IMPACT OF A SHORT-TERM ANTIBIOTIC CYCLE ON GLUCOSE CONTROL IN ADULTS WITH OVERWEIGHT OR OBESITY

by

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APPROVAL

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CHAPTER I: INTRODUCTION

The Gut Microbiome, Obesity, and Insulin Resistance

The prevalence of overweight and obesity in the United States has reached 73.5% of the population[1] and has led to the development of insulin resistance in 40.3% of the cases (NHANES 2018).[2] These high overweight and obesity rates are expected to continue rising.[3] Overweight and obesity are diseases that undermine public health by reducing life expectancy and diminishing quality of life, while also elevating the likelihood of various chronic diseases, consequently inflating national healthcare expenditure.[4] Obesity prevention and treatment is challenging due to its multifactorial etiology. Factors involved in the progression of obesity include, but are not limited to, physical and social environment, genetics, lifestyle, and maternal factors.[5] Lifestyle therapy, composed of dietary counseling, physical activity, and behavioral intervention, is the current recommended treatment for obesity with the addition of pharmacotherapy (for BMI $\geq$ 30 or $\geq$ 27 with one obesity-related disease) and bariatric surgery (for BMI $\geq$ 40 or BMI $\geq$ 35 with at least 1 obesity-related disease) when needed.[6] Lifestyle therapies, while effective, often lead to significant but small and gradual changes in weight, as demonstrated by a recent meta-analysis showing a five-pound weight loss after one year of therapy.[7] Pharmacologic therapies cause more pronounced weight loss results but are often accompanied by significant adverse side effects and require chronic administration.[8] Bariatric surgery has proven more effective than non-surgical treatments for long-term weight loss but includes rare but more severe risks for post-surgery complications including hiatal hernia, hemorrhage, strictures, nutritional deficiencies, stomach leaks, and new addictions.[8] Therefore, given the imminent public health crisis approaching us, research into understanding obesity's etiology is important to develop more efficient and safe preventative therapies and treatments. A

developing area of research with potential for preventative and therapeutic interventions for overweight, obesity, and insulin resistance is the gut microbiome.[9]

The gut microbiome is influenced by a multitude of factors including, but not limited to, genetics, delivery method at birth, method of infant feeding, and diet.[10] Altogether these factors shape the structure and function of the gut microbiome that potentiates beneficial or harmful effects on human health. Beginning in utero and early life, the gut microbiome is developed during this critical period. Maternal overweight or obesity, intrapartum antibiotic exposure, excessive gestational weight gain, and delivery via Cesarean section reduce gut microbiota diversity,[11] while delivery mode and gestational age significantly influence the gut microbiota composition of the infant.[12] Research has shown that delivery mode, preterm gestational age, and excessive gestational weight gain, increase the infant's risk of developing obesity later in life, however, current research is insufficient to determine associated gut microbiome shifts as the mechanism of action.[13-15] Furthermore, breastfeeding promotes gut microbiome diversity, induces a distinct gut microbiota composition, and protects against dysbiosis-induced diarrhea in infancy.[16] The distinct gut microbiome structure of breastfed infants is hypothesized to be a contributing factor in breastfeeding's observed protective effects against obesity and insulin later in life.[17-19] In addition to maternal factors, host genetics are also associated with gut microbiome signatures,[20-22] and can modulate environmental- and diet-induced perturbations and shifts in the gut microbiome.[23] The interactions between the host genome, gut microbiome, and environmental factors such as diet are complex and are one of many factors that determine the gut microbiome and its associated predisposition or protection from the development of obesity.

While novel research continues to reveal the complexity and interactions between factors that shape the gut microbiome, diet remains a consistent factor in determining the overall structure and function of the gut microbiome.[24] Dietary components and their metabolites can

provide accessible nutrients to the gut microbiota that interact with the gut epithelial barrier, altogether shaping the diversity and composition of the gut microbiome, the structure of the intestinal epithelial barrier, and metabolic signaling pathways.[25] Significant compositional and functional shifts in the gut microbiome can take place as quickly as within 24 hours of the dietary modification.[26] Gut microbiome composition and function have been shown to reflect dietary intake metabolism requirements.[27] For example, in mammals, similar gut microbiome structures can be found within herbivores, omnivores, and carnivores across 33 species.[28] Furthermore, people in non-Westernized societies with higher fiber and lower fat, protein, and sugar intake exhibit a gut microbiome equipped with the functional capacity for fiber and carbohydrate digestion as opposed to fat and amino acid metabolism,[29] demonstrating the effect of dietary selection pressures on the gut microbiome's composition and metabolic capacity. In mice, the Western diet has been shown to negatively alter the gut microbiome and induce intestinal inflammation.[30] This alteration in the gut microbiome is defined as dysbiosis in which the gut microbiome reduces its diversity, loses beneficial bacteria, and potentially gains hazardous bacteria.[31] Dysbiosis can induce bacterial translocation and initiate inflammatory responses that contribute to the development of insulin resistance and obesity-associated chronic diseases.[32] Long-term dysbiosis and inflammation are associated with obesity and insulin resistance and are hypothesized to be mechanisms of action for the development of these and other chronic inflammation-induced diseases.[31-33]

In addition to dietary, genetic, and maternal factors, gut microbiome composition and diversity are influenced by body composition, shifting alongside body compositional changes.[34] The relationship between body composition and the gut microbiome is highlighted by the distinction of gut microbiome composition from individuals with obesity compared to normal-weight individuals. Individuals with overweight, obesity, or insulin resistance have less gut

microbial richness (total number of species) and evenness (distribution of species) and have compositional differences compared with healthy individuals.[35,36] Dysbiosis between the two largest phyla in the gut microbiome, Firmicutes and Bacteroidetes, has been demonstrated in individuals with obesity.[37,38] Furthermore, obesity has been positively associated with certain genera that contain pathobionts such as *Escherichia-Shigella* and *Sutterella*, and negatively associated with potential health-promoting genera including *Akkermansia* and *Bifidobacterium*.[35,39] Similarly, the gut microbiome of individuals with insulin resistance is in a state of dysbiosis, with an increase in pathogenic bacteria and a decrease in health-promoting butyrate-producing bacteria.[20] The increased presence of specific genes in individuals with insulin resistance is predictive and discriminative from healthy individuals, altogether demonstrating the taxonomic and functional differences observed in the gut microbiomes of individuals with insulin resistance.[20] Understanding the multi-modal factors contributing to the structure and function of the gut microbiome is necessary to determine associated risks of developing diseases such as obesity and insulin resistance, and creating gut-microbiome targeted prevention and treatments.

