Dairy and Exercise
as a Novel Treatment Strategy for Obesity

by

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Abstract

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as a Novel Treatment Strategy for Obesity

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University of Guelph, 2017

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The purpose of this investigation was to compare the individual and combined effects of dairy and endurance exercise training in reducing weight gain and adiposity in a rodent model of diet-induced obesity. A 6-week feeding intervention of a high fat, high sugar diet was used to rapidly induce obesity in male Sprague Dawley rats (6-weeks of age). Rats were then assigned to one of four weight-matched groups for 6 weeks: 1) casein-sedentary 2) casein-exercise 3) dairy-sedentary 4) dairy-exercise. The exercise training intervention took place 5 days/week (60 minutes of treadmill running: 20m/min, 10% incline). The effects of exercise training in combination with dairy protein were greater than either intervention alone in attenuating increases in weight gain. Adipose tissue and liver mass were reduced to similar extents with casein-exercise, dairy-sedentary and dairy-exercise. Differences in weight gain were not explained by food intake, total energy expenditure nor substrate oxidation. The total amount of feces excreted was higher in the dairy-sedentary group, and this was associated with an increase in the total amount of lipid excreted compared to the casein-sedentary group. This study provides novel evidence that dairy protein attenuated weight gain in obese rats to a similar extent as exercise training, and appeared to exert a partial additive effect when combined with exercise. While exercise training reduces weight gain through increases in energy expenditure with each bout of exercise, dairy would appear to increase the amount of lipid excreted in the feces.
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CHAPTER 1: Literature Review

1.1 Introduction

1.2 The Pathophysiology of Obesity

1.2.1 A Survival Perspective

1.2.2 Adipose Tissue

1.2.2.1 White Adipose Tissue

1.2.2.2 Brown Adipose Tissue

1.2.3 Peripheral Metabolic Tissues Implicated in Obesity-Induced Insulin Resistance

1.2.3.1 Skeletal Muscle

1.2.3.2 Liver

1.2.4 Summary of the Metabolic Complications in Obesity
1.3 Approaches to Prevent and Treat Obesity

1.3.1 Exercise and Adiposity

1.3.2 Skeletal Muscle Metabolic Adaptations with Acute Exercise

1.3.2.1 Skeletal Muscle Glucose Uptake During Exercise

1.3.2.2 Post-Exercise Skeletal Muscle Insulin Sensitivity

1.3.3 Dietary Intervention for Weight Control

1.3.4 Combination Treatment: Dairy and Exercise

1.4 Conclusion

CHAPTER 2: Thesis Rationale, Objectives and Hypotheses

CHAPTER 3: Dairy Attenuates Weight Gain to a Similar Extent as Exercise in Rats Fed a High Fat, high Sugar Diet

3.1 Abstract

3.2 Introduction

3.3 Methods

3.3.1 Animals, Housing and Experimental Diets

3.3.2 Exercise Training

3.3.3 Metabolic Caging

3.3.4 Terminal Tissue Collection

3.3.5 Histological Analysis

3.3.6 Serum Insulin, Glycerol and NEFA

3.3.7 Real-time Quantitative PCR

3.3.8 Lipid Analysis

3.3.9 Statistical Analysis

3.4 Results

3.4.1 Dairy and exercise reduce weight gain in HFHS rats

3.4.2 Dairy and exercise alter adipose tissue mass and morphology in HFHS rats

3.4.3 Dairy and exercise do not alter indices of white adipose tissue inflammation
3.4.4 Exercise training alone, and in combination with dairy, reduces fasting blood glucose in HFHS rats…………………………………………………………35
3.4.5 Dairy and/or exercise training do not increase total energy expenditure in HFHS rats……………………………………………………………………35
3.4.6 Dairy increases lipid excretion in HFHS rats……………………………37

3.5

Discussion………………………………………………………………………………………………40

CHAPTER 4: Integrative Discussion……………………………………………………………………47

References………………………………………………………………………………………………50
List of Figures

CHAPTER 1:

Figure 1.1: Energy balance and weight management ........................................... 4
Figure 1.2: Metabolically healthy versus obese white adipose tissue ................. 7
Figure 1.3: Overview of the mechanisms involved in adipocyte lipid metabolism ... 18

CHAPTER 3:

Figure 3.1: Dairy and exercise attenuate weight gain in HFHS rats .................. 32
Figure 3.2: Dairy and exercise treatment reduce adipose tissue mass and adipocyte size ...... 33
Figure 3.3: Dairy and/or exercise do not reduce indices of adipose tissue inflammation ...... 34
Figure 3.4: Dairy and/or exercise do not increase total energy expenditure .......... 36
Figure 3.5: Dairy protein increases fecal fat excretion ....................................... 38

Supplemental

Figure 3.6: Dairy and/or exercise reduce lipid droplets in liver ....................... 39
List of Tables

CHAPTER 3:

Table 3.1: Experimental diet composition.................................................................45
Table 3.2: Animal characteristics.................................................................................46
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPK</td>
<td>5’AMP activated protein kinase</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<td>BAT</td>
<td>Brown adipose tissue</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>DIO</td>
<td>Diet-induced obesity</td>
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<tr>
<td>eWAT</td>
<td>Epididymal white adipose tissue</td>
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<tr>
<td>FAS</td>
<td>Fatty acid synthase</td>
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<tr>
<td>FFA</td>
<td>Free fatty acid</td>
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<tr>
<td>GLUT1</td>
<td>Glucose transporter protein 1</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Glucose transporter protein 4</td>
</tr>
<tr>
<td>HFHS</td>
<td>High-fat, high-sugar</td>
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<td>HGO</td>
<td>Hepatic glucose output</td>
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<tr>
<td>IL-1β</td>
<td>Interleukin-1 beta</td>
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<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
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<tr>
<td>IRS-1</td>
<td>Insulin receptor susbrate-1</td>
</tr>
<tr>
<td>iWAT</td>
<td>Inguinal white adipose tissue</td>
</tr>
<tr>
<td>JNK</td>
<td>c-Jun amino-terminal kinase</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
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<td>---------</td>
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<tr>
<td>LFD</td>
<td>Low fat diet</td>
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<tr>
<td>MCP-1</td>
<td>Monocyte chemoattractant protein-1</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease</td>
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<tr>
<td>NEFA</td>
<td>Non-esterifed fatty acids</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein Kinase C</td>
</tr>
<tr>
<td>P13K</td>
<td>Phosphatidylinositol 3-kinase</td>
</tr>
<tr>
<td>RER</td>
<td>Respiratory exchange ratio</td>
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<tr>
<td>QUICKI</td>
<td>Quantitative insulin sensitivity check index</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>SMP</td>
<td>Skim milk powder</td>
</tr>
<tr>
<td>SVF</td>
<td>Stromal vascular fraction</td>
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<tr>
<td>TEE</td>
<td>Total energy expenditure</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
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<td>Tumor necrosis factor-α</td>
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<td>Type 2 diabetes</td>
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<td>Uncoupling protein-1</td>
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<tr>
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<td>Uncoupling protein-2</td>
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<td>Carbon dioxide production</td>
</tr>
<tr>
<td>VO₂</td>
<td>Oxygen consumption</td>
</tr>
<tr>
<td>WAT</td>
<td>White adipose tissue</td>
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Chapter 1: Literature Review

1.1 Introduction

In Canada, the prevalence of obesity amongst adults and children is a growing concern and is placing an ever-increasing burden on the health care system (104). In the past three decades, rates of adult obesity have tripled from 6.1% to 18.3% nationwide (104), and in 2014, it was reported that approximately 5.3 million Canadians over the age of 18 are obese (96). Given this obesity pandemic, approaches to prevent and treat obesity and its associated conditions are required. It is indisputable that positive energy balance is a predominant factor underlying the development of obesity (82), and thus interventions aimed at reducing energy intake or increasing energy expenditure are often front line approaches used to control weight gain. Exercise is a well-known approach for attenuating weight gain (58, 69), however it can be challenging for individuals to comply with exercise regimes (58). As such, it is important to invoke additional strategies that can be used to either mimic or augment the protective effects of exercise in the treatment and/or prevention of obesity.

