DECIPHERING DRUG DOSING AND RESPONSE IN SPECIAL PATIENT POPULATIONS

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ABSTRACT OF THE DISSERTATION

Deciphering drug dosing and response in special patient populations

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While drug development has traditionally focused on the general population, the number of individuals falling outside the 'norm' has continued to rise. For example, roughly 40% of the United States population is obese, and 15% is older than 65. Drug dosing is obscure in these patient populations, and a lack of knowledge may contribute to iatrogenesis. This concern is especially concerning with antibiotics due to emerging pathogen resistance and lack of drug development. Similarly, anticoagulant-related iatrogenesis remains one of the most common causes of hospital admission. This concern is heightened in individuals with extreme bodyweight and those of advanced age. Supporting evidence to substantiate concerns, identify mechanisms, and define solutions is urgently needed.

The thesis focused on using various approaches to evaluate drug exposure and response occurring with standard dosing strategies in special populations, namely individuals with obesity and advanced age. In the introductory Chapter (1), an overview

of obesity and advanced age is provided. The epidemiology of these populations and expected pharmacokinetic and pharmacodynamics changes are summarized. Further, information related to critical medications (antibiotics and anticoagulants), and the importance of their dosing is provided. In Chapter 2, a prospective pharmacokinetic study was performed in patients undergoing sleeve gastrectomy surgery. In this study, both plasma and subcutaneous adipose tissue beta-lactam antibiotic exposure was measured after standard surgical prophylaxis dosing. These data were also used to develop a two and three-compartment model describing the disposition of cefoxitin and cefazolin in individuals with obesity. In Chapters 3 and 4, the pharmacokinetics and pharmacodynamics of select anticoagulants were evaluated. First, the pharmacodynamics of subcutaneous unfractionated heparin and enoxaparin was described in individuals with obesity undergoing bariatric surgery. In this study, both anti-factor Xa and measurements of thrombin generation and potential were captured and correlated to measures of body composition. Next, variability in dabigatran exposure in advanced age was investigated, suggesting that monitoring drug exposure may be necessary for this patient population.

In Chapter 5, the influence of body composition on intravenous immunoglobulin response was investigated in individuals with primary immunodeficiency. The relationship between body composition and immunoglobulin pharmacokinetic parameters was established. In Chapter 6, a modified Cockcroft-Gault equation was developed using data obtained from clinical patient data. The influence of obesity, age, and obesity on the accuracy of calculated glomerular filtration rate estimates was established. The modified equation improved the accuracy of estimation. Chapters 7 and 8 provide an overview of the thesis and our future directions, respectively, including an evaluation of the

contributions of drug transporters and the gut microbiome on drug disposition. Collectively, the studies provided essential insights into the shortcomings of the current paradigm of drug dosing.

Obesity should be considered a special population by the Food and Drug Administration. Manufacturers should therefore be required to provide evidence that their dosing recommendations are adequate for extreme body weights. In terms of advanced age, this population is already considered a special population; however, a more rigorous evaluation of drugs is vital as the number of individuals in this group increases. Future studies are aimed at expanding on these data to provide dosing recommendations for special patient populations.

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I dedicate this dissertation to my wife and daughter. Their unwavering support and sacrifices allowed me to succeed in my pursuit of a Ph.D. I wish to thank them for allowing me to turn our home into a library as I prepared for research proposals and grant writing. I am fortunate to have them in my life, and I cannot imagine sharing this moment without them. Finally, to all my family, friends, and mentors along the way, thank you. I believe that each encounter in one's path shapes the future course, and I am confident that you have all helped carve the route towards completing this goal.

DEDICATION

To my wife and daughter.

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CHAPTER 1. Introduction

Overview of special populations

Special patient populations require careful attention when it comes to pharmacotherapy because of altered physiology and pathophysiology. One of the most critical elements of drug development is the selection of a drug regimen that is safe and effective for a given patient.[1] Current drug development practices focus on identifying a dosing regimen that is safe and effective at the population level; however, this may not suffice at the individual level. There is often substantial interindividual variation in drug response, and while this may not be problematic for many medications, it may be crucial for others.[2,3] For example, blood pressure medications may be titrated to patient response. This strategy has been suggested by some authors to account for patient variability in response.[2] This opportunity may not be present with drugs with a narrow therapeutic range or those required for treating critical illness. For example, the incorrect antibiotic dose may lead to suboptimal exposure and subsequent treatment failure and bacterial resistance development. Anticoagulants would also be critical in that the inaccurate dosing can increase bleeding risk or risk of thrombosis. With biologics, the impact of incorrect dosage can be a lack of efficacy, increased toxicity, or even excess expenditure with doses that are too high.

In the Food and Drug Administration Safety and Innovation Act (FDASIA) of 2012, Congress included section 907,[4] which directed the Food and Drug Administration (FDA) to investigate the representation of different demographic subgroups (i.e., sex, age, race,

and ethnicity) in marketing applications for pharmaceutical products and medical devices. This act came about in response to the underrepresentation of the elderly, women, and different ethnic groups in clinical trials. Note that obesity was not included in this Act. The FDA created the *Drug Trials Snapshots* website that makes demographic information in clinical trials for approved pharmaceutical products available to the public.[5] This program also does not contain any information on the number of obese patients enrolled in the trials. Although progress has been made towards ensuring diversity in clinical trials regarding demographic subgroups, it must be extended to include patients with specific clinical characteristics prevalent in our population (e.g. obesity) and have a significant effect on pharmacotherapy.

The focus of the current research was drug dosing in special populations with a focus on obesity and advanced age as the number of individuals within these categories has grown significantly over the past decade. While advanced age is considered a special population by the FDA and obesity is not. This stance implies that manufacturers are required to provide safety data supporting its drug in advanced age. This requirement does not apply to obesity. Many manufacturers do not offer any guidance for dosing their drugs in obesity, leaving a critical knowledge gap. Our pilot study reviewed all FDA approved products in 2015 (n=45) and found that less than 50% included information related to drug dosing in obesity in their product labeling. Moreover, 25% did not perform any studies on individuals with obesity.

Similarly, there are gaps in the optimal drug dosing for drugs in advanced age. While considered a special population by the FDA, individuals over the age of 65 are infrequently included in clinical trials. Further, data in older adults are often extrapolated from small pharmacokinetic studies in relatively healthy individuals. These data are not representative of the aged population who often present with multiple comorbidities and polypharmacy. To summarize, there are knowledge gaps in both obesity and advanced age in terms of drug dosing and response. The gap is more significant in the obese population because it is currently not considered a special population by the FDA. The overall goals of the research presented herein are to provide evidence underscoring the importance of differences in drug response in these populations and to make recommendations for dosage adjustment for some high risk or critical medications.

Scope of obesity

The number of individuals considered obese has reached epidemic proportions (**Figure 1.1**).[6] In the United States, roughly 40% of adults over the age of 20 years of age are considered obese.[7] Moreover, globally, more than 650 million individuals are obese.[8] If current trends continue, estimates suggest that by 2030 up to 57.8% of the world's adult population will be classified as overweight or obese.[9] Furthermore, obesity is associated with various comorbidities such as hypertension, hyperlipidemia, coronary artery disease, abnormal glucose tolerance or diabetes, sleep apnea, nonalcoholic fatty liver disease, musculoskeletal disorders, kidney disease, and a variety of cancers. These comorbidities may also influence drug dosing secondary to pharmacokinetic changes related to polypharmacy or pharmacodynamic changes.[10] Physiological processes may be altered

by obesity, including gut permeability, gastric emptying, cardiac output, hepatic clearance, and renal clearance.[6,11]

Measurement of the degree of obesity and body weight descriptors.

The World Health Organization (WHO) defines obesity as a condition of excessive fat accumulation in the body to the extent that health and well-being are adversely affected.[8] Excessive fat accumulation is related to physiologic normal is not straight forward because "normal" is dependent on age, sex, and body type (i.e., athletic versus non-athletic). A variety of methods for measuring body fat are available such as densitometry, hydrometry, dual-energy X-ray absorptiometry (DXA), chemical multi-compartment models, computed tomography (CT), or magnetic resonance imaging (MRI).

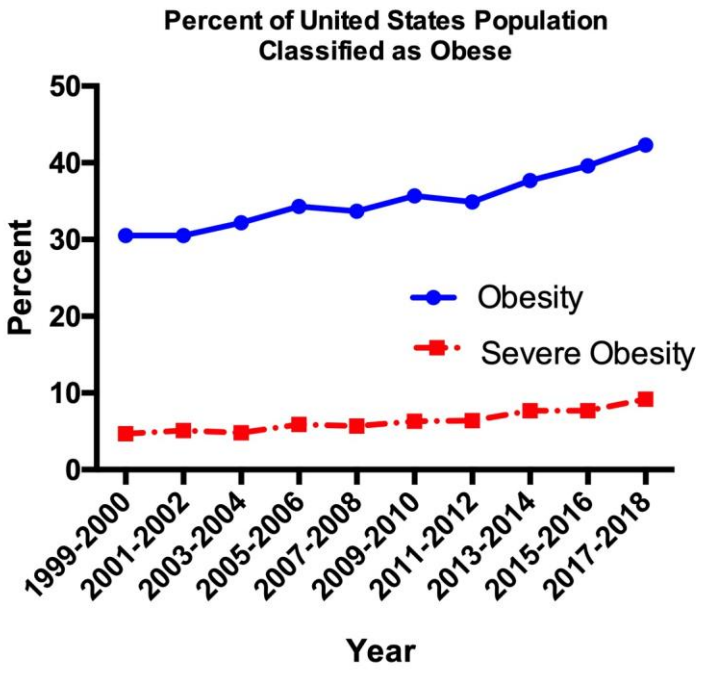

Figure 1.1 Trends in obesity in the United States. Source: NCHS, National Health and Nutrition Examination Survey, 1999–2018.[6]

The major limitations of these approaches time required for measurement, cost, and complexity. More commonly used approaches to predict body fat percentage include skinfold thickness measurements, bioelectrical impedance, body mass index (BMI), waist circumference, and body adiposity index (BAI).[12] Of these methods, BMI is the most commonly used in clinical practice, with a range of 18.5 to 24.9 kg/m^2 representing "healthy". Individuals may be classified according to their BMI, as summarized in **Table 1.1**. While this metric is facile to calculate, it does not account for body type. It cannot differentiate for the wide range of adiposity with the specific body mass index category.

Table 1.1. Classification of obesity by body mass index

	Body mass index (kg/m^2)	**Obesity class**
Underweight	< 18.5	
Normal	18.5 – 24.9	
Overweight	25.0 – 29.9	
Obesity	30.0 – 34.9	I
	35.0 – 39.9	II
Extreme obesity	40.0	III

Bioimpedance (BIA) is the most studied bedside technique for assessing body composition (BC) as it is affordable, easy to transport, and use.[13,14] The method is non-

invasive, relatively inexpensive, and quick to use; therefore, it helps repeat measurements of the same subject.[15] Although, similar to other body composition measurements, it does not directly measure BC, it measures body tissues' resistance to an electric current, an indirect measure of BC.[13,15] The level of precision produced by BIA is reported as good with a 1-to-2% variability between repeat measures.[16-18] Thompson et al. found good absolute and relative agreement between changes in body composition assessed by DXA and BIA with small biases in the estimation fat mass (FM) and percentage body fat (BF%) in overweight women.[19] Note that the limits of agreement were wide for bias and SD but BIA may be a viable option to DXA in clinical practice, especially at the group level.

Finally, body size descriptors (**Table 1.2**) are many, and each has its advantages and disadvantages.[20-23] Total (or actual) body weight is the most straightforward, but other body size descriptors are used clinically for drug dosing (and calculation of creatinine clearance discussed in **Chapter 5**). There are no uniform recommendations of which body weight descriptor should be used for drug dosing. Some drugs are specific in which descriptor to use. For example, the American Society for Clinical Oncology Clinical Practice guideline recommends that total body weight with no dose capping (with few exceptions) be used to dose chemotherapy.[24]

Table 1.2. Summary of commonly used body descriptors and their formulae[20-23]

Bodyweight descriptor	**Formula**
Total body weight (TBW), kg	Patient actual weight
Ideal body weight (IBW), kg	IBW_{male} (kg) = 49.9 + 0.89 (height in cm – 152.4)

	IBW_{female} (kg) = 45.4 + 0.89 (height in cm – 152.4)
Adjusted body weight (ABW), kg	ABW (kg) = IBW + F (TBW – IBW) Where F = drug specific correction factor, ranges from 0.3 – 0.6
Lean body weight (kg), James formula	Male: 1.1 x weight – 128 $(weight/height)^2$ Female: 1.07 x weight – 148 $(weight/height)^2$
Lean body weight (kg), Boer formula	Male: 0.407 x weight + 0.267 x height - 19.2 Female: 0.252 x weight + 0.473 x height - 48.3
Lean body weight (kg), Hume formula	Male: 0.32810 x weight + 0.33929 x height - 29.5336 Female: 0.29569 x weight + 0.41813 x height - 43.2933
Lean body weight (kg), Janmahasatian formula	Male: 9270 x weight / 6680 + 216 x body mass index Female: 9270 x weight / 8780 + 244 x body mass index

Alterations in pharmacokinetics and pharmacodynamics in special populations

Several physiological changes, as discussed earlier, influence pharmacokinetics and pharmacodynamics in obesity. Both gut wall permeability and gastric emptying are increased in obesity.[25,26] One must also consider that individuals with obesity undergo

bariatric surgery, which may also influence the surface area available for drug absorption in the gut. These changes may impact both peak and time to peak drug concentration. Cardiac output is increased as a compensatory mechanism to account for the excess tissue and blood volume.[27] While one would expect enhanced liver clearance due to increased perfusion, non-alcoholic liver disease is prevalent (50-90% of individuals) in obesity and negatively affects the liver's performance and, in turn, hepatic metabolism.[28] In general, free fraction of drug may be unaltered in obesity as both total protein and albumin are unaltered. There may be changes in free fractions of drugs bound to alpha 1 acid glycoprotein; however, there are conflicting data regarding the influence of obesity on this protein.[29,30] Renal clearance in obesity is also unclear, while the literature suggests that overweight and obesity are associated with chronic kidney disease; [31] glomerular filtration is initially augmented in obesity.[32] A detailed summary of pharmacokinetic changes is illustrated in **Figure 1.2**.[33]

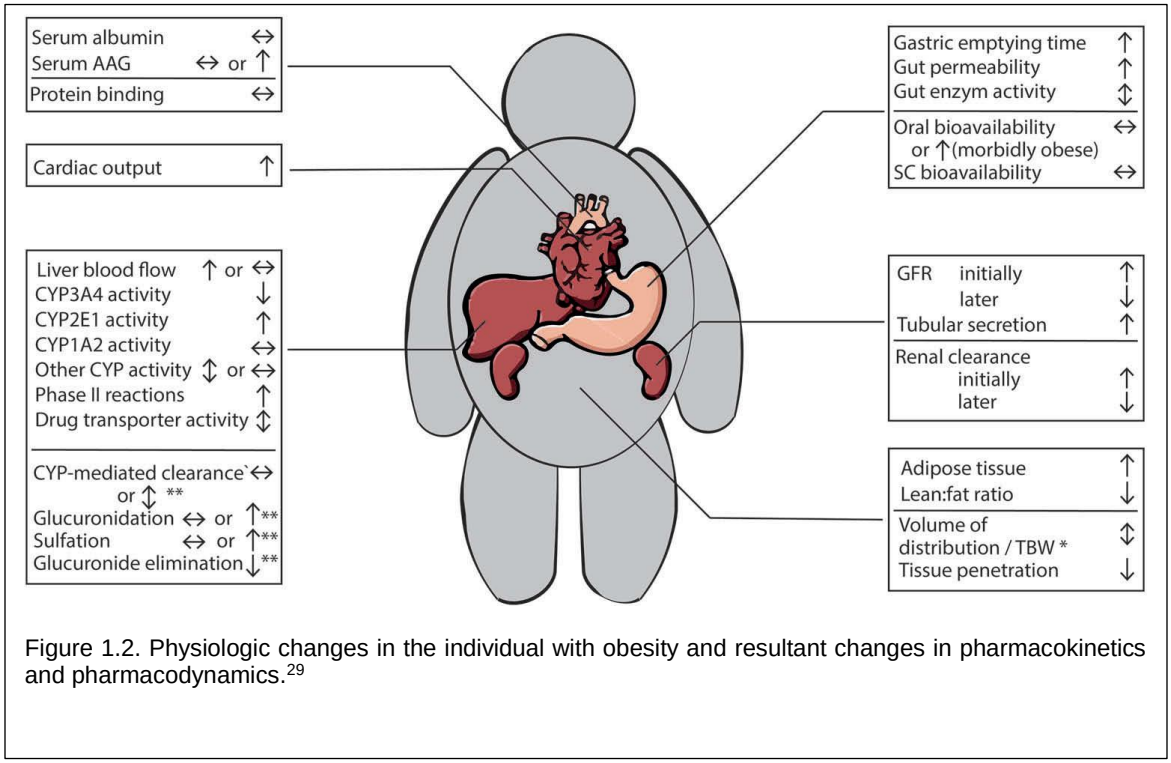

Figure 1.2. Physiologic changes in the individual with obesity and resultant changes in pharmacokinetics and pharmacodynamics.[29]

One must also consider the pharmacodynamic changes present in obesity. There are a variety of examples in the literature. For example, leptin, an adipocyte-derived hormone elevated in obesity,[34] induces platelet aggregation and may blunt antiplatelets' mechanism.[35] Microbiome changes are also evident in obesity and may alter drug metabolism by bacteria in the gut resulting in either augmented or reduced drug bioavailability and effects. The microbiome may also influence platelet reactivity through the production of trimethylamine N -oxide (TMAO), an amine oxide via microbial metabolism, and high levels of TMAO may antagonize clopidogrel and other antiplatelets.[36] Interestingly, leptin reduces macrophage and T-cell differentiation and activity and may contribute to worse outcomes in treating infectious diseases in individuals with obesity than normal-weight individuals.[37] Drugs that affect the central nervous system may also have altered pharmacodynamics. Obstructive sleep apnea is common in obesity, with prevalence estimates at 30%.[38] Obstructive sleep apnea increases the risk of respiratory depression in patients treated with opioids and benzodiazepines. Sensitivity to medications acting in the central nervous system is also increased. In one study, propofol concentrations at half-maximum effect (E_{50}) were reduced in obese individuals.[39]

Finally, drug transporter expression may be altered in obesity. Data evaluating drug transporter changes in obesity is scarce and represents an important area for scientific contribution. Our ongoing human studies (Rutgers Biomedical and Health Sciences IRB number, PRO 2019001020) in this area are important and will inform future research.

Advanced age and its implications on drug response

The elderly and very elderly populations have been steadily increasing in the United States. Between the years 2000 to 2011 (6.3–41.4 million), there was an increase of 18 % in individuals aged 65 years or older in the United States. The number of individuals over 65 years of age is projected to increase to 79.7 million by 2040.[40] In addition, the very elderly population (age 85 years or older) is projected to reach 14.1 million by 2040. Advanced age is a significant risk factor for iatrogenic events secondary to medications.[41] Polypharmacy and the presence of comorbidities influence both pharmacokinetics and pharmacodynamics, placing patients at risk.[42] As a result, hospital utilization in the elderly population related to adverse drug events is common, and up to 30 % of individuals may require emergency care.[43-48]

Advanced age is associated with anatomical and physiological changes as well as functional decline. As a result, an individual's response to drug therapy changes accordingly. There is a significant inter-individual variation in physiologic changes in advanced age to complicate the matter further, making prediction challenging. Nonetheless, various age-related pharmacokinetics and pharmacodynamics changes occur in advanced age, and the consequences of these changes are dependent on the individual.

Changes in physiology in the aging population

Virtually every organ system is altered as we age (**Figure 1.3**).[49] Cardiac structure and function are influenced by advancing age. Higher systolic arterial pressure secondary to reduced elasticity and compliance of the aorta contributes to cardiac function changes.[50,51] Renal mass is decreased with increasing age, and for every decade of life over age 30 years, an individual's glomerular filtration rate is reduced by 8 mL/min. Of course, this reduction may be greater in those with comorbidities.[52,53] Serum creatinine is commonly used in estimates of glomerular filtration rate; however, it may be unreliable in advanced age due to decreased muscle mass. The decline in glomerular filtration rate in elderly patients is not necessarily accompanied by increased serum creatinine due to reduced muscle mass, limiting this parameter's reliability.[54] The prevalence of chronic kidney disease in patients aged 65 years or older was estimated using the National Health and Nutrition Examination Survey (NHANES) data to be 39.4 %.[55] This factor alone is associated with an increase in iatrogenesis.

The gastrointestinal system undergoes a variety of changes as we age. Gastric emptying may be similar to younger patients; however, polypharmacy may increase the risk of reduced gastric emptying.[56] The small intestine, where the majority of drug absorption occurs, has largely unchanged motility.[57,58] Both liver volume and blood flow progressively decline with age.[59] The implications of these changes are unclear.[60]

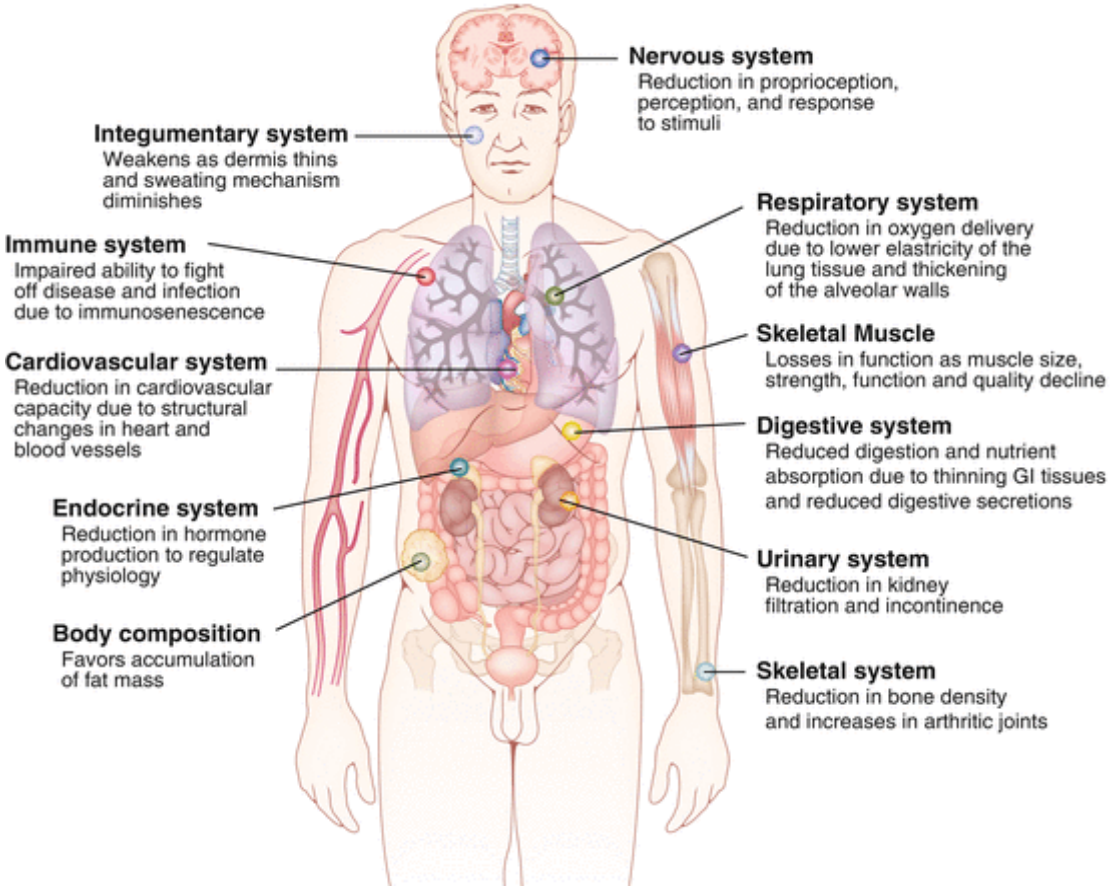

Figure 1.3. Various age-related changes in physiology influence drug pharmacokinetics and pharmacodynamics.[49]

Alterations in pharmacokinetics and pharmacodynamics

Changes in drug disposition are evident in advanced age. Absorption does not appear to be altered in advanced age; however, there are conflicting results in the available studies likely related to differences in the technique used for assessment.[61] Nonetheless, the absorption of iron, calcium, and vitamin B_{12} is reduced.[62] Of note, the absorption of these compounds is via an active transport mechanism.