Human studies have revealed the potential of leveraging the gut microbiome for combating obesity and its associated insulin resistance. These studies have shown a notable enhancement in insulin resistance following fecal microbiota transplantation (FMT) from healthy, lean donors to individuals with obesity and insulin resistance.[40,41] Recent research has underscored the significance of genetics, maternal factors, diet, and medications in shapping the gut microbiome and the risk to develop obesity and insulin resistance. However, conflicting observational and interventional research suggest that antibiotics may be a significant contributing factor to the development of dysbiosis-induced obesity and insulin resistance. Furthermore, murine research has shown fiber to be a potential therapeutic shield that

protects against antibiotic-induced gut microbiome perturbations, promotes gut microbiome structures supportive of healthy body compositions, and is a key factor in glucose control.

Effects of Antibiotics on Glucose Control and the Gut Microbiome

The impact of antibiotic use on metabolism is highlighted in the growing body of evidence linking early-life antibiotic exposure to the development of overweight and obesity later in life,[21] attributed to the antibiotic's perturbation on the gut microbiome.[22] While the relationship between antibiotic exposure in early life and the development of obesity is well-established, recent epidemiologic studies suggest an additional relationship between antibiotic exposure beyond early life with obesity and insulin resistance.[42-44] Overweight and obesity are strong risk factors and physiological triggers for the development of insulin resistance,[45] and previous epidemiological research indicates that the risk of insulin resistance and obesity increases with antibiotic regimens.[42-44] Linking findings from observational and interventional studies that demonstrate an increased risk of insulin resistance due to antibiotic exposure poses challenges. This is primarily because there are a limited number of studies that investigate the effects of repeated real-life, short-term antibiotic exposure on insulin resistance due to ethical concerns, and also due to limitations correlating these interventions to long-term lifetime effects. However, research examining the effect on the gut microbiome of short-term antibiotic exposure may contribute to the growing understanding of the connections between dysbiosis and insulin resistance.

In humans, studies examining the administration of long-term antibiotic regimens in humans for either 20 days or 12 weeks demonstrated either no effects on glycemic control (measured by HbA1c) or worsened insulin sensitivity.[46,47] However, the effects of long-term

antibiotic interventions in human studies may not translate to the effects observed in epidemiologic studies because the most common antibiotic-treated infections are managed over a shorter duration, with the average antibiotic regimen lasting up to 10 days.[48] Therefore, research investigating the effects of individual short-term antibiotic regimens in humans may be more applicable in relating the outcomes observed in epidemiologic studies with outcomes observed in interventional studies.

In humans, short-term antibiotic regimens ranging from 4-7 days have had either no effect[49,50] or harmful effects[51] on insulin sensitivity, fasting glucose, and fasting insulin. Additionally, short-term antibiotic regimens have been shown to perturb the gut microbiome in humans, causing significant reductions in alpha and beta diversity while also modifying microbial composition.[52,53] These changes in taxonomic composition can remain present in the long term, affecting metabolic functions and even increasing the risk of developing antibiotic-resistant bacteria.[54,55] Changes in gut microbiome structure and composition can alter its functional status and metabolome which induces downstream effects on host metabolic physiology, immunity, and signaling pathways.[56] Futhermore, human studies with short-term antibiotic interventions suggest that antibiotic class may play a role in the effects on insulin resistance and antibiotic-induced gut microbiota perturbation. Notably, Reijnders et al illustrated that an antibiotic regimen composed solely of vancomycin produced a significant decrease in insulin sensitivity with accompanied changes in gut microbiota diversity and composition, while amoxicillin had no significant effects on insulin sensitivity or the gut microbiome.[51] Similarly, other human studies demonstrate that antibiotic regimens with vancomycin induced significant changes in the gut microbiota diversity and composition while amoxicillin did not induce any significant changes in the gut microbiome diversity and composition.[49,50,57] However, no significant effects

on insulin sensitivity or glucose control were found with either antibiotic regimen.[49,50,57] The current literature is consistent in demonstrating vancomycin's disruptive effect on the gut microbiome but is conflicting in the associated effects on insulin resistance and glucose control. Thus, the disruption of gut microbiota structure may depend upon antibiotic classification and be liable for downstream metabolic effects on insulin sensitivity and glucose control. This study aims to promote clarity in the literature on Vancomycin's acute effects on glucose control and insulin resistance amidst conflicting findings in the current literature.

Vancomycin is a narrow-spectrum antibiotic that targets gram-positive bacteria.[58] It is often the first-line antibiotic used to treat infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA)[59] and can also be used to treat infections caused by gram-positive bacteria including *Clostridioides difficile* and *staphylococcal* infections.[58] Most recent estimates from the CDC indicate inpatient prescription frequency of vancomycin has increased by 32% from 2006-2012.[60] Vancomycin continues to be widely prescribed for specific local and systemic bacterial infections. Implementing strategies to mitigate or withstand the destabilizing effects of vancomycin on gut microbiome equilibrium and metabolism could potentially lead to more favorable health outcomes in both the short and long term. This study will further the scientific understanding of the metabolic effects of a short-term vancomycin intervention, by measuring its effects on insulin resistance, fasting glucose, and fasting insulin in a population with overweight or obesity with no prior diabetes diagnosis.

Protective Effect of Fiber on Antibiotic-Induced Insulin Resistance and Dysbiosis

While antibiotics have been shown to disrupt the human gut microbiome, research *in vivo* has illustrated the potential for dietary and supplemented fiber to minimize antibiotic-induced gut microbiome disturbances and associated insulin resistance. In mice, fiber supplementation

protected against changes in gut microbiota diversity and composition, intestinal bacterial translocation, *C. difficile* infections, hyperinsulinemia, and insulin resistance.[61-64] Moreover, a fiber-deficient diet in antibiotic-treated mice delayed the stabilization of the gut microbiome when compared to a standard chow diet.[65] The potential protective effects of dietary or supplemented fiber have yet to be observed in humans, therefore, this study will be the first to examine dietary fiber's relationship with antibiotic-induced changes in insulin resistance, fasting glucose, and fasting insulin.

Fiber Intake, Body Composition, and Glucose Control

Dietary fiber by definition cannot be digested by the human digestive system. Some, but not all, fiber molecules are broken down via fermentation by the gut microbiota producing metabolites such as gases, lactate, succinate, and of particular interest, short-chain fatty acids (SCFA).[36] SCFAs are utilized for energy by colonocytes and act as signaling molecules in host metabolic homeostasis, immunological processes, maintenance of the intestinal barrier, neurobiology, skeletal function, and suppression of inflammation and carcinogenesis.[36] Additionally, dietary fiber intake decreases the risk for chronic disease development such as obesity and insulin resistance through selective gut microbial composition changes, diminished activity of starch-degrading enzymes, improved gut epithelial barrier function, reduction in inflammatory pathways, reduction of trimethylamine N-oxide (TMAO) production, and promotion of microbial metabolites such as SCFAs.[66,67] These mechanisms impact human physiological processes by contributing to the gut-brain axis signaling for appetite regulation and reward processing, delaying gastric emptying to promote satiety, inhibiting pathogenic bacteria translocation, regulating nutrient and energy absorption, and modulating bile acid metabolism.[66]

