UNIVERSITY OF CALGARY

Prebiotic Fibre Supplementation In Combination With Metformin Modifies Appetite, Energy Metabolism, And Gut Satiety Hormones In Obese Rats

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTERS OF SCIENCE

FACULTY OF KINESIOLOGY

CALGARY, ALBERTA

JULY 2010

UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Prebiotic Fibre Supplementation In Combination With Metformin Modifies Appetite, Energy Metabolism And Gut Satiety Hormones In Obese Rats" submitted by Kim Alicia Pyra in partial fulfilment of the requirements of the degree of Masters of Science.

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June 30, 2010

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Abstract

The prebiotic fibre, oligofructose (OFS), reduces energy intake and improves glycemic control in rodents and man. Metformin (MT) is a commonly used insulin-sensitizing agent that may limit weight gain in individuals with type 2 diabetes. Our objective was to determine if using OFS as an adjunct to MT therapy (AD) modifies satiety hormone production and metabolism in obese rats. Independently, OFS and MT decreased energy intake, body fat, hepatic triglyceride content, plasma leptin and glucose-dependent insulinotropic peptide (GIP) levels. OFS and AD but not MT rats showed superior glycemic control during an oral glucose tolerance test (OGTT) compared to C. Area under the curve for GIP was lowest in AD<MT=OFS<C and AD rats showed statistically significant synergistic lowering at every time point during the OGTT. AD resulted in advantageous hepatic and intestinal gene expression patterns. Combination therapies have the potential for increased effectiveness over current obesity treatments administered alone.

Acknowledgements

I owe my deepest gratitude to my supervisor, Dr. Raylene Reimer. I will forever value the opportunities and guidance that she has extended. Her exceptional mentorship qualities and abilities have provided the foundation and continued support for my accomplishments.

I would like to express thanks to my supervisory committee members, Dr. Jane Shearer and Dr. Dustin Hittel, for their ongoing support, time and expertise. As well, I am grateful to Dr. David Severson and Dr. Andrea Clark, thank you for serving on the committee for my master's thesis examination.

I would like to recognize Rosalie Kolstad for her advice and ongoing guidance that was so important in assisting me to advance my research experience and attain personal growth and success in the program.

I am very much appreciative of all who furthered my understanding in the lab and aided me with the completion of this research project: Kristine Lee, Jill Parnell, Lindsay Eller, Megan Hallam and Danielle Reid. I have learned a great deal from all of them and offer my regards and blessings. A special mention to Cameron Crossley for his expertise with computers and his patience and understanding in being there for me.

Thanks to all involved in the Faculty of Kinesiology and the University of Calgary for providing a wealth of resources full of unique opportunities, a challenging graduate program and a quality education.

And finally, I would like to thank my family and friends for their encouragement, love and patience with me as we shared this experience.

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List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
WHO	World Health Organization
T2D	Type 2 Diabetes
CVD	Cardiovascular Disease
BMI	Body Mass Index
CCHS	Canadian Community Health Survey
NIH	National Institutes of Health
MT	Metformin Therapy
SDRI	Specific Dietary Reference Intake
OFS	Oligofructose
DIO	Diet-induced Obesity
GLP-1	Glucagon-like Peptide-1
PYY	Peptide YY
GIP	Glucose-dependent Insulinotropic Peptide
tAUC	Total Area Under the Curve
OGTT	Oral Glucose Tolerance Test
HF	High Fat
SCFA	Short Chain Fatty Acids
DPP-IV	Dipeptidyl Peptidase-IV
GLP-1R	GLP-1 Receptor
Ex-4	Exendin-4
TG	Triglyceride
YR	Y Receptor
LDL	Low-density Lipoprotein
HS	High Sucrose
Ex-9-39	Exendin-9-39
IRS-2	Insulin Receptor Substrate-2
IRS-1	Insulin Receptor Substrate-1
SREBP-1C	Sterol Regulating Element Binding Protein-1
FAS	Fatty Acid Synthase
VLDL	Very Low Density Lipoproteins
HFHS	High Fat High Sucrose
ACC	Acetyl CoA Carboxylase
pACC	Phosphorylated ACC
SGLT1	Sodium/Glucose Cotransporter
GLUT5	Glucose Transporter 5
AMPK	Adenosine Monophosphate Protein Kinase
pAMPK	Phosphorylated AMPK
HMGCR	3-Hydroxy-3-methylglutaryl-coenzyme A Reductase
SREBP-2	Sterol Regulatory Element Binding Protein-2
BMD	Bone Mineral Density
BMC	Bone Mineral Content
DEXA	Dual Energy X-ray Absorptiometry
NEFA	Non-Esterified Fatty Acids

Chapter One: **Introduction**

The World Health Organization (WHO) describes obesity as a chronic disease that requires life-long strategies for effective prevention and management [1]. Not only is the global prevalence of obesity alarming and greater than 30% of the worldwide population, but the incidence of overweight in children and adolescents has tripled over the last 20 years and thus threatens the health of future generations [2-4]. Obesity is characterized by an excessive accumulation of adipose tissue and is a known risk to health; predisposing to type 2 diabetes (T2D), cardiovascular disease (CVD), hypertension, certain cancers and disabilities [5-7]. Body mass index (BMI; kg/m^2) is a common tool used to assess an individual's risk of excess body weight with obesity in adults defined as a BMI equal to or greater than 30.0 kg/m^2 and BMI 25.0 to 29.9 kg/m^2 defined as overweight [4, 8]. As BMI increases so does the projected risk to health, therefore, three classification levels of obesity have been further defined in order to better inform therapeutic guidelines for treatment including class I (BMI 30-34.9), class II (BMI 35-39.9) and class III (BMI $\leq$ 40) [8, 9]. Lifestyle modification is the foundation for weight control and is comprised of both increased physical activity and decreased energy intake ultimately aimed at reducing excess energy storage. Pharmacotherapy and bariatric surgery are generally reserved for the increasing prevalence of class II and III obesity to accommodate a more aggressive weight loss approach for individuals resistant to weight loss and /or displaying heightened risk of health problems [9].

According to the 2004 Canadian Community Health Survey (CCHS), 23% of adults are obese and 36 % are overweight [10]. This is likely an underestimate of Canadians at risk

from excess weight as body fat distribution is also a major determinant and has been shown to vary based on ethnicity [11]. In particular, the presence of abdominal obesity greatly increases the risk of the metabolic syndrome's cluster of cardiometabolic risk factors: hypertension, glucose intolerance, insulin resistance and dyslipidemia [12]. The coexistence of three or more of these comorbidities defines the metabolic syndrome and indicates a state of increased risk of developing T2D and CVD [13]. In fact, the National Institutes of Health (NIH) recommends pharmacotherapy, in conjunction with lifestyle modification, for all overweight individuals with a body mass index of >27 kg/m^2 and the presence of at least one comorbidity [14]. For these individuals, use of metformin therapy (MT), a commonly prescribed insulin-sensitizing agent, is recommended to aid in combating CVD risk and morbidity in obese and insulin resistant individuals [15-18].

The Obesity Canada Clinical Practice Guidelines Steering Committee and Expert Panel advocate a five to ten percent reduction in body weight over six months to best manage all states of obesity. Sustained modification of dietary intake is recognized as a primary barrier to success in current obesity treatment [19]. Disturbances in energy balance and the habitual overnutrition is clearly one factor connected to the increased risk of obesity in adults [20]. Consequently, current nutritional strategies recommend reduction in energy intake by 500 to 1000 kcal/d to promote safe weight loss in adults [19]. According to the WHO, growing evidence indicates that energy-dense foods promote excess weight gain and a global strategy to treat nutritional disorders associated with obesity is to reduce the overall energy density of the diet [21]. Today, it is clear that implementation of this strategy will be important for western society based on dietary

intake reports [22]. Epidemiological evidence shows clear associations between habitual dietary intake of either a high-fat and/or low-fibre diet and the increased risk of poor health and obesity [23, 24]. In the 2004 CCHS, dietary fibre was the sole nutrient independently related to obesity in Canadian men [20]. A high fibre diet is linked to improved insulin sensitivity, improved lipid profiles and lower body weight [25]. Therefore it is not surprising that increased dietary fibre intake is prominently recommended for T2D prevention in obese individuals [26-28]. The incidence of overweight or obesity is ~90% in the T2D population and similarly, increasing dietary intake of fibre alongside strict limitations on fat intake is advocated for weight management [3, 29]. To protect against CVD in adults the Specific Dietary Reference Intake (SDRI) endorses 14 g of fibre for every 1000 kcal consumed [24, 30, 31].

Of growing interest is the non-digestible, fermentable prebiotic fibre, oligofructose (OFS). Experimental and clinical data suggest that supplementation with prebiotics may offer an effective treatment option for metabolic disorders related to obesity by acting to dilute the caloric value of the diet while at the same time targeting intestinal function to improve postprandial metabolism and total energy balance [32, 33]. Acting in a similar fashion, the initial physiological interface of orally ingested MT with the body is also in the intestine and clinical studies report that use of this drug may enhance gut hormone release and increase satiety [34, 35]. In turn, a promising therapeutic potential exists that combines oligofructose ingestion with MT therapy to maximize endogenous satiety and glucoregulatory hormone release. However, to fully understand the potential of this physiological response and devise specific dietary strategies and/or ingredients that

maximally impact the secretion of satiety peptides, further work is needed to understand the pathological changes in the release of satiety hormones in response to dietary habits and disease susceptibility.

The purpose of this thesis work was to evaluate the effect of OFS supplementation alone or as an adjunct to MT therapy on food intake, body composition, glucose regulation, and satiety hormone response in a Sprague-Dawley diet-induced obese (DIO) rat model. The primary objective was to determine the effect of OFS and MT, either alone or in combination, on satiety hormone secretion with special emphasis on glucagon-like peptide-1 (GLP-1). The secondary objective was to determine the effect of these treatments on energy intake, body composition, and expression of hepatic genes involved in glucose and lipid metabolism. The overall hypothesis to be tested is that oligofructose as an adjunct to MT therapy generates the greatest degree of intestinal GLP-1 secretion and most beneficial gut hormone profile for weight reduction.

This thesis contains five chapters. Chapter One provides a global introduction to the thesis and the objectives of the work. Chapter Two is a review of the relevant literature and current evidence for the role of prebiotic fibre supplementation and current use of MT therapy in mediating beneficial therapeutic outcomes in obesity treatment. Mechanisms through which dietary fibre and MT act are also highlighted. Chapter Three describes the research methodology employed in this thesis. Chapter Four is a presentation of the results. Chapter Five is a discussion of the major findings from this study including conclusions, future direction and study limitations. References are provided at the end of the thesis document.

Chapter Two: **Literature Review**

2.1 Enteroendocrine Function of the Gastrointestinal Tract

The gastrointestinal tract is the largest endocrine organ that plays a pivotal role in mediating appetite through the production of numerous peptide hormones involved in regulating bodyweight [36]. Targeting the production and activity of gastrointestinal peptides is a promising therapeutic approach to treat metabolic abnormalities associated with defects in the factors mediating the start, frequency and cessation of food intake [37]. The enteroendocrine L cells produce anorexigenic hormones that reduce food intake and include glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) [38]. In contrast, ghrelin is the only known peripheral orexigenic hormone predominantly released from the stomach's oxyntic X/A-like cells and acts to increase food intake [39]. The glucose-dependent insulinotropic peptide (GIP) is secreted from intestinal K cells in the duodenum and proximal jejunum and like GLP-1, is released in response to oral but not intravenous nutrient infusions [40]. Gut-derived peptides work in concert with central and peripheral factors to mediate total energy homeostasis and influence glucose and lipid metabolism in peripheral tissues [36]. Both GLP-1 and GIP peptides are incretin hormones that act to enhance insulin secretion in a glucose-dependant fashion as well as potentiate insulin-mediated glucose uptake in peripheral tissues [40]. While the causes of obesity are multi-factorial, it is clear that the secretion of gut-derived peptides plays a fundamental role in human energy balance and alterations in their secretion are one likely contributor to metabolic abnormalities. A diagram of the gastrointestinal track has been provided in the Appendix A.

2.2 Disturbances in the Enteroendocrine Function of the Obese Gut

2.2.1 GLP-1

Nutrient-stimulated and/or basal secretion of the anorexigenic and insulinotropic GLP-1 hormone is impaired by up to 90% in insulin resistant and obese states; likely contributing to defective energy regulation and impaired glucose metabolism [41-43]. Research suggests that the chronic and habitual overnutrition blunts GLP-1 mediated satiety and increases the energy required to elicit its normal physiological release [44]. Equally important, research also suggests that GLP-1 secretion remains impaired in calorie-restricted individuals who successfully accomplish weight loss compared to normal weight controls [42]. Clinical observations from a wide range of human participants further show that higher fasting concentrations of plasma GLP-1 after adjustments for age, sex and body composition are associated with superior rates of energy expenditure and fat oxidation [45].

2.2.2 GIP

Contrary to GLP-1, investigations with supraphysiological infusions of synthetic GIP show that this peptide hormone can actually decrease resting energy expenditure in healthy weight individuals as well as increase the feeling of hunger [46]. In contrast, findings in individuals with T2D and obesity show no similar effects [46], suggesting GIP resistance. Other evidence for the pathophysiological role of GIP suggests that its reduced insulinotropic effect greatly contributes to insufficient "late phase" 20-120 minute postprandial insulin secretion; a widespread characteristic of most individuals

with diabetes mellitus and not just T2D [47, 48]. Clinical observations made in obese individuals suggest that both fasting and postprandial plasma GIP are enhanced when compared to lean subjects [49], and this most likely is a warning sign related to the developing diabetic state. Others show that elevated total area under the curve (tAUC) for plasma GIP after an oral glucose tolerance test (OGTT) is characteristic of the development of insulin resistance in glucose tolerant lean subjects with polycystic ovary syndrome compared to BMI and age matched healthy controls [50]. However, findings in healthy individuals suggest that GIP is a less potent incretin than GLP-1 on a molar basis [51], and for that reason increased GIP, if acting as a substitute for decreased GLP-1, is also likely inadequate.

2.2.3 Peptide YY

Clinical reports suggest that fasting and postprandial PYY secretion is attenuated in obese versus lean subjects [52]. Young et al. demonstrated that obese subjects required double the calories to evoke similar peak plasma PYY responses when consuming a wide range of calorie doses (250-3000 kcal intake) of a high fat (HF) diet (47% fat) compared to lean subjects [7, 53]. Obese individuals treated with a physiological dose equivalent to the postprandial concentrations in lean individuals did, however, show that infusions of PYY(3-36) may still effectively reduce food intake; this reduction was similar to that measured in lean individuals [52]. Therefore, the potential of PYY appears similar to GLP-1 in that the most effective treatment target would act to correct a defect in the endogenous secretion given that there appears to be no evidence for the development of resistance to the anorectic effects of these peptides in the obese state.

2.2.4 Ghrelin

Having an opposing action to the satiation peptides, plasma concentrations of ghrelin surge before meals and are suppressed after the consumption of food [54]. Increased plasma ghrelin concentration is associated with increased food intake, gastrointestinal motility and gastric acid secretion [55]. The precise role of ghrelin in appetite control is contentious and contradictions in evidence do exist. However, this is likely the result of the several different forms of the ghrelin peptide that exist in vivo as recent evidence suggests that both the acylated and des-acyl forms are unique and elicit opposing effects on insulin secretion and glucose metabolism [56-58]. Despite this, elevated total and acylated ghrelin in humans and rats has been shown to have powerful anabolic effects regulating short and long term energy homeostasis [56]. Low-dose injections of acylated ghrelin, in both lean and obese individuals, are orexigenic and increase food intake [59]. As well, in the obese state the plasma profile of this hormone is decreased, in contrast to what would be expected based on the recognized physiology of this hormone. In obese individuals there is an apparent lack of customary peaks and dips in plasma ghrelin profile throughout the day when compared to lean individuals [55]. Although counterintuitive, the chronic suppression of ghrelin in obesity is tied to abnormal energy regulation. Lower plasma ghrelin levels have been independently associated with T2D and insulin resistance [60]. After adjusting for gender and study group, a hypertensive cohort showed an association between low levels of ghrelin and obesity that corresponded with high fasting insulin and high blood pressure [60]. Other studies suggest that decreased ghrelin concentrations are inversely associated with insulin resistance and/or hyperinsulinemia in obese individuals compared to insulin-sensitive

obese controls [61]. Further research is needed to fully understand the current contradictions and pathophysiological implications of blunted ghrelin secretion in obesity.

2.3 Regulation and Integration of Gut Hormones

2.3.1 GLP-1 Secretion

GLP-1 release is stimulated by individual macronutrients including sugars, fatty acids, essential amino acids, and short chain fatty acid (SCFA) [62-66]. SCFA are by-products of bacterial fermentation of dietary fibre. It remains to be determined, therefore, if the effects of fermentable fibre on GLP-1 secretion are a result of direct stimulatory effects of SCFA, or whether modulation of gut microbiota could also play a role [65]. After consumption of nutrients, GLP-1 secretion occurs in two phases [40]. The early phase occurs within 10-15 minutes and is less likely to be solely stimulated by nutrients given that most L cells are chiefly located in the distal intestine [40, 67]. Rather, the proximal stimulus inducing GLP-1 release is mediated by other somatic signals, one example being the presence of fat in the small intestine, which is thought to mediate GLP-1 production by neurohormonal mechanisms [68]. Innervation by vagal afferent nerve fibres and stimulation by physiological levels of GIP secretion from the duodenum is considered to be a key mechanism regulating GLP-1 release in this neuroendocrine loop [68]. As well, the current understanding of contributors to first phase GLP-1 release has been demonstrated in several experiments showing that it is stimulated by the autonomic nervous system and the neurotransmitters acetylcholine, gastrin-releasing peptide and GIP [40]. Duodenal L cells, although lower in number than those in the distal gut, can

also contribute to plasma GLP-1 concentration [69]. After a low dose glucose infusion in healthy humans, increased plasma GLP-1 from duodenal tissue has been noted at 15 minutes and declines by 30 minutes postprandially [70]. The second phase GLP-1 secretion is a longer lasting 30-60 minutes release, most distinguished, but not limited to direct L cell contact with free fatty acids and carbohydrates [40].

2.3.2 GLP-1 Signalling

Both GLP-1(7-36) amide and GLP-1 (7-37) are secreted from the intestinal L cell and are bio-active forms of this hormone with an estimated half life of between 1 to 2 minutes [71, 72]. These bioactive forms are readily degraded by the biological enzyme dipeptidyl peptidase IV (DPP-IV) [73]. DPP-IV is present on the endothelium of capillaries and it has been estimated that about 50% of intact GLP-1(7-36) amide is degraded to GLP-1(9-36) amide immediately after secretion within vessels of the intestine [74]. Despite its rapid clearance, GLP-1 still signals through a widely distributed GLP-1 receptor (GLP-1R) network that is expressed on specialized cells in a tissue specific manner including the beta cell, heart, brain, lung and intestine [40, 75, 76]. Nutrient-stimulated GLP-1 is released into the hepatic portal vein. Activation of the GLP-1R on nerve terminals in this vicinity is a major mediator for the effects of GLP-1 on glycemic regulation [74]. Vahl et al. demonstrated that low dose GLP-1R antagonism by Exendin-4 (Ex-4) administered in the hepatic portal circulation results in 53% higher glucose excursions, however, a similar effect could not be repeated in systemic circulation [74] highlighting the importance of GLP-1 signalling in portal circulation. Endogenous GLP-1 satiety signalling is mediated through the vagus nerve to feeding circuits in the brain stem and hypothalamus [77].

