IMPACT OF AEROBIC EXERCISE TRAINING ON BODY WEIGHT, ENERGY INTAKE, AND APPETITE-REGULATING HORMONES IN RATS.

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Abstract

Post-exercise energy compensation (EC) through increased energy intake (EI) leads to lower than anticipated weight reduction. This study sought to determine the mechanisms, independent of bodyweight (BW), that are involved in EC over an 8-week period of voluntary wheel running. Twelve-week-old male Sprague Dawley rats *(n=30)* fed an AIN-93M diet were randomized into 3 groups: 1) sedentary control (SED); 2) voluntary wheel exercise (EX); and 3) sedentary, weight-matched to aerobic exercise (SED-WM) for 8 weeks. Measures of BW, adiposity, appetite-regulating hormones, gut microbiota, and NA/VTA volume were assessed. BW was initially reduced in EX, but no differences were present between SED and EX at the end of the study. EI in EX steadily increased over the course of the study. Fat mass, leptin, and insulin were reduced in EX. Exercise-induced improvements in body composition may contribute to reductions in tonic hormones and satiety, potentially contributing to an increase in EI.

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Statement of Contributions

This research was conducted in Exercise and Nutrition Lab at the University of Lethbridge by Isabelle Durocher, with the help of Daniel Grant, and under the supervision of Dr. Marc Bomhof. Isabelle Durocher was the primary contributor to all components of this experiment. The literature review, analysis of the data, and writing of the paper was completed by Isabelle Durocher. Daniel Grant measured daily energy intake and body weight and completed the DNA extraction and purification of the fecal and cecal samples. Dr. Marc Bomhof conceptualized this experiment, helped during the oral glucose tolerance test and euthanasia, and obtained funding for this experiment.

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List of Abbreviations

Symbols	Definition
AgRP	Agouti related peptide
ANOVA	Analysis of variance
AP	Area postrema
ARC	Arcuate nucleus
AUC	Area under the curve
BAT	Brown adipose tissue
BMI	Body mass index
CART	Cocaine and amphetamine regulated transcript
CISI	Composite insulin sensitivity index
DIO	Diet-induced obesity
DVC	Dorsal vagal complex
EX	Exercise
FFAR	Free fatty acid receptors
GLP-1	Glucagon like peptide-1
GPR	G protein-coupled receptors
HF	Hight fat
HIIT	Hight intensity interval training
HR	Heart rate
Kcal	kilocalories
Km	Kilometer
LSD	Least significant difference
NA	Nucleus accumbens
NCD	Non-communicable diseases
NEAT	Non-exercise activity thermogenesis
NTS	Nucleus tractus solitarius
OGTT	Oral glucose tolerance test
POMC	Pro-opiomelanocortin
PYY	Peptide YY
qPCR	Quantitative polymerase chain reaction
SCFA	Short chain fatty acid
SED	Sedentary
SED-WM	Sedentary weight-matched
SEM	Standard error
VO_{2max}	Maximal oxygen uptake
VTA	Ventral tegmental area
WAT	White adipose tissue

CHAPTER 1: INTRODUCTION

1.1 Physical Activity and Exercise-Induced Health Benefits

Physical activity is any body movement that is generated by skeletal muscles that results in elevated energy expenditure (1). Physical activity consists of daily activities such as chores, playing, traveling, working, and recreational activities (2). This type of physical activity is commonly referred to as non-exercise activity thermogenesis (NEAT) (3). On the other hand, physical activity that is structured, repetitive, and undertaken for improvement of physical fitness is referred to as exercise (1). There are many reasons why individuals participate in physical activity. The top cited reasons adults participate in physical activity include weight loss, vanity, enjoyment, fitness, social aspects, stress relief, improved sport performance, and competition (4). Physical activity has a positive, long-term influence on physical and metabolic health, such as improved lipid profiles, glucose homeostasis and insulin sensitivity, blood pressure, blood flow, cardiac function, body composition, and weight control (5). Physical activity also improves psychological well-being (5, 6), stabilizes mood, improves self-esteem and emotional regulation, improves stress tolerance, reduces anxiety and depression, and enhances the individuals' optimism and outlook on life (6).

Physical inactivity accounts for 6-10% of the major non-communicable diseases (NCD) including cardiovascular disease, cancer, chronic respiratory disease, and diabetes (7). It is estimated that 9% of premature mortality globally is related to physical inactivity (7). To combat the negative health outcomes associated with physical inactivity, the World Health Organization has developed physical activity guidelines for adults. Adults are encouraged to participate in 150 minutes of moderate-intensity aerobic physical activity or 75 minutes of vigorous aerobic physical activity per week (8). According to the National Health and Nutrition Examination

Survey, only 5% of individuals meet these guidelines (8). Although the reasons for physical inactivity vary amongst individuals, the top cited reasons for physical inactivity can be attributed to lack of time, lack of motivation, lack of support, and lack of energy which can lead to low adherence rates (9). Additionally, approximately 50% of adults drop out within the first year of starting an exercise program (8).

While exercise is commonly viewed as a strategy for weight loss, research to date suggests that exercise is not effective for long-term reductions in body weight (10). Studies show that weight reduction with supervised exercise programs is often less than anticipated (11). A lower than anticipated reduction in body weight suggests that there is a certain degree of energy compensation that is associated with energy expenditure, wherein the body elicits an opposing physiological response to compensate for the energy expended with physical activity.

1.2 Energy Compensation

To maintain body weight, there must be a state of energy balance, where energy expenditure equals energy intake (12). Energy expenditure consists of basal metabolic rate, the thermic effect on food, and expenditure due to physical activity (exercise and NEAT) (12). Energy intake consists of the chemical energy (carbohydrates, proteins, fats, and alcohol) from consumed food and fluids (12). A negative energy balance is required for weight reduction. To achieve this, individuals can increase energy expenditure or decrease energy intake, or use a combination of both (13).

While physical activity increases energy expenditure, research suggests that increased energy expenditure with physical activity elicits compensatory mechanisms affecting energy intake and/or subsequent energy expenditure (14). Individuals may compensate for energy expenditure by increasing their energy intake or reducing their NEAT(14). In a review of exercise

intervention studies, Riou et al. observed that acute exercise interventions result in very little compensation (10). On the other hand, long term exercise interventions demonstrated an 84% energy compensation, meaning that 84% of increased energy expended through exercise was neutralised through increased energy intake or reduction in physical activity (10).

The degree to which energy compensation is related to an increase in energy intake or an unconscious reduction in NEAT is not completely understood (10). Myers et al. observed after a twelve-week supervised exercise intervention that weight reduction was only ~22% of anticipated weight loss (15). The lower-than-expected weight reduction was attributed to increased energy intake following the exercise intervention (15). Similarly, two previous studies identified a partial energy intake compensation with short term (~ 2 week) exercise interventions (16, 17). This compensation potentially defends the body against severe energy deficits and weight loss (15). Evidence also highlights a reduction in NEAT as a driver of energy compensation. Following a 3-month exercise intervention (low intensity versus moderate intensity) in women with overweight and obesity, energy compensation was ~49% and ~161% in the low and moderate intensity exercise groups, respectively (18). In both groups it was observed that NEAT decreased, whereas energy intake remained similar (18). Findings from Ridgers et al. suggests that in children any additional time spent doing low or moderate to vigorous physical activity resulted in less low intensity physical activity and moderate to vigorous intensity physical activity the following day (19). Evidence from animal research also demonstrates that energy compensation can result from both reduction in spontaneous physical activity and increased energy intake (20).

Research to date demonstrates an increase in energy compensation with prolonged exercise interventions, some of which can be attributed to increased energy intake. The drivers of

increased energy intake following prolonged exercise intervention remains to be determined. While exercise is known to elicit a short-term reduction in appetite (anorectic effects), the prolonged effects of exercise on the appetite-regulatory system as it relates to energy compensation has yet to fully be elucidated.

Thesis Overview

This thesis is comprised of five chapters. Chapter 1 provides an introduction to the thesis. Chapter 2 provides an overview of the homeostatic appetite-regulating system, the hedonic system, and the effects of acute and chronic exercise on appetite, appetite-regulating hormones, the gut microbiota, and the brain structures in both animals and humans. The objectives, rationale, and hypothesis are also presented here. Chapter 3 explains the methodology used for the experimental study Chapter 4 covers the results from the data collected. Chapter 5 provides a discussion on the findings from this research, and includes the study strengths, limitations, future direction, conclusion, and significance. All references are listed at the end of the thesis document.

CHAPTER 2: LITERATURE REVIEW

2.1 Appetite-Regulating Hormones

The homeostatic and hedonic pathways are two complementary drives that influence food intake (21). The homeostatic system is controlled by the hypothalamus and responds to signals of hunger and satiety to ensure balance in energy intake, expenditure, and energy stores (22, 23). The hedonic system is the brain's reward system that is influenced by dopamine and non-dopaminergic signals that stimulate wanting or liking of food (23). Understanding the physiological and neurological mechanisms involved in appetite-regulatory system is important when examining the impact of acute and chronic aerobic exercise on appetite and energy compensation. Energy intake and appetite control are regulated by satiety (24, 25), satiation (24, 25), and hunger (25). Satiety and satiation both contribute to the suppression of energy intake (24). Satiation causes an individual to stop eating, and satiety is the feeling of fullness after eating, preventing overconsumption (24). On the other hand, hunger is the feeling when satiety gradually decreases (25).

2.1.1 Homeostatic Regulatory System

The homeostatic system balances the drive to eat and energy expenditure to maintain energy stores (26). The homeostatic system is regulated by the gut-brain axis (27). The state of satiety influences eating behaviours (28). A low level of satiety will increase the drive to eat by increasing food intake, whereas a high level of satiety will decrease the need to eat and lead to eating cessation. This process is mediated through the arcuate nucleus (ARC) in the hypothalamus by coordinating the neural, nutrient, and short-term and long-term hormonal signals (27). These signals influence the sympathetic and parasympathetic nervous system, gastric function, and episodic and tonic appetite-regulatory hormone secretion (27). The gut-

brain axis depends on the constant communication between the gastrointestinal system, adipose tissue, and the brain to control energy homeostasis (27).