While fiber has many hypothesized mechanisms of action within the gut microbiome for its beneficial effects on body composition,[36,66,67] fiber also has demonstrated physiologic properties that promote improved energy intake control. Higher fat mass is associated with elevated serum leptin levels that lead to leptin resistance.[68] Leptin resistance diminishes postprandial fullness and satiation thereby encouraging increased food intake.[68] Increased short- and long-term fiber intake decreases serum leptin levels in individuals with obesity, thereby reducing leptin resistance and promoting satiety.[69] Decreases in serum leptin levels caused by fiber interventions are attributed to decreasing fat mass and its associated leptin production, increasing the expression of leptin receptors, and stimulating leptin sensitivity. Fiber can also prevent the digestion and absorption of energy-providing dietary macronutrients including protein and fats,[70,71] and may contain antinutritive properties with digestible energy values ranging from -20 to + 10kJ/g dependent upon the dietary fiber source.[72] By adhering to the Recommended Dietary Allowance (RDA), women aged 18-50 are advised to consume 25 g/day of dietary fiber, while men are recommended to intake 38 g/day.[73] This means that the antinutritive impact of fiber can lead to significant energy expenditure, amounting to a maximum of 120 kcals/day for women and 182 kcals/day for men. Supplemented fibers have shown small acute beneficial effects on appetite suppression and acute energy intake, with more beneficial effects from viscous fibers.[74] Satiating effects from fiber may be the result of increasing gastric volume, gastric retention time, small intestinal transit time, and SCFA production that stimulates appetite-suppressing hormones PYY and GLP-1.[75] Obesity arises from and is determined by a chronic disparity between energy intake and expenditure. Dietary and supplemented fiber have demonstrated correlational and interventional evidence to show that fiber intake decreases energy intake and improves body composition parameters. In observational and interventional human studies, higher dietary fiber intake has been consistently associated with decreased

weight, BMI, total fat mass, body fat percentage, and increased lean body mass and lean body percentage.[76-87] This study aims to contribute to the large body of evidence examining the relationship between fiber intake and body composition by using two different body composition measurement methods, air displacement (Bod Pod) and dual X-ray absorptiometry (DEXA). This will allow for precise data and also a variety of body composition variables beyond the most commonly studied body fat and lean mass.

Many longitudinal, cross-sectional, and prospective studies in adults have illustrated the inverse relationship between fiber intake and body fat mass,[76-87] however, few have considered fiber's relationship with lean mass[84,88] and visceral fat mass.[89] Obesity contributes to the accumulation of excess fat around the organs, called visceral fat.[90] Visceral fat accumulation is considered a mechanism for the development of insulin resistance by releasing adipokines that impair insulin sensitivity, inducing lipotoxicity through ectopic lipid accumulation, and the accumulation of macrophages that trigger the release of inflammatory cytokines in metabolically important tissues such as the liver and muscle.[90] Although visceral fat accumulation contributes to the development of insulin resistance, few studies have examined the relationship between fiber, a nutrient that has been inversely associated with insulin resistance, with visceral fat mass. In a previous prospective study, it was observed that for every 10 g rise in soluble fiber consumption within the cohort, there was a dose-dependent reduction in visceral fat mass.[89] Interestingly, total fiber intake was not assessed, and insoluble fiber was not found to significantly affect visceral fat mass.[89] The current study will contribute to the scarcity of research examining the relationships between visceral fat and lean mass, with total fiber intake.

In addition to effects on energy balance, fiber demonstrates physiologic properties that promote improved glucose control via (i) delayed gastric emptying due to changes in luminal viscosity, (ii) increased secretion of glucose-control hormones GLP-1, CCK, and PYY, (iii)

delayed starch metabolism, and (iv) delay of glucose diffusion and absorption.[91] Fiber supplementation interventions in individuals with overweight or obesity who have insulin resistance have been shown to promote improved glucose control and insulin resistance.[92,93] Additionally, higher dietary fiber intake has been associated with decreased fasting glucose, fasting insulin, insulin resistance, and HbA1c alongside increased glucose tolerance and insulin sensitivity.[76,78,94-101]

As a sub-study of a larger clinical trial, we aim to (i) investigate the effect of a short-term antibiotic cycle on insulin resistance and glucose control, (ii) determine for the first time in humans if antibiotic-induced changes in insulin resistance and glucose control are related to total fiber intake, and (iii) explore the relationships between total fiber intake with body composition, baseline acute and chronic glucose control, and baseline insulin resistance in individuals with overweight and obesity. We hypothesize that a short-term antibiotic regimen in individuals with overweight or obesity will increase insulin resistance and that these effects will be related to total fiber intake. Furthermore, we hypothesize that total body fat mass and percentage, visceral fat mass and volume, insulin resistance, and glycemic control (HbA1c) will be negatively correlated with total fiber intake. Finally, we hypothesize that lean mass, fat-free mass (FFM), and FFM percentage will be positively associated with total fiber intake.

CHAPTER III: METHODS

Study Design

This is a sub-study from a larger randomized clinical trial whose main goal is to examine the effect of a next-generation synbiotic on gut and blood microbial composition, body composition, depression, anxiety, food cravings, and glucose control. The primary goal of the sub-study was to evaluate the effects of a 3-day antibiotic intervention on insulin resistance and glucose control. The secondary goal of the substudy was to explore the relationships between fiber intake with body composition, baseline glucose control, and antibiotic-induced changes in glucose control.

Participants with overweight or obesity (25.0-40.0 kg/m^2) who were between 18 and 50 years of age, belonging to any ethnic group, were recruited from TCU and surrounding areas (Figure 2). Participants were screened for inclusion and exclusion criteria through an electronic eligibility survey. Exclusion criteria included: following a vegetarian, vegan, carnivore, or keto diet; pregnancy or planning pregnancy during the study period; lactating women; having a history of inflammatory bowel disease, colon cancer, or chronic polyps; having been diagnosed with T1DM or T2DM; active cancer; currently participating in a weight loss intervention (dietetic or medication); use of antibiotics, antifungals, or antivirals in the last 3 months; currently taking metformin, GLP-1 agonists, insulin, or fiber; having taken PPI, laxatives, probiotics, or immunosuppressants in the last month; history of recent (within 30 days) diarrhea illness (including bacterial or viral enteritis or enteric parasitic infection); having a known hypersensitivity to any component of the study product; having a known allergy to vancomycin; having an acute infection or inflammatory condition over the past 4 weeks; having >10% weight variation in the last 6 months; and history of bariatric surgery in the past. Outcome variables included: fasting glucose and fasting insulin before and after the antibiotic intervention, HbA1c

before the antibiotic intervention, dietary fiber intake before and during the antibiotic regimen, and body composition analyses after the antibiotic intervention (Figure 1).