2.3.3 GIP Secretion

In animal models, GIP secretion is enhanced by the consumption of a high fat diet and leads to increased transcription and proliferation of intestinal K cells [78-80]. Nutrient stimulation of GIP in rats and humans may not be equivocal and it has been suggested that a greater response to carbohydrates in rats, and fats in humans, exists [40]. In general, protein is the least effective macronutrient to trigger GIP release compared to ingestion of fat and simple carbohydrates in healthy individuals [40]. As well, work by Tseng et al. suggests that GIP mRNA and protein expression in the proximal intestine are negatively regulated by insulin [81].

2.3.4 GIP Signalling

The active GIP (1-42) peptide is deactivated into the GIP (3-42) peptide by DPP-IV resulting in a half life of 2 minutes in rats [40, 73]. This differs in humans where the half life in healthy and diabetic individuals is 7 and 5 minutes respectively [40, 82]. Miyawaki et al. demonstrated that GIPR knockout mice show 35% less fat mass accumulation and display increased energy expenditure compared to wild type mice [79]. Furthermore, when fed a high fat diet for 50 weeks, the knockout mice do not develop obesity or insulin resistance and show no alterations in food intake compared to the wild type controls [79]. Kim et al. showed that in the presence of insulin, GIP *in vitro* and *in vivo* promotes adipogenesis and increases lipoprotein lipase activity to enhance the anabolic effect of insulin on glucose uptake and triglyceride (TG) accumulation in epidydimal adipocytes [79, 80]. Moreover, similar experiments were repeated in human

subcutaneous adipocytes and confirm a similar cellular signalling pathway exists with greater sensitivity to the effects of GIP and insulin [80].

2.3.5 PYY Secretion

PYY is stimulated by glucose, lipids, amino acids, certain dietary fibre types and the SCFA, butyrate and propionate, alongside several bile salts, deoxycholate, cholate and taurocholate [83-86]. In the vascularly perfused rat colon, however, both amino acids and glucose are required at supraphysiological doses (250mM) to generate secretion of PYY [85]. The murine STC-1 cell line secretes PYY and has been shown to be responsive to certain free fatty acids with even carbon numbers ranging from C4 to C18 excluding C6 and C12. The SCFA, n-butyrate, also potently stimulated PYY secretion after 30 minutes of incubation which was sustained until 120 minutes in these experiments [87].

2.3.6 PYY Signalling

PYY1(1–36) is released in an active form and remains activated even after it is cleaved by DPP-IV into PYY(3–36) [36, 73]. The former of the two peptides accounts for ~60% and the latter ~40% of circulating PYY [88]. Both peptides cross the blood brain barrier to induce central effects [88, 89]. Important to their effects on energy regulation, differences have been described depending on the location that PYY is administered in the brain as well as the Y receptor (YR) subfamily that is activated with particular differences in the YR1 and YR5 versus the YR2 effects [88, 90]. Experiments in YR2 knockout (-/-) mice suggest that intraarcuate administration of PYY(3–36) is YR2

specific and these mice demonstrate uninhibited food intake compared to controls; further experiments with a YR2 agonist support this effect [91]. In contrast, others report that PYY(3–36) does show a low affinity to both the orexigenic YR1 and YR5 receptors leading to increased food intake similar to the predominant effects of PYY (1–36) [90, 92]. According to Bloom et al. peripheral injections of PYY(3–36) reduce food intake in mice and bodyweight in rats [91]. Observations in both lean and obese rodents receiving intravenous daily infusions of PYY(3–36) (30 pM kg^{-1} administered every other hour for 10 days) support the concept that this hormone reduces food intake and body mass after treatment compared to control [93]. In rabbits, one study suggest that PYY binding to the YR1 is also known to be a potent inhibitor of gastric acid secretion [94]. In general, the YR2 signaling is most critical for enhanced satiety as YR2 signaling is proposed to inhibit neuropeptide Y. Central inhibition of the orexigenic neuropeptide Y signal in turn increases the neuronal activity of the proopiomelanocortin and cocaine-amphetamine-regulated peptides that are anorectic and inhibit food intake [19, 90, 91]. The hypothalamus is the primary site in the brain involved in the regulation of energy homeostasis influenced by both central and peripheral signals [91].

2.3.7 Ghrelin Secretion

Ghrelin secreting cells in the stomach are mainly closed-type cells and do not sense nutrients directly, whereas a greater abundance of opened-type ghrelin secreting cells are present in distal intestinal tissues compared to proximal regions [95]. In the vascularly perfused rat stomach, ghrelin release is reduced by amino acids, insulin, leptin, GLP-1, gastrin, somatostatin and acetylcholine; is unaltered by a 10% lipid infusion; and may be

potently increased by GIP, glucagon and cholecystokinin [96-98]. In rats, regardless of the macronutrient composition, gastric, duodenal and jejunal infusions of 3kcal result in suppression of ghrelin to a similar extent [99]. Glucose and amino acids do appear, however, to have considerably greater effect than lipids on lowering plasma ghrelin 300 minutes postprandial to the 3kcal gavages [99]. In 16 healthy subjects, macronutrients ingested in equicaloric liquid meals result in a lower ghrelin nadir after both carbohydrate and protein ingestion compared to that of lipid [54]. However, the carbohydrate-induced meal suppression of acyl and total ghrelin was shorter and rose past initial preprandial ghrelin values three hours postprandial which is in contrast to both protein and fat [54]. Alternatively, improved suppression of plasma ghrelin has been observed after weight loss with a high carbohydrate rather than a high fat diet [100].

2.3.8 Ghrelin Signalling

The oxyntic cells of the stomach manufacture approximately 2/3 of peripheral ghrelin, although ghrelin is also released throughout the entire length of the intestine from duodenum to colon [90]. The most recognized bioactive form of ghrelin is acylated by the ghrelin-O-acyltransferase enzyme [101]. Post-translational modification of preproghrelin gene also produces obestatin and des-acyl ghrelin and both of these are biologically active peptides with the latter of the two representing ~90% of total plasma ghrelin in rats and humans [57]. In rats the acylated peptide is known to modulate the sympathetic nervous system via central ghrelin receptors, growth hormone secretagogue receptor 1a, thereby involving the neuropeptide Y /Y1 receptor-dependent pathways [102]. Acylated ghrelin also signals through the vagal pathway which is noteworthy

given that binding of these receptors inhibits the anorexogenic actions of GLP-1 and PYY [36]. Furthermore, in rats, ghrelin blunts the inhibitory effects of GLP-1 and PYY on gastric emptying and is a potent antagonist to these peptides [83]. Exogenous daily administration of ghrelin in rodents dose-dependently increases food intake and body weight in addition to enhancing carbohydrate utilization and decreasing fat metabolism when compared to controls [103].

2.4 Diet-Induced Obesity

Positive energy-intake defined as diet intake that continually exceeds energy expenditure over time will promote the development of excess adiposity [23, 104]. A genetic component to obesity also exists that predisposes some individuals to increased risk. For certain individuals an energy dense Western style diet is known to trigger expression of the obese phenotype [105]. Similar to humans, the Sprague-Dawley rat is a commonly used animal model that is characterized by an ~ 50% obesity prone and ~ 50% obesity resistant population when fed a high fat, sucrose rich diet [106, 107]. In this animal model, diet-induced obesity is characterized by excessive fat mass development and deregulation of energy regulating signals including gut hormones [106-108]. Yang et al. showed that feeding DIO prone rats a high fat diet attenuates PYY mRNA levels in the ileum and colon, while switching these rats back to a normal chow diet reverses this effect [107]. This work highlights the potential of altering intestinal signals to modify disease progression in the DIO prone rats [107, 108].

2.5 A High Fat and High Sucrose Diet

Independent of disease susceptibility and excessive food intake, habitual consumption of diets mainly comprised of sucrose or fat are detrimental to health and clearly, the composition of these diets play an influential role. Fat and sucrose both provoke metabolic alterations in fatty acid metabolism in the liver, promoting increased TG storage as well increased serum LDL cholesterol secretion when compared to control rats fed a standard diet [109]. Sumiyoshi et al. demonstrated in lean and obese C57BL/6J mice that consumption of a HF or a high sucrose (HS) diet both independently lead to glucose intolerance after a long-duration (55 weeks) of feeding when compared to a low fat/sucrose diet [110]. The mice fed the HS diet presented early onset hyperglycemia as a result of defective insulin secretion whereas, mice fed the HF diet had insulin resistance in peripheral tissues and subsequent glucose intolerance [110]. Alterations observed in the intestine are indicative of an increased glucose flux that may promote greater blood glucose excursions as a result of the greater glucose absorption in mice fed the HS diet [110]. Mice fed the HF diet showed increased TG storage in peripheral tissues and decreased expression of proteins involved in the metabolism of fat [110]. Taken together, these studies provide evidence that both the intestine and liver are major targets to improve glucose regulation in the postabsorptive state.

2.6 A High Fibre Diet

Diets high in dietary fibre tend to have a lower caloric value along with less added sugar and less fat which must be taken into consideration when understanding how fibre may confer health benefits [23]. In general, a high fibre diet may aid in weight loss by

facilitating greater compliance to caloric restriction through reductions in metabolizable energy and resulting lower energy intake. Dietary fibre, particularly soluble fibre, slows intestinal transit time and therefore, in theory, fibre should reduce postprandial glucose excursions and insulin secretion. However, it is clear that different types of fibre employ diverse mechanisms to improve postprandial metabolism. Certain types of fibres may directly enhance satiety signals from the gut [32, 65]. For example, increased consumption of fermentable prebiotic fibre can trigger endogenous GLP-1 to improve glucose homeostasis in part by enhancing insulin secretion and promoting glucose uptake [111]. GLP-1 may also acts to slow gastric emptying and prevent glucose excursions by decreasing the rate of glucose entry into the blood stream [112, 113] highlighting the fact that other dietary and/or intestinal characteristics influence the fibres postprandial effect. In any case, the potential to manipulate the composition of the diet to achieve weight loss with fibre is a promising strategy to decrease caloric intake. Use of specific fermentable fibres may further enhance compliance via increased production of anorexigenic peptides like GLP-1.

2.7 Prebiotic Chicory-derived Inulin Type Fructans

Not all fibres exert equal benefits with respect to the secretion of anorexigenic peptides [86]. The prebiotic fibres are fermentable and act to promote weight loss by enhancing the production of SCFA via their fermentation which has been shown to promote endogenous GLP-1 and PYY secretion for up to 24 hours when compared to diets supplemented with non-fermentable fibres [86]. Inulin and its partial hydrolysate oligofructose (OFS) are similar prebiotic fibre types derived from the chicory root that in

addition to having a low caloric value (1.5 kcal g^{-1}), play a unique role in promoting human health [114-117]. Most importantly, ingestion of these fibres may attenuate fat mass development [32]. Classification as a prebiotic means that the fibre is not hydrolyzed or absorbed in the proximal gut and rather acts to selectively feed certain beneficial bacterial species such as bifidiobacteria and lactobacilli. This bifidogenic effect has been shown to be health-promoting to the host and encourage a healthier profile of colonic microbiota [114]. In eight healthy volunteers the colonic microflora was favourably altered after 15 days by simply replacing 15 g of dietary sucrose with 15 g of prebiotic fructans yielding decreases in bacteroides (bacteria that was preferentially fed by sucrose) and increases in bifidobacteria. Increased fecal matter and energy and nitrogen excretion were also noted [118]. As several different prebiotic fibres exist, distinction among them includes their structural properties and biological origin, both of which impact their health promoting effect. The degree of polymerization (number of fructose or glucose units of the fibre) influences the fermentation of fructans [119-121]. Inulin has a degree of polymerization of 2 to greater than or equal to 60, while OFS is derived from inulin by partial enzymatic hydrolysis and has a lesser degree of polymerization that is closer to 10 [121]. OFS in comparison to other prebiotic fibre types results in the greatest production of acetic, propionic and lactic acids in vitro as the major fermentation by-products [120]. However, in vivo work by Blay et al. suggests that OFS fed over a long duration leads to continual increases in SCFA with the most prominent being butyrate in cecal tissue [122].

2.8 Prebiotics Modulation of Gut Hormones

Experimental evidence on the use of inulin and/or OFS or a mixture of the two fibre types (50:50 ratio respectively named Synergy 1®) show favourable alterations in glucose and lipid metabolism. For the most part, these effects appear linked to the production of satietogenic gut-derived peptides that promote reductions in food intake that are subsequently linked to reduced body weight and fat mass development in several animal models [32]. The majority of experiments show up-regulation of proglucagon mRNA in the proximal colon corresponding to the marked increase in plasma GLP-1 in several animal models after a 10% fructan dose is added alongside a standard, high fat and high carbohydrate diet [32]. The fermentation of inulin and OFS is linked to elevation of both GLP-1 and PYY gene transcription in the distal intestine [86]. In rats, supplementation of 10% OFS fed alongside a standard diet also led to decreased plasma ghrelin and this contributed to decreased food intake and body weight [123]. Increased plasma GIP has also been observed with OFS supplementation postprandially following an OGTT which may be the consequence of decreased insulin release compared to controls [124].

The physiological benefits of OFS supplementation are greatly influenced by diet composition, duration of feeding and the metabolic state. Pre-treatment of OFS to a standard diet for 35 days can protect rats from a 15 day HF diet challenge with improvements in hepatic TG accumulation that in this case was attributed to decreased food intake when compared to controls on a HF diet [125]. In this study, portal vein GLP-1 response nearly doubled in the OFS HF group and this was associated with an upregulation in proglucagon mRNA in the proximal and medial colon and cecum after accounting for the enlarged total cecum mass compared to control [125]. Delmée et al.

demonstrated that the effectiveness of OFS supplementation to a HF diet in DIO mice is considerably less effective in decreasing fat mass development and promoting growth of the cecum when compared with OFS added to a standard diet [126]. OFS supplementation to a HF carbohydrate free diet decreased food intake, fat mass and improved glycemia by increased insulin secretion after DIO compared to controls [126]. In contrast, no similar effect was observed when OFS was added to a HF and high carbohydrate diet in DIO mice [126]. One important consideration to note, is that the duration of DIO in mice was twice as long in the HF/high carbohydrate group even though the authors did report similar findings in unpublished data when the duration of DIO was identical (8 weeks) [126]. Comparing OFS effects across all three diets (standard, HF carbohydrate free or HF and high carbohydrate) further confirmed that the physiological effects observed on glycemia and fat mass development match the expression of proglucagon mRNA in the proximal colon [126].

OFS supplementation to a standard diet lowers plasma insulin response compared to controls [124], while in HF fed diabetic mice, OFS ingestion led to increased insulin secretion and improved fasting and postprandial glucose tolerance, hepatic insulin sensitivity and reduced body weight compared to controls [127]. In these diabetic mice most, but not all of oligofructose' physiological effects on improved energy status are GLP-1 mediated and require a functional GLP-1R as determined with GLP-1R (-/-) mice and administration of the GLP-1 antagonist, Ex-9-39 [127]. In the absence of a functional GLP-1R, consumption of OFS showed no anti-diabetic effects [127]. Furthermore, Ex-9-39 treated mice demonstrated dose dependent effects on fasting glycemia, glucose

intolerance and endogenous glucose production that correlated with the changes in hepatic insulin sensitivity measured by insulin receptor substrate-2 (IRS2) and Akt phosphorylation [127]. Sustained insulin signalling via IRS2 is physiologically important and highlights the ability of OFS to enhance GLP-1 and insulin action in the diabetic state. Others suggest that in states of nutrient excess and hyperinsulinemia, increased insulin receptor substrate-1 (IRS1) signalling prevails alongside suppressed IRS2 protein expression [128-130]. This abnormal insulin signalling is coupled with increased expression of the sterol regulating element binding protein-1 (SREBP-1C), a transcription factor that regulates lipogenic genes associated with insulin resistance and fatty liver in mice [128-130]. Apart from physiological benefits mediated as a result of enhanced GLP-1 or GLP-1R signalling, ingestion of OFS also leads to decreased intracellular inflammation in the liver in HF fed mice compared to controls [127]. In addition, ingestion of OFS has been shown to promote gut stem cells to differentiate and this may promote the expansion of mature enteroendocrine cell populations [32, 131]. Collectively, these studies suggest that OFS-mediated outcomes would be important to treat several underlying symptoms of the obese state including abnormalities in insulin signalling, inflammation and decreased GLP-1 secretion.

When added at a 10% dose to the diet of rats, OFS supplementation clearly modulates lipid metabolism leading to decreased postprandial serum TG values (% in parentheses) compared to controls on a standard (61), fibre-free (27) or HF (43) diet [132]. The biochemical basis for this effect is attributed, in part, to reduced liver lipogenic capacity and decreased hepatic TG production that has been illustrated *in vitro* by Fiordaliso et al.

and Kok et al. [132-134]. Most distinctively, the decrease in *de novo* fatty acid synthesis is attributed to reduced activity of the fatty acid synthase (FAS) enzyme [132]. Kok et al. demonstrated that a 40% reduction in FAS in the liver of rats fed OFS for 30 days decreased production and secretion of very low density lipoproteins (VLDL) compared to the low-fat fed controls [132, 134]. Importantly, the diet also markedly influenced FAS activity and has been illustrated in rats fed a diet high in saturated fat and cholesterol in which FAS enzyme activity was suppressed by ~95% compared to low-fat fed controls [135]. While OFS ingestion attenuated the pathological degree of hepatic steatosis and led to smaller lipid droplets of TG, cholesterol and phospholipids in the liver compared to HF controls, it was still damaged compared to rats fed a low-fat diet [135]. Moreover, this study depicts that the alterations in the liver do not fully explain the lower postprandial fatty acid profiles observed with OFS supplementation [135]. Rather, increased serum GIP may contribute to the lipid lowering effect of OFS [135]. This has been observed after 30 days following the addition of 10% OFS to a standard diet in rats [124]. Further examination is needed to show a link between lower plasma lipids and increased GIP and lipoprotein lipase activity in response to dietary OFS.

2.9 Fibre Fermentation and Benefits of Short Chain Fatty Acids

Prebiotic fibres are water soluble and readily dispersed in the lower intestine creating an available food source for select gut microbiota that may lead to the continuous production of SCFA [136]. Acidification of cecal contents through the production of SCFA is known to lower the pH of the large intestine which provides protection from a HF diet by decreasing the formation of secondary bile acids and promoting the precipitation of bile

salts [137]. HF diets increase the production of secondary bile acids that, even at physiological doses, may be carcinogenic to the large bowel [138]. In a hypercholesterolemic rat model, observations of decreased plasma cholesterol alongside a dose-dependent increase in bile and cholesterol production in the liver as well as decreased cholesterol absorption in the distal intestine suggest that prebiotic fermentation and production of SCFA renders cholesterol and bile insoluble [139]. Indeed, experimental and human findings advocate ingestion of chicory derived prebiotics in that they deliver bacterial-mediated protection from colon cancer by reducing the intestinal exposure to carcinogens as well as act to suppress tumour cell survival though their fermentation into SCFA [140]. The metabolic ratios of SCFA in the blood are equally important and are altered by a HFHS diet leading to reduced plasma levels of propionate and butyrate that consequently increase plasma ratios of acetic acid when compared to a standard diet [141]. In isolated hepatocytes, others show that acetic acid influences lipid metabolism as this SCFA is lipogenic in the liver while, propionate can competitively inhibit the anabolic actions of acetate and suppress both fatty acid and cholesterol biosynthesis [142]. Thus, increases in propionate acid after the addition of 10% inulin to a HFHS diet in part describe how prebiotic fibre promotes reductions in liver lipids. Normalization of plasma propionate levels can in turn lead to reduced expression of two key lipogenic enzymes, acetyl-CoA carboxylase (ACC) and FAS [141].