The release of gut hormones modulates hunger and satiety (29). The release of episodic or short-term hormones occurs simultaneously or shortly after eating to signal satiation and satiety (30). Ghrelin is the only known orexigenic, hunger, gut hormone (24, 29, 31). Ghrelin is released from the stomach and circulates in both acylated and des-acylated forms (32). Acylated ghrelin only accounts for a small amount of circulating ghrelin (~10-20%) but it is the primary driver in appetite stimulation (33). Ghrelin increases with fasting and decreases with food consumption (29, 31). Mani et al. observed that sedentary, ghrelin-null mice reduced food intake compared to sedentary littermates (34), supporting the role of ghrelin as an orexigenic hormone. The gut-derived, appetite-regulating hormones that work in opposition to ghrelin are called anorexigenic satiety hormones (33). The primary hormones involved are peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) (33). PYY functions to inhibit food intake (22, 29) and is secreted from the enteroendocrine cells, specifically the L cells (22, 24, 26). PYY circulates in two forms, PYY_{1-36} and PYY_{3-36} (31). PYY_{1-36} and PYY_{3-36} produce a dose-dependent inhibition of gastric emptying in rats, however, PYY_{3-36} produces a more potent effect than PYY_{1-36} (35). Secretion usually occurs fifteen minutes after ingestion, peaking after one to two hours, and plateauing for a couple of hours (26, 31). Reinforcing the role of PYY in appetite control, Batterham et al. identified that PYY-null mice had hyperphagia and marked obesity (36). GLP-1 is secreted from L-cells in the gut (29) and is a potent incretin hormone (24, 29). Incretin hormones are gut peptides that trigger insulin secretion and help to control hyperglycemia after food intake (35). GLP-1 acts rapidly to reduce food intake (26, 29). GLP-1 is released in small concentrations within fifteen minutes of macronutrient ingestion but breaks down quickly in circulation (26), due to the quick activation

of dipeptidyl peptidase-4 to inhibit the effects of GLP-1 (36). Evidence in both human and rodent studies show the potent effects of GLP-1. Intracerebroventricular and peripheral GLP-1 administration reduces food intake in rodents and inhibits appetite in rodents and humans (37). GLP-1 and PYY, may contribute to an additive effect on appetite regulation through reduced hunger and increased satiety (29). While macronutrients are understood to be the primary stimuli for secretion of the appetite-regulating hormones, short chain fatty acids (SCFA) may also stimulate the release of GLP-1 and PYY (38). SCFA are produced by the gut microbiota through fermentation of indigestible carbohydrates and account for up to 5-10% of the body's energy requirements (26). The gut microbiota, composed of trillions of bacteria and other microorganisms, is unique to every individual and develops after birth (27, 39). The gut microbiota contains 6 prominent phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia (40). Approximately 90% is composed of the phyla Firmicutes and Bacteroidetes (40). The most prominent genera in the phylum Firmicutes includes: *Clostridium* which makes up around 95% of the phylum, *Lactobacillus, Enterococcus, Roseburia, Faecalibacterium,* and *Ruminicoccus* (40). The main genera in the Bacteroidetes phyla and Actinobacteria phyla are *Bacteroides* and *Bifidobacterium,* respectively (40). The most abundant SCFAs produced in the lumen are acetate, propionate, and butyrate (26). According to a review article conducted by Venegas et al. both Bacteroidetes and Firmicutes are the main contributors to the production of the three main SCFAs (41). Firmicutes, specifically, *Faecalibacterium prausnitizii, Clostridium leptum,* and *Roseburia spp* are the primary bacteria involved in the production of butyrate (41). *Bacteroides* produces mainly acetate and propionate, while *Bifidobacterium* produces acetate, and *Akkermansia muciniphilia* produces propionate and acetate (41). SCFAs activate G-coupled protein receptors (GPR) 41 (GPR41) and

43 (GPR43) (42) in the intestinal L cells to release gut peptides, specifically GLP-1 and PYY, to control satiety (26). Many studies have identified the link between SCFA administration and an increase in release of GLP-1. Tolhurst et al. sought to understand the link between SCFA and GLP-1 secretion in colonic cultures of mice (in vitro) and in knockout mice (in vivo), specifically looking at GPR 41(FFAR2) and GPR43 (FFAR3) (38). In vitro, both acetate and propionate increased the release of GLP-1 (38). On the other hand, in *FFAR 2* knockout mice, a reduction in GLP-1 concentration and SCFA stimulation was observed (38). In vivo similar findings were observed; GLP-1 concentration was significantly reduced in FFAR2 knockout mice (38). These results potentially identify the link between FFAR2, SCFA stimulation, and GLP-1 release.

Long-term appetite hormones, also known as tonic satiety hormones, communicate peripheral adiposity levels to the brain (24). These tonic satiety signals include leptin and insulin (24). These signals act over an extended period to maintain static body weight by adjusting energy intake and energy expenditure (24). Adipose tissue is the primary producer of leptin (21, 24). Leptin is transported through the blood-brain barrier to the receptors in the brain that are associated with appetite control (24). Leptin circulation depends on nutritional status and adipose tissue (24). Leptin concentration is reduced by starvation and weight loss and increased by refeeding (24). An increase in leptin levels reduces food intake and energy storage by stimulating metabolic processes (21), such as increasing oxidation of fatty acids (43) and glucose uptake by muscle (44). Insulin, the second tonic satiety signal, is produced and secreted by the pancreas (24). Insulin crosses the blood-brain barrier and acts on insulin receptors in the brain that control energy intake (24). Postprandially, insulin concentration rises to control glucose levels (24).

Insulin concentration is also influenced by adiposity (24), with an increase in adiposity leading to an increase in insulin levels (24).

The homeostatic appetite regulation is controlled through the brainstem and hypothalamus. Using both neuronal and hormonal signals, the gut-brain axis regulates appetite. The dorsal vagal complex (DVC) relays peripheral signals from the gut to the hypothalamus (45). DVC is made up of the dorsal motor nucleus of vagus (DVN), area postrema (AP), and the nucleus of the tractus solitarius (NTS) (45). Through afferent vagal signalling, signals from peripheral hormones from the gut are relayed to receptors in the brainstem (45). The ARC in the hypothalamus integrates hormonal signals and neuronal signals to regulate appetite (45). The median eminence of the hypothalamus is the site where hormonal signals, such as ghrelin and PYY, may directly pass through the incomplete blood-brain barrier to produce a direct effect on the brain (45). The ARC has two different groups of neurons specific to the anorexigenic and orexigenic pathways (21). The anorexigenic pathway involves neurons that express neuropeptide proopiomelanocortin (POMC) and cocaine-and-amphetamine-related transcript (CART) (24). Orexigenic pathways involve neurons that express neuropeptide Y (NPY) and agouti-related peptide (AgRP) (24). Leptin suppresses energy intake by activating POMC and CART neurons (21, 24) while concurrently producing an inhibitory effect on NPY and AgRP neurons (21). Ghrelin stimulates appetite by activating NPY and AgRP neurons (21, 24). Acting in opposition, GLP-1, PYY_{3-36}, and insulin inhibits NPY and AgRP to reduce appetite and feeding behaviours (24). Furthermore, SCFAs may directly affect central appetite regulation. Studies have shown that acetate can cross the blood-brain barrier and activate anorectic signalling in the ARC by increasing neuronal POMC and reducing AgRP (46).

2.1.2 Hedonic Regulatory System

The hedonic appetite regulatory system increases the wanting and liking of energy-dense, high-fat, and high sugar foods and is largely believed to be responsible for the growing global obesity rates within the context of our current food environment (23). In the presence of highly palatable food, the hedonic reward system will prompt consumption beyond homeostatic requirements (22, 47, 48). In the presence of highly palatable food such as salt, sugar, and fat, the hedonic system influences food-seeking and eating behaviours (49), contributing to an increase in meal frequency and meal size (48). The hedonic system is a reward pathway (24) and involves the forebrain, hindbrain, and the mesolimbic dopamine system (28). The mesolimbic dopamine system influences reward behaviour through the ventral tegmental area (VTA), nucleus accumbens (NA), prefrontal cortex, amygdala, and hippocampus (50). Pre-prandially, dopamine is released from the VTA into the NA (21) and influences decision-making for intake behaviours (50). The ingestion of highly palatable food items containing fat, sugar, and salt also leads to a release of dopamine into the NA, contributing to an increase in reward during and after eating (21). Non-dopaminergic systems also influence reward pathways (22). These non-dopaminergic systems include endogenous cannabinoids, opioids, and serotonin (22). Overall, dopaminergic and non-dopaminergic systems contribute to the hedonic regulatory system to increase food reward and increase food seeking behaviour.

Cross talk between the homeostatic and hedonic system exists. The two systems interact with each other through the gut-brain axis to increase or suppress energy intake. The relationship between these two systems and the effects on palatability may potentially be driven by whether participants are in fasted or fed state. The impact of a 24-hour fast on liking and wanting has been previously studied in rats. In the fasting condition, there is a substantial increase in wanting

and liking of preferred foods, an increase in hunger and desire to eat, and a decrease in fullness compared to the fed state (51). Therefore, an increase in homeostatic hunger may elicit an increase in food reward behaviour (51). When the appetite-regulatory system is exposed to the western diet, which involves energy-dense, high sugar, and high-fat foods, it can affect the homeostatic system. Energy-dense foods are speculated to interfere with the homeostatic ability to maintain balance (29) by overriding the release of appetite-regulating hormones, decreasing vagal response, or increasing adiposity to weaken hormone signalling (52). A review published by Mortan et al. describes an adiposity feedback system where increased adiposity inhibits neuronal activity and increases leptin resistance, which increases feeding and weight (53). Leptin resistance occurs when the brain stops responding to leptin signals (54). In a 4 week diet-induced obesity intervention, mice were fed a high-fat diet to test leptin resistance in the central nervous system (54). After the 4 weeks, leptin signalling decreased in the ARC, suggesting that a high-fat diet contributes to leptin resistance (54). Furthermore, a review conducted by Vincent et al. aimed to determine the influence of appetite hormones on obesity (55). Evidence suggests that postprandially, in individuals with obesity, ghrelin concentrations remain elevated, whereas PYY concentrations remain reduced (55). Taken together, an increase in adiposity may be associated with an increase in central leptin resistance as well as impaired postprandial ghrelin and PYY secretion leading to an increase in energy intake and bodyweight. Increased intake of energy dense foods potentially contributes to a failure in the homeostatic system leading to increased energy intake (52), which may contribute to weight gain and obesity (26, 29).

2.2 Exercise-Induced Energy Compensation in Humans and Rodent Models

Energy balance and weight control are influenced by exercise and appetite regulation. Aerobic exercise influences the secretion of appetite-regulating hormones, sensation of hunger, and energy intake, which may potentially contribute to the energy compensation that is associated with exercise. This compensation may be influenced by exercise duration and intensity or other individual factors that inhibit weight loss efforts. An acute bout of exercise is defined as a single session of low, moderate, or high intensity exercise, whereas chronic exercise is defined as repeated bouts of exercise over a short or long-term period (56). Exercise can be split into three main intensity categories: low (<55% VO_{2max} or HR_{max}), moderate (55%-75% VO_{2max} or HR_{max}), and vigorous (>75% VO_{2max} or HR_{max}) (57).

