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Effect of Prebiotic Fiber Intake on Adiposity and Inflammation in Overweight and Obese Children: Assessing the Role of the Gut Microbiota

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Effect of Prebiotic Fiber Intake on Adiposity and Inflammation in Overweight and Obese
Children: Assessing the Role of the Gut Microbiota

by

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A THESIS

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Abstract

Pediatric obesity is a serious national and global health challenge. Interventions aimed at improving obesity and its metabolic complications in children are critically needed. The aim of this thesis was to assess the effects of 16 weeks of oligofructose-enriched inulin consumption on overweight and obese children 7-12 years old. Compared to placebo, prebiotic fiber consumption normalized body weight gain and significantly reduced body weight Z-score, total body fat and trunk body fat percent. Inflammatory markers interleukin-6 and C-reactive protein were numerically reduced within the prebiotic fiber group, albeit not statistically significantly. Changes in the gut microbiota, specifically significant increases in *Bifidobacterium* spp., were observed with prebiotic fiber and may represent, a mechanism for the metabolic benefits of prebiotic fiber consumption. While future studies are needed, this work highlights a promising role for prebiotic fiber as a dietary obesity intervention in the pediatric population.

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Dedication

This thesis is dedicated to my mother and father for their constant love and support. They deserve all of my successes, even more than I do, because without them my successes would have never happened. From the second I can remember, no matter what, they let me grow to be my own person, learn to make my own mistakes and I would not be me without them. They have trusted me to make my own decisions, but were there for me if I needed guidance. They always made sure that no matter what I decided, I was happy. I especially want to thank them for everything they have done while I have been away from home. Even with the distance and the time difference they learnt about my research so they could edit my work, were always there to talk to, sent me messages of support and faith and most importantly always made sure that I knew my own potential. Words cannot thank them enough for everything they have done for me.

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List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
α	Alpha
β	Beta
γ	Gamma
CVD	Cardiovascular disease
T2D	Type 2 diabetes
SCFA	Short chain fatty acids
TLR	Toll-like receptor
LPS	Lipopolysaccharide
FFA	Free fatty acids
LDL-C	Low-density lipoprotein cholesterol
TG	Triglycerides
HDL-C	High-density lipoprotein cholesterol
GI	Gastrointestinal
CONV-R	Conventionally raised
GF	Germ free
qPCR	Quantitative polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
FB	Firmicutes to Bacteroidetes
BMI	Body mass index
LPL	Lipoprotein lipase
<i>Fiaf</i>	Fasting-induced adipose factor
TNF	Tumor necrosis factor
mRNA	Messenger RNA
IL	Interleukin
MCP	Monocyte chemoattractant
CRP	C-reactive protein
GLUT4	Glucose transporter type 4
IR	Insulin receptor
IRS	Insulin receptor substrate
GPR	G-protein coupled receptor
GLP	Glucagon-like peptide
DXA	Dual-energy-x-ray absorptiometry
A: G	Android to gynoid fat ratio
DNA	Deoxyribonucleic acid
IFN	Interferon
HOMA2-IR	Homeostatic model assessment for insulin resistance 2
QUICKI	Quantitative insulin sensitivity check index
SEM	Standard error of the mean

ANCOVA
RM ANOVA
CHREB
BF
EU
Prop.
MET
IFG
CI
df

Analysis of covariance
Repeated measures analysis of variance
Conjoint Health Research Ethics Board
Body fat
Endotoxin units
Proportion
Metabolic equivalent
Impaired fasting glucose
Confidence interval
Degrees of freedom

Epigraph

“They’re invisible. They’re everywhere. And they rule.”

- Nathan Wolfe “Microbes: Small, Small World”, National Geographic 2013

CHAPTER ONE: INTRODUCTION

Obesity is defined as an abnormal or excessive fat accumulation that may impair health (World Health Organization, 2000). There are inter-individual differences in the amount of excess fat and the distribution of the fat within the body, varying the risk for obesity-associated comorbidities. These comorbidities include cardiovascular disease (CVD) and type 2 diabetes (T2D) which along with obesity are the most common, current non-communicable diseases (World Health Organization, 2014). There is an increased prevalence of obesity in both developed and developing countries and unfortunately, this increase is not only limited to the adult population, but is also observed in children and adolescents (World Health Organization, 2000). Globally in 2013, approximately 42 million children were classified as overweight or obese (World Health Organization, 2015). Since excess adiposity tends to persist into adulthood, these children are at an increased risk of developing obesity-associated comorbidities (Serdula et al., 1993). What is concerning is that these comorbidities, which were once primarily diagnosed in adults, are now also increasingly detected in children and adolescents (Weiss & Caprio, 2005; World Health Organization, 2015). Specifically, components of the metabolic syndrome such as insulin resistance and dyslipidemia which contribute to CVD and T2D are observed. (Weiss & Caprio, 2005).

Positive changes in energy balance are associated with obesity, and factors beyond the reduction in energy consumption and increase in energy expenditure contribute to restoring the energy balance (DiBaise et al., 2008). One such factor that has been of particular interest in the past decade is the role of the microorganisms that reside in the gut which are collectively known as the gut microbiota (Bäckhed et al., 2004). Advancements in gut microbiota research highlight

its importance in affecting metabolism and metabolic diseases such as obesity. The gut microbiota have genes that code for enzymes which are absent from the human genome and are involved in the digestion and metabolism of carbohydrates which the host would have been unable to digest otherwise (Kovatcheva-Datchary et al., 2009; Scott, Gratz, Sheridan, Flint, & Duncan, 2013). These dietary carbohydrates are substrates for the gut microbiota where they are metabolized and fermented into short chain fatty acids (SCFA) (Tan et al., 2014). Within the host, there are various roles for the SCFAs such as providing energy for the colonocytes, and regulating the release of satiety hormones and inflammatory cytokines. Different microbial profiles result in varying concentrations of SCFAs and lead to different host responses, implicating the microbial profile in human metabolic health. Therefore, development of interventions aimed at shifting the microbial profile to provide health benefits is currently a major research focus.

Obesity is not only associated with excess adipose tissue, but is associated with a low-grade chronic inflammatory state (Gregor & Hotamisligil, 2011). Excess adipose tissue contributes to the release of inflammatory cytokines, termed adipokines, which induces a physiological response. Adipose tissue is also comprised of macrophages which are immune cells that infiltrate adipose tissue in obesity (Weisberg et al., 2003). On the surface of adipocytes and macrophages are toll-like receptors (TLR) 4, which when activated promote the inflammatory pathways resulting in the release of the inflammatory cytokines (Kopp et al., 2010; Shi et al., 2006). The resulting inflammation is linked to the development of numerous metabolic comorbidities associated with obesity. Lipopolysaccharides (LPS) from the coat of Gram-negative bacteria are an endogenous ligand for TLR4 and induce the inflammatory response (Glauser, Heumann, Baumgartner, & Cohen, 1994; Shi et al., 2006). Elevated levels of plasma LPS, defined as metabolic endotoxemia, are observed after the consumption of a high-fat diet and increased energy

intake, resulting in the release in inflammatory cytokines (Cani, Amar, et al., 2007). The mechanisms by which high-fat diet promotes metabolic endotoxemia is through increasing the permeability of the intestine by decreasing the expression of endothelial tight junctions (Cani, Bibiloni, Knauf, Neyrinck, & Delzenne, 2008). Metabolic endotoxemia induced by high-fat diet was reversed after antibiotic treatment highlighting a role for the gut microbiota. Inflammation in obesity is linked to insulin resistance which is a precursor for T2D (Kim & Caprio, 2011). In particular, inflammatory cytokines have proposed roles at the level of insulin signaling and at the levels of glucose uptake (Hotamisligil, Budavari, Murray, & Spiegelman, 1994; Hotamisligil, Murray, Choy, & Spiegelman, 1994). The importance of reducing insulin resistance is not only to mitigate the development of T2D, but also because insulin resistance is a risk factor for CVD and is linked to dyslipidemia, a change in the blood lipid profile from that of a healthy state (Caprio et al., 1996; Klop, Elte, & Cabezas, 2013). Insulin is an anabolic hormone that stimulates lipogenesis and inhibits lipolysis. Hence, insulin resistance causes a dysregulation in insulin's action thus increasing lipids in the blood (Saltiel & Kahn, 2001). Conversely, dyslipidemia can induce insulin resistance through the actions of free fatty acids (FFA) (Boden, 2011). The increased FFAs, which are already elevated in obesity due to the enlarged adipocytes, are ligands for the TLR4 receptors inducing an inflammatory response and triggering insulin resistance (Shi et al., 2006; Song, Kim, Yoon, & Kim, 2006). Obesity-associated inflammation is therefore the proposed link between insulin resistance and dyslipidemia. Therefore, attenuating the inflammatory response can improve obesity associated comorbidities.

With the recent insights into the role of the gut microbiota and its roles in metabolic diseases, the development of interventions that could beneficially alter one's microbial profile resulting in improved host health are of particular interest (Delzenne, Neyrinck, & Cani, 2013).

Prebiotic fiber is one such dietary intervention that is unable to be digested by the host and enters the large intestine where it acts as a substrate for the gut microbiota (Roberfroid, 2002). It is selectively fermented by known beneficial bacteria, such as bifidobacteria, potentially reversing the dysbiosis in the gut thereby conferring health benefits (Gibson & Roberfroid, 1995; Gibson et al., 2010). The most common prebiotic fibers are the inulin-type fructans, particularly inulin and oligofructose (Roberfroid, 2007a). Prebiotic fiber consumption has been shown to reduce fat mass in rodent models of obesity and in overweight and obese adults (Cani et al., 2006, 2009; Cani, Neyrinck, et al., 2007; Dewulf et al., 2013; Everard et al., 2013; Parnell & Reimer, 2009; Pyra, Saha, & Reimer, 2012). Risk factors for obesity, metabolic endotoxemia and the subsequent inflammatory response, have also been reduced after prebiotic fiber consumption in both rodent models and in a human clinical trial (Cani et al., 2009; Everard et al., 2011; Lecerf et al., 2012). Prebiotic fiber as a potential treatment option for obesity and obesity-associated comorbidities has been assessed in rodent models and adult humans. However, with the prevalence of childhood obesity, there is a necessity to develop treatment options that can be easily tailored to the child population and are accessible to children of all socioeconomic statuses, and prebiotic fiber is one such treatment option.

The purpose of this thesis was to assess the effect of oligofructose-enriched inulin prebiotic fiber consumption on improving anthropometric measures, adiposity, inflammation, blood lipids, fasting glucose, fasting insulin and indices of insulin resistance in overweight and obese children 7-12 years of age. It also aimed to evaluate the role of the gut microbiota as a mechanism by which these changes occur. The primary objective was to determine the effect of prebiotic fiber consumption on body composition. The secondary objective was to determine the effect of the treatment on levels of inflammatory cytokines, serum lipid profile and on measures and indices of

insulin resistance. The final objective was to determine if gut microbial changes induced by prebiotic fiber consumption was the mechanism by which prebiotic fiber elicits its beneficial effects. The overall hypothesis is that prebiotic fiber consumption in overweight and obese children will reduce adiposity and improve the risk for obesity-associated comorbidities as a result of the beneficial microbial changes.

This thesis contains five chapters. Chapter One provides a general introduction to the thesis, the objectives and hypothesis of the work. Chapter Two is a review of the relevant literature and evidence for the use of prebiotic fiber supplementation as a potential dietary intervention in obesity and obesity-associated comorbidities. The role for the gut microbiota in obesity and the benefits in altering the microbial profile are also highlighted. Chapter Three describes the methodology utilized for the thesis work. Chapter Four presents the results from the randomized clinical trial. Chapter Five is a discussion of the primary results including study limitations, future directions and overall conclusions. References are listed at the end of the thesis document.

CHAPTER TWO: LITERATURE REVIEW

2.1 The Global Obesity Epidemic

The dramatic rise in local, national and global obesity is increasing the need for the development of preventative tools and interventions that are accessible to those of all ages and socioeconomic statuses. Obesity is simply defined as an abnormal or excess increase in adipose tissue such that health may be impaired (World Health Organization, 2000). It increases one's risk for diseases such as, but not limited to, cardiovascular disease (CVD), type 2 diabetes (T2D) and certain cancers (World Health Organization, 2000). The increased obesity prevalence is not only observed in the adult population but is also unfortunately observed in the child population such that globally there was a 47.1% increased prevalence in children classified as overweight or obese between 1980 and 2013 (World Health Organization, 2015). Currently in Canada, approximately one-third of children and youth aged 5 to 17 are classified as overweight or obese (Roberts, Shields, de Groh, Aziz, & Gilbert, 2012). This increased adiposity in childhood tends to persist into adulthood; with approximately one-half of overweight adolescents and over one-third of overweight children remaining obese as adults, their risk for obesity-associated comorbidities being even further increased (Serdula et al., 1993; Weiss & Caprio, 2005).

Of considerable concern is that obesity-associated comorbidities such as T2D and components of the metabolic syndrome, for instance increased levels of fasting glucose, fasting insulin and indices of insulin resistance, which were once primarily associated with obesity in adults, are increasingly observed in the obese child population (Weiss & Caprio, 2005). Overweight and obese children are also increasingly observed with elevated levels of blood lipids

and low-density lipoprotein cholesterol (LDL-C) which are risk factors for CVD (Caprio et al., 1996). Increased blood lipids such as fasting total cholesterol, triglycerides (TG) and LDL-C are significantly higher in obese adolescents compared to non-obese. In particular, intra-abdominal fat was positively correlated with fasting TG and negatively correlated with fasting high-density lipoprotein cholesterol (HDL-C) in these obese children (Caprio et al., 1996). Many of the health complications associated with obesity are related to these physical comorbidities and therefore they are important markers when analyzing the health of those who are overweight and obese, especially children.

Low self-esteem, poor emotional well-being, depression, anxiety and disordered eating behaviours along with other psychosocial comorbidities are also increasingly observed in overweight and obese children (Kalra, De Sousa, Sonavane, & Shah, 2012). Overweight and obese children had a lower overall health-related quality of life when compared to children and adolescents of a healthy weight with a health-related quality of life comparable to children who have been diagnosed with cancer and are currently receiving chemotherapy (Schwimmer, Burwinkle, & Varni, 2003). Therefore treatments targeted to the child population are necessary to combat the obesity epidemic.

2.2 The Role of the Gut Bacteria

2.2.1 Energy Balance in Obesity

Obesity is associated with changes in energy balance; there is an increase in energy intake, a reduction in energy expenditure and alterations in energy storage (DiBaise et al., 2008). Factors

beyond solely reducing calorie consumption and increasing energy output contribute to restoring the energy balance in obesity. One such factor is the microbes that inhabit the gut which are collectively called the gut microbiota. Over the past decade, the roles of the gut microbiota in energy balance have been demonstrated and advancements in microbiota research highlight the importance of the gut microbiota in affecting metabolic diseases such as obesity and obesity-associated comorbidities (Bäckhed et al., 2004).

2.2.2 Gut Bacterial Overview

The human gut contains the largest number of microorganisms in the body and the genes of these microorganisms are collectively termed the ‘metagenome’ (Tagliabue & Elli, 2013; Xu & Gordon, 2003). Currently, there are 10 recognized microbial phyla with Bacteroidetes and Firmicutes representing 92.6% of all the phyla from fecal samples of healthy subjects (Tagliabue & Elli, 2013). There is an increase in complexity of the microbial community when moving down the hierarchical taxonomic ladder from phyla to genera. It is hypothesized there are specific genera that comprise a core microbiome. These proposed genera belong to the Bacteroidetes, Firmicutes and Actinobacteria phyla and are respectively *Bacteroides* and *Alistipes*, *Faecalibacterium*, *Ruminococcus*, *Eubacterium* and *Dorea* and *Bifidobacterium* (Tagliabue & Elli, 2013). Further increasing specificity, it is demonstrated that there can be over 1000 bacterial species in the gut with approximately 160 of them common among individuals (Shoaie & Nielsen, 2014). As well as bacteria, *Methanobrevibacter smithii*, a member of the Archaea is also present in the human gastrointestinal (GI) tract (Tagliabue & Elli, 2013).

The composition of the gut microbiota is developed at birth and changes until the age of three where the composition resembles that of an adult and remains relatively stable (Scott et al., 2013). One's microbial profile is influenced by diet and antibiotic use; however, if such changes are transient, the original profile will return. The gut microbiota and the metagenome have genes that code for enzymes which are absent from the human genome. Many of the enzymes that differ between the host and the residing bacteria are involved in the digestion and metabolism of carbohydrates in the colon, which would have been otherwise non-digestible by the host (Kovatcheva-Datchary et al., 2009). These carbohydrate metabolites are then fermented to short chain fatty acids (SCFA), primarily butyrate, acetate and propionate with varying concentrations depending on diet, bacterial composition and host genotype (Tan et al., 2014). There are various roles for the SCFAs within the host; regulating energy supply for the cells, controlling the pH of the colon and providing resistance to the growth of pathogens (Shoaie & Nielsen, 2014). They also have roles in satiety hormone, insulin and inflammatory cytokine regulation thereby implicating the gut microbiota in human health and disease (Tan et al., 2014). Recently, developments in discerning a 'healthy' versus a 'non-healthy' or dysbiotic microbial profile have occurred such that interventions aimed at shifting the microbial profile toward a healthy profile have become a major research focus (Nieuwdorp, Gilijamse, Pai, & Kaplan, 2014).

2.2.3 The Gut Bacteria- An Environmental Factor Regulating Obesity

One of the first studies to describe the relationship between the gut microbiota and obesity was Backhed et al., (2004) where it was proposed that the coevolution of the gut microbiota with humans was to process and extract energy from the diet. This relationship was revealed after

notable differences in the body fat percent of conventionally raised (CONV-R) mice compared to germ free (GF) mice were identified. Although the CONV-R mice consumed 29% less food than the GF mice, they had 42% more body fat. Colonization of GF mice with microbiota from the CONV-R mice increased the total body fat of the GF mice by 57% and decreased the lean body mass by 7%. They concluded that the gut microbiota is involved in energy extraction from the diet and energy storage for the host, which importantly implicates it in regulating the obese phenotype.

A subsequent study in mice by Ley et al., (2005) used quantitative polymerase chain reaction (qPCR) of the bacterial 16S ribosomal ribonucleic acid (rRNA) to characterize the species that colonized the gut of obese and lean mice. Similar to what is observed in humans, the two main bacterial phyla detected were the Bacteroidetes and the Firmicutes. An increased abundance of Firmicutes compared to the abundance of Bacteroidetes was associated with the obese phenotype whereas an increased abundance in the phyla Bacteroidetes was associated with a lean phenotype. This observed difference between the microbial profile of lean and obese mice was defined as a microbial dysbiosis- a shift in the microbial profile which can be detrimental to the health of the host (Brown, DeCoffe, Molcan, & Gibson, 2012)

These results were verified by a similar study conducted by Turnbaugh et al., (2006) which also characterized a lean and obese profile in mice dependent on the ratio of Firmicutes to Bacteroidetes (FB). Microbial analysis was conducted using shotgun sequencing which unlike qPCR, allows for a broader analysis of the microbial profile and reduces the potential bias associated with primer selection or limitation in available primers. A notable limitation however, was the use of one member of the Bacteroidetes phyla and one member of the Firmicutes phyla to determine the FB ratio which could potentially overestimate or underestimate the ratio. Based on the results of the two mice studies it was hypothesized that the FB ratio is a potential link between

the gut microbiota and its regulation of obesity; however, further research needed to be completed in humans.

Obese adults were assigned to receive either a low-fat or low-carbohydrate diet to promote weight loss and their bacterial profiles were characterized throughout the diet with an aim to investigate the relationship between the gut microbiota profile and increased body fat (Ley, Turnbaugh, Klein, & Gordon, 2006). Results revealed that 70% of the identified species were unique to each person however the gut microbiota was still primarily bacteria from the Firmicutes and Bacteroidetes phyla. They noted that bacterial communities over time were much more similar within the individual than between individuals.

Another critical study analyzed gut bacteria diversity in adult female monozygotic and dizygotic twins as well as their mothers and aimed to address how host genotype, environmental exposure and host adiposity influence the gut microbiota (Turnbaugh et al., 2009). Similar to what was observed by Ley et al., (2006), microbial analysis of an individual at different time points showed specific phylotypes that were consistent, although there were changes in the relative abundance of each phylotype present. Taxonomic and functional data showed that family members had more similar profiles than unrelated individuals and there was significant association between the type of bacteria present and the metabolic profile in an individual. Finally, analysis to determine an identifiable 'core microbiome' was undertaken using the Shannon index which analyzes the diversity and evenness of the bacteria. It was concluded that a core microbiome defined by a set of shared bacteria may be incorrect; however, a core microbiome at the level of shared genes and metabolic functions is more likely. In obesity, analysis of the variable functional groups (those not in the 'core microbiome') identified genes that were significantly different

between lean and obese microbiome which represented any taxonomic differences between the two profiles.

