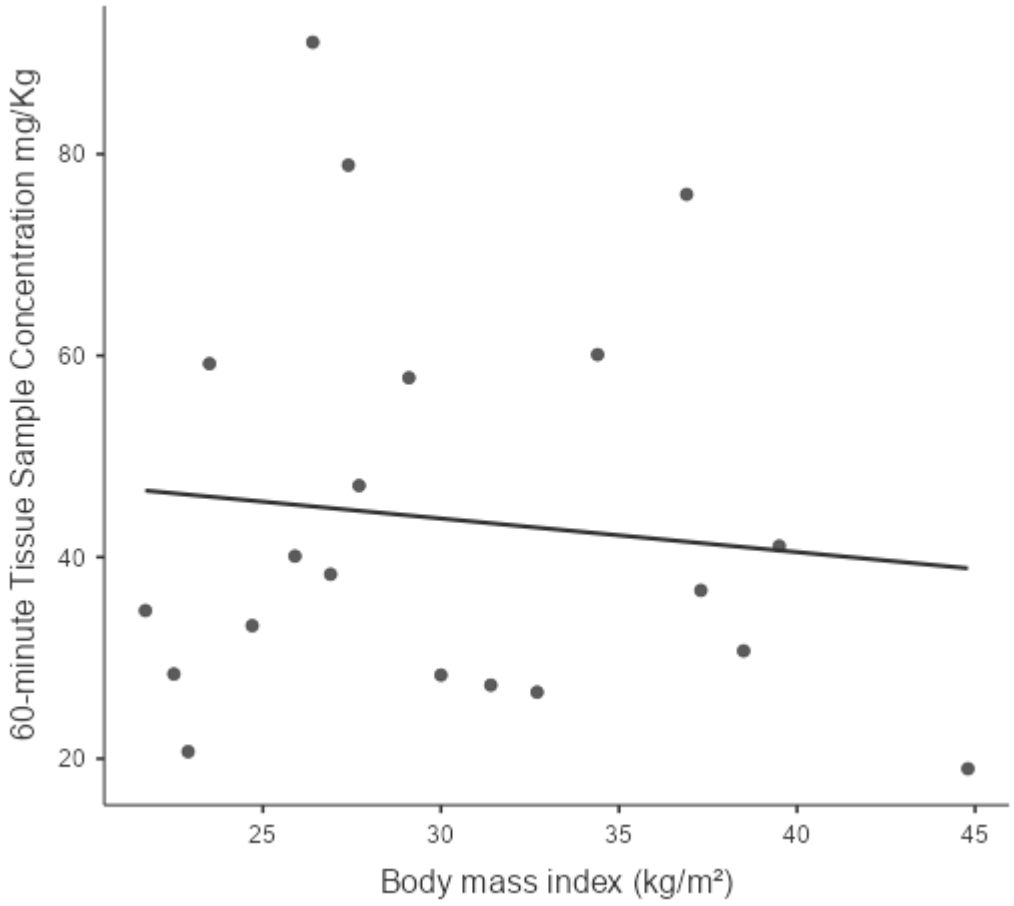


Figure 3.7- Scatterplot graph demonstrating participants' body mass index (x-axis) compared to the 60-minute tissue sample cefazolin concentration (y-axis). Each dot represents a participant's recording. Linear regression line demonstrated. N=20.

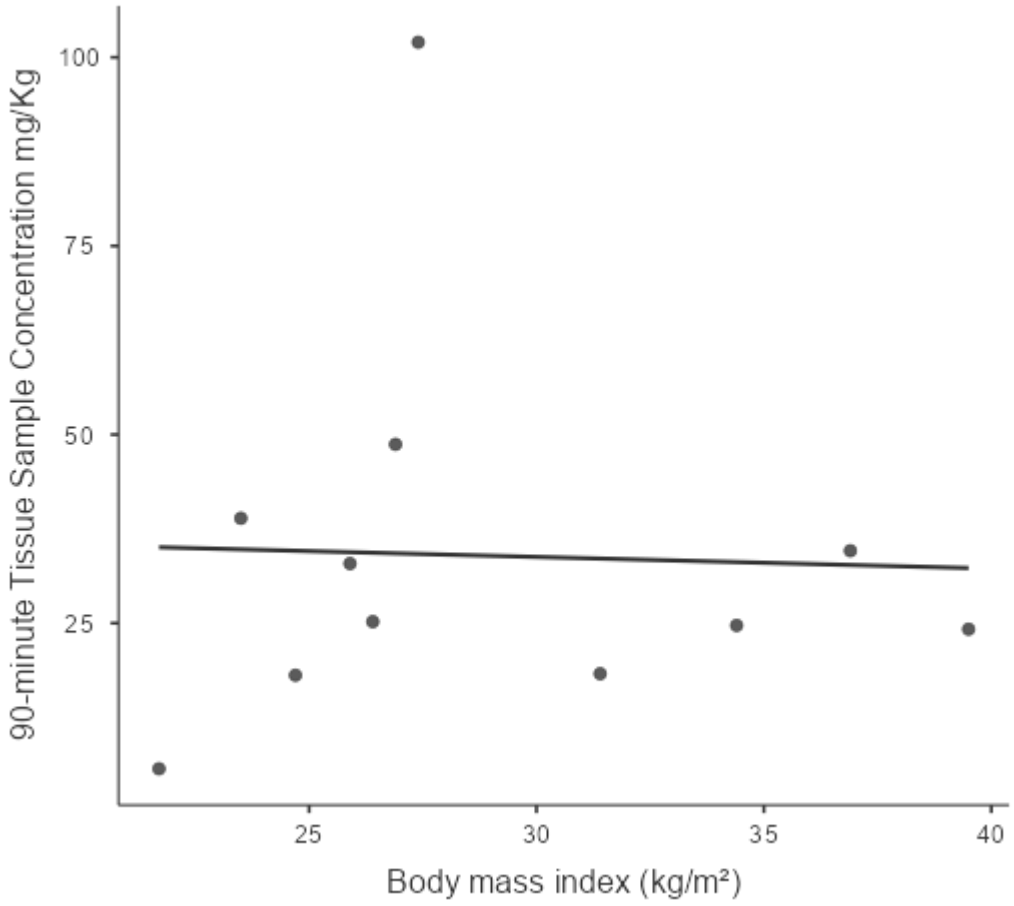


Figure 3.8- Scatterplot graph demonstrating participants' body mass index (x-axis) compared to the 90-minute tissue sample cefazolin concentration (y-axis). Each dot represents a participant's recording. Linear regression line demonstrated. N=11.

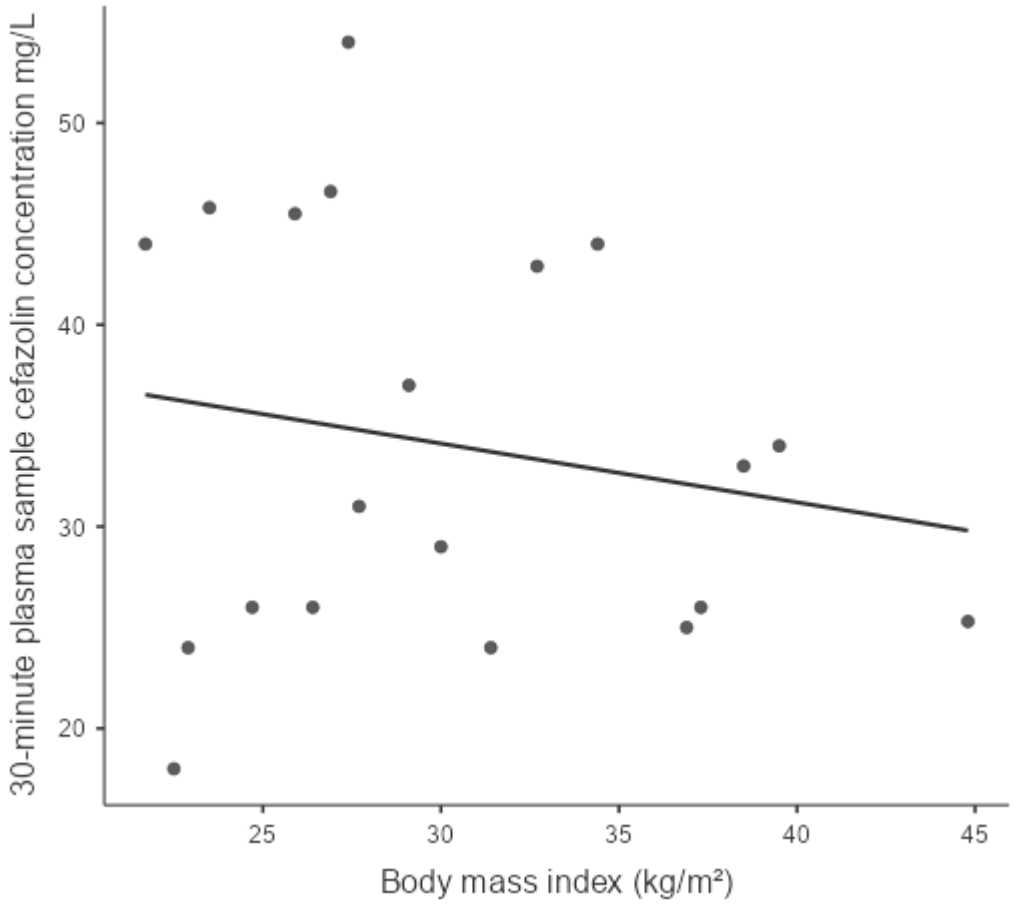


Figure 3.9- Scatterplot graph demonstrating participants' body mass index (x-axis) compared to the 30-minute plasma sample cefazolin concentration (y-axis). Each dot represents a participant's recording. Linear regression line demonstrated. N=30.

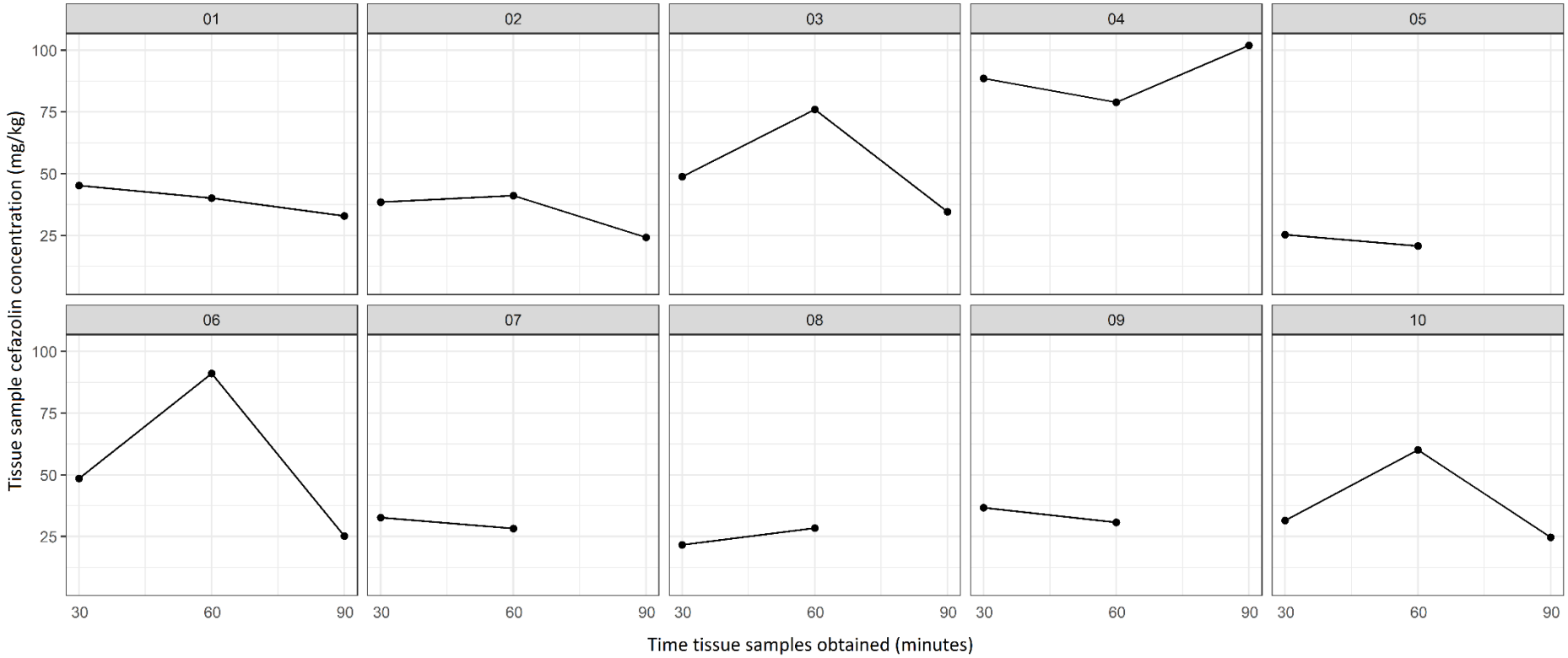


Figure 3.10- graphs demonstrating tissue concentrations (y-axis) compared to time from cefazolin dosing (x-axis) (30, 60 and 90 minutes) for participants 1-10. Participants missing 90-minute plot did not have a 90-minute sample obtained.