2.10 Clinical Evidence of Inulin-derived Fructans

Clinical application of prebiotic fibre supplementation in humans is expanding and improvements in glucose and lipid metabolism are supported in both healthy and disease

states. In healthy subjects, supplementation of Synergy 1 at 16 g/d divided into two equal doses at breakfast and dinner for 14 days has been shown to decrease glucose following a standardized meal in addition to increasing plasma GLP-1 and PYY [143]. Similar findings after OFS consumption of 20 g/d in individuals with gastroesophageal reflux disease also found this fibre increased plasma GLP-1 after a mixed meal compared to a placebo [144]. While in subjects with T2D, 14 days of OFS supplementation at 8 g/d led to decreased fasting glycemia as well reduced total and LDL cholesterol levels when compared to a control group consuming 5 g of sucrose [145]. Human studies of longer duration have also demonstrated that OFS supplementation for 3 months in overweight and obese adults may promote greater weight loss and fat loss when compared to a placebo by increasing plasma PYY and decreasing plasma ghrelin [33]. The link between decreased appetite, improved glucose regulation and alterations in gut hormones most likely result from enhanced SCFA production by gut microbiota [143]. Thus it is promising that a recent randomized single-blind, crossover study in healthy individuals consuming 24 g of inulin showed that prebiotic fibres clearly elevate plasma acetate, propionate and butyrate two hours after consumption of a high caloric liquid test meal compared to controls [146].

2.11 Clinical use of Metformin Therapy

MT is currently used as the first line insulin-sensitizing medication to improve glycemic regulation, insulin resistance and restrain weight gain in individuals with T2D [147, 148]. Currently, MT is widely prescribed and considered safe to manage states of insulin resistance [149, 150]. However, the use of MT to control body weight in overweight and

obese non-diabetic individuals is not standard care unless fasting and/or postprandial glucose intolerance is apparent [151]. Yet, findings from long-term studies advocate its use in metabolic syndrome to reduce CVD risk factors and morbidity [15, 16, 152]. Severe side effects are rare but do include lactic acid and metabolic acidosis, hepatic dysfunction, congestive heart failure and dehydration [148]. The most commonly occurring side effects include nausea, abdominal and gastrointestinal pain and diarrhea [148]. The dose of MT is unique to the individual given its wide range of responses when administered [151, 153], showing the need to assess how this drug interacts with additional dietary prescriptions in a defined model.

2.12 Expanding Clinical Understanding of Metformin Therapy

Clinical research in obese individuals without diabetes suggest MT can favourably increase endogenous GLP-1 [35]. More recently, Mannucci et al. further verified this original finding in 12 obese non-diabetic individuals matched with 22 T2D individuals whom had never been treated with hypoglycaemic drugs [154]. In this report, 4 weeks of MT use (3 daily doses of 850 mg) facilitated greater plasma GLP-1 levels in both study groups compared to initial baseline values, however a single dose of MT did not show immediate influences on plasma GLP-1 in this study [154]. Rather, others show that in a concentration dependant manner, MT enhances the plasma half-life of GLP-1 by inhibiting the actions of the DPP-IV [155-157]. The suggestion that metformin directly triggers the endogenous release of GLP-1 remains controversial. Yasuda et al. in the DPP-IV knockout (-/-) rat model demonstrate a dose-dependent increase in plasma GLP-1 with MT (30, 100, 300 mg kg^{-1}) with no corresponding reductions in blood glucose

during an OGTT that was comparable to the other biguanide drugs phenformin and buformin [158]. In the Fischer rat a synergistic elevation of endogenous GLP-1 was observed after administering a combination of MT (300mg kg^{-1}) with valine-pyrrolidide (30 mg kg^{-1}), a DPP-IV inhibitor, yet neither drug administered alone resulted in increased plasma GLP-1 [158]. Collectively, these experiments do suggest that endogenous production and/or prevention of GLP-1 degradation may be favourably altered by MT and therefore, tailoring dietary treatment to enhance this effect would be most useful in the prevention of T2D.

2.13 Metformin Therapy and the Intestine

Compared to any other tissue, orally ingested MT accrues at highest concentrations in the wall of the gut [159, 160]. MT appears to have a deep compartment in splanchnic tissues and large deposits have been observed in the ileum and colon after both intravenous and oral drug administration [160]. MT absorption occurs throughout the entire length of gastrointestinal tract and findings from a single pass intestinal perfusion study in rats provides some evidence that segment-dependant variations in drug permeability exist. The greatest rate of absorption occurs in the duodenal tissue compared to jejunum and ileum likely resulting from both a saturated and carrier mediated mechanism [161]. Glucose metabolism by the gastrointestinal tissues is mainly anaerobic and influenced by blood glucose whereby, alterations in normal glucose uptake and release by the gut is largely influenced by the composition of the diet, disease state as well as MT [162-164]. In rats, HF feeding for six weeks impedes both basal intestinal glucose uptake and suppresses normal glucose utilization [163]. This is similar to findings in insulin resistant

obese (fa/fa) rats [164]. Addition of MT treatment for 1 week in HF fed rats (starting at week 5 of the HF regime) has been shown to reverse impaired intestinal glucose metabolism and nearly normalize this defect compared to HF controls and starch fed controls respectively [163]. This is comparable to findings in insulin resistant obese (fa/fa) rats where MT was able to lower blood glucose by restoring intestinal glucose uptake and utilization primarily in the stomach and intestinal tissues [164]. Experiments measuring differences in the aerobic and anaerobic effect of MT on various tissues show that improved glucose utilization in the gut promotes a glucose lowering effect in both normal and diabetic rats compared to untreated controls [162]. Here, the decreased plasma glucose was clearly associated with an increased production of plasma lactate measured at highest concentrations in the hepatic portal vein and was supported by experiments in jejunal rings that measured an ~20% increase in lactate production [162]. Lenzen et al. demonstrated that 3 day treatment with oral MT (250 mg kg-1/day) promotes changes in glucose and fructose transport along the brush border membrane in the duodenum and jejunum, increasing the mRNA expression of the energy mediated sodium-glucose cotransporter (SGLT1) and glucose transporter 5 (GLUT5) compared to untreated control rats [165], which likely contribute to increased glucose uptake in the intestine.

2.14 Metformin Therapy and the Liver

Aside from the intestine, MT primarily acts to improve peripheral glucose clearance through activation of adenosine monophosphate protein kinase (AMPK) decreasing gluconeogenesis and increasing peripheral glucose uptake in hepatic and skeletal tissue,

respectively [166, 167]. AMPK is a multi-subunit enzyme that in the liver plays a major role in regulating intracellular energy as well as whole body metabolism [168]. Activated AMPK immediately alters glucose and lipid metabolism by promoting pathways to restore intracellular adenosine triphosphate generated from catabolic processes such as fatty acid oxidation and glycolysis [169]. In turn, AMPK activation prevents the cellular depletion of energy by inhibiting anabolic processes including gluconeogenesis and lipogenesis [169]. Zhou et al. demonstrated *in vivo* that MT stimulates glucose uptake in skeletal muscle coincident with activation of AMPK and reduces the anabolic capacity of liver [167]. In rat hepatocytes, AMPK activation decreased SREBP-1 and ACC protein expression alongside suppressed key lipogenic genes including ACC and FAS [167]. Ultimately, this study provides evidence that MT can change the expression of genes involved in lipolysis and lipid metabolism. This is further supported by experiments in human hepatoma HepG2 cells where the intracellular content of TG and cholesterol was decreased by MT in an AMPK dependant manner [170]. MT pre-treatment in HepG2 cells prevents the increased TG content that can be induced by adding 30mM of glucose (to mimic a state of insulin resistance) in control cells and mechanistically, this effect was abolished by the adenoviral-mediated kinase-inactive AMPKα [170]. Others have also shown that activation of AMPK inactivates 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) , the key rate limiting enzyme in cholesterol synthesis [168]. Correspondingly, MT is known to have an inhibitory effect on cholesterol synthesis in the intestine of diabetic rats [171]. Alternatively, phosphorylation of ACC illustrates the beneficial effects of AMPK on cellular metabolites by decreasing malonyl CoA production and thereby, allows more fatty acids to enter into mitochondria and undergo

fatty acid oxidation [172, 173]. Phosphorylation of the Thr-172 residue site on the catalytic AMPK subunit result in the inhibition of ACC [167, 170]. Overall, hepatic AMPK activation is a plausible target for the treatment of obesity and exhibits potential to influence whole body lipid and glucose metabolism [80, 166, 174].

2.15 Summary of Current Knowledge

Obesity exacerbates metabolic abnormalities including impaired fasting glucose, postprandial glucose intolerance and insulin resistance [175]. Impaired metabolic function associated with obesity is tightly linked to the escalating prevalence of T2D and CVD [176, 177]. While the causes of obesity are multi-factorial, defects in basal and/or nutrient-stimulated GLP-1 secretion is one potential contributor [41-43]. Habitual overnutrition may blunt GLP-1 mediated satiety [44].

The prebiotic fibre OFS has a low caloric value (1.5 kcal g^{-1}) and is a soluble fermentable non-digestible carbohydrate [115, 117, 178]. There is convincing evidence that the hypoglycemic effect of OFS consumption is mediated via enhanced endogenous secretion of GLP-1 in the distal intestine depicted in several animals models [32]. Fermentation of OFS and production of SCFA leads to a daylong elevation of anorexigenic satiety hormones and acts to decrease energy intake and weight loss in the context of a HF diet [86, 179]. In overweight and obese adults, Parnell and Reimer [33] demonstrated a significant reduction in body weight, reduced energy intake and improved glycemic control following 3 months of OFS supplementation (21 g/d); this illustrating the therapeutic potential of this dietary fibre.

MT is the first line drug used to improve glycemic regulation and limit weight gain in individuals with T2D [15, 16, 149, 150, 152]. To decrease gluconeogenesis and improve peripheral glucose clearance, MT primarily targets the activation of AMPK in the liver [166, 167]. This cascade favourably inhibits anabolic processes and promotes the catabolic use of energy [166]. AMPK activation in the liver exhibits potential as a mediator of whole body glucose and lipid metabolism and is proposed to be a reasonable target for the treatment of obesity and its associated metabolic abnormalities [80, 166, 174].

It is well established that habitual overconsumption of a typical Western-style diet comprised of high fat and sugar content and excess calories is detrimental to health and contributes to increased body weight, insulin resistance and impaired regulation of satiety hormone production [180-183]. In addition to the well-established role of oligofructose in enhancing GLP-1 secretion, there is some evidence that metformin also promotes its release and could, therefore, serve as a promising adjunct to dietary interventions that enhance endogenous GLP-1 secretion [35, 154, 158]. Therefore the objective of this study is to determine if the combined therapy of metformin and oligofructose supplementation modifies metabolic perturbations resulting from consumption of a HFHS diet better than either treatment alone. We hypothesize that the combination of MT and OFS will be associated with greater GLP-1 secretion than either treatment alone.

Chapter Three: **Methods**

3.1 Animal Model

Obesity was induced in 8 week old, male Sprague-Dawley rats (n=80) with ad libitum feeding of a HFHS diet for 6 weeks. Full grown rats in the top 50th percentile of weight gain (n=40) were selected and randomized into one of four experimental groups for six weeks: 1) HFHS control (C); 2) HFHS + 10% OFS; 3) HFHS + MT (300mg kg^{-1}); 4) HFHS + 10% OFS + MT (300mg kg^{-1}); (n = 10 rats/ group). MT was administered orally in drinking water according to previous work and adjusted twice weekly to maintain freshness and meet MT dose requirements (300 mg kg^{-1}) [184]. The HFHS diet was commercially prepared and was composed of (g/100g): cornstarch (5); casein (14), sucrose (51), soybean oil (10), lard (10), Alphacel (5), AIN-93M mineral mix (3.5), AIN-93 vitamin mix (1), L-cystine (0.3), and choline bitartrate (0.25). The 10% OFS diet was prepared in house by mixing 90 g of HFHS diet with 10 g of OFS (Raftilose P95, Quadra Chemicals, Burlington, ON, Canada). The energy density of the diets was 4.6 and 4.3 kcal g^{-1} respectively for HFHS and OFS. All animals were purchased from Charles River (St. Constant, PQ). The animals were housed in groups (2-3 per cage) for the initial 6 weeks and then individually (1 per cage) for the 8 week experimental intervention. Ethical approval for the experimental protocol was obtained from the University of Calgary Animal Care Committee and all procedures conformed to the Guide for Care and Use of Laboratory Animals.

3.2 Food Intake

Individual food intake was measured by weighing each food cup to the nearest 0.1 g and then subtracting this weight from the previously measured weight using an electronic scale for the duration of the experiment. Spilled food was collected from the bottom of the cage and accounted for. Water bottles were replaced weekly. The daily and total food consumption was determined for each experimental group.

3.3 Body Weight

Rats were weighed once per week using an electronic scale for the duration of the experiment. All weights were recorded to one decimal place with rats resting in a plastic bucket with lid. The weekly and total body weight change was determined for each experimental group.

3.4 Body Composition

One day prior to sacrifice, rats were lightly anesthetized (Isoflurane inhalation; 1L min^{-1} O_2 flow 5%) and a dual energy x-ray absorptiometry (DEXA) scan performed. Hologic QDR 4500 software for small animals was used to quantify lean and fat mass and bone mineral content and bone mineral density (Hologic ODR 4500; Hologic, Bedford, MA). The body mass measurement provided by the DEXA was used as the final mass of the rats.

3.5 Oral Glucose Tolerance Test

A total of three OGTT were performed. After the induction of obesity but prior to randomization into the protocol, and one week prior to the end of the study, rats were feed-deprived overnight and a baseline blood sample taken via tail nick. Bodyweight was measured the night before to 0.1g using an electronic scale. An oral glucose gavage of 50% dextrose (wt/vol) solution at a dose of 2 g/kg body weight was administered and additional blood samples taken at 15, 30, 60, and 90 minutes via tail nick. Blood glucose was measured immediately at each time point using an Ultra One Touch Blood Glucose Meter (LifeScan, Inc., BD Biosciences). One week prior to the end of the study, a second OGTT was employed following the same tail nick blood draw procedure. At the end of the study, a third OGTT was performed and cardiac blood was collected chiefly for satiety hormone analysis but also included blood glucose measurements. After an overnight fast, rats were anaesthetized with isoflurane and a fasting blood sample taken via cardiac puncture. Rats were allowed to wake and given 50% dextrose (wt/vol) by oral gavage at a dose of 2 g/kg of bodyweight similar to previous work in this lab [185]. Subsequent blood samples were collected at 15, 30, 60 and 90 minutes post-gavage via cardiac puncture. Cardiac puncture was required to obtain sufficient blood volume (1 mL per time point) for satiety hormone analysis. Blood glucose was measured immediately as previously described.

3.6 Plasma Collection

Cardiac blood samples were drawn at five time points for satiety hormones from anaesthetized animals (Isoflurane inhalation; 1L min^{-1} O_2 flow 5%) during the final

OGTT. Blood samples were collected into ice chilled vacutainer tubes (BD Biosciences, Mississauga, Ontario, Canada) containing EDTA (1 mg mL^{-1}; Sigma, Canada), diprotinin-A (0.034 mg/ml blood; MP Biomedicals, Irvine, CA); Sigma protease inhibitor (1 mg/ml blood; Sigma Aldrich, Oakville, ON, Canada) and Roche Pefabloc (1mg mL^{-1} of blood; Roche, Mississauga, ON, Canada). Blood was processed as quickly as possible to prevent degradation. Samples were centrifuged at 1600×g for 15 min at 4°C to separate the plasma layer. Following centrifugation, tubes were kept on ice until the plasma layer was collected by pipette into five separate aliquots and stored at –80°C until later analysis.

3.7 Tissue Collection

Following the final blood draw, rats were killed by over-anesthetization (Isoflurane) and aortic cut. The entire intestine was excised, flushed and weighed to 0.001g. The length of the small intestine and colon was measured under tension from a 5g weight. Three cm segments of the distal duodenum, jejunum, ileum and proximal colon were snap-frozen in liquid nitrogen. Five samples from the liver and the entire stomach and cecum were also collected in a similar fashion. All tissues were stored at –80°C until analysis.

3.8 Plasma Analysis

Active GLP-1 was measured using a commercial ELISA kit with a minimum detection concentration of 2 pM (Millipore, Cillerica, MA, USA). The intra-assay variation is ≤6.5% and inter-assay variation ≤9.5 % as determined by the manufacturer. A monoclonal antibody that binds to the N-terminus of active GLP-1 is utilized in the assay.

A Rat Gut Hormone Milliplex kit was used to measure the following hormones (minimum detectable concentrations in pM in parentheses): insulin (28), active amylin (20), active ghrelin (1.9), leptin (27), total PYY (16) and total GIP (28) (Millipore). The intra-assay variation is <7% and inter-assay variation <24% as determined by the manufacturer. Distinctive antibody-coupled microspheres are utilized in the assay and fluorescence read on a Luminex$^{100\,TM}$ instrument by Eve Technologies (Calgary, AB, Canada).

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using baseline glucose and insulin [HOMA-IR = (I_F x G_F)/22.5] [186]. Plasma non-esterified fatty acids (NEFA) were quantified using the colorimetric WAKO enzymatic HR series NEFA-HR (2) method as per manufactures directions (Wako Chemicals USA Inc., Richmond, VA). Briefly, sample plasma (4µl/well) was added to a 96 well plate with reagent A (225 µl/well) and reagent B (75µl/well) solutions and incubated at room temperature for 10 minutes. The endpoint optical density of samples was read at 595nm and compared to a standard linear curve.

3.9 Hepatic Triglyceride Content Analysis

Triglycerides were measured using TG (GPO) reagent set (Point Scientific. Inc Lincoln Park, Michigan, USA) similar to previous work [139]. Approximately 25mg of wet liver tissue was weighed and the TG extracted with a KOH-EtOH solution. Samples were placed at 70°C for 1 hour and then allowed to rest overnight. The volume was brought up to 500µl with 2M Tris-HCl. Samples were diluted 1:5 with the Tris-HCl. Quantification

of the TG was done colorimetrically with a TG (GPO) liquid reagent set (Point Scientific, Inc. Lincoln Park, Michigan, USA). One ml of GPO was added to a tube for each standard or sample and heated to 37°C for 5 minutes. Standard or sample was added to the GPO and heated for another 5 minutes; 200µl of each was added to a plate and read at 500nm. TG content in mmol/L was determined based on a standard linear curve. Samples were run in duplicate and read twice on a Multiskan EX (Thermo Scientific, Waltham, MA).

3.10 RNA Extraction

RNA extraction was performed using frozen tissue (100 mg) and Trizol Reagent (1 ml) according to manufacture instructions (Invitrogen, Carlsbad, CA). Tissue was homogenized using a Polytron® homogenizer with 4 washes between samples. Following extraction, RNA was dissolved in RNAse/DNAse free water. To verify RNA quality, an agarose gel was run using RNA loading dye (3 µl:10µl/sample) to visualize 18s rRNA and 28s rRNA as two distinct bands.