The identification of a core microbiome and the uncovering of genes unique to an obese or lean microbiome is essential in understanding the gut microbiota influence on energy balance and metabolism. Discerning the functional difference between the two phenotypes, lean and obese, is also essential as it can lead to the development of interventions that improve host health based on restoring a healthy microbial profile.

2.2.4 Firmicutes to Bacteroidetes Ratio- A Marker to Typify a Lean and Obese Gut?

With the understanding that the gut bacteria have roles in energy balance and metabolism, the aforementioned studies used microbial profiling to describe particular bacteria that had observable differences between a lean and obese profile. In particular, the Firmicutes to Bacteroidetes (FB) ratio was an observable marker that discerned a lean and obese gut. This microbial shift, or dysbiosis, between a lean and obese phenotype was initially observed by Ley et al., (2005) and subsequently observed and verified by Turnbaugh et al., (2006) in the rodent population.

The conclusions from microbial profiling in humans are much more variable, in particular the correlation between the FB ratio and obesity. Ley et al., (2006) described the microbial profile of the human gut prior to and after a carbohydrate-restricted or fat-restricted diet in obese individuals. Microbial analysis described similar trends in the proportion of Firmicutes and Bacteroidetes to what was observed previously in rodent models. The relative abundance of Bacteroidetes increased after the diet, whereas the abundance of Firmicutes decreased irrespective

of the diet type. This increase in Bacteroidetes abundance was positively correlated with body fat loss of at least 2% on the low-carbohydrate diet and at least 6% on the low-fat diet. Overall, the diet reduced the FB ratio shifting the microbial profile of these adults from an obese to a lean profile.

Not only did Turnbaugh et al., (2009) aim to functionally characterize the microbial profiles of the lean and obese gut and discern a ‘core microbiome’, analysis of the Bacteroidetes and Firmicutes phyla in relation to obesity was also performed using multiple methods. Collectively, they similarly revealed a lower proportion of Bacteroidetes in obese compared to lean individuals and no significant differences in the Firmicutes. Unlike the study by Ley et al., (2006) a restrictive diet was not implemented and no weight or body fat changes were mentioned in the subjects which limits the scope of the analysis.

Further studies in both humans and mice have analyzed this FB ratio and have typically observed a positive correlation between this ratio and obesity. The primary design of these studies was analysis of microbial profiles after an intervention aiming to reduce weight and/or body fat which is necessary for the development of treatments to shift the microbial profile to promote healthy outcomes. In rodent models of genetically obese mice and rats (Everard et al., 2011; Parnell & Reimer, 2012) and diet-induced obese mice (Everard et al., 2014) prebiotic fiber consumption reduced the FB ratio. There was no mention of weight or body fat loss associated with prebiotic fiber consumption in the studies by Everard et al., (2011 & 2014) which could be due to the fact that it was previously observed that diets enriched with 10% of prebiotic fiber reduced weight gain and fat mass (Cani, Neyrinck, Maton, & Delzenne, 2005). While Parnell & Reimer (2012) observed no changes in body weight or fat mass in genetically obese leptin receptor deficient rats

fed prebiotic fiber, they did observe other metabolic benefits in the rats such as reduced energy intake and an increase in satiety hormones.

In overweight and obese adolescents, the effects of a calorie-restricted diet and physical activity obesity intervention on the microbial profiles was examined (Nadal et al., 2009). All of the participants lost weight and for analysis were stratified by greater than 4.0 kg body weight loss and less than 2.5 kg body weight loss. It was observed that in those with a weight loss of greater than 4.0 kg, proportions of *E. rectale-C. coccoides*, members of the phyla Firmicutes, significantly decreased after the intervention. This reduction significantly correlated with percent body weight loss and body mass index (BMI) Z-score reduction. In contrast, *Bacteroides-Prevotella*, members of the phyla Bacteroidetes, had significantly increased proportions. In those with weight loss less than 2.5 kg, there were no statistically significant differences in bacterial proportions after the intervention and no correlations were detected with either body weight or BMI Z-score. Nadal et al., (2009) concluded that similar to Ley et al., (2006), significant changes in the microbial profile particularly the FB ratio, is correlated with a specific amount of weight loss.

There are studies which refute the reliability of the FB ratio to typify a lean or obese microbial profile. Microbial analysis was conducted on males classified as non-obese or obese by BMI who were randomized to a weight maintenance program; it was observed that Bacteroidetes, expressed as percent of total bacteria, was not significantly different between the two BMI classification groups on the program (Duncan et al., 2008). There was also no significant relationship between BMI and the proportion of *Bacteroides*; however, this correlation could be skewed as *Bacteroides* are only one genera of the Bacteroidetes phyla, although the most abundant. Duncan et al., (2008) also analyzed the microbial profile after weight-loss diets and observed a weak positive correlation between changes in percent of *Bacteroides* and weight-loss which was

significant however, they state this correlation should be higher when comparing to the literature. Bacteria representing the Firmicutes phylum had no observable changes after weight loss therefore contradicting the FB hypothesis. Schwartz et al., (2009) similarly did not observe a positive correlation between the FB ratio and obesity and instead observed a negative correlation. Microbial analysis was performed on individuals who were classified as normal weight, overweight and obese by BMI at one time point. It was observed that there was a significantly increased proportion of bacteria from the phyla Bacteroidetes in those who were overweight compared to normal BMI, but not between obese and normal weight individuals. Bacteria belonging to the Firmicutes phyla were significantly decreased in the overweight and obese individuals compared to lean individuals. These discrepancies in the literature may be due to reduced sample sizes as well as the methodology chosen for microbial profiling. Importantly, these phyla include multiple genera and species of bacteria therefore, depending on the genera or species analyzed, the results may differ. This indicates a need for more wide-spread analysis such as next-generation sequencing to further discern the lean and obese microbial profile. Potential interventions that could shift the obese profile to that of a lean individual or reduce the FB ratio could therefore be beneficial in promoting metabolic homeostasis.

2.2.5 The Gut Bacteria has Roles in Energy Extraction and Storage

Backhed et al., (2004) first demonstrated that the gut microbiota regulates fat storage and promotes energy harvest by the introduction of a conventional microbiota to germ free mice known as conventionalization. Conventionalization resulted in a 57% increase in body fat despite reduced food intake which was accompanied by a proportional increase in the adipocyte hormone leptin

and a significant increase in fasting glucose, fasting insulin and development of an insulin-resistant state. Two mechanisms by which the presence of the gut microbiota affect host energy storage in adipocytes were proposed. First, the presence of a gut microbiota increased processing of dietary polysaccharides and promotes increased monosaccharide uptake from the gut and delivery to the liver resulting in an observed increase in hepatic triglyceride content. Second, conventionalization increased the activity of lipoprotein lipase (LPL) in adipocytes which increases the cellular uptake of fatty acids and storage of triglycerides in adipocytes. This increased activity was a result of the suppressed fasting-induced adipose factor (*Fiaf*) expression, an inhibitor of LPL activity, with the colonization of the gut (Bäckhed et al., 2004).

It was proposed that the dysbiosis observed in obese individuals, particularly the large ratio of Firmicutes to Bacteroidetes, altered the microbiota to promote energy extraction from the diet and increase energy storage (Turnbaugh et al., 2006). To directly test the energy harvest hypothesis and determine if adiposity is transmissible through the gut microbiota, the microbiota of genetically obese mice was transplanted to GF mice which resulted in a significant increase in body fat compared to those that received microbiota from lean mice. Both the lean and obese mice were fed the same diet with no statistical difference in chow consumption; therefore, it was concluded that the presence of the gut microbiota increased the energy extraction from the diet thus resulting in an increase in adiposity. Results were extrapolated to the human population because obese human and obese rodent microbiomes are similar (Turnbaugh et al., 2006). However, as with any animal model, there are limitations, one of which is the potential differences between genetic obesity and the more commonly observed diet-induced obesity.

The gut microbiota's role in increasing energy extraction from the diet and energy storage is proposed to be related to their production of SCFA. It is hypothesized that SCFAs contribute

approximately 10% of the daily energy requirements with estimations that fermentation of dietary fiber or resistant starch to SCFAs provide approximately 2 kcal per gram of fiber/resistant starch compared to the readily digested and absorbed glucose which provides 4 kcal per gram (Bergman, 1990; Ramakrishna, 2013). Therefore, energy extraction from fermentation to SCFA on average is much less efficient than that of glucose. As a result it is hypothesized that obese individuals have a community of bacteria that are more efficient at extracting this energy from the diet, increasing their energy intake, compared to lean individuals (Bäckhed et al., 2004; Turnbaugh et al., 2006).

In an attempt to address this hypothesis, levels of SCFAs and energy content of feces were used as markers of energy harvesting in lean, genetically obese and high-fat fed mice (Murphy et al., 2010). The energy content was modest but significantly reduced in the feces from genetically obese mice compared to the lean controls, however in high-fat fed mice, fecal energy content increased. These differences between the two types of obesity suggest diet has a role in energy excretion and that the gut microbiota has a minor role in energy extraction. This also highlights the differences between diet-induced obese and genetically obese mice and emphasizes the need for research to differentiate between the two. The markers of energy harvesting were further analyzed; specifically in relation to the proportions of Firmicutes, Bacteroidetes and Actinobacteria. It was observed that the proportions of these phyla were not correlated with energy harvesting markers.

There has been further analysis of the major bacterial phyla and their role in energy extraction and energy harvest. In contrast to the conclusions by Murphy et al., (2010), members of both the Bacteroidetes and the Firmicutes have genes that are involved in efficient energy extraction (Flint, Scott, Duncan, Louis, & Forano, 2012). Members of the Bacteroidetes have specific roles in utilizing energy from carbohydrates; in particular members of the *Bacteroides*

genus have a broad saccharolytic potential with some members able to target multiple diverse complex glycans. Evidence of members of the Firmicutes having roles in polysaccharide degradation is emerging; however, these members are major butyrate producers and have roles in conversion of lactate to butyrate and propionate, which are key metabolic conversions. The differences in energy extraction between the members of the two phyla may have roles in defining the Firmicutes to Bacteroidetes ratio and in characterizing the lean and obese profile. A more in-depth analysis of the specific bacterial species in these phyla, in particular the members of Firmicutes, and their role in energy metabolism is required.

2.3 Obesity: The Low-Grade Chronic Inflammatory State

2.3.1 Inflammatory Cytokines and Markers in Obesity

Obesity is not only a state of increased adipose tissue but is also defined as a “low-grade, chronic, inflammatory state” (Gregor & Hotamisligil, 2011). The first molecular link between obesity and inflammation was described in the tissues of lean and genetically obese mice where there was elevated levels of tumor necrosis factor alpha (TNF- α) expression compared with lean controls (Hotamisligil, Shargill, & Spiegelman, 1993). TNF- α was of particular interest to Hotamisligil et al., (1993) due to its direct effects on adipocyte metabolism and the hyperlipidemia that was observed after TNF- α administration in rats and humans (Feingold & Grunfeld, 1987; Sherman et al., 1988). Since adipose tissue is comprised of adipocytes and non-adipose cells, the source of TNF- α expression was also determined. The adipocyte fraction was observed to be

associated with a majority of the TNF- α messenger RNA (mRNA) although some of the TNF- α mRNA was also detected in the fraction of non-adipose cells and less mature adipocytes.

The expression of TNF- α mRNA and TNF- α protein levels were subsequently measured in adipose tissue of lean and obese human adults before and after weight loss (Kern et al., 1995). Similar to what was observed in mice, TNF- α mRNA was detected in human adipose tissue and further analysis also detected TNF- α protein. Adipose tissue biopsies were performed on individuals of different BMIs, sexes and ethnicities to examine the effects of obesity on TNF- α expression. There was a significant positive relationship between TNF- α mRNA and both total body fat percent and total body fat in kilograms. After maintained weight loss in the obese subjects, TNF- α mRNA levels reduced as did TNF- α protein levels, supporting the hypothesis that adipose tissue was associated with the inflammatory response in obesity.

Adipose tissue in obesity is also characterized by increasing concentrations of other inflammatory cytokines such as interleukin (IL)-6 (Fried, Bunkin, & Greenberg, 1998). Importantly, the type of body fat plays a role in IL-6 production such that in severely obese men and women, IL-6 production was three-fold greater from omental adipose tissue than subcutaneous adipose tissue. Production of IL-6 was also greater in isolated adipocytes from the omental tissue than the subcutaneous tissue. This highlights the importance of analysis of the two different fat depots in human obesity. As stated previously, both TNF- α and IL-6 are expressed by adipose tissue; however, to understand if these cytokines have potential roles systemically, the release of these cytokines by subcutaneous adipose tissue in adult humans was measured (Mohamed-Ali et al., 1997). Unlike TNF- α , IL-6 was released by the subcutaneous fat depot, suggesting it was able to signal systemically; whereas TNF- α is suggested to signal through autocrine or paracrine activity.

Chemokine and inflammatory marker, monocyte chemoattractant protein (MCP)-1, which was observed to be expressed and secreted in adipocytes *in vitro*, had elevated expression in adipose tissue from genetically obese mice compared to lean controls (Sartipy & Loskutoff, 2003). In adult women, MCP-1 secretion was approximately ten-fold higher in the adipose tissue of obese participants compared to that of non-obese participants and MCP-1 mRNA was 2-fold higher in obese adipose tissue (Dahlman et al., 2005). There was no observable difference in circulating MCP-1 levels suggesting roles for the chemokine within the tissue.

Since TNF- α , IL-6 and MCP-1 are produced and secreted from adipose tissue, they are collectively known as adipokines (Trayhurn & Wood, 2005). In particular, they are pro-inflammatory adipokines and act as markers of inflammation. Another marker of inflammation is C-reactive protein (CRP), it however is not an adipokine as it is synthesized by the liver in response to adipokines, primarily IL-6 (Ganter, Arcone, Toniatti, Morrone, & Ciliberto, 1989). There is evidence that CRP can be released by human adipocytes *in vitro* in response to inflammatory cytokines, although there is little evidence of this *in vivo* (Calabro, Chang, Willerson, & Yeh, 2005).

2.3.2 Obesity is Characterized by an Inflammatory Response

Adipose tissue is no longer regarded solely as a site of energy storage, but instead as an active tissue that has secretory roles which can induce a physiological response. Adipose tissue produces and secretes adipokines, and its release of inflammatory adipokines led to the hypothesis that the increased amount of adipose tissue observed in obesity is a major source the inflammation associated with obesity (Gregor & Hotamisligil, 2011).

Increased serum levels of inflammatory adipokines TNF- α and IL-6 and inflammatory marker CRP are observed in obesity (Bastard et al., 2000). Markers such as CRP have been shown to be elevated in both overweight and obese adults and children (Bastard et al., 2000; Ford et al., 2001; Visser, Mcquillan, Wener, & Harris, 1999). Importantly, levels of these circulating inflammatory markers such as IL-6 and CRP are reduced after weight loss (Bastard et al., 2000; Esposito et al., 2003).

As well as the adipocytes, adipose tissue is also comprised of non-adipocytes, which are of particular interest in obesity and obesity-associated inflammation, especially macrophages. Macrophages are immune cells that are found to reside in nearly all tissues with tissue-specific roles; however, their functions in adipose tissue are not well described (Weisberg et al., 2003). There are increased number of macrophages observed in obesity; adipocyte size and body mass positively correlated with the number of cells expressing macrophage markers (Weisberg et al., 2003). Activated macrophages from adipose tissue are of interest due to their release of inflammatory molecules such as TNF- α and IL-6 and were the primary source of these inflammatory cytokines in the adipose tissue (Weisberg et al., 2003). This suggests that it is not solely adipose tissue, but also macrophage infiltration, that promotes obesity-associated inflammation.

The primary physiological role of inflammatory cytokine MCP-1 is to act as a chemoattractant for macrophages to areas of inflammation (Melgarejo, Medina, Sánchez-Jiménez, & Urdiales, 2009). In obesity, it was proposed that macrophage infiltration is in part mediated by the expression and release of MCP-1 and other chemoattractants which was supported by the increased expression of MCP-1 in adipose tissue (Kanda et al., 2006; Sartipy & Loskutoff, 2003). Macrophage infiltration is preferential to visceral adipose tissue compared to subcutaneous adipose

tissue with an overall increase in both fat depots in obese subjects compared to lean controls (Harman-Boehm et al., 2007). This was mirrored by increased MCP-1 expression in the visceral adipose tissue depot compared to the subcutaneous adipose tissue and an overall increase in MCP-1 expression in the obese individuals compared to lean controls. Importantly, MCP-1 expression was reduced after an improvement in diet composition and exercise in high-fat fed mice however, improvements were attributed primarily to the change in diet (Ko & Kim, 2013). Similarly, in the adipose tissue of obese adults, low-grade inflammation, MCP-1 and macrophage infiltration was reduced after a lifestyle intervention (Bruun, Helge, Richelsen, & Stallknecht, 2006).

Inflammation is of particular interest in obesity because it is linked to development of many of the metabolic comorbidities associated with obesity. Interventions that aim to attenuate obesity-associated inflammation also have potential to reduce the risk of obesity-associated comorbidities.

2.3.3 Metabolic Endotoxemia- A Role for the Gut Microbiota in Inflammation

With the acceptance that obesity is characterized by a low-grade chronic inflammatory response, one proposed mechanism for the inflammatory response implicates the gut microbiota, in particular, bacterial lipopolysaccharides (LPS) from the cell coat of Gram-negative bacteria (Glauser et al., 1994). On adipocytes and macrophages, the activation of the membrane toll-like receptor (TLR) 4 induces an inflammatory response by promoting the activation of inflammatory pathways and releasing inflammatory cytokines (Kopp et al., 2010; Shi et al., 2006). One such endogenous ligand that activates the TLR4 and induces an inflammatory response is LPS which is elevated after the consumption of a high-fat diet and increased energy intake (Amar et al., 2008;

Cani, Amar, et al., 2007; Shi et al., 2006). Elevated levels of plasma LPS is defined as endotoxemia; however, in response to a high-fat diet, although still elevated, the levels of LPS are less than that which occurs during sepsis and is therefore termed metabolic endotoxemia (Cani, Amar, et al., 2007). Importantly, mice plasma levels of LPS increased with increasing percent of dietary fat. Increased expression of inflammatory cytokine expression was observed in the liver, subcutaneous and visceral adipose tissues after LPS infusion similar to that which is observed after high-fat diet consumption; which suggests LPS is a critical factor connecting a high-fat diet, obesity and the resulting inflammation.

The role of the gut microbiota in metabolic endotoxemia was highlighted with the administration of broad-spectrum antibiotic treatment which reduced plasma levels of endotoxin in high-fat fed mice as well as genetically obese mice compared to those not treated with antibiotics (Cani et al., 2008). Antibiotic treatment also revealed that increased intestinal permeability was the mechanism by which high-fat diet promoted metabolic endotoxemia. High-fat feeding decreased the expression of epithelial tight-junction proteins and increased intestinal permeability; which was restored with antibiotic treatment implicating a role for the gut bacteria in the regulation of intestinal permeability. The increased inflammation, marked by increased expression of TNF- α and other inflammatory cytokines, and macrophage infiltration observed with metabolic endotoxemia and high-fat diet was also reversed after antibiotic treatment. This again suggests the gut bacteria have roles in regulating inflammation in response to a high-fat diet.

2.3.4 Inflammation and Insulin Resistance

There is growing evidence that obesity-associated diseases, such as insulin resistance and T2D, are correlated with increased inflammation. Insulin resistance is defined as an insensitivity to insulin in tissues resulting in greater insulin production to compensate (Jeanrenaud, 1979; Kim & Caprio, 2011). Insulin resistance is a major risk factor for T2D; without intervention, the cells producing insulin will eventually be unable to compensate for the insulin insensitivity resulting in hyperglycemia (Kim & Caprio, 2011). Measuring insulin resistance is therefore imperative in identifying high-risk individuals for the development of T2D, in particular in the child population where the prevalence of insulin resistance in obese children was 52.1% (Lee, Okumura, Davis, Herman, & Gurney, 2006).