As discussed earlier, liver volume and blood flow are reduced in advanced age and reduce first-pass.[63]. As a result, the bioavailability of some drugs may be increased.

If the first-pass metabolism is needed for drug activation (i.e., pro-drugs), there may be a decrease in the bioavailability of active compounds.[64]

Body composition is altered as we age with increased adipose and decreased total body water and lean body mass.[65] Subsequently, the distribution of fat-soluble compounds may be increased in elderly patients and contribute to drug accumulation and increased drug half-life. Some of these compounds, such as benzodiazepines, are associated with harm in this population. Contrary, polar drugs have a reduced volume of distribution.[61] While tissue distribution may be reduced, you may see higher serum concentrations with more polar drugs (i.e., gentamicin, digoxin, theophylline, ethanol) and an increased risk of toxicity. However, typically, the reduction in the volume of distribution for polar drugs is balanced by the decreased clearance (**Figure 1.4**), resulting in a relatively unchanged drug half-life. Protein binding may be reduced in elderly patients who have poor nutritional status increasing the free fraction of acidic compounds that bind to albumin.[66] This phenomenon may increase the risk of toxicity.

$$t_{\frac{1}{2}} = \frac{Ln(2) * V}{Cl}$$

Figure 1.4. Relationship between volume of distribution and decreased clearance in advanced age.

Decreasing volume of distribution of polar drugs is balanced by decreased clearance in advanced age resulting in a net unchanged t1/2. Where Ln(2) = natural log of 2, V = volume of distribution, and Cl = clearance.

$$Cl_{liver} = Q\frac{[C_a - C_v]}{[C_a]} = QE$$

Figure 1.5. Drug clearance by the liver is dependent on liver capacity and hepatic flow.

Both of these variables may be altered in advanced age. Where E= steady-state extraction ratio, Q= liver blood flow, [Ca] = concentration of drug in portal vein and hepatic artery, and [Cv] = concentration of drug leaving the liver and hepatic vein and CL_{liver} = clearance by the liver.

Drugs that are cleared by the kidney may accumulate in the elderly owing to reduced glomerular filtration rate. The risk of toxicity is dependent on the reliance of the drug on the kidney for clearance and its therapeutic window. An important consideration in advanced age is the lack of reliability of serum creatinine-based estimates of renal function to guide drug dosing. Regardless, serum creatinine-based estimates are used despite their limitations, which are discussed further in **Chapter 6**. Finally, many drugs are cleared by the liver, which depends on the liver's capacity and hepatic blood flow (**Figure 1.5**).

Review of antibiotics

Penicillin and sulfonamides were the first antibiotics introduced to the medical market in the 1930s.[67] These life-saving compounds shifted the primary causes of mortality from infection to chronic disease and cancer in future decades. Since the 1930s, numerous antibiotics have made it to the market with various mechanisms of action. Pharmacokinetics and pharmacodynamics understanding are imperative to optimize antibiotic dosing. Achievement of the appropriate therapeutic target for a given antibiotic is often a function of peak concentration or duration of time above the minimum inhibitory concentration for a given pathogen. **Table 1.3** summarizes the appropriate pharmacodynamic targets for specific antibiotic drug classes.[68]

One of the present-day concerns is the emergence of multi-drug resistant pathogens and the slow development of novel antibiotics. In 2016, 270.2 million antibiotic prescriptions were written in the United States.[69] While the overuse of antibiotics is a primary driver of antibiotic resistance, incorrect dosing shares some blame.

Individuals with obesity are at risk of antibiotic underdosing and subsequent colonization with resistant bacteria. Our study found that in a cohort of hospitalized obese individuals versus normal controls, obesity was associated with greater identification of multidrug-resistant gram-negative organisms in routine cultures drawn during hospital visits. While the underlying pathology may contribute to the increased identification of resistant pathogens, one cannot exclude the contribution of inadequate antibiotic dosing for the previous infection. Our cohort study identified a significant association between obesity and multi-drug resistant Enterobacteriaceae.

Like individuals with obesity, elderly patients are frequently not included in antimicrobial clinical trials.[70,71] This population is often excluded because of their inherent risk of adverse reactions, altered clearance, and polypharmacy, which may confound clinical trials results.[71] The general mantra in the elderly is to start low and go slow in terms of dosing. While this may be a viable option for many medications, this option is not feasible with antibiotics, given that the elderly population frequently requires antibiotics for severe infections and dosing. Using too low of a dose may result in treatment failure and the development of antibiotic resistance.

Antibiotics are commonly prescribed in the elderly population, and since the year 2000, there has been a greater than 20% increase in antibiotic prescriptions in this group.[72] Moreover, pneumonia and septicemia are among the top ten causes of death in the elderly, and both of these infectious diseases require the correct antibiotic dose for optimal outcomes.[73]

Table 1.3. Pharmacodynamic parameters or exposure threshold for treatment success[67]

Antibiotic class	Pharmacodynamic target	Pharmacodynamic parameter
Aminoglycosides	Concentration-dependent	*f*C_{max}/MIC ≥10 to 12; total AUC/MIC ≥156
Beta lactams	Time-dependent	*f*T > 30 - 70
Fluoroquinolone	Concentration-dependent	*f*C_{max}/MIC ≥10 to 12; total AUC/MIC > 125 for gram-negatives; *f*AUC/MIC > 30-50 for gram positives
Glycopeptide	Concentration-dependent	*f*AUC/MIC; *f*C_{max}/MIC; total AUC/MIC > 400
Macrolides	Time-dependent	AUC/MIC
Oxazolidinone	Time-dependent	Total AUC/MIC > 100
Polymyxins	Concentration-dependent	*f*AUC/MIC > 12 to 15; total AUC/MIC > 60
Tetracyclines	Time-dependent	AUC/MIC; *f*AUC/MIC

AUC=area under the curve; C_{max}=maximum drug concentration; *f*=free fraction (unbound concentration); MIC=minimum inhibitory concentration

Review of anticoagulants

Unfractionated heparin, low molecular weight heparins, and vitamin K antagonists have been used for over 70 years to treat and prevent venous thromboembolism and stroke. These agents are effective; however, they are fraught with intricacies that make them challenging to use safely, especially in special populations. Anticoagulants are in the top five drugs associated with patient harm in the United States. Some of this association may be related to noncompliance. More than 50% of patients on anticoagulation reporting do not adhere to their medication regimen, likely related complexity in dosing regimens, inconvenience of administration, and frequent monitoring requirements.[74-77] These statistics are concerning because anticoagulants effectively reduce the risk of stroke and systemic embolism and treat venous thromboembolism. When patients do not take these medications, they are at risk of poor outcomes.

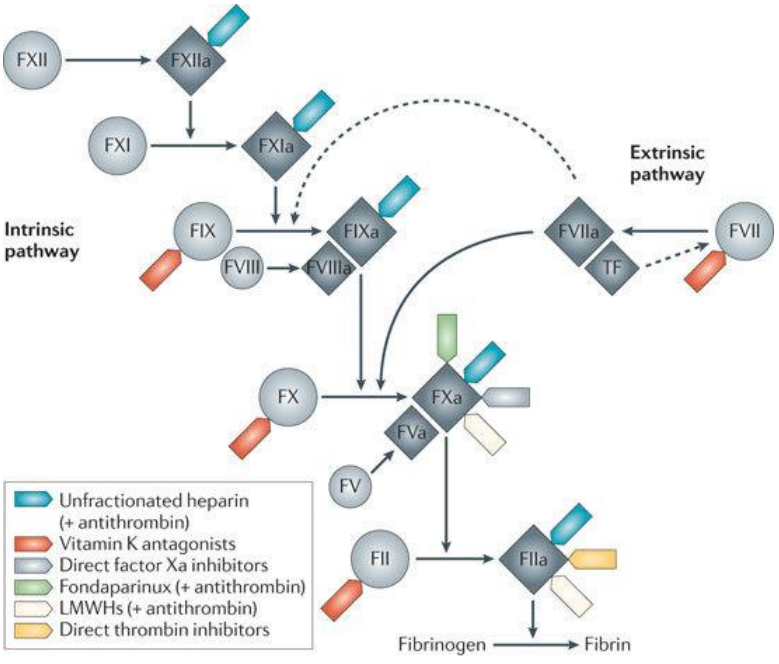

Figure 1.6. The coagulation cascade and therapeutic targets.[73]

Within the past decade, there has been a paradigm shift in anticoagulation with the entrance of direct oral anticoagulants (DOACs). These agents are specific for factor II (dabigatran) or factor X (apixaban, edoxaban, and rivaroxaban) and display more predictable pharmacokinetics. As such, monitoring coagulation parameters

is not routinely recommended. There has been a substantial uptake of these medications, and many of the concerns with the earlier anticoagulants have been remedied. **Figure 1.6** illustrates the mechanism of action of currently available anticoagulants and their mechanisms within the coagulation cascade.[78] Nonetheless, despite this advancement in anticoagulation, optimal dosing (and monitoring) of anticoagulants in special populations is poorly defined. Extreme body weight is associated with several changed increasing the thrombotic risk of patients, including elevated fibrinogen, von Willebrand factor, tissue-type plasminogen activator antigen, factor VII, platelet aggregation, and elevated plasminogen activator inhibitor-1.[79,80] Furthermore, the reduced mobility or immobility in individuals with obesity is a significant risk factor for thrombosis. Anticoagulants are commonly used in this population. Importantly, robust studies to address dosing in obese individuals with anticoagulants are not available.

With heparins and warfarin, clinicians can monitor activated partial thromboplastin time or anti-factor Xa and International Normalized Ratio, respectively. Even with these tools, attaining therapeutic targets may take time. Importantly, anticoagulants are often required acutely, and the rapid attainment of therapeutic targets is associated with improved outcomes. Additionally, excessive dosing may result in bleeding and poor outcomes. Strategies to improve dosing strategies for achieving target range would enhance patient care. In terms of the DOACs, there is a lack of data on whether monitoring coagulation assays improves outcomes and even less on the ideal agent or dose to use in individuals with obesity.

The risk: benefit ratio of anticoagulants in advanced age is shifted owing to drug interactions and frailty, and this population is likely to be exposed to anticoagulants. Anticoagulants are the mainstay for stroke prevention in atrial fibrillation and treatment of venous thromboembolism.[81,82] The mean age of atrial fibrillation onset is 75 to 85 years, and roughly 82% of atrial fibrillation patients in the US are ≥ 65 years old. In terms of venous thromboembolism, age >75 years is an independent risk factor.[81,82] These data suggest that anticoagulants are commonly used in advanced age, yet clinical trials typically do not include this patient population. The direct oral anticoagulants approved by the FDA were investigated in more contemporary clinical trials within the past decade. The mean age of patients in landmark trials leading to the approval of the DOACs ranged from 70 to 73 years.[83-86] While these data are encouraging, the patients included in these studies did not have significant comorbidity as indicated by the $CHADS_2$ (congestive heart failure, hypertension, age ≥75 years, diabetes mellitus, stroke [double weight]) score, a measure of stroke risk in atrial fibrillation, distribution reported in the studies.

To summarize, anticoagulants are an important group of drugs where appropriate dosing can improve outcomes and reduce the risk of iatrogenic events, namely bleeding. Current approaches fall short in obese and elderly patients, and there is an opportunity to enhance therapy by incorporating innovative monitoring strategies or providing definitive dosing recommendations.

Summary

Special patient populations are on the rise, and current paradigms in identifying the best dosing and drug selection based on patient characteristics are not adequate, especially for individuals with obesity and those of advanced age.

Specific Aims

To identify alterations in biodisposition and response in individuals with obesity, focusing on antibiotics, anticoagulants, and protein therapeutics.

To identify alterations in biodisposition and response in individuals of advanced age with a focus on antibiotics, anticoagulants, and protein therapeutics

To provide evidence to support mechanisms contributing to altered biodistribution of drugs in special populations.

CHAPTER 2. Cefoxitin plasma and subcutaneous adipose tissue concentration in patients undergoing sleeve gastrectomy*

Introduction

Obesity is a major public health concern, with more than 35.7% of adults in the United States classified as obese (defined as a body mass index (BMI) of ≥ 30 kg/m^2).[87] Postoperative skin and soft structure infections (SSSIs) are responsible for roughly one-quarter of the estimated 2 million healthcare-associated infections annually.[88] Development of a SSSI is associated with significant morbidity and a mortality rate of 3%.[89] Data supporting the appropriate dosing of many antibiotics for perioperative prophylaxis in the obese population is lacking and often based on expert opinion rather than clinical evidence.

Cefoxitin is a second-generation hydrophilic cephalosporin antibiotic with limited distribution in adipose tissue.[90] Cefoxitin covers a variety of aerobic and anaerobic gram-positive and negative organisms. The bacterial killing of cefoxitin, as with other cephalosporins, is based on the duration of time that free concentrations are above the minimum inhibitory concentration (*f*T > MIC). Optimally, the *f*T > MIC must exceed at least 60–70% of the dosing interval.[91] To prevent postoperative infection, it is ideal to exceed the MIC for the surgical procedure duration. The recommended dose of cefoxitin for surgical prophylaxis is 2 grams administered intravenously as a bolus.[92] Previous studies suggest

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* This chapter has been published. Brunetti L, Kagan L, Forrester G, Aleksunes LM, Lin H, Buyske S, Nahass RG. Cefoxitin Plasma and Subcutaneous Adipose Tissue Concentration in Patients Undergoing Sleeve Gastrectomy. Clin Ther 2016;38:204-10. PMID: 26686826; PMCID: PMC4715936.

that cefoxitin and other cephalosporins' tissue concentrations might be insufficient for providing antibiotic coverage in obese patients.[93,94] The objective of our study was to identify whether currently prescribed doses of cefoxitin achieve adequate and sustained plasma and tissue concentrations in obese patients undergoing sleeve gastrectomy.

Materials and Methods

Study population and drug administration

The Institutional Review Board approved the study protocol. All subjects meeting inclusion criteria and consenting to participate received the institution standard cefoxitin therapy (2 grams as a slow intravenous bolus over 5 minutes) just before induction of anesthesia in the operating room. Patients aged 18 to 65 years old undergoing elective sleeve gastrectomy were eligible to participate. Patients with liver impairment (elevations in liver enzymes of greater than three times the upper limit of normal), reduced renal function (creatinine clearance < 50 mL/min), blood coagulation disorders, or anemia (hemoglobin < 12 mg/dL) were excluded from the study. All patients also received similar preoperative medications, which consisted of dexamethasone, famotidine, fentanyl, metoclopramide, and midazolam. Glomerular filtration rate (GFR) was estimated using serum creatinine (SCr) and cystatin C-based equations.[95-97] SCr levels were extracted from the patient medical record on the day of the surgical procedure. Plasma cystatin C concentrations were obtained using a Quantikine ELISA kit (R&D Systems, Minneapolis, MN).

Sample Collection

Serial venous blood samples were drawn from an antecubital intravenous line opposite to the arm in which cefoxitin was infused. Venous blood samples (5 mL) were collected in EDTA K2 or K3 tubes just before cefoxitin administration and then at 5, 30, 60, 120, and 240 minutes after dose administration, and plasma was separated by centrifugation. Approximately 1 gram of subcutaneous adipose tissue was excised from the surgical site at the time of incision and wound closure. Plasma and tissue samples were stored at –80°C until analysis.

Quantitation of Cefoxitin in Plasma and Adipose Tissue

Quantitation of cefoxitin was adapted from a previously published HPLC method.[98] The control plasma used to prepare calibration standards was obtained from the New Brunswick affiliated hospital blood bank (New Brunswick, NJ). Human Adipose tissue was purchased from Lee Biosolutions Inc.(Saint Louis, MO). Plasma samples and standards (100 µL) were spiked with 10 µL internal standard (cefotaxime sodium 100 µg/mL in methanol) and deproteinized with 400 µL acetonitrile. After centrifugation at 13,000 g for 10 min, the supernatant was evaporated to dryness. The residue was reconstituted in 200 µL of the mobile phase, and 50 µL was injected into the HPLC system. Adipose tissue samples were thawed and cut into pieces about 100 mg for cefoxitin measurement. The tissue was immediately homogenized in 0.5 mL water for 5 minutes using Polytron PT 1300D homogenizer. 10µL cefoxitin stock (1mg/mL) was added to the Eppendorf tube and evaporated under air. Control adipose tissue homogenate (0.5mL) was added and vortexed to prepare 20 µg/mL standard. A series of tissue calibration standards were prepared by dilution with adipose tissue homogenate. The resulting homogenate of the

tissue sample and calibration standard was processed as described under the method for plasma samples.

Chromatographic separation was completed using an ODS-AQ™ column (5µm, 4.1 × 250 mm, YMC America Inc., PA) on a Waters HPLC system equipped with a 717plus autosampler, 486 tunable UV detector, and Hitachi L-7100 pump. The mobile phase consisted of 100mM sodium acetate and acetonitrile (90:10, pH=4.5), and the flow rate was 0.8 mL/min. The detection wavelength was set at 254 nm. The retention times of cefoxitin and the internal standard were 11.6 minutes and 14.7 minutes. The calibration curves were linear between 3.9 to 500 µg/mL for plasma and 2.5 to 100 µg/g for adipose tissue. The acceptable absolute recovery of cefoxitin (85.4%) and internal standard (92.0%) was obtained for the method. Absolute recovery was calculated as the peak area ratio between the tissue homogenate and the solvent standard.

Pharmacokinetic analysis

A standard noncompartmental analysis was performed for each cefoxitin plasma concentration-time profile using Phoenix WinNonlin 6.1 software (Pharsight, Mountain View, CA). Terminal half-life, the area under the concentration-time curve from time zero to infinity (AUC, calculated by linear trapezoidal method), the volume of distribution at steady state (V_{ss}), and systemic clearance (CL) were calculated. Pharmacokinetic parameter values were compared to the reference data from Toma et al. .[93], which evaluated cefoxitin in obese patients undergoing abdominal and pelvic surgery and normal-weight subjects. Also, individual plasma concentration-time profiles were

assessed using a one-compartment model. Plasma concentration at the time of wound closure was determined from the fitted profiles, and the ratio of tissue to plasma concentration was calculated for all patients.

Results

Six patients were enrolled and evaluated for single-dose cefoxitin pharmacokinetics. Patient parameters are summarized in **Table 2.1**. Calculated pharmacokinetic parameters were similar to a historical cohort of obese surgical patients as shown in Table **2.2**.[93] At the time of incision, 4

Table 2.1. Patient characteristics and anthropometrics	
Parameter	Mean ± standard deviation
Age (y)	48.7 ± 6.2
Race (% White)	66.7
Total body weight (kg)	114.1 ± 21.6
Ideal body weight (kg)	48.9 ± 5.87
Height (cm)	163.3 ± 5.4
Body mass index (kg/m2)	42.8 ± 7.1
CrCl (mL/min)*	75.7 ± 18.5
CrCl (mL/min)**	94.0 ± 13.6
Procedure time (min)	78.2 ± 23.6
***Cockcroft-Gault, sCr based** ****CKD-EPI, cystatin C based**	

Table 2.2. Pharmacokinetic parameters with a historical control

Parameters	Normal-weight patients (Toma et al.)	Obese patients (Toma et al.)	Current study
Total body weight, kg	60 ± 10	126 ± 29	114.1 ± 21.6
Dose, g	1 g over 1 min	2 g over 1 min	2 g over 5 min
Number of subjects	13	14	6
AUC (h·mg/L)	81 ± 33	178 ± 40	156 ± 57.9
CL (L/h)	14.4 ± 5.4	11.8 ± 2.6	14.7 ± 6.4
V_{ss} (L)	11 ± 5	18 ± 5	18.9 ± 7.7

Of 6 patients who had tissue cefoxitin concentrations above the MIC for anaerobes (16 mcg/mL) versus 5 of 6 patients had coverage for aerobic infections (8 mcg/mL). At the time of surgical closure, none of the patients enrolled in the study, maintained a therapeutic cefoxitin tissue concentration (**Figure 2.1**, defined as a tissue concentration of > 8 mcg/mL). In **Figure 2.2**, the mean concentration-time profile of cefoxitin in obese

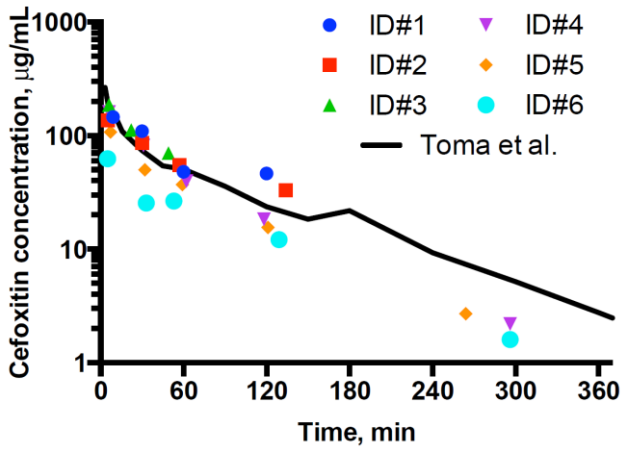

Figure 2.1. Comparison of observed plasma cefoxitin levels to historical data (solid line) in obese patients.

We did not obtain a 240-minute sample for patients 1, 2, 3.

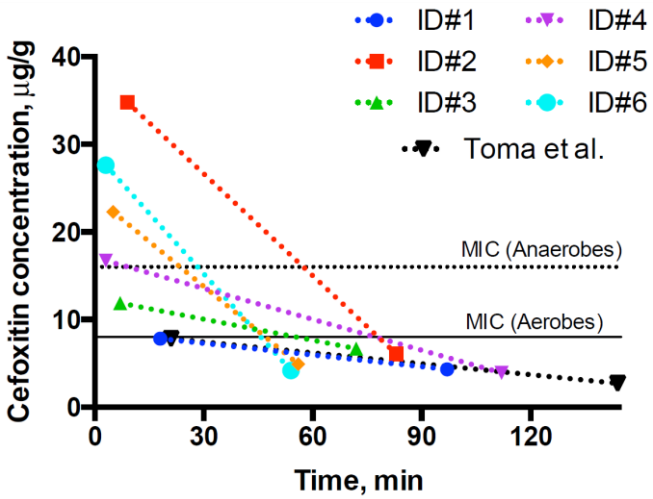

Figure 2.2. Cefoxitin subcutaneous adipose tissue concentrations at incision and closure in relation to minimum inhibitory concentration and compared with historical data.

Note: Only two time points were assessed; therefore, it is unknown when the cefoxitin concentration fell below 8 mcg/mL.

subjects from a previously published study by Toma et al. .[93] is provided for comparison. Individual data profiles were fitted using a one-compartment model with first-order elimination, and the volume of distribution and clearance were determined (19.47 ± 9.75 L and 15.25 ± 2.73 L/h, respectively). The ratio of tissue-to-plasma concentrations at the time of wound closure was 0.12 ± 0.03, which was not statistically different (p=0.13) from the previously published value for obese subjects (0.08 ± 0.07).[93] There was no correlation between estimated GFR (calculated using Cockcroft Gault and Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations) and tissue concentration at the time of surgical closure.