3.11 Real-time RT-PCR

Ribogreen dye was used to quantify RNA and 1 µg of RNA used for reverse transcription with oligo dT primer and reverse transcriptase (RT) SuperScript™ First-Strand Synthesis System (Qiagen, Mississauga, Ontario, Canada). Reverse transcription was used to generate cDNA that was amplified by forward and reverse primers purchased from the University of Calgary Core DNA Services (Calgary, AB, Canada). Real-time PCR was performed in duplicate using the iCycler™ (BioRad; Mississauga, Ontario, Canada). The

PCR reaction was heated for 2 minutes at 95°C and then run for 40 cycles at 95°C, 60 °C and 72 °C for 30 seconds each. The melting point of the PCR product was shown on a melt curve. The fractional cycle number required to amplify each target gene to a fixed threshold was quantified from duplicate PCR reactions; the fixed threshold cycle (C_T) for the internal control and gene of interest was used to determine the relative gene expression using the $2^{-\Delta CT}$ method: $\Delta C_T = C_T$ (gene of interest) - C_T (reference gene) [187]. Beta actin and d-glyceraldehyde-3-phosphate dehydrogenase (GADPH) were selected as appropriate internal loading controls. Primer sequences are provided in **Table 1.**

Table 1. List of Primer Sequences

Gene	5' to 3'Forward primer	5' to 3'Reverse primer
Beta Actin	TATCGGCAATGAGCGGTTCC	AGCACTGTGTTGGCATAGAGG
GADPH	CAAGTTCAACGGCACAGTCAAG	ACATACTCAGCACCAGCATCAC
Proglucagon	ACCGCCCTGAGATTACTTTTCTG	AGTTCTCTTTCCAGGTTCACCAC
Ghrelin	AGAGGCGCCAGCTAACAAGTAA	GCAGGACAGTGCTGGGAGTT
PYY	AGCGGTATGGGAAAAGAGAAGTC	ACCACTGGTCCACACCTTCTG
ACC	CCTTCTTCTACTGGCGACTGAG	TAAGCCTTCACTGTGCCTTCC
CYPT7A1	GCTCTGGAGGGAGTGCCATTTAC	GCTGTGCGGATATTCAAGGATGC
SREBP-1C	TACTCAACAACCAAGACAGTG	AGAGAAGCAGGAGAAGAGAAG
SREBP2	AGAGTTTCCGCCCAGCATAC	CTACTTCTCCGTGTTTGGTGTTC
GLUT2	CTGTCTCTGTGCTGCTTGTG	TTACTCATCCAGGTGAACTTATCC
FAS	GAGGACTTGGGTGCCGATTAC	GCTGTGGATGATGTTGATGATAGAC
HMGCR	GGGACCAACCTTCTACCTCAG	GACAACTCACCAGCCATCAC
AMPKα1	GCCCGACACACCCTAGATG	TCCAACTGCTTGATTGCTCTAC
AMPKα2	ATGAGGTGGTGGAGCAGAG	GAGAGAGCCAGACAGTGAATG

Abbreviations: d-glyceraldehyde-3-phosphate dehydrogenase (GADPH); Peptide YY (PYY); Acetyl-CoA Carboxylase (ACC); Cholesterol 7-α-hydroxylase (CYPT7A1); Sterol Regulatory Element Binding Protein-1C (SREBP-1C); Sterol Regulatory Element Binding Protein-2 (SREBP-2); Glucose Transporter 2 (GLUT2); Fatty Acid Synthase (FAS); 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR); Adenosine Monophosphate Protein Kinaseα1 (AMPKα1); Adenosine Monophosphate Protein Kinase α2 (AMPKα2)

3.12 Statistical Analysis

All data are presented as mean ± SEM. A one-way ANOVA with Tukey's multiple comparison post hoc test was used to identify significant differences between the four groups. To assess multiple comparisons made over time, a repeated measures ANOVA was used to determine significant differences in glucose and satiety hormone levels during the OGTT. Total area under the curve (tAUC) was calculated for glucose and all satiety hormones as described by Massimino et al. [111] and analyzed by one-way ANOVA with Tukey's post hoc test. In cases where unequal variance was identified, Tamhane's T2 post-hoc test was performed. Significance was set at $P \leq 0.05$.

Chapter Four: **Results**

4.1 Food Intake & Body Weight

Food intake and body weight is presented in **Table 2**. Food intake was significantly lower in the MT and AD rats compared to C and OFS rats ($p=0.03$). Energy intake was significantly less in OFS, MT and AD rats than C ($p<0.05$) and less in AD rats than OFS ($p=0.03$). Initial body weights did not differ between groups. At the end of the study, final body weight and total weight gain were significantly lower in MT and AD rats than C and OFS ($p=0.001$). Weekly body weight is depicted in **Figure 1**. At wk 2, body weight was less in AD than C ($p=0.005$) and less than C and OFS at wk 3 ($p=0.008$). For the duration of the study (wk 4-8), AD and MT were lower than C and OFS ($p<0.04$).

Table 2. Food Intake and Body Mass Change

Treatment	C	OFS	MT	AD
Food Intake (g/d)	26.79 ± 1.4^{a}	23.47 ± 1.08^{a}	19.91 ± 1.38^{b}	17.68 ± 1.18^{b}
Caloric Intake (kcal/d)	123.27 ± 6.5^{a}	101.14 ± 4.7^{b}	91.6 ± 6.4^{bc}	76.2 ± 5.1^{c}
Fluid Intake (mL/d)	26.17 ± 1.63^{a}	21.93 ± 0.99^{a}	16.40 ± 1.13^{b}	14.20 ± 0.99^{b}
Initial Body Weight (g)	504.71 ± 7.84	504.84 ± 9.68	507.64 ± 12.49	511.48 ± 8.72
Final Body Weight (g)	648.79 ± 13.73^{a}	624.05 ± 16.43^{a}	494.71 ± 22.38^{b}	498.97 ± 14.90^{b}
Total Weight Change (g)	144.08 ± 10.04^{a}	119.21 ± 10.13^{a}	-12.93 ± 14.15^{b}	-12.51 ± 16.72^{b}

Values are presented as mean ± SEM (n=9-10).

Means with different superscript letters are different ($P<0.05$) from each other within a row as determined with Tukey's Post Hoc Test.

Figure 1: Weekly Body Weight

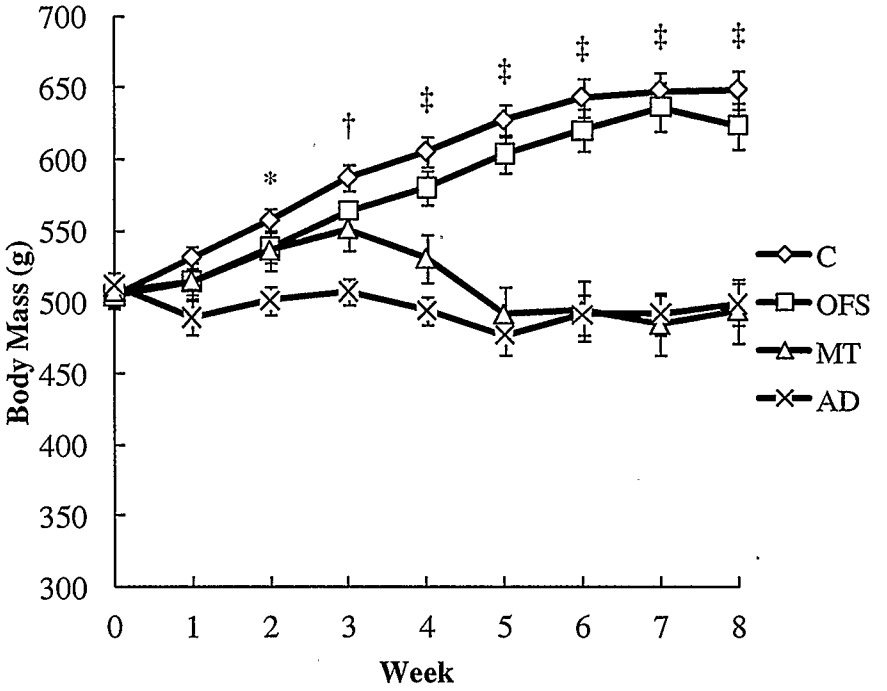


Figure 1. Weekly body weights over the 8 wk intervention. C, control; OFS, oligofructose fibre; MT, metformin therapy; AD, adjunct treatment. Values represent mean ± SEM (n=10). Significance was determined by repeated measures ANOVA with Tukey's post-hoc analysis. * represents a difference ($p<0.005$) between AD and C; † represents a difference ($p<0.008$) between AD and both OFS and C; ‡ represents a significant difference ($p<0.04$) for MT and AD compared to both OFS and C.

4.2 Body Composition

Body composition is presented in **Table 3.** All experimental treatments (OFS, MT and AD) reduced percent body fat compared to C ($p<0.05$). AD and MT rats had less absolute body fat compared to C and OFS ($p=<0.01$) and OFS had less than C ($p<0.02$). OFS rats had greater lean mass than AD and MT ($p<0.05$). OFS rats also had greater bone mineral

density (BMD) than MT but not AD rats (p=0.02). Bone mineral content (BMC) was greater in OFS and C when compared to MT and AD (p<0.02). Hepatic triglyceride content was reduced by all treatments compared to C (p<0.05).

Table 3. Body Composition

	C	OFS	MT	AD
Fat Mass (g)	198.49 ± 14.1^{a}	148.46 ± 12.15^{b}	78.65 ± 3.5^{c}	85.6 ± 9.9^{c}
Lean Mass (g)	433.7 ± 10.3^{ab}	443.6 ± 8.0^{a}	389.7 ± 16.4^{b}	400.7 ± 13.15^{b}
Percent Fat (%)	30.40 ± 1.77^{a}	24.19 ± 1.72^{b}	18.18 ± 1.14^{bc}	16.99 ± 1.76^{c}
BMC (g/cm)	16.67 ± 0.24^{a}	16.30 ± 0.25^{a}	14.14 ± 0.53^{b}	14.76 ± 0.27^{b}
BMD (g/cm^3)	0.176 ± 0.002^{ab}	0.180 ± 0.002^{a}	0.171 ± 0.002^{b}	0.178 ± 0.002^{a}
Liver TG(mg/g)	74.9 ± 5.08^{a}	56.8 ± 3.61^{b}	60.3 ± 5.04^{b}	56.1 ± 2.87^{b}
tTG (mg)] †	1286.0 ± 124.7^{a}	928.4 ± 64.4^{b}	778.5 ± 183.7^{b}	845.3 ± 21.9^{b}

† tTG refers to total liver triglycerides [(mg/g) ×·(g) liver].

Values are presented as mean ± SEM (n=9-10).

Means with different superscript letters are different (P<0.05) from each other within a row as determined with Tukey's Post Hoc Test.

4.3 Physical Characteristics

Physical characteristics of the intestine expressed as absolute values and adjusted values as a proportion of individual body mass are presented in **Table 4.** Absolute liver mass was less in MT and AD rats than C rats (p=0.001); and in MT compared to OFS rats (p=0.02). Absolute small intestine weight was less in MT rats than OFS (p=0.05) and C rats (p=0.001). Absolute and adjusted cecum weight was greater in OFS and AD rats than MT and C rats (p=0.001). Adjusted small intestine length in MT and AD rats was greater than C and OFS rats (p=0.001). Adjusted small intestine weight in AD rats was greater

than in OFS rats (p=0.01). Adjusted colon weight in AD rats was greater than MT (p=0.04) and C rats (p=0.01).

Table 4. Physical Characteristics

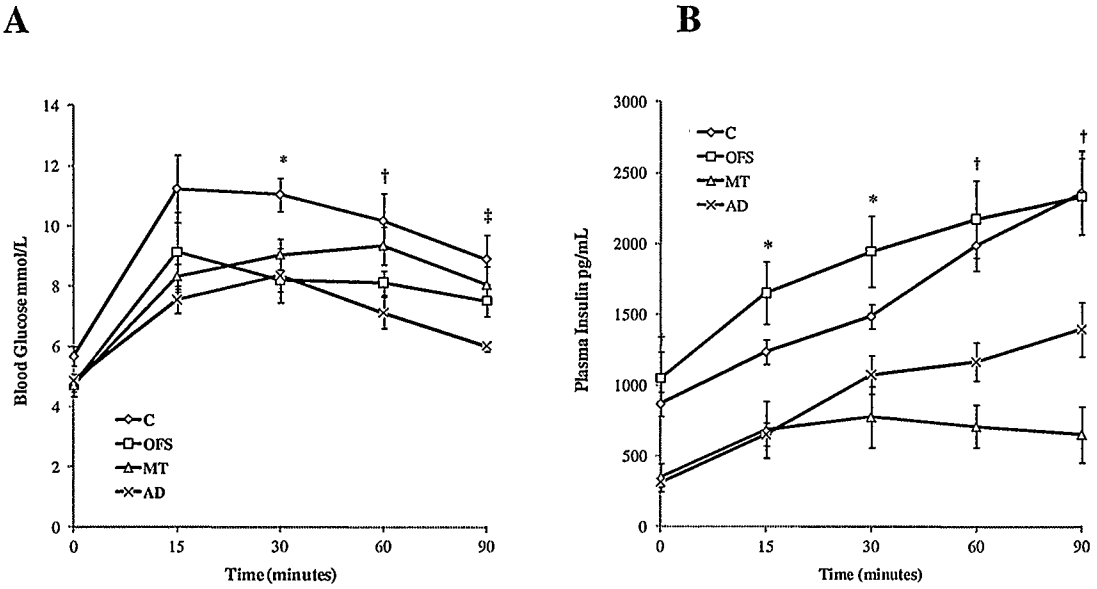
	C	OFS	MT	AD
Absolute				
Stomach Weight (g)	2.96±0.28	3.03±0.29	2.87±0.20	2.86±0.26
Small Intestine Length (cm)	134.6±2.52	129.0±2.17	130.4±2.20	130.9±2.51
Small Intestine Weight (g)	9.31±0.20^{a}	8.25±0.35^{a}	6.99±0.45^{b}	8.15±0.35^{ab}
Cecum Weight (g)	1.01±0.15^{a}	2.31±0.21^{b}	1.02±0.08^{a}	2.09±0.14^{b}
Colon Length (cm)	22.67±1.05^{ab}	25.40±0.48^{a}	22.20±1.50^{ab}	22.25±1.11^{b}
Colon Weight (g)	1.82±0.20^{ab}	2.15±0.08^{b}	1.83±0.29^{a}	2.06±0.19^{b}
Liver Weight (g)	17.75±1.03^{a}	16.90±0.67^{ab}	13.25±0.80^{c}	13.91±0.58^{bc}
Adjusted				
Stomach Weight (mg/g)	0.47±0.04	0.48±0.04	0.58±0.04	0.57±0.04
Small Intestine Length (mm/g)	20.83±1.84^{a}	20.75±1.42^{a}	26.81±3.24^{b}	26.07±2.35^{b}
Small Intestine Weight (mg/g)	1.44±0.04^{ab}	1.33±0.06^{a}	1.42±0.08^{ab}	1.62±0.05^{b}
Cecum Weight (mg/g)	0.15±0.02^{a}	0.37±0.04^{b}	0.21±0.02^{a}	0.42±0.02^{b}
Colon Length (mm/g)	3.55±0.17^{a}	4.08±0.09^{ab}	4.87±0.23^{c}	4.42±0.20^{bc}
Colon Weight (mg/g)	0.30±0.03^{a}	0.34±0.01^{ab}	0.32±0.02^{a}	0.42±0.03^{b}
Liver Weight (mg/g)	2.75±0.13	2.64±0.06	2.67±0.09	2.91±0.08

Results are presented as mean ± SEM (n =9-10). Weight and lengths adjusted using body weight as a denominator. Means with different superscript letters are different (P<0.05) from each other within a row as determined with Tukey's Post Hoc Test.

4.4 Glycemic Response

Glycemic response of the rats after the obesity induction period but prior to randomization in the protocol was assessed via an OGTT and tail nick blood. Total AUC (tAUC) for plasma glucose for all rats was 769.0 ± 31.3 mM L^{-1}. One week prior to the end of the study, the OGTT was repeated using tail nick blood showing lower blood glucose concentration at 30 min in OFS and AD rats compared to C ($p<0.04$). AD rats also had lower glucose at time 60 compared to C ($p<0.03$) and time 90 min compared to MT and C ($p<0.02$; Figure 2A). Blood glucose tAUC in AD and OFS rats was lower than C rats ($p=0.005$; Figure 2C). Glucose measured in conjunction with satiety hormones during the OGTT on the final study day also showed lower tAUC for OFS and AD compared to C ($p=0.05$). Values were 1504.7 ± 151.7, 1102.5 ± 75.9, 1424.8 ± 88.6 and 1027.3 ± 61.8 for C, OFS, MT and AD respectively.

Figure 2. Plasma Glucose and Insulin



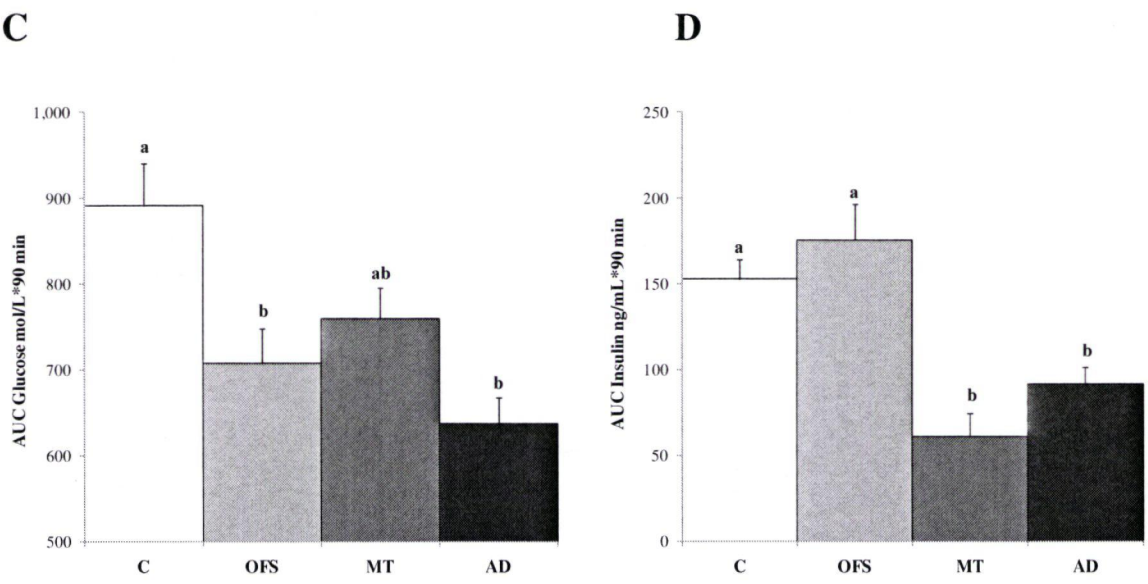


Figure 2. Plasma glucose and insulin during a 90-minute oral glucose tolerance test. C, control; OFS, oligofructose fibre; MT, metformin therapy; AD, adjunct treatment. Panels A and B show serial values of glucose and insulin at individual time points. Panels C and D show tAUC. Values represent mean ± SEM (n=8-10). Significance was determined in panels A and B by repeated measures ANOVA with Tukey's post-hoc analysis; and for panels C and D by ANOVA with Tukey's post-hoc analysis. In panel A for blood glucose, the * represents a difference ($p<0.04$) for OFS and AD compared to C; the † represents a difference ($p<0.03$) in AD compared to C; and the ‡ represents a significant difference ($p<0.02$) for AD compared to both MT and C. In panel B for insulin, the * represents a difference ($p<0.001$) between MT and AD treatments compared to OFS; and the † represents a difference ($p<0.001$) for MT and AD compared to both OFS and C. In panels C and D treatments with different superscripts are different for blood glucose tAUC ($P<0.03$) and for plasma insulin tAUC ($p<0.001$).

4.5 Insulin, NEFA and HOMA-IR

Fasting plasma insulin and NEFA and HOMA-IR are presented in **Table 5**. OFS was associated with greater insulin secretion than MT and AD at 15, 30, 60 and 90 min during the OGTT ($p<0.001$; Figure 2B). Fasting (Table 5) and tAUC for insulin (Figure 2D) was significantly less in AD and MT rats than C and OFS ($p=0.02$). HOMA-IR scores were markedly lower in MT and AD rats compared to C and OFS rats ($p=0.001$). OFS lowered fasting NEFA concentrations with values nearly half that measured in C rats ($p=0.04$; Table 5).