Hotamisligil et al., (1993) not only observed that TNF- α expression was increased in obesity, but also observed that long-term treatment of murine adipocytes *in vitro* with TNF- α resulted in a down-regulation of glucose transporter type 4 (Glut4) mRNA which encodes for the insulin-sensitive glucose transporter. This decreased expression of Glut4 mRNA was similarly observed in adipose tissue of genetically obese rodents, suggesting a role for inflammatory marker TNF- α in glucose homeostasis.

Further studies with low concentrations of TNF- α reduced insulin-stimulated autophosphorylation of the insulin receptor (IR) and more drastically reduced insulin-stimulated phosphorylation of an essential kinase insulin receptor substrate (IRS)-1 after chronic exposure (Hotamisligil et al., 1996, 1994). This reduction in insulin-stimulated phosphorylation was also observed with other cytokines such as IL-6, suggesting inflammatory cytokines interfere with insulin's intracellular signaling actions (Hotamisligil et al., 1994).

Similar to TNF- α , the chemoattractant MCP-1 was also identified as an insulin responsive protein (Sartipy & Loskutoff, 2003). Insulin treatment increased the expression and secretion of

MCP-1 in murine adipocytes *in vitro* and in adipose tissue of wild type and genetically obese mice. Chronic treatment of adipocytes with MCP-1 reduced insulin-stimulated glucose uptake suggesting that similar to other inflammatory cytokines, MCP-1 has roles in the development of obesity-associated insulin resistance.

Mechanisms of insulin resistance induced by inflammatory cytokines are proposed both at the intracellular level with insulin signaling pathway and secondarily at the level of glucose uptake with the GLUT4 transporter. The release of inflammatory adipokines such as TNF- α and MCP-1, from either adipocytes or adipose-associated macrophages, leads to low-grade chronic inflammation which could be an essential role in the development of insulin resistance associated with obesity.

2.3.5 Insulin Resistance and Dyslipidemia- The Inflammatory Link

The role of insulin resistance in obesity-associated comorbidities extends beyond that of solely being a precursor for the development of T2D. Insulin resistance is also a risk factor for the development of CVD, and is linked to dyslipidemia which is defined as a typical increase in blood TGs and free fatty acids (FFA), slight increase in LDL-C and decreased HDL-C (Caprio et al., 1996; Klop et al., 2013). Insulin is involved in promoting the storage of substrates in the tissues such as fat, muscle and liver (Saltiel & Kahn, 2001). One such substrate is lipids where insulin stimulates lipogenesis and inhibit lipolysis. Insulin resistance therefore causes a dysregulation in the storage action of insulin and results in increased levels of glucose and lipids in the blood. In regards to lipids, a state of insulin resistance increases FFAs in the blood, in addition to the already elevated levels of FFAs due to enlarged adipocytes in obesity, which increases the hepatic

production of TG (Boden, 2011; Li et al., 2014). Insulin resistance has also been observed to be associated with low HDL-C (Laakso, Sarlund, & Mykkanen, 1990).

The aforementioned relationship between inflammation and insulin resistance and the relationship between insulin resistance and dyslipidemia brings to question the potential relationship between inflammation and dyslipidemia. It is widely accepted that insulin resistance can lead to dyslipidemia; however, the pathways that link dyslipidemia to insulin resistance are much less understood (Boden et al., 2005; Li et al., 2014). One potential mechanism by which dyslipidemia can induce insulin resistance is through the action of FFAs (Boden, 2011). FFAs, when increased in rats *in vivo* with lipid infusion, resulted in increased expression of the inflammatory pathways and inflammatory cytokines TNF- α and IL-6 and circulating levels of MCP-1 (Boden et al., 2005). Importantly, lipid infusion also inhibits hepatic insulin action; resulting in hepatic insulin resistance through the interruption of intracellular insulin signaling by the FFAs. One mechanism by which FFAs induce this inflammation and insulin resistance is through their role as a ligand for TLR4 on adipocytes and macrophages, which acts similar to activation by LPS (Shi et al., 2006; Song et al., 2006). The activation of inflammatory pathways, which inhibit the actions of insulin, through the binding of FFAs to TLR4 is a proposed candidate for the observed insulin resistance in obesity. This uncovers a relationship between obesity and high-fat diets with insulin resistance and proposes that the inflammatory response is the link.

2.4 The Protective Role of Short Chain Fatty Acids

2.4.1 Short Chain Fatty Acids- Beneficial Roles

SCFAs are the primary metabolites produced from bacterial fermentation of dietary fiber in the colon (Tan et al., 2014). The three major SCFAs produced in the colon are acetate, propionate and butyrate with diverse roles in gut and overall health. The initial energy harvest hypothesis suggested that improved energy extraction, through the bacterial conversion of otherwise non-digestible carbohydrates and dietary fiber to SCFA could contribute to obesity (Blaut, 2014). Conversely, it is also hypothesized that dietary fiber and SCFAs have beneficial roles for the intestinal tract and host. The primary role of SCFAs is to provide energy to the colonocytes; however, of the three SCFAs butyrate is preferred and provides 60-70% of the energy requirements of the colonocytes (Puertollano, Kolida, & Yaqoob, 2014; Tan et al., 2014). SCFAs also have roles in regulating colonic pH such that increased SCFAs result in a decreased pH which modulates the bacterial composition and reduces potentially pathogenic bacteria (Wong, de Souza, Kendall, Emam, & Jenkins, 2006).

The beneficial effects of butyrate have been examined through supplementation of butyrate in the diet of high-fat fed mice (Gao et al., 2009). The development of insulin resistance and diet-induced obesity was prevented and fasting blood glucose, fasting insulin and body fat was preserved with supplementation of butyrate. To test butyrate as a treatment option, obese mice were administered butyrate which not only led to a decrease in their body weight and body fat but also improved markers of obesity-associated disease such as reduced fasting glucose, blood lipids and increased insulin sensitivity. There are limitations to this conclusion since butyrate is not regularly detected in high amounts in the peripheral system and is almost entirely metabolized in the gut or in the liver; suggesting any direct effects of butyrate in the blood is not physiologically expected (Guilloteau et al., 2010).

2.4.2 Gut Hormones and Intestinal Health

Another mechanism by which SCFAs induce their protective effects is their signaling through the G-protein coupled receptors (GPRs) (Tan et al., 2014). The two major receptors that are activated by SCFAs are GPR41 and GPR43 which are expressed on adipocytes and enteroendocrine L cells. One of the best established roles of the activation of GPR43 on the L cells of the intestine is the release of glucagon-like peptide (GLP)-1, a key satiety-promoting hormone that is involved in controlling insulin secretion (Tolhurst et al., 2012). Increased SCFAs resulted in elevated activation of GPR43 and subsequent increased GLP-1 secretion which was reduced in GPR43 knockout mice.

The interaction between SCFAs and GPR43 has also been observed to affect the inflammatory response in models of inflammatory disease (Maslowski et al., 2009). In both acute and chronic colitis-induced mice, *Gpr43* mutants had a discernible difference in their inflammatory response. Acetate administration resulted in a decrease in inflammation but this improvement in inflammation was not observed in *Gpr43* mutants. This suggests the interaction of SCFAs with GPR43 and its resulting stimulation affects the inflammatory response.

In the intestine, enteroendocrine L-cells not only secrete and express GLP-1, but also the gut trophic hormone GLP-2 (Burrin, Petersen, & Stoll, 2001). Nutrient intake is the primary stimulus for GLP-2 release however, SCFAs are also a regulator of GLP-2 release leading to the hypothesis that this increase in GLP-2 is beneficial for host and intestinal health (Cani et al., 2009; Tappenden, Albin, Bartholome, & Mangian, 2003). In particular, regulation of gut permeability and inflammation in obesity was mediated through a mechanism involving GLP-2 (Cani et al.,

2009). Diet-induced changes of the gut bacteria increased plasma levels of GLP-2 in genetically obese mice which was negatively correlated with markers of gut permeability and positively correlated with mRNA of epithelial tight junctions. This improvement was diminished with an antagonist of GLP-2. Therefore, positive changes in the gut microbiota in-part through the actions of GLP-2 can benefit host and intestinal health in obesity.

The evidence presented proposes two roles for SCFAs in obesity; the energy harvest hypothesis suggests they may have harmful roles in the development of obesity. Alternatively, research also suggests that SCFAs are important, not only in colonic health, but also in overall health and may have roles in preventing or managing obesity. With the two polar notions of SCFA there is room to hypothesize that there is a needed balance in the number or type of SCFAs produced and for this reason modulation of the gut microbiota is viewed as a potential intervention. Therefore, interventions which can beneficially modulate the gut microbial profile are of extreme interest.

2.5 Prebiotic Fiber

2.5.1 Prebiotic Fiber Overview

Research into the gut microbiota and its roles in metabolic diseases has led to the interest in developing interventions that could beneficially change the gut microbiota, thereby improving host health (Delzenne et al., 2013). One such dietary intervention is prebiotic fiber which is defined as “selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” (Gibson et

al., 2010). Prebiotic fibers are unable to be digested by host enzymes and are not absorbed in the small intestine, instead they pass into the large intestine where they are fermented by resident gut bacteria (Roberfroid, 2002). Selective resident bacteria ferment the prebiotic fiber, in particular species of bifidobacteria which are recognized beneficial bacteria (Gibson & Roberfroid, 1995). The benefits of increasing *Bifidobacterium* spp. include increasing the acidity of the colon which may exert anti-bacterial effects thereby inhibiting the growth of potentially pathogenic bacteria, as well as vitamin production and restoration of the microbiota during and after antibiotic therapy (Gibson & Roberfroid, 1995). In children, a prospective study observed that those with an increased number of *Bifidobacterium* spp. were less likely to gain excess weight with age, highlighting the importance of the bacteria in health (Kalliomäki, Collado, Salminen, & Isolauri, 2008). Therefore, prebiotic fiber's beneficial effects could be elicited by reversing the dysbiosis in the gut.

The most common prebiotics are non-digestible oligosaccharides, specifically inulin-type fructans (Roberfroid, 2002). They are extracted from chicory root for incorporation into food products; however, inulin-type fructans are also naturally found in foods such as garlic, onion, asparagus and banana, among others (Roberfroid, 2007a). Of the inulin-type fructans, inulin and oligofructose, produced by the partial enzymatic hydrolysis of inulin, are the most common. Although oligofructose is a component of inulin, they each elicit their effects on different portions of the colon. With a majority of the experimental and human clinical trial scientific data, the inulin-type fructans are one of the most researched prebiotics.

2.5.2 Evidence for Prebiotic Fiber as a Dietary Intervention for Obesity

Prebiotic fiber use in the management or treatment of obesity and obesity-associated disease has received considerable interest since the uncovering of the role of the gut microbiota in metabolic health. Normal weight rats (Cani, Hoste, Guiot, & Delzenne, 2007; Cani et al., 2005; Maurer, Chen, McPherson, & Reimer, 2009; Reimer et al., 2012), genetically obese mice (Cani et al., 2009) and diet-induced obese mice and rats (Cani et al., 2006; Cani, Neyrinck, et al., 2007; Everard et al., 2013; Pyra et al., 2012) have all been reported to exhibit reduced fat mass following consumption of prebiotics. In human clinical trials, prebiotic fiber tended to decrease fat mass in obese women after consumption and in overweight and obese men and women resulted in significant weight loss and a greater reduction in trunk fat mass with prebiotic fiber compared to placebo (Dewulf et al., 2013; Parnell & Reimer, 2009). The reduction in body weight and body fat is an important phenotypic change when assessing the relevance of potential obesity therapies and interventions.

Aside from reductions in weight and body fat, prebiotic fiber consumption reduces risk factors associated with obesity-associated disease. In genetically obese mice, along with the decreased fat mass, there was a reduction in the inflammatory response after consumption of prebiotic fiber (Cani et al., 2009). Prebiotic fiber also reduced plasma LPS, a marker of metabolic endotoxemia, in genetically obese mice which was associated with increased expression of epithelial tight junctions and an overall decrease in intestinal permeability (Cani et al., 2009; Everard et al., 2011). Hepatic and colonic expression of inflammatory cytokines and the resulting inflammation was also reduced compared to control diets in genetically obese mice (Cani et al., 2009; Everard et al., 2011). In a healthy, non-obese population, combination of prebiotic fibers inulin and xylo-oligofructose reduced plasma circulating levels of LPS (Lecerf et al., 2012). In addition, in overweight and obese women, a combination of inulin and oligofructose reduced CRP

and resulted in a greater LPS reduction compared to placebo, although neither change was significant (Dewulf et al., 2013).

Blood lipids and measures of insulin resistance are important markers of obesity-associated comorbidities therefore, improvements in these outcomes are important for potential obesity therapies and interventions. Prebiotic fiber supplementation in genetically obese and high-fat fed mice significantly improved glucose tolerance and improved plasma TG levels in genetically obese mice (Everard et al., 2011). An index of insulin resistance was also reduced after prebiotic treatment in high-fat fed mice, which was also observed in control diet mice (Everard et al., 2014). In humans, post-prandial concentrations of insulin and glucose were significantly improved with oligofructose consumption compared to placebo and compared to initial concentrations (Parnell & Reimer, 2009). When using an oral glucose challenge, Dewulf et al., (2013) observed no improvements in insulin post oral glucose load but did detect a significant reduction in serum glucose concentrations. Overall, a systematic review looking at the benefits of prebiotic fiber found that consumption of prebiotic fiber was associated with reductions in postprandial glucose and insulin concentrations (Kellow, Coughlan, & Reid, 2014) but was not associated with improved blood lipid profiles (Dewulf et al., 2013; Kellow et al., 2014; Parnell & Reimer, 2009).

Ongoing research is required as many of the studies assessing the effect of prebiotic fiber are limited by small sample sizes and are also primarily conducted in adult populations (Kellow et al., 2014). In the pediatric population there are two studies, in addition to the present trial, assessing the effect of prebiotic fiber. Abrams et al., (2007) assessed the effects of oligofructose-enriched inulin, in combination with calcium-fortified orange juice on the primary outcome of calcium absorption and bone mineral density in non-obese healthy adolescents. The study, which was not designed to address change in body weight, did show that adolescents taking the prebiotic had a

slower rate of weight gain and lower fat mass. In the overweight and obese pediatric population 7-18 years of age, Liber & Szajewska et al., (2014) assessed the effect of oligofructose on the primary outcome BMI-for-age Z-score and secondary outcomes of body weight and difference in absolute body fat, but observed no significant differences between the prebiotic fiber and placebo control for any of the outcomes. With these two conflicting results and the increased prevalence of childhood obesity, research assessing prebiotic fiber consumption as a potential obesity treatment or management option in the child and adolescent population is necessary. Studies in children and adolescents will broaden the understanding of the gut microbiota and potentially develop predictive measures to diagnose those who are at greater risk of obesity. Overall, it is critical that the effect of prebiotic fiber in improving obesity and its comorbidities be assessed in overweight and obese children. This is especially true since prebiotic fiber is natural, inexpensive, non-invasive and accessible to children of all socioeconomic statuses.

Therefore, the objective of this study was to assess the effects of the prebiotic fiber, oligofructose-enriched inulin, on anthropometric measures, body composition, inflammation, measures of insulin resistance and serum lipid profiles in overweight and obese children 7-12 years old. We hypothesize that similar to what has been observed in obese rodents and overweight and obese adults, prebiotic fiber consumption will improve obesity phenotypes and markers of obesity-associated diseases in overweight and obese children.

CHAPTER THREE: METHODS

This study is designed as a single centre, double blind, placebo controlled, randomized clinical trial. Overweight and obese children 7-12 years old, who are otherwise healthy, were voluntarily recruited using self-selection within Calgary, Alberta, Canada. They were randomized to a 16 week dietary intervention of either prebiotic fiber oligofructose enriched inulin or placebo control maltodextrin. There were two separate cohorts that completed the trial, the first from March to July 2014 and the second from August to December 2014. The outcomes, body composition, pro-inflammatory cytokines, blood lipids and gut microbiota were measured at both baseline and at the end of the study.

3.1 Recruitment

Subjects were recruited from Calgary, Canada and the surrounding area. Written advertisements were placed around Calgary such as posters, local newspaper advertising and community newsletters. Advertisements were also placed on social media and other web-based media. The study was also publicized on the local televised news during an evening broadcast. Parents contacted us on behalf of the participants. Eligibility was determined either by phone or email based on BMI-percentiles and an “Eligibility Questionnaire” (**Appendix A**). The child’s weight, height and date of birth were provided and used to calculate BMI-percentiles via the World Health Organization’s AnthroPlus program at <http://www.who.int/growthref/tools/en/>.

3.2 Subjects

3.2.1 Inclusion Criteria

Subjects were boys and girls between the ages of 7 and 12 years old classified as overweight and obese; $\geq 85^{\text{th}}$ BMI percentile for individual age and sex. Children were at a Tanner developmental stage of ≤ 3 and girls were pre-menarche. Parents provided written informed consent (**Appendix B**) and children provided verbal assent (**Appendix C**) prior to the initial test day.

3.2.2 Exclusion Criteria

Children that received antibiotics < 3 months prior to the study start, with type 1 or type 2 diabetes, liver disease, cardiovascular problems, major gastrointestinal surgery, used supplementation to influence appetite, weight or metabolism, were on a diet designed for weight loss, had lost > 3 kg 12 weeks prior to the start of the study and those that had extreme changes in exercise intensity ≤ 4 weeks prior to study start were excluded from the study.

3.3 Sample Size and Cohort

Since there was no clinical trial looking at the effects of prebiotic fiber on percent body fat in children at the time of study design, the power and sample calculations were based on a similar clinical trial looking at the effects of a dietary and exercise intervention in children on percent

body fat, body weight and BMI-percentile (Savoie et al., 2007) (**Appendix D**). This study was powered on the primary objective, percent body fat, and it was determined that $n = 18$ is required for each group with an alpha level of 0.05 and 80 percent power. With an expected 20% drop out previously observed in human weight loss clinical trials, 4 more children were added to each group (Parnell & Reimer, 2009). Overall there was $n = 22$ for each group, with a total of 44 children required in the study.

The progress of the participants through the trial are presented in the CONSORT 2010 Flow Diagram (**Appendix E**). A total of 119 parents responded to the advertisements on behalf of a total of 139 children; there were 20 sibling pairs. Of these, 33 parents representing 39 children did not remain in contact after initial contact; eligibility of the 100 children was performed using the Eligibility Questionnaire over telephone or by email. After the assessment, 35 children were excluded due to a BMI percentile or age that did not meet inclusion criteria or the use of medications that affect weight and/or appetite. Sixty-five children fulfilled the inclusion criteria and were invited, together with their parents, to an information meeting at the Faculty of Kinesiology, University of Calgary. There were 49 parents that accepted the invitation and attended the information meeting where the children and their parents were provided further information about the study. If a child and their parent wished to participate, written consent was obtained from the parent, verbal assent was obtained from the child and the corresponding materials, questionnaires and forms required for the initial test day were provided. Written consent and verbal assent were obtained for 42 subjects in which there were 5 sibling pairs. There were 22 subjects randomized to the treatment arm and 20 randomized to the control. Throughout the entire trial four participants withdrew. One participant in the placebo group withdrew prior to the initial test day and three participants withdrew during the study because of time commitment. Of those

three participants that withdrew during the study, two participants (one in the placebo arm and one in the treatment arm) withdrew prior to the 8 week visit while the third participant (in the prebiotic arm) withdrew prior to the 12 week visit. Therefore, a total of 38 children completed the study.

3.4 Intervention

Eligible participants were stratified based on their BMI-percentile, sex and age and randomized via computer generated numbers to either the intervention or control group. Those randomized to the intervention consumed 8 g of prebiotic fiber (oligofructose-enriched inulin, Synergy1®; BENEEO-Orafti, Tienen, Belgium) per day. Whereas, those randomized to the control group consumed an equicaloric dose of 3.3 g of maltodextrin per day. Placebo maltodextrin has a similar taste and appearance to Synergy1® and has been used as an acceptable placebo in prebiotic trials (Parnell & Reimer, 2009). Both intervention and placebo control were consumed as a powder and provided to participants in pre-weighed individual foil packets. Participants and their parent(s) were instructed to mix an entire packet with 1 cup (250 mL) of water in a provided reusable water bottle and shake until fully dissolved. They were instructed to consume half the dose for the first 2 weeks and the full dose for the remaining 14 weeks (weeks 3 to 16), 15 to 20 minutes prior to their evening meal. Participants were provided 38 individual packets to consume over 4 weeks before their next visit, which included extra packets to account for anyone unable to schedule a visit at exactly 4 weeks. Empty and unused study packets were returned at their 4 week visits to measure compliance and they were then provided the doses for the next 4 weeks. To determine if participants remained blinded throughout the study, they were asked to complete an exit questionnaire in an informal interview at the end of the study.