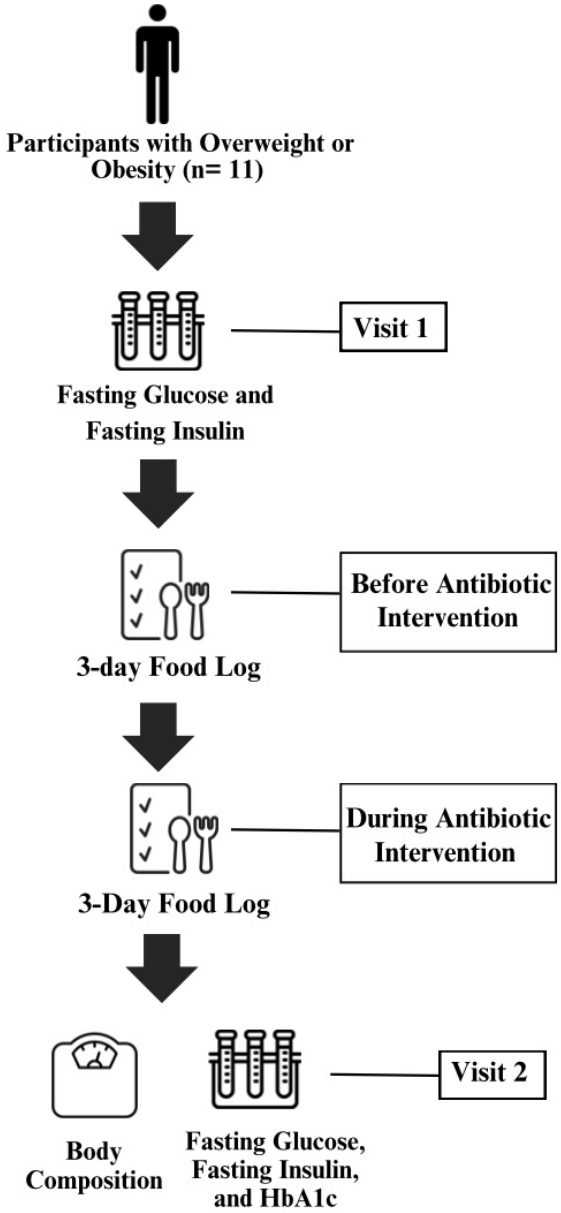


Figure 1. Conceptual framework. Using a pre-test/post-test, we analyzed the effects of a short antibiotic intervention on insulin resistance and whether these effects were modulated by fiber intake. Furthermore, we analyzed the relationships between fiber intake with body composition, insulin resistance, and glycemic control.

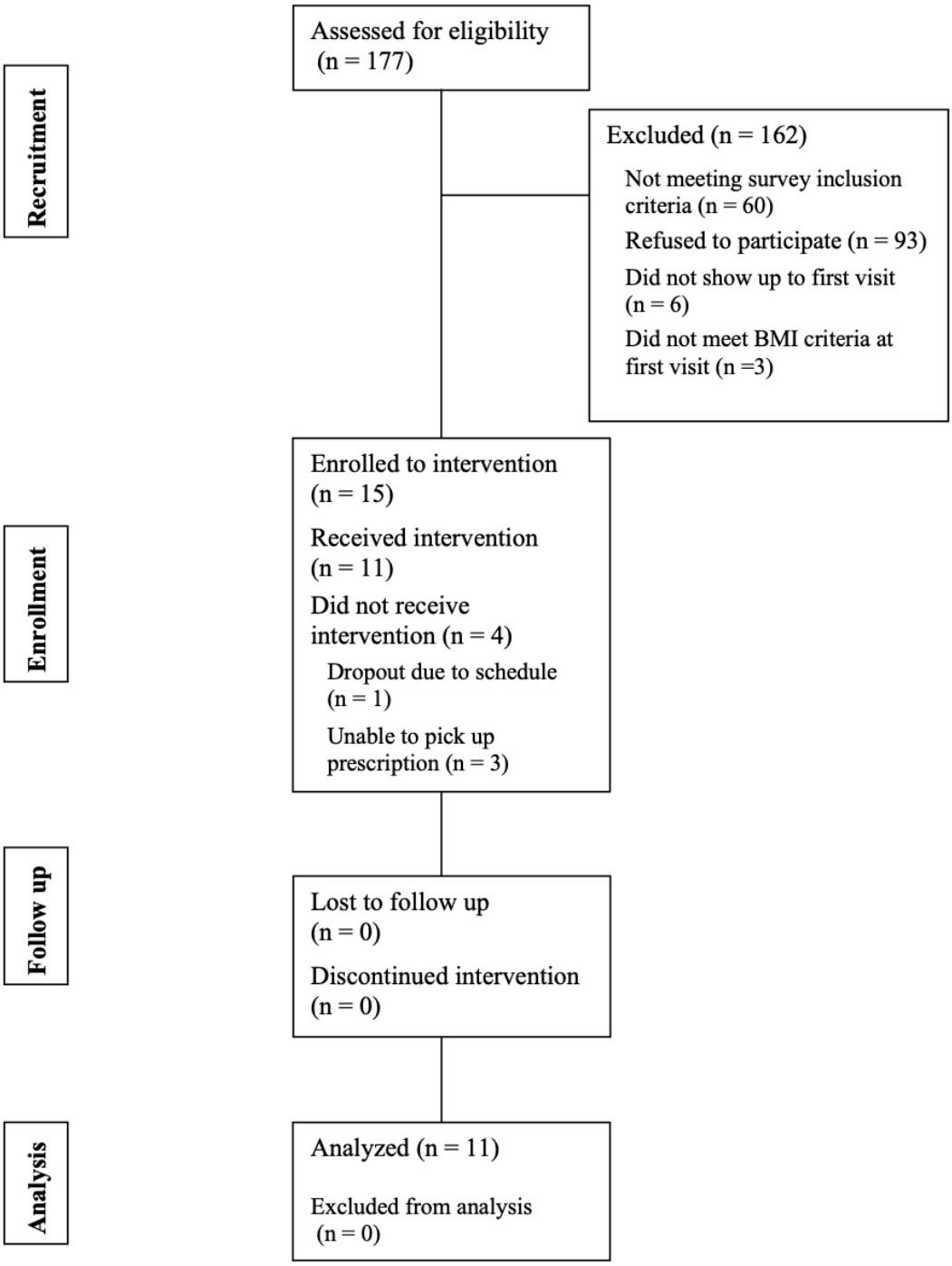


Figure 2. Participant Flow Diagram

Participant Visits

All sub-study data was obtained in a total of two visits per participant. Within the first visit, a 12h fasting blood sample was obtained in which insulin resistance (HOMA-IR) and glycemic control were measured (HbA1c). At the end of the first visit, participants were provided with a 6-day food log (see Appendix A) and a 3-day antibiotic prescription for 500 mg of Vancomycin taken every eight hours. Participants were instructed to record all food and beverages consumed the three days prior to and the three days of the antibiotic intervention (these food log time periods captured the dietary intake of the three days prior to each of the two stool sample collections which took place as part of the main study). Participants were scheduled to complete the second visit within 12-24 hours after finishing the 3-day antibiotic intervention. At the second visit, participants returned their self-administered food logs for nutritional analysis, a second 12h fasting blood sample was taken (HOMA-IR), and two body composition tests were conducted (DEXA and Bod Pod). At this second visit, participants also showed research members the pharmacy ticket of the acquired antibiotic and/or the empty antibiotic bottle to ensure that the antibiotic was obtained on their own and to proceed with antibiotic cost reimbursement ($50 per participant in the form of an Amazon gift card). This study protocol was approved by the Texas Christian University Institutional Review Board for Human Subjects Research as part of a larger randomized clinical trial. Informed consents were obtained from each participant.