2.2.1 Acute Aerobic Exercise

Acute exercise and appetite control have been thoroughly examined in humans. The majority of research suggests that the acute effects of aerobic exercise on appetite may be intensity-dependent (58). Consistent findings have demonstrated that an acute bout of moderate to vigorous aerobic exercise induces anorectic effects on appetite (30, 59-62). These satiety-inducing effects, however, are transient in nature as appetite recovers to resting values within thirty to sixty minutes after exercise (59, 62). The following day after an acute bout of aerobic exercise, King et al. observed that appetite was neither increased or decreased relative to the control group (63). Although there have been consistent findings showing acute anorectic effects with moderate to vigorous exercise, not all studies come to the same conclusion. One study observed that female participants' hunger increased after a one-hour moderate aerobic session (64). Other studies suggest that appetite is not affected by exercise. Pomerleau et al. identified no

change in hunger after a high-intensity exercise session (65). Additionally, several studies have demonstrated that hunger is not impacted after low-intensity aerobic exercise (62, 66).

While exercise elicits acute, satiety-inducing effects, the impact of exercise on energy intake yields mixed findings. The majority of short-term, aerobic studies identify no change in absolute energy intake following exercise (30, 33, 58, 62, 64, 66). A suppression of energy intake after a single bout of moderate-intensity aerobic exercise has been observed in individuals with obesity (67), normal weight (68), and sedentary lifestyles (69). In contrast, partial or full compensation has been demonstrated after moderate to vigorous (70% VO_{2max}) bouts of aerobic exercise (60, 65). However, according to Maraki et al. relative energy intake may be a better measure of exercise-induced energy compensation (64). Relative energy intake compares energy intake to exercise energy expenditure. It is measured by subtracting exercise-induced energy expenditure from food intake for the remainder of the day (64). When individuals increase their absolute energy intake above their normal resting intake but have a lower relative energy intake, only a partial compensation occurs (60). In contrast, if absolute energy intake stays constant after exercise, there is no energy compensation, leading to lower relative energy intake and a greater energy deficit (62). Overall, previous research suggests that after an acute bout of aerobic exercise, relative energy intake decreases (58, 61, 64, 66, 67, 70), leading to an increased energy deficit and greater weight loss or weight maintenance. Based on the literature, energy intake compensation is highly variable. While some individuals show no compensation, others show partial or complete compensation (71).

The alteration in appetite and energy compensation with exercise is believed to be mediated primarily through the secretion of appetite-regulating hormones (33). Appetite-regulating hormones are released from the digestive tract to modulate the feeling of satiety and postprandial

satiation (29). The gut hormones include orexigenic hormone, acylated ghrelin, which increases appetite, and the anorexigenic hormones, PYY, and GLP-1 (29). While many early studies concluded that total ghrelin concentration remained unaffected by aerobic exercise (72-77), some studies observed an increase in total ghrelin (78). Other studies have observed a suppression of total ghrelin after acute bouts of aerobic exercise (79). More recently, the identification of acylated ghrelin and des-acylated ghrelin has become the primary focus of acute aerobic exercise studies. Evidence consistently demonstrates a transient suppression of acylated ghrelin after an acute bout of aerobic exercise (59, 80-83). After an acute bout of exercise an increase in the anorexigenic hormones PYY (59, 61, 67, 68, 83, 84), and GLP-1(59, 61, 67, 68, 83, 84) have been observed. A single bout of exercise has been shown to stimulate POMC neurons while also inhibiting arcuate NPY neurons (85), thus potentiating appetite suppression. Confirming these findings, a study observed that after high-intensity, acute exercise, activation of POMC was elevated and remained high in rats (86). Overall, while there is some contrasting evidence, it is largely believed that activation of POMC neurons within the hypothalamus mediates the acute, exercise-induced reductions in hunger and appetite.

Acute exercise may also influence the secretion of tonic hormones. Olive & Miller aimed to understand the effects of a single bout of endurance exercise training of either long duration moderate-intensity or short-duration maximal-intensity in trained males on leptin secretion (87). It was identified that leptin concentrations showed no change immediately after exercise but instead had a delayed decrease, 24 to 48 hours post-exercise (87). However, these changes were only observed after a long duration, moderate-intensity bout of exercise (87). Furthermore, insulin was also shown to be reduced 24 hours post exercise, while glucose showed no change (87). In contrast, Vatansever-Ozen et al. conducted two 4 hour trials, one control trial where

individuals rested for 4 hours and an exercise trial, where individuals ran for 105 minutes at 50% VO_{2max} with the last 15 minutes at 70% VO_{2max} followed by a rest period of 120 minutes (58). Following each trial, a buffet meal was consumed by all participants (58). In response to acute aerobic exercise, it was observed that glucose, insulin, and leptin concentrations remained unchanged (58).

The impacts of aerobic exercise on energy compensation have been examined extensively in humans. Several mechanisms are predicted to be involved in acute exercise-induced effects on appetite-regulatory hormones. These factors include blood flow redistribution, lactate production, high body temperature from exercise (88), and gastric emptying (89). Exercise stimulates a redistribution of blood flow from the stomach to the skeletal muscles (88). Given that ghrelin is released from the stomach, reduced blood flow may be a potential factor in the suppression of ghrelin after exercise (88). During and after exercise, lactate production initiates anorexigenic effects in the body, as lactate binds to receptors that prevents the release of ghrelin reducing hunger (88). Gastric emptying increases with an acute bouts of low intensity exercise such as walking and decreases with moderate to high intensity bouts of exercise (89). It has been demonstrated that a decrease in gastric emptying is associated with an increase in GLP-1 release, whereas an increase in gastric emptying is associated with an increase in acylated ghrelin (88). Therefore, after an acute bout of high intensity aerobic exercise, gastric emptying and acylated ghrelin are supressed leading to a transient suppression of appetite and a decrease in energy intake.

The differences observed in appetite-regulating hormones and energy intake after acute bouts of exercise may be impacted by sex, adiposity, and energy status. Hazell et al. conducted a study to identify whether males and females demonstrated the same appetite-regulating response to

acute exercise (84). It was observed that females had a higher increase in GLP-1, whereas males had a higher increase in PYY (84). However, no difference in hunger between sexes was observed (84). Alajmi et al. conducted a study with 10 males and 10 females who participated in two 7-hour trials (control and exercise) with a test meal given 2 hours into the intervention (90). The exercise trial consisted of sixty minutes of running at ~70% VO_{2max} followed by participants resting for the remainder of the day, whereas the control trial consisted of individuals resting for the seven hours (90). It was observed that regardless of sex, no change to appetite, acylated ghrelin, and energy intake occurred after exercise (90). Douglas et al. sought to understand the effects of acute exercise on appetite control in individuals who were lean compared to individuals who were obese and overweight (91). Each participant completed two 8 hour trials, one control and one exercise (91). The exercise trial consisted of a 60 minute treadmill exercise session at ~60% VO_{2max} (91). Similarly, exercise reduced appetite and increased PYY and GLP-1 concentrations in both groups (91), however, individuals with obesity had higher GLP-1 concentrations, whereas lean individuals had higher PYY concentrations (91). In contrast, Ueda et al. identified that young adults with obesity increase their energy deficit after an acute bout of aerobic exercise compared to age-matched, normal weight control subjects (67). This reduction was independent of hormone changes as there was no difference between ghrelin, PYY, and GLP-1 between groups (67). Bachman, Deitrick & Hillman aimed to identify exercise effects on energy intake in the fasted vs. fed state (92). Twelve healthy males were assigned to a breakfast group and a non-breakfast group, and were instructed to run for 60 minutes at 60% VO_{2max} (92). Food was recorded for 24 hours after exercise (92). Hunger increased for the non-breakfast group before exercise, after exercise, and before lunch (91). These findings, however, did not correlate with energy intake, meaning that hunger did not predict energy intake (92). The fasted

group consumed less food and had increased fat oxidation over the 24-hours relative to the fed group (92). These results suggest that training in the fasted state may elicit greater weight reduction (92). On the other hand, Deighton, Zahra & Stensel had twelve males perform three trials: sedentary control, fasted exercise, and postprandial exercise (93). It was identified that after a 60 minute run at ~ 70% VO_{2max}, the postprandial trial had a greater appetite reduction than the fasted-state trial (93). It was concluded that there was no difference in energy intake when exercising before breakfast or after breakfast (93). Additional research needs to be conducted to understand the true effects of these potential modulating factors as they relate to changes after exercise in energy intake, perceptions of hunger, and appetite-regulating hormones.

2.2.2 Chronic Aerobic Exercise

To date, the effects of chronic exercise on appetite, energy intake, and energy compensation are still relatively unclear. Previous research suggests that compensatory behaviours occur more frequently after prolonged exercise. A systematic review conducted by Riou et al. identified that one of the main predictors of energy compensation is the duration of the intervention (10). Riou et al. suggest that energy deficits produced from exercise can only be maintained for a short period (10). Longer duration exercise studies have demonstrated exercise-induced energy compensation to be ~84% (10). This may be why exercise on its own is not the most effective weight loss strategy (15). Compensation occurs through an increase in energy intake or a reduction in NEAT. After a prolonged exercise intervention, the degree to which an individual compensates varies immensely. To identify this variation in exercise-induced compensatory response, some researchers retrospectively separate participants into two groups, responders (i.e., non-compensators) and non-responders (i.e., compensators), after the completion of an exercise intervention (14, 94). Non-responders/compensators are individuals who do not achieve

predicted weight loss after an intervention, while responders achieve their predicted weight loss (94). This is calculated by estimating predicted weight loss from an exercise intervention and comparing it to actual weight loss after the intervention (94). Therefore, understanding who the non-responders are at the beginning of an intervention may allow programs to be adapted to individual needs.

After continuous aerobic exercise training, it is observed that energy compensation differs among studies. Prolonged aerobic exercise studies show either no compensation (95-98), partial compensation (15-17, 99-101), or full compensation (18). When no compensation or partial compensation occurs, an individual fails to increase their intake to match exercise energy expenditure, creating an energy deficit that may lead to weight loss. However, when full compensation occurs after an exercise bout, it negates the exercise-induced energy deficit leading to an increase or maintenance of weight. Myers et al. conducted an exercise intervention with thirty-two females who were obese or overweight (15). The intervention consisted of 5 aerobic exercise sessions per week for 12 weeks (15). After the 12 weeks, partial compensation occurred as most participants lost weight (15). However, two-thirds of participants did not reach predicted weight loss; therefore, these participants would be identified as non-responders (15).

Long-term energy compensation may be influenced by multiple factors, such as exercise intensity, type of exercise (i.e., interval or continuous), anthropometrics, exercise history, and sex. Chronic exercisers in an energy deficit have shown an ability to fine-tune appetite control to achieve energy balance (69). This suggests that habitual exercisers have a greater ability to control food intake, therefore preventing overconsumption past the energy deficit produced by exercise. On the other hand, non-exercisers at baseline displayed a greater tendency to binge eat and increase food intake, consequently exhibiting weaker appetite control and greater energy

compensation (102). Martins et al. conducted a twenty-four week exercise intervention with 171 participants who were obese and overweight (99). Participants were separated into three groups: 1) a sedentary control group; 2) a supervised exercise group of 8kcal/kg/week (8KKW); or 3) a 20 kcal/kg/week (20 KKW) supervised exercise group (99). Compensation occurred in both exercising groups, by ~76% and ~90%, in the 8KKW group and 20 KKW group, respectively (99). Additionally, weight loss was variable between groups. Almost half (~42%) of the participants in the 20 KKW group and a quarter (~24%) of participants in the 8 KKW group showed no weight changes (99). Furthermore, the compensators had increased hunger, prospective food consumption, and cravings for sweet foods, contributing to elevated energy intake by ~90kcals/day (99). Overall, this suggests that full energy compensation is common with exercise and occurs from an increase in energy intake (99).