Discussion

Cefoxitin pharmacokinetics in obese subjects undergoing sleeve gastrectomy has not been evaluated before. *In vitro*, cefoxitin exhibits a MIC of 8 mcg/mL or less for aerobic organisms and 16 mcg/mL or less for anaerobic organisms. Although we found that plasma cefoxitin concentrations exceeded the MIC for the duration of the sleeve gastrectomy procedure, this was not the case for subcutaneous adipose tissue. Tissue concentrations at the surgical incision time were above a MIC of 8 mcg/mL for 5 of 6 patients. Reaching an adequate tissue concentration is essential at the time of incision to prevent infection; however, ideally, antibiotic concentrations should remain above the desired MIC for the procedure's duration for optimal benefit.[89,99,100] In all patients, the tissue concentration at the time of surgical closure was below a MIC of 8 mcg/mL. Several studies suggest a negative correlation between the end of procedure tissue and serum concentrations of antibiotics and infection rates.[94,101,102] Of note, the mean procedure time was 78.2 ± 23.6 minutes, which is less than the time where redosing of cefoxitin is advocated. There was some variation in the initial plasma cefoxitin concentrations; however, the plasma concentration of a compound administered intravenously is expected to change rapidly within the first few minutes after administration due to the distribution processes. Therefore, some of the variability can be attributed to the timing of the first sample, which varied from 5 to 9 minutes. Relying on antibiotic plasma concentrations as a metric for success is short-sighted and does not assure adequate tissue levels throughout the dosing interval. Rather, tissue concentrations are more appropriate to identify the optimal dosing for infections involving the skin and soft structures. A better understanding of antibiotic tissue disposition is essential to optimize antibiotic dosing and

choose the most effective therapies in the obese population. Ultimately, optimization of dosing should reduce SSSI occurrence; an endpoint that requires a large sample to assess.

The majority of cefoxitin pharmacokinetic studies have included normal weight or healthy subjects with little data in the obese population.[93,103,104] Typical cefoxitin plasma concentrations depending on renal function range from 14.3 mcg/mL to 86.0 mcg/mL (after a single dose of 30 mg/kg) at 2 hours post-dose.[103] Peak cefoxitin concentrations after intravenous administration in healthy adults have been reported as 244 mcg/mL.[104] Our findings affirm those reported by Toma and colleagues who evaluated the tissue penetration and pharmacokinetic parameters of cefoxitin in obese patients undergoing abdominal and pelvic surgery.[93] Despite increased prophylactic doses of cefoxitin from 1 to 2 grams before surgery, obese patients enrolled in their study had inadequate cefoxitin tissue concentrations. Adipose tissue concentrations were 7.8 mcg/g and 2.7 mcg/g at incision and closure, respectively. Of note, the time the tissue sample at closure was excised was at a mean of 2.4 hours post dose. By contrast, our tissue sample at closure was excised at approximately a mean of 78 minutes post dose. Thus, an even a shorter surgery length was insufficient in maintaining tissue cefoxitin concentrations above the MIC at the time of closure.

Several pharmacokinetic parameters may be altered in the obese population due to altered volume of distribution, total body clearance, and plasma protein binding (alpha-1 acid glycoprotein).[105] In obesity, variation in blood flow within adipose tissue, cytochrome

P-450 enzyme activity, and GFR may all contribute to altered pharmacokinetics and inadequate drug concentrations in the target tissue.[106] Isla and colleagues performed a prospective study involving 56 patients undergoing colorectal surgery who received 2 grams of prophylactic cefoxitin to develop a population pharmacokinetic model.[107] They determined that to maintain adequate cefoxitin plasma concentrations, redosing would depend on renal function CrCl (redose at 1.5 hours if CrCl 60 – 80mL/min and at 1 hour if CrCl > 100 mL/min). This finding is intriguing, as obese patients often have augmented GFR.

Collectively, the literature on antibiotic dosing in obese surgical patients is scarce. The majority of available data supporting the use of higher antibiotic doses have evaluated cefazolin. In a small two-phase study evaluating 1 gram of prophylactic cefazolin administered before gastroplasty in morbidly obese adults, plasma and tissue cefazolin concentrations were lower in the obese patients compared to normal-weight patients.[108] Furthermore, cefazolin concentrations did not surpass the MIC required to prevent morbidly obese patients. In the second phase of the study, the dosage was increased to 2 grams of prophylactic cefazolin. The dose increase resulted in adequate plasma and tissue concentrations and a significant reduction in SSSIs (5.6% versus 16.5%; p=0.03) compared to patients in the first phase. Edmiston and colleagues evaluated perioperative cefazolin (2 grams) prophylaxis in 38 patients undergoing a Roux-en-Y gastric bypass procedure. Patients were stratified into three groups depending on BMI, and both serum and tissue cefazolin concentrations were evaluated.[109] There was an inverse relationship between BMI and serum cefazolin concentration. Tissue concentrations were below the target level (8 mcg/mL) at all time points measured except for the lowest BMI group at the

time of closure. This finding is expected as cefazolin tissue distribution is lower in morbidly obese patients.[110] Likewise, our results, similar to those reported by Toma and colleagues, suggest that tissue distribution of cefoxitin are lower in obese patients.[93]

Our study had some limitations, including small sample, measurement of total cefoxitin concentration rather than free cefoxitin, and measurement of tissue concentrations at only two time points. If free cefoxitin concentrations were measured, they would be expected to be lower than total cefoxitin concentrations due to the binding of cefoxitin to plasma and cellular proteins and therefore strengthen our argument of the inadequacy of current cefoxitin dosing practices in obese patients. Regarding the limited time points of tissue sampling, this strategy was chosen due to the feasibility within the surgical procedure framework and intended to provide minimal interference in clinical care. As a result, we cannot determine the exact time point when the tissue concentrations became subtherapeutic. Despite these limitations, this study intended to provide preliminary data to support larger-scale clinical studies. Inadequate antibiotic concentrations may place patients at risk for treatment failure and allow bacteria to develop resistance. There is an urgent need to identify appropriate antibiotic dosing strategies in obese patients.

Considering the wide therapeutic range of cefoxitin, it is reasonable to consider cefoxitin 3 grams for surgical prophylaxis in obese patients, especially those with a BMI of greater than 40 kg/m^2. Three-gram doses of cefoxitin have been used for the treatment of gas gangrene infections. However, additional data are warranted to confirm whether

this dosing would achieve adequate tissue concentrations. Furthermore, the impact and importance of duration of therapeutic concentration in tissue on rates of SSSI are still to be determined. Given the low postoperative infection rates in laparoscopic procedures, surrogate markers such as tissue concentrations at incision and closure are an important initial step to understand appropriate doses in obese patients better. These data also provide important insight into the treatment of infections in obese patients.

Conclusion

Currently, recommended doses of cefoxitin used for surgical prophylaxis in obese patients are inadequate to achieve therapeutic tissue levels through the duration of the surgical case and may predispose patients to surgical site infections.

CHAPTER 3. Anticoagulant activity of enoxaparin and unfractionated heparin for venous thromboembolism prophylaxis in obese patients undergoing sleeve gastrectomy*

Introduction

While bariatric surgical procedures have shown improvements in safety, the mitigation of venous thromboembolism (VTE) risk, and iatrogenic events associated with anticoagulant chemoprophylaxis requires further investigation. Obesity is a significant risk factor for VTE development, and the proposed mechanisms include increased inflammation,[111] increased thrombin generation,[112], and greater platelet reactivity compared to individuals without obesity.[113,114] Chemoprophylaxis has been shown to reduce VTE risk but also increases bleeding risk, complicating surgery, and increases transfusion requirements.[115] Identification of the optimal dosing strategy is essential to balance risks and benefits. A challenge in patients with obesity is the altered body composition and the effects of this alteration in drug biodisposition. Some have advocated higher doses of enoxaparin and unfractionated heparin (UFH) to account for an increase in the adipose compartment; however, data to support these recommendations are limited.

Currently, both unfractionated heparin and low molecular weight heparins (LMWH) are recommended for VTE chemoprophylaxis in conjunction with mechanical prophylaxis in bariatric surgery despite a lack of class I clinical guideline-based evidence.[116] Many studies have measured anti-Xa activity as a surrogate biomarker of adequate LMWH or UFH exposure for prophylaxis;[117-123] however, this strategy may not adequately correlate with patient outcomes.[124] The American Society for Metabolic and Bariatric Surgery

(ASMBS) does not endorse any specific anticoagulant or specific dosage for VTE chemoprophylaxis due to lack of evidence.[125] In general, higher doses are recommended based on expert opinion and observational studies.[121,126]

While anti-Xa monitoring in patients with obesity receiving LMWH or UFH for VTE prophylaxis has been proposed, several targets have been suggested despite a lack of correlation to clinical outcomes.[124] A previous study suggested anti-Xa activity < 0.1 IU/mL as a sub-therapeutic for enoxaparin in surgical intensive care patients.[127] Alternative monitoring methods, such as measurement of area under the TGA (thrombin generation assay; **Figure 3.1**)[128] curve (endogenous thrombin potential [ETP]) may be superior to anti-Xa-based monitoring to enable clinicians to determine adequate

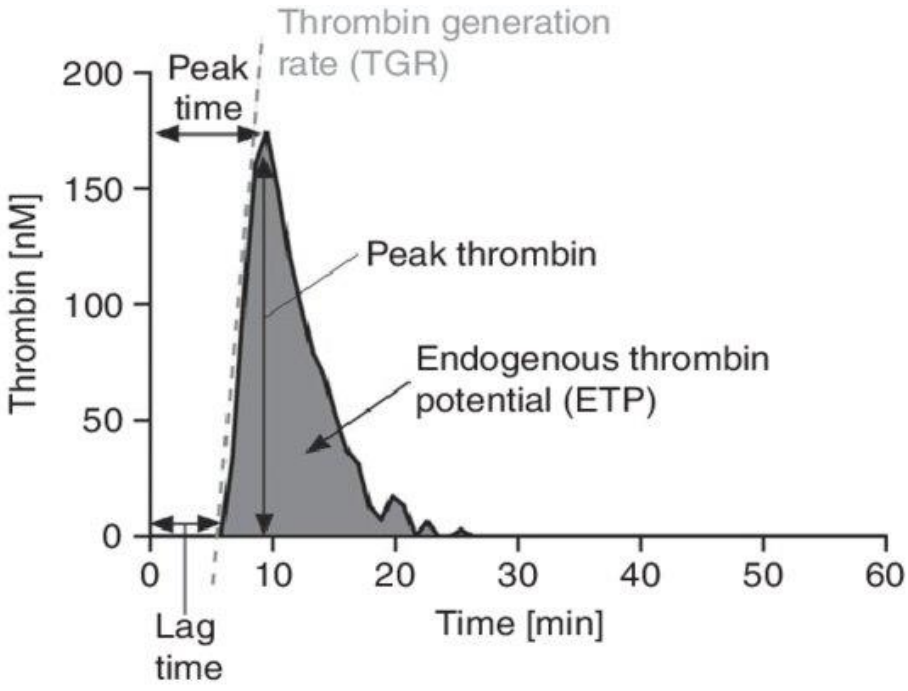

Figure 3.1. Interpretation of the thrombin generation curve.[127]

response to therapy.[129,130] Furthermore, previous studies in special populations (i.e., end-stage renal disease) have provided evidence of discordance between the level of anticoagulation when comparing TGA and anti-Xa measurements.[131] Thrombin is essential for maintaining the platelet plug formed by the primary hemostasis process,[132] and an important consideration in surgical patients. The establishment of the platelet plug is vital to reduce bleeding risk post-operatively. Previous studies also highlight the superiority of TGA potential as a marker of overall anticoagulation compared to anti-Xa.[133,134]

The purpose of this study was to 1) evaluate the attainment of anti-Xa goal levels in sleeve gastrectomy patients managed with the institution standard VTE chemoprophylaxis, 2) analyze discordance between anti-Xa and TGA in terms of adequacy of anticoagulation, and 3) evaluate the correlation of various measures of body composition with coagulation parameters.

Materials and methods

Study Design

Our prospective, non-interventional study enrolled consecutive sleeve gastrectomy patients receiving the institution standard VTE chemoprophylaxis (participation in the study did not alter drug selection or dosage). Patients receiving either enoxaparin or UFH were included. The standard institutional practice is to administer the first anticoagulant dose 2 to 3 hours before surgery. The subcutaneous injection is administered in the side or back of the upper arm to avoid administration close to the site of surgery. Enoxaparin 40 mg (Lovenox®, Sanofi-Aventis, Bridgewater, NJ) was

administered subcutaneously every 12 hours. UFH (heparin sodium, Hospira, Inc., Lake Forest, IL) was administered subcutaneously every 8 hours, depending on body weight. Patients weighing more than or equal to 120 kg received 7500 units, while others

received 5000 units. All patients were placed on pneumatic compression devices as part of the institution standard for VTE prophylaxis. The Rutgers Biomedical and Health Sciences Institutional Review Board approved the study (Protocol number Pro20160000203).

Patient population

A convenience sample of all consecutive patients scheduled for a sleeve gastrectomy was screened and approached for study participation. To minimize bias related to surgical technique, we only included surgeries performed by one bariatric surgeon. Approximately 80 percent of the stomach was removed during this laparoscopic procedure. The enrollment goal of 60 patients was achieved. Consenting patients were included and evaluated for up to 30 days after hospital discharge. After discharge, evaluation occurred with the patient's routine followed-up at the bariatric clinic after one week and repeated at 30 days. Patients with liver impairment (elevations in aspartate aminotransferase [AST, normal range, 10 – 55 units/L] or alanine aminotransferase [ALT, normal range 10 – 50 units/L] greater than three times the upper limit of normal), mild to moderate renal disease (creatinine clearance < 50 mL/min), or those with a documented UFH allergy (i.e., heparin-induced thrombocytopenia, HIT) were excluded.

Sample Collection

Blood samples were drawn from a dedicated antecubital saline lock device. Before each sample, a 3 mL aliquot was drawn and discarded. Samples were collected in Vacutainer tubes containing 0.106 M sodium citrate (9:1 v/v). Platelet poor plasma (PPP) was prepared by centrifugation at 3000 x g for 20 minutes. Samples were aliquoted into cryovials within 1 hour of collection and stored at -80°C until analysis. Three samples were collected from each patient. Sample 1 (baseline sample) was drawn before the administration of any anticoagulants. For patients receiving enoxaparin 40 mg every 12 hours, sample 2 was drawn three hours after the first dose, and sample 3 was drawn 3 hours following the second dose. For the patients receiving UFH, sample 2 was drawn 3 hours after the first dose, and sample 3 was drawn 3 hours after the third dose.

Patient variables

Bioelectrical impedance analysis (BIA) was performed for each patient using the Tanita MC-780 (Tanita Corporation, Arlington Heights, IL) body composition analyzer. No specific protocol was in place except for standard food restrictions before surgery (nothing by mouth for 12 hours before surgery except water). A complete body profile, including total body weight, body fat percentage, body fat mass, body mass index (BMI), estimated muscle mass, total body water, and basal metabolic rate (BMR), was collected. BMR was assessed with a multiple regressive analysis using fat-free mass by the device as specified by the manufacturer. A strong relationship has been reported with the estimated BMR value using fat-free mass compared to actual breath analysis resting energy expenditure (REE; $r=0.9$; $p<0.0001$).[135,136] Factors such as age, height, race, gender, comorbidities, and relevant laboratory values were extracted from the patient's medical record (**Table 3.1**).

Table 3.1. Demographic, clinical and dosing information for patients receiving venous thromboembolism prophylaxis with enoxaparin or UFH

Characteristic	**All (n=60)**	**Enoxaparin (n=16)**	**UFH (n=44)**	**P-Value***
Mean age, (years)	42.9 ± 11.6	44.2 ± 7.9	42.4 ± 12.7	0.603
Female, n (%)	32 (53.3)	10 (62.5)	22 (50)	0.391
Race, n (%)				
Caucasian	44 (73.3)	12 (75)	32 (72.7)	1.000
African-American	8 (13.3)	1 (8.3)	7 (15.9)	0.669
Other	7 (11.7)	2 (12.5)	5 (11.4)	1.000
sCr, (mg/dL)	0.78 ± 0.21	0.71 ± 0.12	0.80 ± 0.23	0.070
CrCl (mL/min)†	99.8 ± 32.8	85.1 ± 23.0	105.1 ± 34.4	*0.035*
LDL-C, (mg/dL)	104.9 ± 28.1	112.7 ± 26.8	101.7 ± 28.1	0.196
HDL-C, (mg/dL)	48.55 ± 10.6	49.6 ± 7.4	48.4 ± 11.6	0.569
TG, (mg/dL)	100.7 ± 40.6	88.4 ± 20.0	104.7 ± 44.8	0.162
Total body weight, (kg)	136.3 ± 23.4	124.3 ± 25.5	140.6 ± 21.2	*0.016*
BMI, (kg/m^2)	44.7 ± 6.8	41.8 ± 5.9	45.8 ± 6.9	*0.049*
Body fat percentage (%)	43.8 ± 7.3	42.4 ± 6.1	44.3 ± 7.7	0.359
Basal metabolic rate (Kcal)	2430.8 ± 481.9	2237 ± 511.0	2501.1 ± 456.7	0.060
Comorbidities, n (%)				
Diabetes mellitus	20 (33.3)	5 (31.3)	15 (34.1)	1.000
Dyslipidemia	33 (55.0)	2 (12.5)	31 (70.5)	*<0.0001*
Hypertension	33 (55.0)	11 (68.8)	22 (50.0)	0.248
Non-alcoholic fatty liver	6 (10.0)	1 (6.3)	5 (11.4)	1.000
Obstructive sleep apnea	19 (31.7)	5 (31.3)	14 (23.3)	1.000
Anticoagulant dose				
Enoxaparin 40 mg	16	16	-	-
UFH 5000 units	8	-	8	
UFH 7500 units	37	-	37	

Data presented as mean ± SD or number of subjects (%)**.** Abbreviations: BMI, body mass index; CrCl, creatinine clearance; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; sCr, serum creatinine; SD, standard deviation; TG, triglycerides; UFH, unfractionated heparin

*enoxaparin versus UFH**;** †Estimated using the Cockcroft-Gault equation using an adjusted body weight [ideal body weight + (1.4 x (Actual body weight – Ideal body weight)]

Goal anti-Xa was defined as 0.1 to 0.5 IU/mL for both enoxaparin and UFH. There have been various ranges suggested in the literature;[126,137,138] however, this range was selected for simplicity and encompass ranges reported in the literature. Goal reduction in ETP from baseline was defined as a reduction of > 20%.[139] For context, approximately a 30% reduction in ETP has been reported in patients receiving therapeutic doses of warfarin after six weeks of therapy.[139] A non-inferiority margin of 20% has been used to establish similarities with rivaroxaban and warfarin based on ETP.[140] Since we were interested in prophylaxis and not treatment doses, we selected a 20% reduction as our target decrease in ETP versus baseline.

The VTE incidence within 30 days of hospital discharge was extracted from the medical record, and subjects were queried at each postoperative follow-up visit. Clinicians assessed both major and minor bleeding during and after the surgical procedure. Bleeding was assessed during the procedure by direct visualization of the surgical site. Major bleeding was defined based on the International Society on Thrombosis and Haemostasis (ISTH) criteria (fatal bleeding, symptomatic bleeding in a critical area or organ (intracranial, intraspinal, intraocular, retroperitoneal, intraarticular, pericardial, or intramuscular), or bleeding causing a fall in the hemoglobin of 2 g/dL or more requiring transfusion, surgical bleeding requiring a second intervention, or unexpected and prolonged surgical bleeding associated with a drop in hemoglobin of greater than 2 g/dL for evaluating anti-hemostatic agents in surgical patients.[141] Minor bleeding included all other bleeding, including bleeding at the site of surgery during the procedure requiring manipulation.

Determination of coagulation parameters

All coagulation parameters were measured on the STA Compact Max automated benchtop coagulation analyzer using dedicated reagents, calibrators, and controls as applicable (Diagnostica Stago Inc., Parsippany, NJ). Assay precision and accuracy were monitored by analyzing assayed quality control vials with each new day or change in lot number for any of the assays or reagents. The assay accuracy and precision parameters were within the predefined acceptance limits (10%) for the instrument as provided by the manufacturer. Activated partial thromboplastin time (aPTT) was measured using STA PTT A 5 (normal range 26.0 – 40.0 seconds), and thrombin time was determined using the STA Thrombin reagent (normal range 14.0 – 21.0 seconds). STA Liquid Anti-Xa was used to measure chromogenic anti-Xa activity. The manufacturer-defined working ranges for enoxaparin and UFH were 0.1 to 2.0 and 0.1 to 1.1 IU/mL, respectively. A hybrid calibration curve for both enoxaparin and UFH was generated and displayed a strong correlation versus either UFH or LMWH calibrated curves (r^2= 0.997 to 0.999).

Fluorescence-based ETP determination

TGA determinations were made using standardized methods on a dedicated Thrombinoscope platform (also known as the Calibrated Automated Thrombogram, CAT) as recommended by the manufacturer (Diagnostica Stago Inc., Parsippany, NJ). Briefly, frozen PPP was thawed in a 37 °C water bath for exactly 10 minutes, followed by pipetting 80 µL of each sample plasma into each of six wells of a 96 well Immulon 2HB plate. To three of the six wells, 20 µL of PPP Reagent HIGH was added,[142] along with 20 µL of

Thrombin Calibrator to the other three wells of each six well groupings. The plates were then inserted into a Fluoroskan Ascent instrument equipped with one liquid dispenser unit set to a nominal temperature of 37 °C for 10 minutes, followed by dispensing of 20 µL of FluCa reagent into each well. Fluorescence measurements were collected for 60 minutes, followed by determination of ETP, peak height, time to max peak height, lag time, and velocity index by the Thrombinoscope software. The method used was consistent with previously published methods.[143,144]

Data Analysis

All data were analyzed using descriptive and inferential statistics. The Shapiro-Wilk test, combined with a visual inspection of histograms, was used to assess the normality of paired data. The Wilcoxon test for paired samples was used for nonparametric data, and the paired t-test was used for normally distributed data. Coagulation parameters (anti-Xa, TT, PT, aPTT, and components of the TGA) were correlated with various patient characteristics (body composition) using Spearman's Rho method. A secondary analysis evaluating concordance in attaining adequate anticoagulation between the anti-Xa and the ETP assay was performed. Clinical endpoints were collected in all patients, including minor and major bleeding and symptomatic VTE within 30 days of hospital discharge. All statistical analyses were performed with SPSS for Windows, release 24.0 (IBM SPSS Statistics, Armonk, NY), and GraphPad Prism 7.0 (GraphPad Software, San Diego, CA).

Results

Sixty patients were enrolled, with 16 receiving enoxaparin and 44 receiving UFH (**Figure 3.2**). Baseline demographics were similar between groups (**Table 3.1**), except for BMI and weight, which were significantly higher in the UFH group. When using the anti-Xa target for prophylaxis, those in the enoxaparin group achieved the goal significantly more frequently than the UFH group (93.8 % versus 4.5%, respectively; $p<0.0001$; **Figure 3.3**). Similarly, the target ETP reduction from baseline was more frequently obtained in the enoxaparin group versus UFH (50% versus 27.7%, respectively; $p=0.12$; **Figure 3.3**). ETP and anti-Xa were concordant in 68.3% of cases based on the goal definitions used.

Compared to sample 1, there was a significant reduction in ETP, anti-Xa, and TT in the enoxaparin group at sample 3 compared to baseline ($p<0.05$, $p<0.001$, $p<0.001$; respectively; **Table 3.2 and Figure 3.4**). Similarly, a significant change at sample 3 in ETP, TT, and aPTT was demonstrated in the UFH group compared to baseline ($p<0.001$, $p<0.001$, $p<0.001$, respectively; **Table 3.2 and Figure 3.4**).