Nutritional strategies for weight loss are of increasing importance and often represent an alternative approach to preventing obesity. Indeed, one approach that could be used to attenuate weight gain is manipulating dietary protein intake. For instance, individuals consuming diets high in protein had a greater preservation of lean body mass during energy restriction than those eating normal amounts of protein (99). Of note, increasing the consumption of dairy, a food that is rich in protein, has shown promising effects in preventing the development of obesity. In a recent meta-analysis Lu et al. (62) reported that children consuming the most dairy were 38% less likely to develop obesity than those consuming the lowest amounts of dairy. These findings are consistent
with other work demonstrating a modest protective effect of dairy consumption against the development of obesity in adolescents (24). Accumulating evidence, especially in rodent models, has shown a beneficial effect of dairy products in modulating adiposity. For example, dairy protein supplementation in the form of skim milk powder, which includes casein, whey, and calcium, a micronutrient linked to weight loss (25), has been reported to attenuate weight gain in obese rats fed a high-fat, high sucrose (HFHS) diet (26) and in mice fed a high fat diet (101). While not a universal finding (16), the inclusion of dairy into the diet has been reported to improve indices of glucose homeostasis in overweight but otherwise healthy subjects (81) and to reduce trunk fat and markers of inflammation in subjects with obesity and the metabolic syndrome (91).

Given these important findings, there is growing interest in understanding the effects of dairy’s anti-obesogenic effects in preventing weight gain. However, to date, very few studies have looked at the combined benefits of exercise with dairy intake in the treatment of pre-existing obesity, and precisely, the potential synergistic effects in altering fat mass and body weight.

This literature review will provide an overview of the pathophysiological mechanisms involved in the development of obesity and insulin resistance, and will describe the mechanisms through which exercise training and dairy can induce metabolic adaptations, although through distinct pathways, in support of treating obesity.
1.2 The Pathophysiology of Obesity

1.2.1 A Survival Perspective

To fully understand the major contributing factors of obesity in today’s society, it is important to first explore this from an evolutionary and survival standpoint. The idea that adipose tissue has the ability to expand and accommodate for additional storage of calories in periods of excessive food intake proved to be a benefit during the hunter-gatherer epoch (90). For instance, extra calories, which were stored as fat, provided the hunter-gatherers with an energy reserve for which they could rely on in periods of under-nutrition and/or famine, and thus enabled them to survive in these nutrient deprived times (90). In addition, the hunter-gatherers also had to rely heavily on their locomotive abilities for food procurement, and to evade predators in order to ensure their own survival (90). However, this is not the case anymore in today’s Western society. The readily accessible and overabundance of calorie dense foods and inactive lifestyle have become health risks for many individuals (43, 107). Over time, chronic over-consumption of nutrient-dense and high fat foods, with little to no energy output, contributes to weight gain, and eventually obesity (43, 107). In a state of prolonged positive energy balance, the excess intake of energy is stored as fat, where this disrupts the normal function of adipose tissue, while also leading to metabolic perturbations in skeletal muscle and hepatic glucose homeostasis (66).
Energy intake and energy expenditure are the two main components of energy balance. When energy intake exceeds energy expenditure, adipocytes enlarge for the excess storage of energy and results in weight gain. When energy intake is less than energy expenditure, the body mobilizes its energy reserves to meet the energy deficit and results in weight loss.

1.2.2 Adipose Tissue

There exist two functionally different types of adipose tissue in the body: 1) white adipose tissue (WAT) and 2) brown adipose tissue (BAT). The following sections will discuss the physiological functions of these two distinct fat tissues.
### 1.2.2.1 White Adipose Tissue

WAT is no longer recognized as an inert organ. Rather, WAT plays a central role in whole-body energy homeostasis and serves as the primary energy storage depot (12, 15, 50, 54). Predominantly composed of adipocytes, as well as fibroblasts, endothelial cells and immune cells that make up the stromal vascular fraction (SVF), WAT is known to be a highly active heterogeneous endocrine and metabolic organ that secretes a plethora of adipokines and cytokines to regulate energy intake and coordinate metabolic activity in peripheral tissues (50, 93). Specifically, WAT is comprised of two major depots: 1) the subcutaneous depot and 2) the intra-abdominal visceral depot, the latter having a stronger association to the metabolic perturbations of obesity (5, 54, 57, 92).

WAT is a plastic tissue that is continually remodeling itself in response to the systemic environment (54). In principle, fat mass can expand by increasing the number of adipocytes (hyperplasia) and/or fat cell volume (hypertrophy) (5). During the progression of obesity, it has been observed that adipose depots will first expand by hypertrophic growth (up to a maximal size of ~0.7-0.8 ug/cell) in order to meet the need for additional fat storage capacity, upon which signals are then turned on to induce the proliferation and differentiation of pre-adipocytes (53, 83).

#### 1.2.2.1.1 White Adipose Tissue Inflammation in Obesity

A healthy expansion of WAT is associated with angiogenesis and sufficient vascularization in order to support the tissue’s growth (54, 73). In this condition, WAT is characterized by an anti-inflammatory state, with a higher ratio of M2-polarized macrophages to M1-polarized macrophages (37, 65). The M2 macrophage phenotype is characterized by higher levels of anti-inflammatory cytokines that mediate angiogenesis, and promote tissue repair (37, 65). In contrast,
M1 macrophages are implicated in the production of pro-inflammatory cytokines which can induce insulin resistance and inflammation in adipocytes (37, 65).

Due to this diverse and dynamic population of resident macrophages, WAT is a major tissue involved in systemic obesity-associated inflammation (14). During the development of obesity, i.e., when WAT expands, there is an increase in the number of macrophages that infiltrate adipose tissue, and a phenotypic switch towards a pro-inflammatory profile occurs (39, 73). For example, hypertrophic obesity development increases the diffusion distance of oxygen within the tissue’s surface, and consequently reduces blood flow and oxygen delivery to WAT (5). This hypoxic condition induces a heightened influx of inflammatory mediators and chemo-attractant molecules into the tissue, where adipocytes become necrotic, and stimulate the activation of stress-sensing pathways that consequently intensify the inflammatory state within WAT (73). Adipose tissue inflammation is characterized by increases in the expression and secretion of cytokines such as tumor necrosis factor (TNF-alpha), interleukin 6 (IL-6), monocyte chemoattractant protein (MCP-1) and interleukin 1-beta (IL-1 beta) (73) that stimulate lipolysis and impair adipocyte insulin signaling. The development of inflammation in adipose tissue not only impacts adipose tissue metabolism, but is also a major contributor to systemic inflammation in obesity.
Figure 1.2 Metabolically healthy versus obese white adipose tissue. In a healthy, lean animal, WAT is in an anti-inflammatory state with a predominance of M2-macrophages. In this condition, there is normal vasculature and WAT displays high insulin sensitivity. During weight gain, adipocytes enlarge and there is an increased infiltration of pro-inflammatory M1-macrophages. These events impair WAT function, by promoting a pro-inflammatory tissue milieu.

1.2.2.2 Brown Adipose Tissue

In contrast to white adipocytes, brown fat cells are characterized by multilocular lipid droplets, high mitochondrial content and a robust expression of uncoupling protein 1 (UCP 1) (98). This protein plays an important role in thermogenesis by uncoupling mitochondrial respiration from ATP synthesis to produce heat (28).

Originally, brown adipose tissue (BAT) was thought to be physiologically irrelevant in adult humans, but with the usage of positron emission tomography (PET) and computed tomography (CT) technologies, functional BAT has been identified in humans (28). Yet, the
detection of BAT is minimal and only found in a small percentage of humans. In one study by Cypess et al (18), the prevalence of detectable BAT was only seen in 7.5% of women and 3.1% of men. While not without controversy, it has been suggested that increasing thermogenesis, via BAT activation, will increase energy expenditure and lead to a negative energy balance (76). This in turn would have important metabolic implications towards decreasing blood glucose levels and fat mass, and limiting weight gain (76). Indeed, there is evidence to indicate that BAT transplantation into the visceral cavity of rodents improves glucose tolerance and insulin sensitivity as well as decreases fat mass (94). These metabolic improvements were also found to be present in humans. For example, two hours of daily cold exposure (17°C) for 6 weeks led to increases in BAT activity and cold induced increases of energy expenditure, with a concomitant decrease in body fat mass (108). However, targeting BAT thermogenesis in the treatment of obesity is still at a preliminary stage. More studies are needed to understand the specific mechanisms that lead to increases in energy expenditure, independent of changes in physical activity, and its exact role in energy balance as a way to control body mass.