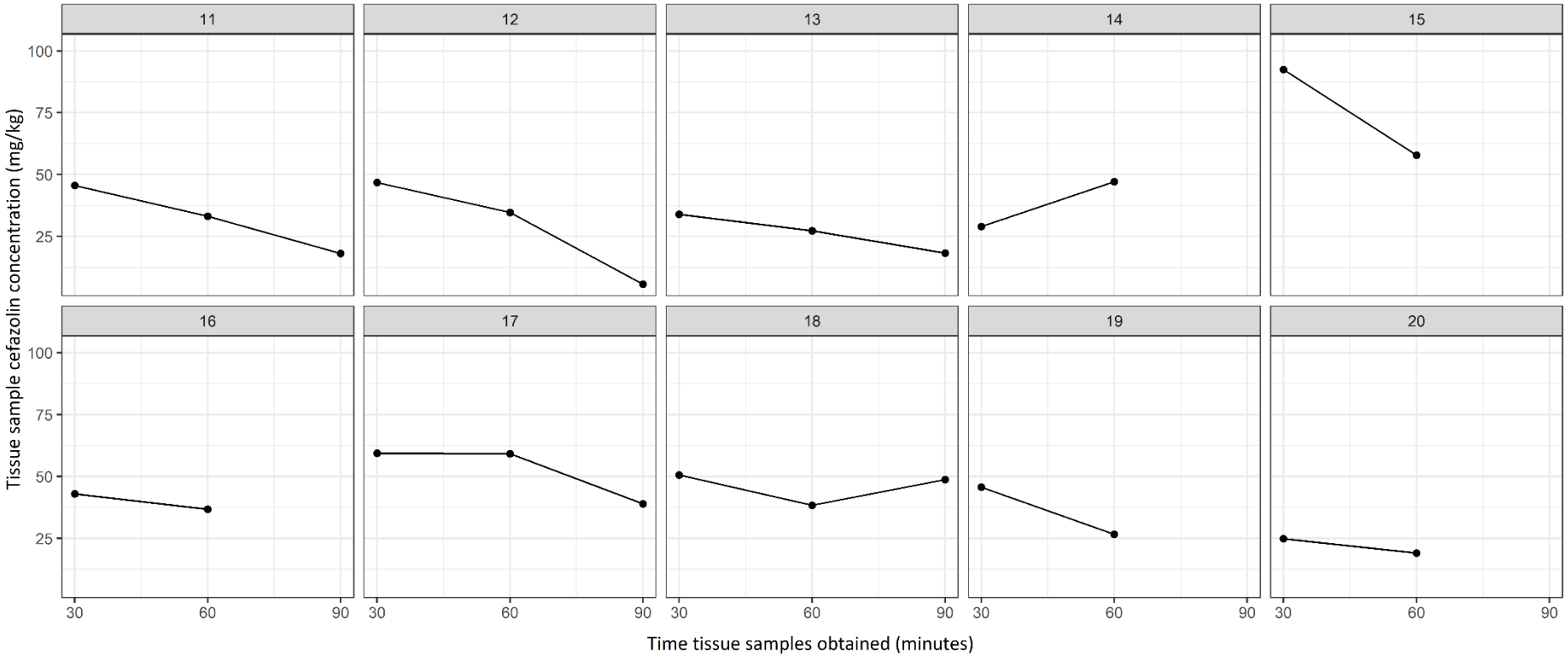


Figure 3.11- graphs demonstrating tissue concentrations (y-axis) compared to time from cefazolin dosing (x-axis) (30, 60 and 90 minutes) for participants 11-20. Participants missing 90-minute plot did not have a 90-minute sample obtained.

### 3.5 Removal of potential confounder for statistical analysis of predictors

Of the 20 participants, 18 received 2 g of cefazolin. Of the remaining 2 participants, 1 received 1 g cefazolin and the other 3 g of cefazolin.

Due to the potential confounding effect that this could have on analysis, it was determined to use the data of only the 18 participants who received 2 g of cefazolin for any further analysis that follows.

Analysis of the tissue samples was limited to the 30-minute and 60-minute tissue sample, as there was a large quantity of missing data for the 90-minute tissue sample. This is because for only 55% of participants a 90-minute tissue sample was obtained.

### 3.6 Univariate Analysis

A Pearson Correlation matrix was used for simple univariate analysis to look for any correlation between plasma and tissue cefazolin levels and the predictors obtained. Correlation matrix analysis was done for the 18 participants who received 2 g of cefazolin.

#### 3.6.1 Plasma sample Pearson Correlation Matrix

A Pearson Correlation matrix for plasma concentration using the 18 participants who received 2 g cefazolin found multiple predictors significantly associated with plasma concentration, all of them having a negative association to cefazolin concentration.

The following list is of the significant predictors using the participants who received 2 g of cefazolin. Confidence Intervals (CI) in brackets.

- Lean mass weight r-value was -0.705 (CI -0.882 to -0.355, p=0.001)
- Dry lean mass weight r-value was -0.688 (CI -0.874 to -0.326, p=0.002)
- Absolute body weight r-value was -0.589 (CI -0.828 to -0.168, p=0.01)
- Height r-value was -0.633 (CI -0.849 to -0.236, p=0.005)

Lean mass weight was the largest and most significant finding over any other significant predictor, including absolute body weight, or insignificant predictor, including BMI r-value -0.327 (CI -0.689 to 0.165, p=0.185) (tables 3.4 and 3.5).

### 3.6.2 Tissue sample Pearson Correlation Matrix

A Pearson Correlation matrix for tissue concentration found multiple predictors significantly associated with both the 30- and 60-minute tissue samples, having both positive and negative correlation to cefazolin concentration.

The following list is of the significant predictors, with the timed tissue sample they are significant for identified after the brackets. Confidence Intervals (CI) in brackets.

- 30-minute plasma cefazolin concentration r value was 0.527 (CI 0.079 to 0.797, p=0.025) for 30-minute tissue samples
- Height r-value of -0.552 (CI -0.810 to -0.115, p=0.017) for the 60-minute tissue samples
- 30-minute tissue cefazolin concentration r-value 0.556 (CI 0.120 to 0.812, p=0.017) for the 60-minute tissue samples

No body morphology measures used for current cefazolin dosing were significant, including BMI and absolute weight, as well as lean or fat mass measurements (tables 3.4 and 3.5).

| *Variable* | *ABW* | *Ht* | *BMI* | *Waist /hip ratio* | *FMW* | *LMW* | *FMP* | *LMP* | *DLM* | *TBW* | *TBW-P* | *ECW* | *ECW-P* | *ICW* | *ICW-P* | *BCM* | *30-TS* | *60-TS* | *PS* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *ABW* | | 0.46 | **0.86** | 0.09 | **0.76** | **0.77** | 0.24 | -0.24 | **0.85** | **0.67** | **-0.49** | **0.80** | **-0.70** | **0.82** | -0.29 | **0.82** | -0.37 | -0.35 | **-0.58** |
| *Ht* | 0.46 | | -0.02 | 0.10 | -0.15 | **0.86** | **-0.67** | **0.67** | **0.77** | **0.86** | 0.43 | **0.80** | 0.19 | **0.81** | **0.59** | **0.81** | -0.34 | **-0.55** | **-0.63** |
| *BMI* | **0.86** | -0.02 | | 0.02 | **0.95** | 0.38 | **0.64** | **-0.64** | **0.52** | 0.28 | **-0.78** | 0.46 | **-0.89** | **0.48** | **-0.64** | **0.48** | -0.27 | -0.12 | -0.32 |
| *Waist/ hip ratio* | 0.09 | 0.10 | 0.02 | | -0.01 | 0.15 | -0.08 | 0.08 | 0.07 | 0.19 | 0.08 | 0.19 | 0.07 | 0.13 | 0.04 | 0.13 | 0.17 | 0.10 | 0.24 |
| *FMW* | **0.76** | -0.15 | **0.95** | -0.01 | | 0.18 | **0.79** | **-0.79** | 0.38 | 0.06 | **-0.91** | 0.26 | **-0.95** | 0.28 | **-0.79** | 0.28 | -0.27 | -0.14 | -0.19 |
| *LMW* | **0.77** | **0.86** | 0.38 | 0.15 | 0.18 | | -0.41 | 0.41 | **0.93** | **0.97** | 0.14 | **0.96** | -0.14 | **0.98** | 0.33 | **0.98** | -0.30 | -0.39 | **-0.70** |
| *FMP* | 0.24 | **-0.67** | **0.64** | -0.08 | **0.79** | -0.41 | | **-1.00** | -0.20 | **-0.52** | **-0.94** | -0.33 | **-0.80** | -0.33 | **-0.97** | -0.33 | -0.02 | 0.18 | 0.25 |
| *LMP* | -0.24 | **0.67** | **-0.64** | 0.08 | **-0.79** | 0.41 | **-1.00** | | 0.20 | **0.52** | **0.94** | 0.33 | **0.80** | 0.33 | **0.97** | 0.33 | 0.02 | -0.18 | -0.25 |

Table 3.4 - description on page 72

| *Variable* | *ABW* | *Ht* | *BMI* | *Waist /hip ratio* | *FMW* | *LMW* | *FMP* | *LMP* | *DLM* | *TBW* | *TBW-P* | *ECW* | *ECW-P* | *ICW* | *ICW-P* | *BCM* | *30-TS* | *60-TS* | *PS* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *DLM* | **0.85** | **0.77** | **0.52** | 0.07 | 0.38 | **0.93** | -0.20 | 0.20 | | **0.84** | -0.11 | **0.86** | -0.38 | **0.92** | 0.10 | **0.92** | -0.35 | -0.37 | **-0.68** |
| *TBW* | **0.67** | **0.86** | 0.28 | 0.19 | 0.06 | **0.97** | **-0.52** | **0.52** | **0.84** | | 0.29 | **0.96** | 0.01 | **0.96** | 0.46 | **0.96** | -0.25 | -0.38 | **-0.67** |
| *TBW-P* | **-0.49** | 0.43 | **-0.78** | 0.08 | **-0.91** | 0.14 | **-0.94** | **0.94** | -0.11 | 0.29 | | 0.09 | **0.94** | 0.07 | **0.95** | 0.06 | 0.16 | -0.06 | -0.03 |
| *ECW* | **0.80** | **0.80** | 0.46 | 0.19 | 0.26 | **0.96** | -0.33 | 0.33 | **0.86** | **0.96** | 0.09 | | -0.15 | **0.96** | 0.25 | **0.96** | -0.28 | -0.42 | **-0.69** |
| *ECW-P* | **-0.70** | 0.19 | **-0.89** | 0.07 | **-0.95** | -0.14 | **-0.80** | **0.80** | -0.38 | 0.01 | **0.94** | -0.15 | | -0.22 | **0.81** | -0.22 | 0.29 | 0.05 | 0.15 |
| *ICW* | **0.82** | **0.81** | **0.48** | 0.13 | 0.28 | **0.98** | -0.33 | 0.33 | **0.92** | **0.96** | 0.07 | **0.96** | -0.22 | | 0.28 | **1.00** | -0.33 | -0.40 | **-0.69** |
| *ICW-P* | -0.29 | **0.59** | **-0.64** | 0.04 | **-0.79** | 0.33 | **-0.97** | **0.97** | 0.10 | 0.46 | **0.95** | 0.25 | **0.81** | 0.28 | | 0.28 | 0.01 | -0.17 | -0.17 |
| *BCM* | **0.82** | **0.81** | **0.48** | 0.13 | 0.28 | **0.98** | -0.33 | 0.33 | **0.92** | **0.96** | 0.06 | **0.96** | -0.22 | **1.00** | 0.28 | | -0.34 | -0.40 | **-0.69** |