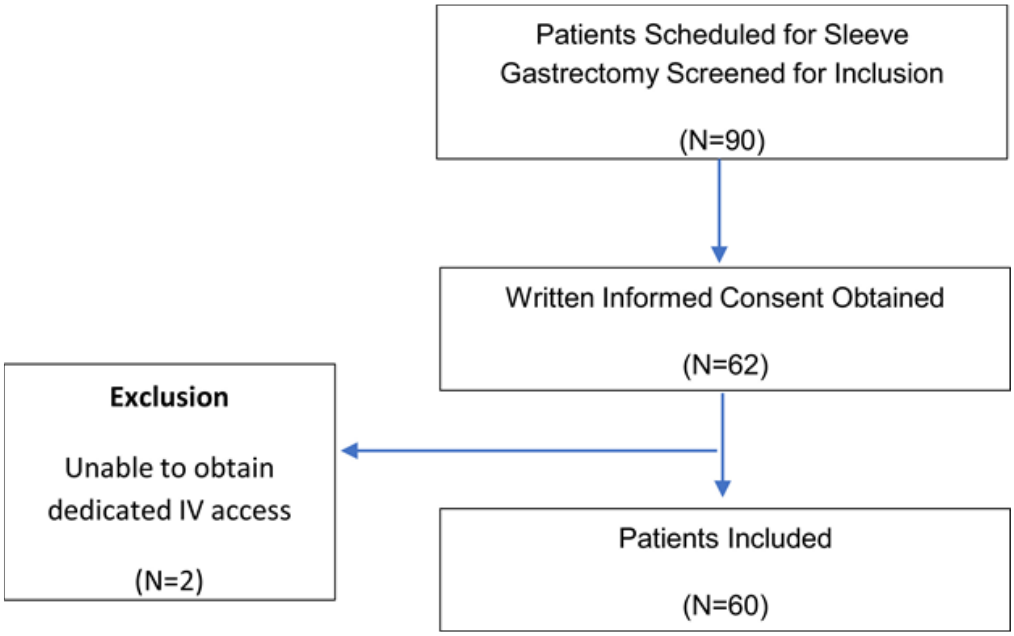

Figure 3.2. Flow chart for patient enrollment in the enoxaparin versus unfractionated heparin study.

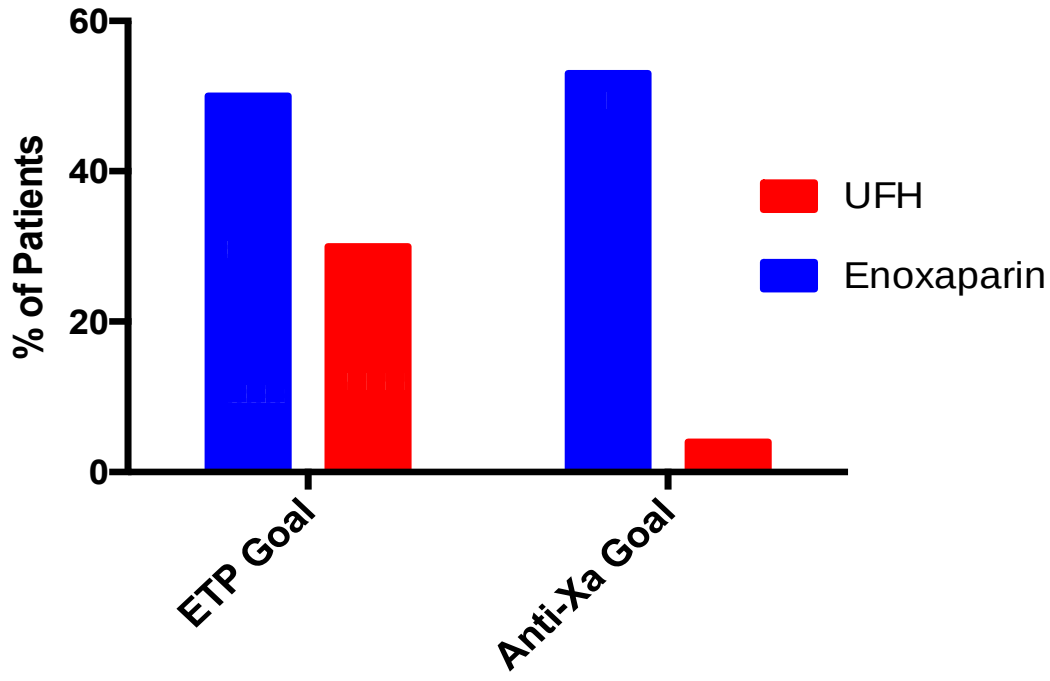

Figure 3.3. Comparison of anti-Xa and ETP goal attainment between enoxaparin and UFH.

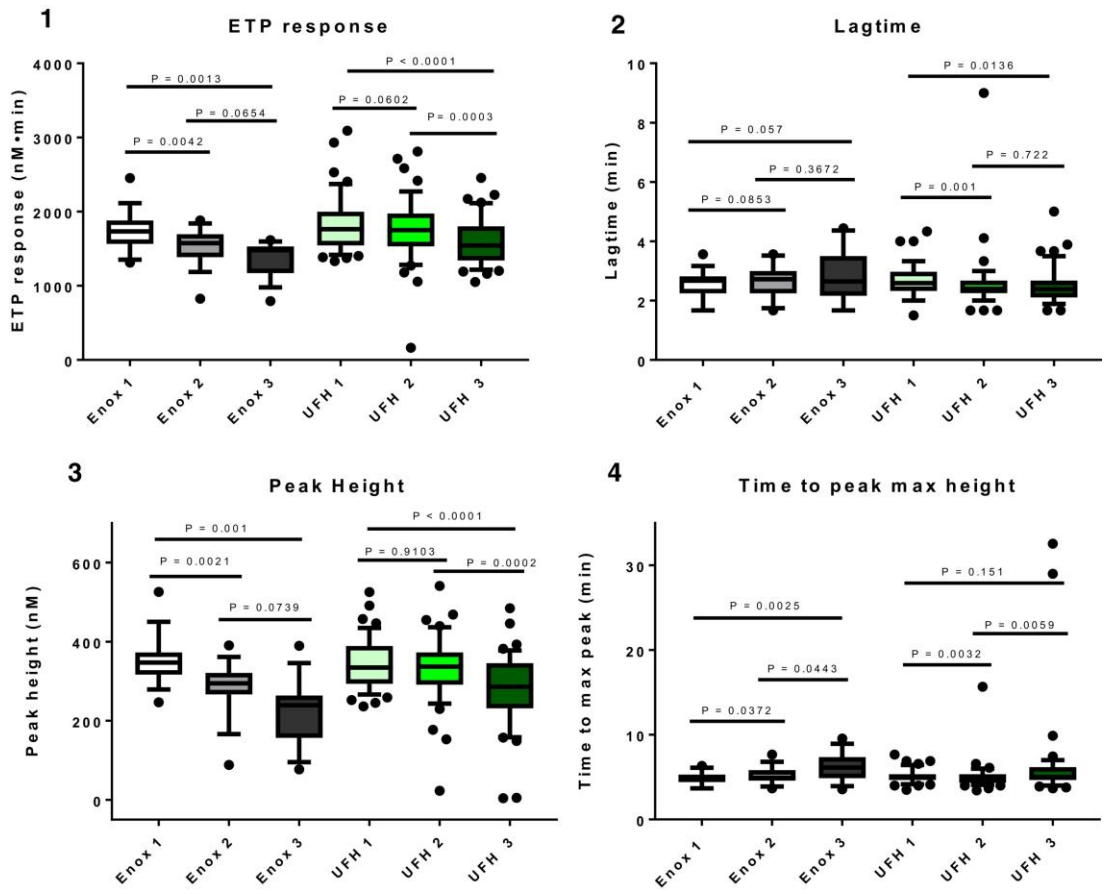

Figure 3.4. Thrombin generation assay parameter measurements at specified time points in the enoxaparin (enox) and unfractionated heparin (UFH) groups.

The values represent the median [whiskers, 25th – 75th percentile] for each respective plot. ETP=endogenous thrombin potential. Dots represent outliers in the dataset.

Table 3.2. Coagulation parameters at baseline and after administration of enoxaparin or UFH

	Enoxaparin (n=16)			**UFH (n=44)**		
	Sample 1 (baseline)	*Sample 2*	*Sample 3*	*Sample 1 (baseline)*	*Sample 2*	*Sample 3*
anti-Xa (IU/mL)	<0.01	0.18 [0.12 – 0.23]‡	0.20 [0.13 – 0.23]‡	<0.01	<0.01	<0.01 [0.0 – 0.02]
aPTT (sec)	31.7 [29.9 – 36.1]	34.1 [31.4 – 37.7]	33.2 [31.2 – 34.8]	33.1 [30.6 – 37.0]	31.0 [28.1 – 36.2]†	31.0 [28.1 – 34.6]‡
TT (sec)	16.0 [15.7 – 16.7]	16.5 [15.3 – 17.7]	19.7 [18.6 – 19.8]‡	16.5 [16.0 – 17.0]	17.8 [16.9 – 18.7]‡	18.5 [17.1 – 19.8]‡
Thrombin generation assay (TGA)						
ETP (nM·min)	1732.7 [1564.0 – 1881.4]	1574.0 [1387.6 – 1694.5]*	1480.3 [1170.7 – 1529.3]*	1762.4 [1545.9 – 1999.6]	1750.5 [1531.5 – 1974.7	1543.0 [1342.7 – 1799.8]‡
Lag time (sec)	2.67 [2.25 – 2.82]	2.73 [2.25 – 3.00]	2.7 [2.2 – 3.5]	2.59 [2.33 – 2.97]	2.33 [2.33 – 2.67]†	2.39 [2.11 – 2.67]*
Peak height (nM)	347.2 [316.8 – 372.6]	294.8 [267.1 – 321.1]†	239.1 [156.9 – 263.9]†	334.5 [293.9 – 389.0]	337 [291.4 – 372.8]	286.3 [231.5 – 435.4]‡
Time to peak (sec)	4.75 [4.42 – 5.3]	4.92 [4.59 – 5.82]*	6.14 [4.88 – 7.36]†	5.04 [4.67 – 5.33]	4.75 [4.33 – 5.33]†	5.00 [4.67 – 6.17]

Abbreviations: aPTT, activated partial thromboplastin time; ETP, endogenous thrombin potential; TT, thrombin time. Notes: median [25th to 75th percentile]. All comparisons versus baseline.*p<0.05; †p<0.01; ‡p<0.001

The change in ETP from baseline to sample 3 significantly correlated with several body composition parameters. In general, correlations were stronger for the enoxaparin group as compared to UFH (**Figure 3.5**). The strongest correlation was obtained for the enoxaparin group and basal metabolic rate reported by the Tanita device (R_S=0.782; p<0.0001). Estimated creatinine clearance was negatively associated with ETP change from baseline (enoxaparin Rs= - 0.656, p=0.006; UFH Rs= - 0.191, p=0.214). No significant correlations were observed with ETP change and lipid values.

None of the patients were readmitted with a VTE within 30 days of hospital discharge. Major bleeding during surgery occurred in one patient in the enoxaparin group. Minor bleeding was significantly more common in the enoxaparin group (87.5% versus 27.3%, respectively; p<0.0001).

Discussion

The current study found that minor bleeding was more common with enoxaparin compared to UFH VTE prophylaxis. Of the 16 patients who received enoxaparin, one had a major bleed. Nonetheless, no VTE occurred in the study, regardless of coagulation parameter attainment. While this study's sample size limits interpretation, these data support further investigation in a large randomized study.

In a recent review, the prevalence of VTE after bariatric surgery ranged from 0 to 6.4%.[116] In addition, average direct medical costs associated with VTE range from $12,000 to $15,000, and subsequent complications are estimated at $18,000 to $23,000.[145] Strategies to reduce the

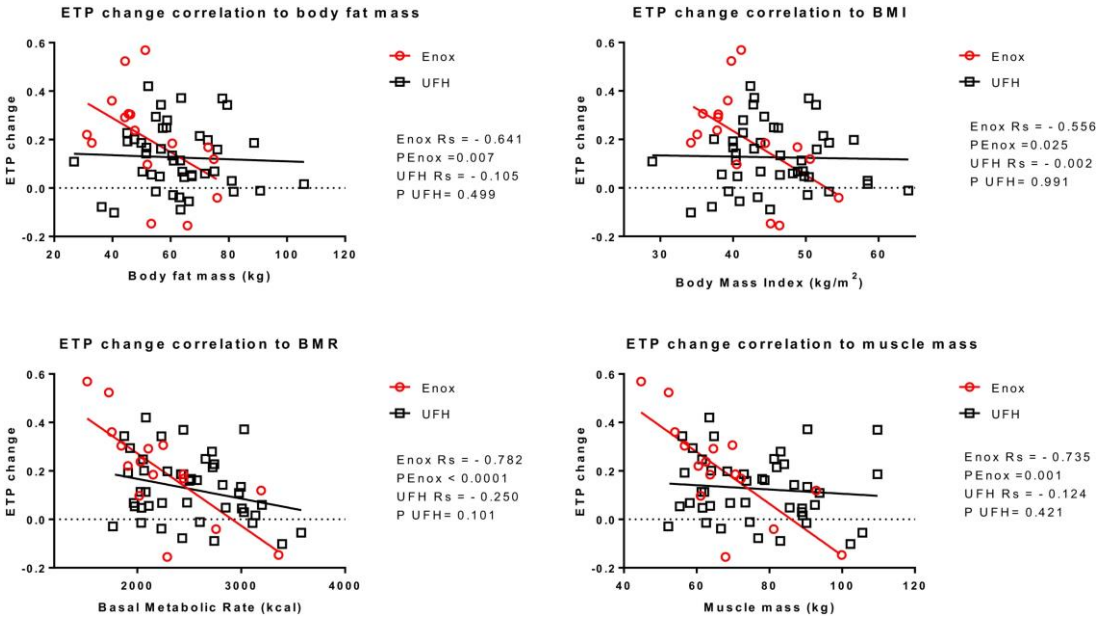

Figure 3.5. Correlation of endogenous thrombin potential (ETP) change (expressed as sample 3 ETP – sample 1 (baseline) ETP / sample 1 (baseline) ETP) to various body composition parameters.

BMI=body mass index, kg/m^2; BMR=basal metabolic rate, kcal; Body fat mass, kg; muscle mass, kg.

the occurrence of post-operative VTE is essential to improve patient outcomes; however, the optimal goal and approach for VTE anticoagulant chemoprophylaxis in bariatric surgery patients are currently undefined. While earlier studies suggested more aggressive anticoagulant dosing strategies, these studies were also performed in the setting of higher incidence rates of VTE likely related to a variety of factors, including duration of the procedure, adequacy of dosing, slower recovery time, and invasiveness of surgical procedures. The occurrence of VTE in the post-operative procedure is associated with

significant morbidity, and bleeding during the surgical procedure may influence procedure success. Identification of strategies aimed at defining the optimal drug and dosing strategy is essential to improve patient care. Further, the influence of body composition on anticoagulation adequacy has not been well defined. It is largely unknown in which coagulation assays offer better performance in situations of extreme bodyweight. The current study aimed to provide additional insight into these concerns, as mentioned earlier.

Subjects receiving prophylaxis with enoxaparin were frequently within the presumed target anti-Xa range. Conversely, those receiving UFH seldom reached the target range, and many subjects had an anti-Xa activity below the limit of quantification. Providing support for our findings, a study by Shepherd et al. suggested that the median dose of UFH required to produce adequate prophylaxis based on anti-Xa target in individuals with obesity was 8000 units subcutaneously every 12 hours (range 3000 to 19000 units).[138] Importantly, the mean BMI in their study was 28 kg/m^2 and only 25% of patients had a BMI of > 35 kg/m^2 compared to 44.7 kg/m^2 and 96.7%, respectively in our study. Together, these data suggest current doses of UFH used for VTE prophylaxis in obesity require better evidence-based definition. Despite the failure of patients to reach the target anti-Xa range, no patients receiving UFH prophylaxis developed a VTE within 30 days of hospital discharge. While many studies evaluating VTE prophylaxis in surgical patients with obesity have focused on major bleeding, minor bleeding during sleeve gastrectomy may increase surgical time and require intervention during surgery to control bleeding.

Previous studies in medical patients with obesity suggest enoxaparin and UFH doses used in the current study are adequate for preventing VTE. Wang and colleagues performed a cohort study evaluating 9241 inpatients who received VTE prophylaxis with either enoxaparin or UFH.[146] In patients weighing more than 100 kg, high dose VTE prophylaxis (enoxaparin 40 mg every 12 hours and UFH 7500 units every 8 hours) was associated with an approximate 50% reduction (0.77% (12/1559) high dose versus 1.48% (35/2369) standard dose; odds ratio 0.52, 95% Confidence Interval, 0.27-1.00; p=0.050) in VTE without increasing the risk of bleeding. Predictors of VTE included surgery, male sex, cancer, and increasing BMI. Otaib et al. evaluated the efficacy of enoxaparin 0.5 mg/kg subcutaneously once daily for VTE prophylaxis in patients with obesity (n=50; BMI > 35 kg/m^2) undergoing surgery, with 88% of patients achieving the proposed target anti-Xa concentration (0.2 – 0.6 IU/mL).[120] None of the patients enrolled experience any minor or major bleeding. The findings in Otaib et al. contrast starkly with our findings showing the occurrence of minor bleeding was common in patients receiving enoxaparin. Several surgical procedures were included in this study (8/50 were sleeve gastrectomy) and may be associated with different bleeding and VTE risks. Even within the same surgical procedure, the technique may also influence bleeding. For example, less bleeding was observed in one study using a powered versus automatic stapler in bariatric surgery.[147] The difference in results may also be related to the definition of bleeding used between studies.

Furthermore, the authors found no associated with peak anti-Xa and weight or BMI despite using a weight-based regimen of 0.5 mg/kg once daily. Although many studies suggest higher doses of enoxaparin in individuals with obesity, Lalama and colleagues reported the attainment of anti-Xa targets with lower doses in this population.[148] They

reported equal attainment of goal anti-Xa concentration with a 25% reduction in dosage than standard VTE treatment doses of enoxaparin, further adding to the ambiguity in optimal dosing for enoxaparin.

The use of anti-Xa to measure the adequacy of VTE prophylaxis is often advocated despite the lack of a definitive target range. Few studies report the measurement of TGA in the bariatric surgery setting. Evaluation of alterations in TGA in surgical patients with obesity is an important consideration as thrombin generation is necessary to maintain the hemostatic plug's integrity. In addition, studies have shown that TGA potential may be a better marker of overall anticoagulation compared to anti-Xa.[129,133] We did find approximately 32% discordance between the attainment of ETP goal versus anti-Xa goal in our study. In addition, while many patients did not achieve target anti-Xa in the UFH group, many patients did have a reduction in ETP. Further studies evaluating ETP change from baseline and clinical outcomes are necessary to validate this measure's ability to predict VTE and bleeding. We used an ETP target of > 20% reduction from baseline based on previous literature reporting >30% reductions in ETP with treatment doses of warfarin.[139] The implications of leveraging coagulation testing are significant in that they may help individualize patient dosing necessary to prevent VTE and minimize bleeding.

While transfusions were unnecessary for any of the subjects in this study, additional surgical clips and intraabdominal surgical gauze were necessary to mitigate bleeding to maintain patient safety during the surgical procedure. Intraoperative bleeding and subsequent blood transfusions have been associated with VTE risk in bariatric

surgery.[149] The association of minor to moderate bleeding not requiring transfusion, and VTE risk is unknown, yet does not mitigate the possibility of a likely positive correlation. Naturally, with an increase in the complexity of the surgery, operative time can increase significantly, thereby potentially increasing the risk of VTE caused by side effects of robust chemoprophylaxis. We did not design the study to detect changes in surgery duration; however, there is a high likelihood that the management of these minor bleeding events required additional surgical time. Global coagulation assays such as TGA may prove able to more robustly assess the adequacy of VTE prophylaxis.

There have been several attempts to correlate body composition parameters to the attainment of coagulation targets in patients receiving prophylaxis with enoxaparin, namely total body weight or BMI. While these values are easy to obtain in the clinical setting, they may not adequately describe the adipose compartment. We provide evidence of parameters obtained from BIA measurement of body composition that may correlate more closely to ETP change from baseline. BMR was strongly correlated to change in ETP from baseline in subjects who received enoxaparin, a finding previously not reported. The mechanism of the observation is unclear but may be related to altered hepatic clearance of clotting factors. Hyperthyroidism is associated with hypercoagulability as assessed by TGA (increased ETP and peak thrombin versus controls)[150] and is known to alter the hepatic clearance of clotting factors.

Limitations

Several limitations must be considered when interpreting the findings of this study. First, the number of subjects included in the current study receiving enoxaparin was small, making it difficult to perform more robust regression analyses in this subgroup. Further, BMI was significantly lower in the enoxaparin group. While one could consider that the lower attainment of coagulation parameters was related to the higher BMI in the UFH group, there was no significant difference in body fat percentage between groups. BMI is a surrogate for adiposity and may not accurately capture an individual's excess body fat level.[151] We were able to identify significant correlations with several body composition parameters and change in ETP from baseline. Next, blood samples were obtained at 3 hours post anticoagulant dose administration. Previous studies have suggested that peak blood samples be drawn between 3- and 5-hours post-dose. Still, it is unknown to what extent obesity may influence time to peak after subcutaneous administration. After the study started, the institution standard for VTE prophylaxis in sleeve gastrectomy changed from enoxaparin to UFH. As a result, the majority of patients included in the study received UFH. Despite this change, the primary was an objective measurement (coagulation parameters) and unlikely to have been influenced by this change. Finally, the goal anti-Xa and ETP change from the baseline used in this study are based on a review of the current literature, and these targets have not been formally validated. Future studies using the targets described (or variations) to guide chemoprophylaxis are essential to better define the appropriate anticoagulant course and identify a biomarker target best correlated to safety and efficacy.

Important considerations when extrapolating findings to contemporary bariatric procedures from older literature suggesting more aggressive VTE chemoprophylaxis

dosing include the duration of surgical procedure and subsequent length of stay. While earlier procedures may have been longer than 1 hour in duration and length of stay post-operatively several days, current procedures require approximately 30 to 60 minutes of surgical time, are less invasive, and patients are typically discharged within 24 hours. As a result, VTE rates reported in earlier studies may not illustrate the current standard of patient care. Length of surgical procedure and length of hospital stay influence the risk of VTE.[152]

Additional considerations when interpreting findings concerning VTE chemoprophylaxis dosing are the use of intermittent pneumatic compression therapy systems, as well as post-operative time to ambulation. Several studies have indicated the benefit of implementing IPCs as a standard for VTE mechanical prophylaxis, and as such, can be a confounding factor that reduces the incidence of VTE.[153,154] Likewise, a decrease in time to ambulation postoperatively has been shown to decrease VTE incidence.[155] Many bariatric programs implement a range of 6-to-24 hours to ambulation post-operatively, yet patients at our bariatric center are encouraged to ambulate 2 hours postoperatively, which can potentially decrease VTE incidence. All patients in our study received both IPC therapy as well as early ambulation post-operatively, both of which are known to influence the risk of VTE.

Conclusion

High-dose enoxaparin achieves target anti-Xa and ETP more frequently than high dose UFH prophylaxis at the expense of greater minor bleeding rates. Change in ETP is correlated to several body composition measures, particularly basal metabolic rate, in

patients receiving enoxaparin. Further study is needed to determine if ETP guided therapy is a more appropriate coagulation status measure and better correlated to patient outcomes. Ultimately, a randomized controlled trial is necessary to identify which prophylaxis regimen provides the greatest benefit-to-risk ratio and whether monitoring coagulation parameters provides any clinical utility.