Body Composition

Body composition was measured through DEXA and Bod Pod scans. In the DEXA scans, total body fat mass and percentage, visceral fat mass and volume, fat-free mass (FFM), and lean mass were analyzed after completion of the 3-day antibiotic intervention. Before conducting the

DEXA scan, participants were instructed to void their bladder and remove all metal objects and shoes. Female participants who were of child-bearing age were required to complete a pregnancy questionnaire (see Appendix B). Any participants who indicated a possibility of pregnancy were prohibited from receiving a DEXA scan and withdrawn from the study. All DEXA scans were completed by a General Electric Certified DEXA operator. All DEXA scans were analyzed and interpreted by our General Electric Certified DEXA operator to decrease inter-rater error. In the Bod Pod, total fat mass, body fat percentage, FFM, and FFM percentage were similarly analyzed after the completion of the 3-day antibiotic. For this test, participants were required to wear tight clothing, no shoes, and a swim cap to ensure minimal air pockets were trapped within hair, clothing, or shoes that may interfere with the air displacement readings of the Bod Pod.

Blood Samples

Blood samples were taken via venipuncture before and after the 3-day antibiotic intervention. Participants were required to fast for 12 hours before each sample collection (no food or beverages except water). The blood samples were collected using a traditional blood draw needle to extract blood from a vein in the antecubital region of the arm. At each visit, four vials of blood were collected, two of which were used for the sub-study: one 10 mL vial for attaining the serum that was used to measure fasting insulin and fasting glucose (used to calculate HOMA index), and one EDTA 3mL vial for attaining the plasma that was used to measure HbA1c. Both vials were collected and centrifuged at the Exercise Physiology Laboratory and then transported to BioReference for analysis.

Diet

Participants' food logs were transcribed into the ESHA Food Processor Nutrition Analysis Software to complete a full dietary compositional analysis. All food logs (n= 22) were analyzed by a single research member to decrease potential inter-rater differences in data

interpretation. For this study, the average value of caloric intake, daily fiber, insoluble fiber, and soluble fiber intake were utilized for statistical analyses.

Statistical Methods

AIM1: To investigate the effect of a short-term antibiotic regimen on insulin resistance and glucose control we used a paired T-test comparing baseline to post-intervention fasting glucose, fasting insulin, and HOMA-IR values. Prior to conducting the t-tests, we assessed our data to ensure satisfaction of assumptions. Specifically, we confirmed that: 1) our dependent variables (fasting glucose, fasting insulin, and HOMA-IR) were continuous variables; 2) each paired measurement was acquired from the same subject; 3) the dependent variables exhibited normal distribution, as confirmed by Q-Q plots; and 4) the dependent variables did not include outliers, as suggested by analysis of BoxPlots.

AIM 2: To explore for the first time in humans if antibiotic-induced changes in insulin resistance and glucose control are related to total fiber intake we used Pearson and Spearman correlation coefficients for parametric and non-parametric data respectively. Prior to conducting correlation analyses, we assessed our dataset to determine which variables were suitable for Pearson versus Spearman analyses. This involved several steps: 1) confirming that variables were either on a continuous scale (for Pearson) or an ordinal scale (for Spearman); 2) examining scatterplots with lines of best fit to identify any linear relationships between the variables; 3) using BoxPlots to identify any spurious outliers; and 4) employing Q-Q plots to assess the normal distribution of the data.

AIM 3: To explore the relationships between total fiber intake with body composition, baseline acute and chronic glucose control, and baseline insulin resistance in individuals with

overweight and obesity, we used Pearson and Spearman correlation coefficients respecting the assumptions indicated in AIM 2. SPSS was utilized for all statistical analysis. A p-value of <0.05 was considered statistically significant.

CHAPTER IV: RESULTS

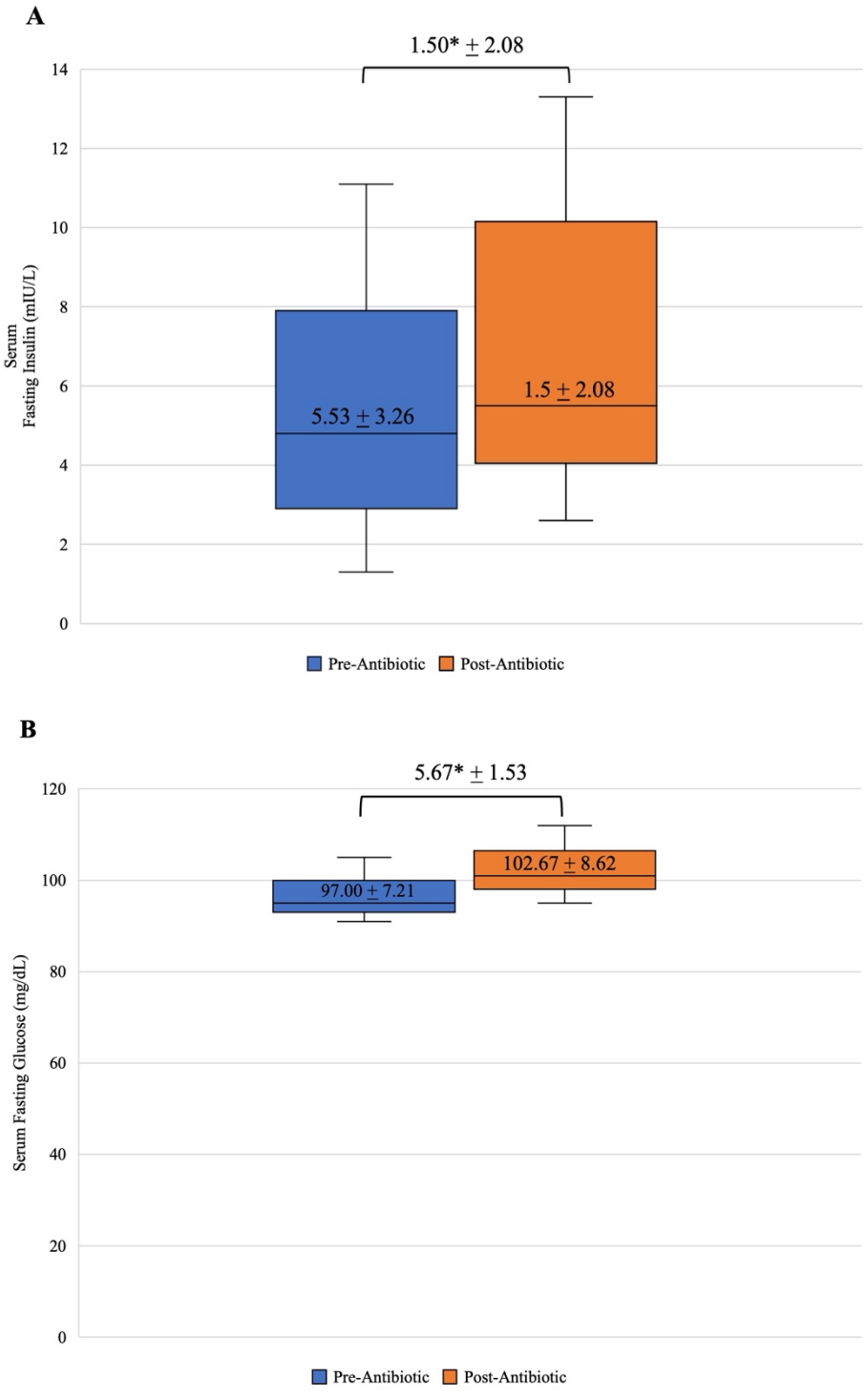
A total of 11 participants finalized the two visits pertaining to the substudy. Both sexes were well represented in the sample (Table 1). The baseline average glucose control parameters including HbA1c, fasting glucose, fasting insulin, and HOMA-IR, all fell within normal clinical ranges[102] and therefore did not introduce an additional variable that may affect changes in glucose control levels and insulin resistance (Table 1). Serum fasting glucose levels (and thus HOMA-IR) were only obtained from 3 participants due to commercial laboratory miscommunication regarding serum sample processing.