Exercise intensity may be an influential factor on energy compensation, however, previous research exhibits ambiguous findings. Alkahtani et al. observed that after a 4-week high-intensity and a 4-week moderate-intensity training intervention, males who were overweight and obese had a greater tendency to increase compensatory responses after moderate-intensity interval training (103). Participants had increased desire to eat, fat intake, and liking of high-fat non-sweet foods compared to high-intensity interval training (103). In females with obesity or overweight, lower-intensity aerobic training for 3 months led to lower energy compensation (49%) compared to the moderate-intensity group (161%) (18). In another study, Martins et al. examined the effects of exercise intensity on energy compensation in forty-six sedentary males and females with obesity over a 12-week exercise intervention (104). The high-intensity interval training (HIIT) group completed 8 second sprints with 12 second recovery to expend 250 kcals/session, the moderate-intensity continuous training group performed continuous cycling at

70% VO_{2max} to expend 250kcal/session, and the ½ HIIT group performed the same protocol as the HIIT group but only expended half the calories (125kcals/session) (104). This study identified no change in appetite, energy intake, or food hedonics, suggesting that a difference in exercise intensity has no major impact on energy intake or appetite (104). The full effects of exercise intensity on energy compensation have yet to be fully elucidated.

Sex differences may also influence exercise-induced compensatory behaviours. Stubbs et al. performed two identical studies, one with males (98) and the other with females (16). The studies resulted in partial compensation of ~30% for females (16) and no compensation for males (98). However, in response to a 12 week supervised exercise intervention, Caudwell et al. identified that when energy expenditure is controlled (i.e., with both males and females expending 10.5 MJ/week at 70% HR_{max}), there were no differences in energy compensation or body fat change between males or females (95). Differential changes in appetite-regulating hormones between males and females with exercise training may potentially influence the degree of energy compensation. Hagobian et al. conducted an exercise trial with 9 females and 9 males who were previously sedentary and overweight or obese (105). Satiety, hunger perceptions, and appetite-regulating hormones were assessed after three different conditions: 1) in energy balance, 2) after four days of exercise with consumption of food after exercise to achieve energy balance, and 3) after four days of exercise without consuming food to produce an energy deficit (105). After both exercise trials, females had increased acylated ghrelin and reduced insulin, contributing to an elevation in hunger (105). In males, acylated ghrelin concentrations were not altered (105). This variation of appetite-regulating hormone concentrations in males and females may contribute to energy compensation differences after chronic exercise.

The effects of prolonged exercise on compensatory behaviours remains unclear in humans. It is important to understand the limitations with assessment of energy intake with prolonged exercise interventions in human studies. Unlike short-term interventions where researchers can use buffets and fixed meals to record and control energy intake, in long-term exercise interventions, studies utilize food journals and 24-hour recall or a combination of both (106). Underestimating food intake frequently occurs in self-reported dietary intake, as individuals are likely to underreport their food intake by 20 to 30% and up to 50% (106). Additionally, there can also be error with data entry and food analysis software. This includes errors with food composition, such as incorrect identification of food items, the use of analytical methodologies (estimates made by the software when data is not available), and incorrect data entry (107). The errors and biases that occur with food intake records make it very difficult to accurately measure energy intake and predict compensatory behaviours over an extended period.

2.2.3 Energy Compensation in Rodent Models

Another strategy to examine exercise-induced compensatory behaviours is to employ a rodent model. With rodents, researchers can control food intake, track body weight changes, and assess the total amount of physical activity (exercise and NEAT), which allows for a more comprehensive understanding of exercise effects on appetite. Foright et al. aimed to examine the energy compensatory mechanisms in male and female rats (108). Over a four-week trial, rats engaged in five days of forced running, with running duration and speed increasing each week (108). On exercise days, male rats reduced their energy intake, whereas females increased their energy intake (108). On rest days, independent of sex, energy intake was similar between exercise and sedentary rats (108). Other studies have demonstrated that rodents will increase their food intake when provided with access to a running wheel (20, 109-112). Evidence suggests

that body weight may decrease (20, 109, 112) or stay constant (110) in response to long-term voluntary exercise, despite an increase in energy intake. After 10 weeks of voluntary running, de Carvalho et al. observed that mice with access to a running wheel increased energy intake but had no change in body mass (110). In another study conducted by McMullan et al. aimed to understand the long-term exercise effects on metabolic changes such as body mass, body composition, and food and water intake, using 30 female and 30 male mice (113). The mice were split into two groups, a control group, and a running group with access to a voluntary running wheel (113). Relative to sedentary, control mice, the mice with access to the running wheel had increased food intake, reduced body weight by ~16%, lowered body fat by ~50%, and increased lean mass by ~15% (113). Overall, rodent models, like human studies, provide mixed evidence on compensatory behaviours following exercise training, with some studies demonstrating a high degree of compensation while others show a low degree of compensation.

2.3 Mechanisms of Energy Compensation in Humans and Rodents

The mechanisms involved in prolonged exercise-induced energy compensation are not fully elucidated. Potential mechanisms include a change in body composition (fat mass and fat free mass), fasting and postprandial hunger, episodic satiety hormones, increased sensitivity to tonic hormones (114), the hedonic system (83), the gut microbiota (109, 111, 115), and SCFA production (116). In response to an energy deficit, these mechanisms may interact to influence overall energy compensation, contributing to a lower than anticipated weight reduction with exercise.

Exercise-induced energy compensation may be influenced by episodic satiety hormones (14). In a study conducted by Gibbons et al. individuals who were responders had a greater reduction in acylated ghrelin and increase in GLP-1 and PYY than non-responders (14). Supporting these

findings, previous research has identified an increase in PYY after chronic aerobic exercise (97, 117) and an increase in late postprandial release of GLP-1 (118, 119), leading to a greater satiety effect. On the other hand, Flack et al. identified a positive correlation between ghrelin and fat loss, where after 12 weeks of aerobic exercise, exercise reduced ghrelin area under the curve (AUC) and reduced body fat (100). Habitual exercisers and responders may secrete different concentrations of appetite-regulating hormones after prolonged exercise, therefore increasing their ability to control appetite. Overall, the effects of prolonged exercise on the secretion of short-term, appetite-regulating hormones are still relatively unknown.

After prolonged exercise training, tonic hormones have a more pronounced effect on appetite control than episodic hormones. Long-term body changes, such as fat loss, are more prevalent in chronic exercisers; therefore, both leptin and insulin may be affected. Fasting and postprandial leptin concentrations have been observed to decrease after chronic exercise, potentially due to fat loss (117, 120, 121). In a systematic review, Fedewa et al. identified that regardless of a change in body fat, chronic exercise reduces leptin concentration (121). However, evidence from Koshki et al. demonstrates that a 12-week, high-intensity exercise intervention in 25 females with obesity or overweight elicits no change in leptin concentration, despite reduced body weight and adiposity (96). In a recent randomized control study conducted by Flack et al. fifty-two adults with overweight or obesity, were split into three groups: 1) exercise 6 days/week, 2) exercise 2 days/week, or 3) sedentary for a 12-week intervention. Both exercise groups were given heart rate monitors to assess exercise intensity (100). Participants in the 6d/wk group exercised between 40-60 minutes/session whereas the participants in the 2d/wk group exercise for 90-120 minutes/session, both groups exercised at a self-selected intensity (100). All groups were told to make no dietary adjustments. Energy compensation was assessed based on body composition

changes. Hormones were assessed after an overnight fast and a standardized breakfast with serial blood draws for 2 hours (100). After 12 weeks, body weight and fat were reduced in the 6d/week group but not in the 2d/week group or control. As well, both exercise groups compensated ~50% (100). Energy compensation was positively associated with leptin AUC, meaning that a reduction in leptin was also associated with a reduction in energy compensation (100). Additionally, body fat loss was positively related with leptin (100). This study suggests that energy compensation may be influenced by changes in leptin AUC such that, an increase in leptin sensitivity, results in less energy intake and therefore, less energy compensation (100). In rodent models, this ambiguity has also been shown. It has been observed that exercised mice have reduced leptin concentration compared to the sedentary control group (112, 122). Furthermore, it has also been observed that plasma leptin concentrations were unaffected when minimal weight loss occurred (123). Bi et al. studied the effects of a twelve-week wheel running intervention on leptin concentration in hyperphagic, obese Long Evan Tokushima (OLETF) rats and lean Long Evan Tokushima (LETO) rats (122). Both strains of rats were separated into a running group and a sedentary control group (122). After 6 weeks, the OLETF running rats were further split into two groups: 1) continued running and 2) sedentary (running wheels were locked) for further 6 weeks (122). As running wheel activity increased over the 12 weeks, leptin concentration decreased in both the LETO and OLETF rats compared to the sedentary controls (122). When running wheels were locked after 6 weeks, OLETF rats increased leptin concentration, however this increase was still below the sedentary control OLETF rats (122). In conclusion this study suggests that exercise produces satiety reducing effects by lowering leptin which continues after exercise was stopped.

Insulin is the other tonic hormone that impacts appetite control and glucose homeostasis. Bradley et al. designed a 10 week study to identify the effects of exercise on insulin concentration and sensitivity (124). Mice were divided into two groups: 1) a regular chow diet, or 2) a high-fat diet (124). After 4 weeks, the mice were further divided into non-wheel running or wheel running groups (124). After 6 weeks of access to voluntary wheels, mice in the high-fat diet group showed reduced glucose and insulin concentrations, reduced adiposity, and increased insulin sensitivity (124). It has been established in rodents and humans that regular exercise increases sensitivity to insulin (125), and reduces fasting and postprandial insulin secretion (97, 119, 126).