CHAPTER 4. Evaluation of the chromogenic anti-factor IIa assay to assess dabigatran exposure in geriatric patients with atrial fibrillation in an outpatient setting[*]

Introduction

Dabigatran possesses many of the attributes of an ideal anticoagulant for stroke prevention in nonvalvular atrial fibrillation (NVAF), including predictable pharmacokinetics and lack of the requirement for routine monitoring.[156-158] While routine monitoring may be unnecessary, assessing the degree of anticoagulation may be important in populations at risk of altered pharmacokinetics.[159,160] Since the FDA approval of dabigatran etexilate in 2010, several regulatory agencies have issued warnings regarding bleeding risk, analogous to other target-specific oral anticoagulants vitamin K antagonists. The majority of hemorrhagic events linked to dabigatran have been reported in geriatric patients with renal dysfunction.[161-164] Although the landmark Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial found dabigatran etexilate 150 mg twice daily to be superior to warfarin; it has been difficult to extrapolate the results to the geriatric population or patients with severe renal impairment. A post-doc analysis of the RE-LY trial revealed that patients ≥ 75 years of age had a greater incidence of gastrointestinal bleeding (but not intracranial) compared with patients on warfarin (1.85%/year versus 1.25%/year,

[*] This chapter has been published. Brunetti L, Sanchez-Catanese B, Kagan L, Wen X, Liu M, Buckley B, Luyendyk JP, Aleksunes LM. Evaluation of the chromogenic anti-factor IIa assay to assess dabigatran exposure in geriatric patients with atrial fibrillation in an outpatient setting. Thromb J 2016;14:10. PMID: 27158246.

respectively, p<0.001).[165] Furthermore, an increased risk of bleeding was identified in elderly patients irrespective of renal function.[166] Dabigatran etexilate is underutilized in geriatric patients because of insufficient clinical experience with dosing recommendations in severe renal impairment and post-marketing reports of bleeding complications.[161-163,167-173] The mean age of RE-LY patients was 71.5 years old, and the mean creatinine clearance (CrCl) was approximately 70 mL/min.[84] Patients with a CrCl < 30 mL/min were excluded from RE-LY. Moreover, the FDA approval of dabigatran etexilate dosing regimen for patients with severe renal dysfunction was supported by pharmacokinetic modeling based on data from middle-aged patients rather than actual clinical outcome.[174-177] The European Medicines Agency (EMA) considers dabigatran etexilate is contraindicated in patients with a CrCl < 30 mL/min and patients with a CrCl < 50 mL/min should receive 110 mg twice daily.[173] Collectively, these data suggest that the ability to gauge the degree of anticoagulation in the geriatric patient population may be beneficial.

Several routine coagulation tests are used in clinical practice; however, few are useful for quantitative assessment of dabigatran.[160,178] The chromogenic anti-factor IIa assay has been successfully used for therapeutic drug monitoring of parenteral direct thrombin inhibitors and is insensitive to lupus anticoagulant or genetic coagulation deficiencies.[179,180] Very little data have been published on the use of chromogenic anti-factor IIa assay and its correlation with HPLC-MS-MS measurement of dabigatran.[181] this prospective pilot study aimed to evaluate the utility of the chromogenic anti-factor IIa assay for monitoring dabigatran therapy and the intra- and interpatient variability of trough concentrations in elderly patients with atrial fibrillation.

Materials and methods

A prospective study of nine geriatric patients was performed to assess dabigatran plasma trough concentrations using HPLC-MS/MS and the chromogenic anti-factor IIa quantification methods on two separate visits to the clinic. Male and female patients ≥ 75 years of age with NVAF currently receiving dabigatran etexilate mesylate (dabigatran prodrug) to prevent stroke were eligible for inclusion. Patients with a creatinine clearance of less than 15 mL/min were excluded since data are extremely limited. The use of dabigatran etexilate is contraindicated in this population (based on the United States product labeling).[182] Patients with hemorrhagic disorders or baseline platelet count of less than 100,000 per liter, on hemodialysis, or with moderate or severe liver impairment (Child-Pugh score of B or greater) or those on strong P-glycoprotein inhibitors and inducers (i.e., amiodarone, clarithromycin, dronedarone, ketoconazole, quinidine, rifampin, verapamil, and St. John's wort) were excluded. Dabigatran etexilate should be avoided with rifampin due to a significant reduction in area under the curve (AUC) and maximum serum concentration (C_{max}) (66% and 67%, respectively).[182] While not contraindicated with P-glycoprotein inhibitors, the use of dabigatran etexilate with these agents should be carefully monitored due to increased AUC and C_{max}. Furthermore, in the setting of moderate-to-severe renal dysfunction and a P-glycoprotein inhibitor, dabigatran etexilate dose reductions should be considered.[182] The protocol was approved by the Rutgers University Institutional Review Board (Protocol # 13-503), and all patients signed informed consent before participating in the study.

Patient Dosing

On the morning of study initiation, consenting patients were instructed to hold the morning dabigatran etexilate dosage until a blood sample was obtained at the physician's office. Once venous blood samples were drawn, the patient was instructed to take his/her dose. Patient demographics and concomitant medications were collected. The process was repeated on the patient's next scheduled visit, a minimum of 1 month apart.

Sample Collection

Venous blood samples were taken just before the morning dose. Approximately 5 mL was collected in EDTA tubes for dabigatran plasma concentration measurement by HPLC-MS/MS. Another 5 mL was collected in 3.2% tri-sodium citrate tubes (blood:citrate ratio 9:1) as recommended by the manufacturer for chromogenic assay. The samples were centrifuged at 2500 x g for 20 minutes, and the plasma was kept on ice for a max of 1 hour. Samples were kept frozen at –80°C until assessment.

Quantitation of Dabigatran

Dabigatran concentration in plasma samples was directly measured using a validated HPLC-MS/MS technique (modified from Delavenne et al.)[183] and estimated using a chromogenic anti-factor IIa assay (Hyphen Biomed, Neuville-sur-Oise, France). Plasma samples or standards (100µL) were mixed with 10µL of an internal standard ($^{13}C_6$-dabigatran 1µg/mL). Analytes were isolated from plasma using protein precipitation with 400µL methanol/0.1N HCl (90:10). After centrifugation, a 100µL aliquot of the supernatant was taken for the injection, and the injection volume was 20µL. A Thermo LTQ mass

spectrometer was interfaced with a Finnigan Surveyor Autosampler plus and Finnigan Surveyor MS Pump plus for separation and quantitation of dabigatran. The separation was completed using Betasil Phenyl/Hexyl column (3 µm, 100 x 4.6mm, Thermo Scientific) and a gradient flow of water and methanol with 0.1% formic acid. Electrospray ionization source was used to ionize the dabigatran before introduction into the mass spectrometer. Quantification was performed by addition of 472.2->324.2 and 472.2->306.1 and 472.2->289.1 m/z for dabigatran and 478.3->330.2 and 478.3->295.1 m/z for the internal standard. The calibration curves were linear over a concentration range of 4-1000 ng/mL.

Chromogenic anti-IIa assay

Dabigatran activity was quantified using a BIOPHEN DTI kit (Aniara, West Chester, OH). Plasma samples, dabigatran calibrators, or quality controls (50 µl) were mixed with 50 µl of thrombin chromogenic substrate at 37°C for 1 min in a 96-well plate. The mixture was then incubated at 37°C for 2 min after adding 50 µl of pre-heated purified human thrombin. The activity was measured spectrophotometrically at 450 nm (SpectraMax 5, Molecular Devices, Sunnyvale, CA) in the presence of 20% of acetic acid and adjusted for sample blanks, and extrapolated from a standard curve. Samples were run in duplicate. The limit of detection was 14.6 ng/mL, and the dynamic range from 0 to 500 ng/mL.

Assessment of renal function

Both serum creatinine and cystatin-C were measured in order to estimate renal function using the Cockcroft-Gault ([140 – age [years] x total body weight]/0.72 x sCr (mg/dL)) x 0.85 [if female]) and CKD-EPI ($127.7 \times \text{Cystatin C}^{-1.17} \times \text{age}^{-0.13} \times 0.91$ [if female]

x 1.06 [if African American]) equations, respectively.[184,185] Of note, Cockcroft-Gault was the method used to estimate renal function in RE-LY,[84] the landmark trial leading to the approval of dabigatran etexilate for the prevention of stroke and systemic embolism in patients with NVAF. Serum creatinine levels were measured using a kit based on the Jaffe reaction (Pointe Scientific, Canton, MI). Briefly, 190 µl of pre-heated working reagent, including five volumes of alkaline buffer and 1 volume of picric acid (40 mM), were added to 10 µl of samples, creatinine standard or blank serum. The mixture was incubated at 37°C for 1 min, and the change in optical density was measured at 510 nm over 3 min.

Cystatin C levels were quantified using a Quantikine ELISA kit according to the manufacturer's recommendations (R&D Systems, Minneapolis, MN). Samples or cystatin C standards (50 µl) were added to a 96-well plate coated with an antibody specific for human cystatin C and incubated at 2-8°C for 3 hours. After washing, cystatin C conjugate was then added to compete for binding with the antibody. Following incubation, washing, and addition of substrate solutions (stabilized hydrogen peroxide and tetramethylbenzidine), the stop solution (2 N sulfuric acid) was added, and the optical density was measured at 450 nm and 570 nm. Concentrations of cystatin C were extrapolated from the standard curve. Samples were run in duplicate. Renal function was assessed at each visit.

Data Analysis

All data were analyzed using descriptive statistics. Categorical data were reported as proportions and continuous data as the mean or median as appropriate. Pearson

correlation coefficients were calculated for the relationship between HPLC-MS/MS and chromogenic assay dabigatran trough levels and renal function estimates. Bland-Altman analysis and linear regression were performed to assess the strength of agreement and proportionality bias between HPLC-MS/MS and chromogenic anti-IIa measures of dabigatran levels. Correlation of dabigatran trough levels between visits was also evaluated. Trough levels were also compared to proposed dabigatran on target range (30 ng/mL – 130 ng/mL).[186] Analysis was performed using SAS 9.2 (SAS Institute, Cary, NC) or SPSS version 21 (IBM Corporation, Armonk, NY).

Results

Nine patients were enrolled, seven patients returned for a second visit. All patients were on dabigatran etexilate therapy for a minimum of one month before the study's initiation. Patient characteristics are summarized in **Table 4.1**. Blood was collected at 13.1 ± 2.3 hours (mean ± SD) post-dose from patients receiving dabigatran etexilate 150 mg twice daily (5/9 patients) or dabigatran etexilate 75 mg twice daily (4/9 patients). Results from the anti-IIa chromogenic assay

Table 4.1. Summary of patient characteristics	
Characteristic	**Value**
Mean Age ± SD (years)	81.3 ± 4.5
Female (%)	44.5
Mean time after last dabigatran dose ± SD (hours)	13.1 ± 2.3
Mean weight ± SD (kg)	83.0 ± 21.1
Body mass index ± SD (kg/m^2)	28.9 ± 4.7
Baseline Renal Clearance ± SD (mL/min)	
Cockcroft-Gault	68.4 ± 28.4
CKD-EPI	40.9 ± 12.3
Dabigatran dosage (n,%)*	
75 mg twice daily	3, 33.3
150 mg twice daily	6, 66.6
Comorbidities (n, %)	
Chronic obstructive pulmonary disease	3, 33.3
Diabetes Mellitus	4, 44.4
Heart Failure	3, 33.3
Malignancy	2, 22.2
Thyroid Disease	4, 44.4
Coronary Artery Disease	2, 22.2
Mean HPLC MS/MS dabigatran level ± SD (ng/mL)	161.1 ± 104.1
Mean chromogenic anti-IIa dabigatran level ± SD (ng/mL)	161.9 ± 104.8
*Dosing was consistent with product labeling	

correlated with dabigatran concentrations as assessed by HPLC-MS/MS (**Figure 4.1**; r^2=0.81, n=15). In addition, Spearman's rho yielded similar results (rho=0.91). The Bland-Altman plot shows a very high limit of the agreement defined by the mean ± 1.96*SD (**Figure 4.2**). The mean bias present was 0.86, and the limits of agreement were 93.0

and – 91.0. The Bland-Altman plot's linear regression did not suggest any significant proportionality bias (equation; Y = 0.006545*X – 0.1945; p=0.9583). High intrapatient variability in dabigatran trough plasma concentrations was observed (r=0.04, p=ns; n=7; **Figure 4.3**). All the patients enrolled in the study were not within the proposed on-therapy range[186] during at least one study visit. Seven patients had a dabigatran level exceeding 130 ng/mL, and three patients had a level of less than 30 ng/mL during at least one of the recorded visits. Baseline creatinine-based (Cockcroft-Gault) and cystatin-C based estimates (CKD-EPI) of renal function had a no-to-poor correlation with plasma dabigatran concentrations (r=0.07 and – 0.26, p=ns for both; respectively).

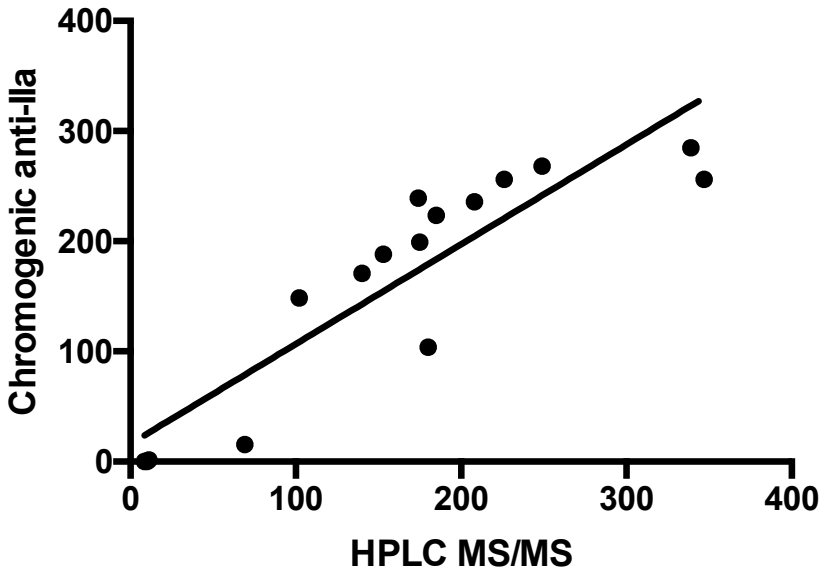

Figure 4.1. The relationship between plasma dabigatran concentrations (ng/mL) determined by chromogenic anti-IIa assay and HPLC-MS-MS.

Solid line – linear regression y = 0.9053x + 16.11, r^2 = 0.81.

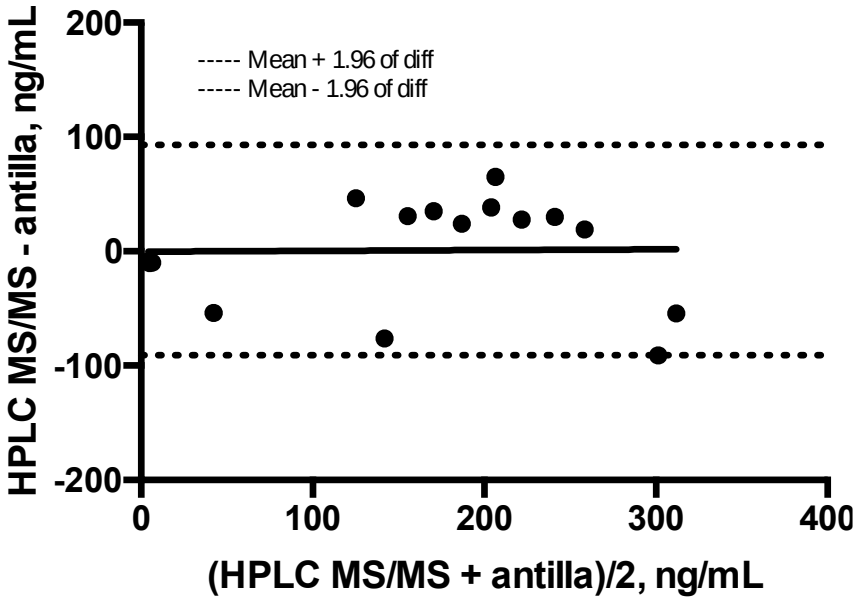

Figure 4.2. Bland-Altman plot is shown for dabigatran levels by HPLC MS/MS and chromogenic anti-factor IIa (diff, difference; n=16)

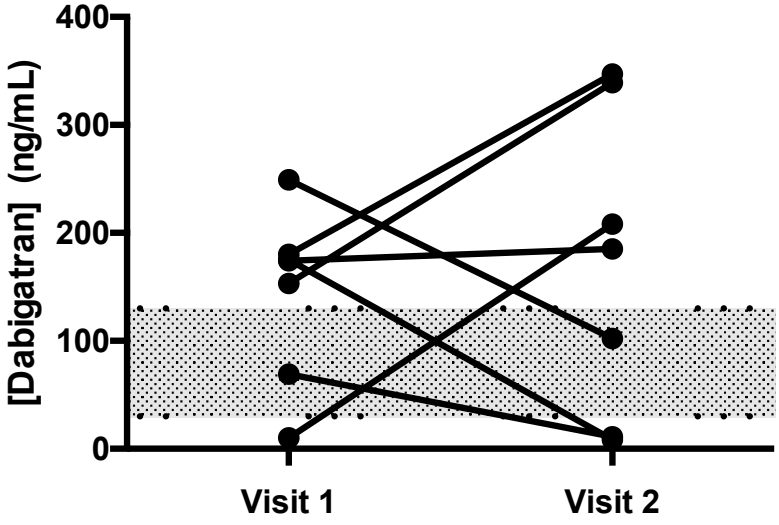

Figure 4.3. Individual dabigatran plasma trough concentration was measured on two separate occasions using HPLC MS-MS (n=7).

High inter- and intrapatient variability was observed. Conceptually, due to this variability, the clinical application of therapeutic drug monitoring is challenging. The shaded area represents the on-therapy range (30 ng/mL to 130 ng/mL).

Discussion

There is a widely held view that the target-specific oral anticoagulants, including dabigatran etexilate, have a predictable response and do not require monitoring; however, data suggest significant interpatient variability in pharmacokinetics.[187,188] In addition, the landmark RE-LY trial suggests low trough concentrations (rapid decrease in the probability of stroke from a concentration of zero through approximately 70 ng/mL)[189,190] were associated with reduced efficacy, and high concentrations were associated with an increased risk of bleeding.[159] Chan and colleagues measured the Hemoclot® assay at baseline and every two months for up to 4 visits in 100 patients (mean age 69.9 years) with atrial fibrillation.[188] They reported a large intrapatient variability in Hemoclot® levels (geometric coefficient of variation 32 – 40%). The authors concluded that a single Hemoclot® measurement is not reliable in identifying patients with consistently high or low dabigatran exposure. Some concerns have been raised regarding the large variation in trough dabigatran levels seen between visits in the Chan and colleagues study.[191] These concerns included timing of trough sample, the stability of plasma stored at -80°C, the performance of the analysis on the same run, and lack of outcome data. Arguably, the most important concern is the lack of stringent timing of trough levels. While measuring trough levels at 12 ± 1 hour is ideal, this criterion is difficult to enforce when relying on patient-reported drug administration in clinical practice. Patient-reported adherence is inherently a limitation and may result in measurement bias. Similar to the Chan and colleagues study, our pilot study found that there was large intrapatient variability in dabigatran exposure in geriatric patients as measured by chromogenic anti-IIa assay and HPLC MS/MS.

Vulnerable populations, such as the elderly and patients with renal impairment, can exhibit exaggerated responses to dabigatran.[166,192,193] Dabigatran etexilate, a prodrug, completes its bioconversion in the liver, and approximately 20% is conjugated with glucuronic acid excreted via the biliary system.[157,194] Dabigatran etexilate requires conversion via esterase hydrolysis to the active form (dabigatran).[157] Genetic factors, such as polymorphisms in carboxylesterase 1, may also be responsible for interpatient variability.[195] There may also be variability in drug exposure secondary to inhibition or induction of the efflux transporter P-glycoprotein, as dabigatran etexilate is the substrate of this transport protein.[182,196] While these factors explain the interpatient variability present; they do not account for the intrapatient variability observed between clinic visits in this study.

No therapeutic range has been established for dabigatran; however, Chin and colleagues have suggested a target plasma dabigatran trough concentration of 30 – 130 ng/mL.[186] Some limitations to using this range include derivation from pharmacokinetic simulations and lack of prospective studies confirming that the range predicts clinical outcomes. However, with the lack of definitive data, this range provides a good starting point. There are data from landmark trials confirming dabigatran levels may be predictive of thrombosis and bleeding.[159] For example, patients in the RE-LY trial with any major bleeding had a higher dabigatran trough concentration (113 ng/mL) compared to patients without a bleeding event (72.8 ng/mL).[159] Furthermore, age was the most important covariate. Collectively, these data may be used to construct a dabigatran concentration-to-assay result curve to predict drug exposure and predict the risk of bleeding.[197] In our analysis, we found that all patients were not in the on-therapy range on at least one of the two visits. Furthermore, 4 out of 9 patients had dabigatran trough levels exceeding 200

ng/mL during at least one visit, and trough levels above 200 ng/mL are associated with an increased risk of bleeding.[198] These results are concerning and suggest geriatric patients may be at an unecessary risk of treatment failure and/or bleeding.

Estimating renal function in the elderly is challenging, and many of the currently available methods are inaccurate.[199] Unlike creatinine, cystatin C levels are unaffected by age, muscle mass, gender, and race.[185] We could not appreciate any significant correlation with either creatinine or cystatin C-based estimates of renal function with dabigatran trough concentrations. Based on this finding, additional research is warranted to identify which estimate renal function leads to selecting the most appropriate dose or if age alone is sufficient to suggest a dosage reduction.[95,200] Current FDA and EMA recommendations for dosing dabigatran etexilate in renal disease advocate using the Cockcroft-Gault equation to estimate renal function, and clinicians should not deviate from this strategy.[173,182] Hellden and colleagues investigated the impact of using the Modified Diet in Renal Disease 4 (MDRD4 equation to estimate glomerular filtration rate and subsequent dose adjustment in the elderly population (defined as age > 65 years).[201] Their findings suggest that the MDRD4 would result in higher recommended doses of dabigatran etexilate to elderly patients versus Cockcroft-Gault, particularly in women. The increased dose may increase the risk of toxicity; hence these findings suggest continued use of Cockcroft-Gault to estimate renal function for dabigatran etexilate dosing.