Table 3.4 continued - description on page 72

| *Variable* | *ABW* | *Ht* | *BMI* | *Waist /hip ratio* | *FMW* | *LMW* | *FMP* | *LMP* | *DLM* | *TBW* | *TBW-P* | *ECW* | *ECW-P* | *ICW* | *ICW-P* | *BCM* | *30-TS* | *60-TS* | *PS* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *30-TS* | -0.37 | -0.34 | -0.27 | 0.17 | -0.27 | -0.30 | -0.02 | 0.02 | -0.35 | -0.25 | 0.16 | -0.28 | 0.29 | -0.33 | 0.01 | -0.34 | | **0.55** | **0.52** |
| *60-TS* | -0.35 | **-0.55** | -0.12 | 0.10 | -0.14 | -0.39 | 0.18 | -0.18 | -0.37 | -0.38 | -0.06 | -0.42 | 0.05 | -0.40 | -0.17 | -0.40 | **0.55** | | 0.24 |
| *PS* | **-0.58** | **-0.63** | -0.32 | 0.24 | -0.19 | **-0.70** | 0.25 | -0.25 | **-0.68** | **-0.67** | -0.03 | **-0.69** | 0.15 | **-0.69** | -0.17 | **-0.69** | **0.52** | 0.24 | |

Table 3.4- Pearson correlation matrix for the measurements obtained from weight, height BMI and BIA. Absolute body weight (ABW), height (Ht), body mass index (BMI), waist-to-hip ratio (waist/hip ratio), fat mass weight (FMW), lean mass weight (LMW), fat mass percentage (FMP), lean mass percentage (LMP), dry lean mass weight (DLM), Total body water volume (TBW), total body water percentage (TBW-P), extracellular water percentage (ECW), extracellular water volume (ECW-V), intracellular water volume (ICW), intracellular water percentage (ICW-P), body cell mass weight (BCM), 30-minute tissue cefazolin concentration (30-TS), 60 minute tissue cefazolin concentration (60-TS), and 30-minute plasma cefazolin concentration (PS). Results that are highlighted in **bold** have a significant p-value which can be found in table 3.5.

| *Variable* | *ABW* | *Ht* | *BMI* | *Waist /hip ratio* | *FMW* | *LMW* | *FMP* | *LMP* | *DLM* | *TBW* | *TBW-P* | *ECW* | *ECW-P* | *ICW* | *ICW-P* | *BCM* | *30-TS* | *60-TS* | *PS* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *ABW* | | 0.051 | **<0.0001** | 0.712 | **0.000** | **0.000** | 0.342 | 0.342 | **<0.0001** | **0.002** | **0.039** | **<0.0001** | **0.001** | **<0.0001** | 0.244 | **<0.0001** | 0.123 | 0.156 | **0.010** |
| *Ht* | 0.052 | | 0.920 | 0.678 | 0.541 | **<0.0001** | **0.002** | **0.002** | **0.000** | **<0.0001** | 0.072 | **<0.0001** | 0.443 | **<0.0001** | **0.009** | **<0.0001** | 0.168 | **0.017** | **0.005** |
| *BMI* | **<0.0001** | 0.920 | | 0.916 | **<0.0001** | 0.110 | **0.004** | **0.004** | **0.027** | 0.251 | **0.000** | 0.054 | **<0.0001** | **0.043** | **0.004** | **0.042** | 0.274 | 0.624 | 0.185 |
| *Waist/hip ratio* | 0.712 | 0.678 | 0.916 | | 0.970 | 0.546 | 0.725 | 0.725 | 0.754 | 0.450 | 0.729 | 0.435 | 0.767 | 0.592 | 0.847 | 0.595 | 0.492 | 0.687 | 0.330 |
| *FMW* | **0.000** | 0.541 | **<0.0001** | 0.970 | | 0.452 | **<0.0001** | **<0.0001** | 0.118 | 0.813 | **<0.0001** | 0.291 | **<0.0001** | 0.259 | **<0.0001** | 0.253 | 0.266 | 0.578 | 0.430 |
| *LMW* | **0.000** | **<0.0001** | 0.110 | 0.546 | 0.452 | | 0.088 | 0.088 | **<0.0001** | **<0.0001** | 0.574 | **<0.0001** | 0.576 | **<0.0001** | 0.172 | **<0.0001** | 0.220 | 0.106 | **0.001** |
| *FMP* | 0.336 | **0.002** | **0.004** | 0.725 | **<0.0001** | 0.088 | | **<0.0001** | 0.426 | **0.027** | **<0.0001** | 0.179 | **<0.0001** | 0.178 | **<0.0001** | 0.181 | 0.934 | 0.473 | |
| *LMP* | 0.336 | **0.002** | **0.004** | 0.725 | **<0.0001** | 0.088 | **<0.0001** | | 0.426 | **0.027** | **<0.0001** | 0.179 | **<0.0001** | 0.178 | **<0.0001** | 0.181 | 0.934 | 0.473 | 0.303 |

Table 3.5 - description on page 75

| *Variable* | *ABW* | *Ht* | *BMI* | *Waist /hip ratio* | *FMW* | *LMW* | *FMP* | *LMP* | *DLM* | *TBW* | *TBW-P* | *ECW* | *ECW-P* | *ICW* | *ICW-P* | *BCM* | *30-TS* | *60-TS* | *PS* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *DLM* | **<0.0001** | **0.000** | **0.027** | 0.754 | 0.118 | **<0.0001** | 0.426 | 0.426 | | **<0.0001** | 0.642 | **<0.0001** | 0.114 | **<0.0001** | 0.691 | **<0.0001** | 0.145 | 0.130 | **0.002** |
| *TBW* | **0.002** | **<0.0001** | 0.251 | 0.450 | 0.813 | **<0.0001** | **0.027** | **0.027** | **<0.0001** | | 0.240 | **<0.0001** | 0.948 | **<0.0001** | 0.054 | **<0.0001** | 0.310 | 0.116 | **0.002** |
| *TBW-P* | **0.038** | 0.072 | **0.000** | 0.729 | **<0.0001** | 0.574 | **<0.0001** | **<0.0001** | 0.642 | 0.240 | | 0.704 | **<0.0001** | 0.782 | **<0.0001** | 0.788 | 0.525 | 0.814 | 0.899 |
| *ECW* | **<0.0001** | **<0.0001** | 0.054 | 0.435 | 0.291 | **<0.0001** | 0.179 | 0.179 | **<0.0001** | **<0.0001** | 0.704 | | 0.551 | **<0.0001** | 0.305 | **<0.0001** | 0.260 | 0.078 | **0.001** |
| *ECW-P* | **0.001** | 0.443 | **<0.0001** | 0.767 | **<0.0001** | 0.576 | **<0.0001** | **<0.0001** | 0.114 | 0.948 | **<0.0001** | 0.551 | | 0.370 | **<0.0001** | 0.366 | 0.241 | 0.839 | 0.544 |
| *ICW* | **<0.0001** | **<0.0001** | **0.043** | 0.592 | 0.259 | **<0.0001** | 0.178 | 0.178 | **<0.0001** | **<0.0001** | 0.782 | **<0.0001** | 0.370 | | 0.247 | **<0.0001** | 0.169 | 0.098 | **0.001** |
| *ICW-P* | 0.241 | **0.009** | **0.004** | 0.847 | **<0.0001** | 0.172 | **<0.0001** | **<0.0001** | 0.691 | 0.054 | **<0.0001** | 0.305 | **<0.0001** | 0.247 | | 0.250 | 0.962 | 0.488 | 0.481 |
| *BCM* | **<0.0001** | **<0.0001** | **0.042** | 0.595 | 0.253 | **<0.0001** | 0.181 | 0.181 | **<0.0001** | **<0.0001** | 0.788 | **<0.0001** | 0.366 | **<0.0001** | 0.250 | | 0.167 | 0.096 | **0.001** |

Table 3.5 continued - description on page 75

| *Variable* | *ABW* | *Ht* | *BMI* | *Waist /hip ratio* | *FMW* | *LMW* | *FMP* | *LMP* | *DLM* | *TBW* | *TBW-P* | *ECW* | *ECW-P* | *ICW* | *ICW-P* | *BCM* | *30-TS* | *60-TS* | *PS* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *30-TS* | 0.122 | 0.168 | 0.274 | 0.492 | 0.266 | 0.220 | 0.934 | 0.934 | 0.145 | 0.310 | 0.525 | 0.260 | 0.241 | 0.169 | 0.962 | 0.167 | | **0.017** | **0.025** |
| *60-TS* | 0.155 | **0.017** | 0.624 | 0.687 | 0.578 | 0.106 | 0.473 | 0.473 | 0.130 | 0.116 | 0.814 | 0.078 | 0.839 | 0.098 | 0.488 | 0.096 | **0.017** | | 0.332 |
| *PS* | **0.010** | **0.005** | 0.185 | 0.330 | 0.430 | **0.001** | 0.303 | 0.303 | **0.002** | **0.002** | 0.899 | **0.001** | 0.544 | **0.001** | 0.481 | **0.001** | **0.025** | 0.332 | |

Table 3.5- table demonstrating the respective p-value for the Pearson correlation matrix in table 3.4 for the measurements obtained from weight, height BMI and BIA. Absolute body weight (ABW), height (Ht), body mass index (BMI), waist-to-hip ratio (waist/hip ratio), fat mass weight (FMW), lean mass weight (LMW), fat mass percentage (FMP), lean mass percentage (LMP), dry lean mass weight (DLM), Total body water volume (TBW), total body water percentage (TBW-P), extracellular water percentage (ECW), extracellular water volume (ECW-V), intracellular water volume (ICW), intracellular water percentage (ICW-P), body cell mass weight (BCM), 30-minute tissue cefazolin concentration (30-TS), 60 minute tissue cefazolin concentration (60-TS), and 30-minute plasma cefazolin concentration (PS). Note that significant p-values are in **bold.**

*This page is left purposely blank*

### 3.7 Linear Regression analysis

Linear regression using a stepwise model, incorporating significant variables from univariate analysis was used to determine predictive equations for cefazolin concentration. This was done for both plasma and tissue samples.

#### 3.7.1 Plasma sample Linear Regression Analysis

For plasma sample cefazolin concentration, linear regression using the 18 participants who received 2 g of cefazolin was performed for the following predictors: lean mass weight, dry lean mass weight, weight, absolute body weight and height. Lean body mass weight was noted to be the most significant predictor.

Lean mass weight fitted regression model was:

$$\text{Plasma cefazolin concentration 30 minutes (mg/L)} = 62.686 - 0.515 * (\text{Lean mass weight})$$

The lean mass weight regression was statistically significant ($R^2$=0.497, F=15.8, p=0.001).

Table 3.4 demonstrates the use of the lean mass weight regression model with the 18 participants, comparing the predicted values with the actual recorded values.

#### 3.7.2 Tissue sample Linear Regression Analysis

##### 3.7.2.i 30-minute tissue concentration regression analysis

Linear regression using the 18 participants who received 2 g of cefazolin was performed for 30-minute plasma sample concentration (as a predictor) for the 30-minute tissue sample.

30-minute plasma sample fitted regression model was:

$$\text{Tissue cefazolin concentration at 30 minutes (mg/L)} = 11.437 + 0.991 * (30\ minute\ plasma\ sample)$$

30-minute plasma sample was statistically significant ($R^2$=0.277, F=6.14, p=0.025).

Table 3.5 demonstrates the use of the 30-minute plasma sample fitted regression model with the 18 participants, comparing the predicted values with the actual recorded values for the 30-minute tissue cefazolin concentration.

### 3.7.2.ii 60-minute tissue concentration regression analysis

Linear regression was done for height and 30-minute tissue cefazolin concentration for the 60-minute tissue sample.

30-minute tissue cefazolin concentration (as a predictor) fitted regression model was:

$$Tissue\ cefaozlin\ concentration\ at\ 60\ minutes\ \left(\frac{mg}{L}\right) = 15.471 + 0.637 *)30\ minute\ tissue\ cefaozlin\ concentration)$$

30-minute tissue cefazolin concentration was statistically significant ($R^2$=0.339, F=9.25, p=0.007).