Table 1: Characteristics of Participants at Baseline

Baseline characteristic	Value (n= 11)
Gender	
Female	55%
Male	45%
Race	
White	55%
Hispanic or Latino	18%
Asian	9%
Two or more races	18%
Age (years)	27 $\pm$ 9.2
BMI (Kg/m^2)	28.6 $\pm$ 2.7
Fasting Glucose (mg/dL)	97.0 $\pm$ 7.2[a]
Fasting Insulin (mIU/L)	5.5 $\pm$ 3.3
HOMA-IR	1.3 $\pm$ 0.60[a]
HbA1c (%)	5.0 $\pm$ 0.61

[a] Sample size of 3

As part of our aim 1, we investigated the effect of a short-term antibiotic cycle on insulin resistance and glucose control finding a significant increase in fasting insulin (p= 0.037) and fasting glucose (p= 0.023) in response to the antibiotic intervention (Figure 3). The antibiotic intervention, however, did not significantly affect HOMA-IR levels.



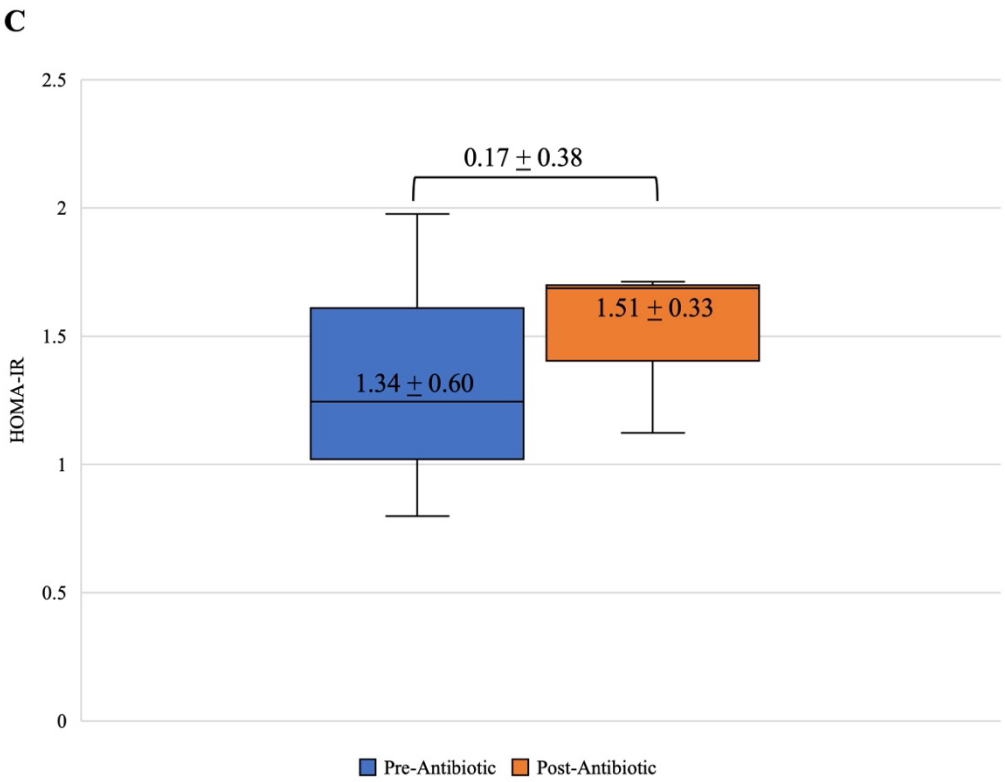


Figure 3: Effect of a 3 short-term antibiotic cycle on glucose control and insulin resistance. Pre-antibiotic (blue color) and post-antibiotic (orange color) levels of (**A**) fasting glucose (n=3), (**B**) fasting insulin (n=11), and **(C)** HOMA-IR (n=3) are shown. Values are given as mean ± SD. *p <0.05

As part of our aim 2, we evaluated for the first time in humans if antibiotic-induced changes in insulin resistance and glucose control were related to total fiber intake, however, we saw no evidence of a relationship between total fiber intake and glucose control. Similarly, as part of our aim 3 we explored the relationships between total fiber intake with body composition, baseline acute and chronic glucose control, and baseline insulin resistance in individuals with overweight and obesity, finding no significant relationships between total fiber intake and body fat mass, body fat percentage, fat-free mass, lean mass, visceral fat mass, or visceral fat volume irrespective of the method used to analyze body composition (DEXA versus BodPod) (Table 2 and Table 3).