These data support further evaluation of strategies to individualize treatment. The literature on coagulation monitoring to guide dabigatran therapy is evolving with several studies and comprehensive reviews now published.[159,160,187,197,202-207] Evidence supports that dabigatran levels are correlated to bleeding risk and efficacy.[159] Furthermore, in a

sub-analysis of the RE-LY trial, a plasma concentration at trough between 90 and 140 ng/mL provided the best benefit/risk ratio in patients with NVAF,[208] although other authors have suggested other on-target ranges.[186,209] Tailoring dabigatran etexilate dose according to patient risk (i.e., age, renal function) is essential to balance the benefit:risk of thrombosis and bleeding.[210] Adding the ability to assess the degree of anticoagulation can further improve the benefit:risk ratio of dabigatran and warrants consideration, especially in special populations such as the geriatric population.[202,211]

This study provides important information obtained from the 'real world' use of dabigatran etexilate in geriatric patients. Chromogenic anti-IIa assay correlates with HPLC MS/MS measured dabigatran concentrations and may be useful for quantitative measurement; however, the intrapatient variability of dabigatran concentrations may make clinical application challenging. The frequency of patients outside a proposed therapeutic window suggests an opportunity to improve dosing strategy to further enhance the risk versus benefit ratio of dabigatran. Glucuronidation is the major metabolic pathway of dabigatran. The major metabolite of dabigatran, 1-O-acylglucuronide, and its isomers result in the equipotent prolongation of the activated partial thromboplastin time.[194,212] Acylglucuronides accounted for 2.0% of the dose in plasma at 2 hours and 4.3% at 4 hours post-administration of intravenous dabigatran.[194] The acylglucuronide metabolites may contribute to the overall clinical effect of dabigatran. They can explain some of the difference between HPLC-MS/MS detection of dabigatran and the chromogenic measurement of anti-IIa activity if there is interpatient variability in glucuronidation. Of note, previous studies suggest that age does not significantly influence glucuronidation.[213,214]

Certain limitations of our study should be acknowledged. Although the chromogenic anti-factor II assay may be performed manually or using an automated coagulometer as indicated in the assay specifications, manual methods may be a potential source of measurement bias. The timing of trough levels was often not within 1 hour of the next scheduled dose due to patient availability, as suggested to be optimal for pharmacokinetic studies.[215] Our data reflects a practical scenario that resembles the 'real world' clinical setting. Furthermore, data support that sampling within 6 hours of the next scheduled dose will still provide a value within the 80% confidence interval for the true trough value, as was discussed by Chan and colleagues.[176] When planning to measure dabigatran levels, it is paramount to educate the patient on the importance of accurately documenting the last intake of medication. In addition, scheduling patient visits according to their usual drug administration schedule may enhance the accuracy of trough levels. Another strategy involves the collaboration of clinicians with laboratories or anticoagulation clinics. Patients can be instructed to hold their dabigatran etexilate dose until their office visit, where the administration can be directly observed. Following directly observed administration of dabigatran etexilate, the office staff can schedule an appointment for the patient to present to the laboratory or clinic for their blood to be drawn.

This study found no correlation between dabigatran trough levels taken at two different patient visits; however, the limited sample size requires future studies to confirm this finding. Ultimately, a large controlled study is necessary to confirm if a monitoring strategy will improve dosage selection and dabigatran treatment outcomes.

Conclusion

Chromogenic anti-factor IIa assay demonstrated similar performance in quantifying dabigatran plasma trough concentrations to HPLC-MS/MS. All geriatric patients were not within the on-therapy trough range during at least one visit. Routine adjustment of dosages based on a single measurement of trough concentration may not be appropriate due to significant intrapatient variation. Given the large proportion of patients falling outside the on-therapy range and the high variability observed in this pilot study, larger clinical studies can be recommended to determine the clinical utility of concentration monitoring in the outpatient setting.

CHAPTER 5. The impact of body composition on immune globulin exposure after administration of IVIG in primary immunodeficiency*

Introduction

Approximately 250,000 patients in the United States are diagnosed with primary immunodeficiency, and immune globulin G replacement is the mainstay of therapy.[216] Current dosing practices for immunoglobulin G (IgG) may be inadequate in extreme bodyweight.[217-219] Total, ideal, and adjusted body weight-based dosing strategies are suggested in the literature,[219-221] but these recommendations are based on expert opinion rather than high-quality evidence. The adoption of a specific strategy is highly variable depending on the clinician and/or institutional setting.[220] Recently, payors have also adopted strategies to reduce IgG therapy costs by capping doses. These recommendations are often based on the presumption that IgG distribution is limited to the vascular space.[222] While this assertion is logical, it does not account for changes adipose tissue may confer on target sites, nor does it account for adipose tissue's potential to function as a metabolic sink or a source of inflammatory mediators. The latter would be especially important in patients receiving subcutaneous immune globulin administration.

Moreover, IgG distribution is not only a function of the partition coefficient but may be influenced by active transport and inflammation.[223] Several observational studies have evaluated IgG dosing in obese patients and have been the source of support for dosing

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* This chapter has been published in part. Chapy H, Kagan L, Na A, Moore R, Nahass RG, Brunetti L. The influence of body composition on intravenous immune globulin half-life in patients with primary immunodeficiency. Pharmacotherapy 2017; 37(12):E205.

strategies.[217,224-226] Many of these studies were not representative of the general population, contained a wide variety of patients with different IgG indications, and had sparse serum sampling.

Total body weight for dose calculation is the current recommendation in the FDA-approved prescribing information for intravenous IgG products. Using total body weight to dose IgG in obese patients may increase the risk of thrombosis owing to increased blood viscosity, activation of platelets, or vasospasm;[224,225] and the increase in blood viscosity have been reported as IgG dose-dependent.[224] However, serum immunoglobulin concentration has been reported to be associated with hyperviscosity.[227,228] The use of ideal or adjusted body weights has been advocated to reduce the side effects and drug expenditures.[217] It is currently unknown the clinical impact of using body weight measures other than total body weight to calculate IgG doses, and the effect of obesity on IgG pharmacokinetics has not been experimentally evaluated. Although some data suggest total body weight for IgG dosing, in a survey (2015) of 92 academic institutions in the United States, approximately 60% of respondents indicated they do not use total body weight to dose IgG.[220] This choice is likely related to the cost of IgG treatment, which has a tremendous impact on the healthcare system. The cost of intravenous IgG therapy for patients with primary immunodeficiency is approximately $30,000 per patient annually,[229] which corresponds to a total of more than $3.5 billion/year for primary immunodeficiency alone (there are more than 150 unlabeled uses for IgG reported in the literature).[230] Moreover, IgG shortages are common, and using ideal or adjusted body weight for dosing may conserve an important resource.[231] To this end, doses are frequently rounded to the nearest vial size.[232]

The mechanisms by which obesity affects the pharmacokinetics of protein therapeutics, including IgG, have not been sufficiently investigated. It was recently suggested that IgG should be dosed based on the patient response in primary immunodeficiency rather than body weight.[218] However, even this approach involves a loading dose based on ideal body weight and then adjustment of 0.15 mg/kg/month when patients present with a serious infection or three or more moderate infections over one year.[218] This assertion has been challenged by some authors who suggest adjusted body weight is more appropriate for individuals with obesity.[219] Furthermore, dosing recommendations (initiation and adjustment) were not based on strong clinical data but rather on the presumption that the volume of distribution of IgG is limited to the intravascular space. This proof-of-concept study's objective was to identify the association between body composition and intravenous IgG pharmacokinetic parameters, namely half-life.

Materials and methods

Study population

Male and female patients aged 18 to 75 years receiving the institutional standard intravenous IgG dosing (400- 600 mg/kg) were eligible for study enrollment. All patients received intravenous IgG for at least six months and were considered to be at steady state. Serum IgG concentration at steady state after intravenous administration is typically achieved after the fourth to sixth infusion. Demographic and clinical data were obtained at the time of enrollment.[233] Patients with liver impairment (elevations in liver enzymes of greater than three times the upper limit of normal) or reduced renal function (CrCl < 50 mL/min) were excluded. Patients with a pacemaker or an automatic implantable

cardioverter-defibrillator were not eligible to participate. These exclusions were necessary because the bioelectrical impedance analysis (BIA) device may interfere with these medical devices.

Measurement of serum immunoglobulins

Serum immunoglobulin measurements were taken immediately before and after administering intravenous IgG on two consecutive treatments (trough 1, peak 1, trough 2, peak 2; approximately one month apart). Another measurement was taken at approximately two weeks post-infusion (after treatment 1). Briefly, 6 mL of blood was collected in a serum vacutainer, blood was allowed to clot, and the serum was separated by centrifugation. The samples were divided into aliquots and stored at -80 ºC until analysis. Serum IgG subtypes, IgA, IgE, IgM, were measured using a magnetic bead multiplex assay (Antibody Isotyping 7-Plex Human ProcartaPlex™ Panel, Invitrogen, Carlsbad, CA) on a Luminex platform (Magpix, Thermofisher, USA) according to manufacturer protocol. Total serum IgG concentration was calculated as a sum of four IgG subtypes. For comparison, we also measured total IgG using ELISA (Human IgG ELISA (ab195215), Abcam, Cambridge, MA).

Assessment of body size and composition

Total body weight and height were measured at the time of enrollment. Body mass index, body surface area, ideal, lean (Hume equation), and [22] adjusted body weight were calculated using standard equations.[234] Body composition of all subjects was measured using a multi-frequency segmental body composition analyzer (BIA, Tanita MC-780U). This method can measure body fat percentage, body fat mass, BMI, fat-free mass, estimated muscle mass, total body water, and basal metabolic rate by measuring body

resistance to the current. BIA is the most studied bedside technique for assessment of body composition as it is affordable, easy to transport and use.[13,15] Although, similar to other body composition measurements, it does not directly measure body composition, it measures the resistance of body tissues to an electric current, an indirect measure of body composition.[13,15] The level of precision produced by BIA is reported as good with a 1-to-2% variability between repeat measures.[17,18] Thompson et al. found good absolute and relative agreement between changes in body composition assessed by dual-energy X-ray absorptiometry (DXA) and BIA with small biases in the estimation fat mass and percentage body fat in overweight women.[19]

Data analysis

All data were analyzed using descriptive statistics. Counts and proportions were calculated for all categorical data and mean and standard deviation for continuous data. The mean change in total serum IgG and subtypes of IgG was calculated by subtracting the trough serum concentration from the peak concentration on each occasion for each subject and calculating the mean. Mean trough and peak total serum IgG and subtypes were obtained by calculating the mean of each of the available troughs and peak serum IgG. Pearson's correlation coefficient and Spearman's Rho were calculated to describe the correlation between various measures of body composition and IgG pharmacokinetic parameters. The half-life of total serum IgG and subtype was calculated for each subject using Phoenix Winnonlin (v8, Certara, Princeton, NJ) using peak 1, 2-week measurement, and trough 2.

Results

A total of 8 subjects with primary immunodeficiency were included in this pilot study, and the mean weight normalized intravenous IgG dose administered was 0.413 g/kg (dose/total body weight) monthly. **Table 5.1** provides a summary of the patients characteristics. The mean age was 63 years, and 6 of 8 subjects were female. The median BMI was 26.8 kg/m^2 (range 20.5 kg/m^2 – 34.4 kg/m^2). **Table 5.2** summarizes the mean serum trough, peak, and change after IgG administration in total IgG and each of the subtypes. As expected, IgG_1 was the most abundant subtype. The mean half-life was longest in the IgG_2 and IgG_4 subtypes. Various descriptors of

Table 5.1. Individual and Summary Characteristics of patients with PID enrolled in the study.										
Overall Characteristic	**Overall**	**Individual Characteristics**	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**	**Patient 8**
Median age (range)	63 (58 – 71)	**Age, years**	71	57	55	67	61	72	58	63
Percent female	75	**Sex**	Female	Female	Female	Male	Male	Female	Female	Female
Mean dose, g/kg (SD)	0.41 (0.04)	**Dose, g/kg**	0.47	0.39	0.43	0.43	0.37	0.46	0.36	0.40
Product Name	-	**Product Name**	O	GG	O	O	O	GC	O	O
Mean height, cm (SD)	161.6 (13.1)	**Mean height, cm**	144.8	165.1	163.8	175.3	182.9	148.6	152.4	160.0
Mean weight, kg (SD) **Weight range, kg**	71.9 (17.1) 43 – 93	**Weight, Kg**	43	64	58	93	81	65	83	88
Ideal body weight, kg (SD)	57.9 (9.4)	**Ideal body weight, kg**	46	60	59	68	74	49	51	57
Mean body mass index, kg/m^2 (SD)	27.5 (5.8)	**Body mass index, kg/m^2**	20.5	23.5	21.6	30.3	24.2	29.4	35.7	34.4
Mean body fat percentage (SD)	37.8 (8.7)	**Body fat percentage**	35.5	31.5	35.2	34.8	23.7	46.6	49.0	46.3
Mean fat mass, kg (SD)	27.3 (10.0)	**Fat mass, kg**	15.3	20.0	20.2	32.2	19.1	30.4	40.4	40.8
Total body water, kg (SD)	32.6 (7.8)	**Total body water, kg**	22.5	28.9	28.1	45.3	43.3	28.1	31.6	33.0
Bone mass, kg (SD)	2.25 (0.56)	**Bone mass, kg**	1.5	2.2	1.9	3.0	3.1	1.8	2.2	2.4
Basal metabolic rate, kCal (SD)	1346 (317.5)	**Basal metabolic rate, kCal**	882	1293	1138	1784	1770	1105	1331	1465

O=Octagam; GG=Gammagard; GC=Gamunex-C

Table 5.2. Key pharmacokinetic parameters for total serum IgG and subtypes.					
Parameter	**IgG_{Total}**	**IgG_1**	**IgG_2**	**IgG_3**	**IgG_4**
[C_{min}], mg/dL ± SD	575.6 ± 254.7	460.0 ± 223.0	50.6 ± 18.5	58.0 ± 26.5	8.0 ± 3.3
[C_{max}], mg/dL ± SD	1358.5 ± 295.3	1106.0 ± 261.2	97.8 ± 22.0	137.9 ± 39.5	16.9 ± 5.1
[Δ], mg/dL ± SD	783.0 ± 209.3	646.0 ± 180.8	47.2 ± 23.4	79.9 ± 23.6	8.9 ± 5.1
$T_{1/2}$ day ± SD	22.2 ± 7.4	21.2 ± 6.8	28.1 ± 8.8	24.4 ± 7.5	34.4 ± 20.8

body composition were correlated with IgG pharmacokinetic parameters, and those that were significant are summarized in **Table 5.3**. The half-life of total and all IgG subtypes measured displayed a negative correlation with body mass index and fat mass (**Figure 5.1**). Other body composition descriptors were tested; however, no significant correlations were identified. The mean change in total serum IgG concentration was significantly correlated to body mass index and fat mass. The trend in IgG and subtype response after intravenous IgG administration is illustrated in **Figure 5.2**. Serum IgA, IgE, and IgM

Table 5.3. Pearson's correlation coefficient between available IgG pharmacokinetic parameters and select descriptors of body composition.					
Body Mass Index					
Parameter	**IgG_{Total}**	**IgG_1**	**IgG_2**	**IgG_3**	**IgG_4**
$T_{1/2}$	-0.594	-0.553	-0.574	-0.772*	-0.498
[C_{max}]	0.218	0.229	0.276	-0.066	0.221
[C_{min}]	-0.362	-0.316	-0.372	-0.534	-0.287
[Δ]	0.748*	0.721*	0.554	0.489	0.409
Body fat mass					
$T_{1/2}$	-0.538	-0.501	-0.477	-0.729*	-0.423
[C_{max}]	0.288	0.299	0.337	-0.047	0.288
[C_{min}]	-0.277	-0.229	-0.275	-0.531	-0.182
[Δ]	0.743*	0.715*	0.534	0.518	0.408
***p<0.05**					

concentrations were also evaluated (**Figure 5.3)**. Very little change in IgA and IgM were observed; however, a notable change in serum IgE concentration was evident after the administration of commercially available intravenous IgG.

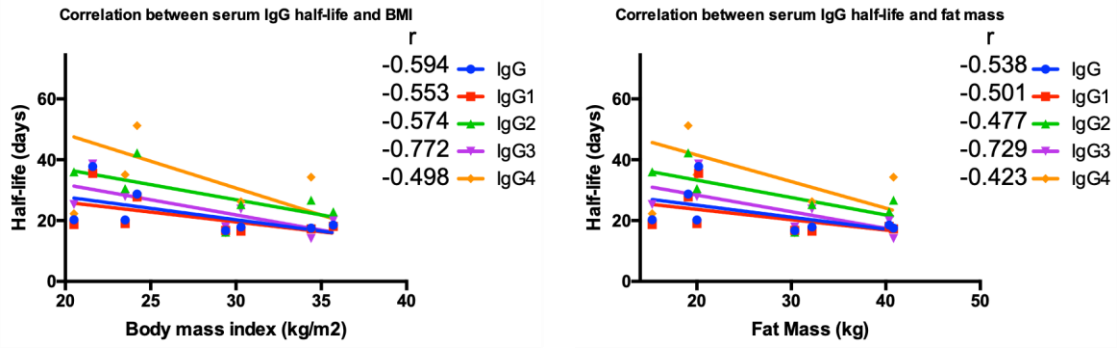

Figure 5.1. Panels A and B: Pearson's correlation coefficient evaluating serum IgG and subtype half-life versus body mass index and fat mass.

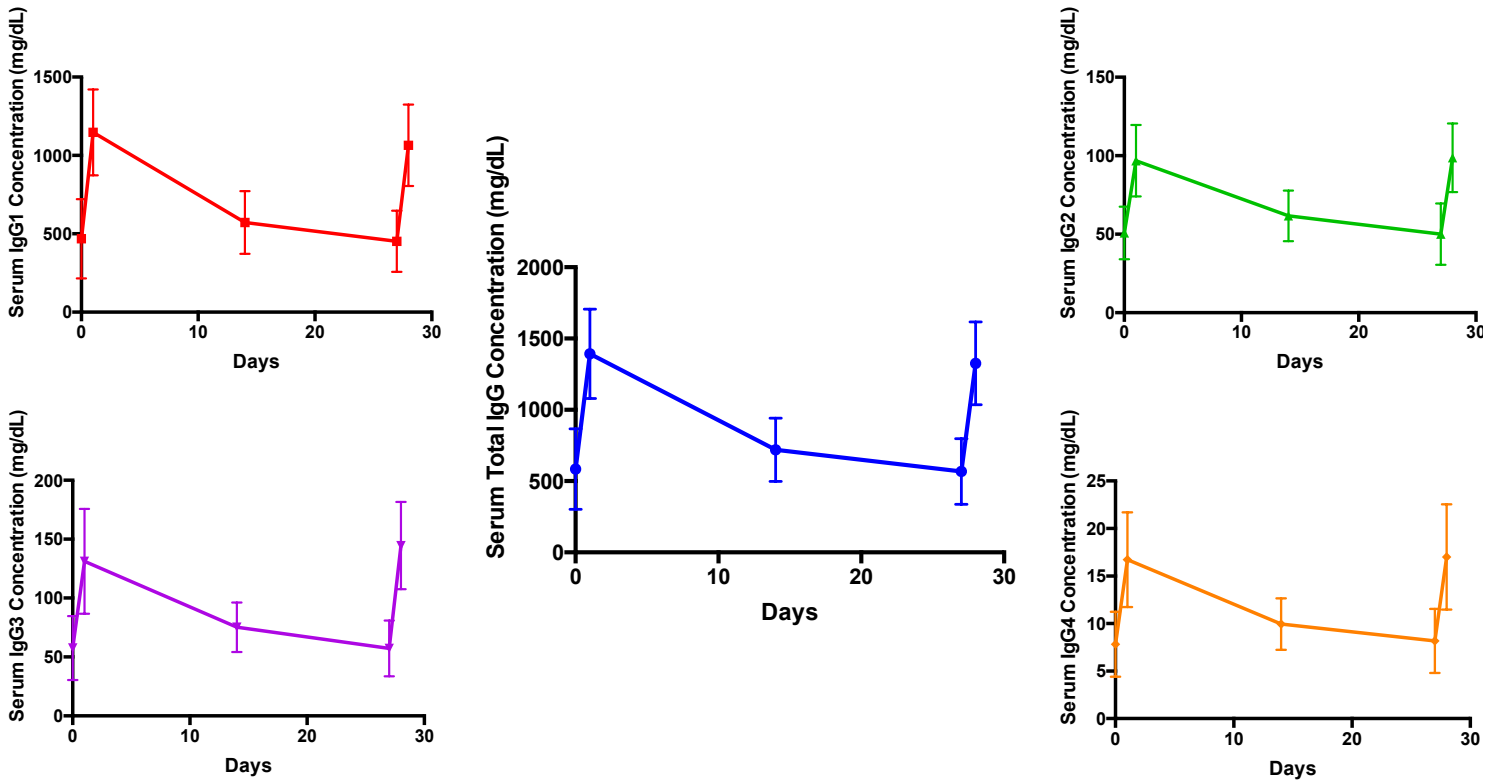

Figure 5.2. Serum total IgG and subtype profile after dosage administration on day 1 and 28. P<0.05 for change in serum concentration from baseline to immediately after adminsinistration for total and each IgG subtype (day 1 and 28).

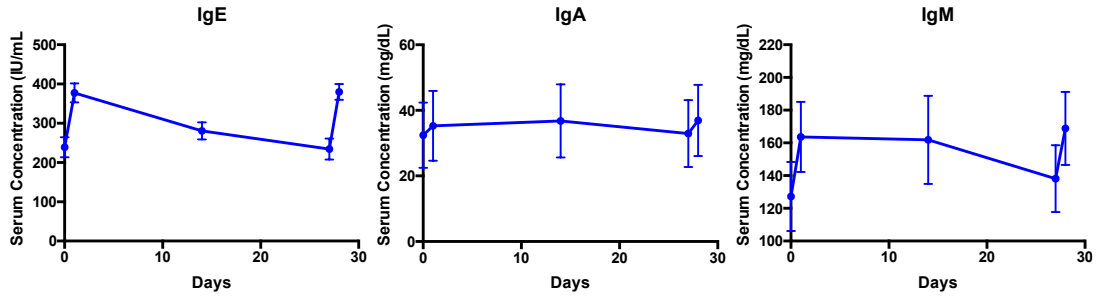

Figure 5.3. Serum IgE, IgA, and IgM concentration after intravenous IgG dosage on days 1 and 28. P<0.05 for change in serum Ig concentration before and after IVIG administration on day 1 and 28 with the exception of IgA day 1.

Discussion

IgG pharmacokinetic parameters displayed a correlation with body composition after intravenous administration in this pilot study. Specifically, the half-life for IgG decreased as BMI, and body fat mass increased. This finding suggests that the optimal dosing interval for individuals with obesity may be shorter than for non-obese individuals. In addition, the mean change in serum IgG concentration was significantly correlated with BMI and body fat mass. These findings suggest that individuals with obesity have a greater increase in serum IgG concentration following the administration of standard doses. One could postulate that this increase could result in hyperviscosity; however, additional data are needed to confirm this hypothesis. Collectively, these data suggest lower and more frequent dosing may be ideal in this patient population, a postulation that requires evaluation in further larger studies.

Intravenous IgG infusions are typically administered every 3 to 4 weeks at an initial dose of 300 to 800 mg/kg. All of the patients in this study were administered IVIG every four weeks, and the mean mg/kg dosage was within this range. None of the patients included in the study experienced treatment failure or adverse events. Current

recommendations suggest an IgG trough concentration of at least 500 mg/dL, and some clinicians prefer to target 300 mg/dL above the patient's pretreatment concentration.[235] All of the patients in this study were above the 500 mg/dL threshold. In agammaglobulinemia, a form of primary immunodeficiency, IgG trough concentrations above 800 mg/dL prevented serious bacterial infections.[236] Importantly, while IgG concentration is a surrogate for success, treatment adjustments should not be based solely on this value but rather on the patient's symptoms or response to therapy.[218]

Approximately 25% of individuals with primary immunodeficiency are obese.[237] Understanding the effects of obesity on IVIG exposure and treatment outcomes is critical. Data examining the impact of obesity on primary immunodeficiency outcomes is scarce, and very few studies have evaluated drug concentrations prospectively. One study reported an increase in sepsis (12% versus 6%; p=0.05) in patients with obesity and common variable immune deficiency versus normal-weight patients.[238] Other studies suggest no difference in IgG exposure between obese and non-obese individuals.