1.2.3 Peripheral Metabolic Tissues Implicated in Obesity-Induced Insulin Resistance

It has become clear that WAT plays a key role in glucose homeostasis, and this can subsequently affect insulin sensitivity in peripheral tissues (66). Specifically, as seen in an insulin resistant state, hypertrophied adipocytes become resilient to further fat accretion, an insulin dependent-process (73). As lipid storage is driven to excess in WAT, this increases ectopic lipid deposition in peripheral tissues, i.e., fat accumulation in non-adipose tissues (66, 73). This is
harmful as ectopic lipid storage, as discussed below, is linked to impairments in insulin action in both skeletal muscle and liver.

**1.2.3.1 Skeletal Muscle**

Skeletal muscle is one of the primary fuel consuming organs, and the principal site of insulin-mediated glucose uptake and glycogen synthesis in the postprandial state (19) and is regulated by glucose transporters. Specifically, glucose transporter type 1 (GLUT 1) allows for the diffusion of glucose across the plasma membrane in the basal state, while glucose transporter type 4 (GLUT 4) is the primary glucose transporter isoform that facilitates the insulin and/or contraction induced facilitated diffusion of glucose into myocytes (19, 64, 80). Glucose transport into skeletal muscle can be stimulated through either insulin-dependent or independent mechanisms, such as contractions/exercise (48) (reviewed in a subsequent section). The pathways through which these two stimuli trigger glucose transport into muscle are distinct (48). In support of this concept, the effects of maximally effective insulin and muscle contractions on muscle glucose uptake are additive (44). In obesity, the insulin stimulated glucose uptake of skeletal muscle is reduced. The following section will discuss skeletal muscle insulin stimulated glucose uptake in the healthy and obese states.

**1.2.3.1.1. Insulin Stimulated Glucose Uptake**

In the non-diabetic state, plasma glucose values are maintained between 4 and 7 mM, via an intricate coordination of intestinal absorption, hepatic glucose production and uptake into the peripheral tissues, namely adipose tissue, skeletal muscle, and liver (31, 77, 84). Insulin, a hormone recognized as one of the principle regulators of blood glucose, promotes the storage of glucose in
skeletal muscle, adipose tissue, and liver, while inhibiting hepatic glucose output (HGO) (22, 31, 84). Following the ingestion of a meal, the rise in blood glucose signals the pancreas to produce and release insulin which stimulates glucose uptake into insulin-responsive tissues. Given that skeletal muscle accounts for ~ 80% of insulin-stimulated glucose uptake (70), skeletal muscle’s responsiveness to insulin is therefore highly important in the regulation of whole-body insulin sensitivity.

Briefly, upon insulin binding to the insulin receptor on the surface of the muscle cell, a phosphorylation cascade is activated, which includes the tyrosine phosphorylation and activation of insulin receptor substrate 1 (IRS-1) (19). The phosphorylation of IRS-1 results in the activation of phosphatidylinositol-3-kinase (PI3-K), which in turn activates protein kinase B (also known as Akt) to mediate the translocation of GLUT4 to the plasma membrane, and the subsequent uptake of glucose into the cell (19). However, increases in intramuscular lipid content, as seen in obesity, has been shown to reduce skeletal muscle and whole-body insulin sensitivity (1, 48). Specifically, the accumulation of intramuscular lipid impairs the insulin induced activation of IRS-1 and PI3-Kinase leading to impairments in GLUT4 translocation (1, 35, 48). Lipid-induced reductions in muscle insulin action are thought to be mediated by serine/threonine kinases such as Protein Kinase C (PKC) and/or c-Jun amino-terminal kinase (JNK) (84).

1.2.3.2 Liver

The liver also regulates glucose homeostasis through balancing glucose storage (glycogenesis) with glucose production. HGO occurs via the breakdown of glycogen (glycogenolysis) and the conversion of non-carbohydrate substrates (gluconeogenesis) to glucose (66, 68). The liver responds directly to insulin by suppressing HGO (33), however, in the obese
and insulin-resistant state, ectopic lipid deposition blunts the inhibitory action of insulin on HGO (52), consequently resulting in elevated hepatic glucose production, a contributor to hyperglycemia. As in skeletal muscle, this is likely mediated through the activation of serine/threonine kinases such as PKC and JNK (84).

1.2.3.2.1 Lipolysis and Gluconeogenesis

Insulin’s ability to regulate HGO is also partly linked to its inhibitory role on adipose tissue lipolysis (33). Briefly, insulin induces a decrease in the release of non-esterified fatty acids (NEFA) and glycerol from adipose tissue, thus reducing the delivery of gluconeogenic precursors to the liver (33). In obesity and insulin resistance, the anti-lipolytic effects of insulin are attenuated resulting in an increased supply of gluconeogenic precursors to the liver, which could be a contributing factor to increases in HGO and hyperglycemia (33).

1.2.4 Summary of the Metabolic Complications in Obesity

In summary, the pathophysiology of the metabolic alterations that are associated with obesity, such as dyslipidemia, hepatic fat accumulation, and hyperglycemia, are part of a multi-organ disease network. A common underlying feature of these metabolic complications is the resistance to insulin action in 1) WAT (i.e., impaired insulin-mediated suppression of lipolysis); 2) the liver (i.e., impaired insulin-mediated suppression of glucose production); and 3) in skeletal muscle (i.e., impaired insulin-mediated glucose uptake into muscle). The following sections will discuss the metabolic health promoting effects of both exercise and dairy as treatment modalities for obesity and its associated co-morbidities.

1.3 Approaches to Prevent and Treat Obesity
1.3.1 Exercise and Adiposity

The metabolic health promoting effects of exercise in both the prevention and treatment of obesity are well established, and constitutes an important strategy in targeting weight loss (73). Repeated bouts of exercise training leads to beneficial adaptations in whole body health, including improvements in glucose tolerance and insulin sensitivity, and reducing hyperlipidemia (92). These beneficial adaptations have important implications for individuals seeking ways to improve their metabolic profile and prevent adiposity. In addition, regular exercise is also an important component of energy balance (103). As such, exercise increases energy expenditure, and as long as there are no compensatory changes in energy intake, this generates an energy deficit (103). The accumulated net product, over a long-term period from an exercise-induced energy deficit, leads to reductions in adipose tissue mass (103). Exercise induces specific morphological adaptations in WAT, in regulating fat mass (8, 92, 93 103). It is believed that these exercise-induced adaptations in adipose tissue phenotype during weight loss are partially explained by a decrease in adipocyte size and lipid content (34). Indeed, this was demonstrated in two separate studies by Després et al. (20, 21) using a 20-week endurance training program. A significant reduction in the diameter of adipocytes and in fat cell weight, subsequent to a loss in body mass, was reported at the end of the training period (20, 21).

The decrease in adipose tissue mass is also associated with a reduction in the production and release of inflammatory adipokines in WAT. WAT is a target tissue for the anti-inflammatory effects of exercise, specifically in obese individuals (73). For example, 6-weeks of exercise training reduced the expression of TNF-alpha and MCP-1 in diet-induced obese (DIO) mice, when compared to their lean counterparts (7). A similar observation was reported in obese humans,
where 15-weeks of daily exercise reduced markers of inflammation in adipose tissue, such as the expression of IL-6 and TNF-alpha (8). Taken together, this highlights the importance of exercise as a therapeutic tool in preventing adiposity and adipose-tissue inflammation.

1.3.2 Skeletal Muscle Metabolic Adaptations with Acute Exercise

Exercise (muscle contraction) improves the metabolic regulation of glucose via two separate pathways: exercise/contractions and insulin (40). The following sections will discuss how exercise increases muscle glucose uptake during exercise (insulin independent) and enhances muscle insulin sensitivity post exercise (insulin dependent).