Table 5. Fasting Plasma Hormones

	C	OFS	MT	AD
Glucose (mmol/L)	5.68±0.3	4.73±0.3	4.73±0.2	4.88±0.1
Insulin (ng/mL)	0.87 ± 0.1^{a}	1.05 ± 0.2^{a}	0.35 ± 0.1^{b}	0.31 ± 0.0^{b}
HOMA-IR	258.9 ± 18.9^{a}	230.2 ± 34.4^{a}	70.8 ± 16.2^{b}	77.0 ± 9.1^{b}
NEFA (mEq/L)	0.44 ± 0.0^{a}	0.28 ± 0.0^{b}	0.32 ± 0.0^{ab}	0.35 ± 0.0^{ab}
Leptin (ng/mL)	20.4 ± 1.7^{a}	14.6 ± 1.7^{ab}	6.5 ± 1.5^{b}	7.4 ± 1.4^{b}
Amylin (pg/mL)	36.04 ± 7.0^{ab}	48.59 ± 6.5^{a}	25.75 ± 6.7^{ab}	22.07 ± 5.7^{b}
GIP (pg/mL)	86.18 ± 13.6^{a}	48.02 ± 6.8^{b}	49.67 ± 8.6^{b}	30.29 ± 6.6^{c}
PYY (pg/mL)	29.74 ± 1.9^{ab}	35.99 ± 3.4^{a}	20.04 ± 2.3^{c}	22.30 ± 1.8^{bc}
Ghrelin (ng/mL)	0.23±0.01	0.16±0.01	0.22±0.01	0.20±0.01
GLP-1(pmol/mL)	5.18±0.3	5.38±0.5	4.50±0.1	5.40±0.3

Results are presented as mean ± SEM (n =7-10). Means with different superscripts are different ($P<0.05$) from each other within a row as determined by Tukey's post hoc.

4.6 Satiety Hormone Profiles

Fasting levels for the satiety hormones are presented in Table 5. Fasting leptin was lower in MT and AD compared to C ($p<0.05$) and correlated with body mass ($r=0.74$; $p=0.001$) as expected [188]. At every time point during the OGTT, leptin was greater in C compared to all other treatments ($p<0.02$; Figure 3A). Leptin was also greater in OFS compared to MT and AD at all time points ($p<0.03$). Leptin tAUC was lower in OFS compared to C rats and lower in MT and AD compared to OFS and C ($p<0.05$; Figure 3C). Amylin was greater in OFS rats than AD rats at fasting ($p=0.001$), and greater than AD and MT at 15, 30 and 90 min during the OGTT ($p=0.001$; Figure 3B). Amylin tAUC in OFS rats was greater than MT and AD rats ($p<0.05$; Figure 3D). Fasting PYY was greater in OFS rats than MT and AD ($p<0.001$). At 15 and 30 min during the OGTT, PYY was greater in OFS and C compared to MT and AD ($p<0.001$; Figure 4A). tAUC for PYY was lower in MT and AD compared to OFS and C ($p<0.001$; Figure 4C). GIP was higher in C than all other treatments at every time point during the OGTT ($p<0.002$; Figure 4B). GIP in AD rats was also lower than OFS and MT at each time point ($p<0.03$). tAUC for GIP was lowest in AD<MT=OFS<C ($p<0.001$; Figure 4D). Ghrelin was lower in OFS than C rats ($p=0.001$) at 15 and 30 min during the OGTT (Figure 5A). At 60 min, both OFS and AD were lower than C and at 90 min, OFS, MT and AD were lower than C ($p<0.05$). tAUC for ghrelin was lower in OFS and AD compared to C ($p<0.05$; Figure 5C). GLP-1 was higher in OFS rats compared to MT at 30 and 60 min during the OGTT, and at 90 min both OFS and AD were higher than MT ($p<0.05$; Figure 5B). tAUC for GLP-1 was lower in MT compared to all other groups ($p<0.05$; Figure 5D).

Figure 3:Plasma Leptin and Amylin

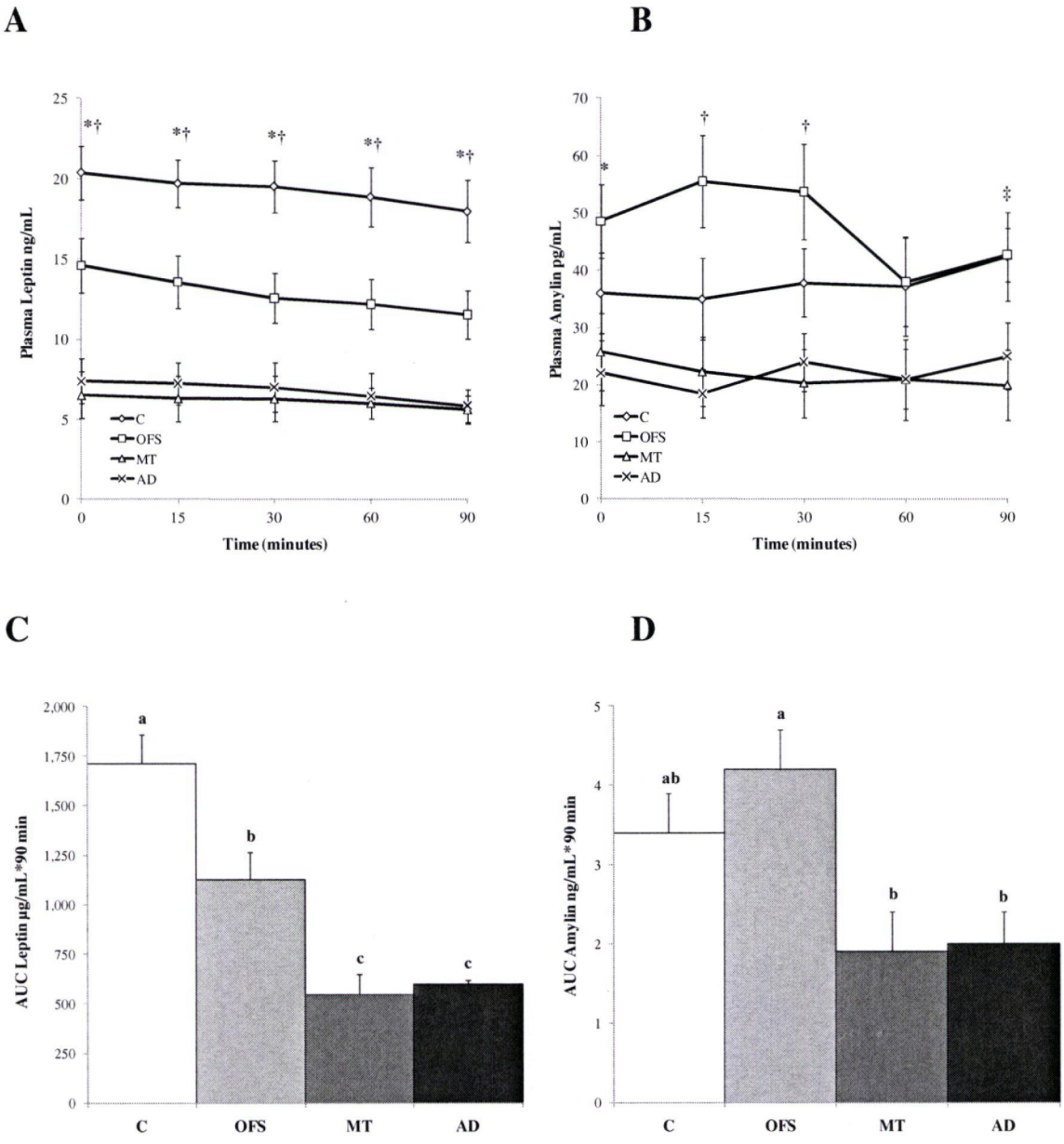
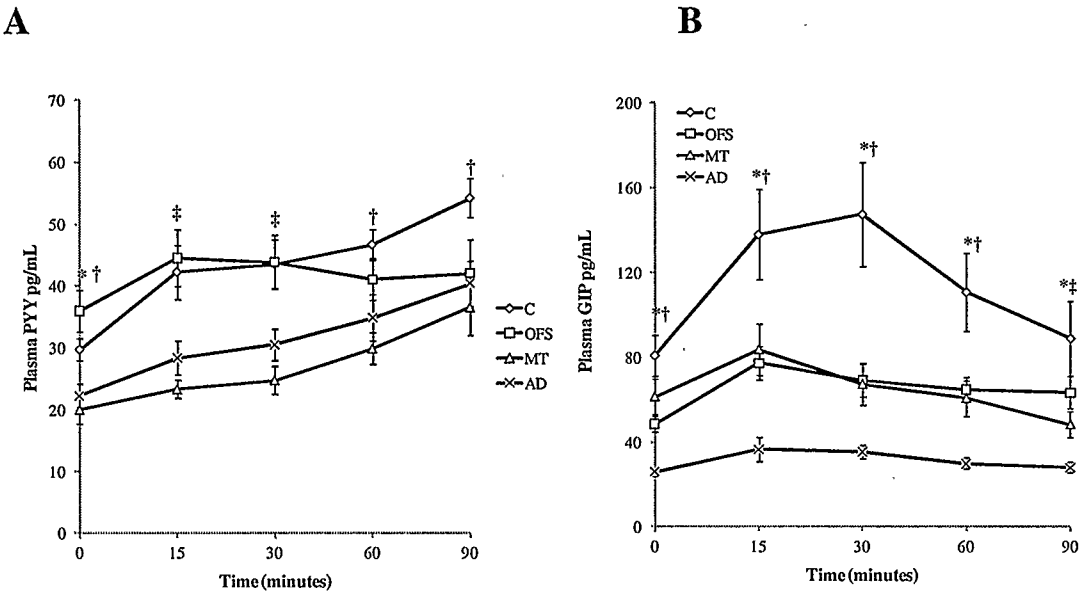


Figure 3. Plasma leptin and amylin during a 90-minute oral glucose tolerance test. C, control; OFS, oligofructose fibre; MT, metformin therapy; AD, adjunct treatment. Panels A and B show serial values of leptin and amylin at individual time points. Panels C and D show tAUC. Values represent mean ± SEM (n=8-10). Significance was determined for

panels A and B by repeated measures ANOVA with Tukey's post-hoc analysis and for panels C and D by ANOVA with Tukey's post-hoc analysis. In panel A for leptin, the * represents a significant difference ($p<0.02$) for all treatments compared to C; and the † represents a significant difference ($p<0.03$) for OFS compared to both MT and AD. In panel B for amylin, the * represents a significant difference ($p<0.01$) for OFS compared to AD; the † represents a significant difference ($p<0.01$) for OFS compared to both MT and AD; and the ‡ represents a significant difference ($p<0.01$) for both OFS and C compared to MT and AD. In panels C and D treatments with different superscripts are significantly different ($p<0.001$).

Figure 4. Plasma PYY and GIP



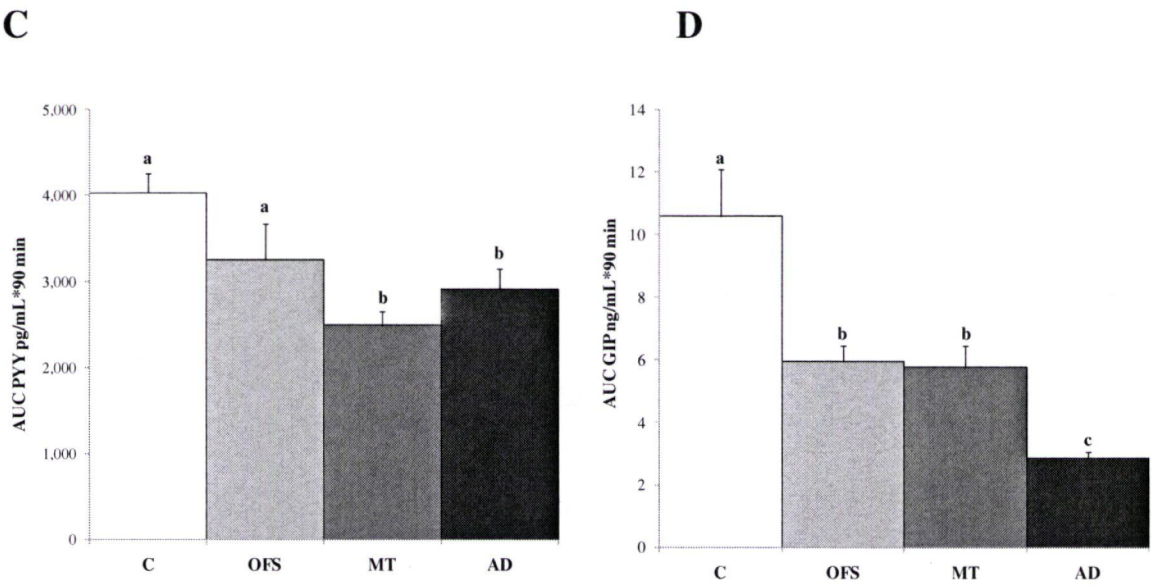


Figure 4. Plasma PYY and GIP during a 90-minute oral glucose tolerance test. C, control; OFS, oligofructose fibre; MT, metformin therapy; AD, adjunct treatment. Values represent mean ± SEM (n=8-10). Significance was determined for panels A and B by repeated measures ANOVA with Tukey's post-hoc analysis and for panels C and D by ANOVA with Tukey's post-hoc analysis. In panel A for PYY, the * represents a significant difference ($p<0.001$) for MT and AD compared to OFS; and the † represents a significant difference ($p<0.05$) for MT compared to C; and the ‡ represents a significant difference ($p<0.001$) for MT and AD compared to both OFS and C. In panel B for GIP, the * represents a significant difference ($p<0.002$) for all treatments compared to C; and the † represents a significant difference ($p<0.03$) for AD compared to both OFS and MT. In panels C and D treatments with different superscripts are significantly different ($p<0.001$).

Figure 5: Plasma Ghrelin and GLP-1

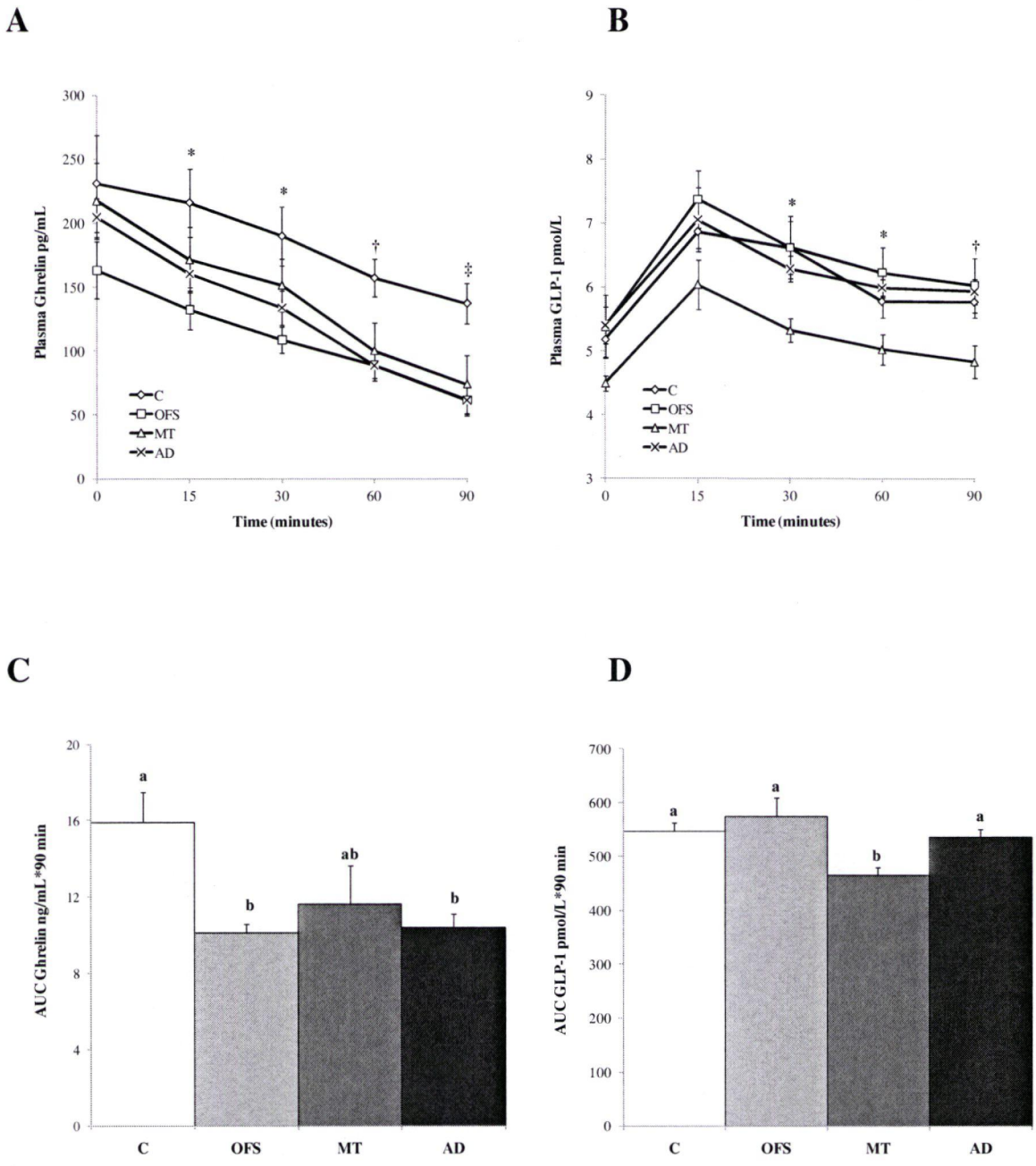


Figure 5. Plasma ghrelin and GLP-1 during a 90-minute oral glucose tolerance test. C, control; OFS, oligofructose fibre; MT, metformin therapy; AD, adjunct treatment. Panels A and B show serial values for ghrelin and GLP-1 at individual time points. Panels C and

D show tAUC. Values represent mean ± SEM (n=6-9). Significance was determined for panels A and B by repeated measures ANOVA with Tukey's post-hoc analysis; and for panels C and D by ANOVA with Tukey's post-hoc analysis. In panel A for ghrelin, the * represents a significant difference ($p<0.05$) for OFS compared to C; the † represents a significant difference ($p<0.05$) for both OFS and AD compared to C; and the ‡ represents a significant difference ($p<0.001$) for all treatments compared to C. In panel B for GLP-1, the * represents a significant difference ($p<0.01$) for OFS compared to MT; and the † represents a significant difference ($p<0.04$) for OFS and AD compared to MT. In panels C and D treatments with different superscripts are significantly different ($p<0.05$).

4.7 Intestinal Gene Expression

Gene expression data is presented in **Table 6.** Duodenum proglucagon mRNA level was greater in AD than MT ($p=0.05$). Ghrelin mRNA levels in the duodenum were less in AD than C rats ($p=0.02$). Glucose transporter 2 (GLUT2) mRNA levels were doubled in AD compared to OFS ($p=0.008$). Duodenum PYY expression was greater in C than AD rats ($p=0.05$). In the jejunum, ghrelin expression was doubled in C rats compared to all other treatments ($p<0.05$). GLUT2 mRNA levels were greater ($p=0.02$) in C than MT rats and PYY expression was greater in C rats compared to MT and AD ($p<0.05$). In the cecum, proglucagon expression was greater ($p<0.05$) in OFS and AD compared to MT. In the colon, OFS up-regulated proglucagon mRNA levels compared to MT ($p=0.02$) and AD ($p=0.04$) whereas, PYY mRNA levels were greater in C ($p<0.02$) compared to OFS, MT and AD.