There are various potential concerns with IgG disposition in obesity, and some may explain the reduced half-life observed in our study. First, IgG is a polar molecule that has a small volume of distribution. As such, IgG would not accumulate in adipose but rather accumulate preferentially in the plasma.[239] We did not observe an increase in IgG peak or trough concentration in individuals with increased body fat. This finding is not surprising because IgG distribution is complex and influenced by both inflammation and active transport.[240] Notably, obesity is associated with chronic inflammation. The neonatal Fc receptor (FcRn) protects IgG from lysosomal degradation through a recycling process in which IgG is circulated into an intracellular protein reservoir and eventually back into circulation.[241] Expression of FcRn is lower in adipose tissues versus other tissues.[242] This

observation may explain the inverse association of body fat mass and IgG half-life in our study. The clearance of protein drugs may also increase with body weight. Notably, clearance may be non-linear, especially at higher body weights.[243] But, the current study population did not include patients with more severe obesity (i.e., BMI > 40 kg/m^2). Catabolism of IgG may also be increased due to a greater number of activated macrophages in excess adipose tissue. The primary elimination route for IgG is via the reticuloendothelial system.[244] As such, half-life may be reduced in obese individuals relative to lean individuals.

We also measured serum IgA, IgM, and IgE at specified time points throughout the study. There did not appear to be a significant change in IgA or IgM after IVIG administration; however, there was a substantial change in IgE. Our finding related to IgE was unexpected and warranted further investigation. None of the commercially available products list IgE content on their FDA approved product labeling. There are primary immune deficiencies associated with elevated IgE concentrations, including Job syndrome, immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX), Wiskott-Aldrich syndrome, Omenn syndrome, and atypical complete DiGeorge syndrome.[245] Elevations in IgE are possible in this population; however, the elevations we observed were immediately after the IVIG administration. Only a few reports measured IgE in commercially available IVIG products and change in serum IgE concentration after IVIG administration. [246,247] Paganelli and colleagues observed a wide range of IgE in various products and within different lot numbers of the same product (ranging from approximately 20 to 900 IU/mL per 5 to 6 grams of IVIG product). Similarly, Toro and colleagues reported varying amounts of IgE in commercially available products after observing a change in serum IgE after IVIG in children with hypogammaglobulinemia.[246]

IVIG has been postulated to decrease IgE production in vitro,[248], but this finding has not translated to human studies.[249] Further longitudinal studies are necessary to confirm whether repeated administration of IVIG results is short term elevations in IgE. While IgG represents more than 90% of the proteins present in IVIG, trace amounts of IgA, IgM, and other immune globulins may be present.[250]

Despite the pilot study's limitations relative to its small sample size, we provide evidence of reduced half-life of IgG with increasing body fat mass and body mass index. This finding warrants further study and could provide the impetus for obesity specific dosing. Moreover, intravenous IgG had been previously titrated to achieve the target steady-state IgG concentration (serum IgG concentration > 500 mg/dL) in the study population. Nonetheless, this study's finding may apply to individuals starting intravenous IgG since this population is at greater risk of dosing inadequacy and negative outcomes.

Conclusion

IgG half-life is associated with body composition. Individuals with obesity may require more frequent IgG administration and requires further study. Future studies are needed to establish outcomes achieved with more frequent dosing in obese individuals with primary immunodeficiency.

CHAPTER 6. Evaluation of Renal Function Estimation in Individuals with Components of Metabolic Disease

Introduction

Creatinine clearance (CrCl) is used to estimate the glomerular filtration rate (GFR) to assess renal function. Numerous medications require dosage adjustment in the setting of reduced GFR, and CrCl is often used to guide the dosing of medications cleared by the kidney. The most accurate and clinically feasible estimate of CrCl is the measured 24-hour urine CrCl; however, this method is not often practical and is time-consuming. The Cockcroft-Gault equation published in 1976,[95] is the most commonly used formula to calculate creatinine clearance (CrCl) using serum creatinine in the clinical setting. Owing to its simplicity, it is easily calculated and applied at the bedside to gauge renal function and guide subsequent drug dosage adjustments for medications cleared by the kidneys. This equation was derived from a primarily Caucasian male population aged 18–92 years. While simple and in clinical use for more than four decades, there are several limitations one must acknowledge with the use of this formula. First, the population in 1976 was very different from the present-day population, where over one-third of US adults are considered overweight or obese.[7] Further, in 2015, an estimated 30 million Americans were diagnosed with type 2 diabetes mellitus, and continued increases in prevalence are expected.[251,252] Both animal and human studies provide evidence that atherogenic lipid profile influences glomerular sclerosis and renal dysfunction, respectively, making dyslipidemia a relevant consideration in evaluating renal function estimates.[253,254] Both obesity and diabetes are associated with altered muscle mass,[255,256] which may influence serum creatinine, a key variable in the Cockcroft-Gault equation. Another consideration

is that the population used to derive the equation was primarily male (96%), and the extrapolation of the equation to females was based on estimates rather than objective data. Finally, the relatively small sample included in the study (n=249) did not allow for subgroup analysis to determine what populations predicted CrCl was not accurate.

The appropriate assessment of renal function is critical for drug dosing. The kidneys clear more than half of all medications, and inappropriate dose adjustment to account for potential drug accumulation may lead to drug toxicity. Most drugs cleared by the kidney require renal dose adjustment once the CrCl is below 50-to-60 mL/min. This group may be at greater risk of incorrect renal function estimation resulting in incorrect dosage adjustment and subsequent lack of desired effect or toxicity. Therefore, an adept understanding of the Cockcroft-Gault equation's accuracy, reliability, and nuances is necessary. Moreover, strategies to correctly classify the degree of renal dysfunction are likely to improve patient outcomes.

The reliability and application of the Cockcroft-Gault equation (see equation below)

$$\frac{(140-age\ in\ years)*total\ body\ weight\ in\ kg}{72*sCr\ (mg/dL)}$$ *0.85 if female

in clinical practice may be influenced by several factors. First, the impact of weight must be considered. The physiological max GFR is approximately 120 mL/min; however, if the total body weight of an obese patient is entered into the equation, the resultant value for CrCl often exceeds this threshold.[257-259] The question arises as to whether we should use ideal, lean, adjusted, or total body weight; a question that has been frequently tested with

different answers.[257-262] The original Cockcroft-Gault equation suggested total body weight – but this was before the worldwide obesity epidemic. Second, serum creatinine may be influenced by malnutrition, cachexia, liver disease, and other conditions leading to lower muscle mass.[261,263,264] In clinical practice, serum creatinine is often below 1.0 mg/dL in these populations; therefore, GFR tends to be overestimated by the Cockcroft-Gault equation. Some have suggested rounding serum creatinine to 0.8 mg/dL or 1 mg/dL to account for this concern.[265] There is no substantial evidence to support these suggestions.[265] Overall, the limitations mentioned above are evident in individuals with cardiometabolic disease (i.e., obesity and diabetes). These individuals have altered body composition, and as such, estimates of renal function may be less accurate. This study's primary objective aims to test patient factors, with a focus on cardiometabolic disease, influencing the performance of the Cockcroft-Gault equation in estimating GFR. The secondary objectives were to determine if the development of a new CrCl equation incorporating disease states and race improved estimation relative to measured CrCl (a 24-hour urine).

Research Design

Data source and patient selection

A retrospective cohort study was performed using data extracted from the electronic discharge database and medical records at an academic medical center between January 2009 and July 2019. All consecutive adult patients (aged 18 years or older) with both a 24-hour urine creatinine collection and a serum creatinine obtained within 24 hours of each other were screened for inclusion. Only patients with comorbidities recorded in the electronic health record were further considered for inclusion. Patients

must have a stable serum creatinine defined as less than a 20% fluctuation between two serum creatinine values measured within 48 hours. Patients who were pregnant had undergone amputations, in acute renal failure, or those on hemodialysis were excluded. Individuals with serum creatinine greater than 2.5 mg/dL were excluded from the analysis since this would suggest Stage 5 Chronic Kidney Disease. Previous data reported inaccuracies in the estimation of CrCl using traditional equations in this population.[266,267] Patients with a serum creatinine < 0.6 mg/dL were excluded since low serum creatinine may significantly overestimate renal function. Individuals with a measured 24-hour urine CrCl below 10 mL/min were excluded since it would be expected that those in this group would receive dialysis.[268]

Data extraction and collection

All data were extracted from the patient discharge database and electronic health records (Cerner Millennium and Allscripts, Sunrise Clinical Manager). Patient height, weight, age, sex, race, and laboratory data were extracted from the medical record. All patient comorbidities were identified using International Classification of Diseases, 9th Revision or Clinical Modification or International Classification of Diseases, 10th Revision, Clinical Modification codes depending on availability. Once data were extracted, lean body weight, ideal body weight, and adjusted body weight were calculated based on standard equations.[20,22,23,226,269-271] Subsequently, CrCl was calculated using the Cockcroft-Gault equation. Various weight descriptors were then used for different versions of the Cockcroft-Gault equation. Some authors suggest rounding the sCr to 1.0 mg/dL if less than 1.0 mg/dL, especially in patients > 65 years of age. Therefore,

calculated CrCl with the original Cockcroft-Gault equation was computed using sCr rounded to 1.0 mg/dL if less than 1 mg/dL. CrCl was also computed using the Modified Diet in Renal Disease (MDRD) and CKD-EPI equations, other commonly used equations for estimating renal function.[272-274] All the standard equations used to estimate GFR are summarized in **Table 6.1**.[95,272,273,275]

Table 6.1. Methods for calculating or measuring creatinine clearance

Method	Equation
Cockcroft-Gault	$\frac{(140 - age\ in\ years) * weight\ in\ kg}{72 * sCr\ (^{mg}/_{dL})}$ Multiply by 0.85 if female Original equation used total body weight
CKD-EPI	$141 * \min\left(\frac{sCr}{k}, 1\right) \alpha * \max(\frac{sCr}{k}, 1)^{-1.209} * 0.933^{age} * 1.018^{if\ female} * 1.159^{if\ African\ American}$ Where: sCr is serum creatinine in mg/dL k is 0.7 for females and 0.9 for males α is -0.329 for females and -0.411 for males min indicates the minimum of sCr/k or 1 max indicates the maximum of sCr/k or 1
MDRD	$175 * sCr^{-1.54} * Age^{-.203} * 0.742\ (if\ female) * 1.212\ if\ African\ American$
24-hour urine CrCl	$\frac{Urinary\ creatinine\ \left(\frac{mg}{dL}\right) * urine\ volume\ (L) * 1000}{sCr\ \left(\frac{mg}{dL}\right) * 1440\ (\frac{min}{day})}$

New equation development

Measured 24-hr urine CrCl was used as the gold standard to construct a prediction equation. All the equations derived for the prediction of CrCl were estimated using nonlinear regression. To consider the sex effect of equation performance, the coefficient

values for each male and female were estimated. Further, the effect of disease status on the equation performance was tested and selected based on the calculated *p*-value from nonlinear regression. To develop a new CrCl equation, two strategies were considered. First, we considered a modification of the Cockcroft-Gault equation. The coefficient values in the original Cockcroft-Gault equation (72, 140, and 0.85 for women) were re-estimated using total body weight or lean body weight for each sex. Then, the effect of disease state (obesity, diabetes, and dyslipidemia) was included.

We also considered substitution of various estimates of lean body weight in the Cockcroft-Gault equation. While the James equation for lean body weight estimation is commonly used due to its brevity, the Hume equation has been suggested as the optimal choice in special populations, including obese patients (those with a BMI index above 37 kg/m^2).[276] We placed preference on this equation; however, tested others through construction and visual inspection of surface area plots and the influence this weight descriptor had on the Cockcroft-Gault equation's performance versus measured 24-hr urine CrCl.

Second, we performed a multivariate regression analysis. For multivariate equation 1, measured 24-hr urine CrCl was divided by body surface area, and then coefficient values for serum creatinine, age, and sex were estimated. For multivariate equations 2, 3, and 4 total, adjusted, or lean body weight was included with coefficient values to develop an equation, respectively, and serum creatinine, age, and sex. After estimating coefficient values from base equations, disease states (obese, diabetes, and

dyslipidemia) and race (white or non-white) were tested and included if $p<0.05$ in the regression analysis. Only disease states present in at least 15% of patients in the analytic dataset were considered for evaluation in the regression.

Statistical Analysis

All results were summarized using descriptive statistics. Mean and the standard deviation was reported for normally distributed continuous data, and median and range were reported for data that were not normally distributed. The normality of data was assessed using visual inspection of histograms and the Kruskal-Wallis test. Binary data were reported as counts and proportions. Pearson's correlation coefficient between each of the calculated CrCl values and the measured 24-hour urine CrCl and corresponding 95% confidence intervals were calculated using Fishers Z methods. The mean bias between the measured 24-hour CrCl and various methods for calculated CrCl was defined as the difference between the two values, and the precision was described using the 95% confidence interval for the difference. Root mean square error (RMSE) was calculated for each outcome to assess the degree of bias. Calculated CrCl not deviating more the 30% from the 24-hour urine CrCl was considered to be accurate. This definition of accuracy was based on the original study validating the Cockcroft-Gault equation, which reported that Cockcroft-Gault calculated CrCl was within 30% of the 24-hour urine CrCl[95] others suggesting that if calculated CrCl is within 25% of measured 24-hour urine CrCl, it is considered accurate.[266] All analyses were conducted using SPSS, version 26 (IBM Corporation, Somers, NY) and R (R Core Team, Vienna, Austria).

Results

Figure 6.1 provides an overview of the patient selection process. After the initial screening, 687 patients were included in the dataset; however, upon applying the inclusion and exclusion criteria, the final analytic dataset included 484 patients. **Table 6.2** provides a summary of the patient characteristics. In the overall population, 44.2% of patients were obese, 44.0% had diabetes, and 30.8% had dyslipidemia. The mean calculated CrCl ranged from 55.8 ± 28.0 mL/min to 77.8 ± 37.2 mL/min depending on the method used. For comparison, the mean measured 24-hour urine CrCl was 85.1 ± 47.5 mL/min.

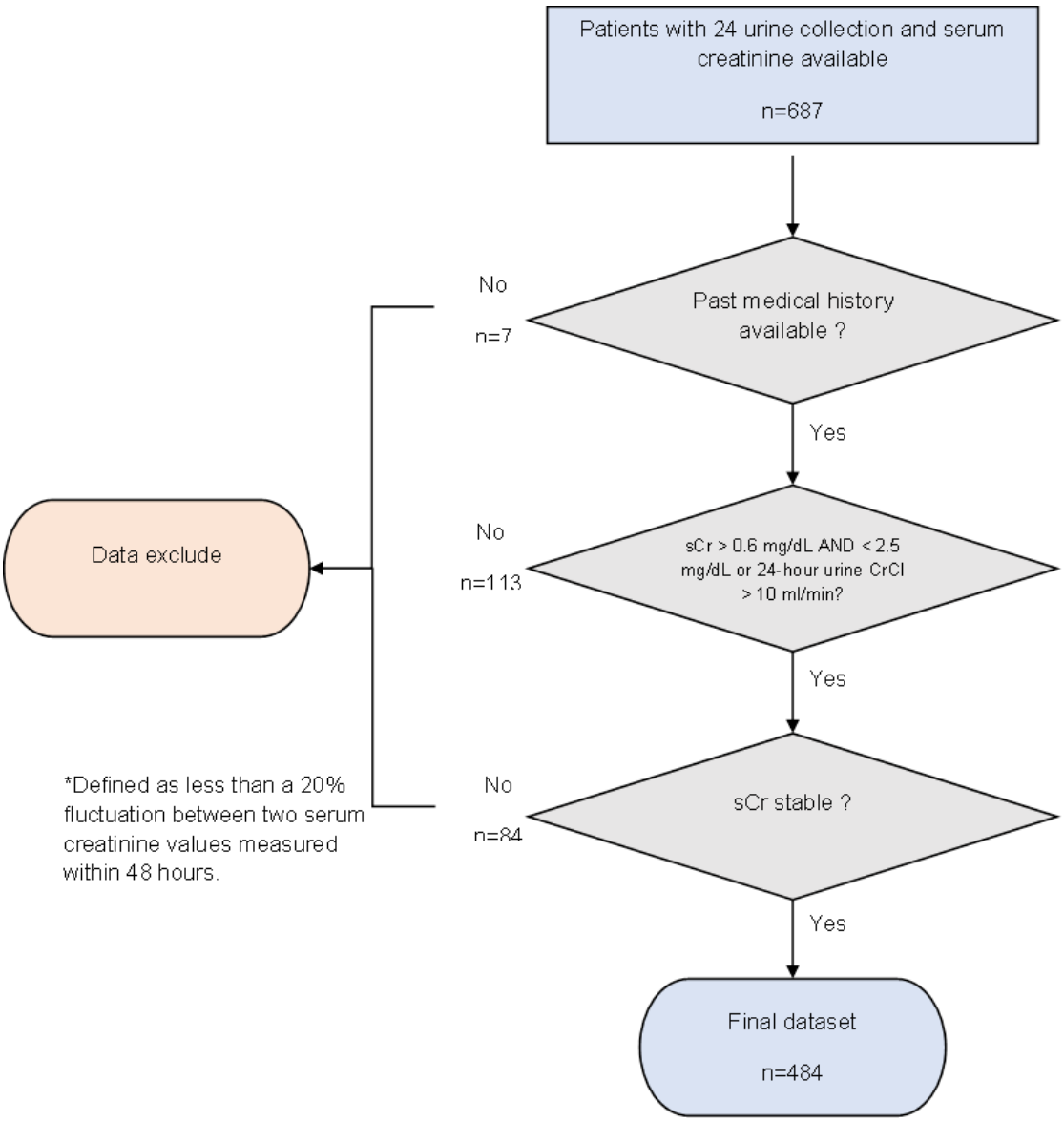

Figure 6.1. Data inclusion decision tree for evaluating various methods of calculating creatinine clearance.

Depending on the subgroup (obese, diabetes, or dyslipidemia), there was variation in the mean calculated CrCl (**Table 6.2**). Using the traditional equations, adjusted and lean body weight produced the strongest correlation coefficients in the overall population and subgroups. Total body weight was similar and outperformed the other weight descriptors in terms of mean bias. Finally, adjusted body weight produced the greatest accuracy in most cases (**Table 6.3**). Of these equations, MVA 4, which incorporated lean body weight, select diseases, sex, and race, performed the best in correlation to measured 24-hour urine CrCl, accuracy, and RMSE value. This equation was further tested versus the other methods. In addition, a modified Cockcroft-Gault equation was developed using the available data (**Figure 6.2**). This equation incorporated obesity, diabetes, dyslipidemia, sex, and lean body weight.

$$CrCl_{Male} = \frac{(194.385 - age) * LBW * 1.022^{DM} * 0.949^{Obese} * 0.931^{Dyslipidemia} * 1.046^{Race}}{82.812 * sCr}$$

$$CrCl_{Female} = \frac{(182.643 - age) * LBW * 1.013^{DM} * 1.036^{Obese} * 1.010^{Dyslipidemia} * 0.959^{Race}}{79.138 * sCr}$$

Figure 6.2. Suggested modified CrCl calculation where LBW=lean body weight calculated using the Hume formula, DM=1 if patient has diabetes, obese=1 if patient is obese, dyslipidemia=1 if patient has dyslipidemia, and race=1 if patient is Caucasian.

The correlation coefficient, mean bias, accuracy, and RMSE of each calculated CrCl method (including newly developed methods) versus the measured 24-hour urine CrCl for the overall population and each subgroup is summarized in **Table 6.4**. The modified Cockcroft-Gault equation using lean body weight calculated using the Hume

method performed best in the overall population, the obese subgroup, and the dyslipidemia subgroup in terms of strength of the correlation, mean bias, and accuracy. The Cockcroft-Gault equation using adjusted body weight performed best in the diabetes subgroup.

Table 6.2. Comparison of patient characteristics and renal function estimates in the study population

Subject characteristic		Obese			Diabetes			Dyslipidemia		
	All Patients (n=484)	**None (n=270)**	**Yes (n=214**	**p-value***	**None (n=271)**	**Yes (n=213)**	**p-value***	**None (n=335)**	**Yes (n=149)**	**p-value***
Age (years, mean ± SD)	59.2 ±17.8	60.0 ± 19.0	58.07 ± 16.2	0.23	60.2 ± 18.4	57.8 ± 17.0	0.14	58.1 ± 18.9	61.5 ± 14.9	0.03
Age greater than 65 years (n,%)	212 (43.8)	127 (47.0)	85 (39.7)	0.11	127 (47.0)	85 (40.0)	0.13	140 (41.8)	72 (48.3)	0.18
Female (n, %)	275 (56.8)	142 (52.6)	133 (62.1)	0.04	160 (59.0)	115 (54.0)	0.27	198 (59.1)	77 (51.7)	0.13
Race (n, %)				0.07			0.15			0.55
White	327 (67.6)	180 (66.7)	147 (68.7)		193 (71.2)	134 (62.9)		222 (66.3)	105 (70.5)	
Black	60 (12.4)	27 (10.0)	33 (15.4)		25 (9.2)	35 (16.4)		41 (12.2)	19 (12.8)	
Hispanic	45 (9.3)	27 (10.0)	18 (8.4)		24 (8.9)	21 (9.9)		35 (10.4)	10 (6.7)	
Asian	36 (7.4)	27 (10.0)	9 (4.2)		19 (7.0)	17 (8.0)		24 (7.2)	12 (8.1)	
Other	16 (3.3)	9 (3.3)	7 (3.3)		10 (3.7)	6 (2.8)		13 (3.9)	3 (2.0)	
Serum creatinine (mg/dL, mean ± SD)	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	0.64	1.2 ± 0.4	1.1 ± 0.4	0.19	1.1 ± 0.4	1.2 ± 0.4	0.64
Weight (kg, mean ± SD)	86.0 ± 25.6	70.5 ± 14.0	105.6 ± 23.3	<0.01	84.1 ± 24.8	88.5 ± 26.4	0.06	83.5 ± 24.3	91.7 ± 27.4	<0.01
Body mass index (kg/m^2, mean ± SD)	30.4 ± 8.1	24.8 ± 3.6	37.4 ± 6.7	<0.01	30.3 ± 8.2	30.1 ± 8.1	0.85	29.8 ± 8.2	31.6 ± 8.0	0.03

Body surface area (m^2, mean ± SD)	1.9 ± 0.3	1.8 ± 0.2	2.1 ± 0.3	<0.01	1.9 ± 0.3	2.0 ± 0.3	<0.01	1.9 ± 0.3	2.0 ± 0.3	<0.01
Obese (n, %)	214 (44.2)	-	-	-	117 (43.2)	97 (45.5)	0.60	141 (42.1)	73 (49.0)	0.16
Heart failure (n, %)	53 (11.0)	22 (8.1)	31 (14.5)	0.03	7 (2.6)	46 (21.6)	<0.01	17 (5.1)	36 (24.2)	<0.01
Diabetes (n, %)	213 (44.0)	116 (43.0)	97 (45.3)	0.60	-	-	-	97 (29.0)	116 (77.9)	<0.01
Dyslipidemia (n, %)	149 (30.8)	76 (28.1)	73 (34.1)	0.16	33 (12.2)	116 (54.5)	<0.01	-	-	-
Measured $CrCl_{24\text{-hr urine}}$ (mL/min, mean ± SD)	85.1 ± 47.5	77.8 ± 43.1	94.3 ± 51.2	<0.01	81.3 ± 47.3	90.0 ± 47.5	0.05	85.1 ± 48.0	85.2 ± 46.6	0.98
CrCl CG_{TBW} (mL/min, mean ± SD)	89.3 ± 49.7	72.6 ± 33.7	110.4 ± 58.0	<0.01	85.5 ± 50.2	94.1 ± 48.7	0.06	88.1 ± 49.1	91.9 ± 51.1	0.44
CrCl CG_{IBW} (mL/min, mean ± SD)	64.0 ± 31.5	64.6 ± 32.1	63.3 ± 30.8	0.65	60.6 ± 31.3	68.4 ± 31.3	<0.01	64.4 ± 32.5	63.1 ± 29.2	0.66
CrCl CG_{AdjBW} (mL/min, mean ± SD)	74.1 ± 37.0	67.8 ± 32.2	82.2 ± 40.9	<0.01	70.5 ± 37.1	78.7 ± 36.3	0.02	73.9 ± 37.2	74.6 ± 36.4	0.85
CrCl CG_{LBW} (mL/min, mean ± SD)	55.8 ± 28.0	50.9 ± 24.1	62.0 ± 31.2	<0.01	53.1 ± 28.1	59.3 ± 27.6	0.02	55.6 ± 28.1	56.3 ± 27.8	0.81
CrCl $CG_{rounded\ sCr}$ (mL/min, mean ± SD)	77.8 ± 37.2	62.9 ± 25.1	96.5 ± 41.4	<0.01	74.1 ± 37.2	82.5 ± 36.8	0.01	76.3 ± 36.8	81.0 ± 38.0	0.21

CKD-EPI (mL/min, mean ± SD)	70.6 ± 28.7	71.3 ± 28.7	69.7 ± 28.8	0.55	68.3 ± 29.3	73.5 ± 27.7	0.05	71.4 ± 29.6	68.8 ± 26.7	0.35
MDRD (mL/min, mean ± SD)	71.8 ± 29.6	73.0 ± 29.6	70.5 ± 29.6	0.36	69.9 ± 30.6	74.3 ± 28.2	0.10	72.6 ± 30.5	70.3 ± 27.4	0.43
***p-value for comparison between-group (i.e., no obesity versus obesity). Independent samples t-test for continuous data and chi-square for binary data.**										

Table 6.3. Development and testing of multivariate equations to calculate creatinine clearance

Model	Equation	Correlation coefficient / RMSE
MVA 1	$154.727 \times BSA \times Scr^{-0.937} \times age^{-0.301} \times 0.872^{Sex} \times 0.983^{Race} \times 1.042^{Diabetes} \times 1.050^{Obesity} \times 0.971^{Dyslipidemia}$	0.78 / 29.89
MVA 2	$5.466 \times TBW^{0.976} \times Scr^{-0.919} \times age^{-0.359} \times 0.926^{Sex} \times 1.003^{Race} \times 1.034^{Diabetes} \times 0.804^{Obs} \times 0.957^{Dyslipidemia}$	0.79 / 29.25
MVA 3	$0.985 \times AdjBW^{1.356} \times Scr^{-0.924} \times age^{-0.314} \times 1.014^{Sex} \times 0.970^{Race} \times 0.998^{Diabetes} \times 0.924^{Obesity} \times 0.964^{Dyslipidemia}$	0.80 / 28.65
MVA 4	$1.431 \times LBW^{1.360} \times Scr^{-0.920} \times age^{-0.318} \times 1.043^{Sex} \times 0.976^{Race} \times 0.999^{Diabetes} \times 0.913^{Obesity} \times 0.963^{Dyslipidemia}$	0.80 / 28.65

Abbreviations: AdjBW=adjusted body weight, BSA=body surface area, LBW=lean body weight calculated using the Hume equation, MVA=multivariate, TBW=total body weight. MVA 4 was selected for further development based on strength of correlation coefficient and RMSE value versus measured 24-hour urine CrCl.