3.5 Food Intake and Physical Activity

The overall purpose of the study was to determine the efficacy of prebiotic fiber consumption and analyze its effects independent of other variables such as physical activity and prescribed diet. As a result, participants were asked to maintain similar levels of physical activity and eat until comfortably full throughout the study. To assess participant's levels of physical activity and ensure a similar level of physical activity was maintained throughout the study, participants and their parent(s) were asked to complete the "Godin's Leisure-Time Exercise Questionnaire" (**Appendix F**) (Godin & Shephard, 1997). This questionnaire is reliable in adults (Godin & Shephard, 1985) and validated and reliable in children and adolescents (Sallis, Buono, Roby, Micale, & Nelson, 1993). The Godin's Leisure-Time Exercise Questionnaire was reported as MET hours/ week analyzed as the following: $[(\text{mild duration} \times \text{mild frequency} \times 3 \text{ METs}) + (\text{moderate duration} \times \text{moderate frequency} \times 5 \text{ METs}) + (\text{strenuous duration} \times \text{strenuous frequency} \times 9 \text{ METs})] / 60$. To assess participant's diet, they were provided a "Three Day Food Record" (**Appendix G**) and food scale. Parent(s) were instructed to record all meals, beverages and snacks consumed by the child over two weekdays and one day on the weekend; food scales were used to increase the accuracy of reporting portions and amounts. This thesis was a part of a larger clinical trial, as a result energy intake was assessed but is not reported in this thesis (**Table 1**). Both the Godin's Leisure Time Questionnaire and the Three Day Food Record were completed a week prior to baseline (week 0), the midpoint (week 8) and the final (week 16) test days.

3.6 Tanner Developmental Staging

Pubertal development was assessed using Tanner staging at the baseline test day, after the parent/s provided consent. The physical exam was conducted by a pediatric endocrinologist from the Alberta Children's Hospital, Calgary (**Appendix H**).

3.7 Body Composition and Physical Characteristics

At baseline and final test days, fat mass and lean mass were measured using a whole-body dual-energy-x-ray absorptiometry (DXA) scan (Hologic QDR 4500, Hologic, Inc., Bedford, MA, USA). Indices for lean and fat mass were generated using the respective equations [lean or fat mass (g)/height (cm)²]. Android and gynoid fat was estimated using the Hologic QDR software according to Arnberg et al., (2012). The lower limit of the android region was set at the upper border of the pelvis and the upper limit of the android region was set at 20% of the distance from the upper border of the pelvis to the base of the neck. The upper limit of the gynoid region was set below the pelvis such that the distance from the upper border of the pelvis to the upper limit of the gynoid region was 1.5 times the length of the android region. The lower limit of the gynoid region was set at a level such that the length of gynoid region was twice the length of the android region. The android to gynoid fat ratio (A: G) was calculated as [android fat mass (g)/gynoid fat mass (g)].

Height, weight and waist circumference were measured in duplicate at baseline and every 4 weeks thereafter (4, 8, 12 and 16 weeks) by the same person to minimize inter-rater variability. Height was measured using a standard stadiometer and body weight was measured using a standard

balance beam scale. Height and weight Z scores were calculated using the Baylor College of Medicine- Body Composition Laboratory: Pediatric Body Composition Reference Charts online calculator at:

<https://www.bcm.edu/bodycomplab/Flashapps/AllDXArefsChartpage.html>.

Waist circumference was measured using a non-elastic measuring tape at both the top of the iliac crest and 2 cm below the umbilicus.

3.8 Stool Specimens

Participants and parent(s) were instructed to collect a stool sample 3 days prior to the baseline and final test days (week 16). Parent(s) and participants were provided a stool collection kit and were instructed on proper methods for stool collection. One tablespoon of stool was placed in a sterile conical tube, placed in a biohazard bag and stored in the participant's home freezer (-20°C). Stool samples were brought to the laboratory on ice on the test days and stored in the laboratory freezer (-80°C).

3.9 Gut Microbiota Profiling

Microbial profiling was performed according to previous work in the Reimer lab (Parnell & Reimer, 2012). Total bacterial DNA was extracted from a subsample of stool samples using FastDNA Spin Kit for Feces (MP Biomedicals, Lachine, QC, Canada) and quantified using PicoGreen DNA quantification kit (Invitrogen, Carlsbad, CA, USA). All samples were brought to a concentration of 4 ng/μL prior to storage at -30°C for future analysis. Quantitative PCR (qPCR)

of the bacterial 16S rRNA was used to quantify the major microbial groups in the human gut. Amplification and detection was conducted in 96-well plates with SYBR Green qPCR Master Mix (BioRad, Hercules, CA). Samples were run in duplicate with a final volume of 25 μ L containing 0.3 μ M primer and 20 ng template DNA. The specificity of the primers and the limit of detection was determined according to Louie et al., (2012). Purified template DNA from reference strains was used to generate standard curves for each primer set using 10-fold serial dilutions of DNA. Melting curve analysis was performed following each assay to confirm the specificity of the PCR products. The 16S rRNA gene copies value was calculated according the following webpage: <http://cels.uri.edu/gsc/cndna.html> using reference genome sizes. Standard curves were normalized to the copy number of the 16S rRNA gene (Stoddard, Smith, Hein, Roller, & Schmidt, 2014). .

3.10 Blood Biochemistry- Pro-inflammatory Cytokines, CRP and LPS

A 12 hour fasted blood sample was obtained from the participants at the baseline and final test days by a nurse from the Alberta Children's Hospital. Approximately 10 ml of blood was collected in Serum Separation Tubes with clot activator and serum separator or in Sodium Fluoride (5mg) Potassium Oxalate (4mg) tubes for glucose determination. Serum Separation Tubes were allowed to clot at room temperature (20°C) for 30 minutes and then centrifuged at 1200 rpm for 10 minutes at 20°C to separate serum. Calgary Lab Services (Calgary, AB, Canada) measured triglycerides, total cholesterol, low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C) cholesterol and C-reactive protein (CRP) in the serum and calculated the total cholesterol to HDL ratio. LDL-C was calculated using the Freidewald equation (Ahmadi, Boroumand, Gohari-Moghaddam, Tajik, & Dibaj, 2008). Inflammatory cytokines, interleukin (IL)-1 β , IL-4, IL-6, IL-

10, IL-33, interferon (IFN)- γ , monocyte chemoattractant protein (MCP)-1 and tumor necrosis factor (TNF)- α were analyzed from the serum using Human Adipokine Milliplex kits (Millipore, St. Charles, MO, USA). Lipopolysaccharides (LPS) were measured in the plasma using the Pyro-Gene Recombinant Factor C Endotoxin Detection assay (Lonza Group Ltd., Basel, Switzerland) according to manufacturer's directions. Fasted blood glucose concentrations were also measured from the plasma in duplicate using a glucose trinder assay (Stanbio Laboratory, Boerne, TX). Fasted insulin was quantified using a Human Insulin ELISA kit (Millipore). The approximation of insulin resistance was determined using the homeostatic model assessment for insulin resistance 2 (HOMA2-IR) calculated via the University of Oxford HOMA2 Calculator (<https://www.dtu.ox.ac.uk/homacalculator/>) and the quantitative insulin sensitivity check index (QUICKI = $1/\log I_F (\mu\text{U/mL}) + \log G_F (\text{mg/dL})$); where I denotes insulin, G denotes glucose and subscript F denotes the fasting concentrations. All serum and plasma used for analysis and additional serum was stored at -80°C prior to analysis.

3.11 Summary of Outcomes

This thesis was a part of a larger clinical trial that assessed multiple outcomes, only some of which are presented in this thesis. The list of all outcomes and the thesis they are reported in are presented in **Table 1**.

Table 1. Summary of all outcomes assessed in the clinical trial

Thesis Presented In:	Outcomes
This thesis	Anthropometrics <ul style="list-style-type: none"> • Body weight (kg) • Body weight Z-score • Height (cm)

	<ul style="list-style-type: none"> • Height Z-score • Waist circumference (cm) <ul style="list-style-type: none"> ○ Iliac crest ○ 2 cm below the umbilicus • BMI <p>Body composition</p> <ul style="list-style-type: none"> • Total body & Trunk fat (%) • Lean & Fat mass index (g/cm³) • Absolute lean & fat mass (kg) • Android & Gynoid body fat composition (%) • Android to Gynoid ratio <p>Blood biochemistry- Fasting</p> <ul style="list-style-type: none"> • Inflammatory markers and cytokines- Serum • Serum blood lipids • Plasma glucose • Serum insulin • HOMA2-IR, QUICKI <p>Microbial analysis qPCR</p>
(Hume, 2015) (Unpublished Master of Science Thesis)	<p>Anthropometrics</p> <ul style="list-style-type: none"> • BMI Z-score <p>Self-reported food intake- 3 day food records</p> <ul style="list-style-type: none"> • Energy Intake (kcal/day) • Macronutrients & micronutrients <p>Objective measure of energy intake- <i>ad libitum</i> breakfast buffet</p> <p>Subjective appetite ratings –Visual Analog Scale</p> <p>Child eating behaviours – Children’s Eating Behaviour Questionnaire</p> <p>Blood biochemistry- Fasting</p> <ul style="list-style-type: none"> • Serum satiety hormones <ul style="list-style-type: none"> ○ Gastric inhibitory polypeptide (GIP), glucagon-like peptide (GLP-1), peptide YY (PYY) & ghrelin ○ Insulin • Serum adipokines <ul style="list-style-type: none"> ○ Adiponectin, resistin & leptin

* Physical activity- Godin’s Leisure-Time Exercise Questionnaire, participant & parental demographics, compliance, adverse events, blinding and acceptability are reported in both theses

3.12 Statistical Analysis

All data is presented as mean $\pm$ SEM. Data analysis was performed using SPSS 21.0 software (IBM, Armonnk, NY, USA) and results were considered statistically significant with a two-tailed, $p \leq 0.05$. The primary outcome of body fat percent and the secondary outcomes of pro-inflammatory cytokines, lipids, CRP and LPS were measured at two time points, baseline and the final test day, with importance placed on a change from baseline. Normality was verified using a Shapiro-Wilk test prior to statistical analysis. Any skewed data was logarithmically transformed prior to analysis and reassessed for normality. If transformed data was not normally distributed, corresponding non-parametric tests were run. Analysis was performed on an intent-to-treat basis, regardless of subject compliance or completion. Cases with missing outcome data were excluded from analysis for that outcome. Baseline measurements between the control and prebiotic fiber intervention group were compared using an independent t-test. Between-group differences of fiber intervention and control, using the mean differences from the two time points of the within-group analysis, were analyzed and compared using an independent t-test. Within-group differences were analyzed using a dependent t-test. Primary outcome measure percent body fat, at baseline and final test day, was analyzed further using an analysis of covariance (ANCOVA), controlling for confounding factors (sex) and assessing potential covariates (age and initial BMI). Similarly, secondary outcomes of gut microbiota, pro-inflammatory cytokines and anthropometrics, were also analyzed with an ANCOVA. Height, body weight and waist circumference were measured

multiple times throughout the 16 week study (baseline, 4, 8, 12 and 16 weeks) as was physical activity (baseline, 8 and 16 weeks). These data were analyzed using repeated measures analysis of variance (RM ANOVA) with a Bonferroni adjustment to correct for the multiple comparisons.

3.13 Ethics

Ethics approval from the Conjoint Health Research Ethics Board (CHREB) was received December 2013, REB13-0975. Parents provided informed written consent and children provided verbal assent prior to the research study. Two mitigation strategies were used to reduce any potential side effects from consuming the fiber such as bloating or flatulence. First, the participants were asked to consume half a dose for the first two weeks of the study to allow for adaptation. Secondly, they were asked to consume their dose prior to their evening meal such that potential side effects would occur while sleeping. Finally, all data and data analysis was completed and stored on a password protected computer.

CHAPTER FOUR: RESULTS

4.1 Baseline Measures

Overweight and obese children classified as such using BMI percentile, between the ages of 7 to 12, from Calgary, Alberta, Canada were eligible to participate in the clinical trial. Consent to participate in the study was obtained from 42 children and their parents, although 41 children attended the baseline test day. Of those 41 children, 22 were randomized to the prebiotic fiber group and 19 were randomized to the placebo control group. The prebiotic fiber intervention was oligofructose-enriched inulin and the placebo control was maltodextrin.

Baseline characteristics are provided in **Table 2**; sex and age represent the 42 subjects that signed consent, the remaining outcomes represent the 41 subjects that attended the baseline test day. There were no significant differences between the groups. The distribution of sexes amongst the groups was similar; 12 boys in each group, 10 girls in the prebiotic group and 8 girls in the placebo group. The average age of the participants in both groups was 10 years. At the baseline test day the two groups were similar in body weight, body weight Z-score, height, height Z-Score and body mass index (BMI). Percent total body fat was also similar between the two groups and on average the participants of the study had approximately 42% total body fat.

Table 2. Baseline characteristics of overweight and obese children 7-12 years old

Characteristics	Prebiotic Fiber Group	Placebo Group	p value
Sex, Number			
Male	12	12	0.721
Female	10	8	
Age, y	10.4 ±1.6	10.2 ±1.6	0.724
Body weight, kg	58.5 ±3.1	59.6 ±4.5	0.837
Body weight Z-score	2.25 ±0.12	2.14 ±0.16	0.573
Height, cm	148.1 ±2.4	147.1 ±2.8	0.783
Height Z-score	1.31 ±0.24	0.97 ±0.22	0.304
BMI, kg/m ²	26.3 ±0.7	26.9 ±1.3	0.653
% Total Body Fat	42.5 ±1.3	41.8 ±1.2	0.688

Abbreviations; Body Mass Index (BMI)

Values are presented as mean ±SEM (n = 22 prebiotic fiber, n = 19 placebo)

4.2 Anthropometric Measures

4.2.1 Height, Weight and Body Mass Index

Anthropometrics measures, including Z-Score for height and weight, are presented in **Table 3**. There was a significant main effect of time on height ($p < 0.001$), although there was no main effect of treatment ($p = 0.704$) and no significant interaction between treatment and time ($p = 0.091$) (**Figure 4-1A**). Similarly, there was no significant interaction between treatment group and time on height Z-score ($p = 0.216$); analysis of the main effects showed no main effect of treatment group ($p = 0.247$) but a significant main effect of time ($p < 0.001$) (**Figure 4-1A**). There were no significant differences in change in height or height Z-score (**Figure 4-2A & C**) between the two groups. Within the prebiotic and placebo group there was a significant increase in height from baseline (**Figure 4-3A**) but no significant change in height Z-score within the prebiotic fiber group and placebo groups (**Figure 4-3B**).

Body weight was significantly affected by the interaction between treatment group and time ($p = 0.011$) (**Figure 4-1B**). There was also a statistically significant interaction between treatment group and time on body weight Z-Score ($p = 0.033$) (**Figure 4-1B**). Changes in body weight were significantly different between the groups and there were significant changes in body weight Z-Score (**Figure 4-2B & D**). Within the prebiotic fiber and placebo groups there were significant increases in body weight from baseline (**Figure 4-3C**). Body weight Z-Score significantly decreased within the prebiotic group but did not change within the placebo group (**Figure 4-3D**).

The effect of treatment on BMI was also assessed however, it is not an optimal method to classify overweight or obesity in children and adolescents as it is not age or sex specific (Must & Anderson, 2006). BMI was significantly influenced by the interaction between treatment groups and time ($p = 0.009$) (**Figure 4-1C**). There was no significant change in BMI within the prebiotic fiber group from baseline to final test day however, there was a significant increase in BMI within the placebo group (**Figure 4-3G**). There was also a significant difference between treatments when BMI was expressed as change in BMI (**Figure 4-2E**).

4.2.2 Waist Circumference

As a measure of central adiposity, waist circumference was measured at the iliac crest and at 2 cm below the umbilicus. There was no significant interaction between treatment group and time for either measure of waist circumference ($p = 0.786$ and $p = 0.828$ respectively) (**Figure 4-1D**). Analysis of the main effects showed a significant effect of time for both waist circumference at the iliac crest ($p < 0.001$) and below the umbilicus ($p < 0.001$), however there was no significant

main effect of treatment group for either measurement ($p = 0.809$ and $p = 0.731$ respectively).

The change from initial to final test day was not significantly different between the treatment groups for either waist circumference at the iliac crest or below the umbilicus (**Figure 4-2F**). Within the prebiotic fiber and placebo treatment groups, waist circumference at the iliac crest significantly increased; whereas below the umbilicus, it significantly decreased within the prebiotic fiber and placebo groups (**Figure 4-3E & F**).

Table 3. Anthropometric measures of overweight and obese children 7-12 years old

Outcome	Prebiotic Fiber				Placebo				Between groups p value
	Initial	Final	Within group p value	Change	Initial	Final	Within group p value	Change	
Height (cm)	148.1 ±2.4	150.5 ±2.4	< 0.001	2.3 ±0.3	147.1 ±2.8	149.1 ±2.9	< 0.001	2.0 ±0.2	0.354
Height Z-Score	1.31 ±0.24	1.37 ±0.24	0.139	0.06 ±0.04	0.97 ±0.22	0.99 ±0.23	0.481	0.03 ±0.04	0.509
Body Weight (kg)	58.5 ±3.1	59.6 ±3.1	0.009	1.1 ±0.4	59.6 ±4.5	62.2 ±4.8	< 0.001	2.6 ±0.4	0.009
Body Weight Z-Score	2.25 ±0.10	2.18 ±0.12	0.006	-0.07 ±0.02	2.14 ±0.16	2.14 ±0.16	0.790	0.01 ±0.02	0.024
Waist- Iliac Crest (cm)	87.3 ±2.1	92.0 ±2.2	< 0.001	4.8 ±0.9	88.1 ±3.0	93.7 ±3.5	< 0.001	5.6 ±0.1	0.520
Waist- Umbilicus (cm)	91.2 ±2.2	89.2 ±2.1	0.012	-2.1 ±0.8	92.6 ±3.3	90.9 ±3.2	0.009	-1.7 ±0.6	0.814
BMI (kg/m ²)	26.2 ±0.7	26.0 ±0.7	0.199	-0.3 ±0.2	26.9 ±1.3	27.3 ±1.3	0.020	0.4 ±0.2	0.004

Abbreviations; Body Mass Index (BMI)

Values are presented as mean ±SEM (n = 22 prebiotic fiber, n = 19 placebo)

Figure 4-1. Anthropometric measures over the 16 week intervention.

Mean + SEM values for anthropometric measures of height and height Z-Score (A), body weight and body weight Z-Score (B), body mass index (BMI) (C) and waist circumference measured at the iliac crest and two centimeters below the umbilicus (D) measured at monthly visits over the 16 week intervention.

Figure 4-2. Anthropometric changes from baseline between groups.

Changes from baseline between treatment groups in anthropometric measures height (A), body weight (B), height Z-score (C), body weight Z-score (D), body mass index (BMI) (E) and measures of waist circumference at the iliac crest and below the umbilicus (F). Data are presented as mean + SEM. * represents $p < 0.05$ and † represents $p < 0.01$ between groups.

Figure 4-3. Anthropometric changes from baseline within groups.

Anthropometric changes within treatment groups for height (A), height Z-score (B), body weight (C), body weight Z-score (D), waist circumference measured at the iliac crest (E), waist circumference measured below the umbilicus (F) and Body Mass Index (BMI) (G). Data are presented as mean + SEM. * represents $p < 0.05$ and † represents $p < 0.01$ within groups.

4.3 Body Composition

All measures of body composition are reported in **Table 4**. Changes in whole-body percent body fat and percent trunk body fat were significantly different between the treatment groups (**Figure 4-4A**). Within the prebiotic fiber group, there were significant decreases in total body fat and trunk body fat from initial test day to final, whereas there were no significant changes in either of these outcomes within the placebo group (**Figure 4-5A & B**). There were no significant between group differences for changes in lean mass and lean mass index (**Figure 4-4B & C**). Within the prebiotic fiber and placebo group, lean mass significantly increased over the 16 weeks of the study (**Figure 4-5E**). Change in fat mass and fat mass index were significantly different between the treatment groups (**Figure 4-4B & C**). Within the prebiotic fiber group, there was no significant change in fat mass but there was a significant decrease in fat mass index (**Figure 4-5D & F**). Within the placebo group, there was a significant increase in fat mass and no significant change in fat mass index.