Table 6. Gene Expression

		C	OFS	MT	AD
Stomach	Ghrelin	6.21±2.24^{a}	14.03±5.77^{ab}	19.92±5.46^{b}	11.83±4.33^{ab}
Duodenum	Proglucagon	0.29±0.09^{ab}	0.31±0.08^{ab}	0.10±0.03^{a}	0.93±0.45^{b}
	PYY	2.14±0.39^{a}	1.37±0.42^{ab}	0.89±0.21^{ab}	0.75±0.31^{b}
	Ghrelin	0.85±0.23^{a}	0.56±0.13^{ab}	0.56±0.11^{ab}	0.19±0.08^{b}
	GLUT2	4.94±0.21^{ab}	2.96±0.58^{a}	3.97±0.47^{ab}	6.07±1.02^{b}
Jejunum	Proglucagon	40.26±4.91	36.69±5.68	35.48±5.02	33.47±3.25
	PYY	52.29±8.59^{a}	42.13±12.93^{ab}	19.91±5.50^{b}	21.92±4.25^{b}
	Ghrelin	4.16±0.98^{a}	1.34±0.36^{b}	1.04±0.22^{b}	1.89±0.52^{b}
	GLUT2	3.21±0.52^{a}	2.01±0.49^{ab}	1.17±0.25^{b}	1.84±0.37^{ab}
Ileum	Proglucagon	1.40±0.52	1.67±0.53	1.19±0.34	0.93±0.25
	PYY	0.89±0.34	0.67±0.39	0.38±0.11	0.60±0.27
Cecum	Proglucagon	1.59±0.25^{ab}	2.22±0.40^{a}	0.96±0.21^{b}	2.25±0.54^{a}
	PYY	1.14±0.17	1.07±0.13	0.83±0.18	0.92±0.22
Colon	Proglucagon	0.12±0.03^{ab}	0.23±0.06^{a}	0.08±0.03^{b}	0.09±0.02^{b}
	PYY	1.58±0.20^{a}	0.66±0.13^{b}	0.77±0.23^{b}	0.66±0.17^{b}
Liver	SREBP-1C	1.47±0.33^{a}	0.78±0.11^{b}	0.66±0.10^{b}	0.61±0.09^{b}
	FAS	2.83±0.55^{a}	1.88±0.31^{ab}	2.97±0.64^{a}	0.93±0.29^{b}
	ACC	2.00±0.30^{a}	1.39±0.19^{ab}	1.23±0.29^{ab}	0.78±0.16^{b}
	SREBP2	1.17±0.18^{a}	2.19±0.40^{a}	1.49±0.30^{a}	3.62±0.48^{b}
	HMGCR	1.73±0.30^{ab}	1.58±0.23^{a}	3.00±0.54^{b}	2.21±0.29^{ab}
	CYPT7A1	2.54±0.38^{ab}	2.33±0.39^{a}	4.49±0.78^{b}	3.20±0.57^{ab}

Results are presented as mean ± SEM (n =6-10). Means with different superscript letters are different ($P<0.05$) from each other within a row as determined with Tukey's Post Hoc Test.

4.8 Hepatic Gene Expression

Hepatic SREBP-1C expression was less ($p<0.05$) in OFS, MT and AD rats than C rats. This was accompanied by a down-regulation of hepatic ACC and FAS mRNA levels in AD rats compared to C rats ($p<0.01$). Sterol regulatory element binding protein-2 (SREBP-2) expression was greater with AD compared to all other treatments ($p<0.04$). Lastly, MT increased 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and cholesterol 7-α-hydroxylase (CYPT7A1) expression compared to OFS ($p=0.04$). Hepatic AMPKα1 mRNA levels were upregulated with OFS ($p=0.03$) and AD ($p=0.03$) compared to C (**Figure 6A**). AMPKα2 expression was up-regulated with AD compared to all other treatments ($p=0.02$; Figure 6B).

Figure 6. Liver AMPKα1 and AMPKα2

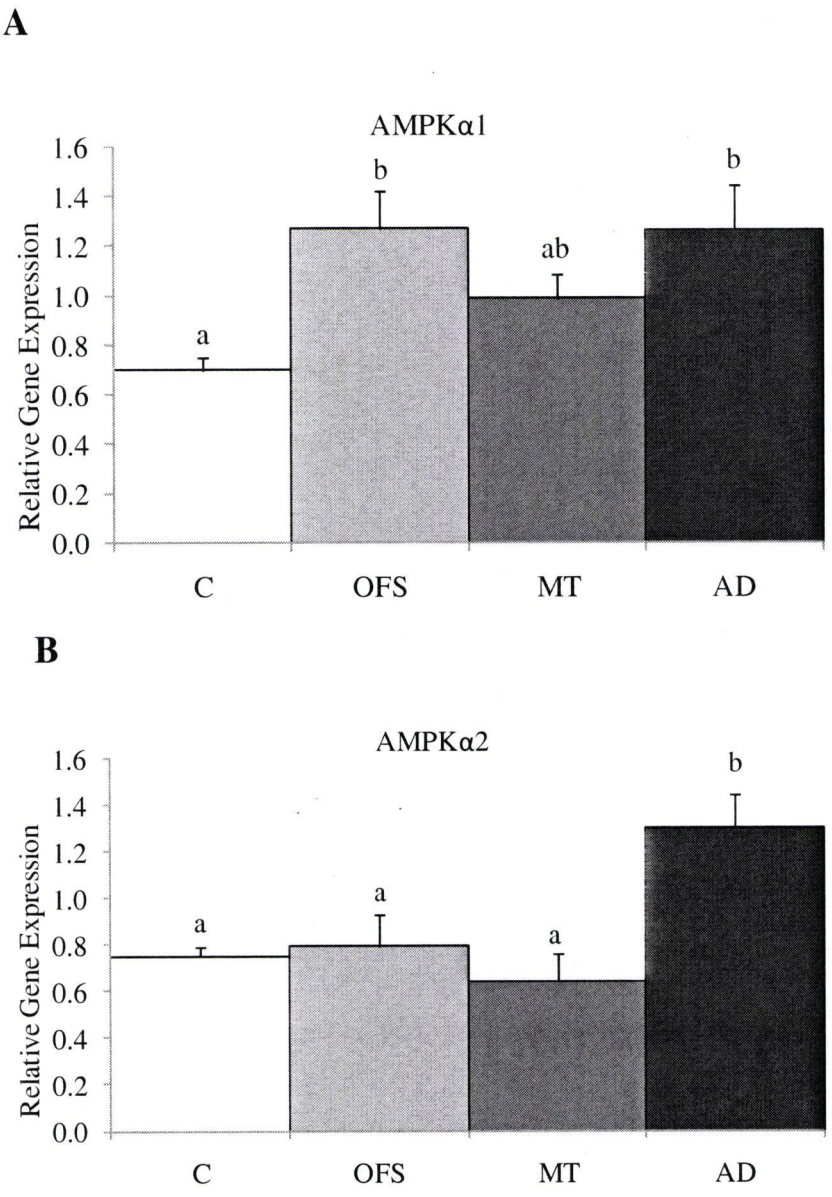


Figure 6. Hepatic AMPKα1 and AMPKα2 gene expression. C, control; OFS, oligofructose fibre; MT, metformin therapy; AD, adjunct treatment. Values represent mean ± SEM (n=7-10). Significance was determined by ANOVA with Tukey's post-hoc analysis. Treatments with different superscripts are significantly different ($p<0.03$).

Chapter Five: **Discussion**

Obesity is driving the increased prevalence of T2D. The inextricable link between excess body weight and T2D highlights the need to develop cost effective solutions to prevent, treat and reverse the progression of obesity to overt metabolic disease. Clearly, dietary intake plays a fundamental role in promoting health and is directly influential to the gastrointestinal regulation of hormones mediating food intake, glycemia and energy homeostasis. Evidence based strategies involving gut-based therapeutics that target diet-induced defects in metabolism are promising although incompletely understood. A summary of the major findings of this study are presented in **Table 7** below that highlight the key differences in response to the treatments employed in this study alone or in conjunction.

Table 7. Summary of Major Findings

Oligofructose	Metformin	Combination
Physical Characteristics		
↓ Energy Intake	↓ Energy Intake	↓ Energy Intake (>OFS)
≈ Body Weight	↓ Body Weight	↓ Body Weight (>OFS)
↓ Body Fat	↓ Body Fat	↓ Body Fat (>OFS)
≈ Lean Mass	↓ Lean Mass	↓ Lean Mass (<OFS)
≈ Bone Mineral Density	↓ Bone Mineral Density	≈ Bone Mineral Density
↑ Cecum & Colon Weight	≈ Cecum & Colon Weight	↑ Cecum & Colon Weight
Glycemic Control		
↓ Glucose AUC	≈ Glucose AUC	↓ Glucose AUC
≈ HOMA-IR	↓ HOMA-IR	↓ HOMA-IR (<OFS)
≈ Insulin (fasting & AUC)	↓ Insulin (fasting & AUC)	↓ Insulin (fasting & AUC) (<OFS)
↓ NEFA (fasting)	≈ NEFA (fasting)	≈ NEFA (fasting)
Plasma Satiety Hormone Levels		
↑ PYY (fasting)	↓ PYY (fasting)	↓ PYY (fasting)
↓ Ghrelin AUC	≈ Ghrelin AUC	↓ Ghrelin AUC
≈ GLP-1 AUC	↓ GLP-1 AUC	≈ GLP-1 AUC
↓ GIP (fasting & AUC)	↓ GIP (fasting & AUC)	↓ GIP (fasting & AUC) (<MT & OFS)
Intestinal Gene Expression		
≈ Ghrelin mRNA (stomach)	↑ Ghrelin mRNA (stomach)	≈ Ghrelin mRNA (stomach)
≈ Proglucagon mRNA (duodenum)	≈ Proglucagon mRNA (duodenum)	↑ Proglucagon mRNA (duodenum) (>MT & OFS)
≈ Ghrelin mRNA (duodenum)	≈ Ghrelin mRNA (duodenum)	↓ Ghrelin mRNA (duodenum) (<MT & OFS)
Hepatic Gene Expression		
↑ AMPKα1	≈ AMPKα1	↑ AMPKα1
≈ AMPKα2	≈ AMPKα2	↑ AMPKα2 (>MT & OFS)
≈ FAS	≈ FAS	↓ FAS (<MT & OFS)
≈ACC	≈ACC	↓ACC (<MT & OFS)
≈ SREBP-2	≈ SREBP-2	↑ SREBP-2 (>MT & OFS)
≈ HMGCR	↑ HMGCR	≈ HMGCR
≈ CYPT7A1	↑ CYPT7A1	≈ CYPT7A1
Hepatic Triglyceride		
↓ Triglyceride content	↓ Triglyceride content	↓ Triglyceride content

* Direction of change is indicated in comparison to the control group.

Magenta: Represents an outcome where OFS or MT performs better or worse (depending on the outcome) than the other.

Blue: Represents a synergistic effect wherein AD performs better than OFS and MT.

Green: Represents an outcome where AD performs better than OFS

Yellow: Represents an outcome where AD performs better than MT.

5.1 Food Intake and Body Weight

A primary objective of the current study was to determine if using OFS as an adjunct to MT would improve energy homeostasis more so than either treatment administered alone. A reduction in energy intake is crucial to the successful loss of excess adiposity. Very recently, a link between the resident intestinal microbial flora and energy extraction from food has been proposed. It is hypothesized that the obese state is characterized by enhanced caloric release from food during digestion and is associated with an approximate 50% decrease in Bacteriodetes and corresponding increase in Firmicutes bacteria in obese compared to lean mice [189]. Similar observations have been made in lean and obese humans [189]. Other work by the group of Gordon et al. has shown that reductions in caloric intake over the duration of one year, irrespective of diet composition, promoted a favorable shift in these two dominant bacterial divisions by increased Bacteriodetes and decreased Firmicutes in obese individuals that correlated with body weight loss [189]. In the current study a decrease in food intake was also demonstrated with all experimental treatment groups that paralleled changes in body weight. Although bacterial populations were not assessed in this study, the possibility remains that the treatments, and particularly the OFS, could have altered the gut flora. This remains to be elucidated in future work.

We observed the greatest reduction in body weight with the AD treatment compared to OFS which suggests that a decrease in energy intake is likely mediated largely by the effects of MT. Cani et al. previously demonstrated that addition of 10% OFS to a HF diet decreased food intake and led to favorable modulation of gut satiety hormones, chiefly

enhanced GLP-1. Decreased food consumption protected these mice from the accumulation of excess body weight compared to controls [125]. Alternatively, Quaile et al. have shown that the anorectic effect of MT results in a dose-dependent reduction in food intake evident after 1 week and lasting up to 7 weeks; a 200 mg/kg daily dose promoted a 11% reduction in the amount of calories consumed and a 12 % reduction in mean body weight after 36 days of oral MT administration [190]. The >25% decrease in average daily calories in rats administered MT in our study is greater than the magnitude of response seen by Quaile et al. While at the same time, the approximate 3% reduction in body weight was less than the previous report, collectively suggesting that administration of MT to DIO rats consuming a HFHS diet likely influences the overall drug effect.

Supporting this suggestion, Kim et al. showed that MT at a daily dose of 300mg/kg for 4 weeks markedly reduced food intake compared to untreated controls after 6 months of HF feeding in DIO rats and although a similar trend was shown in chow fed rats, this effect was not significant [184]. Differences in the amount of food consumed (size and/or meal frequency) as well as differences in the energy density of the HF versus chow diets are both contributing factors that help explain the greater differences seen between the treated and untreated HF fed rats and treated and untreated chow fed rats [184]. In humans, metformin has been associated with a neutral effect on body weight or as found in smaller trials in patients with T2D or polycystic ovary syndrome, significant weight loss in part due to reduced caloric intake [191].

Our findings with OFS supplementation are similar to Daubioul et al. showing the reductions in food intake are greatest in the first 4 weeks of fibre supplementation and this difference compared to control progressively dissipates [192]. In accordance with the reductions in food and energy intake, AD rats showed a rapid response to treatment, however, no differences in body mass were shown between MT and AD rats and by week 4 both groups weighed less than C and OFS rats until the end of the study. Observations in obese Zucker rats fed a standard diet have demonstrated that supplementation with 10% (wt/wt) Synergy 1®, a combination of the prebiotic fibres inulin and oligofructose, reduces total energy intake to a greater extent than an equivalent dose of indigestible bulk (cellulose) [193]. Likewise, OFS fibre when administered alone in our study, did not alter total grams of food consumed compared to C, but did significantly reduce daily calorie intake due to dilution of energy. A similar trend, although not statistically significant was also observed after the combination therapy compared to MT wherein AD rats consumed 15.4 kcal/d less than MT rats. The difference between the two MT groups, although small in magnitude, could provide crucial deficits in energy intake over the long term that help sustain weight loss. A recent clinical trial points to this potential showing that OFS supplementation in overweight and obese free living individuals reduced self-reported food intake by 29% during a 12 week placebo controlled intervention [33].

5.2 Body Composition

The evaluation of body composition is an equally important measure taking into account changes in lean mass and bone mineral content which accompany body weight loss. Preserving lean mass, which is more metabolically active than fat mass, during weight

loss, is critical for preventing sharp declines in energy expenditure which further undermine weight loss or attempts at weight maintenance. We observed a 10g increase in lean mass between the C and OFS groups and between the MT and AD groups, but this trend was not statistically significant taking into account that BMC was also greater in controls compared to both MT treatment groups. Although this finding contradicts others who have shown that supplementation of prebiotic fibres can lead to improved bone mineral content (BMC)[194], the most logical explanation for the changes observed in this study would be in the alterations in body mass; mainly since both AD and MT rats had a decreased body mass and BMC compared to OFS and C rats.

Rather, a physiological meaningful effect observed in this study was the negative impact MT treatment had on bone mineral density (BMD) that in addition, may be of clinical relevance considering that the combined approach appeared to mitigate this decline. Insight into the beneficial nutrient interactions occurring with prebiotic supplementation has recently emerged showing enhanced intestinal mineral absorption [194-196]. Others have shown that consumption of the prebiotic fibre inulin, in place of sucrose, results in similar gains in BMD[194]. Although not tested in the current study, OFS likely played an influential role in the preservation of BMD observed after the AD treatment compared to MT treatment alone via enhanced bioavailability of minerals (magnesium and calcium) in cecal tissue [196]. Greater calcium absorption has been linked in a dose-dependent manner to prebiotic fibre intake and this has been shown to promote increased calcium content in bones via enhanced bone mineralization [195]. Taken together, the findings on food and energy intake, body mass, body fat and body composition point to a greater

therapeutic efficacy, albeit modest in many respects, from the combination approach. In particularly, its use is supported by clinical studies evaluating bone health in the later years of chronic disease management for T2D [197-199].

5.3 Body Fat and Leptin

When considering improved energy balance after HF feeding, a reduction in adipose tissue is the most critical physical target. Adipose tissue is an active endocrine organ secreting numerous adipokines, including leptin, a long term regulator of body mass and fat stores [200]. In this study all experimental treatments facilitated a >25% reduction in grams of absolute body fat as well as led to improved percent body fat when compared only to C rats. Therefore, unlike body mass, a clear reduction in adiposity in OFS rats was observed and linked to the alteration in the composition of the diet. OFS has been shown to protect rats from fat mass accumulation in part by diluting the energy content of a HF diet [125]. No differences in final adiposity were observed between the MT and AD treated rats and this likely reflects their similarly reduced energy intake. When taking into account that AD rats had a twofold leaner phenotype measured in percent body fat compared to OFS rats, it is probable that the greater reduction in adiposity was again associated with the dramatic reductions in food intake and largely driven by MT [184, 201]. MT, administered at the same dose employed in this study, decreased visceral fat mass in genetically obese rats as well as in their wild-type lean littermates by 41 and 29% respectively, compared to untreated rats [184]. The greater fat loss observed in obese compared to lean rats was an effect coupled to greater reductions in cumulative food intake and greater declines in plasma leptin [184]. In the present study, all treatments

reduced hyperleptinemia compared to controls, an observation likely explained by the reductions in adiposity.

MT treatment played an influential role in further reducing plasma leptin compared to OFS. It has been suggested that metformin (at a physiological dose) can directly inhibit leptin production and secretion by modulation of glucose metabolism in adipocytes by diverting pyruvate into lactate and away from oxidation and lipogenesis [202]. Reductions in body mass with a low dose of MT (75mg/kg) administered for 14 days has been shown to correlate with pre-treatment fasting leptin concentrations [203]. Moreover, low-dose MT treatment administered to chow fed controls, HF DIO and HF diet resistant rats only reduces food intake in DIO and diet resistant rats fed the HF diet where use of this drug was associated with suppressed plasma levels of insulin but not leptin [203]. Importantly, several lines of evidence speculate that decreased ObRb gene expression is related to HF feeding and connected to central leptin resistance attenuating its beneficial regulation of satiety, although this is not always consistently shown [200]. In essence, it is evident that MT can mitigate hyperinsulinemia and hyperleptinemia in the obese state [200, 203]. Kim et al. further established that MT potentiates leptin-induced central signaling via proopiomelanocortin protein expression in the hypothalamus and it is to be expected that increases in this peptide mediated some of the drugs anorectic effects [184]. Additional findings show MT treatment for 4 weeks reduced plasma leptin but did not normalize levels to that of the chow fed healthy weight controls [184]. Further work would be needed to verify if MT was driving reduced hunger through increased leptin signaling in this study however, in normal weight humans, MT yielded a 30% decrease in

plasma leptin (24% from baseline) despite no reductions in bodyweight or hunger scores compared to placebo controls [204]. Similar to our findings, others have shown similar reductions in plasma leptin and bodyweight in rodents and obese individuals after use of MT [16, 205].