Table 3.6 demonstrates the use of the 30-minute tissue cefazolin concentration fitted regression model with the 18 participants, comparing the predicted values with the actual recorded values for the 60-minute tissue cefazolin concentration.

## 3.8 Summary of results

The results from univariate analysis demonstrate four variables associated with plasma cefazolin concentration; lean mass weight, dry lean mass weight, absolute body weight and height.

Univariate analysis for tissue cefazolin concentrations demonstrated three variables associated with different time points; 30-minute plasma cefazolin concentration (30-minute tissue sample), 30-minute tissue cefazolin concentration (60-minute tissue cefazolin concentration) and height (60-minute tissue cefazolin concentration).

Regression analysis demonstrated lean mass weight to be the best predictor for 30-minute plasma cefazolin concentrations. In turn, the 30-minute plasma concentration was the best predictor for the 30-minute tissue cefazolin concentration and the 30-minute tissue cefazolin concentration was the best predictor for the 60-minute tissue cefazolin concentration using regression analysis.

*This page is left purposely blank*

| | Lean weight mass (kg) | Actual 30-minute plasma cefazolin concentration (mg/L) | Predicted 30-minute plasma cefazolin concentration (mg/L) | Residual | Std. dev. on pred. (Mean) | Lower boundary Confidence Interval 95% (Mean) | Upper boundary Confidence Interval 95% (Mean) |
|---|---|---|---|---|---|---|---|
| Participant 1 | 44.200 | 45.500 | 39.905 | 5.595 | 2.112 | 35.428 | 44.381 |
| Participant 2 | 49.600 | 34.000 | 37.122 | -3.122 | 1.795 | 33.315 | 40.928 |
| Participant 3 | 68.500 | 25.000 | 27.380 | -2.380 | 2.573 | 21.926 | 32.835 |
| Participant 4 | 39.700 | 54.000 | 42.224 | 11.776 | 2.498 | 36.929 | 47.519 |
| Participant 5 | 61.800 | 24.000 | 30.834 | -6.834 | 2.010 | 26.572 | 35.096 |
| Participant 6 | 39.500 | 26.000 | 42.327 | -16.327 | 2.517 | 36.992 | 47.662 |
| Participant 7 | 54.600 | 29.000 | 34.545 | -5.545 | 1.719 | 30.901 | 38.188 |
| Participant 10 | 44.100 | 44.000 | 39.956 | 4.044 | 2.119 | 35.463 | 44.449 |
| Participant 11 | 65.200 | 26.000 | 29.081 | -3.081 | 2.272 | 24.265 | 33.898 |
| Participant 12 | 47.900 | 44.000 | 37.998 | 6.002 | 1.873 | 34.028 | 41.968 |
| Participant 13 | 73.800 | 24.000 | 24.649 | -0.649 | 3.119 | 18.036 | 31.261 |
| Participant 14 | 48.600 | 31.000 | 37.637 | -6.637 | 1.838 | 33.740 | 41.534 |
| Participant 15 | 53.100 | 37.000 | 35.318 | 1.682 | 1.717 | 31.679 | 38.957 |
| Participant 16 | 86.200 | 26.000 | 18.258 | 7.742 | 4.549 | 8.614 | 27.901 |
| Participant 17 | 35.400 | 45.800 | 44.440 | 1.360 | 2.928 | 38.233 | 50.648 |
| Participant 18 | 52.400 | 46.600 | 35.678 | 10.922 | 1.723 | 32.026 | 39.331 |
| Participant 19 | 39.600 | 42.900 | 42.276 | 0.624 | 2.507 | 36.961 | 47.590 |
| Participant 20 | 62.500 | 25.300 | 30.473 | -5.173 | 2.059 | 26.107 | 34.838 |

Table 3.6 – description on following page

Table 3.6- Prediction table for plasma cefazolin concentration (mg/L) using fitted regression model demonstrating the lean mass weight, the actual 30-minute plasma cefazolin concentration recorded from the sample, the predicted 30-minute plasma cefazolin concentration using the fitted linear regression model for lean mass weight, the residual, the standard deviation of the predicted mean (Std. dev. on pred.) and the upper and lower boundary confidence interval for the predicted mean (95%).

Actual cefazolin concentration is the cefazolin concentration measure from the plasma sample. Predicted plasma cefazolin concentration is the cefazolin concentration predicted with use of the fitted regression model. Residual is the difference between the actual and predicted concentration. Std. dev. on pred. (mean) is the standard deviation of the predicted cefazolin mean. Lower/Upper confidence interval 95% (mean) is the lower and upper boundary of the confidence interval for the predicted cefazolin mean.

| | 30-minute Plasma cefazolin concentration (g/L) | Actual 30-minute tissue cefazolin concentration (mg/kg) | Predicted 30 minute tissue cefazolin concentration (mg/kg) | Residual | Std. dev. on pred. (Mean) | Lower boundary Confidence Interval 95% (Mean) | Upper boundary Confidence Interval 95% (Mean) |
|---|---|---|---|---|---|---|---|
| Participant 1 | 45.500 | 45.200 | 56.513 | -11.313 | 5.706 | 44.418 | 68.609 |
| Participant 2 | 34.000 | 38.500 | 45.120 | -6.620 | 3.887 | 36.880 | 53.361 |
| Participant 3 | 25.000 | 48.800 | 36.204 | 12.596 | 5.564 | 24.410 | 47.999 |
| Participant 4 | 54.000 | 88.600 | 64.934 | 23.666 | 8.522 | 46.867 | 83.001 |
| Participant 5 | 24.000 | 25.300 | 35.214 | -9.914 | 5.858 | 22.796 | 47.632 |
| Participant 6 | 26.000 | 48.500 | 37.195 | 11.305 | 5.283 | 25.995 | 48.395 |
| Participant 7 | 29.000 | 32.700 | 40.167 | -7.467 | 4.551 | 30.519 | 49.815 |
| Participant 10 | 44.000 | 31.500 | 55.027 | -23.527 | 5.280 | 43.833 | 66.221 |
| Participant 11 | 26.000 | 45.600 | 37.195 | 8.405 | 5.283 | 25.995 | 48.395 |
| Participant 12 | 44.000 | 46.800 | 55.027 | -8.227 | 5.280 | 43.833 | 66.221 |
| Participant 13 | 24.000 | 33.900 | 35.214 | -1.314 | 5.858 | 22.796 | 47.632 |
| Participant 14 | 31.000 | 29.000 | 42.148 | -13.148 | 4.185 | 33.277 | 51.020 |
| Participant 15 | 37.000 | 92.500 | 48.093 | 44.407 | 3.948 | 39.724 | 56.461 |
| Participant 16 | 26.000 | 42.900 | 37.195 | 5.705 | 5.283 | 25.995 | 48.395 |

Table 3.7 – continues with description on following page

| | 30-minute Plasma cefazolin concentration (g/L) | Actual 30-minute tissue cefazolin concentration (mg/kg) | Predicted 30 minute tissue cefazolin concentration (mg/kg) | Residual | Std. dev. on pred. (Mean) | Lower boundary Confidence Interval 95% (Mean) | Upper boundary Confidence Interval 95% (Mean) |
|---|---|---|---|---|---|---|---|
| Participant 17 | 45.800 | 59.300 | 56.811 | 2.489 | 5.795 | 44.527 | 69.095 |
| Participant 18 | 46.600 | 50.600 | 57.603 | -7.003 | 6.037 | 44.806 | 70.400 |
| Participant 19 | 42.900 | 45.600 | 53.938 | -8.338 | 4.991 | 43.357 | 64.518 |
| Participant 20 | 25.300 | 24.800 | 36.502 | -11.702 | 5.478 | 24.889 | 48.114 |

Table 3.7- Prediction table for 30-minute tissue cefazolin concentration using fitted regression model demonstrating the 30-minute Plasma cefazolin concentration, the actual 30-minute tissue cefazolin concentration recorded from the sample, the predicted 30-minute tissue cefazolin concentration using the fitted linear regression mode for the 30-minute plasma cefazolin concentration, the residual, the standard deviation of the predicted mean (Std. dev. on pred.) and the upper and lower boundary confidence interval for the predicted mean (95%).

Actual cefazolin concentration is the cefazolin concentration measure from the tissue sample. Predicted tissue cefazolin concentration is the cefazolin concentration predicted with use of the fitted regression model. Residual is the difference between the actual and predicted concentration. Std. dev. on pred. (mean) is the standard deviation of the predicted cefazolin mean. Lower/Upper confidence interval 95% (mean) is the lower and upper boundary of the confidence interval for the predicted cefazolin mean.

| | 30-minute tissue cefazolin concentration (mg/kg) | Actual 60-minute tissue cefazolin concentration (mg/kg) | Predicted 60-minute tissue cefazolin concentration (mg/kg) | Residual | Std. dev. on pred. (Mean) | Lower bound 95% (Mean) | Upper bound 95% (Mean) |
|---|---|---|---|---|---|---|---|
| Participant 1 | 45.200 | 40.100 | 44.775 | -4.675 | 4.227 | 35.815 | 53.736 |
| Participant 2 | 38.500 | 41.100 | 40.617 | 0.483 | 4.576 | 30.916 | 50.318 |
| Participant 3 | 48.800 | 76.000 | 47.010 | 28.990 | 4.267 | 37.964 | 56.055 |
| Participant 4 | 88.600 | 78.900 | 71.713 | 7.187 | 10.724 | 48.979 | 94.446 |
| Participant 5 | 25.300 | 20.700 | 32.424 | -11.724 | 6.415 | 18.825 | 46.023 |
| Participant 6 | 48.500 | 91.100 | 46.824 | 44.276 | 4.257 | 37.799 | 55.849 |
| Participant 7 | 32.700 | 28.300 | 37.017 | -8.717 | 5.245 | 25.898 | 48.136 |
| Participant 10 | 31.500 | 60.100 | 36.272 | 23.828 | 5.415 | 24.793 | 47.752 |
| Participant 11 | 45.600 | 33.200 | 45.024 | -11.824 | 4.223 | 36.071 | 53.976 |
| Participant 12 | 46.800 | 34.700 | 45.769 | -11.069 | 4.224 | 36.814 | 54.724 |
| Participant 13 | 33.900 | 27.300 | 37.762 | -10.462 | 5.085 | 26.982 | 48.541 |
| Participant 14 | 29.000 | 47.100 | 34.720 | 12.380 | 5.796 | 22.433 | 47.008 |
| Participant 15 | 92.500 | 57.800 | 74.134 | -16.334 | 11.561 | 49.625 | 98.642 |
| Participant 16 | 42.900 | 36.700 | 43.348 | -6.648 | 4.287 | 34.260 | 52.435 |
| Participant 17 | 59.300 | 59.200 | 53.527 | 5.673 | 5.213 | 42.476 | 64.579 |
| Participant 18 | 50.600 | 38.300 | 48.127 | -9.827 | 4.348 | 38.911 | 57.344 |
| Participant 19 | 45.600 | 26.600 | 45.024 | -18.424 | 4.223 | 36.071 | 53.976 |
| Participant 20 | 24.800 | 19.000 | 32.114 | -13.114 | 6.503 | 18.328 | 45.899 |

Table 3.8 – description on following page

Table 3.8- Prediction table for 60-minute tissue cefazolin concentration using fitted regression model demonstrating the 30-minute tissue cefazolin concentration, the actual 60-minute tissue cefazolin concentration recorded from the sample, the predicted 60-minute tissue cefazolin concentration using the fitted linear regression model for 30-minute tissue cefazolin concentration, the residual, the standard deviation of the predicted mean (Std. dev. on pred.) and the upper and lower boundary confidence interval for the predicted mean (95%). Actual cefazolin concentration is the cefazolin concentration measure from the tissue sample. Predicted tissue cefazolin concentration is the cefazolin concentration predicted with use of the fitted regression model. Residual is the difference between the actual and predicted concentration. Std. dev. on pred. (mean) is the standard deviation of the predicted cefazolin mean. Lower/Upper confidence interval 95% (mean) is the lower and upper boundary of the confidence interval for the predicted cefazolin mean.