Android fat, gynoid fat and the ratio of the two fat depots are measures of body fat distribution. There were no significant differences in changes in percent android fat, gynoid fat or the android to gynoid (A: G) fat ratio between the treatment groups (**Figure 4-4D**). Within the prebiotic fiber group, percent android body fat tended to decrease after the 16 weeks however there were no statistical differences in percent gynoid fat (**Figure 4-5G & H**). Within the placebo group, there were no statistical differences in android fat or gynoid fat from initial to final test days.

Table 4. Measures of body composition in overweight and obese children 7-12 years old

Outcome	Prebiotic Fiber				Placebo				Between group p value
	Initial	Final	Within group p value	Change	Initial	Final	Within group p value	Change	
Total BF (%)	42.5 ±1.3	41.6 ±1.2	0.005	-1.0 ±0.3	41.8 ±1.2	41.8 ±1.3	0.958	0.02 ±0.30	0.027
Trunk BF (%)	37.0 ±1.2	35.6 ±1.2	0.019	-1.4 ±0.6	35.2 ±1.1	35.1 ±1.1	0.876	-0.1 ±0.4	0.029
Lean Mass (kg)	31.7 ±1.4	33.1 ±1.5	<0.001	1.3 ±0.3	32.6 ±1.9	34.0 ±2.0	0.001	1.3 ±0.3	0.977
Lean Mass Index (g/cm ²)	1.41 ±0.02	1.43 ±0.03	0.115	0.02 ±0.01	1.45 ±0.03	1.48 ±0.04	0.017	0.02 ±0.01	0.944
Fat Mass (kg)	25.4 ±2.0	25.3 ±1.8	0.519	-0.2 ±0.3	25.8 ±2.6	27.0 ±2.8	0.001	1.2 ±0.3	0.003
Fat Mass Index (g/cm ²)	1.10 ±0.06	1.07 ±0.05	0.015	-0.04 ±0.01	1.10 ±0.07	1.12 ±0.08	0.210	0.02 ±0.01	0.002
Android BF (%)	44.2 ±1.2	43.3 ±1.2	0.055	-1.0 ±0.5	42.1 ±1.4	41.7 ±1.3	0.481	-0.4 ±0.5	0.379
Gynoid BF (%)	45.1 ±1.3	44.9 ±1.0	0.586	-0.3 ±0.3	46.2 ±1.3	46.3 ±1.3	0.983	0.1 ±0.4	0.445
A:G Ratio	0.97 ±0.02	0.95 ±0.02	0.212	-0.02 ±0.01	0.91 ±0.02	0.91 ±0.02	0.467	-0.003 ±0.01	0.441

Abbreviations; Body Fat (BF), Android to Gynoid (A: G)

Values are presented as mean ±SEM (n = 22 prebiotic fiber, n = 19 placebo)

Figure 4-4. Body composition changes from baseline between groups.

Changes from baseline between treatment groups in body composition measures total and trunk body fat percent (A), lean and fat mass (B), lean mass and fat mass indices (C) and android fat, gynoid fat and the ratio of the two outcomes (D). Data are presented as mean + SEM. * represents $p < 0.05$ and † represents $p < 0.01$ between groups.

Figure 4-5. Body composition changes from baseline within groups.

Body compositional changes within treatment groups for total body fat (A), trunk body fat (B), lean mass (C), fat mass (D), lean mass index (E), fat mass index (F), android fat (G) and gynoid fat (H). Data are presented as mean + SEM. * represents $p < 0.05$ and † represents $p < 0.01$ within groups.

4.4 Cytokines and Inflammatory Markers

Measures of the markers of the inflammatory response are presented in **Table 5**. Pro-inflammatory marker IL-6 was significantly different between the prebiotic fiber and placebo groups (**Figure 4-6A**). Within the prebiotic group there was no significant change after the 16 weeks of intervention, whereas within the placebo group, IL-6 tended to increase (**Figure 4-7A**). CRP tended to be reduced between treatment groups (**Figure 4-6B**), but there was no significant changes within the prebiotic fiber or the placebo control groups (**Figure 4-7B**). There was no significant change between the two treatment groups for TNF- α (**Figure 4-6A**) or MCP-1. There were no within group differences for TNF- α (**Figure 4-7C**) and MCP-1 over the 16 week intervention.

LPS concentrations are reported in **Table 5**. There were no significant between group or within group differences for LPS over the 16 weeks of intervention.

Table 5. Inflammatory markers in overweight and obese children 7-12 years old

Outcome	Prebiotic Fiber				Placebo				Between group p value
	Initial	Final	Within group p value	Change	Initial	Final	Within group p value	Change	
CRP (mg/L)	2.3 ±0.4	1.6 ±0.2	0.155	-0.7 ±0.4	2.4 ±0.5	2.6 ±0.6	0.285	0.2 ±0.2	0.081
IFN γ (pg/mL)	16.58 ±3.95	16.33 ±3.98	0.800	-0.25 ±0.98	11.89 ±3.02	11.94 ±3.40	0.953	0.06 ±0.92	0.822
IL-10 (pg/mL)	3.72 ±0.65	3.61 ±0.59	0.513	-0.10 ±0.17	4.35 ±1.19	3.86 ±0.85	0.475	-0.49 ±0.47	0.799
IL-1 β (pg/mL)	0.94 ±0.11	0.84 ±0.13	0.245	-0.10 ±0.08	1.07 ±0.21	1.07 ±0.21	0.875	0.001 ±0.05	0.384
IL-6 (pg/mL)	0.76 ±0.16	0.66 ±0.15	0.092	-0.10 ±0.06	0.51 ±0.12	0.64 ±0.14	0.055	0.13 ±0.06	0.010
TNF- α (pg/mL)	4.26 ±0.46	4.36 ±0.40	0.653	0.10 ±0.25	3.93 ±0.45	4.19 ±0.51	0.433	0.26 ±0.22	0.961
IL-4 (pg/mL)	2.27 ±0.78	1.55 ±0.40	0.114	-0.71 ±0.61	3.46 ±1.97	2.20 ±0.99	0.657	-1.26 ±1.08	0.135
IL-33 (pg/mL)	3.7 ±1.3	4.6 ±1.4	0.220	0.9 ±0.6	7.4 ±3.4	6.7 ±3.9	0.674	-0.7 ±2.6	0.332
MCP-1 (pg/mL)	1331.7 ±110.2	1235.8 ±129.4	0.194	-95.9 ±71.0	1628.5 ±112.3	1582.9 ±122.4	0.458	-45.6 ±59.8	0.604
LPS (EU/mL)	4.1 ±0.7	4.1 ±0.4	0.609	-0.03 ±0.6	4.1 ±0.6	3.6 ±0.5	0.347	-0.5 ±0.6	0.215

Abbreviations; C-reactive protein (CRP), interferon (IFN), interleukin (IL), tumor necrosis factor (TNF), monocyte chemoattractant protein (MCP), lipopolysaccharide (LPS)

Values are presented as mean ±SEM (n = 19-17 prebiotic fiber, n = 16-14 placebo)

Figure 4-6. Inflammatory marker changes from baseline between groups.

Changes from baseline treatment groups in inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor- alpha (TNF- α) (A) and inflammatory marker C-reactive protein (CRP) (B). Data are presented as mean + SEM. † represents $p < 0.01$ between groups.

Figure 4-7. Inflammatory marker changes from baseline within groups.

Inflammatory marker changes within treatment groups for interleukin- 6 (IL-6) (A), C-reactive protein (CRP) (B), tumor necrosis factor alpha (TNF- α) (C). Data are presented as mean + SEM.

4.5 Blood Lipids

Concentrations of blood lipids are presented in **Table 6**. There were no significant differences in the blood lipid outcomes between groups (**Figure 4-8**). Changes in blood lipid levels within groups showed no significant changes in total cholesterol within either the prebiotic fiber or the placebo group (**Figure 4-9A**), nor in other fractions of cholesterol. There was a significant decrease in blood triglycerides within the prebiotic group whereas in the placebo group there was no significant change (**Figure 4-9B**).

Table 6. Blood lipid levels in overweight and obese children 7-12 years old

Outcome	Prebiotic Fiber				Placebo				Between group p value
	Initial	Final	Within group p value	Change	Initial	Final	Within group p value	Change	
Total Cholesterol (mmol/L)	4.54 ±0.20	4.58 ±0.22	0.671	0.04 ±0.10	4.59 ±0.23	4.60 ±0.27	0.944	0.01 ±0.11	0.800
LDL-C (mmol/L)	2.76 ±0.18	2.86 ±0.19	0.184	0.11 ±0.08	2.74 ±0.18	2.75 ±0.23	0.904	0.02 ±0.14	0.556
HDL-C (mmol/L)	1.18 ±0.05	1.23 ±0.04	0.181	0.05 ±0.03	1.10 ±0.06	1.12 ±0.07	0.829	0.01 ±0.06	0.620
TG (mmol/L)	1.31 ±0.16	1.06 ±0.09	0.024	-0.25 ±0.11	1.66 ±0.24	1.60 ±0.23	0.826	-0.06 ±0.22	0.400
Total to HDL Ratio	3.95 ±0.20	3.68 ±0.17	0.113	-0.27 ±0.16	4.33 ±0.31	3.97 ±0.34	0.301	-0.37 ±0.34	0.785

Abbreviations; Low-density lipoprotein- cholesterol (LDL-C), high-density lipoprotein- cholesterol (HDL-C), Triglycerides (TG)

Values are presented as mean ±SEM (n = 21 prebiotic fiber, n = 15 placebo)

Figure 4-8. Blood lipid changes from baseline between groups.

Changes from baseline between treatment groups in blood lipid levels of total cholesterol, low-density lipoprotein- cholesterol (LDL-C), high-density lipoprotein- cholesterol (HDL-C) and triglycerides (TG). Data are presented as mean + SEM.

Figure 4-9. Blood lipid changes from baseline within groups.

Blood lipid levels within treatment groups for total cholesterol (A) and triglycerides (B). Data are presented as mean + SEM. * represents $p < 0.05$ within groups.

4.5 Glucose, Insulin and Measures of Insulin Resistance

Fasting levels of plasma glucose, serum insulin and two indices of insulin resistance, homeostatic model assessment for insulin resistance 2 (HOMA2-IR) and quantitative insulin sensitivity check index (QUICKI), are presented in **Table 7**. The change in fasting levels of glucose and insulin was not significantly different between the prebiotic fiber and placebo group (**Figure 4-10A & B**). There were also no significant changes within the prebiotic fiber or placebo group for either fasting glucose or fasting insulin (**Figure 4-11A & B**). Changes in HOMA2-IR and log transformed HOMA2-IR numerically decreased within the prebiotic fiber group and increased within the placebo group however, the changes were not significantly different. QUICKI also changed in the direction hypothesized but similarly this change was not significantly different between the groups.

Table 7. Measures of insulin resistance in overweight and obese children 7-12 years old

Outcome	Prebiotic Fiber				Placebo				Between group p value
	Initial	Final	Within group p value	Change	Initial	Final	Within group p value	Change	
Fasting Glucose (mmol/L)	5.43 ±0.14	5.53 ±0.13	0.431	0.10 ±0.13	6.23 ±0.29	6.17 ±0.24	0.780	-0.06 ±0.20	0.493
Fasting Insulin (μU/mL)	11.3 ±1.4	9.9 ±1.2	0.486	-1.4 ±1.2	11.3 ±1.4	11.4 ±1.3	0.937	0.1 ±0.8	0.838
HOMA2-IR	1.36 ±0.16	1.31 ±0.15	0.879	-0.05 ±0.20	1.63 ±0.20	1.75 ±0.56	0.701	0.11 ±0.14	0.869
Log HOMA2-IR	0.08 ±0.05	0.07 ±0.04	0.870	-0.01 ±0.06	0.16 ±0.06	0.18 ±0.06	0.606	0.02 ±0.04	0.666
QUICKI	3.06 ±0.06	3.11 ±0.05	0.500	0.05 ±0.07	3.09 ±0.07	3.06 ±0.05	0.975	-0.03 ±0.06	0.729

Abbreviations; Homeostatic Model Assessment 2- Insulin resistance (HOMA2-IR), Quantitative Insulin Sensitivity Check (QUICKI)

Values are presented as mean ±SEM (n = 20 prebiotic fiber, n = 17 placebo)

Figure 4-10. Fasting glucose and fasting insulin changes from baseline between groups.

Changes from baseline between treatment groups in fasting glucose (A) and fasting insulin (B). Data are presented as mean + SEM.

Figure 4-11. Fasting glucose and fasting insulin changes from baseline within groups.

Within treatment group changes for fasting glucose (A) and fasting insulin (B). Data are presented as mean + SEM.

4.6 Gut Microbial Composition

Microbial abundance measured by quantitative polymerase chain reaction (qPCR) was reported as absolute values and as proportions of the total bacteria. The proportions of total bacteria are reported in **Table 8**. There was no significant change in the absolute abundance of any of the bacterial groups or species between the prebiotic fiber group and the placebo groups. There was a significant change in the proportion of *Bifidobacterium* spp. between the prebiotic fiber and placebo groups (+1.71% vs +0.13% respectively; $p = 0.049$) (**Figure 4-12A**). The change in proportion of *Bacteroides* spp. tended to differ between the two treatment groups (+4.83% vs -0.82%; $p = 0.077$) however, this was not statistically significant (**Figure 4-12B**).

Within group analysis, prebiotic fiber significantly increased the proportion of *Bifidobacterium* spp. from baseline to 16 weeks (**Figure 4-13A**). *Clostridium* cluster XI tended to decrease with prebiotic fiber consumption and significantly decreased within the placebo group after the 16 weeks (**Figure 4-13B**).

Table 8. Proportion of total bacteria assessed by quantitative PCR in overweight and obese children 7-12 years old

	Prebiotic Fiber				Placebo				
	Initial	Final	Within group p value	Change	Initial	Final	Within group p value	Change	Between group p value
<i>Bifidobacterium</i> spp.	2.44 ±0.59	4.14 ±0.72	0.023	1.71 ±0.80	2.10 ±0.38	2.23 ±0.84	0.469	0.13 ±0.94	0.049
<i>Bacteroides</i> spp.	2.73 ±0.64	7.50 ±2.43	0.218	4.77 ±2.39	2.36 ±0.54	1.55 ±0.48	0.278	-0.80 ±0.74	0.077
<i>Lactobacilli</i> spp.	0.002 ±0.004	0.01 ±0.01	0.658	0.01 ±0.01	0.002 ±0.001	0.002 ±0.001	0.605	0.0002 ±0.0004	0.888
<i>Clostridium</i> cluster XIV	49.44 ±2.74	48.26 ±4.40	0.433	-1.19 ±4.52	49.96 ±3.12	47.84 ±3.74	0.796	-2.12 ±4.59	0.765
<i>Clostridium</i> cluster IV	35.93 ±2.84	31.75 ±3.73	0.232	-4.18 ±4.29	38.15 ±2.99	39.17 ±4.44	0.836	1.02 ±5.37	0.519
<i>Clostridium</i> cluster XI	1.56 ±0.37	0.82 ±0.14	0.079	-0.74 ±0.38	1.22 ±0.23	0.75 ±0.25	0.044	-0.47 ±0.20	1.00
<i>Clostridium</i> cluster I	0.27 ±0.06	0.37 ±0.13	0.538	0.10 ±0.14	0.47 ±0.20	2.39 ±1.70	0.679	1.92 ±1.72	1.00
<i>Roseburia</i> spp.	6.18 ±1.16	5.49 ±0.87	0.940	-0.69 ±1.43	4.35 ±0.90	4.30 ±0.66	0.569	-0.05 ±1.00	0.789
<i>M. smithii</i>	0.04 ±0.02	0.12 ±0.08	0.279	0.08 ±0.06	0.02 ±0.02	0.01 ±0.002	0.408	-0.02 ±0.02	0.352
<i>Enterobacteriaceae</i> spp.	0.13 ±0.05	0.18 ±0.09	0.627	0.05 ±0.09	0.04 ±0.01	0.07 ±0.02	0.352	0.02 ±0.02	0.789
<i>A. muciniphila</i>	0.14 ±0.08	0.35 ±0.24	0.339	0.21 ±0.16	0.06 ±0.02	0.20 ±0.14	0.594	0.14 ±0.13	0.765
<i>F. prausnitzii</i>	1.14 ±0.18	1.00 ±0.14	0.332	-0.14 ±0.24	1.27 ±0.21	1.48 ±0.22	0.163	0.22 ±0.20	0.124
FB Ratio	87.60 ±31.49	174.71 ±85.32	0.913	87.11 ±90.31	195.08 ±60.02	345.61 ±186.3	0.650	79.16 ±192.43	0.808

Data are presented as the proportion of total microbes attributed to each group

Abbreviations; Firmicutes to Bacteroidetes ratio (FB Ratio)

Values are presented as mean ±SEM (n = 22 prebiotic fiber, n = 19 placebo)

Figure 4-12. Changes in microbial proportions from baseline between groups.

Changes in the proportion (prop.) of *Bifidobacterium* spp. (A) and proportion of *Bacteroides* spp. (B) between groups. Data are presented as mean + SEM. * represents $p < 0.05$.

Figure 4-13. Changes in microbial proportions from baseline within groups.

Changes in the proportion of *Bifidobacterium* spp. (A) and *Clostridium* Cluster XI (B) within groups. Data are presented as mean + SEM. * represents $p < 0.05$.

4.7 Demographics

The demographics of the study population are presented in **Table 9**. The parents of the participants were primarily married or common law (81.8%) while the remainder of the parents were divorced or separated (18.2%). The families of the participants were affluent with 75.8% of the families earning more than \$80,000. The education level of the parent of primary contact was generally very high with 57.6% of the parents having completed university or college and 18.2% of the parents having some or completed graduate school. The parent was also primarily full-time employment status (63.6%). The parent of primary contact was the mother for 69.7% of the participants and the average age for that parent was 44 years old. The ethnicity of the participants were 81.8% Caucasian and 18.2% categorized as “Other” which represented Black and Hispanic.

Table 9. Demographics of children and parents who completed the 16 week intervention

Demographic	Frequency	Percent (%)
Marital Status		
Married/Common Law	27	81.8
Divorced/Separated	6	18.2
Widowed	0	0
Never Married	0	0
Education Level		
Some High School	0	0
Completed High School	2	6.1
Some University/College	6	18.2
Completed University/College	19	57.6
Some OR Completed Graduate School	6	18.2
Annual Income		
< \$20, 000	0	0
\$20,000 - \$39,999	2	6.1
\$40,000 - \$59,999	1	3.0
\$60,000 - \$79,999	5	15.2
> \$80,000	25	75.8
Employment Status		
Full-Time	21	63.6
Homemaker	3	9.1
Retired	0	0
Part-time	8	24.2
Unemployed	1	3.0
Disability/Sick Leave	0	0
Race		
Caucasian	27	81.8
Other	6	18.2
Gender		
Male	10	30.3
Female	23	69.7

4.8 Physical Activity

Physical activity measurements are presented in **Table 10**. There was no statistically significant interaction between treatment group and time on Godin's measure of physical activity ($p = 0.502$), and analysis of main effects determined there was no main effect of time ($p = 0.781$) or main effect of group ($p = 0.495$) (**Figure 14**).

Table 10. Measures of physical activity in overweight and obese children 7-12 years old

Outcome	Prebiotic Fiber	Placebo
Godin's Leisure Time Score (MET hours/week)		
Initial Week	18.26 $\pm$ 3.15	16.09 $\pm$ 3.19
Week 8	21.70 $\pm$ 2.68	21.31 $\pm$ 4.15
Final Week	22.14 $\pm$ 2.89	26.81 $\pm$ 4.70

Abbreviations; Metabolic Equivalent (MET)

Values are presented as mean $\pm$ SEM (n = 18 prebiotic fiber, n = 15 placebo)

Figure 4-14. Measure of physical activity over the 16 week intervention

Change in measure of physical activity measured in metabolic equivalent (MET) hours/week over the 16 week intervention between and within treatment groups. Data is presented as medians.

4.9 Gastrointestinal Feelings, Acceptability and Compliance

Responses to the questionnaire on gastrointestinal symptoms and the acceptability of the treatments for those who completed the study are presented in **Table 11**. In both the prebiotic group and the placebo group, a majority of the participants reported the supplement was very acceptable (55% and 66.7% respectively) with the remaining reporting the supplement was moderately acceptable.