One factor that may mediate exercise-induced energy changes in appetite and energy compensation is the gut microbiota. The gut microbiota changes substantially due to nutrition status and exercise. Gut microbiota composition can influence body weight regulation by controlling energy extraction from food, mediating the inflammatory response, and influencing energy intake through the activation of the gut-brain axis (115). The gut microbiota, therefore, plays an important role in body weight regulation and obesity (115). Through fermentation, the gut microbiota may also increase energy extraction through the production of SCFA (116). While the impact of exercise on SCFA production is relatively new and unknown, emerging evidence shows that exercise may increase the production and utilization of SCFA (116). Independent of diet, exercise influences a change in the gut microbiota (109). Exercise impacts the balance of major bacterial phyla (Bacteroidetes and Firmicutes), alters SCFA production and utilization (111), and alters the diversity of bacterial species (111, 127). Evidence has shown that body weight and body composition are highly correlated with certain bacteria species in the gut. Bacteroidetes has been positively associated with lean body mass and weight loss (128). As well,

body fat and BMI have been found to be negatively correlated with *Bifidobacterium* (129), *Lactobacillus* (129), and *Faecalibacterium* (116, 130). Aerobic exercise has also shown to impact bacteria species in the gut, Mahdieh et al. observed after 10 weeks of aerobic training an increase in the abundance of *Bifidobacterium*, however the abundance of *lactobacillus* was not affected (129). Evans et al. observed that an increase in aerobic exercise was associated with an increase in percentage of *Bacteroidetes, Lachnospiraceae, Ruminococcaceae*, and trends towards increase in *Clostridiaceae* and a reduction in *Lactobacillaceae* and *Bifidobacteriaceae* (111). Additionally, exercise was negatively associated with the Bacteroides:Firmicutes ratio (111). An increase in both Bacteroidetes *(Bacteroidales*) and Firmicutes (*Lachnospiraceae, Ruminococcaceae, and Clostridiaceae*) increased butyrate-producing bacteria (111). Allen et al. conducted a 14 week longitudinal study with thirty-two sedentary females and males (116). The participants were split into lean and obese groups (116). Each group performed supervised aerobic exercise three days per week for 30-60 minutes at moderate to vigorous intensity (116). Post-exercise, in lean individuals, there was an increase in SCFA concentration, however, this change was not observed to the same degree in individuals with obesity (116) After aerobic exercise, an increase in butyrate producing bacteria (*Clostridiales spp, Lachnospira spp, Roseburia spp.,* and *Faecalibacterium spp.*) was observed (116). Additionally, Barton et al. conducted a study in profession rugby players and sedentary individuals to examine potential differences in the gut microbiota, microbial diversity, and SCFA profiles between groups (131). It was observed that the athletes had increased microbial diversity and enhanced profiles of SCFAs compared to the sedentary controls, a finding that the authors speculated could contribute to improved overall health in the athletic group (131). Queipo-Ortuna et al. conducted a 6-day study, looking at nutritional status, exercise and its effects on the gut microbiota composition and

serum leptin and insulin. Forty male Sprague Dawley rats were split into four groups: 1) activity based anorexia (ABA), where rats were restricted to eating for only 1 hour/day and the other 23 hours had free access to running wheels, 2) control to ABA, where rats followed the same eating restriction as ABA but had no access to a running wheel, 3) exercise group, where rats ate *ad libitum* and had free access to running wheel, or 4) *ad libitum* group, where rats had free access to food but no access to wheel (132). Using a Real-Time PCR, an increase in Actinobacteria and Bacteroidetes and decrease in Firmicutes was seen in EX relative to *ad libitum* group (132). *Clostridium, Enteroccocus,* and *Bacteroides* were also reduced, whereas *Bifidobacterium* and *Lactobacillus* were increased in exercise compared to *ad libitum* (132). Additionally, Queipo-Ortuna et al. identified a positive correlation between *Bifidobacterium* and *Lactobacillus* and serum leptin, and a negative correlation with *Clostridium, Bacteroides* and serum leptin (132). Furthermore, ghrelin levels were positively correlated with *Bacteroides* and negatively correlated with *Bifidobacterium* and *Lactobacillus* (132). To date, no studies have examined the relationship between long-term aerobic exercise (>7 days) and the effect on exercise-induced changes in the gut microbiota, satiety-regulating hormones, and appetite control.

Exercise may influence appetite by stimulating the hedonic response to food. Exercise potentiates wanting and liking for highly palatable foods and increases pleasure from food consumption (133). Non-responders/compensators showed an increase in wanting and liking of palatable foods (133), craving for sweets (99), preference for high-fat sweet foods (133), and high-fat non-sweet foods (103) immediately post-exercise. In contrast, Martin et al. identified that after high-intensity interval training and moderate-intensity continuous training, food hedonics did not change (104). Additionally, Moody et al. conducted a study to identify food preference after aerobic exercise in Sprague Dawley rats (134). The rats were randomized into

one of four groups: 1) sedentary, high-fat diet, 2) sedentary, high sugar diet, 3) wheel running, high-fat diet, or 4) wheel running, high sugar diet (134). The researchers identified that running wheel access decreased preference for both high-fat and high sugar diets in male and female rats (134). Additionally, Liang et al. observed the effects of wheel running and preference for a high-fat diet (135). Before rats were given access to a running wheel, preference was given to the high-fat diet compared to the high carbohydrate, chow diet (135). Over the 16 day running intervention, the rats reduced their intake of the high-fat diet suggesting that exercise reduces intake of highly palatable foods (135). Taken together, this evidence suggests that preference for highly palatable food may be influenced by physical activity (134). However, the mechanisms behind how physical activity may influence food choice are still relatively unknown. It is speculated that the NA and VTA may play a large role. Studies using fMRI have shown an increase in NA activity in response to food cues that predict weight gain and are positively associated with overeating behaviours (136). Additionally, a study conducted by Rapuano et al. identified that children who are genetically prone to obesity have a larger reward response, along with a larger NA volume (137). On the other hand, Samara et al. identified that people with obesity had larger NA volumes than individuals of normal weight, however it was not associated with eating behaviours (138). The microstructure in the NA, specifically, the axonal density, has been demonstrated to mediate emotional eating and is correlated with adiposity measures (138). Based on this, it could be speculated that the larger the NA, or potentially the higher axonal density within the NA, the stronger the food reward signalling and likelihood of increased food intake (138). This association between NA volume and eating behaviours has also been observed in individuals with eating disorders, where individuals with binge eating disorder had a larger left volume NA compared to non-binge eaters (139). This would suggest that the NA may play a

prominent role in controlling energy intake and eating behaviours. While there is evidence to suggest an association between the volume of the NA and body composition or body weight, evidence is still inconclusive. NA volume has been associated positively with BMI (140, 141) and negatively with body fat % (142). Additionally, some studies show no relationship between BMI and NA volume (143). A review conducted by Garcia-Garcia et al. aimed to elucidate the association between volume measures of the NA and obesity measures (144). It was concluded that age was a large predictor of the association between volume and obesity (144). Younger individuals had a positive association between NA volume and BMI, whereas, older adults had a negative association between NA volume and BMI (144). Taken together, evidence suggests that the volume of the NA may be an important indicator in food reward, and therefore, research is still needed to understand whether exercise may impact the volume of the NA and potentially contribute to energy compensation.

Exercise-induced reduction in NEAT has also been studied in humans and rodents. In rodents, access to voluntary running wheels have been shown to increase energy compensation by reducing the energy expended through other means (i.e., climbing, and other locomotor activities) (20, 110, 145). Furthermore, Levin & Dunn-Meynell observed that when rats were calorically restricted, rats lowered the amount of running they did to achieve energy balance (146). In another study, Lark et al. sought to determine how voluntary wheel running (VWR) and off wheel activity (OWR), also referred to as NEAT, influences energy balance in mice. It was observed after unlocking running wheels an increase in voluntary wheel running occurred followed by a subsequent decrease in off wheel running (147). This compensatory behaviour negated ~ 45% of the energy deficit produced by VWR (147). These results suggest that an increase in voluntary aerobic exercise can lead to a reduction in NEAT which potentially may

limit weight loss. Human studies have demonstrated that an increase in energy expenditure may contribute to energy compensation by reducing NEAT (18, 19, 148). In a recent 2019 study, Riou et al. aimed to understand the effects of a 3-month low and moderate intensity exercise intervention (1500 kcal/week in both groups) on energy compensation in twenty-two females who were obese or overweight (18). An overall energy compensation of ~49% and ~161% in LOW and MOD groups, respectively was identified (18). Two notable effects occurred throughout the three months: 1) food intake did not change, and 2) regardless of intensity, NEAT decreased early and remained low throughout the duration of the study (18). Additionally, a substantial increase in sedentary time was observed in the MOD group compared to the LOW group (18), suggesting that energy compensation was largely due to a decrease in NEAT and not due to a change in energy intake. The variation in compensatory energy expenditure response is still relatively unknown, but individual differences, sex differences, exercise intensity, and duration may mediate this response.

Table 2.1 Summary of acute and chronic effect of exercise on appetite-regulating hormones

Hormones	Secreted	Target	Function	Acute Exercise	Chronic Exercise	
					Compensators	Non-Compensators
Acylated Ghrelin	Stomach	NPY/AgRP	Hunger	↓	↓ ↔ ↑	↓
GLP-1	L Cells in small intestine/colon	POMC/CART	Satiety	↑	↔	↑
PYY	L Cells in small intestine/colon	POMC/CART	Satiety	↑	↓	↑
Insulin	Pancreas	POMC/CART	Satiety	↑	↓	
Leptin	Adipose Tissues	POMC/CART	Satiety	↑	↓	

ARC, arcuate nucleus of the hypothalamus; GLP-1, glucagon like peptide-1; PYY, peptide YY

Table 2.2 Summary of neurotransmitters involved in the hedonic appetite-regulatory system

	Secreted	Target	Function
Dopamine	VTA	NA	↑ Wanting
Endogenous Cannabinoids	N-arachidonoyl phosphatidyl ethanol (NAPE) 2-AG from 2-arachidonoyl-containing phospholipids	CB_1 receptors	↑ Reward behaviours
Opioids	Brain, Pituitary	Mu-opioid receptors	↑ Reward behaviours
Serotonin	Neurons in the raphe nuclei	5-HT Receptors	↓ Reward behaviours

NA, nucleus accumbens; VTA, ventral tegmental area

2.4 Rationale for Study

It has been established that body weight is regulated by energy balance, which is a function of energy intake and energy expenditure. While exercise influences energy expenditure, evidence shows that exercise also influences energy intake. Short-term, aerobic exercise, elicits a transient suppression of appetite by influencing gut-derived, appetite-regulatory hormones, contributing to a short-term energy deficit. However, after long-term exercise, the majority of studies show a partial or complete energy compensation, leading to lower than predicted weight loss. The mechanisms mediating this energy compensation are not completely understood. To date, research has not thoroughly examined the relationship between long term aerobic exercise and the gut microbiota, appetite-regulating hormones, the hedonic system, and energy compensation. Most long-term exercise interventions that seek to examine energy compensation and appetite control have been completed in humans. The results from human studies may be confounded by short-term weight loss and poor control of energy intake measures. By using a rat model, we can strictly measure and control food intake, weight changes, and assess appetite regulation in response to chronic exercise to gain a more complete understanding of how chronic exercise

affects the homeostatic appetite-regulatory hormones and influences energy compensation, independent of weight loss.