Table 6.4. Correlation, bias, and accuracy between measured (24-hour urine) glomerular filtration rate and estimated glomerular filtration rate in select populations.

Method	Mean CrCl ± SD	Correlation coefficient (95% CI)	Mean bias (95% CI)	Accuracy within ± 30% n (%)	RMSE
Overall (n=484)					
CrCl CG_{ABW}	89.3 ± 49.7	0.77 (0.73 – 0.80)	-4.2 (-7.2 – 1.2)	295 (61.0)	33.49
CrCl CG_{IBW}	64.0 ± 31.5	0.72 (0.67 – 0.76)	21.1 (18.2 – 24.1)	224 (46.3)	39.13
CrCl CG_{AdjBW}	74.1 ± 37.0	0.78 (0.74 – 0.81)	11.0 (8.3 – 13.6)	298 (61.6)	31.56
CrCl CG_{LBW}	55.8 ± 28.0	0.78 (0.74 – 0.81)	29.3 (26.5 – 32.1)	162 (33.5)	42.72
CrCl $CG_{rounded\ sCr}$	77.8 ± 37.2	0.73 (0.69 – 0.77)	7.3 (4.4 – 10.3)	289 (59.7)	62.26
CKD-EPI	70.6 ± 28.7	0.68 (0.63 – 0.73)	14.5 (11.4 – 17.6)	256 (52.9)	37.88
MDRD	71.8 ± 29.6	0.64 (0.58 – 0.69)	13.3 (10.0 – 16.5)	250 (51.7)	40.45
CrCl $CG_{modified}$	85.4 ± 37.2	0.78 (0.74 – 0.81)	0.2 (-2.4 – 2.9)	300 (62.0)	28.48
MVA	75.9 ± 26.9	0.77 (0.73 – 0.80)	4.2 (1.2 – 7.2)	282 (58.3)	28.65
Obesity (n=214)					
CrCl CG_{ABW}	110.4 ± 58.0	0.78 (0.72 – 0.83)	-16.1 (-21.0 – -11.2)	129 (60.3)	39.89
CrCl CG_{IBW}	63.3 ± 30.8	0.76 (0.70 – 0.81)	31.0 (26.4 – 35.6)	75 (35.0)	46.06
CrCl CG_{AdjBW}	82.2 ± 40.9	0.79 (0.73 – 0.84)	12.2 (7.9 – 16.4)	131 (61.21)	33.69
CrCl CG_{LBW}	62.0 ± 31.2	0.78 (0.72 – 0.83)	32.3 (27.9 – 36.8)	71 (33.2)	46.21
CrCl $CG_{rounded\ sCr}$	96.5 ± 41.4	0.76 (0.70 – 0.81)	-2.2 (-6.7 – 2.3)	135 (63.1)	67.24
CKD-EPI	69.7 ± 28.8	0.72 (0.65 – 0.78)	24.6 (19.7 – 29.5)	102 (47.7)	44.04
MDRD	70.5 ± 29.6	0.67 (0.59 – 0.74)	23.9 (18.7 – 29.1)	102 (47.7)	47.68
CrCl $CG_{modified}$	94.3 ± 41.4	0.79 (0.73 – 0.84)	0.01 (-4.2 – 4.2)	137 (64.0)	30.38
MVA	83.7 ± 30.1	0.77 (0.71 – 0.82)	16.1 (11.2 – 21.0)	128 (59.8)	30.59
Diabetes (n=213)					
CrCl CG_{ABW}	94.1 ± 48.7	0.73 (0.66 – 0.79)	-4.1 (-8.9 – 0.7)	121 (56.9)	35.75
CrCl CG_{IBW}	68.4 ± 31.3	0.73 (0.66 – 0.79)	21.6 (17.2 – 26.0)	101 (47.4)	39.02
CrCl CG_{AdjBW}	78.7 ± 36.3	0.77 (0.71 – 0.82)	11.3 (7.2 – 15.4)	131 (61.5)	32.36
CrCl CG_{LBW}	59.3 ± 27.6	0.76 (0.70 – 0.81)	30.7 (26.4 – 35.0))	80 (37.6)	44.21
CrCl $CG_{rounded\ sCr}$	82.5 ± 36.8	0.70 (0.62 – 0.76	7.5 (2.9 – 12.1)	124 (58.2)	61.57
CKD-EPI	73.5 ± 27.7	0.66 (0.58 – 0.73)	16.5 (11.7 – 21.3)	101 (47.4)	39.29

MDRD	74.3 ± 28.2	0.61 (0.52 – 0.69)	15.7 (10.6 – 20.7)	100 (46.9)	42.62
CrCl $CG_{modified}$	90.2 ± 37.4	0.76 (0.70 – 0.81)	0.1 (-4.0 – 4.3)	129 (60.6)	28.96
MVA	80.2 ± 27.4	0.77 (0.71 – 0.82)	4.1 (-0.7 – 8.9)	128 (60.1)	29.71
Dyslipidemia (n=149)					
CrCl CG_{TBW}	91.9 ± 51.1	0.75 (0.67 – 0.81)	-6.7 (-12.3 – -1.1)	91 (61.1)	35.07
CrCl CG_{IBW}	63.1 ± 29.2	0.75 (0.67 – 0.81)	22.2 (17.1 – 27.2)	72 (48.3)	38.29
CrCl CG_{AdjBW}	74.6 ± 36.4	0.78 (0.71 -0.84)	10.6 (5.9 – 15.4)	95 (63.8)	30.76
CrCl CG_{LBW}	56.3 ± 27.8	0.78 (0.71 – 0.84)	29.0 (24.0 – 33.9)	55 (36.9)	41.97
CrCl $CG_{rounded\ sCr}$	81.0 ± 38.0	0.72 (0.63 – 0.79)	4.3 (-1.0 – 9.6)	95 (63.8)	58.70
CKD-EPI	68.8 ± 26.7	0.67 (0.57 – 0.75)	16.5 (10.8 – 22.1)	76 (51.0)	38.52
MDRD	70.3 ± 27.4	0.62 (0.51 – 0.71)	15.0 (9.0 – 20.9)	76 (51.0)	41.29
CrCl $CG_{modified}$	84.6 ± 37.5	0.79 (0.72 – 0.84)	-0.6 (-5.3 – 4.0)	101(67.8)	27.70
MVA	76.5 ± 27.8	0.78 (0.71 – 0.84)	6.7 (1.1)	98 (65.8)	27.22

Abbreviations: AdjBW=adjusted body weight, CG=Cockcroft-Gault, CrCl=creatinine clearance, LBW=lean body weight, RMSE=root mean square error, TBW=total body weight.

Green box = best performer in each stratum for each metric. Yellow = second-best performer in each stratum. Based on the criteria evaluated, CrCl $CG_{modified}$ outperforms all other tested equations versus measured 24-hour urine CrCl in the overall population, individuals with obesity, and individuals with dyslipidemia.

Discussion.

The estimation of GFR using calculated CrCl is critical to select optimal medication dosing regimens. Individuals with obesity, diabetes, and dyslipidemia often are on many medications and have altered kidney function, making calculating CrCl more critical. The current study identified several limitations with current practices in the calculation of CrCl. First, the Cockcroft-Gault equation uses the total body weight in calculating CrCl. There has been conflicting data regarding substituting adjusted body weight into the equation. We provide evidence that adjusted body weight is a reasonable consideration. Using this weight produced similar and, in some situations, better correlation, lower bias, and

improved accuracy. Moreover, we demonstrate that the inclusion of obesity, diabetes, dyslipidemia, and race into a modified Cockcroft-Gault equation improves performance relative to the original Cockcroft-gault equation.

Some limitations of our study must be considered. First, 24-hour CrCl was used as the gold standard. There are potential sources of bias to this method, including urine collection errors, increased creatinine secretion, and increased extrarenal degradation.[277] For example, up to 20% of creatinine is not cleared through the kidney but rather through active secretion. This inherent bias will be present in any method using sCr in its estimation of GFR. Nonetheless, this strategy represents the most accurate clinically used method for measuring CrCl as an estimate of GFR. Second, we excluded patients with very high (> 2.5 mg/dL) or low (0.6 mg/dL) serum creatinine and those with a 24-hour urine CrCl < 10 mL/min; therefore, extrapolating our results to these populations may not be appropriate. Regardless, drug dosing in patients in these extremes should be based on clinical context rather than calculated CrCl alone. The population age in this study was between (18 to 94 years) and included many patients over the age of 65 years (43.8%). While this limits the external application to a younger population, advanced age represents a special population at an increased risk of drug toxicity. Renal impairment is frequently reported in older patients experiencing drug-related iatrogenesis, and improved renal function assessment in this population is highly relevant.

Moreover, hospitalized patients are often of advanced age, and this population is more likely to have reduced CrCl requiring dosage adjustment. Finally, as with any retrospective study, there is potential for information bias. Despite these limitations, our findings provide important information for the clinician. Total body weight is the appropriate body weight descriptor to use in the Cockcroft-Gault equation to calculate

CrCl. Using adjusted body weight is reasonable in individuals with obesity but provides modest benefits. Our modified Cockcroft-Gault equation using lean body weight outperforms all current methods and warrants further evaluation and validation.

Despite the shortcomings of the original Cockcroft-Gault equation, renal dysfunction classification based on its values is sufficient. Ultimately, one could argue that renal dysfunction classification is a more important metric because it would affect drug dosing (i.e., most drugs require renal dose adjustment when CrCl falls below 50-to-60 mL/min). Given that the kidney clears half of all medications or their metabolites, and roughly 3 billion prescriptions for medications are written annually, many individuals may be at risk for underdosing (or overdosing) if renal function isn't appropriately assessed.[278,279] While clearly renal dysfunction places patients at risk for adverse events when dosing is not appropriately adjusted,[280] dosage reductions when not necessary may increase the risk of treatment failure, which is especially concerning with antibiotics or chemotherapeutic agents.[281]

Conclusions

Total body weight is the appropriate weight descriptor to use in the original Cockcroft-Gault equation. Our modified Cockcroft-Gault equation using lean body weight outperforms other methods of calculating CrCl in terms of correlation, accuracy, mean bias, and RMSE value. Additional research is warranted to determine if this equation is correlated with drug exposure, toxicity, and efficacy.

CHAPTER 7. General Discussion

In this Ph.D. project, both retrospective and prospective human clinical studies were designed to evaluate drug exposure and response in special populations, namely obesity and the aging population. Model drugs, cefoxitin, anticoagulants (dabigatran, enoxaparin, heparin), and intravenous immune globulin were chosen based on the risk for poor outcomes with incorrect dosing. In addition, current practices for renal function estimates were evaluated, given the lack of reliability of available methods in estimating GFR in obesity and advanced age. Despite the rapid growth of both the obese and advanced age population in the United States, these populations are seldom incorporated into clinical trials. While advanced age is considered a special population by the FDA, obesity is not resulting in a greater concern over the evidence gap in drug dosing.

Beta-lactam antibiotics are among the most commonly used anti-infectives, and inadequate dosing in obesity results in poor outcomes. We have provided evidence that cefoxitin, a commonly used beta-lactam antibiotic used for surgical prophylaxis, does not achieve sufficient concentration at the target tissue (surgical site) in obese patients. The potential implications of inadequate exposure include surgical site infection and the development of multidrug resistance. Novel dosing strategies may help overcome this concern, including prolonging a beta-lactam infusion to take advantage of time above minimum inhibitory concentration killing properties of this drug class. Our study found that the institution standard 2 grams cefoxitin was inadequate; however, administration of cefoxitin 4 grams over 30 minutes in a large prospective cohort study in bariatric surgery patients reported inadequacy in achieving target concentrations.[282] Rather than

increasing dosage alone, a strategy incorporating a bolus dose, followed by a prolonged infusion, may provide a greater chance of attaining pharmacodynamic targets. Thus, understanding pharmacokinetic and pharmacodynamic principles are essential in improving dosing for special populations. Importantly, the target site in our study was the epidermis and dermis. We cannot make any conclusions related to drug concentration on other tissues at the studied dose. Plasma extraction ratios for other tissues may be different and dependent on various factors, including drug transporters. Additional studies are warranted to investigate the mechanisms of altered antibiotic tissue distribution in obese individuals. Incorporating microdialysis techniques and using novel approaches such as population pharmacokinetics using remnant tissue from surgical procedures may provide much-needed data. We are currently planning studies using remnant tissue from various procedures, including bariatric, appendectomy, and orthopedic surgery.

Anticoagulants are often among the drugs most commonly leading to hospitalization for iatrogenic events, namely bleeding.[41] Despite this risk, they are essential for preventing and treating venous thromboembolism, which can have devastating consequences. Our studies in both obese and elderly populations highlight the challenges with currently utilized anticoagulants. They also highlight the inadequacy of current approaches to care related to anticoagulation in these populations. For heparins, most report the success of anti-Xa concentrations to guide dosing, particularly in the obese population.[120,124] However, anti-Xa may not adequately capture an individual's coagulation status. Our study provides evidence that ETP is a better marker.[129,133,139,283] Additional studies are warranted evaluating this biomarker prospectively. The search for drugs that require less frequent monitoring is always

desirable; however, the use of good judgment should not cloud our enthusiasm for drugs with more favorable pharmacokinetic profiles. Our evaluation of dabigatran in the elderly demonstrates that despite a favorable pharmacokinetic profile versus warfarin, dabigatran produces variation within and between individuals. While plasma concentration or coagulation parameter monitoring is not recommended with this drug, it seems prudent. Dabigatran is not recommended for elderly patients due to its increased association with bleeding versus other anticoagulants in this population.[162,284] Perhaps the use of therapeutic drug monitoring would improve the safety profile of dabigatran. However, the current environment may not lend itself to this notion since newer agents have now been developed with better safety profiles. Nonetheless, the story of dabigatran highlights the importance of appropriate assessment of high-risk medications in special populations.

The assessment of protein therapeutics in special populations requires a significant amount of attention. Protein therapeutics represent a growing class of therapeutics, which places a tremendous economic strain on healthcare resources owing to their high costs. Therefore, appropriate dose selection is paramount to enhance drug response and avoid excess dosing. Our preliminary study found a longer half-life of IgG after intravenous administration in obese patients versus normal-weight patients as assessed by body mass index and body fat. While the study was limited in sample size, our ongoing prospective cohort study evaluating IVIG in patients currently on therapy will provide additional information.

CHAPTER 8. Future directions

While deciphering altered pharmacokinetics has largely focused on traditional parameters (altered absorption, the volume of distribution, metabolism, clearance), future studies should focus on other contributors to gain a better understanding of drug disposition in special populations. Two such areas include the gut microbiome and drug transporters. Both can have a significant impact on drug disposition and, ultimately, drug response.

There is a consensus that obesity and related metabolic diseases are associated with low-grade inflammation,[285] though the exact mechanisms by which obesity triggers low-grade inflammation remain poorly understood.[286] Gut dysbiosis has been reported in individuals with obesity, and in advanced age,[287-289] and loss of gut microbiota diversity have been associated with chronic inflammation.[290,291] Inflammation may contribute to a variety of physiological changes and alter drug response.[292] Studies have also linked obesity and a high-fat diet to increases in gut membrane permeability[293] and circulating serum lipopolysaccharide (LPS) concentration.[294] The gut microbiome has been reported to influence intestinal permeability and subsequently alter absorption of various substances, including drugs. The microbiome may also influence drug response. For example, trimethylamine N-oxide (TMAO), an amine oxide produced via microbial metabolism in the gut,[295] may antagonize the effects of antiplatelets.[36] The gut microbiome also metabolizes a variety of drugs.[296,297] Our preliminary data identified that the gut microbiome can metabolize anthocyanins, flavonoids with potent anti-oxidant and anti-inflammatory properties into a variety of pharmacologically active compounds (**Figure 8.1**). Collectively, the gut microbiome significantly contributes to variability in drug

exposure and response. The development of *in silico* models that incorporate gut microbiome diversity may capture the variability in drug response in special populations (or among individuals

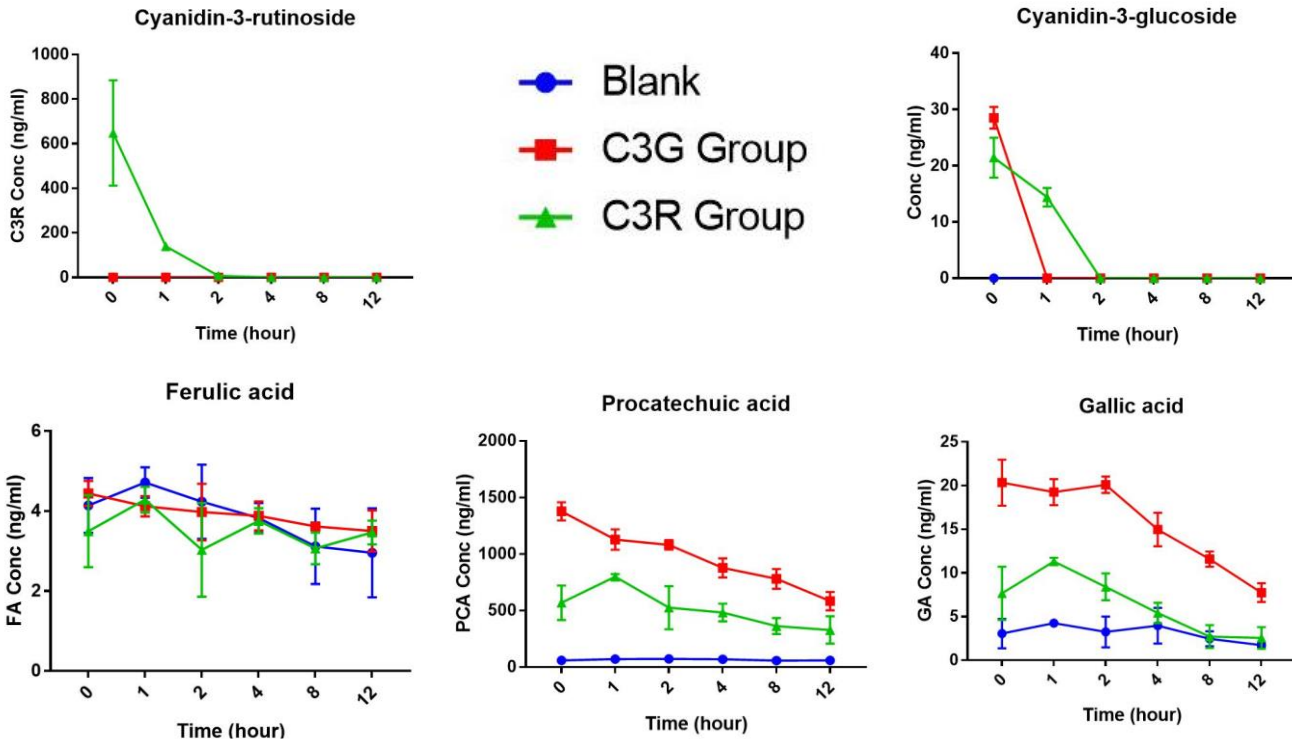

Figure 8.1. . In vitro culture of cyanidin-3-glucoside (C3G) and cyandin-3-rutinoside (C3R) in human fecal samples.

Within 2 hours of incubation the parent compounds were nearly completely degraded as detected using UPLC-MS of fecal supernatant over 12 hours. Metabolites (ferulic acid, procatechuic acid, and gallic acid were observed immediately and were also degraded over time.

Transporters must also be considered as part of the mechanism of altered drug response in special populations. Beta-lactam antibiotics were shown to be substrates for organic anion transporter (OAT)1, OAT2, and OAT3 located in the human renal proximal tubule cells.[298,299] Certain disease states may affect (increase or decrease) the expression level of these transporters in the kidney.[300-302] The elimination rate constant of cefazolin significantly correlated with OAT3 mRNA expression;[303] and mRNA expression for Oat2 was decreased in obese mice.[304] P-glycoprotein expression may be altered in obesity after bariatric surgery, and gut dysbiosis and many oral anticoagulants and other medications are substrates of this transporter. Collectively, the clinical relevance of studying drug transporter changes in special populations is high and warrants more attention. There is a myriad of transporters that must be considered depending on the target tissue. In terms of biologics, the neonatal FcRn (FcRn) is a major determinant affecting IgG disposition.[305] Monoclonals antibodies utilize IgG as their backbone and may be affected by altered FcRn expression and function.

Overall, we must look beyond traditional pharmacokinetic parameters to advance our understanding of altered drug disposition in special populations. We must also advocate for the importance of including a more robust analysis of drug in these high risk, high prevalence, yet underrepresented populations in clinical studies. Finally, collaborations between preclinical and clinical researchers can yield high impact research directly applicable to clinical practice. Incorporating *in silico* approaches to optimal drug regimen design using preclinical data and validation with clinical data provides an ideal platform for enhancing drug dosing in the future.

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