Pearson Correlation Coefficients	Total fiber (g)	HbA1c (%)	Baseline Fasting Insulin (mIU/L)	Fasting Insulin Change (mIU/L)
Total Fiber (g)	1.000	0.240	-0.199	-0.319
HbA1c (%)	0.024	1.000	0.561	0.111
Baseline Fasting Insulin (mIU/L)	-0.119	0.561	1.000	0.175
Fasting Insulin Change (mIU/L)	-0.319	0.111	0.175	1.000
Bod Pod Body Fat Mass (kg)	0.314	0.510	0.159	-0.970
Bod Pod Fat-Free Mass (kg)	-0.051	-0.385	-0.389	-0.170
DEXA Body Fat Mass (kg)	0.247	0.448	0.517	-0.002
DEXA Fat-Free Mass (kg)	-0.065	-0.476	-0.620*	-0.286
DEXA Lean Mass (kg)	-0.075	-0.479	-0.626*	-0.277
DEXA Visceral Fat Mass (kg)	-0.126	0.270	0.296	0.121
DEXA Visceral Fat Volume (kg)	-0.125	0.271	0.296	0.121

Table 2: Pearson correlations among fiber intake, body composition, and glucose control. Daily total fiber (g), baseline fasting insulin (mIU/L), fasting insulin change (mIU/L), baseline HbA1c (%), Bod Pod free fat mass (kg), DEXA body fat mass (kg), DEXA free fat mass (kg), DEXA lean mass (kg), DEXA visceral fat mass (kg), and DEXA visceral fat volume (in^3) realtionships are shown using Pearson correlation coefficients

Spearman Correlation Coefficients	Total fiber (g)	HbA1c (%)	Baseline Fasting Insulin (mIU/L)	Fasting Insulin Change (mIU/L)
Bod Pod Body Fat Percentage (%)	0.237	0.478	0.333	-0.091
Bod Pod Fat-Free Mass Percentage (%)	-0.237	-0.478	-0.333	0.091
DEXA Body Fat Percentage (%)	0.437	0.315	0.564	0.059

Table 3: Spearman correlations among fiber intake, body composition, and glucose control. Daily average daily total fiber intake (g), baseline HbA1c (%), baseline fasting insulin (mIU/L), fasting insulin change (mIU/L), Bod Pod body fat percentage (%), and DEXA body fat percentage (%) realtionships are shown using Spearman correlation coefficients

CHAPTER V: DISCUSSION

Effects of Antibiotics on Glucose Control

The present study demonstrated that treatment with a three-day antibiotic cycle of Vancomycin can induce significant increases in fasting insulin and fasting glucose. Insulin resistance (HOMA-IR), however, was not affected in response to the antibiotic treatment which could be the result of the small sample size for this particular variable (n= 3). Our study demonstrated the potential for antibiotics to cause acute harmful effects on glucose regulation. These findings align with the previous study completed by Vrieze *et al.* that demonstrated the ability of short-term antibiotic administration, particularly Vancomycin, to cause harmful effects on insulin sensitivity in adults with obesity and metabolic syndrome.[51] Vrieze also discriminated that while Vancomycin induced harmful effects on glucose regulation, Amoxicillin did not. [51] Although our study did not investigate other antibiotic agents, it did corroborate existing evidence regarding Vancomycin's potential for adverse effects on glucose regulation. Furthermore, it underscored the importance of conducting studies that assess multiple antibiotics to differentiate which ones may contribute to insulin resistance and glucose dysregulation.

The findings from this study contradict previous findings from longer human antibiotic interventions that showed no effect on glucose control or insulin resistance in populations with insulin resistance or prediabetes in addition to obesity.[49,51] This suggests that baseline glucose dysregulation may be a confounding variable on the effects of antibiotic interventions on glucose control parameters and insulin sensitivity. Obesity is a risk factor for insulin resistance and glucose dysregulation,[45] and Vancomycin theoretically may perturb glucose metabolism enough to affect a vulnerable population and induce harmful effects as seen in the current study. Our study detected significant glucose effects with a comparably shorter intervention duration (3 days) compared to previous antibiotic interventions with longer durations (4-7 days).[49-51] This

sets a novel minimum antibiotic duration threshold that has shown to induce signficiant disruptions in glucose regulation.

Interestingly, HOMA-IR did not have a significant change post-antibiotic intervention despite significant changes in its formula constituents, fasting glucose, and fasting insulin. This result may be due to the reliance on a small group of available fasting glucose values (n= 3), limitating its power to detect significant differences. Fasting insulin had significant and more consistent changes likely due to a larger sample size (n=11). Meanwhile, fasting glucose elicited a significant change despite a small sample size (n=3), demonstrating the potential for a large effect size once a larger sample size is obtained. Overall, this sub-analysis of data found significant harmful effects of antibiotics on glucose regulation in individuals with overweight or obesity who have no diabetes diagnosis. Furthermore, our data exposed the need for research examining (i) antibiotic agent-specific effects on glucose regulation, (ii) the differential effects of antibiotics on glucose regulation in populations with vs. without insulin resistance, and (iii) the potential mechanisms of actions through which antibiotics exert their glucoe regulating effects (i.e. gut microbiome).

Protective Effect of Fiber on Antibiotic-Induced Insulin Resistance and Dysbiosis

No relationships between the daily average total fiber intake and antibiotic-induced changes in fasting insulin were shown in correlational analyses. Assessing the potential moderating impact of fiber intake on antibiotic-induced alterations in glucose regulation while controlling for confounding factors was unfeasible due to the limited sample size. However, we plan to conduct this analysis upon completion of the main study.

Previous studies showing the protective effects of fiber against antibiotic-induced disruptions on the gut microbiota and glucose dysregulation were conducted in murine models[61-

[65] and therefore the translation to the minimum fiber dosage to produce a protective effect is difficult to compare directly to human research. Every participant's average daily total fiber intake was below the recommended daily allowance for males (38 g/day) and females (25 g/day) set by the United States Department of Agriculture.[103] On average, females met 65.5% of the RDA for fiber, while males met only 38.5%. In mice, the absence of dietetic fiber significantly elevated compositional disturbances in the gut microbiome caused by antibiotics.[65] The current sub-analysis did not explore the impact of the antibiotic on gut microbiota. However, upon completion of the main study, gut microbial composition will be analyzed. Our preliminary findings suggest a significant adverse effect of antibiotics on glucose regulation, which theoretically could be mitigated by sufficient fiber intake, as supported by animal studies. Nevertheless, the insufficient fiber intake observed in our participants, ranging from 9.0 to 20.8 g/day with an average of 15.58 g/day, may mask a potential protective effect of fiber on insulin resistance following antibiotic treatment. Therefore, future investigations should include participants with both adequate and inadequate fiber intake to ascertain whether fiber adequacy yields divergent effects on glucose dysregulation post-antibiotic intervention.