The participants reported if they experienced bloating or flatulence throughout the 16 weeks of the study. In the prebiotic fiber group, 70% of the participants did not experience any side effects, 25% experienced mild increases in gas and bloating and 5% experienced moderate increases. In the placebo group a majority of the participants also did not experience any side effects (61.1%) whereas 27.8% experienced mild increases in gas and bloating and 11.1% experienced moderate increases.

The participants were then asked which group, either the prebiotic fiber group or the placebo, they thought they were assigned to in order to determine if they remained blinded. Half of the participants in the prebiotic group believed they were in the placebo group and the other half believed they were in the prebiotic fiber group. In the placebo group, 72.2% of the participants believed they were in the placebo group while the remaining 27.8% believed they were in the prebiotic fiber group.

Finally, throughout the clinical trial compliance was assessed. Those in the prebiotic fiber group had on average 87% compliance whereas those in the placebo group had 91% compliance. This difference between the two groups was not statistically significant ($p = 0.213$).

Table 11. Measures of Acceptability in participants who completed the 16 week intervention

Outcome	Prebiotic Fiber		Placebo	
	Frequency	Percent (%)	Frequency	Percent (%)
Acceptability				
Very Acceptable	11	55	12	66.7
Moderately Acceptable	9	45	6	33.3
Not at all acceptable	0	0	0	0
Experience Bloating/Flatulence				
None	14	70	11	61.1
Mild Increase	5	25	5	27.8
Moderate Increase	1	5	2	11.1
Severe Increase	0	0	0	0
Guessed Treatment Group				
Prebiotic Fiber	10	50	5	27.8
Placebo	10	50	13	72.2

CHAPTER FIVE: **DISCUSSION**

With a 47.1% increased prevalence in global pediatric obesity since 1980 and with approximately 31% of children 5-17 years old classified as overweight or obese in Canada, pediatric obesity is both a global and national health concern (Roberts et al., 2012; World Health Organization, 2015). As a result there is an increasing need for preventative tools and interventions that target pediatric obesity. This work was designed to evaluate the effects of a prebiotic fiber supplement in overweight and obese children. The primary outcome was change in body composition, while the secondary outcomes were serum inflammatory markers, lipid profile and insulin, as well as plasma glucose. Gut microbiota were examined as a possible mechanistic link between prebiotic consumption and metabolic health outcomes.

5.1 Anthropometrics

5.1.1 Absolute Measures of Height and Weight

The primary objective of this study was to determine if prebiotic fiber consumption improved body composition in overweight and obese children compared to placebo control. While DXA provides the most objective assessment of body composition in our participants and is discussed below, there were a number of additional weight-related outcomes assessed in our study. Anthropometric measures are used to assess the growth and development of children with the two most common measures being height and body weight; both of which increased linearly with age and show minor differences between the sexes in early childhood (Malina, 1999). As expected

there was an increase in height within both our treatment group and control group. This increase over the 16 week intervention was approximately 2 cm, following the yearly expected 5-8 cm growth for those 6-10 years old (Malina, 1999). With the average age of the study population being 10 years old at the beginning of the study and expected trends in height to follow for the remainder of the year, children in both treatment groups were meeting expected growth rates for height.

Importantly, body weight is expected to increase yearly, with an average body weight gain of 2-3 kg in children aged 6-10 years (Malina, 1999). If trends for body weight observed over the 16 weeks continued for the year, children in the prebiotic fiber group would follow expected trends. This is in contrast to children in the placebo group where the projected increase in body weight is 8 kg, almost triple the expected yearly increase. Prebiotic fiber consumption normalized the body weight gain observed in overweight and obese children allowing them to meet expected growth trends for both height and body weight.

5.1.2 Age-sex Specific Outcomes

In the pediatric population, height and body weight are not only observed as absolute values but also as age-sex-specific measures, allowing the values to be compared across age and sex for a particular outcome (Y. Wang & Chen, 2012). Height as a Z-score was not significantly different within or between groups. Change in body weight Z-score however, was not only significantly different between the groups, but there was a significant decrease in body weight Z-score within the prebiotic fiber group after 16 weeks of the intervention. Not only was prebiotic fiber consumption able to maintain absolute body weight gain within the expected yearly increase, it

was able to reduce the average age-sex-specific body weight gain which remained the same in the placebo control.

5.1.3 Body Mass Index

With measures of height and body weight, body mass index (BMI) was also calculated as an anthropometric outcome. BMI aims to assess body weight status and is commonly used in adults however, it cannot distinguish between an individual being overweight due to excess fat mass or higher lean mass (Must & Anderson, 2006). The measure can be used to assess overweight and obesity in children, but the values are not age-sex specific and can therefore be relatively inaccurate. However, in children 5-18 years old with a BMI-for-age $\geq 85^{\text{th}}$ percentile, BMI was an accurate measure and the accuracy of BMI increased with increases in BMI-for-age or body fat (Freedman et al., 2005). With the inclusion criteria for our clinical trial being children $\geq 85^{\text{th}}$ BMI-for-age percentile and the average percent total body fat for the treatment groups being 40%, the use of BMI within this cohort can be considered an accurate predictor of obesity.

In a population of healthy, non-obese 9-13 year olds, BMI increased following one year of prebiotic fiber supplementation in combination with increased calcium consumption (Abrams et al., 2007). This increase over the year-long intervention however, was significantly lower than the increase seen with control and was within the expected yearly increase of 0.6 to 0.8 kg/m² for normal weight children in this age range. In contrast to these findings, prebiotic fiber consumption in our study prevented increases in BMI and resulted in a numerical decrease in BMI, although this was not statistically significant. In our placebo group, there was a significant increase in BMI over the 16 week clinical trial. In a normal weight population, the normalization of BMI increase

over time is important as a preventative measure, whereas in overweight or obese children, the reduction or maintenance of BMI is important for potential interventions. This highlights a beneficial role for prebiotic fiber in both a normal weight, overweight and obese pediatric population in BMI maintenance. Notably, in the study by Abrams et al., (2007), the dose of prebiotic fiber and the combination of oligofructose and inulin were the same as the present study.

Since BMI is a measure that does not account for the distribution of body fat, which is important in understanding disease risk, anthropometric measurements that aim to assess central adiposity are important measures for identifying those at risk.

5.1.4 Waist Circumference

Waist circumference is one anthropometric measure of central adiposity that estimates body-fat distribution. In children, waist circumference may be used to help identify children with potential abnormal blood lipid and insulin concentrations as well as those at risk for cardiovascular disease (CVD) (Freedman, Serdula, Srinivasan, & Berenson, 1999; Maffei, Pietrobello, Grezzani, Provera, & Tato, 2001). Waist circumference measurements however, are subject to a large amount of measurement error, which is especially true in those who are obese (Dalton et al., 2003). In particular, measurements directly above the iliac crest, which is most correlated with percent body fat, are technically difficult due to incorrect positioning and the amount of tension needed to apply with the measuring tape (J. Wang et al., 2003). This is a measurement limitation which could explain the large variation we observed in the waist circumference at the iliac crest and the reason why waist circumference increased in the prebiotic fiber group despite a reduction in trunk body fat measured via DXA.

Waist circumference was also measured below the umbilicus in an attempt to control for the error associated with measures at the iliac crest. Similarly however, there was a large variance and issues with measurement error, particularly the tension applied and participant compliance. This could explain the sudden decrease in waist circumference between the 12 week and final 16 week visit. It could also explain the difference in trends observed between the iliac and the umbilicus waist circumference measurements.

In normal weight, obese children (Abrams et al., 2007; Liber & Szajewska, 2014) and in adults (Dewulf et al., 2013; Lecerf et al., 2012; Parnell & Reimer, 2009) the effect of prebiotic fiber on waist circumference has not been reported. The effect of prebiotic fiber on the waist to hip circumference ratio however was assessed in one study in adults (Dewulf et al., 2013). After the 12 week intervention there were no observed changes in the waist to hip circumference ratio. Given that in the present study there were no observed improvements in waist circumference specific to prebiotic fiber consumption, in combination with the lack of clinical trials similarly assessing waist circumference, the effect of prebiotic fiber on waist circumference remains inconclusive.

5.2 Body Fat and Regional Body Composition

5.2.1 Proportional Changes in Body Fat

The primary outcome in this study was change in whole and regional body composition after prebiotic fiber consumption in overweight and obese children. The definition of obesity is excess body fat that may impair health, therefore the reduction of this body fat is necessary to

promote healthy outcomes (World Health Organization, 2000). As mentioned previously, anthropometric measures such as BMI and waist circumference are used as surrogate markers of changes in body fat but they do not always accurately reflect adiposity. DXA is a technology that more accurately measures total and regional body fat (Goran, 1998). The assessment of regional body fat is equally important as the assessment of total body fat due to the particular negative health outcomes that are associated with intra-abdominal adipose tissue. Although DXA analysis cannot distinguish between visceral and subcutaneous fat, it is much more clinically accessible and can highlight regional changes. DXA has been validated and its use in the pediatric population is proven accurate and reproducible (Gutin et al., 1996; Sopher et al., 2004).

In the present study, prebiotic fiber consumption not only maintained body fat, but more importantly, it significantly reduced total and trunk regional percent body fat in the overweight and obese children. Whereas, the percent of total and trunk body fat in the placebo group was maintained over 16 weeks. In overweight and obese adults, prebiotic fiber consumption similarly reduced percent trunk fat over the 12 week intervention (Parnell & Reimer, 2009). In normal weight children, the effect of year-long prebiotic fiber consumption on reducing percent body fat was a secondary outcome and within group analysis was not statistically assessed (Abrams et al., 2007). However, the outcome at baseline and after a year was reported, and similar to the observations in the present study, there were reductions in percent total body fat in those that consumed prebiotic fiber. In contrast body fat percent was maintained in the placebo group. These results, as well as the results from the present study, highlight the potential for prebiotic fiber as a dietary intervention to reduce adiposity in childhood obesity.

5.2.2 Absolute Changes in Fat and Lean Mass

Absolute fat and lean body mass was also assessed using DXA. Lean mass consists of muscle and soft tissue organs, hence there are expected lean mass increases in children as they grow in height. The absolute increase in lean mass was identical between the prebiotic fiber (1.3 kg) and placebo group (1.3 kg) over the 16 weeks and followed the expected increases in lean mass in this age group. The absolute change in fat mass was a decrease in the prebiotic arm (-0.19 kg) and an increase in the placebo arm (+1.16 kg). Therefore, it is hypothesized that the observed increase in absolute body weight in the prebiotic fiber group was a result of the increased lean mass; whereas the increase in absolute body weight in the placebo group was due to increases in both fat mass and lean mass. Although the magnitude of fat mass reduction in the adult prebiotic study by Parnell and Reimer (2009) was greater (-1.1 kg) than in the present study in children, it also showed a preservation of lean mass in the face of weight loss. These results are in contrast to the null findings of Liber & Szajewska (2014) that showed no changes in total body fat in overweight and obese 7-18 year olds consuming oligofructose for 12 weeks. The conflicting results may be attributed to differences in the duration of intervention, the study populations, the type of prebiotic administered or dosage. In particular, comparing the present study to the clinical trial by Liber & Szajewska (2014), differences may lie in the age of the cohort. In the present study, attempts were made to control for puberty by restricting the age inclusion criteria and assessing Tanner development stage. Whereas in the study by Liber & Szajewska (2014), the cohort was 7-18 years old and no developmental staging was undertaken to assess pubertal status. As a result, puberty could be a potentially uncontrolled confounding factor. The addition of inulin in the present study may also contribute to the differences in the effects of prebiotic fiber. With some

exceptions, the majority of studies favor prebiotic fiber in reducing fat mass and preserving lean mass.

5.2.3 Regional Composition– Android and Gynoid Body Fat

Regional composition, particularly fat mass in the android and gynoid region, was also assessed using DXA. Android fat, which defines a fat depot in the truncal region, is another measure to assess intra-abdominal adipose tissue (He et al., 2002). The gynoid region however, describes fat located in the thighs and hips and is associated with less metabolic risk. A ratio of the android to gynoid fat depots is a secondary assessment of the regional body composition. This was the first clinical trial assessing the effect of prebiotic fiber on the android and gynoid fat depots. However, the importance of assessing these fat depots in overweight and obese children is highlighted in the positive association observed between android fat distribution and insulin resistance (Taillardat & Duche, 2009). It was also observed that the android to gynoid fat ratio was a useful method to assess body fat distribution which is important in metabolic risk assessment. Similarly, in a population of normal weight, overweight and obese children, it was observed that the android to gynoid (A: G) ratio, in particular increased percent android fat, was correlated with increased insulin resistance and dyslipidemia (Samsell, Regier, Walton, & Cottrell, 2014). Therefore, improvements in the A: G ratio, particularly the reduction of android fat, is an important outcome for proposed future clinical trials.

In the present study, prebiotic fiber consumption tended ($p = 0.055$) to decrease android body fat and decreased gynoid fat, although not significantly, whereas placebo had no significant effect on either outcome. Although the defined android fat region is part of the trunk region, it

does not encompass the entire trunk. Hence, this could explain the difference between the change in percent trunk fat and percent android fat. Due to the decreases in the android and gynoid fat depots with prebiotic fiber consumption, albeit not significant, prebiotic fiber can potentially be used as a treatment option for overall and regional fat reduction. Importantly, both anthropometric and body compositional changes occurred in the absence of statistically significant changes in physical activity, thereby attributing these changes to the prebiotic fiber consumption.

5.3 Cytokine Profiles and the Inflammatory Response

A secondary outcome of the present study was to assess changes in the inflammatory profile, particularly serum interleukin (IL)-6, tumor necrosis factor- alpha (TNF- α) and C-reactive protein (CRP) in overweight and obese children consuming prebiotic fiber compared to placebo control. Inflammation in obesity is associated with the increased adipose tissue that defines obesity and is of importance as it is linked to the development of many of the obesity-associated metabolic comorbidities. Inflammatory markers of obesity are therefore hypothesized to decrease as one reduces adiposity, particularly the adipokines. Since there was a reduction in percent body fat observed in those that consumed the prebiotic fiber it was similarly hypothesized that there would be a reduction in adipokines. Both serum IL-6 and TNF- α have been observed to be increased in obesity however, the effect of prebiotic fiber on the reduction of these inflammatory cytokines is not well understood in the otherwise healthy obese population.

In the present study, there were no observed changes in TNF- α after prebiotic fiber consumption. This was similarly observed in healthy, normal weight adults after prebiotic fiber consumption (Lecerf et al., 2012). Serum IL-6 however, was reduced in the present population of

overweight and obese children after prebiotic fiber consumption, whereas in the absence of any intervention IL-6 increased, albeit neither change was statistically significant. Although the effect of prebiotic fiber is not well established, the effect of weight loss on IL-6 has been reported (Bastard et al., 2000; Bruun, Verdich, Toubro, Astrup, & Richelsen, 2003). In obese men and women, body weight and fat mass loss after dietary restriction resulted in significant reductions in IL-6 which was correlated with improved insulin sensitivity (Bastard et al., 2000; Bruun et al., 2003). The observed differences in the changes in adipokine levels is proposed to be a result of their different signaling activity (Mohamed-Ali et al., 1997). Since TNF- α is hypothesized to signal through autocrine or paracrine activity, changes of a lesser magnitude are less likely to be observed in the circulation. Whereas, IL-6 has been observed to signal systemically (Mohamed-Ali et al., 1997).

Notably, the role of IL-6 in obesity, and in particular insulin sensitivity, remains controversial (Carey & Febbraio, 2004). Evidence suggests that IL-6 may stimulate the production of anti-inflammatory cytokines and suppress TNF- α production which would beneficially affect insulin sensitivity (Pedersen & Febbraio, 2007). Moreover, it is hypothesized that TNF- α is the inflammatory cytokine that is detrimental in obesity. In contrast and in response to this hypothesis, it is theorized that similar to what is observed in the aforementioned clinical trials, IL-6 is detrimental in obesity and is positively correlated with insulin resistance (Kern, Ranganathan, Li, Wood, & Ranganathan, 2001; Mooney, 2007). Increased IL-6 levels in the long-term has also been associated with coronary heart disease (Danesh et al., 2008). Based on what is observed in previous clinical trials and the present study, the latter hypothesis that IL-6 is detrimental seems more likely however, both hypotheses are probable.

Inflammatory marker CRP, although not an adipokine, is synthesized in the liver in response to IL-6 and therefore proposed to change with shifts in the degree of obesity (Ganter et al., 1989). CRP has been observed to be reduced after weight loss (Esposito et al., 2003). In adults, elevated IL-6 and CRP levels predict the development of type 2 diabetes (T2D) (Pradhan, Manson, Rifai, Buring, & Ridker, 2001). In children, CRP was correlated with cardiovascular risk factors, highlighting that long-term exposure to inflammation could increase risk factors for CVD (Cook et al., 2000). Therefore, although not significant, the 31% reduction in CRP observed in the children who consumed prebiotic fiber compared to the 8% increase in the placebo arm, could beneficially reduce their risk of metabolic comorbidities associated with obesity.

5.4 Lipid Profile and Measures of Insulin Resistance

5.4.1 Blood Lipid Profile

Another aim of this study was to assess if prebiotic fiber consumption in overweight and obese children improved the serum lipid profile. Components of the metabolic syndrome such as insulin resistance and dyslipidemia can already be observed in overweight and obese children and adolescents (Weiss & Caprio, 2005). These comorbidities, along with other components of the metabolic syndrome, strongly predict CVD and T2D thereby warranting therapies and interventions to reduce one's risk. Hypertriglyceridemia is a hallmark of dyslipidemia in obesity and impairs lipid metabolism at multiple sites throughout the pathway (Klop et al., 2013). The average fasting triglyceride (TG) levels at baseline for those children randomized to the prebiotic fiber and the placebo group exceeded the upper limit of the Calgary Lab Services' reference range

(0.40-1.30 mmol/L), although not a clinically defined state of hypertriglyceridemia consistent with the International Diabetes Federation criteria for metabolic syndrome in children and adolescents (Zimmet et al., 2007). In adults, significant reductions in triglyceride levels were observed after year long interventions that induced weight loss (Howard, Ruotolo, & Robbins, 2003). The effect of prebiotic fiber consumption on serum lipids was inconclusive in overweight and obese adults and no changes were observed after the 12 week intervention (Parnell & Reimer, 2009). In the present study in overweight and obese children, prebiotic fiber significantly reduced fasting TG over the 16 week intervention and importantly, this value was reduced to within the desired reference range. Lipid analysis after prebiotic fiber consumption was also assessed in overweight and obese children by Liber & Szajewska (2014). The number of participants with dyslipidemias was improved in both the prebiotic fiber group and the placebo control with no significant difference in this improvement between groups (Liber & Szajewska, 2014). As a result they concluded that prebiotic fiber did not significantly improve dyslipidemia. Importantly, the improvement of dyslipidemia was the outcome and individual measures of the blood lipid profile were not assessed which could uncover specific improvements in particular lipid outcomes with prebiotic fiber consumption.

In a meta-analysis of trials examining the effect of childhood obesity prevention programs on blood lipids, the combination of physical activity and dietary intervention induced the most beneficial changes (Cai, Wu, Cheskin, Wilson, & Wang, 2014). There was a significant reduction in low-density lipoprotein- cholesterol (LDL-C) and increase in high-density lipoprotein- cholesterol (HDL-C) with no significant changes in total cholesterol (TC) or TGs. Overall, it was observed that interventions of any kind that significantly reduced adiposity had greater improvements in HDL-C, LDL-C and TGs (Cai et al., 2014). In combination with our results, it is

hypothesized that prebiotic fiber in combination with a physical activity and dietary intervention program may elicit the most beneficial effects on levels of blood lipid. Although there were no significant improvements in LDL-C or HDL-C, the improvements in TG highlight that in the absence of either physical activity or dietary intervention programs, prebiotic fiber supplementation alone beneficially alters the blood lipid profile which is likely in part due to its improvements to adiposity.

5.4.2 Measures of Insulin Resistance

Another outcome of the study was the assessment of insulin resistance with HOMA2-IR derived from fasting serum insulin and fasting plasma glucose concentrations. Insulin resistance has been observed to play a major role in dyslipidemia as well as being a precursor for T2D (Klop et al., 2013; Weiss & Caprio, 2005). The homeostatic model of insulin resistance-2 (HOMA2-IR) was the primary assessment of insulin sensitivity in this cohort, and a threshold of a HOMA2-IR > 2.10 , the 97.5th percentile of normal-weight children, was selected as a defined state of insulin resistance (Manios et al., 2008). It was observed that neither the prebiotic fiber group nor the placebo group were at a state of insulin resistance at baseline or the final test day. Although on average those in the placebo group were not at a defined state of insulin resistance, decreases in insulin sensitivity were observed in the placebo arm; whereas there were numerical improvements in insulin sensitivity after prebiotic fiber consumption over the 16 week intervention.