1.3.2.1 Skeletal Muscle Glucose Uptake During Exercise

Exercise requires an increase in glucose uptake by skeletal muscle in order to provide substrate for energy production (36). This insulin independent process is facilitated by an acute increase in glucose transport activity (36, 64). Briefly, during muscle contractions, GLUT 4 translocates to the cell surface from intracellular storage depots, in order to mediate glucose delivery to the exercising muscle (6, 64, 80). However, the glucose transport process reverses rapidly upon cessation of exercise (30).

As mentioned, the mechanism mediating this effect is distinct from the pathway used by insulin. In support of this, wortmannin, an inhibitor of PI3-kinase, does not attenuate contraction stimulated glucose uptake (56). Although the precise mechanisms involved in regulating contraction stimulated glucose uptake have not been clearly defined, there is evidence to suggest that the energy sensing enzyme 5’-AMP-activated protein kinase (AMPK) is involved. AMPK is
activated by increases in the AMP to ATP ratio and various knockout mouse models have reported reductions in contraction/exercise stimulated glucose uptake in mice lacking AMPK (70).

1.3.2.2 Post-Exercise Skeletal Muscle Insulin Sensitivity

Following exercise, glucose transporter levels at the membrane return to resting levels. As the residual effects of the bout of exercise on insulin independent glucose uptake subside, there is a marked increase in muscle insulin sensitivity that can persist upwards to 48 hours (106). The sensitivity of the response to insulin has been previously defined as the concentration of insulin to induce 50% of its maximal effect (49), whereas states of decreased sensitivity require a higher concentration of insulin to produce the same effect. While the mechanisms that govern exercise-induced increases in insulin sensitivity remain unclear there is evidence to suggest that similar to the regulation of contraction stimulated glucose uptake, that AMPK is involved in this process (51).

1.3.3 Dietary Intervention for Weight Control

The therapeutic role of exercise in the prevention and treatment of obesity is well documented. Weight loss through exercise confers important metabolic benefits in obese individuals (58, 69). Yet, despite these numerous benefits that are associated with exercise, many individuals are unwilling, or unable to comply to exercise training and to commit to this long-term lifestyle intervention as a mean to achieve these health effects (58). The increased prevalence of obesity, and it’s associated deleterious metabolic consequences, underscores the imperative need to identify additional treatment approaches that can regulate body mass and improve the metabolic state of individuals. As such, individuals will often incorporate a specific dietary regimen to further
their weight loss goals and improve their metabolic profile. In particular, dietary protein is known to be an effective nutritional strategy for weight loss, presumably because it increases satiety to a greater degree than an iso-energetic intake of fat or carbohydrate (71). A particular emphasis has been placed on protein-rich dairy products.

1.3.3.2 Dairy

Dairy, and particularly milk products are a source of high-quality protein which contain all nine essential amino acids (4, 11). The two main protein fractions found in milk are casein (80%) and whey (20%), and are found in dairy and while they both contribute to suppressing short-term food intake and increasing satiety (4, 9), these two proteins differ mainly in the way they are digested and absorbed. Casein has a slower rate of digestion with amino acids being slowly released into the bloodstream, whereas whey is digested more rapidly, thus resulting in a greater increase in plasma amino acids (4). Furthermore, dairy is also a rich source of calcium, an essential micronutrient that must be acquired from food sources, or from a supplement (23).

Currently, the Canadian Food Guide recommends 3 to 4 servings of milk per day for adolescents aged 14 to 18 to allow for healthy growth and development, and 2 to 3 servings for adults. While dairy products are an easily accessible food source, it has recently been reported that only 1 in 3 Canadian adults are meeting the minimum recommended daily servings of milk per day (95). However, in the past decade, dairy consumption has garnered notice for its suggested anti-obesity effect in facilitating weight loss and improving weight management (26, 42, 100). The very first empirical evidence demonstrating an inverse association between a calcium-rich dairy-based diet and body mass index (BMI) was reported in the 1980s in a study of hypertensive humans (67). This study showed a significant correlation between calcium intake and body mass index.
(BMI), where a higher intake of calcium from dairy products was negatively correlated with BMI (67). Since then, the ability of calcium and dairy products to regulate energy metabolism and body weight, specific to changes in fat mass, has gained considerable attention. Yet, evidence of calcium enriched dairy products in regulating adiposity is equivocal in both rodent and human studies (55, 61). Some studies show a reduction in both weight and fat mass during an energy restriction period, and an attenuation in weight gain during an ad libitum feeding period, in both humans and rodents (25, 26, 47, 102), whereas others have reported no association or even an inverse correlation between dairy intake and weight gain (41, 55, 75). A plausible explanation for these inconsistent findings could be attributed to an overall increase in total energy intake with the added dairy.

Regardless of this inconclusive evidence, it would appear that calcium in dairy sources yields a greater effect in further augmenting weight loss and fat mass changes than equal amounts of calcium in the form of supplements. In particular, one study compared calcium intake in dairy and non-dairy sources of calcium (supplemental calcium) in weight management in adults with obesity, where it was shown that the dietary calcium exerted a greater reduction in fat mass and weight than the supplemental calcium (111). Given these findings, an additional bioactive compound of dairy is likely acting in concert with calcium to stimulate these effects.

Although to date, the molecular and physiological pathways that underlie these metabolic effects of dairy remain elusive, different mechanisms have been proposed to explain how calcium, and specifically calcium consumed with protein from dairy products, influence energy partitioning and weight management. A first line of suggestion, introduced by Zemel et al (109) highlighted a calcium-controlled pathway in adipose tissue. Specifically, they suggested that dietary calcium and dairy foods regulate adipocyte lipid metabolism and energy partitioning between adipose tissue and skeletal muscle (109, 110, 111). In fact, calcitriol, the active form of vitamin D (1,25-
dihydroxyvitamin D) is recognized as a potent mediator of adipocyte lipid metabolism. In response to a low-calcium diet, calcitriol stimulates a rapid influx of intracellular calcium into adipocytes, which in turn promotes lipogenesis and inhibits lipolysis via an increase in fatty acid synthase (FAS) activity (109, 110, 111). On the contrary, these effects can be reversed with a high-calcium diet. Specifically, it appears that calcium decreases 1.25-dihydroxyvitamin D levels. This induces a shift from energy storage to energy expenditure, in the partitioning of dietary energy, as lower levels of 1.25-dihydroxyvitamin D levels promotes a decrease of adipocyte intracellular calcium, thus stimulating adipocyte lipolysis and inhibiting lipogenesis (109). Interestingly, dietary calcium has also shown to increase the up-regulation of WAT mitochondrial uncoupling protein 2 expression (UCP 2) in an obese mouse model, a protein that is implicated in thermogenesis (86). Although the mice on the high-calcium diet had an increase in core temperature in comparison to their low-calcium fed mice counterparts, Shi et al (86) note that the increased expression of WAT UCP 2 could merely be a physiological consequence of lipolysis.
Figure 1.3 Overview of the mechanisms involved in adipocyte lipid metabolism. A low calcium diet leads to increases in adipocyte intracellular Ca$^{2+}$ levels via the actions of 1,25 dihydroxyvitamin D, which increases FAS activity and lipogenesis.

Another possible mechanism for the decrease in weight associated with the intake of dairy products and calcium is an increase in fecal fat excretion. As reported in a short-term study by Jacobsen et al (45), there was a difference of 8.2 g/day in total fat excreted between the high calcium (1800 mg) and low calcium (500mg) diet, in overweight, but otherwise healthy individuals. Both diets were iso-caloric and had equivalent amounts of dairy protein. In agreement with these results, another study was able to show a significant 2-fold increase in fecal fat excretion in Wistar rats consuming a high-calcium diet containing dairy protein (non-fat skim milk powder) to the control casein diet. In this study, the amount of sucrose added to the high-calcium diet was
reduced to provide equivalent macronutrient composition between the high-calcium and control casein diets (72).

The mechanism by which calcium can increase lipid excretion is likely the interaction between calcium and fatty acids, via calcium binding to dietary fat in the intestine to form insoluble calcium fatty acid soaps. This subsequently reduces the digestible energy of the diet and decreases the amount of lipids that are absorbed.