Fiber Intake, Body Composition, and Glucose Control

Similarly, no significant relationships were observed between fiber intake and body composition or glucose control parameters. Although not the aim of the current study, other interesting findings were observed. Specifically, a significant strong, inverse correlation was found between fasting insulin levels and FFM/lean mass (DEXA) meaning that higher FFM levels were associated with lower insulin concentrations. A similar strong, inverse relationship was found between insulin levels and caloric intake which could be sex-mediated. Nonetheless, control for potential confounding variables was not possible in this sub-analysis of data due to the small sample size. Visceral fat mass and volume were correlated with total body fat mass,

which is supported in the literature demonstrating the increased risk for visceral fat accumulation with obesity and excess total body fat.[90]

Strengths and Limitations

As this substudy is a component of a broader, ongoing project, its design was tailored to distinct objectives, resulting in notable limitations in the subanalysis of data. Firstly, the lack of a control group limits our ability to rule out random effects when comparing baseline and post-intervention data. Although participants are instructed to not change their lifestyle through the duration of the study, we cannot dischard the possibility that changes in fasting glucose, fasting insulin, and insulin resistance, measured within <1 week apart, may also reflect changes in physical activity and other covariates not measured and controlled for in this study. Secondly, the small sample size limits the statistical power to detect significant changes and associations. A lab miscommunication error limited the available fasting glucose samples for analysis (n=3) and prevented the analyses of eight acquired values contributing to the changes of fasting glucose and HOMA-IR. This commercial laboratory error further limited our ability to examine fasting glucose and insulin resistance changes with a stronger sample size. While strong statistical techniques were used, significant associations may not have been detected due to this limitation. Thirdly, a short antibiotic regimen of Vancomycin may produce alternative effects when compared to longer antibiotic interventions and compared to other antibiotics.[51] Thus, further research is warranted to identify antibiotic-specific effects on metabolic parameters and gut microbiome composition. Lastly, the lack of accompanying gut microbiota compositional and metabolomic analysis restricts our ability to determine whether changes in fasting glucose, fasting insulin, and insulin resistance were associated with compositional and functional metabolic changes in the gut microbiome caused by the antibiotic. This study, however, possesses several strengths: 1) this was the first human study analyzing the potential relationship

between fiber intake and glucose regulation following an antibiotic intervention, 2) body composition was measured by two reliable body composition equipments, 3) nutritional information was obtained prospectively and thus the accuracy of the information is considered to be higher compared to retrospective logs, and 4) both food logs and body composition scans were analyzed by a single research member each to decrease inter-rater error.

CONCLUSION

The present study illustrated that a short-term antibiotic cycle of Vancomycin has significant effects on acute glucose control in adults with overweight or obesity without diabetes. Fiber intake did not show a significant relationship with either antibiotic-induced changes in glucose regulation biomarkers or with baseline body composition parameters. However, the current preliminary analysis of data consisted of a small sample size that limits the statistical power to detect significant differences and correlations. Hence, upon completion of our primary study and analysis of larger sample size, we will repeat all analyses to ascertain whether humans exhibit protection against antibiotic-induced disruptions in glucose control in response to fiber intake, in comparison to findings from previous murine studies. Overall, this study supports previous human studies using longer antibiotic durations in which Vancomycin has shown harmful effects on insulin sensitivity and manifests the need for further research on the prevention and treatment of antibiotic-induced perturbations in glucose control.

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APPENDICES

APPENDIX A

24h Food Log

Instructions: Fill out the 24h food logs reporting everything you eat and drink for **3 days prior to the stool sample collection** providing as much information as possible. Use a different sheet for each day. You may write in the back of the sheet for that day (if needed). Please be specific, follow the provided example. Fill it out by hand, and bring it the day of your appointment. Contact Jessica Mrosla or Dr. Marroquin if you have questions (J.MROSLA@tcu.edu or E.Marroquin@TCU.edu)

ID: Example				
Date:				
Time	**Amount (Specify household measure or units)**	**Ingredients**	**Brand**	**Preparation method**
8 am	2 slices	100% whole wheat bread	Nature's Own	Grilled
	1 slice	Lean, smoked ham	Oscar Mayer	Grilled
	1 slice	American cheese	Kraft (singles)	Grilled
	1 slice	tomato		
	1 cup	Whole milk	Great value	
11 am	1 piece (big)	Red apple	Fuji	
12 pm	2 piece	Chicken legs	Popeyes	Breaded
	1 can	Diet coke	Coca Cola	

APPENDIX B

Pregnancy Questionnaire
Texas Christian University Exercise Physiology Lab

ID: _____

Participation in this study involves the use of low levels of x-ray to determine bone density and body composition. As discussed in the Consent Form, this risk is minimal; however, pregnant women should not undergo this procedure. Radiation may harm a developing fetus. Therefore, it is important for us to determine whether you are pregnant, or if you believe there is a chance that you are pregnant. Please respond below by checking the response that applies to you, and then sign this form.

A. I am not pregnant _____

B. There is a possibility that I could be pregnant _____

C. I am pregnant _____

First day of last menstrual cycle: _____

_____ Date: _____
Signed

_____ Date: _____
Witness

VITA

Jessica Marie Mrosla was born July 24, 2001, in Irving, Texas. She is the daughter of Laura Elizabeth Mrosla and Nathan Michael Mrosla. A graduate of Lakeville North High School, Lakeville, Minnesota, she completed her requirements for a Bachelor of Science degree with a major in Dietetics from Texas Christian University in 2023. During her time pursuing her Bachelor of Science, she was accepted into the Combined Program in Dietetics at Texas Christian University in 2021.

Throughout her undergraduate academic career, she has been an Army ROTC cadet in the 73rd Horned Frog Battalion. She will be commissioned into the Army Reserve as an Officer, with the rank of 2nd Lieutenant, in May 2024.

Upon completion of requirements for her Bachelor of Science degree in Dietetics from Texas Christian University in 2023, she continued her education at Texas Christian University to pursue her Master of Science in Dietetics. While working on her Bachelor of Science and Master of Science degrees at Texas Christian University, she has completed internships accredited by the Accreditation Council for Education in Nutrition and Dietetics in community nutrition, food service management, and clinical nutrition. She is continuing to work towards completing all required competencies to become eligible to take the Registered Dietitian exam. She is a member of the Academy of Nutrition and Dietetics and Phi Upsilon Omicron National Honor Society.

ABSTRACT

IMPACT OF A SHORT-TERM ANTIBIOTIC CYCLE
ON GLUCOSE CONTROL IN ADULTS WITH
OVERWEIGHT OR OBESITY

by Jessica Mrosla
Department of Nutritional Sciences
Texas Christian University

Thesis Advisor: Elisa Marroquin, Assistant Professor of Nutritional Sciences and Director of Graduate Studies

Epidemiologic evidence suggests that antibiotic frequency increases insulin resistance and reduces glucose control. However, interventional human studies have produced conflicting results on the effects of short-term antibiotic cycles on glucose control, demonstrating either no effect or harmful effects on glucose control. Our study aimed to contribute to the current body of literature examining the effects of short-term antibiotic cycles on glucose control in order to better clarify and understand the accumulated exposure risk of chronic insulin resistance and glucose dysregulation. Despite a small sample size (n=11), a short antibiotic cycle (3 days of therapeutic dosge of Vancomycin) significantly increased serum fasting insulin (p=0.037) and fasting glucose (0.023) levels from baseline. Our study indicates that short antibiotic cycles can induce acute harmful effects and warrants for further research examining the chronic and accumulated risk of antibiotic exposures. Furthermore, this study highlights the need for research on methods to prevent acute antibiotic-induced glucose control disruption.