# Chapter 4

# Discussion

## 4.1 Introduction

The causes of SSI are variable and often the exact source was not identified. Sources can include the patient's own skin flora, introduced bacteria from contaminated environments and haematogenous spread, among others (4). The burden of disease from SSI can be variable depending on the severity of infection, but all have a negative impact upon and cost to the patient and the healthcare system (7, 13).

The treatment required for SSIs are variable. SSI that involves the superficial wound are often treated successfully with antibiotics and have no long-term implications. In orthopaedics, more severe SSIs include prosthetic joint infections (PJI). Diagnosis of PJI alone can be a difficult task and sometimes require surgical procedures simply for a diagnosis. Treatment often involves multiple revision surgeries in an attempt to treat the infection, having a large burden upon the healthcare system as well as the impact on the patient (17). In certain cases treatment can be unsuccessful, leaving the patient with a chronic infection which requires lifelong treatment and suppression.

Several methods are used to attempt to reduce the occurrence of surgical site infections but there is no method or collection or methods that can prevent all SSIs. One of the most essential steps is the use of prophylactic antibiotics prior to surgery (39). In the modern-day era, cephalosporins such as cefazolin are commonly used in orthopaedics surgery as a prophylactic antibiotic.

Cefazolin initially was dosed at 1 g when successfully used as a prophylactic agent, but it was noted that patients of higher weight and BMI were at increased risk of SSI (59). In a landmark trial by Forse et al. it was proven that cefazolin had a weight related relationship in preventing SSI. It was shown that higher doses of cefazolin at 2 g in patients of a higher weight or BMI helped reduce the SSI incidence (59). Despite the now regular use of 2 g cefazolin in the large majority of patients, those who have a high BMI or absolute body weight retain higher rates of SSI.

Whilst evidence exists regarding cefazolin delivery and BMI there is no reported relationship between cefazolin delivery and other measurements of body composition, including lean and fat mass body composition.

## 4.2- Aims of study

Therefore the aims of this study included;

1. Measure tissue and plasma concentrations of prophylactic cefazolin in elective lumbar spine surgery
2. Determine the prevalence of cefazolin MIC in elective lumbar spine surgery using the hospital prophylactic regimen
3. Define the relationship between tissue and plasma cefazolin concentrations and measures of body composition (weight, BMI, BIA)
4. Determine which measure of body composition is best associated with tissue and plasma concentration of cefazolin

## 4.3 Measurement of tissue and plasma cefazolin concentrations in lumbar spine surgery

Measuring of the tissue and plasma cefazolin levels at the set time frame was successful in providing various recordings of cefazolin concentration for all participants. Only 55% of participants had a 90-minute tissue sample obtained due to closure of the wound prior to the 90-minute post cefazolin mark.

This is the first known study where multiple muscle tissue sample cefazolin concentrations have been measured, in any form of surgery in an adult population. Himebauch et al., in a study into paediatric spine surgery, collected plasma and muscle samples for cefazolin concentration analysis as a prophylactic agent. The muscle samples were collected by micro-dialysis and processed with High

Performance Liquid Chromatography (HPLC) (90). In the literature there are studies that have evaluated the penetration and measurement of prophylactic cefazolin and other antibiotics into the lumbar spine region (89, 91). A study by Capoor et al. in spine surgery patients undergoing lumbar micro-discectomy, demonstrated successful measurement of cefazolin concentrations in the removed lumbar discs using a similar method to our study with HPLC (89). Gregory et al. demonstrated the measurement of prophylactic vancomycin levels in paediatric spinal patients. This was with muscle samples collected in a similar method to our study, with direct visualisation and sampling and evaluation done using liquid chromatography-tandem mass spectrometry (91).

### 4.4 Achievement of the MIC for tissue and plasma cefazolin concentration

For cefazolin to be successful as a prophylactic antibiotic it must be able to reach the MIC (51, 52). The MIC is different for each bacteria that is susceptible to the antibiotic being used (52). For cefazolin, a common MIC is that for *S. aureus*, being 2 mg/kg for tissue and 2 mg/L for plasma (52). The reason behind the use of *S. aureus* as the MIC used for cefazolin is that it is the most common cause of SSI.

All participants' tissue and plasma samples in our study achieved the set MIC of 2 mg/kg and 2 mg/L respectively. All participants' concentrations demonstrate that the set Waikato Hospital policy for cefazolin is successful in achieving the MIC for *S. aureus*.

Previous studies have investigated the achievement of cefazolin MIC in surgical patients. In paediatric spine surgery, Himebauch et al demonstrated plasma cefazolin levels achieved a satisfactory MIC of 2 mg/L for *S. aureus* and in micro-dialysis muscle tissue samples demonstrated 98.9% of all samples achieved a satisfactory MIC of 2 mg/kg for *S. aureus* (90). This was done using a dosing regimen of 30 mg of cefazolin per kg to a maximum of 2 g cefazolin.

Gregory et al. investigated prophylactic vancomycin levels in paediatric spinal patients with weight based dosage at 15 mg/kg. They demonstrated achievement of the required MIC for vancomycin as a

prophylactic antibiotic (2 mg/L for *S. aureus*) in plasma samples. No achievement of the required MIC was demonstrated in any of the muscle tissue samples (91). Other studies have looked at the achievement of MIC of other antibiotics in lumbar spine surgery in porcine models. Hvistendahl et al. investigated cefuroxime concentrations in porcine lumbar spines, collecting samples by microdialysis, demonstrating MIC levels being achieved in the anterior (vertebral body) and posterior (posterior arch) lumbar columns for a mean time of 123 minutes and 93 minutes, respectively (92). Kaspersen et al. also used porcine lumbar spine models to look at epidural and plasma concentrations of cefuroxime using microdialysis. The mean time above the MIC was 115 minutes for epidural tissue and 123 minutes for plasma (93).

Walters et al. used sheep as models to measure cefazolin levels in lumbar spine discs, amongst other aims. They demonstrated that the discs had varying cefazolin concentrations depending on the location in the disc, with the outer annulus fibrosus having significantly higher concentrations compared to the inner nucleus pulposus (94). 2 mg/L was achieved in the annulus fibrosus in all sheep who received 2 g of cefazolin or greater.

These studies in spine surgery in both human and animal models demonstrate that multiple antibiotics, including cefazolin, have satisfactory penetration into multiple tissue in the spine region. The nucleus pulposus was shown to have levels below an acceptable cefazolin concentration in one study and vancomycin demonstrated not reaching satisfactory MIC levels in spinal skeletal tissue. Our study demonstrates that there is adequate penetration of cefazolin in the erector spinae muscles around the lumbar spine in those undergoing primary lumbar spine surgery.

Our study supports the current Waikato Hospital policy of cefazolin dosing in achieving the MIC for prophylactic lumbar spine surgery. Furthermore, our evidence further supports the use of 2 g dosing being successful in achieving the required MIC to target *S. aureus* regardless of weight of BMI and that 3 g of cefazolin was not supported by any evidence in the literature.

## 4.5 Relationship between tissue and plasma cefazolin concentrations and measures of body composition

Three techniques were used to assess body composition: absolute body weight, BMI and BIA. The associations between cefazolin concentration and these measures was analysed.

### 4.5.1 Relationship between plasma cefazolin concentrations and measures of body composition

No previous studies have looked at a potential relationship between measurements of BIA and plasma cefazolin concentration, nor have they compared those measurements with BMI or weight with plasma cefazolin concentrations. Univariate analysis demonstrated four significant predictors for plasma cefazolin concentration: lean mass weight, dry lean mass weight, absolute body weight and height. Linear regression showed that mean mass weight was the most significant predictor.

Young et al. demonstrated in caesarean patients an association between BMI and plasma cefazolin levels, whilst other studies in caesarean patients by Maggio et al. and Ho et al. found no correlation between weight or BMI with plasma cefazolin concentrations (67, 68, 69). No previous studies are able to support our findings of absolute body weight being associated with plasma cefazolin levels.

Interestingly, our results show that whilst absolute body weight and height are independently significant correlated with plasma cefazolin concentration, BMI, which is calculated from absolute body weight and height, was not found to be a significant correlated predictor.

### 4.5.2 Relationship between tissue cefazolin concentration and measures of body composition

Our study results demonstrated three significant predictors associated with muscle tissue cefazolin concentrations: 30-minute plasma cefazolin concentration (for 30-minute tissue cefazolin concentration), and 30-minute tissue cefazolin concentration (for 60-minute tissue cefazolin). In effect the muscle concentration appears entirely dependent on adequate entry to the vascular system.

Studies that have evaluated both plasma and tissue cefazolin levels in spine surgery have not commented on the association between plasma concentration and concentration at the surgical site (90, 95, 96). This potential linkage has relevance if larger studies correlate it. This would allow future studies of cefazolin concentrations to not require tissue samples, potentially saving time and money for these future studies.

Swank et al. showed that caesarean patients that increased weight and BMI were associated with decreased adipose tissue cefazolin concentrations (65). Young et al also demonstrated in caesarean patients that increased BMI was associated with decreased adipose tissue cefazolin concentrations (67). In contrast, Maggio et al. did not find an association between BMI and adipose tissue concentrations of cefazolin in caesarean patients (68). The applicability of these studies to our findings can be considered limited due to the tissue samples being adipose tissue as compared to the muscle tissue samples in our study.

Himebauch et al. did also investigate any correlation between BMI to muscle and unbound plasma cefazolin ratio, with $r^2$=0.14 (90). A key difference to this study is that the patients were of paediatric age and received individualised per-kg weight-based dosing of cefazolin. This causes higher weight, and potentially higher BMI, patients to receive higher doses and therefore it is more likely to correlate BMI increases with increase cefazolin concentrations.

Our study did not find any significant association between any measure of body composition and tissue cefazolin concentrations. Dry lean mass weight was found to be the strongest correlated measurement of BIA associated insignificantly with muscle tissue cefazolin concentration (table 3.4). Prior to the start of the study measurements of lean mass were thought most likely to be significant in being associated with the muscle tissue samples, the reasoning being that muscle mass makes up part of the lean mass measurement.

Our study is unique as no previous studies have compared analysed body composition with measurements of BIA nor compared the findings with tissue cefazolin levels or other prophylactic antibiotics. This study provides benchmarks for future studies to compare to in similar research.

### 4.6 Determine which measure of body composition is best associated with tissue and plasma concentration of cefazolin

Linear regression was used on the predictors found to be significant from the Pearson Correlation Matrix to see which one had the strongest association with cefazolin concentration. For plasma this was lean mass weight and for tissue it was the 30-minute plasma cefazolin concentration.

Our results demonstrate that whilst association exists between both weight and lean mass weight (a measurement of BIA), lean mass weight is more accurate at predicting plasma cefazolin concentration. A potential hypothesis of why our results demonstrate this is that cefazolin is a hydrophilic antibiotic, therefore the hydrophilic properties of muscle (which partially makes up lean mass) may potentially absorb increased amounts of cefazolin. This is in comparison to fat, which is hydrophobic. This hypothesis correlates with the negative association.

Lean body mass and BIA is a feasible procedure to be done on nearly all elective surgical patients. The association with lean mass weight would allow for more precise dosing of cefazolin if benefit was to

be achieved from it. Further studies with a larger study group would first be needed to demonstrate the association.

### 4.7 Limitations of study

This study's findings are limited by the small number of participants. They are further limited by only 18 participants receiving 2 g of cefazolin, who were used as the basis for the analysis of predictors. The small numbers make it difficult to take into account potential confounders, including comorbidities and their effect upon the results.

As the analysis only had participants who received 2 g of cefazolin, there is limitation in its findings of lean mass weight being a successful predictor for other doses than 2 g of cefazolin.

A further limitation is that none of our participants had an extreme BMI of above 40 kg/m², limiting the results application to the extreme BMI population.

No assessment was done of adipose tissue or bone tissue in the lumbar spine region to evaluate cefazolin concentrations. This limits our results to the erector spinae muscles of the lumbar spine.

With our participants being solely from an elective pool of primary lumbar surgery, there is a limit in the application of results to other orthopaedic cases. Issues that could arise from other surgical sites around the body include; vascular supply to the tissue, as well as surgical incision size.