Fasting glucose and fasting insulin levels were measured primarily to estimate insulin sensitivity as they are the values utilized by HOMA2-IR. Fasting glucose levels are also used to assess those with pre-diabetes; an intermediate stage of hyperglycemia which is characterized by

impaired fasting glucose (Kim & Caprio, 2011). According to the American Diabetes Association, impaired fasting glucose (IFG) is defined as a fasting plasma glucose value of 5.6-6.9 mmol/L (100 mg/dL to 125 mg/dL), with the increased risk for diabetes also extending to values below the lower limit of the range (American Diabetes Association, 2010). Importantly, the use of this definition of IFG in a pediatric population has also been established (Williams et al., 2005). At baseline, those in the placebo group on average were in the defined state of IFG or pre-diabetic. In the prebiotic group, the average fasting plasma glucose was not in the defined state of IFG but just beyond the lower limit, indicating similar diabetes risk. Over the 16 weeks of the study, both groups remained at high risk for diabetes and were in a defined state of IFG. These changes over the 16 weeks however, were not significant in either group. Although not statistically significant, prebiotic fiber consumption increased insulin sensitivity. Therefore, it is hypothesized that any benefits elicited by prebiotic fiber with respect to insulin sensitivity is done so through changes in fasting insulin levels but not fasting plasma glucose.

5.5 A Proposed Mechanistic Role of the Gut Microbial Profile

The final outcome of the present study was to assess if prebiotic fiber consumption induced beneficial changes in the gut microbial profile and if these changes were a possible mechanistic link between prebiotic consumption and metabolic health outcomes. Bifidobacteria has been reproducibly shown to be decreased in obesity and this reproducibility is the reason it is hypothesized to be the best bacterial candidate to have anti-obesity effects (Angelakis, Armougom, Million, & Raoult, 2012). One of the proposed mechanisms by which bifidobacteria elicits its beneficial effects is through the growth inhibition of other potentially pathogenic bacteria

associated with obesity and thereby reversing dysbiosis in the gut (Gibson & Roberfroid, 1995). Although there are conflicting results as to which bacterial species would collectively be defined as a “healthy” or “dysbiotic” profile, bifidobacteria is a well-established beneficial bacteria that served as a model for the concept of prebiotic fiber (Delzenne, Neyrinck, Bäckhed, & Cani, 2011). Prebiotic fiber is selectively fermented by bifidobacteria and this is highlighted in clinical trials (Gibson & Roberfroid, 1995). In normal weight young adults and similarly in obese women, consumption of prebiotic fiber significantly increased the abundance of *Bifidobacterium* spp. (Dewulf et al., 2013; Lecerf et al., 2012). It was therefore hypothesized that prebiotic fiber consumption would have significant increases in the proportion of bifidobacteria in the present study, over the 16 week intervention. These changes were observed in the present study and prebiotic fiber consumption doubled the proportion of *Bifidobacterium* spp., which was statistically significant. This result is of importance because changes in the microbial profile, specifically dysbiosis, are associated with metabolic diseases; hence, increases in bifidobacteria due to prebiotic fiber consumption is a marker for improved intestinal and metabolic health (Roberfroid et al., 2010).

Unlike bifidobacteria, and similar to many of the other bacteria quantified, *Bacteroides* spp. is not definitively defined as “beneficial” or “harmful”. The Firmicutes to Bacteroidetes (FB) ratio hypothesis brings to question the role of *Bacteroides*, as it is the most abundant genera in the Bacteroidetes phylum (Karlsson, Ussery, Nielsen, & Nookaew, 2011). Hence increasing *Bacteroides*, in the absence of changes in the overall abundance of Firmicutes, would reduce the FB ratio which is hypothesized to have metabolic benefits (Ley et al., 2006; Nadal et al., 2009). Significant increases in *Bacteroides-Prevotella* abundance were observed in overweight and obese adolescents on a dietary and physical activity obesity intervention after weight loss of > 4.0 kg,

but no changes were observed in those that lost < 2.5kg (Nadal et al., 2009). Similarly, in the present study, increases were observed in *Bacteroides* with prebiotic consumption over the 16 week intervention however, these changes were in the absence of weight loss, but rather a slowing of weight gain. Meta-analysis of the gut microbial profile associated with lean/normal weight and obese individuals revealed no significant differences in *Bacteroides* abundance between the groups (Angelakis et al., 2012). More importantly, in an analysis of the effect of prebiotic fiber consumption on microbial profiles, two studies observed changes in the counts of *Bacteroides* in adults using bacterial culturing for quantification (Roberfroid, 2007b). Gibson et al., (1995) observed a reduction in *Bacteroides* counts in those that consumed oligofructose with no changes in *Bacteroides* counts in those that consumed inulin. Conversely, Rao (2001) observed increases in *Bacteroides* counts after oligofructose consumption. In the present study, the change in *Bacteroides* spp. tended to be significant between the groups and within the prebiotic fiber group there was an increase in the proportion of *Bacteroides* spp. over the 16 week intervention, albeit not statistically significant. As a result, the effect of prebiotic fiber consumption on *Bacteroides* remains inconclusive.

This study revealed changes in the gut microbiome that occurred with the consumption of prebiotic fiber. There were beneficial changes in the microbial profile, particularly an increased proportion of *Bifidobacterium* spp. over the 16 weeks. The gut microbiota has important roles in the inflammatory response, energy extraction from the diet, regulation of intestinal permeability and the production of short chain fatty acids (SCFA) which are involved in the modification and secretion of satiety hormones (Kellow et al., 2014). In a diseased state such as obesity, these roles are dysregulated due to the dysbiotic gut microbial profile. Therefore, the beneficial changes in

the gut microbiota through the consumption of prebiotic fiber, specifically the increase in the proportion of *Bifidobacterium* spp., lead to benefits in host health (Slavin, 2013).

The benefits of increased bifidobacteria are highlighted in infant studies with *Bifidobacterium* spp. dominating the gut of breast-fed babies (Di Gioia, Aloisio, Mazzola, & Biavati, 2013). Importantly, breastfeeding is associated with reduced likelihood of overweight and obesity in childhood. Moreover in a prospective study, children with increased abundance of *Bifidobacterium* spp. at infancy were more likely to remain at a normal weight (Kalliomäki et al., 2008). This highlights the importance of bifidobacteria in weight maintenance and suggests that differences in the gut microbial composition may precede the development of overweight and obesity. With the mounting evidence that increased bifidobacteria abundance is beneficial, research is not only directed at assessing the effect of prebiotic fiber, but also to assess the introduction of bifidobacteria as a probiotic either alone or in combination with prebiotic fiber. In infants, different species, subspecies and strains of bifidobacteria are used as a probiotic for the prevention of diverse pathologies, some of which are gut dysfunctions such as acute diarrhea and obesity (Di Gioia et al., 2013). These benefits could be attributed to the lack of microbial diversity and the simplicity of the diets of the infants. In contrast to the benefits observed in infants, a study which aimed to assess the effect of the probiotic *Bifidobacterium animalis* combined with prebiotic fiber on gut microbiota and metabolism in rats observed that prebiotic fiber, but not probiotic, reduced energy intake, weight gain and fat mass (Bomhof, Saha, Reid, Paul, & Reimer, 2014). It was also observed that modifications of the gut microbiota and greater increases in *Bifidobacterium* spp. occurred after prebiotic fiber consumption compared to probiotic consumption. This further highlights the beneficial role of prebiotic fiber, specifically its “bifidogenic” effect, in improving obesity and metabolic outcomes in obesity.

As there is limited data assessing the effects of prebiotic fiber in the overweight and obese child population and this is the first study to specifically address gut microbiota changes in overweight and obese children, the present study adds to the current understanding about prebiotic fiber and its beneficial metabolic effects.

5.6 Strengths and Limitations

This study was designed as a double-blind, placebo-controlled randomized clinical trial which allowed us to assess the effectiveness of prebiotic fiber. Double-blind, randomized controlled clinical trials are a scientifically rigorous method of hypothesis testing and is regarded as a gold standard however, there are also limitations (Kaptchuk, 2001). There is a volunteer bias associated with the recruitment of participants for a research study. There is also a reduced generalizability of our study findings as the parents of the participants that responded were primarily Caucasian, affluent and well-educated.

A particular strength of the clinical trial was the monthly meetings for anthropometric measures and compliance assessment. This monthly contact with participants and parents motivated the participants to comply with the intervention and improved adherence to the clinical trial. As a result there was a 10% dropout which is less than the previously observed 20% dropout in an adult human weight loss clinical trial (Parnell & Reimer, 2009)

Physical activity was assessed using the Godin's Leisure-Time Exercise Questionnaire which is a subjective measure that was reported by the parents' of participants. Although physical activity was solely assessed to ensure there were no large changes in energy expenditure which could explain changes in the study outcomes, the use of a subjective measure of physical activity

is a limitation of the study. Another limitation of the assessment of physical activity throughout the clinical trial is the seasonal differences between the cohorts which could account for the changes in physical activity and might not be properly accounted for by subjective measures.

Clinical trials in pediatrics must consider the effect of puberty and the associated changes which could confound the outcomes. A strength of this clinical trial was the assessment of pubertal status using Tanner staging at the beginning of the study. All the Tanner stages were recorded and children were at a Tanner stage of ≤ 3 which allows for some control over pubertal status and its impact on the outcomes.

As previously mentioned, there were limitations to the measurement of waist circumference. To limit inter-rater variability, one investigator measured and reported the outcome to mitigate the measurement errors associated with the waist circumference outcome. In another attempt to mitigate the error, two measurements at different locations on the waist were taken. A particular strength of the anthropometric measures was the calculation of Z-scores which allows for the comparison of the anthropometric measures across different ages and sexes. Since this is the one very few studies looking at the effect of the prebiotic fiber in children, and the only study to report its effects of body weight Z-score, the results from future studies can more easily be compared.

The calculation of Z-scores using the World Health Organization- Anthro and AnthroPlus calculators are the gold standard in global pediatric research however, the calculators are limited. Using the Anthro tool, the calculation of body weight Z-score was limited to a maximum of 3 standard deviations (SD) from the mean and any value above 3 SD was not provided and considered 3+ SD. As a result, changes in body weight Z-score were unable to be observed in multiple participants who were 3+ SD. The AnthroPlus tool was limited because the calculation

of body weight Z-score was restricted to children under the age of 10, which excluded analysis for many of our participants. As a result, the WHO calculators were not suited for this cohort or clinical trial. Instead, the Baylor College of Medicine, Body Composition Laboratory- Pediatric Body Composition Calculator was used to calculate height and body weight Z-score. This measurement tool was used by Abrams et al., (2007) to calculate the Z-score for their cohort of children.

Another strength of this study was the analysis of the inflammatory cytokines and markers which have roles in obesity-associated comorbidities. Multiple cytokines and other markers of inflammation were assessed in this population in an attempt to obtain a well-rounded understanding of the inflammatory profile. Inflammatory markers, such as CRP, can also be elevated in a state of acute illness which is common in children. To control for this, parents were asked to inform us if their child was ill prior to the blood draw. It was also possible that the child was ill without visible symptoms, therefore in an attempt to control for this, statistical outliers were removed prior to analysis. One limitation of the measurement of inflammatory cytokine CRP is a statistical limitation. Calgary Lab Services provided the measurements for CRP however, any values less than 1 mg/L were reported as <1 mg/L and not provided as a numerical value. This has implications in the statistical analysis and could have resulted in the lack of statistical differences in CRP since there were statistical trends.

The real world application for the prebiotic fiber is a key strength of this clinical trial. The aim was to solely assess the effect of prebiotic fiber supplement, in the absence of changes in physical activity or dietary interventions. Finally, an additional strength was the consumption of prebiotic fiber as a beverage mixed with water. The intervention could be prepared by parents quickly and with ease. This intervention can be taken on-the-go with the children while they

participate in extra-curricular evening activities limiting major lifestyle changes. Importantly, the children could also prepare the intervention themselves giving them a sense of autonomy and control of their health. This highlights prebiotic fiber's potential as a dietary intervention that can easily be tailored to children and introduced easily into their everyday activities.

5.7 Future Directions

Further work is needed to understand the effects of prebiotic fiber on reducing adiposity and improving metabolic outcomes in overweight and obese children. Since this is one of the first clinical trials aiming to do so in the pediatric population, future clinical trials should have a larger study population. It is hypothesized that this would uncover more statistical differences and benefits of prebiotic fiber consumption. Future clinical trials should also extend the treatment time which is similarly hypothesized to uncover more benefits and importantly the sustainability of the intervention for families. Further, in regards to study design, recruitment should also aim to include diverse races and those of diverse socio-economic status to understand the effect of prebiotic fiber and increase the generalizability of the results. Not only are the effects of prebiotic fiber consumption in overweight and obese children not fully understood, but it is also not well known in normal weight or lean children. Future clinical trials should aim to include normal weight children, not only as a control but also to assess the benefits of prebiotic fiber, especially on the microbial profile of the healthy weight children.

This study was unable to observe any differences in lipopolysaccharides between prebiotic fiber consumption and placebo control. Cani et al., (2009, 2008) however, showed that obese mice were characterized by increased gut permeability and decreased glucagon-like peptide 2 (GLP-2)

and this could be reversed with prebiotic fiber consumption. Hence, future clinical trials should also assess gut permeability which could be assessed in the serum with a lactulose/mannitol/3-O-methylglucose protocol.

With regards to the microbial profiles, Illumina sequencing should be undertaken to confirm the results from qPCR analysis. Although the primers used in the present study represent the major groups of the gut microbiota, sequencing would allow for a more global representation of the microbial profiles which could be limited by the use of self-selected primers in qPCR. Future clinical trials should also extend beyond taxonomic analysis of the microbes and assess the metagenomics changes. Research regarding the gut microbiota highlighted that although individual microbes are of interest, their functions within the gut are also important to assess the mechanism and benefits of prebiotic fiber consumption (Schwartz et al., 2009; Turnbaugh et al., 2009). Furthermore, in association with the microbiota, metabolomics in both the feces and the blood would be important for future clinical trials. Both metagenomics and metabolomics could provide mechanistic insight into the roles of the prebiotic fiber and the microbial profiles.

Finally, future studies should not only aim to assess solely prebiotic fiber, but also assess the effect of prebiotic fiber in combination with dietary and physical activity interventions. This research could be conducted in a clinical setting as part of an established pediatric obesity program. One such possibility would be for a randomized clinical trial in which pediatric weight centre patients are randomized to placebo or prebiotic fiber in conjunction with the standardized clinical program. This would show prebiotic fiber's full potential as an intervention in obesity. It is hypothesized that in combination with other interventions, the benefits observed consuming solely prebiotic fiber would be even greater.

5.8 Overall Conclusions

The overall objective of this thesis was to assess the effects of prebiotic fiber in the absence of physical activity or dietary interventions. There were no statistical differences in physical activity over the 16 weeks in either groups, therefore the metabolic benefits observed can be attributed primarily to prebiotic fiber consumption. Importantly, prebiotic fiber consumption improved anthropometric and body composition measures as well as components of the inflammatory and lipid profile. These changes can be potentially attributed to the improvement in the microbial profile, in particular, the increased proportion of the beneficial *Bifidobacterium* spp. Since the number of studies specifically designed to examine the effect of prebiotic fiber on adiposity and inflammation in overweight and obese children is limited, much more research is needed. However, this research provides a necessary and important basis to understand the effects of prebiotic fiber as a potential dietary intervention in obese and overweight children.

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APPENDIX A: ELIGIBILITY QUESTIONNAIRE

Name: _____

Weight: _____ Height: _____ Age: _____ DOB: _____ Gender: _____

	Yes	No
1. Does your child have cardiovascular problems?	☐	☐
2. Does your child have type 1 or 2 diabetes?	☐	☐
3. Does your child have liver disease?	☐	☐
4. Has your child ever had major gastrointestinal surgery?	☐	☐
5. If your child is female has she began her menstrual cycle?	☐	☐
6. Do you believe your child has reached puberty?	☐	☐
7. Is your child currently receiving insulin or oral hypoglycemic treatment?	☐	☐
8. a. Is your child on drugs that may influence appetite or weight? (This includes herbal supplements)	☐	☐
b. Is your child on drugs or herbal supplements that may affect their metabolism? (eg. Ginseng, green tea extract, chromium)	☐	☐
9. Is your child following a diet designed for weight loss, and/or has their weight changed >3kg in the past 12 weeks?	☐	☐
10. Have there been any changes in your child's exercise intensity or frequency over the past 4 weeks?	☐	☐
11. Has your child taken antibiotics within the past 3 months?	☐	☐
12. Does your child have any allergies (eg. Milk, other food, latex)? If yes, please list them _____	☐	☐
13. Do you know of any reasons why your child should not be eligible for this study including medical conditions not listed here? If yes, please list them _____	☐	☐
14. Do you know of any reasons why participation in this study could negatively affect your child's health? If yes, please explain _____	☐	☐
15. Please rate your child's intake of Prebiotic and/or Probiotic and/or Fiber-Enriched Foods. _____ 0 Intake _____ 1-2 servings/week _____ 3-5 servings/week _____ >5 servings/week (Includes yogurts, prebiotic bread, Catelli smart pasta, V8 juice with inulin, Oasis Probiotic juice, Yogen Fruz probiotic frozen yogurt, high fiber bread or breakfast cereals, high fiber snack bars, Metamucil, etc.).		

Thank you for filling out this questionnaire. The information you have provided is essential to determine eligibility for this study and helps ensure your safety.

APPENDIX B: PARENTAL CONSENT FORM

Effect of Prebiotic Fiber Intake on Satiety and Gut Microbiota in Overweight and Obese Children

Principal Investigator: [REDACTED]

PARENT INFORMED CONSENT

This consent form, a copy of which has been given to you, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what you and your child's participation will involve. If you would like more detailed information about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

This form seeks your informed consent for your child to participate in the study. The form provides specific information on the procedures to be used in this study, so that you can know the nature of the risks involved and can decide if your child will participate or not participate in a free and informed manner.

Please read this entire form carefully. If you decide to give your informed consent for your child to participate in this study, you will be asked to indicate your consent by signing the form on the last page. You will also be asked to initial each page to indicate that you have read it.

BACKGROUND

Your child is being asked to participate in a research study because they have a body weight above the healthy range. We are trying to determine if supplementing the diet with dietary fiber (called a prebiotic fiber) can help with reducing body fat and controlling appetite.

Obesity can cause many health problems as is it linked to many chronic diseases including heart disease and type 2 diabetes. Although there are many possible causes for obesity, it is known that diet plays an important role. Eating enough fiber helps control body weight, however, the majority of Canadians eat too many processed foods and do not meet their daily fiber recommendation.

Inulin and oligofructose are two prebiotic fibers that are approved as ingredients that can be added to foods in Canada. Research has found that adding these fibers to the diet can reduce body fat and lower food intake in adults. There is also the potential for prebiotic fibers to help control appetite. Finding out if prebiotic fiber supplementation can reduce body fat and improve appetite control in children will be tested in this study.

Your child will be randomly assigned to one of two groups that receive either the fiber or placebo control. To make sure that this research is not biased neither you, nor your child, nor the researcher will know who will be given the fiber or who will be given the placebo control. However, should there be a medical emergency and information as to which group your child is in becomes necessary, you and your child can become informed of their grouping. At the end of the study, there will be a final meeting where you and your child will be told which group they were in and the results of the study.

This study is significant because it will determine if adding this type of fiber to regular diet in children can cause a reduction in body fat and improve appetite control. *This information is directly applicable to treating diseases such as obesity, type 2 diabetes and heart disease early in life.*

WHAT IS THE PURPOSE OF THE STUDY?

The main purpose of this study is to investigate the fiber supplement and its effects on body fat, appetite, food intake and gut bacteria in overweight or obese children.

WHAT WOULD MY CHILD HAVE TO DO?

You will need to sign this informed consent document before any procedures specific to this study are performed. If your child also agrees to be in this study, various procedures will be performed. This study will run for a total of 16 weeks from baseline (the start of the study), and will require you and your child to visit the research sites at least 5 times over that period. Parking costs will be covered. As part of the study process, your child will be required to undergo the following tests and procedures during your child's visits to the study site:

Fiber Supplement: Your child will be randomly assigned to one of two groups receiving prebiotic fiber (oligofructose-enriched inulin) or placebo (maltodextrin). Those in the fiber group will receive a pre-weighed packet of oligofructose-enriched inulin powder to be mixed into water and consumed within 30 minutes before the evening meal. Those in the placebo group will receive a similar packet of maltodextrin powder and similarly consume it in water prior to the evening meal. This will continue for 16 weeks. At the end of the study, there will be a final meeting where you will be told which group you were in and the results of the study.