1.3.4 Combination Treatment: Dairy and Exercise

As dairy has been reported to induce similar effects to that of exercise in attenuating the development of obesity (25, 26, 46, 102), incorporating dairy as a nutritional strategy in conjunction with exercise represents an ideal treatment approach to further potentiate the beneficial effects of exercise in reducing/attenuating weight gain and WAT mass. To date, few human trials have examined the therapeutic treatment combination of dairy and exercise in the context of weight loss. Based on findings from Josse et al, (47) increasing the consumption of dairy foods with an exercise and diet-induced weight loss intervention promoted greater losses in total and visceral fat in obese women, than those who consumed lower amounts of dairy while exercising. However, all participants consumed a hypo-energetic diet, and dairy had no additional further effect in body weight loss between both groups. As such, future work is required to validate these findings as well as identify the long-term implications that can be drawn from these conclusions. Ideally, what remains to be examined is the potential ability of dairy to augment the effects of exercise in attenuating further weight gain.
1.4 Conclusion

The incidence of obesity is growing at epidemic proportions. Adipose tissue, skeletal muscle and liver are largely implicated in glucose uptake and disposal, and are therefore paramount in the development of obesity and obesity-induced insulin resistance. Despite convincing evidence that exercise training is still the most effective approach to regulate weight gain and promote improvements in insulin sensitivity and glucose homeostasis, commitment to regular exercise remains a challenge for many individuals.

In an attempt to mitigate obesity and its perpetual health problems, dairy has been identified as an adjunct dietary strategy for weight management, with several studies showing its unique potential in modulating body weight. However, further studies are warranted to clarify more fully what individual bioactive ingredients in dairy products contribute to regulating body weight and changes in adipose mass. Given its accessibility and affordability, dairy represents a feasible approach and effective strategy, to that of exercise, in facilitating weight loss.
Chapter 2: Aims of Thesis

The purposes of this thesis were to:

1. Compare the individual and combined effects of dairy protein and endurance exercise training on reducing weight gain, adipose tissue accretion and indices of glucose homeostasis and inflammation in a rodent model of DIO;

2. Examine potential mechanisms such as substrate oxidation, energy expenditure and lipid excretion, that could be mediating the effects of dairy protein alone and in combination with exercise on weight gain.

It was hypothesized that:

1. The addition of dairy to a HFHS diet would attenuate weight gain and accretion of WAT mass, similar to the effects of exercise training;

2. The combined effects of dairy and exercise would be greater than that of either intervention alone.
Chapter 3: Dairy Attenuates Weight Gain to a Similar Extent as Exercise in Rats Fed a High Fat, High Sugar Diet

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3.1 Abstract

**Objective:** To compare the individual and combined effects of dairy and endurance exercise training in reducing weight gain and adiposity in a rodent model of diet-induced obesity.

**Methods:** A 8-week feeding intervention of a high fat, high sugar diet was used to induce obesity in male Sprague Dawley rats. Rats were then assigned to one of four groups for 6 weeks: 1) casein sedentary (casein-S), 2) casein exercise (casein-E) 3) dairy sedentary (dairy-S), and 4) dairy exercise (dairy-E). Rats were exercised trained by treadmill running 5 days/week.

**Results:** Dairy-E prevented weight gain to a similar extent than either intervention alone. Adipose tissue and liver mass were reduced to a similar extent in dairy-S, casein-E and dairy-E groups. Differences in weight gain were not explained by food intake or total energy expenditure. The total amount of lipid excreted was greater in the dairy-S compared to casein-S and dairy-E groups.

**Conclusions:** This study provides evidence that dairy limits weight gain to a similar extent as exercise training and the combined effects are greater than either intervention alone. While exercise training reduced weight gain through increases in energy expenditure, dairy appears to increase lipid excretion in the feces.
3.2 Introduction

It is indisputable that positive energy balance is a predominant factor underlying the development of obesity (82) and interventions to reduce energy intake or increase energy expenditure are front line approaches used to curb weight gain. While exercise is a well-known approach for attenuating weight gain (69), it can be challenging for individuals to comply with exercise regimes (58). As such, it is important to seek strategies that can be used to either mimic or augment the effects of exercise in the prevention and treatment of obesity.

One approach that could be used is manipulating dietary protein intake. For instance, individuals consuming diets high in protein had a greater preservation of lean body mass during energy restriction than those eating normal amounts of protein (99). Of note, increasing the consumption of dairy, a food rich in protein, has shown promising effects in preventing the development of obesity. In a recent meta-analysis, Lu et al (62) reported that children consuming the most dairy products were 38% less likely to develop obesity than those consuming the lowest amounts of dairy. These findings are consistent with other work demonstrating a protective effect of dairy consumption against the development of obesity in adolescents (24). Accumulating evidence, especially in rodent models, has shown a beneficial effect of dairy products in modulating adiposity. For example, dairy supplementation in the form of skim milk powder, which is composed of the proteins casein and whey, but also lactose and, among other minerals, calcium, a micronutrient linked to weight loss (25), has been reported to attenuate weight gain in obese rats fed a high-fat, high sucrose (HFHS) diet (27) and in mice fed a high fat diet (101). While not a universal finding (16), the inclusion of dairy into the diet has been reported to improve indices of glucose homeostasis in overweight but otherwise healthy subjects (81) and to reduce trunk fat and
markers of inflammation in subjects with obesity and the metabolic syndrome (91). Few studies have examined the combined effects of dairy and exercise in the context of weight loss. Parr et al (74) found that dairy did not augment the effects of exercise and a hypocaloric diet on weight loss in subjects with obesity. However, what remains to be seen is if dairy can augment the effects of exercise in slowing the development of obesity. The primary objective of this investigation was to compared the individual and combined effects of dairy and endurance exercise training on reducing weight gain, adipose tissue accretion and indices of glucose homeostasis and inflammation in a rodent model of diet-induced obesity. We were further interested in examining potential mechanisms such as substrate oxidation, energy expenditure and lipid excretion (13), that could be mediating the effects of dairy alone and in combination with exercise on weight gain. We hypothesized that the addition of dairy, via non-fat skim milk powder, to a HFHS diet would attenuate weight gain and accretion of white adipose tissue (WAT), similar to the effects of exercise training, and that the combined effects would be greater than that of either intervention alone.
3.3 Methods

3.3.1 Animals, Housing and Experimental Diets

All experimental protocols were approved by the University of Guelph Animal Care Committee and were consistent with the Canadian Council on Animal Care guidelines. Four-week old male Sprague Dawley rats were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed 3-4 per cage, in a temperature (22°C) and humidity controlled room with a 12:12h reverse light-dark cycle (lights on at 21:00), and had free access to food and water. After a one-week acclimatization period with standard chow, rats (n= 47) were fed a HFHS diet for 8-weeks to induce obesity (Table 1). A separate group of rats (n = 16) were fed a low-fat control diet (AIN-93M) as a reference for HFHS-induced weight gain. The dietary ingredients were purchased from Dyets Inc. (Bethlehem, PA), diets prepared in-house, and formulated to meet the nutritional requirements of rodents (79). Body weight of all rats was measured twice per week with a digital scale. At ~12 weeks of age, rats were assigned to one of four weight-matched, HFHS groups (4.59 kcal/gram casein, 4.52 kcal/gram dairy) for 6 weeks (Figure 3.1 A): 1) casein-sedentary (casein-S) 2) casein-exercise (casein-E) 3) dairy-sedentary (dairy-S), and 4) dairy-exercise (dairy-E). Non-fat skim milk powder (MP Biomedical) was used as the sole protein source in the dairy diet and casein was the protein source in the control casein diet (Table 3.1). The calcium content of the casein diet was matched to the amount of calcium that is naturally found in skim milk powder (0.67%). As the skim milk powder contains lactose, the amount of sucrose added to the dairy diet was reduced in order to have the same macronutrient composition between the dairy and casein diets. Rats had ad libitum access to their respective diets and water throughout the study. Body
weight and food intake were determined bi-weekly. Food intake was estimated by subtracting the weight of the food remaining in the feed cup from the initial amount in the cup plus any food recovered from the cage that was spilled. The amount consumed was then averaged per day, per number of rats in each cage and averaged over the duration of the intervention.