2.5 Objective and Hypothesis

This study aimed to determine the weight-independent effects of prolonged aerobic exercise on appetite control and energy compensation in male Sprague Dawley rats. Our primary outcome was to assess the impact of 8 weeks of prolonged aerobic exercise training on appetite control signals including anorexigenic (GLP-1), orexigenic (acylated ghrelin), and tonic (leptin, insulin) hormones. The secondary outcome was to examine the effects of exercise on the gut microbiota to look at the potential mechanisms mediating the change in appetite-regulating hormones through the collection of fecal and cecal matter. The third outcome was to assess how prolonged aerobic exercise impacts the hedonic reward pathway by identifying differences in the volume of the NA and area of the VTA. Based on previous literature, we hypothesized that prolonged aerobic exercise would improve appetite control by increasing the concentration of short-term satiety hormones, however these effects on appetite may be negated by reductions in long term homeostatic hormones leptin and insulin. Additionally, prolonged aerobic exercise would improve the gut bacteria composition as well as, increase the volume and area of the NA and VTA, respectively.

CHAPTER 3: METHODS AND MATERIALS

3.1 Animals & Experiment

This study was approved by the Animal Welfare Committee at the University of Lethbridge (Protocol #2104) and was completed in accordance with the ethical standards outlined in the Canadian Council on Animal Care. Thirty, 12-week-old male Sprague Dawley rats were obtained from the Charles Rivers Laboratories (Charles River, St. Constant, PQ). The Sprague Dawley rats were individually housed in techniplast cages with solid floors, shredded paper towels, crinkle paper bedding, and PVC tubes. The rats were housed in temperature-controlled (20-22ºC) and humidity-controlled rooms with controlled 12-hour light-dark cycles (7:00 am - 7:00 pm). Cages were cleaned weekly.

A seven-day acclimatization period took place where rats were given *ad libitum* access to water and introduced to AIN-93M purified diet (Dyets Inc., Bethlehem, PA). The AIN-93M diet has uniform energy density and conforms to the recommendations set forth by the American Institute of Nutrition (149).The rats were weighed daily throughout this period, and food intake was monitored. The nutrient breakdown from the AIN-93M diet is provided in Table 3.1.

The Sprague Dawley rats (n=30) were randomized based on initial body weight into one of three groups: 1) sedentary control (SED); 2) voluntary aerobic exercise (EX); or 3) sedentary and weight-matched to aerobic exercise (SED-WM) for 8 weeks ($n=10$ rats/group). The SED-WM group was included as a means to isolate the weight-independent effects of exercise-induced energy expenditure on appetite-regulating hormones. Randomization was completed to control starting group body weight (400 ± 6g). Initial fecal samples were taken prior to randomization and were placed on ice and stored at -80°C.

Table 3.1 Composition of AIN-93M Diet

(g/kg)	
Casein	140
L-Cystine	1.8
Sucrose	100
Dyetrose	155
Corn-starch	465.7
Soybean Oil	40
t-butylhydroquinone	0.008
Cellulose	50
Mineral Mix	35
Vitamin Mix	10
Choline Bitartrate	2.3
Energy (Kcals/g)	3.61
Protein (% energy)	14.1
Carbohydrate (% energy)	76
Fat (% energy)	9.9

AIN-93M diet based on Reeves et al. (1993a)

3.2 Measurement of Exercise Distance

Rats in the EX-group had voluntary access to running wheels (Techniplast, Philadelphia, USA). Activity tracking (running distance) was monitored daily with Cateye Velo 9 cycling computers (Cateye Co. LTD, Osaka, Japan). In the first two weeks, six revolution counters were used to test the reliability of the cycling computers. Distance (km) was calculated and compared to the Cateye Velo 9 cycle computers. The circumference of the wheel was 96 cm. The Cateye Velo 9 cycle computers recorded 1.05km-1.14km for every 1 km on the revolution counter.

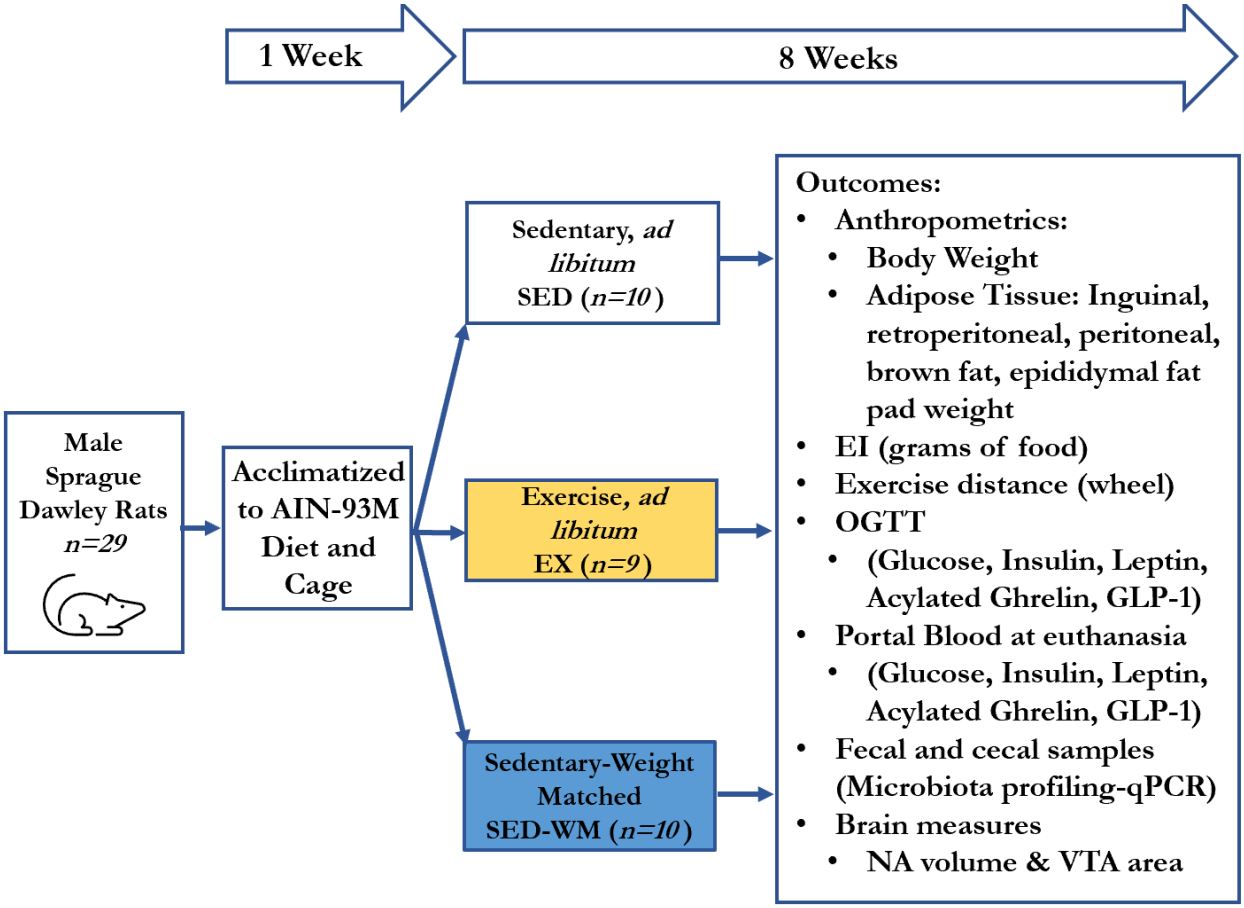


Figure 3.1 Study Schematic.
EI, energy intake; EX, exercise; GLP-1, glucagon like peptide-1; OGTT, oral glucose tolerance test; SED, sedentary; SED-WM, sedentary weight matched.

3.3 Measurement of Energy Intake and Diet Manipulation

Energy intake and body weight were monitored daily. Daily energy intake was measured by subtracting the remaining food from the total food given from the day prior. SED and EX groups received food *ad libitum*. Daily food intake from SED was weighed, and body weight was compared between EX and SED-WM. Food provisions for SED-WM were adjusted accordingly to ensure equal weight between EX and SED-WM. Based on previous research with exercise training, it was estimated that rats in SED-WM would require ~80% of the energy intake of SED (150).

3.4 Oral Glucose Tolerance Test

Fasting conditions were in accordance with previous studies (151). One week prior to euthanasia, an Oral Glucose Tolerance Test (OGTT) was completed after an overnight fast and 12 hours of locked running wheels. Research has clearly demonstrated an anorectic effect on appetite after an acute bout of aerobic exercise, through a suppression of acylated ghrelin and an increase in GLP-1 and PYY. To examine the impact of chronic exercise on appetite-regulating hormones and to mitigate the effects of acute exercise on the satiety hormones, the wheels were locked for 12 hours before the OGTT. Blood collection was completed according to the methods described by Lee & Goosens (152). Briefly, rats were placed on a heating pad to increase tail vein dilation. Blood was sampled by placing an indwelling butterfly catheter in the lateral tail vein. The rats were restrained in plastic tubing, making sure the rat was comfortable but immobile. Next, the lateral tail vein was identified, and sampling area was wiped with chlorhexidine antiseptic solution. Holding the tail firmly on the table, the butterfly catheter was inserted into the tail vein at a shallow angle and secured to the rat's tail using tape. A 300 ul baseline blood sample was collected. After the baseline blood draw, rats received an oral glucose

load (2 mg/kg) via gavage with 50% dextrose solution for a total gavage volume of 4 mg/kg. Additional serial 300ul blood collections were completed at the time points of 15, 30, 60, 90, and 120 minutes. Patency of the catheter was maintained through injection of 0.1 mL of heparin after all blood draws. Collected blood was immediately transferred into cooled Ethylenediaminetetraacetic acid spray-coated microcentrifuge tubes (BD, Mississauga, ON, Canada) containing diprotinin-A (10 μL/mL of blood; MilliporeSigma Corp., ON, Canada), Sigma protease inhibitor (1 mg/mL of blood; SigmaFast, MilliporeSigma Corp), and Roche Pefabloc (1mg/mL of blood; MilliporeSigma Corp) to prevent degradation of the hormones of interest. After each blood draw, blood glucose was also measured using a OneTouch Glucose Meter (OneTouch Glucose Meter, Lifescan Inc.). Blood samples were centrifuged at 2000 rpm for 10 minutes at 4°C, and aliquoted plasma was stored at -80°C until analysis. The composite insulin sensitivity index (CISI) was calculated using the AUC of OGTT fasting and average glucose and insulin concentration, with a higher score meaning improved insulin sensitivity (153). The following formula was used:

$$CISI = \frac{1000}{\sqrt{(AUCGlucosebase\ x\ AUCInsulinbase)x(AUCGlucosemean\ x\ AUCInsulinmean)}}$$

3.5 Tissue and Plasma Collection

The day prior to euthanasia fecal samples were collected from clean rat cages. Fecal samples were placed on ice and stored at -80°C. Following a 12 hour overnight fast and a period of 12 hours of locked wheels, rats were over-anesthetized in an induction chamber with 5% isoflurane. Once rats were in a state of deep anaesthesia, the rats were switched to a nose cone with 2% isoflurane. To assess the state of anesthesia the paw pinch reflex and eyelid reflex were used. For the pinch reflex, if a withdrawal response was observed, it was assumed the rat was still capable

of feeling pain and the rat was placed back into the isoflurane induction chamber until no reflex response was observed.