5.4 Glycemic Response

The second objective of this study was to determine if the combination therapy would foster improvements in the regulation of blood glucose in glucose intolerant DIO rats. In HF-fed obese Sprague-Dawley rats, hyperinsulinemia results in a near doubling of circulating insulin compared to standard chow fed controls and addition of MT for 4 weeks normalizes these levels [184]. In vivo, it has been shown that MT directly attenuates glucose-stimulated insulin secretion via the activation of AMPK in isolated pancreatic islets, and this was unlike the effects of leptin [206]. It is well established that use of MT results in a dramatic lowering of insulin and improves blood glucose regulation in states of glucose intolerance in rodents and humans [147, 184, 203, 207]. Similarly, in this study use of MT significantly reduced serum insulin compared to that of C and OFS.

To the contrary, increased insulin secretion led to improved glucose tolerance after feeding a fermentable fibre compared to a non-fermentable fibre diet in dogs [111]. In streptozotocin-treated diabetic rats, a fermentable dietary fibre also increased insulin secretion and improved blood glucose control compared to controls [208]. This increased insulin and improved glucose response to oral glucose has been linked to increased GLP-

1 secretion with fermentable fibre consumption [111, 126, 208]. Blocking GLP-1 signalling with Exendin-9 or abolishing it in the GLP-1R knockout mouse has been shown to abrogate OFS-induced improvements in glucose tolerance [127]. While in sucrose-fed rats addition of OFS decreased blood glucose compared to HS fed controls but showed no differences in plasma insulin [209]. Moreover, the addition of inulin to a HFHS diet has been shown to decrease portal plasma glucose in obese rats, but did not reduce elevated insulin compared to HFHS fed controls [141]. A potential explanation for these divergent effects may be the enhanced glucose uptake via insulin-mediated and insulin-independent mechanisms facilitated by incretin secretion and signalling respectively [210]. This effect is further supported by findings that show OFS feeding can increase serum concentrations of the two insulinotropic hormones GLP-1 and GIP [125]. These finding support the suggestion that prebiotics may improve glucose tolerance via an insulin secretagogue mechanism as opposed to the insulin sensitizing effects of MT.

In conjunction with the marked lowering of HOMA-IR in the current study, it is apparent that MT whether alone or in combination with OFS, was an effective insulin sensitizer which was largely driven by reduced fasting insulin levels. Indeed, recent experiments *in vivo* and *in vitro* by He et al. show that both MT and insulin collectively circumvent insulin resistance via the atypical protein kinase C (aPKCι/λ) resulting in the phosphorylation of the cAMP response element binding (CREB) protein and inhibition of hepatic gluconeogenesis in obese, hyperglycemic and insulin resistant states [129, 211]. It is interesting, however, that glycemic response to an oral glucose load was significantly lower in OFS and AD rats but not MT compared to C. At the 90 minute time point during

the OGTT, glycemic values were reduced in AD compared to MT rats. This would suggest that OFS is the major driver of reduced glucose appearance and/or utilization following an acute glucose bolus in our rats. This also confirms previous work showing a hypoglycaemic effect of OFS [33, 126]. In essence, these findings suggest that the complementary action exerted in the combination therapy resulted in the most favourable postprandial glycemic response.

5.5 Physical Characteristics of the Intestine

Alterations to the size and physical characteristics of the intestine occur in response to changes in diet composition which in turn impact gut function. Parnell and Reimer [212] demonstrated that prior to the onset of excess adiposity (3.5 wk), obese (*cp/*cp) JCR:LA-cp rats have greater gut mass and length compared to lean (+/?) counterparts. This increase in functional absorptive area was suggested to allow the obese rats to consume and digest greater amounts of food at a young age. Once the rats reach adulthood and exhibit well-established obesity, the size of the intestine becomes proportionally smaller compared to lean rats with greater modifications to nutrient-responsive genes noted in the proximal compared to distal regions [212]. An increased proximal absorption of nutrients has been noted in obese compared to lean individuals [213] suggesting less nutrients reach the distal portions of the gut where the majority of satiety hormone production occurs. In the current study, after adjustments for body weight, both MT and AD rats displayed greater small intestine length compared to OFS and C rats, supporting previous observations following DIO in rats [210]. Moreover, this increased length is illustrative of the fact that treatment with MT promotes reduced food intake despite increased

proximal intestinal capacity. Since orally administered MT is absorbed at a high rate from the duodenum and accumulates in the wall of the intestine [160, 161, 214], this may also be a contributor to the increased small intestine length.

In the current study, AD rats showed a 22% increase in adjusted small intestine weight compared to OFS. In contrast, MT rats showed a decrease in the absolute small intestine weight compared to OFS and C rats and although this was not statistically different after adjustments for body weight, the incongruity in MT treatment groups may suggest that alterations in diet also played an influential role. In dogs, addition of OFS and beet pulp to the diet for 6 weeks was associated with a 35% heavier proximal intestine with a 37% increase in mucosal mass alongside a 28% increase in intestinal surface area compared to controls [215]. On the other hand, the decreased weight of the intestine after MT may be indicative of a potential repercussion to the intestinal cell integrity and this may alter the endocrine capacity of the gut [212]. The implications of this suggestion are discussed further in the intestinal gene expression section below.

Rather unique to many nutrients, dietary OFS reaches the large intestine intact requiring bacterial degradation to release its carbohydrate energy stores through fermentation [141]. In particular, extensive fibre fermentation following consumption of OFS results in an expansion of the cecum compared to the effects of cellulose, a non-digestible non-fermentable fibre source [216]. Subsequent growth of epithelial cells, proliferation of the distal intestine and promotion of indigenous bifidobacteria species is attributed to this effect [216]. Cani et al. reported that enlargement of cecal contents and tissue weight is

distinct 8 hours after ingestion of a 10 % OFS supplemented HF diet [125]. However, compared to a standard diet, HF feeding attenuates this proliferative effect [126]. Delmée et al. showed in DIO mice that the carbohydrate content of a HF diet may influence the degree of cecal tissue enlargement with a considerably greater effect on full cecal contents observed after OFS addition to a carbohydrate-free diet compared to a high–carbohydrate diet [126]. In the current study, both the absolute and adjusted empty cecum mass were enlarged in OFS and AD rats compared to the MT and C rats, which concurs with the present literature on OFS feeding.

After adjustment for body weight, AD rats also showed an increased mass of the colon compared with MT and C rats which is again consistent with previous work showing the proliferative effects of fibre enriched diet in the distal gut [65]. Alternatively, MT and AD rats both showed a greater adjusted colon length than C rats which could point to an independent effect of MT. In fact, others have shown that oral or intravenous use of this drug leads to the accumulation of MT in the ileum and colon [160]. Further work is needed to quantify differences in fibre excretion, bacterial colonization, and production of SCFA following these treatments in order to determine if OFS fermentation per se was a factor contributing to body weight reductions observed in this study. Failure to ferment fibre is linked to consumption of a HF diet in obese mice and the consequences of this are evident in minimal to no loss of body fat [217].

5.6 Physical Characteristics of the Liver

It is well established that TG infiltration in hepatic tissues is a common pathology associated with fatty liver disease and that consumption of excess energy can alter the metabolic efficiency of the liver depending on the composition of the diet [130, 132, 141]. The consumption of a HF and/ or HS diet is linked to the manifestation of fatty liver disease and insulin resistance [132, 135, 141, 212]. Within 3 weeks of feeding a HFHS diet, obese rats display an increased liver mass and TG content compared to animals consuming a standard diet with or without supplementation of fibre [141]. Supplementation of OFS in a HS diet has been shown to reduce liver weight and TG levels compared to controls [209]. In the current study, liver mass was significantly reduced alongside bodyweight by MT treatment compared to C rats with statistical differences no longer apparent after adjusting for body weight. There was a 25% reduction in whole liver TG content in the treated rats compared to C rats which may be reflective of the reduced caloric intake. Kok et al. suggest that the 10% addition of OFS to a HF diet does not significantly prevent and/or reverse the infiltration of dietary TG into hepatic tissues [135]. In contrast to Kok et al., our study was of longer duration and had greater differences between groups in daily energy intake which could account for the discrepancies.

5.7 Satiety Hormone Profiles

The gastrointestinal tract is prominently involved in the regulation of food intake via the production of gut-derived satiety hormones that work in an integrated fashion. The lower energy intake with OFS alone was associated with enhanced PYY and suppression of

ghrelin secretion, both events that have been similarly linked to satiation in rodents fed a standard diet [123]; as well in overweight adult individuals who showed improvements in plasma hormone profiles after 12 weeks of treatment [33]. Increased plasma amylin, normally co-secreted with insulin, has been shown to reduce gastric emptying, appetite and food intake in healthy humans similar to the effects associated with GLP-1. Increased secretion of this hormone in OFS rats may have also played a role in reducing appetite [218]. In contrast, the anorectic effects of MT are not logically linked to plasma PYY and GLP-1 given their decreased levels relative to OFS and C rats in this study. Based on Yasuda et al. [158] we hypothesized that MT would increase GLP-1 secretion; it did not appear to do so in our DIO rats.

Although numerous satiety hormones were measured, it appears that decreased secretion of GIP was the only circulating peptide with a consistent relationship to the gross behavioural measurements of food and energy intake. GIP is mainly secreted from the intestinal K cells and released in the duodenum and proximal jejunum [40]. Others have shown that a diet comprised of saturated fat is pathological to the intestine and exacerbates GIP secretion by increasing K cell transcription and proliferation [219]. In healthy subjects, increased consumption of a HF diet compared to a habitual diet comprised of less than 125g/day of fat enhances the postprandial GIP response to an oral glucose challenge [220]. In this study, the synergistic effect of OFS and MT observed in AD treated rats supports previous observations with reduced fat intake and suggests that combined actions of OFS and MT maximally attenuate GIP release.

Research suggests deregulation of GIP is involved in the development of impaired insulin signalling and elevated basal and nutrient-stimulated GIP has been associated with glucose intolerance and hyperinsulinemia in obese individuals [221]. GIP is an incretin [40] and alongside the anabolic actions of insulin, it also plays a prominent role in regulating fat metabolism. GIP receptor (GIPR) knockout (-/-) mice exhibit increased energy expenditure and are resistant to obesity and insulin resistance when fed a HF diet for 50 weeks, unlike wild type controls [79]. Kim et al. demonstrated that GIP infusions elevate the activity of lipoprotein lipase and consequently increase TG uptake into and storage in epididymal adipose tissue [174].

Mechanistically, GIP stimulates release of resistin, an intermediate signalling adipokine that leads to the increased activity of lipoprotein lipase and protein kinase B with consequences on the activity of AMPK and it upstream regulator protein LKB1 [174]. In essence, GIP signalling promotes storage rather than utilization of fat, while at the same time decreases basal energy expenditure. Highlighting the involvement of this hormone in facilitating lipogenic processes, GIPR (-/-) mice display decreased expression of the required catalyst for TG synthesis in the adipocyte: Acyl CoA:diacylglycerol transferase 1 (Dgat1) compared to wild type controls [79]. Zhou et al. illustrated that on a standard diet, GIP signalling, despite compromised insulin action, still promotes increased adiposity in knockout mice lacking insulin receptor 1 (IRS-1) compared to knockouts lacking both GIP and IRS-1 [222]. Here, the double knockout mice showing abolished GIP signalling had increased energy expenditure, glucose tolerance and greater fat oxidation in skeletal and liver tissues compared to mice with GIP [222]. More recently,

clinical findings show that the elevated plasma GIP in obese and diabetic individuals following a saturated fat load is characteristic of nonalcoholic steatohepatitis (NASH) liver disease which is correlated with plasma adipokine imbalance and elevated resistin [223].

5.8 Gene Expression of the Intestine

In the genetically obese JCR:LA-cp rat, gene expression of satiety hormones is altered during the progression of obesity [40]. Here, alteration in the expression of proglucagon mRNA was chiefly noted in the proximal compared to the distal intestine, which may alter levels of circulating GLP-1 given that the highest abundance of L cell are located in the distal and not proximal region of the gut [40]. In accordance with this regional distribution, the discussion has been divided into the proximal and distal segments of the intestine to better clarify several potentially meaningful differences in satiety hormone secretion.

5.8.1 Proximal Intestine

Ghrelin is primarily secreted from the stomach and is typically suppressed following food intake [224]. In obese rats, others have shown blunted ghrelin secretion in response to a HF diet challenge compared to the profile obtained during habitual consumption of a standard chow diet [125]. Decreased ghrelin mRNA in the stomach has been previously noted early in the development of obesity in JCR:LA-corpulent rats while, in adulthood the differences in ghrelin gene expression between lean and obese rats were no longer evident in the stomach [212]. In the present study, ghrelin mRNA levels in the stomach

were reduced in C compared to MT rats and this may be reflective of the greater intake of energy in C versus MT rats. In contrast to this speculation, findings in male Wistar rats show decreased expression of ghrelin mRNA in the stomach after five months of 35% dietary restriction compared to rats fed ad libitum [225]. Furthermore, clinical findings have shown that in individuals with T2D, use of MT for 6 weeks actually led to a 24% increase in plasma ghrelin after a 350kcal meal that did not correspond to changes in subjective appetite [226]. When considering that AD rats ate similar amounts of energy (grams and calories) as MT and had comparable body weight to MT rats, it is possible that OFS supplementation may have prevented a similar effect. Others have shown that the addition of OFS to a standard diet reduced ghrelin secretion but this effect was no longer evident when added to the HF diet and it was suggested that the HF diet suppressed ghrelin secretion completely [125]. Ultimately, further investigation is needed to fully understand the divergent regulation of this hormone in the context of this study.

The modification of proglucagon, PYY, ghrelin and GLUT2 mRNA levels in the duodenum was relatively similar among the C, OFS and MT groups. The combined therapy, although not statistically significant, was associated with greater proglucagon and GLUT2 and lower ghrelin mRNA compared to the other treatments. The lack of change in GLUT2 by MT alone is consistent with findings from Lenzen et al. [165]. GLUT2 receptor knockout (-/-) mice are characterized by impaired intestinal secretion of glucose-induced GLP-1 emphasizing the importance of the recruitment of this glucose transporter for normal GLP-1 secretion [227, 228]. Duodenal release of GLP-1 is characterized by an early peak and rapid fall and is regulated by low-dose infusions of

glucose in humans [70]. The rapid early peak in GLP-1 observed in our AD rats during the OGTT could be mediated by duodenal L cells [69]. In addition, ghrelin suppression alongside these findings is physiologically important, as ghrelin is a potent antagonist of GLP-1 [83]. Taken together, these findings indicate that enhanced duodenal GLP-1 production in AD may be another important factor optimizing serum concentrations of this peptide compared to MT alone.

Orally administered MT is absorbed at a high rate from the proximal intestine [160, 161, 214] and has been shown to dose-dependently decrease glucose absorption [229]. On the other hand, ingestion of fermentable fibre may increase the absorption of glucose by enhancing intestinal glucose transport capacity and expression of brush border and basolateral glucose transporter proteins [111]. Alternatively, in the jejunum, our findings suggest this was not the case and rather C rats displayed the greatest glucose transport capacity with increased expression of GLUT2 compared to MT; most likely linked to the habitual intake of HFHS diet in C. In addition to this, a twofold greater expression of ghrelin mRNA in C rats clearly suggests that the pervasive effects of the HFHS diet are detrimental in the jejunum considering again, this peptide is a potent antagonistic to the action of both GLP-1 and PYY [83]. PYY expression was still greater in C rats compared to AD in the duodenum and AD and MT in the jejunum, similar to what Parnell and Reimer showed in well-established obesity in the JCR-LA cp rat [212].

5.8.2 Distal Intestine

It was initially anticipated that proglucagon mRNA expression in the distal intestine would be markedly upregulated alongside plasma GLP-1 as in the study by Cani et al. [125]. Here, a 35 day pre-treatment with OFS supplemented standard diet was used to protect animals from fat accretion during a 15 day HF dietary challenge and was linked to both enhanced proglucagon mRNA and the GLP-1 (7-36) peptide content in intestinal tissues [125]. In the face of the HF feeding, GLP-1 was mainly produced in the proximal and medial colon in both the HF and OFS-HF fed animals. After accounting for cecal tissue enlargement, GLP-1 concentration was nearly twofold higher with OFS consumption [125]. Differences in proglucagon gene expression between the HF and OFS-HF fed animals showed the intestine was favourably modified by OFS leading to increased proglucagon mRNA which was expressed to a greater extent in the cecum and to a lesser extent in the proximal colon [125]. In the current study, given that the increased mRNA in OFS and AD rats was only apparent in the cecum compared to MT; and in the colon in OFS rats compared to MT and AD; it is clear that the effects of OFS in this study were severely attenuated. This blunted effect could be attributed to the HF feeding similar to work by Zhou et al. wherein a HF diet was linked to the attenuated fibre fermentation [217]. Delemée et al. also demonstrated that OFS-induced changes in proglucagon expression may be greatly influenced by the composition of the diet and/or ensuing diet-induced disease state with the former of the two most probable [126]. They demonstrated that the ability of OFS to upregulate proglucagon expression is eliminated when added to a HF diet rich in simple carbohydrates and is severely reduced by a HF diet compared to that of a standard control [126]. In the present study, both disease state

and background diet may be contributing factors to our findings but, unlike Delemée et al., our findings suggest that OFS supplementation in the face of a HFHS diet may still foster some improvements in proglucagon gene expression when compared to MT and reduced caloric intake alone. It is therefore apparent that in this study, the 10 % dilution of metabolizable energy from the background HFHS diet contributed to the reductions in body fat percent observed after OFS supplementation compared to C. Since alterations in the composition of gut microbiota may also be a contributing factor to intestinal modification, this is an area that warrants further investigation [230].

5.9 Liver Gene Expression

Our findings suggest that the physical measure of hepatic TG content is equally reduced by all three experimental treatments, yet there are alterations at the gene level that, if they persisted, would give the AD treatment an advantage in hepatic lipid processing. The down regulation of SREBP-1C in the liver by all experimental treatments supports improvements in liver metabolism especially when considering increased activity of this transcription factor is linked to continual IRS-1 signalling and has been associated with the development of fatty liver in two different models of diabetic mice [130]. SREBP-1C governs transcription of enzymes involved in lipid synthesis [141, 173]; while FAS (transcription and activity) governs endogenous lipid flux [132, 231]. In this work, the combined treatment significantly attenuated hepatic FAS mRNA, illustrating a reduced capacity of enzymes required for *de novo* lipogenesis when compared to control and MT. Independently acting, both decreased hyperinsulinemia by MT and addition of OFS are likely factors that suppress FAS gene expression [132, 141] and it is likely that this led to

alterations in hepatic gene expression and reductions in hepatic TG content seen across all treatments. Kok et al. suggest that OFS attenuates liver TG accumulation compared to control when fed a standard diet for 30 days as well as after a 48 hour high fructose drink challenge and this protective effect was attributed to decreased FAS enzyme activity [132]. Agheli et al. also observed an 11% decrease in liver weight, improved plasma TG and free fatty acids after addition of OFS to a sucrose-enriched diet for 3 weeks and this was also associated with lower FAS activity compared to control [209]. Similar to the previous report, addition of inulin to a HFHS diet for 3 weeks has also been shown to decrease liver weight and TG levels alongside improved plasma TG and VLDL concentrations compared to controls. This was further associated with lower FAS and ACC gene expression and probably due to differences in dietary intake [141] similar to our report. *In vitro* experiments further confirm that OFS can decrease the lipogenic capacity of hepatocytes depicted by reduced de novo TG synthesis [132].