Significant challenges were found during the undertaking of this study. As Waikato Hospital is the tertiary trauma centre for the central North Island of New Zealand, it receives a high load of patients requiring acute orthopaedic care. Due to limitations of the healthcare system in dealing with this burden this often leads to planned elective lists being cancelled to give priority to the acute surgical cases. This caused potential participants who had been recruited from the elective waitlist to not undergo surgery and caused delays in tissue collection.

### 4.7.1 Impact of COVID-19

Another impact is that of COVID-19 and its effect upon the healthcare system. This was due to a lack of hospital beds available due to an increase in admissions for patients with COVID-19, causing elective patients to be unable to undergo surgery. Elective patients who had contacted COVID-19 also faced delays due to guidelines recommending delays of 7 weeks from time of infection until non-urgent elective surgery.

## 4.8 Areas of future research

Further research is required in several areas, identified from the results of this study, to optimise the use of cefazolin in reducing SSI rate:

- The concentration of cefazolin in other lumbar spine tissue, including adipose and bone, and its relation to measurements of body composition
- The concentration of cefazolin in lumbar spine patients of an extreme BMI with comparison to body composition to evaluate the effects on cefazolin delivery in plasma and tissue
- The relationship between plasma and tissue cefazolin concentration at the same time point in collection to see if plasma successfully predicts tissue levels
- The relationship between blood markers, including albumin and renal function, and plasma and tissue cefazolin concentration and their potential influence of cefazolin delivery and removal

## 4.9 Conclusion

2 g of cefazolin in lumbar spine patients is successful at achieving tissue and plasma cefazolin MIC levels required to be a safe prophylactic antibiotic at the time of surgery.

Lean mass weight is the most accurate predictor of plasma cefazolin concentration, more so than absolute body weight or BMI. There is a potential relationship between plasma and tissue cefazolin concentration from the same time point. This could allow for ease of measuring cefazolin in future studies if it is shown to exist in larger studies and at consistent time periods.

Due to the small size of the recruited study population, larger studies would be required to see if any other predictors could be found to be significant due to the potential removal of unknown confounders facilitated by having a larger study population.

# Appendix 1
# Participant Information sheet

# Participant Information Sheet

**Comparing body tissue antibiotic concentration levels to lean and fat mass using bioimpedance analysis**

Name of Principal Investigator/Supervisor: Mr Joseph Baker

Name of Student Researcher: Dr Thomas Pett

Study Site: Waikato Hospital

Contact phone number: 07 839 8899

Ethics committee ref.: AH23409

---

You are invited to take part in a Masters in Health Science study on tissue concentration of antibiotics compared to the lean and fat mass of your body, to be calculated by bioimpedance analysis. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason, and it won't affect the care you receive. If you do want to take part now, but change your mind later, you can pull out of the study at any time prior to surgery and up to one month after surgery.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is 6 pages long, including the Consent Form. Please make sure you have read and understood all the pages.

## Voluntary Participation and Withdrawal From This Study

Your participation in this study is voluntary and you are free to decline to participate. You are also free to withdraw from the research at any time prior to surgery and up to one month after surgery without any impact on your care. If you do withdraw, all your data collected and tissue samples taken will be deleted, destroyed or returned to you as appropriate.

## WHAT IS THE PURPOSE OF THE STUDY?

This Masters study is to look at the affects that tissue proportions (and amount of fat and muscle mass people have) have on the levels of antibiotics given at the time of surgery.

When you have your surgery, antibiotics are given to help prevent infections. Currently we use your weight to calculate the dose for antibiotics. This study is looking to see if we can find a better way of calculating the antibiotics that you should receive at the time of surgery using bioimpedance analysis and comparing this to tissue samples taken at the time of surgery. The aim of this study is to assist in preventing surgical wound infections.

This study does not affect or change the surgery that you are going to have, nor when you would be having your surgery.

What is bioimpedance analysis?

Bioimpedance analysis is a way of measuring your fat and lean muscle mass. It involves attaching some small electrodes to your arms and legs to pulsate a small electrical current through your body whilst you lie flat. We plan of using this to calculate your fat and lean muscles mass prior to surgery.

It does not hurt to have this done. Some people report feeling a small buzzing feeling in your arms and legs. It takes about 5-10 minutes to do.

What tissue samples are going to be taken and what will they be used for?

During your surgery, several small tissue samples will be taken after 30 and 60 minutes from your back muscles called your erector spinae, which sit in the middle of your back. These samples will not affect your muscles after surgery nor affect the type of surgery that you are receiving. We will also take some blood samples at the same time.

These samples will then be taken to a lab and analysed to look at the concentration of antibiotics in them. These tissue samples will not be used for any other purposes. Once finished with, the samples will be either disposed of or returned to you as per your wishes.

Once surgery is done, you will continue your normal post operative cares in hospital as normal.

## WHO IS INVOLVED WITH THIS STUDY?

This study is part of a Masters in Health Science being done by Dr Thomas Pett at the University of Auckland. He currently works as an Orthopaedic Registrar at Waikato Hospital.

The supervisor of this study is Mr Joseph Baker, who currently works as an Orthopaedic Spine Surgeon at Waikato Hospital and an Associate Professor at the University of Auckland.

## HOW IS THE STUDY DESIGNED?

This study will take place at Waikato Hospital and will involve 20 patients. All patients will be undergoing a form a lumbar spine surgery.

The study will run from 1st December 2021 until 20 patients have been collected for the study.

For this study, your body lean and fat mass will be calculated using a bioimpedance machine. Then during the surgery, blood and tissue samples will be taken to be analysed to see the concentration of antibiotics.

During study, two muscle tissue samples will be taken by the surgeon(s) and/or registrar involved with the surgery. Two blood samples will also be taken by the anaesthetists during the surgery.

After surgery you will continue with your normal post-operation rehabilitation.

## WHO CAN TAKE PART IN THE STUDY?

To take part in this study you must meet the following criteria:

- Over the age of 18 years
- Undergoing lumbar spine surgery of at least 1 hour in length
- Not had any infections or antibiotics 3 days prior to surgery
- No known allergy to cefazolin (the antibiotic used in surgery)

- Of not had any previous lumbar spine surgeries

## WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

Once you have consented to being part of the surgery the following will be required of you:

- On the day of surgery you will have both your height and weight measured
- You will have bioimpedance analysis performed on you prior to being put to sleep
- You will have 2 tissue samples and 2 blood samples taken at 30 and 60 minutes after you were given antibiotics. This will occur whilst you are asleep for surgery.

## WHAT WILL HAPPEN TO MY TISSUE AND BLOOD SAMPLES?

All tissue and blood samples will be sent to a laboratory for further analysis to calculate the antibiotic concentration. These samples will not have any identifying details about you on them.

Once these samples have been used, they will either be safely disposed of or returned to you as per you wishes.

We respect Tikanga Māori practices when it comes to handling of tissue and blood samples.

## WHAT ARE THE POSSIBLE RISKS OF THIS STUDY?

There are no further added risks from your surgical procedure that you are already having. It is normal in lumbar surgery for a small amount of muscle tissue to be removed (debrided) to clean up the wound. The blood samples will be taken from a venous line placed in your arm, which you are required to have for surgery in the first place.

The bioimpedance machine carries no increased chance of risks and is a relatively safe procedure to undergo.

There are no benefits to yourself from this study.

## WHAT ARE THE POSSIBLE BENEFITS OF THIS STUDY?

There are no direct benefits to yourself from this study.

There are potential indirect benefits to other people having future surgery if we can find more appropriate ways to calculate a patient's antibiotic dosage.

## WILL ANY COSTS BE REIMBURSED?

There is no cost for you to be involved with this study.

## WHAT IF SOMETHING GOES WRONG?

If you were injured in this study, you would be eligible to apply for compensation from ACC just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

## WHAT WILL HAPPEN TO MY INFORMATION?

During this study the researchers will record information about you and your study participation. This includes the results of any study assessments such as bioimpedance analysis. If needed, information from your hospital records and your GP may also be collected. You cannot take part in this study if you do not consent to the collection of this information.

Identifiable Information

Identifiable information is any data that could identify you (e.g. your name, date of birth, or address). Only researchers will have access to your identifiable information for the purpose of this study.

De-identified (Coded) Information

To make sure your personal information is kept confidential, information that identifies you will not be included in any report generated by the researcher. Instead, you will be identified by a code. The researcher will keep a list linking your code with your name, so that you can be identified by your coded data if needed.

The results of the study may be published or presented, but not in a form that would reasonably be expected to identify you.

Security and Storage of Your Information.

Your identifiable information is held at the Waikato Clinical Campus (part of the University of Auckland) during the study. After the study it is transferred to a secure archiving site and stored for at least 10 years, then destroyed. Your coded information will be entered into electronic case report forms and sent through a secure server to the sponsor. Coded study information

will be kept by the sponsor in secure, cloud-based storage indefinitely. All storage will comply with local and/or international data security guidelines.

Risks.

Although efforts will be made to protect your privacy, absolute confidentiality of your information cannot be guaranteed. Even with coded and anonymised information, there is no guarantee that you cannot be identified. The risk of people accessing and misusing your information (e.g. making it harder for you to get or keep a job or health insurance) is currently very small, but may increase in the future as people find new ways of tracing information.

Rights to Access Your Information.

You have the right to request access to your information held by the research team. You also have the right to request that any information you disagree with is corrected.

Rights to Withdraw Your Information.

You may withdraw your consent for the collection and use of your information at any time prior to surgery, by informing your Study Doctor. You may withdraw your consent for participating in the study up to one month after the surgery.

If you withdraw your consent, your study participation will end, and the study team will stop collecting information from you.

If you agree, information collected up until your withdrawal from the study will continue to be used and included in the study. You may ask for it to be deleted when you withdraw, unless you withdraw after the study analyses have been undertaken.

## WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

Your participation in this study is voluntary and you are free to decline to participate. You are also free to withdraw from the research at any time prior to surgery, and up to one month after surgery, without any impact on your care. If you do withdraw all your data collect and tissue samples taken will be deleted, destroyed or returned to you as appropriate.

You have the right to access any information collected about you as part of the study. You will be told of any new information (positive or negative) related to the study that may have an impact on your health as it becomes available.

Your privacy will be respected and information will be kept confidential as per the usual hospital system. Any research information collected will be deidentified for storage and analysis and you will not be able to be identified from it.

## CAN I FIND OUT THE RESULTS OF THE STUDY?

Once the study is complete you will be able to receive a summary of the study results if you wish to.

## WHO IS FUNDING THE STUDY?

This study is currently no sponsored but is actively seeking sponsorship and you will be updated if any changes occur to this.

## WHO HAS APPROVED THE STUDY?

This study has been approved by an independent group of people called Auckland Health Research Ethics Committee (AHREC), who check that studies meet established ethical standards. The AHREC has approved this study.

## WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

| | |
|---|---|
| *Name, Position* | Dr Thomas Pett, Orthopaedic Registrar |
| *Telephone number* | 07 839 8899 |
| *Email* | thomas.pett@waikatodhb.health.nz |

For Maori health support please contact:

*Kaitiaki Cultural Support Team*

*021 806 171*

For concerns of an ethical nature, you can contact the Chair of the Auckland Health Research Ethics Committee at ahrec@auckland.ac.nz or at 373 7599 x 83711, or at Auckland Health Research Ethics Committee, The University of Auckland, Private Bag 92019, Auckland 1142.