Once in a state of deep anaesthesia, an incision into the abdomen was made. The portal vein was then located, and a 3 ml blood sample was collected. Portal vein blood was used to assess the concentration of fasting appetite-regulating hormones. Protease inhibitors were added to portal blood immediately after collection and mixed to prevent degradation of samples. Once the blood draw was completed, an incision into the thoracic cavity (pneumothorax) was made, and the heart was removed to complete the euthanasia. The retroperitoneal, peritoneal, epididymal, inguinal, and brown fat pads were precisely excised and weighed. Relative fat mass was calculated by summing the five fat pads and diving by end body weight.

Follow a similar protocol as Soueid et al. the brain was removed through decapitation (154). A razor blade was then used to make a midline incision in the skin to free the skull (154). Using forceps, the interparietal bone was removed along the sagittal suture (154). The parietal bone was then removed using forceps to break off the bone. Once the parietal bones were removed, a cut was made through the most anterior part of the skull between the eyes (154). The frontal bone plate was removed using forceps (154). Throughout the entire procedure, meninges that surrounded the brain were cut, as they could rupture the brain when removing the brain from the skull (154). When the brain was clear of meninges, forceps were inserted under the anterior of the brain (olfactory bulb) and used to separate any additional underlying tissues, optic nerve, and other cranial nerves from the brain (154). Once free from all nerves and other tissues, the brain was removed from the skull and placed in a solution of 10% formalin and sucrose and stored at 2-8°C until analysis.

Cecal matter was collected, placed into liquid nitrogen, and stored at -80°C until analysis. The liver, cecum, colon, and ileum were excised, placed in liquid nitrogen, and then stored at -80°C for future analysis.

3.6 Analysis of Gut Microbiota

Microbial profiling was performed according to previous research (155, 156). Fecal samples were collected from clean rat cages on the day prior to randomization and before euthanasia. A cecal sample was collected post-euthanasia. Fecal and cecal samples were placed on ice and stored at -80°C. DNA was extracted from fecal and cecal samples using the FastDNA spin kit for Feces (MP Biomedical, LLC, Solon, OH, USA). Once extracted, the DNA was purified following the Ethanol Precipitation Protocol. DNA was purified by adding 10 μL of 5 M NaCl samples and inverted 3-5 times to mix. Next, 250 μL of 100% cold ethanol was added to DNA samples and inverted. The samples were left to incubate at -20°C overnight. The next day, the samples were centrifuged at 10,000g for 15 minutes at 4°C, and the liquid was decanted. Next, 100 μL of 75% cold ethanol was added to the DNA pellet, and the samples were centrifuged at 10,000g for 15 minutes at room temperature and decanted again. The samples were left to air dry for 30-60 minutes. The DNA pellet was dissolved in 100 μL TES and vortexed. Using the Nanodrop 1000 (Thermo Fisher Scientific Inc., Waltham, MA, USA), the DNA concentration of the samples was analyzed and recorded. Fecal and cecal samples were normalized to 4 ng/μL for a total volume of 200 μL and placed in -20°C freezer. The gut microbiota bacteria detection was determined using a qPCR. The bacteria being examined were *Bacteroides, Bifidobacterium, Akkermansia muciniphila,* Enterobacteriaceae, *Methanobrevibacter, Lactobacillus, Clostridium coccoides, Clostridium leptum, Clostridium cluster XI, Clostridium cluster I, Faecalibacterium prausnitzii,* and *Roseburia*. Additionally, Firmicutes (*C. Cluster XI & I, Roseburia,*

Lactobacillus, C. coccoides, C. Leptum) was analyzed. Purified DNA from reference strains for bacteria of interest (ATCC, Manassas, VA, USA & DSMZ Gmbh, Germany) was serially diluted to create standard curve. Group specific standards were obtained from Cedarlane (Burlington, ON, Canada) (*Bifidobacterium adolescentis* ATCC 15703D-5; *Bacteroides thetaiotaomicron* ATCC 29148D-5, *Akkermansia muciniphila* ATCC BAA-835D-5, *Escherichia coli* ATCC 8739D-5, *Methanobrevibacter smithii* DSM861D, *Lactobacillus* DSM20557D, *Blautia producta* ATCC 27340D-5, *Clostridium leptum* DSM753D, *Clostridium difficile* ATCC9689D-5, *Clostridium perfringens* ATCC 13124D-5, *Faecalibacterium prausnitzii* DSM17677D, and *Roseburia hominis* DSM16839*)*. Using the reference strain, 16S rRNA copy number and genome size were obtained from the following database https://rrndb.umms.med.umich.edu/. Primers were prepared to 100uM solution by adding appropriate amount of purified water to primer, then diluted by 10 to yield 10uM solution. Table 3.2 provides the gut microbial group specific primers and genomic DNA standards for qPCR according to previous work (155, 156). Using a 96-well plate (Bio-Rad, Hercules, CA, USA), 20ng (5uL) of DNA from standards and samples were added in duplicate. Each well had 20 μL of master mix, including 1 ul/well of both forward and reverse bacteria primers, 12.5 μL/well of supermix (SYBR Green), 5.5 μL/well of molecular water, and 5μL/well of standard or samples for a total volume of 25 μL/well. Using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad laboratories, Hercules, CA, USA) a longitudinal gut microbiota analysis was completed using the fecal and cecal samples. The qPCR was heated for 2 minutes at 95°C, followed by 35 cycles of 95°C for 20 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and a final cycle for 2 minutes at 72°C. Additionally, a melt curve analysis was run at 60°C and increased by 1°C every 8 seconds.

Table 3.2 Gut microbial group specific primers and genomic DNA standards for qPCR

Microbial Group	Primer Sequence, 5'-3' (Forward, F and Reverse, R)	Genomic DNA Standard	Reference
Firmicutes			
Clostridium coccoides (cluster XIV)	F: ACTCCTACGGGAGGCAGC R: GCTTCTTAGTCARGTACCG	Blautia Producta	Amann, Krumholz, & Stahl, 1990; Franks et al., 1998
Clostridium leptum (cluster IV)	F: GCACAAGCAGTGGAGT R: CTTCCTCCGTTTGTCAA	Clostridium leptum	Matsuki, Watanabe, Fujimoto, Takada, & Tanaka, 2004
Clostridium group (cluster I)	F: ATGCAAGTCGAGCGAKG R: TATGCGGTATTAATCTYCCTTT	Clostridium perfringens	Rinttila, Kassinen, Malinen, Krogius, & Palva, 2004
Clostridium group (cluster XI)	F: ACGCTACTTGAGGAGGA R: GAGCCGTAGCCTTTCACT	Clostridium difficile	Song, Liu, & Finegold, 2004
Faecalibacterium prausnitzii	F: AACCTTACCAAGTCTTGACATC R: TTGCGTAGTAACTGACCATAAG	Faecalibacterium prausnitzii	Beacon Designer 3.0
Lactobacillus	F: GAGGCAGCAGTAGGGAATCTTC R:GGCCAGTTACTACCTCTATCCTTCTTC	Lactobacillus jensonii	Delroisse et al., 2008
Roseburia	F: TACTGCATTGGAAACTGTCG R: CGGCACCGAAGAGCAAT	Roseburia hominis	Larsen et al., 2010
Bacteroidetes Bacteroides/Prevotella	F: TCCTACGGGAGGCAGCAGT R: CAATCGGAGTTCTTCGTG	Bacteroides thetaiotaomicron	Bernhard & Field, 2000; Nadkarni, Martin, Jacques, & Hunter, 2002
Actinobacteria Bifidobacterium	F: CGCGTCYGGTGTGAAAG R: CCCCACATCCAGCATCCA	Bifidobacterium adolescentis	Delroisse et al., 2008
Archaea Methanobrevibacter	F: CTCACCGTCAGAATCGTTCCAGTC R: ACTTGAGATCGGGAGAGGTTAGAGG	Methanobrevibacter smithii	Bomhof et al., 2014
Proteobacteria Enterobacteriaceae	F: CATTGACGTTACCCGCAGAAGC R: CTCTACGAGACTCAAGCTTGC	Escherichia coli	Bartosch, Fite, Macfarlane, & McMurdo, 2004
Verrucomicrobia Akkermansia muciniphila	F: TCTTCGGAGGCGTTACACAG R: AGTTGATCTGGGCAGTCTCG	Akkermansia muciniphila	Beacon Designer 3.0

3.7 Measures of the Nucleus Accumbens and Ventral Tegmental Area

Brains were coronally sliced using a Reichert-Jung, Frigocut 2800E Cryostat Microtome (Leica microscopes, Apeldoorn, Netherlands) in series of 8 at 50 μm sections and were placed on super-frost plus microslides (VWR, Radnor, PA, USA). The charged slides were subbed with 2 g of gelatin (G8-500 Fisher) (Thermo Fisher Scientific Inc., Waltham, MA, USA), 0.4 g of chrome alum (0.2%), and 200 ml of deionized water to ensure proper tissue adherence to the slides. The brain slices were stained using a Crystal violet stain (Nissl Stain) protocol. A series of initial ascending alcohol baths and HemoDe baths were used to remove fats and chemicals and dehydrate the tissues. Descending alcohol baths of 100% alcohol, 95% alcohol, and 70% alcohol were used to rehydrate the tissues before staining. After rehydration, tissues were placed in the dH_20 bath for the stain to hold. The tissues were placed in the Cresyl Violet stain solution (1% Cresyl Violet Acetate in dH20). To destain the tissues so differentiation could be seen, tissues were placed in acetic acid-alcohol (2 ml glacial acetic acid in 200ml of 70% ETOH). Finally, the tissues were placed in a HemoDe bath until coverslips were mounted. Excess HemoDe was wiped off, and coverslips were mounted on slides using Permount. After the brain samples were stained and mounted, the Northern Light Imaging Precision Illuminator Model B90 (Imaging Research, St. Catharines, Ontario, Canada), Dino-Lite Camera (Dunwell Tech, Inc, Torrance, CA, USA), and DinoCapture 2.0, version 1.5.37 software (Dunwell Tech, Inc, Torrance, CA, USA), were used to photograph each section of the NA and VTA. The rat brain atlas was used for reference (157) as follows: 1) NA: bregma, 0.48mm to 2.70mm; and 2) VTA: bregma -4.80mm. Images were analyzed using imageJ software (U. S. National Institutes of Health, Bethesda, Maryland, USA) (158). The final volume measure was the sum of all the areas

measured multiplied by the number of sections (8) then multiplied by the thickness of each slice (0.05mm).