3.3.2 Exercise Training

Rats underwent two low-intensity acclimation sessions (10 minutes, 10 m/min/ at 5% incline) prior to commencing training. Exercise training took place 5 days/week on a motorized treadmill followed by a 2-day recovery period. We used treadmill running to ensure an equal distance run between all rats. All training occurred during the rats’ dark cycle. The animals trained at 10m/min at 0% incline for 60 minutes for the first week, and the speed and incline were gradually increased to 15 m/min at 0% incline for 60 minutes by the second week. By the third week of training, the speed and incline of the treadmill were increased to 20 m/min at 5% incline for 60 minutes and maintained for the remainder of the exercise intervention.

3.3.3 Metabolic Caging

During the fifth week of the intervention, and ~24 hours following their last bout of exercise, rats were placed in a Comprehensive Lab Animal Monitoring System (CLAMS, Columbus Instruments, Columbus, OH) to assess whole body energy expenditure and substrate oxidation. The first 12 hours in the CLAMS served as an acclimation period, and data collected over the following 24 hours was used to measure oxygen consumption, carbon dioxide production, respiratory exchange ratio (RER, \(\frac{VCO_2}{VO_2}\)), total energy expenditure, and activity levels. The reverse light-dark cycle described above remained the same for the CLAMS, and the rats were provided with their respective experimental diet and water ad libitum throughout the duration of
this experiment. Total energy expenditure (TEE) was calculated using the modified Weir equation as we have described previously (89). Activity levels were determined by summing the total number of beam breaks in both the X and Z planes. All metabolic caging data were averaged separately over the light and dark phases. Food intake was estimated by subtracting the weight of the food remaining in the hopper from the initial amount in the hopper plus any food recovered from the cage that was spilled. Feces were collected in the metabolic caging at the end of the 36-hour measurement period. Due to time spent in the metabolic cages two less training sessions were completed during the 5th week.

3.3.4 Terminal Tissue Collection

In order to avoid any of the residual effects from the last bout of exercise, the animals had a 48-hour recovery period following the last training session. Rats were fasted for 12 hours and anaesthetized with 5% isoflurane. Inguinal, epididymal and brown adipose tissue, and liver were excised, weighed and flash frozen in liquid nitrogen or fixed in formalin. Blood was collected from the left ventricle and centrifuged at 4,000 g for 10 minutes at 4°C. The serum was collected, and all tissues were stored at -80°C until further analysis.

3.3.5 Histological Analysis

Inguinal and epididymal adipose tissue and liver were fixed in 10% neutral-buffered formalin (VWR, Mississauga, ON), dehydrated in 70% ethanol and embedded in paraffin. Five micrometer sections were stained with modified Harris hematoxylin and eosin stock, and imaged under 40X magnification (Olympus FSX 100 light microscope, Olympus, Tokyo, Japan). Four images (150 cells/image) from each animal were analyzed to determine cross-sectional area (ImageJ software, National Institute of Mental Health, Bethesda, MD) (2).
3.3.6 Serum Insulin, Glycerol, and NEFA

Commercially available kits were used to measure serum glycerol (Sigma, Cat # FG0100), nonesterfied fatty acids (NEFA) (Wako Chemicals) and insulin (Millipore, Cat # EZRMI-13K). All samples were run in duplicate and the average coefficient of variation for these assays in our laboratory is <10%. The quantitative insulin sensitivity check index (QUICKI) (inverse log sum of fasting plasma glucose and fasting plasma insulin) was used as an index of whole body insulin sensitivity (10).

3.3.7 Real Time Quantitative PCR

RNA was extracted from inguinal and epididymal adipose tissue using the Qiagen RNeasy kit according to the manufacturer’s instructions (Qiagen) followed by DNase free treatment (Ambion). The quantity and the purity of the RNA were assessed with a NanoDrop system 21 (NanoDrop 2000 Spectro-photometer; Thermo Scientific). Complementary DNA (cDNA) was synthesized from total RNA (1 µg) using SuperScript II Reverse Transcriptase, random primers and dNTP (Invitrogen). Real time PCR was carried out using a CFX Connect Real-Time PCR Detection System (Bio-Rad). Samples were loaded in triplicate on a 96-well plate. Primer sequences as previously published (17, 59), were synthesized by the Genomics Facility at the University of Guelph. Primers spanned an exon-exon junction. The relative differences between the experimental groups were determined using the $2^{-\Delta\Delta CT}$ method (60). The endogenous control gene, beta-actin, did not change with treatment.
3.3.8 **Lipid Analysis**

Frozen feces were homogenized in 2.5 mL double distilled water and the resultant homogenate was transferred into Kimax tubes and total lipids were extracted using chloroform/methanol (2:1) as previously described (29). Extracted lipids were dried down using nitrogen gas and weighed. Liver triglycerides were analyzed as described in detail previously (78).

3.3.9 **Statistical Analysis**

Data that was not normally distributed was log transformed. Differences between groups were analyzed using a one-way ANOVA, a one-way ANOVA on ranks or ANCOVA with body weight as a covariant. A P value of $\leq 0.05$ was considered significant.
3.4 Results

3.4.1 Dairy and exercise reduce weight gain in HFHS rats.

Rats fed a HFHS diet were heavier than LFD control animals demonstrating the effectiveness of the diet to cause weight gain (HFHS 608.2 ± 7.3 g, LF 536.3 ± 11.9 g, p<0.05). The HFHS rats were assigned to one of four treatment groups that did not differ in pre-treatment body weight. At the end of the 6-week treatment period, rats in the casein-S, dairy-S and casein-E groups were heavier than before the intervention (Figure 3.1 B). Conversely, dairy-E rats were not heavier than pre-treatment (Figure 3.1 B). When assessing individual weight gain within each animal, rats in the dairy-S, casein-E and dairy-E groups all gained less weight than the casein-S animals with dairy-E gaining less weight than dairy-S and casein-E (p=0.053) groups (Figure 3.1 C). There were no differences between groups for food intake as measured in the rats’ standard cages (Figures 3.1 D). Liver weight was reduced in all treatment groups, which occurred alongside reductions in liver triglyceride content in casein-E (p=0.054), dairy-S (p<0.05), and dairy-E (p=0.08) (Table 2). Similarly, the number of lipid droplets appeared to be reduced with treatment (Supplemental Figure 3.6). There were no significant differences in brown adipose tissue between groups (Table 3.2).
**Figure 3.1 Dairy and exercise attenuate weight gain in HFHS rats.** Rats were fed a HFHS diet for 8 weeks and then were exercise trained and/or had their diet supplemented with dairy protein for an additional 6 weeks (A) and changes in body weight (B), weight gain (C), and daily food intake (D) measured. Data are presented as means ± SEM for 9-15 samples per group. In (B) * denotes a significant (p<0.05) difference from pre-treatment and in (C) significant difference compared to casein. ‡ Significantly (p<0.05) different compared to dairy.

### 3.4.2 Dairy and exercise alter adipose tissue mass and morphology in HFHS rats.

Having shown marked effects of dairy and exercise on reducing weight gain we next assessed indices of adipose tissue morphology. Inguinal adipose tissue mass (Figure 3.2 A) and adipocyte area (Figure 3.2 B) were reduced in all treatment groups compared to casein-S animals. Similarly, epididymal adipose tissue mass (Figure 3.2 C) was decreased in all treatment groups whereas epididymal adipocyte area was only reduced in the dairy-S and dairy-E groups compared to casein-S control animals (Figure 3.2 D).
Figure 3.2 Dairy and exercise treatment reduced adipose tissue mass and adipocyte size. Rats were fed a HFHS diet for 8 weeks and then were exercised trained and/or had their diet supplemented with dairy protein for an additional 6-weeks and inguinal adipose tissue mass (A), inguinal adipocyte area (B), epididymal adipose tissue mass (C), and epididymal adipocyte area (D) determined. Data are presented as means ± SEM for 9-15 samples per group for Figure 3.2 A and 2C and 5 samples per group for figures 5B and 5D. * denotes significant (p<0.05) difference compared to casein alone. # denotes a significant (p<0.05) difference with casein and exercise. Representative H&E are shown below the quantified data.
3.4.3 **Dairy and exercise do not alter indices of white adipose tissue inflammation.**

Adipose tissue inflammation typically tracks with the degree of adipose tissue accretion (105) and thus we wanted to determine if the blunted weight gain with dairy and/or exercise altered indices of adipose tissue inflammation. There were no differences in the mRNA expression of the inflammatory markers IL-6, TNF-alpha, MCP-1 and IL-1 beta, in either the inguinal or epididymal adipose depots (Figure 3.3).