**Approved by the Auckland Health Research Ethics Committee on [date] for three years. Reference number AH23409.**

# Appendix 2
# Participant Consent Form

**CONSENT FORM**

THIS FORM WILL BE HELD FOR A PERIOD OF 10 YEARS

**Project title: Comparing body tissue antibiotic concentration levels to lean and fat mass using bioimpedance analysis**

Name of Principal Investigator/Supervisor: Mr Joseph Baker

Name of Student Researcher: Dr Thomas Pett

- I have read the Participant Information Sheet, have understood the nature of the research and why I have been selected. I have had the opportunity to ask questions and have had them answered to my satisfaction.
- I agree to take part in this research.
- I understand that I am free to withdraw my participation at any time prior to surgery, and to withdraw any data or tissue traceable to me up to one month after surgery
- I understand that as part of the study my hospital records will be accessed and data obtained from them. I also understand that my GP/usual doctor may be contacted for further information.
- I wish/ do not wish to have my tissue samples returned to me after they have been used (please cross out one)
- I wish / do not wish to receive the summary of findings (please cross out one)
  Address for a summary of findings to be sent out to;
  _____

Name: _____ Signature: _____
Date: _____

Approved by the Auckland Health Research Ethics Committee on [date] for three years. Reference number AH23409

# Bibliography

1. National Healthcare Safety Network, Centers for Disease Control and Prevention. Surgical site infection (SSI) event [PDF on internet]. Published January 2017. Accessed November, 2021. Available from http://www.cdc.gov/nhsn/pdfs/pscmanual/9pscssicurrent.pdf.
2. Berríos-Torres, S. I., Umscheid, C. A., Bratzler, D. W., Leas, B., Stone, E. C., Kelz, R. R., Reinke, C. E., Morgan, S., Solomkin, J. S., Mazuski, J. E., Dellinger, E. P., Itani, K. M. F., Berbari, E. F., Segreti, J., Parvizi, J., Blanchard, J., Allen, G., Kluytmans, J. A. J. W., Donlan, R., Schecter, W. P., … Healthcare Infection Control Practices Advisory Committee (2017). Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection, 2017. *JAMA surgery*, *152*(8), 784–791.
3. Saadatian-Elahi, M., Teyssou, R., & Vanhems, P. (2008). Staphylococcus aureus, the major pathogen in orthopaedic and cardiac surgical site infections: a literature review. *International journal of surgery (London, England)*, *6*(3), 238–245.
4. Altemeier, W. A., Culbertson, W. R., & Hummel, R. P. (1968). Surgical considerations of endogenous infections--sources, types, and methods of control. *The Surgical clinics of North America*, *48*(1), 227–240.
5. Rakow, A., Perka, C., Trampuz, A., & Renz, N. (2019). Origin and characteristics of haematogenous periprosthetic joint infection. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, *25*(7), 845–850.
6. Zimmerli, W., Trampuz, A., & Ochsner, P. E. (2004). Prosthetic-joint infections. *The New England journal of medicine*, *351*(16), 1645–1654.
7. Ban, K. A., Minei, J. P., Laronga, C., Harbrecht, B. G., Jensen, E. H., Fry, D. E., Itani, K. M., Dellinger, E. P., Ko, C. Y., & Duane, T. M. (2017). American College of Surgeons and Surgical Infection Society: Surgical Site Infection Guidelines, 2016 Update. *Journal of the American College of Surgeons*, *224*(1), 59–74.
8. Viqueira, A. Q., Caravaca, G. R., Quesada Rubio, J. A., & Francés, V. S. (2014). Surgical site infection rates and risk factors in orthopedic pediatric patients in Madrid, Spain. *The Pediatric infectious disease journal*, *33*(7), 693–696.
9. Starčević, S., Munitlak, S., Mijović, B., Mikić, D., & Suljagić, V. (2015). Surgical site infection surveillance in orthopedic patients in the Military Medical Academy, Belgrade. *Vojnosanitetski pregled*, *72*(6), 499–504.
10. Zhou, J., Wang, R., Huo, X., Xiong, W., Kang, L., & Xue, Y. (2020). Incidence of Surgical Site Infection After Spine Surgery: A Systematic Review and Meta-analysis. *Spine*, *45*(3), 208–216.

11. Morris, A. J., Roberts, S. A., Grae, N., Hamblin, R., Shuker, C., & Merry, A. F. (2018). The New Zealand Surgical Site Infection Improvement (SSII) Programme: a national quality improvement programme reducing orthopaedic surgical site infections. *The New Zealand medical journal*, *131*(1479), 45–56.
12. Yao, R., Zhou, H., Choma, T. J., Kwon, B. K., & Street, J. (2018). Surgical Site Infection in Spine Surgery: Who Is at Risk?. *Global spine journal*, *8*(4 Suppl), 5S–30S. https://doi.org/10.1177/2192568218799056
13. Leaper, D., & Ousey, K. (2015). Evidence update on prevention of surgical site infection. *Current opinion in infectious diseases*, *28*(2), 158–163.
14. Plowman, R., Graves, N., Griffin, M. A., Roberts, J. A., Swan, A. V., Cookson, B., & Taylor, L. (2001). The rate and cost of hospital-acquired infections occurring in patients admitted to selected specialties of a district general hospital in England and the national burden imposed. *The Journal of hospital infection*, *47*(3), 198–209.
15. Staats, A., Li, D., Sullivan, A. C., & Stoodley, P. (2021). Biofilm formation in periprosthetic joint infections. *Annals of joint*, *6*, 43.
16. Dobson, P. F., & Reed, M. R. (2020). Prevention of infection in primary THA and TKA. *EFORT open reviews*, *5*(10), 604–613.
17. New Zealand Joint Registry, New Zealand Orthopaedic Association. The New Zealand Join Registry 23 Year Report January 1999 to December 2023. Published 2022. Accessed December 2022. Available from https://www.nzoa.org.nz/sites/default/files/16Feb23%20NZJR_Twenty%20Three%20Year%20Report_Jan1999%20to%20Dec2021.pdf
18. Cheadle W. G. (2006). Risk factors for surgical site infection. *Surgical infections*, *7 Suppl 1*, S7–S11.
19. Lepelletier, D., & Lucet, J. C. (2013). Controlling meticillin-susceptible Staphylococcus aureus: not simply meticillin-resistant S. aureus revisited. *The Journal of hospital infection*, *84*(1), 13–21.
20. Edmiston, C. E., Jr, Ledeboer, N. A., Buchan, B. W., Spencer, M., Seabrook, G. R., & Leaper, D. (2016). Is Staphylococcal Screening and Suppression an Effective Interventional Strategy for Reduction of Surgical Site Infection?. *Surgical infections*, *17*(2), 158–166.
21. Uçkay, I., Lübbeke, A., Harbarth, S., Emonet, S., Tovmirzaeva, L., Agostinho, A., Longtin, Y., Peter, R., Hoffmeyer, P., & Pittet, D. (2012). Low risk despite high endemicity of methicillin-resistant Staphylococcus aureus infections following elective total joint arthroplasty: a 12-year experience. *Annals of medicine*, *44*(4), 360–368.

22. Sporer, S. M., Rogers, T., & Abella, L. (2016). Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus Screening and Decolonization to Reduce Surgical Site Infection in Elective Total Joint Arthroplasty. *The Journal of arthroplasty*, *31*(9 Suppl), 144–147.
23. Yuwen, P., Chen, W., Lv, H., Feng, C., Li, Y., Zhang, T., Hu, P., Guo, J., Tian, Y., Liu, L., Sun, J., & Zhang, Y. (2017). Albumin and surgical site infection risk in orthopaedics: a meta-analysis. *BMC surgery*, *17*(1), 7.
24. Martin, E. T., Kaye, K. S., Knott, C., Nguyen, H., Santarossa, M., Evans, R., Bertran, E., & Jaber, L. (2016). Diabetes and Risk of Surgical Site Infection: A Systematic Review and Meta-analysis. *Infection control and hospital epidemiology*, *37*(1), 88–99.
25. Caputo, A. M., Dobbertien, R. P., Ferranti, J. M., Brown, C. R., Michael, K. W., & Richardson, W. J. (2013). Risk factors for infection after orthopaedic spine surgery at a high-volume institution. *Journal of surgical orthopaedic advances*, *22*(4), 295–298.
26. Cheong, C. M., Golder, A. M., Horgan, P. G., Roxburgh, C. S. D., & McMillan, D. C. (2022). Relationship between pre-operative glycated haemoglobin and surgical site infection in patients undergoing elective colon cancer surgery. *Oncology letters*, *24*(3), 296.
27. Zhuang, T., Feng, A. Y., Shapiro, L. M., Hu, S. S., Gardner, M., & Kamal, R. N. (2021). Is Uncontrolled Diabetes Mellitus Associated with Incidence of Complications After Posterior Instrumented Lumbar Fusion? A National Claims Database Analysis. Clinical orthopaedics and related research, 479(12), 2726–2733.
28. Hikata, T., Iwanami, A., Hosogane, N., Watanabe, K., Ishii, K., Nakamura, M., Toyama, Y., Matsumoto, M., & Kamata, M. (2014). High preoperative hemoglobin A1c is a risk factor for surgical site infection after posterior thoracic and lumbar spinal instrumentation surgery. Journal of orthopaedic science : official journal of the Japanese Orthopaedic Association, 19(2), 223–228.
29. Webster, J., & Osborne, S. (2015). Preoperative bathing or showering with skin antiseptics to prevent surgical site infection. *The Cochrane database of systematic reviews*, (2), CD004985.
30. Franco, L. M., Cota, G. F., Pinto, T. S., & Ercole, F. F. (2017). Preoperative bathing of the surgical site with chlorhexidine for infection prevention: Systematic review with meta-analysis. *American journal of infection control*, *45*(4), 343–349.
31. Kowalski, T. J., Kothari, S. N., Mathiason, M. A., & Borgert, A. J. (2016). Impact of Hair Removal on Surgical Site Infection Rates: A Prospective Randomized Noninferiority Trial. *Journal of the American College of Surgeons*, *223*(5), 704–711.

32. Shi, D., Yao, Y., & Yu, W. (2017). Comparison of preoperative hair removal methods for the reduction of surgical site infections: a meta-analysis. *Journal of clinical nursing*, *26*(19-20), 2907–2914.
33. Tanner, J., & Melen, K. (2021). Preoperative hair removal to reduce surgical site infection. *The Cochrane database of systematic reviews*, *8*(8), CD004122.
34. World Health organization (WHO) Global guidelines on the prevention of surgical site infection, November 2016. Accessed on November 2022. Available from http://who.int/gpsc/ssi-prevention-guidelines/en/
35. Lidwell, O. M., Lowbury, E. J., Whyte, W., Blowers, R., Stanley, S. J., & Lowe, D. (1982). Effect of ultraclean air in operating rooms on deep sepsis in the joint after total hip or knee replacement: a randomised study. *British medical journal (Clinical research ed.)*, *285*(6334), 10–14.
36. Charnley J. (1972). Postoperative infection after total hip replacement with special reference to air contamination in the operating room. *Clinical orthopaedics and related research*, *87*, 167–187.
37. Hooper, G. J., Rothwell, A. G., Frampton, C., & Wyatt, M. C. (2011). Does the use of laminar flow and space suits reduce early deep infection after total hip and knee replacement?: the ten-year results of the New Zealand Joint Registry. *The Journal of bone and joint surgery. British volume*, *93*(1), 85–90.
38. Gastmeier, P., Breier, A. C., & Brandt, C. (2012). Influence of laminar airflow on prosthetic joint infections: a systematic review. *The Journal of hospital infection*, *81*(2), 73–78.
39. Benard HR, Cole WR. The Prophylacis of Sugical Infection: The Effect of Prophylactic antimicrobial Drugs on the Incidence of Infection Following Potentially Contaminated Operations. Surgery. 1964 Jul;56:151-7.
40. Feltis JM Jr, Hamit HF. Use of prophylactic antimicrobial drugs to prevent postoperative wound infections. Am J Surg. 1967 Dec;114(6):867-70.
41. de Jonge SW, Gans SL, Atema JJ, Solomkin JS, Dellinger PE, Boermeester MA. Timing of preoperative antibiotic prophylaxis in 54,552 patients and the risk of surgical site infection: A systematic review and meta-analysis. Medicine (Baltimore). 2017 Jul;96(29):e6903.
42. McKittrick LS, Wheelock FC Jr. The routine use of antibiotics in elective abdominal surgery. Surg Gynecol Obstet. 1954 Sep;99(3):376-7.
43. Blowers R, Mason GA, Wallace KR, Walton M. Control of wound infection in a thoracic surgery unit. Lancet. 1955 Oct 15;269(6894):786-94.