Physical Characteristics: Once informed consent has been obtained, a baseline visit will take place and the following assessments made: age, height, weight, and waist circumference. These measurements will be repeated every 4 weeks. Your child's stage of development (called a Tanner scale) will be assessed by a physician from the Alberta Children's Hospital in a confidential manner at the baseline visit. Your child's body composition (amount of muscle, fat and bone) will be assessed at the beginning, and at the end of the study, 16 weeks later, using a full body, low emission x-ray called dual energy x-ray absorptiometry (DXA).

Food Intake and Exercise: Food and beverage intake will be assessed using 3-day weighed food records. Your child will not be prescribed specific diets but will continue to consume your regular family foods. Prior to the start of the study you and your child will attend a training session delivered by a Registered Dietitian where you will be instructed on the use of the food scale and how to record their food intake. You will then be asked to weigh and record all food and beverages consumed by your child for 2 weekdays and 1 weekend day at baseline, 8 and 16 weeks. You will also be asked to fill out a questionnaire regarding your child's physical activity at baseline, 8 and 16 weeks.

Appetite: You will fill out a short questionnaire at baseline, 8 and 16 weeks to measure your child's hunger and appetite. You will complete this questionnaire at home and on your own time. At baseline and at the end of the study your child will come to the University of Calgary and be given a breakfast buffet. Research staff will help your child complete a visual analogue scale, to assess their feelings related to appetite, immediately before and after the buffet.

Stool Sample: At baseline and the end of the study your child will be asked to provide a stool sample. We will supply you with an easy to use kit to collect a small amount (approximately 1 tablespoon) of stool at home and then bring it to the study site when your child comes for their baseline and final appetite testing.

Fasted Blood Sample: Your child will have a fasted blood sample drawn at baseline and the end of the study by a registered nurse from the Alberta Children's Hospital. We ask that your child refrains from eating food 12 hours prior to giving the blood sample. Drinking water is permitted.

EXPECTED LENGTH OF PARTICIPATION IN THE STUDY

Your child's involvement in the study will include a minimum of 5 visits to the study site over the course of 16 weeks. Taking into account the information sessions, body weight measurements, appetite measures and food weighing, it is projected that you and your child will dedicate at least 12 hours of your time over the next 16 weeks to this project.

WHAT ARE THE RISKS

The oligofructose-enriched inulin fiber may cause some mild side effects such as gas, nausea, thirst, or diarrhea. Usually these symptoms are minimized by starting the fiber slowly and they subside once the body adjusts to the dietary change. If your child takes part in this research, your child will be exposed to a very small amount of radiation. The risk from this amount of radiation has been categorized by the Radiation Safety Committee as 'very low'.

WILL MY CHILD BENEFIT IF HE/SHE TAKES PART?

Participation in this study could help your child lose body fat and decrease your child's caloric intake. Your child will receive information on their current diet and suggested diet changes. By participating in this study, you and your child will also be helping others because you will be giving us information that can help find treatments for obesity and other related diseases such as type 2 diabetes and heart disease.

If you and your child agree to participate in this study there may or may not be a direct medical benefit to you. Your child's body composition and appetite control may be improved during the study but there is no guarantee that this research will help your child. The information we get from this study may help us to provide better treatments in the future for patients with obesity and related diseases such as type 2 diabetes.

WHAT ELSE DOES MY CHILD'S PARTICIPATION INVOLVE?

Participation requires that your child does not make any major lifestyle changes (e.g. start new sports) during the study or participate in any weight management programs.

DOES MY CHILD HAVE TO PARTICIPATE?

If you and your child do not wish to participate in any procedure mentioned above, you may choose to not participate in this study.

Participation in this study is completely voluntary. You are free to participate or refuse to participate in this study. If you decide not to allow your child to participate, or if you and your child begin the study and subsequently decide to withdraw, your decision will involve no penalty or loss of benefits to which you would otherwise be entitled. You should contact the primary investigator Dr. Reimer at 403-220-8218 if you decide to withdraw from the study.

The investigators or the Ethics Committee can stop your participation in the study at any time without your consent if participation appears to be medically harmful to you. You may also be withdrawn from the study without your consent if you fail to follow directions for participating in the study, if it is discovered that you do not meet the study requirements, if the study is canceled, or for administrative reasons.

WILL WE BE PAID FOR PARTICIPATING, OR DO I HAVE TO PAY FOR ANYTHING?

You will have to pay for you and your child's transport to the University of Calgary however; the investigator will reimburse any parking fees you incur during the site visits. You will be provided with the supplement packets however, you will be responsible for buying you and your child's own food throughout the study. The researchers will provide the breakfast buffet on the test day as part of the study. The researcher will cover all other tests fees. Your child will be given a \$50 gift certificate to Toys R' Us for their participation upon completion of the study.

WILL MY CHILD'S RECORDS BE KEPT PRIVATE?

To protect your child's confidentiality only the researchers, study sponsor, and University of Calgary Conjoint Health Research Ethics Board will have access to the data files. Your child will be given an identification number in all analysis. Only the researchers and University of Calgary Conjoint Health Research Ethics Board will have access to the list that contains actual names. Any published information will contain only the number of participants of each gender in each group and the average values for each group. All data will be presented in terms of averages, percentages or ratios. Your child's identity or personal facts unrelated to this research will never be mentioned or published. The data will be stored at the University of Calgary for 5 years in a locked filing cabinet and any computer files will be password protected.

IF MY CHILD SUFFERS A RESEARCH-RELATED INJURY, WILL WE BE COMPENSATED?

In the event that your child suffers injury as a result of participating in this research, no compensation will be provided to you by the manufacturer of the supplements, the University of Calgary, Alberta Health Services or the Researchers. You still have all your legal rights. Nothing said in this consent form alters your right to seek damages.

AGREEMENT TO PARTICIPATE IN THE STUDY

If you consent for your child to participate in the research study, please sign and date this form in the spaces provided below. Your signature below indicates your agreement with the following statements:

1. You have read the information provided in the preceding pages about the study supplement and the procedures to be used in this study. You have been informed about the purpose of the study, the benefits and risks involved. You have understood to your satisfaction the information regarding participation in the research project and agree that your child can be a participant in this study. You

have been informed that you can withdraw your consent for your child to be a research subject and stop participation in this study at any time without affecting your right to seek medical care for your child.

2. It is your responsibility to ask questions to clarify any points you do not clearly understand regarding your child's participation in this study. You have asked all such questions of the study coordinator and you have received answers to your satisfaction.
3. You are not assured or guaranteed a place in this study.
4. You have not concealed or distorted any current medical condition or any medical history that might impair or affect your child's health. You have answered all questions concerning your child's health accurately and truthfully to the best of your knowledge.
5. You agree that representatives of the organizations co-ordinating this study, the sponsor (or their representatives), national health authorities, the ethics committee, and government institutions charged with checking the correct execution of studies such as this one, may have access to your child's data. By signing this form, you agree that the data collected during this study will be used for the purposes as mentioned above, inclusive of forwarding of the data to the national health authorities if required. This will be done in an anonymous way in order to help ensure the confidentiality of the information. You do not waive any rights which you may have under Canadian Law by signing this form.
6. You are 18 years of age or older, and you are legally capable of giving this consent and authorization.
7. You voluntarily consent for your child to participate in this study. You understand that refusing to participate, or discontinuing participation at any time, will involve no penalty or loss of benefits to which you or your child are otherwise entitled.

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time without jeopardizing your health care. Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation. If you have further questions concerning matters related to this research, please contact:

If you have any questions concerning your rights as a possible participant in this research, please contact The Chair, Conjoint Health Research Ethics Board, University of Calgary _____

Name of Participant (please print)

Signature of Participant

Date dd/mm/yy

Signature of Parent/Guardian

Date dd/mm/yy

Signature of Witness

Date dd/mm/yy

Name of Investigator/Designee (please print)

Signature of Investigator/Designee

Date dd/mm/yy

The University of Calgary Conjoint Health Research Ethics Board has approved this research study. A copy of this consent form has been given to you to keep for your records and reference.

APPENDIX C: CHILD INFORMATION ASSENT FORM

Child Information Sheet

Research Title: Effect of Prebiotic Fiber Intake on Satiety and Gut Microbiota in Overweight and Obese Children

Principal Investigator:

What is this study for?

- This a study for children to see how your body responds to eating more fiber.
- You will be randomly placed into one of two groups. If you are in Group A, you will receive a fiber supplement, and if you are in Group B you will receive a control supplement. You will not know which group you are in because both supplements will look and taste similar.
- You will be participating with other 8 to 12 year old children in this study.
- We will be looking at how prebiotic fiber can affect your body weight and your appetite.
- This study will take place at the University of Calgary.
- You will receive a health report at the beginning and end of the study. It will show how you have improved and what areas you can focus on.

What do I have to do?

- Before the study begins, we will ask you for your age. We will measure your height, weight, and the size of your waist 4 times during this study.
- You will lay down on a machine that will measure your body's level of fat, muscle and bone density. This will be done 2 times during the study.
- A doctor from the Alberta Children's Hospital will perform a physical assessment one time at the beginning of the study to indicate your stage of development.
- With your parents, you will write down all the food and drinks you have for 3 days (3 times).
- With your parents, you will fill out a form about your physical activity (3 times).
- You will have your blood drawn 2 times during the study.
- You will provide a stool sample 2 times during the study.

Are there any safety issues?

- A registered nurse from the Alberta Children's Hospital will take your blood two times during the study. You may feel a little poke or get a small bruise on your arm.
- The machine used to measure your body fat composition has a small amount of radiation (much smaller than a normal x-ray machine). We will take that test in a safe way.

Do I have to be and stay in the study?

- You do not have to be in the study.
- You can stop the study at any time during the study.
- If the researcher thinks it is better that you do not participate, they will take you out of the study.

Will my results stay private?

- You will be given a special number and that is the only way your information will be stored.
- Only the research staff directly working with the study will be able to see any information.

If you have any questions about this study, please ask us anytime.

😊 **Thank you for your help !!** 😊

APPENDIX D: SAMPLE SIZE

Data was provided as geometric mean with 95% confidence intervals. Using the provided confidence interval the midpoint of both the treatment and control groups were determined for each outcome. Using this data, sample size and assuming t-value confidence intervals were reported based on the large sample size the standard deviation was determined.

The equation used to determine the standard deviation based on the provided confidence interval (CI) was:

$$CI = x \pm t_{df, \alpha/2} * \frac{s}{\sqrt{n}} ; x = \text{mean}, s = \text{standard deviation}$$

E.g. Standard deviation of % Body fat for the treatment group (df = 104 and $\alpha = 0.05$)

$$-2.8 = -4 + 1.98 * \frac{s}{\sqrt{105}} \quad \text{AND} \quad -5.2 = -4 - 1.98 * \frac{s}{\sqrt{105}}$$

In solving for s the standard deviation for both equations is 6.2

Outcome of interest	Midpoint - treatment (n=105)	Midpoint - Control (n=69)	Standard Deviation	Alpha	Power	Number of Subjects (for each sample)	# subjects + 20% for drop-out	Citation
% Body fat	-4.0	2.0	6.2	0.05	0.8	18	22	Savoye et al. 2007 ⁵¹ (Table 2)
BMI	-1.7	1.55	3.13	0.05	0.8	15	18	
Body weight	0.3	7.65	9.81	0.05	0.8	28	34	
HOMA-IR	-1.47	0.99	4.42	0.05	0.8	51	62	

Sample Size was calculated using Dr. Rollin Brant's web based sample size calculator "Inference for Means: Comparing Two Independent Samples" (Brant, 2006) using the equation provided in the textbook Fundamentals of Biostatistics (Rosner, 2010).

The mean for both treatment group and control group were input into the online calculator. The standard deviation chosen was the greater value between the two groups to obtain a more conservative number for sample size.

APPENDIX E: CONSORT 2010 FLOW DIAGRAM

APPENDIX F: GODIN'S LEISURE TIME QUESTIONNAIRE

We would like you to recall your child's *average weekly exercise* over the *PAST MONTH*.
How many times per week on average did your child do the following kinds of exercise over the past month?

When answering these questions please remember to:

- Consider your average over the **past month**
- Only count exercise sessions that lasted **15 minutes or longer** in duration
- Only count exercise that was done during **free time** (i.e., not during physical education class at school)
- Note that the main difference between the three categories is the **intensity** of the exercise
- Write the average number of times on the first line and the average duration on the second line – please write “0” in each line if it does not apply

A. STRENUOUS EXERCISE (Heart beats rapidly, sweating)

(e.g., running, jogging, hockey, football, soccer, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)

In an average week my child was involved in strenuous exercise _____ times/week for an average duration of _____ minutes/each session.

B. MODERATE EXERCISE (Not exhausting, light perspiration)

(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, downhill skiing, popular and folk dancing)

In an average week my child was involved in moderate exercise _____ times/week for an average duration of _____ minutes/each session.

C. MILD EXERCISE (Minimal effort, no perspiration)

(e.g., easy walking, yoga, archery, fishing, bowling, golf, snowmobiling)

In an average week my child was involved in mild exercise _____ times/week for an average duration of _____ minutes/each session.

APPENDIX G: THREE DAY FOOD RECORD

THREE-DAY DIETARY INTAKE RECORD

Name: _____

Phone Number: _____

Date of Birth: _____ _____ _____

(Day) (Month) (Year)

Height: _____ Weight: _____

Record Dates: _____ (Date – Weekday)

_____ (Date – Weekday)

_____ (Date – Weekend)

University of Calgary

Faculty of Kinesiology

INSTRUCTIONS FOR RECORDING DAILY FOOD INTAKE

The purpose of this study is to discover everything you eat and drink during a three-day period – 2 weekdays and 1 weekend day. It is important to record ALL foods and beverages – whether it is a full course meal at home or a quick can of pop at school/work. Before you start recording your intake, please read the following instructions and the Sample Day.

The Three-Day Dietary Intake Record has a separate section for every day (see Day 1, Day 2, Day 3 on top each page). Each day is broken up into 3 eating times:

1. Morning meal
2. Midday meal
3. Evening meal

It is a good idea to carry your Dietary Intake Record book with you and record your entries as soon after eating as possible. Foods and beverages consumed away from home – at a friend's house, at the mall, at a restaurant- are just as important as those eaten at home. Please include the following information on your food record:

1. **FOOD AND BEVERAGE ITEMS** Column: Enter all foods and beverages consumed at the meal or snack time. Please record the specific type of food (for example: *WHOLE WHEAT* bread, *FROSTED FLAKES* cereal). In the same column, record all toppings or items added at the time of eating (for example: sugar, syrup, jam, butter, mayonnaise, gravy, milk, salt, etc.). For combination foods, please include detailed information on each item. For example: If you had a tuna sandwich, you would list the following foods and include detailed information for each of them: white bread, mayonnaise, celery, solid white tuna packed in water, salt.
2. **DESCRIPTION OF ITEM** Column: For every food or beverage item listed, include the following (if applicable):
 - **Brand:** *MIRACLE WHIP* mayonnaise, *PIZZA HUT DEEP DISH* pizza, *OREO* cookie
 - **Type of flavour:** *BLUEBERRY* muffins, *STRAWBERRY* yogurt
 - **Method of cooking:** *FRIED*, *BAKED*, *BBQ'D*, *HOMEMADE*

All other relevant information included on food label:
LOW FAT ranch salad dressing, *28% M.F. (MILK FAT)* cheddar cheese, *LEAN* Ground Beef

3. **AMOUNT** Column: For every item consumed, enter the amount eaten using specific units of measure that are convenient for you. For example: enter the word “cup”, “grams”, “piece”, “ounce”, “number”, “teaspoon”, or “tablespoon”. Enter a unit of measure not only for the menu item, but for toppings or items added as well. Each entry must have its own unit of measure. Use measuring cups and spoons whenever possible during the 3-day period to record the most accurate estimation of the amount of each food you consume.

Fill in the two blanks on the bottom of each record. Indicate the time of your meal or snack and where it was eaten (for example: at home, at a restaurant, in class). If you did not eat a meal or snack, please place a check mark (✓) in the space provided on the bottom of the page, so that we do not think you forgot to record it.

Daily check: in the evening, after you have recorded everything for the day, go back over your entries to make sure you have included as much detail as possible for each item. Also check to ensure the blanks are completed on the bottom of the page.

All foods and beverages you consume every day are important and your Dietary Intake Record should be as accurate as possible. It should also reflect the way you usually eat. Please do not change your normal eating habits for the 3 days you are recording your food intake. Your honesty is crucial to the success of this research study. Thank you for your participation and cooperation in helping with this study. Please look closely at the Sample Day before beginning your Dietary Intake Record. **If you have any questions about filling out your Three-Day Dietary Intake Record, please phone:**

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	AMOUNT
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: <ul style="list-style-type: none"> - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label 	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon
Spaghetti with tomato/meat sauce:		
Pasta	Spaghetti, cooked	2 Cups
Tomato sauce	Hunt's canned sauce, roasted garlic flavour	1 Cup
Meat balls	Made with extra lean ground beef	5 (1 oz/ball)
Parmesan cheese, grated	Kraft, 30% Milk Fat (M.F.)	1 Tablespoon
Garlic Bread:		
Italian Bread	Toasted	3 large slices
Garlic Butter		3 Teaspoon
Caesar salad:		
Lettuce	Romaine	1 Cup
Croutons	Safeway brand, garlic flavor	2 Tablespoons
Bacon bits	Simulated flavour, No Name Brand	2 Tablespoons
Caesar salad dressing	Kraft, Fat free	2 Tablespoons
Milk	1%	1 Cup
Tiramisu	Sarah Lee	1 Slice
Coffee	Black	1 Cup

Time of meal/snack: 6:00 pm **Location meal/snack was consumed:**
at home

Please CHECK (✓) if you did not eat or drink at this meal or snack time: _____

Day 1 – Morning Meal

<i>Food and Beverage Items</i>	DESCRIPTION OF ITEM	UNIT OF MEASURE
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: <ul style="list-style-type: none"> - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label 	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon

Time of meal/snack:_____ **Location meal/snack was consumed:**_____

Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 – Midday Meal

<i>Food and Beverage Items</i>	DESCRIPTION OF ITEM	UNIT OF MEASURE
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: <ul style="list-style-type: none"> - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label 	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon

Time of meal/snack:_____ **Location meal/snack was consumed:**_____

Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 – Evening Meal

<i>Food and Beverage Items</i>	DESCRIPTION OF ITEM	UNIT OF MEASURE
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: <ul style="list-style-type: none"> - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label 	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon

Time of meal/snack: _____ Location meal/snack was consumed: _____
 Please CHECK (✓) if you did not eat or drink at this meal or snack time: _____

APPENDIX H: TANNER DEVELOPMENTAL STAGING

Female Tanner Staging

Tanner JM. Growth at Adolescence, Oxford, England: Blackwell Scientific Publications; 1962; 30-36

Male Tanner Staging

I		3 ←<2.5
II		4 ←2.5-3.2
III		10 ←3.6
IV		16 ←4.1-4.5
V		25 ←>4.5

Tanner JM. Growth at Adolescence, Oxford, England: Blackwell Scientific Publications; 1962;30-36

Prader Orchidometer

<http://www.teenforumz.com/images/orchidometer.gif>

APPENDIX I: DEMOGRAPHICS QUESTIONNAIRE

DEMOGRAPHICS (please answer in relation to your current status):

Please check **only one** option:

1. Marital status:

- Married/common law: _____
- Divorced/separated: _____
- Widowed: _____
- Never Married: _____

2. Education Level (please check highest level attained):

- Some high school: _____
- Completed high school: _____
- Some University/College: _____
- Completed University/College: _____
- Some OR completed Grad school: _____

3. Annual Family Income:

- < 20,000: _____
- 20,000 – 39,000: _____
- 40,000 – 59,999: _____
- 60,000 – 79,000: _____
- > 80,000: _____

4. Employment Status:

- Full-time: _____
- Homemaker: _____
- Retired: _____
- Part-time: _____
- Unemployed: _____
- Disability/Sick Leave: _____

5. Race

- Caucasian: _____
- Other: _____

6. Gender:

- Male: _____
- Female: _____

7. Age: _____