Inactivation of ACC by activated AMPK can lead to immediate alterations in lipid metabolism [173]. Essentially, by activating AMPK, the two ACC enzymes that mediate different cellular distribution of synthesized malonyl-CoA can be inhibited and therefore, facilitate increased energy use and decreased fatty acid synthesis via decreased malonyl-CoA production [173]. In the present study, the addition of OFS in either group led to increased hepatic expression of AMPKα1 mRNA. On the cellular level, this is suggestive of improved metabolic capacity and greater ability to deactivate ACC and malonyl-CoA production that ultimately, would aid in decreased hepatic lipid accumulation. Seo et al. demonstrated that the suppression of ACC activity is distinctly reduced by 7 day

adenovirus-mediated overexpression of AMPKα1 subunit in the liver and subsequently, this reduces blood and liver TG concentrations as well as the degree of hepatic steatosis in hyperlipidemic and hypercholesterolemic T2D rats when compared to untreated controls [232]. The suppression of ACC mRNA in AD compared to C supports the notion that important cellular changes were occurring although, again this was not statistically different from either treatment alone and in contrast to other reports with inulin supplementation to a HFHS diet [141].

The increased AMPKα2 expression in AD compared to all other experimental groups further suggest that the greatest alterations in energy regulation at the gene level were promoted in the combination therapy. As GIP receptors are not expressed in liver tissues [222], GIP-mediated inhibition of LKB1 via resistin [173, 174] may explain the decreased gene expression of the AMPKα2 subunit measured in MT compared to AD rats. Therefore, decreased GIP in AD compared to all treatments could lead to amplified AMPKα2 expression especially since MT requires the activation of LKB1 to mediate AMPK activity and its therapeutic effect on blood glucose [173]. Indeed, Banerjee et al. observed increased AMPK activation in the liver and decreased gluconeogenesis in mice lacking adipocyte derived resistin [173]. Therefore, it is not unreasonable to further suggest that decreased GIP may also lead to greater AMPK activity and fat oxidation in the liver via decreased resistin signaling. Others suggest that the increased β-oxidation of fat in the liver contributes to the increased energy expenditure in IRS-1(-/-) and GIP(-/-) knockout mice compared to IRS-1(-/-) and GIP(+/+) mice and this was supported by increased transcriptional activity of peroxisome proliferator-activated receptor-α [222].

Moreover, short-term activation of AMPKα2 in the liver of mice led to decreased SREBP-1 protein expression as well as fatty liver that resulted from the accumulation of fatty acids released from adipocytes [233], and therefore may suggest that the similarities in liver TG content across all treatments may be misleading in regards to the improved fatty acid utilization. As well, since synergistic differences existed between the treatments, it is likely that the daily reductions in food intake and reduced energy availability in AD rats also contributed to the long-term alterations we observed in energy partitioning.

In the diabetic state, a common pathology leading to glucose intolerance is increased endogenous production of glucose in the liver, whereas treatment with MT improves fasting blood glucose levels by decreasing gluconeogenesis [234]. With respect to the drug actions of MT, the changes we observed in hepatic gene expression would also support an improved metabolic state for the AD rats. Activation of the AMPK enzyme is the projected link for MT's therapeutic effect in the liver where it activates this metabolic switch to improve excursions in blood glucose by reducing hepatic de novo glucose production [167, 173, 234]. Zhou et al. demonstrated *in vitro* that this drug can activate both AMPK catalytic subunits to inhibit glucose production in primary rat hepatocytes and AMPK inhibition by compound C prevents this effect [167]. In the present study, MT treatment alone did not lead to enhanced mRNA expression of either AMPK catalytic subunits and in part, may explain the blunted effects of MT in our rats consuming a HFHS diet making the drug less successful in improving hyperglycaemia compared to the control. Overexpression of the α1 subunit (CA-AMPKα1) in HF DIO mice has been

shown to decrease hepatic gluconeogenesis and lead to moderate improvements in the regulation of blood glucose after an intravenous glucose challenge compared to wild type controls [232]. Here, the reduced insulin secretion and decreased expression of glucose-6-phosphatase (G6Pase) provided further support for a role of the AMPKα1 subunit in glucose regulation yet, considering this subunit is constitutively active and still did not normalize impaired glucose tolerance compared to lean controls [232], the influence of the AMPKα1 enzyme to glucose regulation may be minimal. Even though the AMPK catalytic subunits are equally expressed in the liver, there is elevated blood glucose in liver specific AMPKα2 (-/-) knockout mice that still retain AMPKα1 expression [235]. Since consumption of a HF diet down regulates hepatic expression of the AMPKα1 enzyme in rats [236], this may lead to increased glucose output in the liver whereby, the reductions in fat intake mediated by OFS fibre attenuate this effect.

Alternatively, substantial evidence from liver specific AMPKα2 (-/-) knockout mice show that the AMPKα2 subunit is physiologically critical for the regulation of blood glucose. These mice have up to 50% greater glucose output alongside increased activity and expression of the gluconeogenic enzymes phosphoenolpyruvate carboxykinase and G6Pase, promoting hyperglycemia in the fasted and fed state compared to controls [235]. Therefore, increased AMPKα2 mRNA expression in the AD rats compared to all treatments further supports the observed improvements in glycemic regulation likely resulted from alterations in the liver. Several lines of evidence would suggest that these changes may be mediated by adipocyte derived hormones such as leptin and adiponectin, but not insulin that independently shut off gluconeogenic enzymes [235]. This suggests

that improvements we observed in OFS and AD may be mediated via independent mechanisms.

5.10 Alterations to Cholesterol Metabolism

The unique non-viscous yet soluble properties of OFS allow this fibre to readily disperse in the distal gut and preferentially promote the growth of health-enhancing gut bacteria [136]. These fibres improve gut health through their fermentation and production of SCFA by-products [136] and via the reduction in the growth of pathogenic gut bacteria [114]. The production of acetate, propionate and butyrate decrease the pH of cecal contents and this acidification is known to increase the turnover of bile by promoting its precipitation and limiting the formation of secondary bile acids [137]. Evidence from hypercholesterolemic rats show that prebiotics can reduce total plasma cholesterol in the face of a HFHS diet by reducing cholesterol absorption from the gut as well as modifying cholesterol metabolism in the liver [139]. The liver followed by the intestine are the two main sites for cholesterol biosynthesis whereby, cholesterol excretion in the form of bile salts leads to compensatory alterations in the activities of key enzymes involved in cholesterol homeostasis [171]. SREBP transcription genes govern cholesterol metabolism exclusively, with the expression of SREBP-2 showing preference in maintaining adequate cellular cholesterol by downstream activation of HMGCR, the rate limiting catalyst mediating cholesterol synthesis [231]. Supplementation of prebiotic fibres dose-dependently increase gene expression of SREBP-2 as well as CYPT7A1, the rate limiting enzyme in bile synthesis, with a 20% fibre dose yielding the most significant increases in both lean and obese JCR:LA-cp rat [139]. Unlike a previous report, the 10% dose of OFS

we used did not significantly alter genes involved in cholesterol or bile acid synthesis, however, this could reflect the relatively lower dose administered.

Alternatively, others show that MT increases bile salt absorption in the jejunum but also acts to decrease active bile salt absorption from the ileum thus, overall MT induces decreased intestinal bile salt absorption from the intestine [237]. It was therefore hypothesized that the combination of the two treatments in this study would act synergistically. With the exception of SREBP-2 mRNA in AD, our results suggest this was not the case and rather gene expression of SREBP-2 may be the result of increased production of one of the SCFA, propionate. Others have reported that supplementation with inulin normalizes the HFHS feeding-induced suppression of this SCFA in the liver of rats compared to controls and this was linked to decreased expression of ACC and FAS enzymes [141], with the latter of the two similar to the present observation made in AD rats. MT administered alone promoted the greatest increase in hepatic HMGCR and CYPT7A1 gene expression compared to OFS and when considering that this was not significantly different from AD and controls rats, the long-term changes between groups again appear quite similar. Scott and Tomkin show this may also be reflective of disease state as only diabetic rats treated with MT display increased hepatic expression of HMGCR alongside decreased activity of HMGCR and acyl-CoA-cholesterol acyltransferase (ACAT), the rate-liming enzyme mediating cholesterol esterification and storage in the intestine, compared to untreated diabetic rats [171]. Whereas, MT treated normal rats only showed reduced ACAT activity compared to untreated controls [171]. Although, the decrease in genes governing bile synthesis in AD compared to MT rats could also be indicative of an unfavorable interaction between the OFS and MT and

suggest that alterations in the distal intestine may impede the cholesterol lowering actions of MT by changing the resident microflora, pH and/ or limiting the drugs direct exposure to intestinal enterocytes and its effect on bile salts [161, 237, 238]. Since increased synthesis and excretion of bile salts is not the only proposed mechanism exerting a cholesterol lowering effect of metformin [171]; or oligofructose [139]; further investigation in a more appropriate model is warranted.

5.11 Hypothesis Conclusion

Since the primary objective of this research was to determine if the combination of OFS and MT could synergistically enhance endogenous GLP-1 it is important to emphasize that these findings refute the null hypothesis. Moreover, several factors that may have contributed to this outcome have been identified. The first source of error may be the cardiac puncture technique employed during the final blood collection in this study, especially considering the extremely short half life of the GLP-1 peptide and the fact that this hormone is chiefly active in portal circulation. Findings from the Cani et al. [125] lab show blood samples collected directly from the portal vein may be an important technique to exercise for future research investigation and despite the difficulty of this technique, it has generated the utmost evidence for the physiological effect of oligofructose on GLP-1 secretion. Research also suggests that a more accurate means of studying the secretion of GLP-1 *in vivo* may be to use an intestinal lymphatic sampling technique as portal vein GLP-1 concentrations compared to intestinal lymph GLP-1 concentrations are greater for both the fasting and postprandial state: 7.1 ± 0.7 vs. 16.8 ± 5.1 ($p=0.08$) and 315 ± 131 vs. $44{,}443 \pm 4{,}748$ pM/min ($p<0.001$), respectively with

tAUC determined after 240 minutes post intragastric infusions[239]. One precaution however to the intestinal lymphatic GLP-1 sampling technique would be that this research did not differentiate between the active and inactive forms of GLP-1 even though, this study did show decreased DPP-IV concentrations in the intestinal lymph when compared the portal plasma values measured for this enzyme. Alternatively, Yasuda et al. [158] work collected plasma samples (200μl) from the caudal vein that may also provide a more accurate method for determination of plasma GLP-1 concentrations. The second factor that may have been overlooked in the development of the main hypothesis was the HFHS diet as Delemée et al.[126] has shown the proglucagon gene expression and GLP-1 secretion decrease as a result of altering the composition of the diet from a standard chow to a high fat and even more so with a high fat high carbohydrate diet. This was previously discussed above in the gene expression of the intestine section, however exemplifies that this experimental design is inadequate to determine the role of MT alone or in combination on promoting endogenous GLP-1 alongside any other diet than the HFHS diet. Following DIO feeding a standard diet may be optimal conditions to further test this hypothesis. Lastly, considering that significant differences were evident in the caloric intake of food between MT treated and untreated rats, the insight gained from this study would recommend that future experiments use a pair-fed control to determine if the decreased caloric intake observed in this study also played a role in the decreased secretion of GLP-1.

5.12 Strengths and Limitations

While our ultimate goal is to identify new therapeutic strategies for the management of obesity and metabolic syndrome in humans, there are several limitations in doing so from this study alone. We acknowledge that it is inappropriate to directly extrapolate findings obtained in rat studies to humans because of numerous physiological differences and above all else differences in precise disease etiology. Nevertheless, there is sufficient evidence to suggest that the DIO rat is a suitable animal model that closely mimics human obesity. In addition, the treatment doses used in this study equate to a greater concentration, particularly of the OFS fibre, than what would be used in humans. While humans would not be expected to tolerate an equivalent 10% dose of OFS, largely because of gastrointestinal side effects, Parnell and Reimer [33] have successfully demonstrated weight loss with a 21 g/d dose in overweight and obese adults. In terms of the outcomes measured in this study, an important component to understanding the functional changes associated with the hepatic AMPK gene expression measured would have been protein expression measured with Western blots. While not a part of this thesis, our lab now has data showing increased pAMPK in OFS compared to control that is amplified in the MT and AD treatments. Complete examination of these findings will provide an important functional dimension to the findings of this thesis. A noted strength of the study is that independently, both MT and prebiotic fibres, are widely used today and could be easily combined into an adjunct therapy. To our knowledge this is the first in vivo study to evaluate if these two therapeutics strategies could be administered safely in combination and act to promote improved metabolic outcomes.

5.13 Overall Conclusions

Independently, OFS and metformin have been shown to increase GLP-1 secretion and improve glycemic control. Our objective was to determine if combining these two therapies, one dietary and one pharmacological, could improve satiety hormone secretion and glucose homeostasis in obese rats. Administered alone, both OFS and metformin decreased energy intake, body fat, hepatic TG content, and plasma leptin and GIP levels. The combined therapy was more effective than either treatment alone for several outcomes although, statistically significant synergistic effects were evident solely in plasma GIP concentrations and hepatic AMPKα2 and SREBP-2 expression. From a physiological standpoint, the greater reduction in plasma GIP and ghrelin mRNA and the greater increase in duodenum proglucagon mRNA and hepatic AMPKα2 and SREBP-2 mRNA seen with the combined therapy has the potential to reduce metabolic abnormalities with prolonged exposure. From a clinical perspective, the reversal of metformin-associated declines in BMD with the addition of OFS to the diet could have practical implications for bone health. Interestingly, although metformin is a highly effective insulin sensitizer, glucose AUC was significantly lower than C in OFS and AD rats but not MT rats suggesting much of the glycemic control in response to an acute oral glucose load was mediated via OFS in these rats.

Taken together, the observations in this study suggest that decreased consumption of the HFHS diet resulting from our dietary and/or pharmacological treatment is likely a key mediator promoting improved metabolic regulation in our obese rats. This occurs alongside a complementary reduction in endogenous lipid synthesis in the liver with all treatments compared to control. Increasing dietary fibre intake forms one strategy in the

goal of reducing energy intake that serves as a first line defence for treating obesity and its associated metabolic abnormalities. While many of the mechanisms associated with the beneficial effects of OFS and MT have been identified, it is also important to consider the independent effects that reduced food intake had in this study. OFS, MT and AD all resulted in lower intake of the background HFHS diet and this cannot be ruled out as playing an important role in the metabolic outcomes observed in our rats in conjunction with the physiological and cellular changes noted. Clinically, the preservation of BMD seen in the AD rats compared to MT alone and the synergistic reduction in GIP point to the potential in further defining effective dietary and pharmacological adjunct therapies. Identification of these combined therapies has the potential to increase the effectiveness of current treatments administered alone.

5.14 Future Direction

Further work is needed to evaluate the energy content in fecal matter, bacterial colonization and production of SCFA as this may have facilitated some of the body weight reductions observed in our study. Others have show that the alterations in the composition of gut microbiota may also be a contributing factor attenuating weight gain and this is an area that warrants further investigation. Cani et al. has demonstrated that increases in bacterial lipopolysaccharide (LPS) more than doubles after HF feeding in mice and consequently leads to a state of metabolic endotoxemia [240]. Elevated plasma LPS, which characterizes metabolic endotoxemia, is a triggering factor promoting glucose intolerance, hepatic insulin resistance, TG accumulation, weight gain and whole body inflammation [230, 240]. Further work is required to evaluate if some of the weight

reduction observed in this study could be the result of prebiotic-induced alterations in gut microbiota [230, 240].

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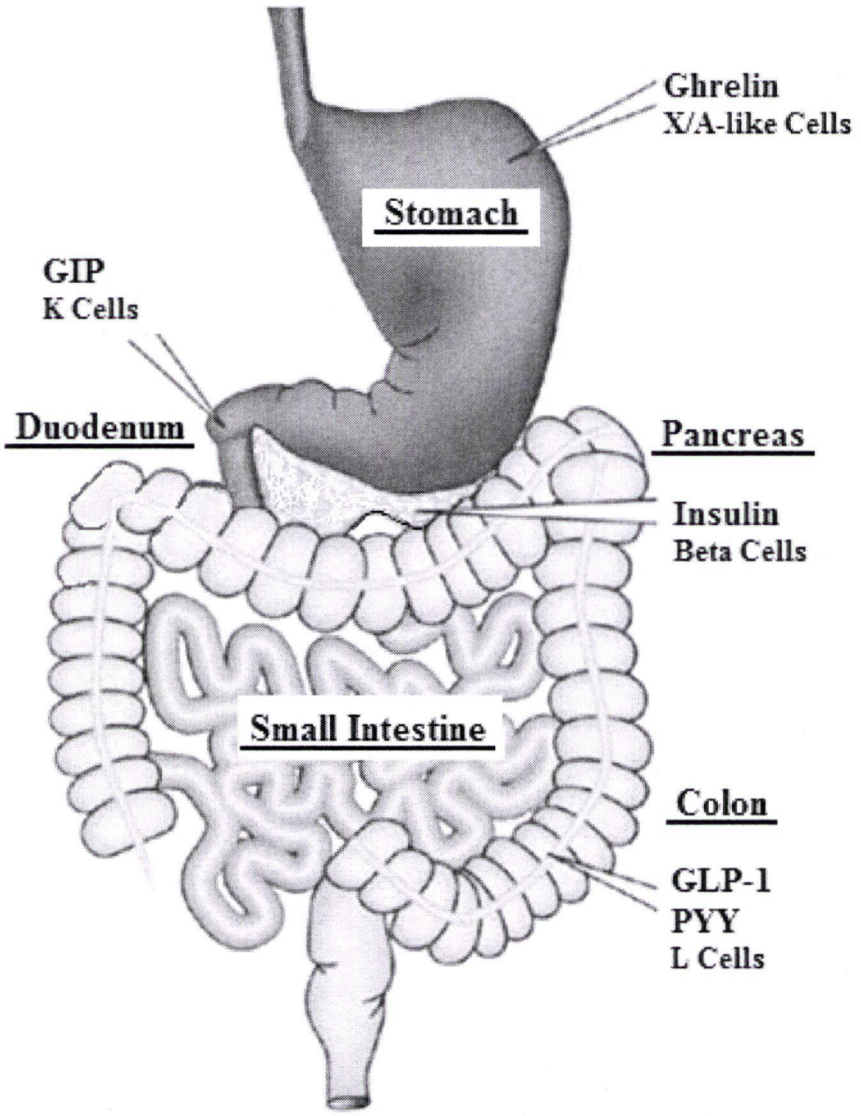
APPENDIX A: DIAGRAM OF THE GASTROINTESTINAL TRACK



Adapted from Dr.Sharrna's diagram retrieved from http://www.drsharma.ca/wp-content/uploads/sharma-obesity-guthormones1.jpg

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COPY

Protocol M07069

Certification of Animal Protocol Approval

Applicant: Dr. R. Reimer

Faculty/Department: Kinesiology

Project Title: Dietary modulation of integrated gut hormone response to manage obesity

Sponsoring Agency(s): Canadian Institutes of Health Research

Effective: October 31, 2008 Expires: October 31, 2009 (renewal date)

The Animal Care Committee,
having examined the animal care and treatment protocol,
approves the experimental procedures proposed and certifies
with the applicant that the care and treatment of animals
used will be in accordance with the principles
outlined in the most recent policies and
"Guide to the Care and Use of Experimental Animals"
By The Canadian Council on Animal Care.

R. Reimer — Applicant — Oct. 21/08 — Date

Chair of Animal Care Committee or University Veterinarian — Oct 28/08 — Date

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