44. Siddiqi A, Forte SA, Docter S, Bryant D, Sheth NP, Chen AF. Perioperative Antibiotic Prophylaxis in Total Joint Arthroplasty: A Systematic Review and Meta-Analysis. J Bone Joint Surg Am. 2019 May 1;101(9):828-842.
45. Ryan SP, Kildow BJ, Tan TL, Parvizi J, Bolognesi MP, Seyler TM; American Association of Hip and Knee Surgeons Research Committee. Is There a Difference in Infection Risk Between Single and Multiple Doses of Prophylactic Antibiotics? A Meta-analysis. Clin Orthop Relat Res. 2019 Jul;477(7):1577-1590.
46. Khalooeifard, R., Oraee-Yazdani, S., Keikhaee, M., & Shariatpanahi, Z. V. (2022). Protein Supplement and Enhanced Recovery After Posterior Spine Fusion Surgery: A Randomized, Double-blind, Placebo-controlled Trial. *Clinical spine surgery*, *35*(3), E356–E362.
47. Abola, M. V., Lin, C. C., Lin, L. J., Schreiber-Stainthorp, W., Frempong-Boadu, A., Buckland, A. J., & Protopsaltis, T. S. (2021). Postoperative Prophylactic Antibiotics in Spine Surgery: A Propensity-Matched Analysis. *The Journal of bone and joint surgery. American volume*, *103*(3), 219–226.
48. Kim, J. L., Park, J. H., Han, S. B., Cho, I. Y., & Jang, K. M. (2017). Allogeneic Blood Transfusion Is a Significant Risk Factor for Surgical-Site Infection Following Total Hip and Knee Arthroplasty: A Meta-Analysis. The Journal of arthroplasty, 32(1), 320–325.
49. Zhang, X., Liu, P., & You, J. (2022). Risk factors for surgical site infection following spinal surgery: A meta-analysis. Medicine, 101(8), e28836.
50. Falsetto, A., Roffey, D. M., Jabri, H., Kingwell, S. P., Stratton, A., Phan, P., & Wai, E. K. (2022). Allogeneic blood transfusions and infection risk in lumbar spine surgery: An American College of Surgeons National Surgery Quality Improvement Program Study. Transfusion, 62(5), 1027–1033.
51. Prokuski L. Prophylactic antibiotics in orthopaedic surgery. J Am Acad Orthop Surg. 2008 May;16(5):283-93.
52. 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 Sep;20(6):439-443.
53. Clinical and Laboratory Standards Institue. Performance Standards for Antimicrobial Susceptibility Testing [PDF on internet]. 31st Edition, published 2021; cited November 2021. Available from: https://clsi.org/standards/products/microbiology/documents/m100/
54. European Committee on Antimicrobial Susceptibility Testing, European Society of Clinical Microbiology and Infectious Disease. MIC EUCAST, published February 2023. Accessed February 2023. Available at

https://mic.eucast.org/search/?search%5Bmethod%5D=mic&search%5Bantibiotic%5D=27&search%5Bspecies%5D=-1&search%5Bdisk_content%5D=-1&search%5Blimit%5D=50

55. Burke JF. The effective period of preventive antibiotic action in experimental incisions and dermal lesions. Surgery. 1961 Jul;50:161-8.
56. Classen DC, Evans RS, Pestotnik SL, Horn SD, Menlove RL, Burke JP. The timing of prophylactic administration of antibiotics and the risk of surgical-wound infection. N Engl J Med. 1992 Jan 30;326(5):281-6.
57. The World Health Organisation, The United Nations. Global Guidelines for the Prevention of Surgical Site Infection [PDF on internet]. Published 2016. Cited November, 2021. Available from https://www.who.int/gpsc/global-guidelines-web.pdf
58. Horizon Strategic Partners Ltd, Microguide (version 7.1.6) [Mobile app]. Google App store https://play.google.com/store/apps/details?id=com.xancu.utreat&hl=en_NZ&gl=US&pli=1
59. Forse RA, Karam B, MacLean LD, Christou NV. Antibiotic prophylaxis for surgery in morbidly obese patients. Surgery. 1989 Oct;106(4):750-6; discussion 756-7.
60. Rondon AJ, Kheir MM, Tan TL, Shohat N, Greenky MR, Parvizi J. Cefazolin Prophylaxis for Total Joint Arthroplasty: Obese Patients Are Frequently Underdosed and at Increased Risk of Periprosthetic Joint Infection. J Arthroplasty. 2018 Nov;33(11):3551-3554.
61. Morris AJ, Roberts SA, Grae N, Frampton CM. Surgical site infection rate is higher following hip and knee arthroplasty when cefazolin is underdosed. Am J Health Syst Pharm. 2020 Mar 5;77(6):434-440.
62. Hussain Z, Curtain C, Mirkazemi C, Gadd K, Peterson GM, Zaidi STR. Prophylactic Cefazolin Dosing and Surgical Site Infections: Does the Dose Matter in Obese Patients? Obes Surg. 2019 Jan;29(1):159-165.
63. Peppard WJ, Eberle DG, Kugler NW, Mabrey DM, Weigelt JA. Association between Pre-Operative Cefazolin Dose and Surgical Site Infection in Obese Patients. Surg Infect (Larchmt). 2017 May/Jun;18(4):485-490.
64. Unger NR, Stein BJ. Effectiveness of pre-operative cefazolin in obese patients. Surg Infect (Larchmt). 2014 Aug;15(4):412-6.
65. 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 Sep;213(3):415.e1-8.
66. Sharareh B, Sutherland C, Pourmand D, Molina N, Nicolau DP, Schwarzkopf R. Effect of Body Weight on Cefazolin and Vancomycin Trabecular Bone Concentrations in Patients Undergoing Total Joint Arthroplasty. Surg Infect (Larchmt). 2016 Feb;17(1):71-7.

67. Young OM, Shaik IH, Twedt R, Binstock A, Althouse AD, Venkataramanan R, Simhan HN, Wiesenfeld HC, Caritis SN. Pharmacokinetics of cefazolin prophylaxis in obese gravidae at time of cesarean delivery. Am J Obstet Gynecol. 2015 Oct;213(4):541.e1-7.
68. 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 May;125(5):1205-1210.
69. Ho VP, Nicolau DP, Dakin GF, Pomp A, Rich BS, Towe CW, Barie PS. Cefazolin dosing for surgical prophylaxis in morbidly obese patients. Surg Infect (Larchmt). 2012 Feb;13(1):33-7.
70. Stitely M, Sweet M, Slain D, Alons L, Holls W, Hochberg C, Briggs F. Plasma and tissue cefazolin concentrations in obese patients undergoing cesarean delivery and receiving differing pre-operative doses of drug. Surg Infect (Larchmt). 2013 Oct;14(5):455-9.
71. Chen X, Brathwaite CE, Barkan A, Hall K, Chu G, Cherasard P, Wang S, Nicolau DP, Islam S, Cunha BA. Optimal Cefazolin Prophylactic Dosing for Bariatric Surgery: No Need for Higher Doses or Intraoperative Redosing. Obes Surg. 2017 Mar;27(3):626-629.
72. Anderson DJ, Podgorny K, Berríos-Torres SI, Bratzler DW, Dellinger EP, Greene L, Nyquist AC, Saiman L, Yokoe DS, Maragakis LL, Kaye KS. Strategies to prevent surgical site infections in acute care hospitals: 2014 update. Infect Control Hosp Epidemiol. 2014 Jun;35(6):605-27.
73. National Institute for Health Care Excellence. Surgical site infections: prevention and treatment NICE guideline [Available online]. Updated August 2020; cited November 2021. Available from: https://www.nice.org.uk/guidance/ng125/chapter/recommendations
74. Parvizi J, Gehrke T, Chen AF. Proceedings of the International Consensus on Periprosthetic Joint Infection. Bone Joint J. 2013 Nov;95-B(11):1450-2.
75. Morita S, Nishisho I, Nomura T, Fukushima Y, Morimoto T, Hiraoka N, Shibata N. The significance of the intraoperative repeated dosing of antimicrobials for preventing surgical wound infection in colorectal surgery. Surg Today. 2005;35(9):732-8.
76. Scher KS. Studies on the duration of antibiotic administration for surgical prophylaxis. Am Surg. 1997 Jan;63(1):59-62.
77. Zanetti G, Giardina R, Platt R. Intraoperative redosing of cefazolin and risk for surgical site infection in cardiac surgery. Emerg Infect Dis. 2001 Sep-Oct;7(5):828-31.
78. Reynolds, K., & He, J. (2005). Epidemiology of the metabolic syndrome. *The American journal of the medical sciences*, *330*(6), 273–279.
79. Daniels S. R. (2006). The consequences of childhood overweight and obesity. *The Future of children*, *16*(1), 47–67.

80. WHO Expert Consultation (2004). Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet (London, England)*, *363*(9403), 157–163.
81. WHO Global InfoBase team. The SuRF Report 2. Surveillance of chronic disease Risk Factors: Country-level data and comparable estimates. Geneva, World Health Organization, 2005. Available at https://apps.who.int/iris/bitstream/handle/10665/43190/9241593024_eng.pdf
82. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. (2000). *World Health Organization technical report series*, *894*, i–253.
83. Daniels S. R. (2009). The use of BMI in the clinical setting. *Pediatrics*, *124 Suppl 1*, S35–S41.
84. Sun, S. S., Chumlea, W. C., Heymsfield, S. B., Lukaski, H. C., Schoeller, D., Friedl, K., Kuczmarski, R. J., Flegal, K. M., Johnson, C. L., & Hubbard, V. S. (2003). Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. *The American journal of clinical nutrition*, *77*(2), 331–340.
85. Frisard, M. I., Greenway, F. L., & Delany, J. P. (2005). Comparison of methods to assess body composition changes during a period of weight loss. *Obesity research*, *13*(5), 845–854.
86. Demura, S., Sato, S., & Kitabayashi, T. (2004). Percentage of total body fat as estimated by three automatic bioelectrical impedance analyzers. *Journal of physiological anthropology and applied human science*, *23*(3), 93–99.
87. Mulasi, U., Kuchnia, A. J., Cole, A. J., & Earthman, C. P. (2015). Bioimpedance at the bedside: current applications, limitations, and opportunities. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition, 30(2), 180–193.
88. Moonen, H. P. F. X., & Van Zanten, A. R. H. (2021). Bioelectric impedance analysis for body composition measurement and other potential clinical applications in critical illness. Current opinion in critical care, 27(4), 344–353.
89. Capoor, M. N., Lochman, J., McDowell, A., Schmitz, J. E., Solansky, M., Zapletalova, M., Alamin, T. F., Coscia, M. F., Garfin, S. R., Jancalek, R., Ruzicka, F., Shamie, A. N., Smrcka, M., Wang, J. C., Birkenmaier, C., & Slaby, O. (2019). Intervertebral disc penetration by antibiotics used prophylactically in spinal surgery: implications for the current standards and treatment of disc infections. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society*, *28*(4), 783–791.
90. Himebauch, A. S., Sankar, W. N., Flynn, J. M., Sisko, M. T., Moorthy, G. S., Gerber, J. S., Zuppa, A. F., Fox, E., Dormans, J. P., & Kilbaugh, T. J. (2016). Skeletal muscle and plasma

concentrations of cefazolin during complex paediatric spinal surgery. *British journal of anaesthesia*, *117*(1), 87–94.

91. Gregory, J. R., Smith, J. C., Brown-Riley, S. M., Elward, A. M., & Luhmann, S. J. (2018). Vancomycin Concentrations in Paraspinal Muscles During Posterior Spinal Fusions for Neuromuscular Scoliosis. *Spine deformity*, *6*(6), 736–740.
92. Hvistendahl, M. A., Bue, M., Hanberg, P., Kaspersen, A. E., Schmedes, A. V., Stilling, M., & Høy, K. (2022). Cefuroxime concentrations in the anterior and posterior column of the lumbar spine - an experimental porcine study. *The spine journal : official journal of the North American Spine Society*, *22*(9), 1434–1441.
93. Kaspersen, A. E., Hanberg, P., Hvistendahl, M. A., Bue, M., Schmedes, A. V., Høy, K., & Stilling, M. (2023). Evaluation of cefuroxime concentration in the intra- and extrathecal compartments of the lumbar spine - an experimental porcine study. *British journal of pharmacology*, 10.1111/bph.16045. Advance online publication.
94. Walters, R., Rahmat, R., Fraser, R., & Moore, R. (2006). Preventing and treating discitis: cephazolin penetration in ovine lumbar intervertebral disc. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society*, *15*(9), 1397–1403.
95. Yan, D., Li, J., Zhang, Z., & Zhu, H. (2012). Determination of cephazolin, ceftazidime, and ceftriaxone distribution in nucleus pulposus. *Archives of orthopaedic and trauma surgery*, *132*(7), 969–973.
96. Walters, R., Moore, R., & Fraser, R. (2006). Penetration of cephazolin in human lumbar intervertebral disc. *Spine*, *31*(5), 567–570.