**Figure 3.3** Dairy and/or exercise did not reduce indices of adipose tissue inflammation. Rats were fed a HFHS diet for 8-weeks and then were exercise trained and/or had their diet supplemented with dairy protein for an additional 6-weeks and the mRNA expression of IL-6, TNF-alpha, MCP-1, and IL-1 beta determined in eWAT (A-D) and iWAT (E-H). Data are presented as means ± SEM for 6-7 samples per group.
3.4.4 Exercise training alone, and in combination with dairy, reduces fasting blood glucose in HFHS rats.

Casein-E and dairy-E rats had lower fasting blood glucose levels in comparison to the casein-S group. (Table 2) There were no differences observed in fasting serum glycerol, NEFA, insulin concentrations or QUICKI between treatment groups (Table 2).

3.4.5 Dairy and/or exercise training do not increase total energy expenditure in HFHS rats.

As dairy and/or exercise training attenuated weight gain in HFHS fed rats in the absence of alterations in food intake we next wanted to determine if this could be explained by alterations in energy expenditure. To this end respiratory exchange ratio (RER), total energy expenditure (TEE) and activity levels were measured over 24-hours during both the light and dark phase. Dairy-E rats had a lower RER than casein-S and dairy-S groups in the light phase, while no differences in RER were observed in the dark phase between groups (Figures 3.4 A and B). Absolute TEE was similar between groups in both the light and dark phases (Figures 7C-F). When analyzing the data by ANCOVA with body mass as a covariant, there were no differences between groups (light phase p=0.458, dark phase p=0.185). Similarly, when TEE was expressed relative to body weight no differences between groups were seen (light phase p=0.317, dark phase p=0.376). Food intake measured over 36-hours in the metabolic cages was not different between groups (data not shown) confirming our measurements made under regular housing conditions.
Figure 3.4 Dairy and/or exercise did not increase total energy expenditure (TEE). Rats were fed a HFHS diet for 8-weeks and then were exercise trained and/or had their diet supplemented with dairy protein for an additional 4-weeks and RER in the light (A) and dark phase (B), TEE in the light (C) and dark phase (D), and total activity in the light (E) and dark phase (F) determined over 24 hours. Data are presented as means ± SEM for 10-15 rats per group. * denotes a significant difference with casein alone. ‡ denotes a significant difference with dairy alone.
3.4.6 *Dairy increases lipid excretion in HFHS rats.*

Dairy-E gained less weight, yet food consumption and energy expenditure were similar between groups and thus we wanted to determine if dairy could be altering the amount of lipid that was excreted in the feces (13). To assess this, feces were collected in the CLAMS caging (12-hour acclimation, 24-hour measurement). The total weight of feces excreted over a 36-hour period was significantly higher in dairy-S rats compared to the casein-E and dairy-E groups (Figure 3.5 A). While there were no differences in the concentration of lipid in the feces amongst groups (Figure 3.5 B), the total amount of lipids excreted in the dairy-S protein group was increased compared to all other groups (p=0.055 compared to casein-E) (Figure 3.5 C).
Figure 3.5 *Dairy protein increases fecal fat excretion.* Rats were fed a HFHS diet for 8-weeks and then were exercise trained and/or had their diet supplemented with dairy protein for an additional 4-weeks and the total amount of feces excreted (A), the lipid content in the feces (B) and the total amount of lipid excreted (C) over a 36-hour time period determined. Data are presented as means ± SEM for 5-10 samples per group. ‡ denotes a significant difference compared with dairy alone.
Supplemental Figure 3.6 Lipid droplets are reduced in livers from dairy and/or exercise trained rats given a HFHS diet. Rats were fed a HFHS diet for 8 weeks and then were exercise trained and/or had their diet supplemented with dairy for an additional 4 weeks. At the end of the intervention, livers were harvested, fixed and stained with H&E. Representative images are shown at 40X magnification. L is used in the casein-S image to identify lipid droplets.
3.5 Discussion

The effects of dairy on the prevention of weight gain have been previously investigated in rodents (26, 27, 100, 101) and humans (46, 74). However, the mechanism(s) through which dairy reduced weight gain was not identified. For example, diet-induced obese rats fed a HFHS diet supplemented with complete dairy protein, the same diet as used in the current study, had a greater attenuation in weight gain when compared to rats that were fed the HFHS diet with casein or whey alone as the protein source (26). Dietary modification is often prescribed in combination with exercise and thus we were interested in comparing the effects of dairy, to that of exercise, both alone and in combination. We demonstrate that 6-weeks of dairy supplementation reduces further weight gain in HFHS fed rats to a similar extent as endurance exercise training and that when given in combination with exercise, weight gain was completely prevented.

Despite the attenuated weight gain in rats receiving dairy this was not associated with reductions in fasting blood glucose concentrations, nor increases in the QUICKI index, a marker of whole body insulin sensitivity (10). These findings are consistent with previous work demonstrating equivalent glucose infusion rates during a euglycemic-hyperinsulinemic clamp in HFHS rats given dairy compared to casein (27). Exercise training, which blunted weight gain to a similar extent as dairy, led to subtle decreases in fasting blood glucose, however this was not associated with reductions in serum insulin or increases in insulin sensitivity calculated using the QUICKI index. While surprising that insulin sensitivity was not improved, similar effects have been reported in obese Zucker rats (88) and in high fat fed rats (97) following training. This could perhaps be related to the timing of assessment following the last bout of exercise, the sensitivity of the technique compared to more robust measures (i.e. hyperinsulinemic-euglycemic clamps), or stress related to treadmill running and concomitant increases in cortisol, a reputed mediator of
insulin resistance (87). Despite an attenuation of further weight gain, exercise training did not significantly reduce indices of adipose tissue inflammation. We speculate that weight loss, and not just an attenuation of further weight gain could be required for exercise to elicit more robust reductions in adipose tissue inflammation.

While dairy and exercise training yield comparable results in regards to modulating weight gain in the context of obesity, the mechanisms regulating these alterations in fat mass and body weight would appear to differ. The attenuation of weight gain with exercise training, in the absence of reductions in food intake is likely explained by increases in energy expenditure that were incurred with each bout of training. In contrast dairy led to a similar attenuation in weight gain as exercise but independent of reductions in food intake or increases in energy expenditure, at least at the time-point measured. Given this, we reasoned that dairy, as suggested previously (13), could be leading to alterations in the amount of fat that was being excreted in the feces. In fact, the reduction in weight gain in the dairy-S group corresponded with increases in total fecal fat loss over a 36-hour period. This is consistent with previous work demonstrating increases in fecal energy loss in mice fed a high fat diet supplemented with calcium and skim milk (102).

It has been proposed that dietary calcium from dairy sources augments the excretion of fecal fat, as calcium and fatty acids have the ability to form insoluble calcium fatty acid soaps in the intestine, which subsequently reduces fat absorption (3). Increasing calcium intake through the consumption of low fat dairy foods increases fecal lipid excretion in moderately overweight subjects (3, 45). Similarly, total fecal fatty acid excretion is increased in rats given a diet supplemented with lactase-treated whole milk powder (38). However, in our study, given that the amount of calcium (0.67%) was equivalent between the casein and dairy diets, we cannot conclude
that calcium is the sole contributing factor to explain this significant protective effect of weight gain with dairy. Thus, it would appear that additional, yet unidentified, bioactive components in milk-derived products might act in concert with calcium to increase fecal fat loss.

One of the most intriguing findings of this study is that the prevention of further weight gain observed in the dairy-E was not paralleled by an increase in fecal lipid excretion, differences in food intake, or alterations in total energy expenditure. Although speculative, this data would perhaps suggest that the energetic cost of exercise was increased, or that energy expenditure following exercise was enhanced in rats given dairy. While we cannot measure energy expenditure during exercise with our metabolic caging, earlier work from Melanson et al. (63), would argue against this supposition. These authors found that energy expenditure following exercise was similar in individuals given high compared to low dairy diets for 1 week prior to exercise (63).

The differences in weight gain between the dairy-E compared to dairy-S and casein-E groups was ~30 grams. While food intake was not different between groups, measured in both home and metabolic caging, there are caveats to both approaches that should be mentioned. First, rats were group housed in their home caging. Thus we cannot determine the amount of food consumed per rat, rather only the amount consumed per cage. Second, although we measured food intake in individually housed rats in metabolic caging, this form of housing can be a stress and thus might not be representative of the home cage condition (32). With these points in mind we cannot exclude the possibility that subtle differences in food intake (~5% differences in daily intake ~1 g/day) over the course of the study could be explaining some of the prevention of weight gain with our experimental treatments.

In conclusion, we have provided novel data demonstrating the role of dairy, adjunct to an
endurance exercise training program, in preventing further weight gain during a high energy, *ad libitum* feeding period. Notably, dairy was as effective as exercise training at preventing weight gain and appeared to exert a partial additive effect when combined with exercise. As dairy increased lipid excretion it is possible that this could have been associated with changes in the gut microbiota, an idea that deserves further attention. Our results provide evidence that dairy is an effective alternative strategy that could be an affordable and accessible approach, especially in combination with exercise in managing obesity.
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Disclosure

The authors declared no conflict of interest.

Author Contributions

Wright DC, Shearer J, and Reimer RA conceived and designed the experiments. Trottier SK, MacPherson REK, Knuth CM, Peppler WT, Mikhaeil JS, Leveille CF, LeBlanc PJ performed the animal training, experiments, and harvested tissues. Trottier SK, Knuth CM, Townsend LK and Peppler WT collected and assembled the biochemical data. Trottier SK and Wright DC analyzed and interpreted the data. Trottier SK and Wright DC drafted the manuscript. All authors revised the article for intellectual content and approved the final version of the manuscript.

Acknowledgements

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### Table 1.1 Experimental diet composition (g/kg)

<table>
<thead>
<tr>
<th></th>
<th>HFHS-casein</th>
<th>HFHS-dairy</th>
<th>LFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>514.2</td>
<td>314.7</td>
<td>100</td>
</tr>
<tr>
<td>Dyestrose</td>
<td>0</td>
<td>0</td>
<td>155</td>
</tr>
<tr>
<td>Lard</td>
<td>100.0</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>100.0</td>
<td>100.0</td>
<td>40</td>
</tr>
<tr>
<td>t-Butylhydroquinone</td>
<td>0</td>
<td>0</td>
<td>0.008</td>
</tr>
<tr>
<td>Casein</td>
<td>140.0</td>
<td>0</td>
<td>140</td>
</tr>
<tr>
<td>Skim milk powder</td>
<td>0</td>
<td>343.0</td>
<td>0</td>
</tr>
<tr>
<td>α-cellulose</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>4.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>42.2</td>
<td>43.0</td>
<td>465.7</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>55.6</td>
<td>53.3</td>
<td>72.1</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>12.4</td>
<td>12.2</td>
<td>12.4</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>20</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>

Skim milk powder contains 51,000 mg/100 gram lactose, 21 mg/100 gram galactose and 53 mg/100 gram glucose. The skim milk and casein contain 35% and 87% protein respectively. A complete description of skim milk powder can be found at: [http://www.mpbio.com/includes/msds/02902887/MP_DS_02902887.pdf](http://www.mpbio.com/includes/msds/02902887/MP_DS_02902887.pdf)
Table 1.2 Animal characteristics in sedentary and exercise trained rats fed a high fat, high sugar diet with casein or skim milk powder (dairy) as the source of protein. Data are presented as means ± SEM for 9-15 rats/group. * P<0.05 compared to sedentary casein animals.

<table>
<thead>
<tr>
<th></th>
<th>Sedentary</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Casein</td>
<td>Dairy</td>
</tr>
<tr>
<td>Serum NEFA (mmol/L)</td>
<td>0.57 ± 0.05</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td>Serum glycerol</td>
<td>0.36 ± 0.05</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>2.88 ± 0.90</td>
<td>4.01 ± 1.01</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.72 ± 0.28</td>
<td>4.46 ± 0.27</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.29 ± 0.01</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>18.3 ± 1.4</td>
<td>14.7 ± 0.8*</td>
</tr>
<tr>
<td>BAT (g)</td>
<td>0.44 ± 0.05</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>Liver TG (mg/g)</td>
<td>23.94 ± 4.42</td>
<td>8.95 ± 1.64*</td>
</tr>
</tbody>
</table>
Chapter 4: Integrative Discussion

The overarching objective of this thesis was to examine the individual and combined effects of a 6-week dairy supplementation and endurance exercise training intervention on reducing weight gain, adipose tissue accretion, and indices of WAT inflammation and glucose homeostasis in a rodent model of DIO.

The main finding of this thesis was that dairy combined with exercise was greater than either dairy or exercise alone in reducing weight gain, and independent of reductions in food intake. Consistent with our hypothesis, iWAT and eWAT mass were reduced with either dairy or exercise alone, and with both interventions combined. However, what was interesting to note was that epididymal adipocyte area was only reduced in the dairy and dairy exercise groups. The next steps were to examine mechanism(s) by which weight gain is attenuated in the dairy-fed rats. Dairy did not increase energy expenditure, but did significantly increase the total amount of lipids excreted.

Although these findings have generated new insight into dairy’s ability to attenuate excessive weight gain and fat mass, to a similar extent to exercise, and without caloric restriction, prospective studies should consider evaluating the long-term impact of these findings. For instance, in our study, we did not see a reduction in indices of adipose tissue inflammation in both iWAT and eWAT with exercise training alone or combined with dairy. This could be due to the fact that the duration of the exercise protocol was not long enough to induce a weight loss effect in our obese model to elicit a robust reduction in WAT inflammation, and that the blunted weight
gain with exercise is not sufficient to uncover exercise’s anti-inflammatory effect. However, one intriguing finding from this study that merits further attention was dairy’s ability to reduce cell size in the epididymal region (a representation of the abdominal depot). As previously discussed in Chapter 1, visceral fat accumulation is positively correlated with the metabolic dysfunctions that are associated with obesity. Given these results, it could be implied that dairy exerts its anti-obesity effect by specifically targeting fat losses in the visceral depot (i.e., both a reduction in size of adipocytes and fat pad mass), which proves to be a beneficial adaption in altering the metabolic characteristics of adipocytes. Although eWAT fat mass was also reduced with exercise there was no change in cell size. This could be explained by a reduction in cell number.

Lastly, although briefly discussed in this thesis, it is worth reiterating in further detail the ~30 grams weight loss differences between the dairy plus exercise groups compared to dairy and exercise alone. As reported, we did not detect any differences in energy intake between groups, in both home and metabolic caging, however, we cannot completely disregard the possibility of subtle differences in energy intake that may account for some of the weight gain prevention we observed in our study. For instance, assuming that a 3500 kcal difference in energy balance equates to an ~455 gram (1 pound) change in weight gain, then an ~230 kcal difference in food intake, equivalent to approximately 50 grams of food (4.6 kcal/gram), over the 6-week treatment could perhaps explain the additional weight loss in the dairy plus exercise group. However, in this present study, we did not have the sensitivity to detect this, as rats were group housed over the course of the intervention.

Overall, this thesis provides new knowledge into dairy’s unique ability in being as effective as exercise in attenuating weight gain, specifically in the context of obesity and without an energy restricted diet. While additional studies are warranted to further clarify what specific individual
bioactive ingredient(s) in dairy products contribute to regulating body weight and changes in WAT mass, we were able to demonstrate dairy’s mechanistic action in reducing weight gain through increases in lipid excretion. Taken together, this work provides convincing evidence of dairy to be part of an efficient nutrition based, cost-effective and easily implementable intervention for the prevention and management of obesity. However, it remains to be seen if these results from our animal model would be applicable to human obesity. The future utility of pursuing this research in human trials would add considerable value to human health in the prevention and treatment of obesity.
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