3.8 Biochemical Analysis of Appetite-Regulating Hormones

All appetite-regulating hormones, acylated ghrelin (active), glucagon like peptide-1 (total), insulin, and leptin were analyzed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (MilliporeSigma Corp, Billerica, MA) according to the manufacturer's directions. Corticosterone concentration was determined using Corticosterone ELISA Kit (Arbor Assays, Ann Arbor, MI, USA). All blood samples from the OGTT were assayed in singles, as according to the National Centre for the Replacement, Refinement, & Reduction of Animals in Research, less than 2 mL of blood could be safely collected over 24 hours (159). Portal blood samples were assayed in duplicates. Blood glucose was assessed using a OneTouch blood glucose meter (OneTouch Glucose Meter, Lifescan Inc.) immediately after each blood draw.

3.9 Statistical Analysis

IBM SPSS 27.0 software was used to analyze all data. Using G power, a power calculation was used to determine sample size (power level 0.8; alpha level of 0.05) to detect a difference in satiety hormones concentration of 20% based on the standard deviation of ~16% from experiments previously conduced in our lab. To assess normality of data, the Shapiro-Wilk (S.W) test was used with P <0.05 and S.W. <0.8 statistics defined as significantly skewed. The appropriate transformation was used for data that were not normally distributed. Missing values for appetite-regulating hormones were filled using the highest concentration. To determine the difference for all data involving serial measures, mixed-design ANOVA was used to assess time as the within-condition variable and group as the between-condition variable. If a significant

main effect of time was observed, the least significant difference (LSD) test was used to determine the differences for the within condition. A one-way ANOVA was used to determine differences between the treatment groups and time if a significant interaction was identified. A *post hoc* LSD test was used to determine differences between treatment groups if a significant group effect was observed. AUC measure was used to assess the total changes in appetite-regulating hormones after the OGTT. AUC measures were calculated by trapezoidal sums. Additionally, Pearson correlation or Spearman correlation was used to assess the correlation between outcome variables. For all statistical tests, $P<0.05$ was considered statistically significant. All data is represented as the mean ± standard error mean (SEM).

CHAPTER 4: RESULTS

4.1 Body Weight & Body Composition

4.1.1 Body Weight

Baseline body weights were the same between groups (SED: 402.4g ± 11.2; EX: 394.1g ± 6.85; and SED-WM: 400.3g ± 9.11, P=0.819). A main effect of time (P<0.001) and interaction effect of time x group (P=0.043) were observed. No main effect of group was observed (P=0.059) (Figure 4.1A & Table 4.1). A follow up one-way ANOVA showed that body weight was different in week 3 (P=0.043), week 4 (P=0.026), and week 5 (P=0.038). EX had lower body weight than SED in week 3 (P=0.014), week 4 (P=0.012), and week 5 (P=0.016). Body weight in SED-WM was lower than SED in weeks 4 (P=0.033) and 5 (P=0.035). No differences in body weight were observed in week 1 (P=0.316), week 2 (P=0.127), week 6 (P=0.052), week 7 (P=0.073), and week 8 (P=0.106). Overall, it was initially observed that body weight reduced in EX, but no differences were present between SED and EX at the end of the study.

4.1.2 Fat Pads and Relative and Total Fat Mass

A group difference was observed between epididymal (P=0.007), retroperitoneal (P=0.013), peritoneal (P=0.002) and inguinal (P=0.039) fat pad mass (Table 4.2). A *post hoc* test identified that EX had reduced epidydimal (P=0.002), retroperitoneal (P=0.004), peritoneal (P<0.001), and inguinal (P=0.015) fat pad mass relative to SED. EX had reduced epididymal (P=0.038), retroperitoneal (P=0.042), and peritoneal (P=0.013) relative to SED-WM.

A difference was observed for total fat mass (P=0.007) and relative fat mass (P=0.003) between groups (Table 4.2). A *post hoc* test identified that EX had lower total fat mass relative to SED (P=0.002) and SED-WM (P=0.027). Additionally, EX had lower relative fat mass compared to both SED (P=0.002) and SED-WM (P=0.005).

4.2 Food and Energy Intake

To weight match SED-WM to EX group, food intake was restricted within the range of 83.8%-97.8% of SED or an average of 89.6% of SED energy intake throughout the duration of the study. A main effect of time (P<0.001) and time x group interaction (P<0.001) were observed (Table 4.1 & Figure 4.1B). A follow up one way ANOVA demonstrated a difference in food intake at week 1 (P=0.003), week 3 (P=0.001), week 4 (P<0.001), week 5 (P<0.001), week 6 (P=0.032), and week 7 (P=0.002). A *post hoc* test showed an early reduction in EI in EX relative to SED, in week 1 (P=0.001) and week 3 (P=0.016), followed by a steady increase in EI in EX where EI was the same between EX and SED. SED-WM had lower EI than SED in week 1 (P=0.009), week 3 (P<0.001), week 4 (P<0.001), week 5 (P<0.001), week 6 (P=0.042), and week 7 (P=0.050). Additionally, SED-WM had lower EI than EX in week 3 (P=0.044), week 4 (P<0.001), week 5 (P<0.001), week 6 (P=0.011), and week 7 (P<0.001). A main effect of group (P=0.010) was observed. A *post hoc* test identified a difference between SED-WM and both SED (P=0.003) and EX (P=0.034), with SED-WM having lower EI relative to SED and EX. There was no overall difference observed for EI between SED and EX. There was a positive Pearson's Correlation between total energy intake and final body weight (r =0.892, P<0.001), total fat (r = 0.774, P=0.001), and relative fat mass (r =0.619, P=0.005) (Figure 4.2).

Table 4.1 Weekly body weight, food intake, energy intake, and exercise distance for SED, EX, and SED-WM over an 8-week period

		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Body Weight (g)	SED	423.6 ± 11.9	464.7 ± 13.8	501.5 ± 16.0[a]	534.4 ± 18.1[a]	561.8 ± 20.2[a]	587.7 ± 21.8	610.5 ± 23.6	625.5 ± 23.8
	EX	403.1 ±7.19	432.9 ± 9.93	454.3 ± 12.62[b]	478.6 ± 15.90[b]	501.5 ± 18.76[b]	525.8 ± 21.02	550.2 ± 22.46	565.1 ± 24.27
	SED-WM	409.6 ± 8.34	444.2 ± 7.25	471.9 ± 7.69[ab]	488.9 ± 7.70[b]	511.0 ± 7.87[b]	534.7 ± 8.09	555.5 ± 8.68	576.5 ± 8.75
Food Intake (g)	SED	27.4 ± 1.02[a]	26.2 ± 0.88	27.5 ± 1.08[a]	27.3 ± 0.83[a]	27.8 ± 0.93[a]	27.2 ± 1.05[a]	27.0 ± 0.99[a]	25.8 ± 0.74
	EX	23.1 ± 0.89[b]	24.0 ± 0.79	24.9 ± 0.47[b]	26.9 ± 0.59[a]	27.4 ± 0.79[a]	28.0 ± 0.89[a]	29.2 ± 0.70[a]	26.3± 0.58
	SED-WM	24.4 ± 0.13[b]	25.7 ± 0.46	22.7 ± 0.23[c]	23.0 ± 0.18[b]	23.3 ± 0.28[b]	24.8 ± 0.37[b]	24.8 ± 0.51[b]	24.9 ± 0.23
Energy Intake (kcal)	SED	99.0 ± 3.67[a]	94.7 ± 3.21	99.1± 3.89[a]	98.5 ± 2.98[a]	100.4 ± 3.36[a]	98.2 ± 3.79[a]	97.3 ± 3.57[a]	93.2 ± 2.66
	EX	83.3 ± 3.22[b]	86.7 ± 2.83	89.7 ± 1.69[b]	97.1 ± 2.12[b]	98.7 ± 2.86[a]	100.9 ± 3.22[a]	105.3 ± 2.53[a]	94.9 ± 2.08
	SED-WM	88.0 ± 0.47[b]	92.6 ± 1.64	82.0 ± 0.83[c]	82.8 ± 0.66[b]	84.1 ± 1.00[b]	89.4 ± 1.34[b]	89.5 ± 1.82[b]	89.9 ± 0.85
Exercise Distance (km)	EX	1.01 ± 0.13	3.47 ± 0.72	4.80 ± 0.87	4.61 ± 0.78	4.11 ± 0.81	3.78 ± 0.81	2.83 ± 0.63	2.82 ± 0.71

Values are means ± SEM, n = 9-10/gp. Mixed design repeated measures ANOVA performed to determine main effect of time, group, and their interaction. When an interaction effect was observed between time x group, superscripts indicate significant differences between groups. Labeled means at a time without a common letter differ, $P<0.05$. EX, exercise; SED, sedentary; SED-WM, sedentary weight-matched.

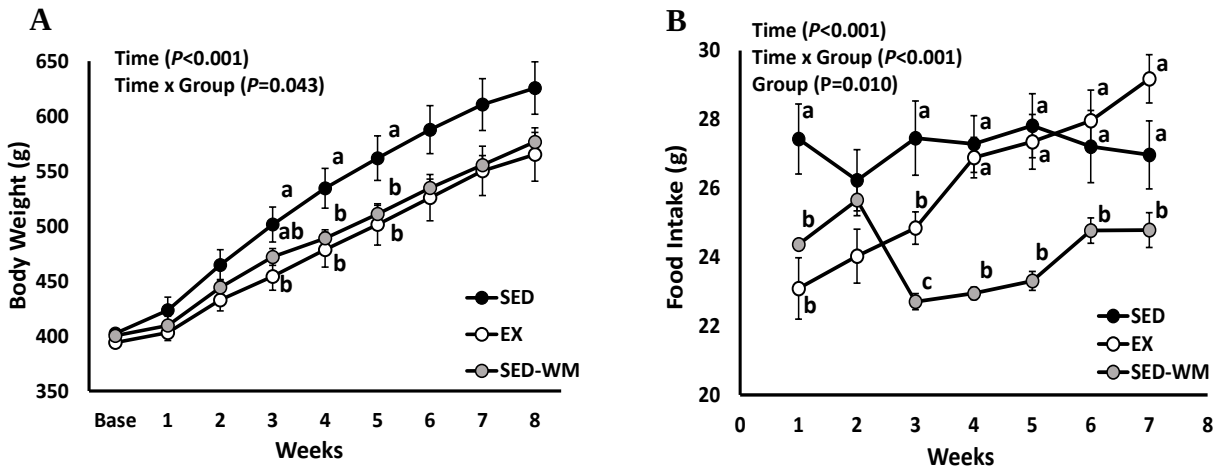


Figure 4.1 (A) weekly body weight and (B) food intake for SED, EX, and SED-WM over 8 weeks.

Values are means ± SEM, *n* = 9-10/gp. When an interaction effect was observed between time x group, superscripts indicate significant differences between groups. Labeled means at a time without a common letter differ, *P*<0.05. EX, exercise; SED, sedentary; SED-WM, sedentary weight-matched.

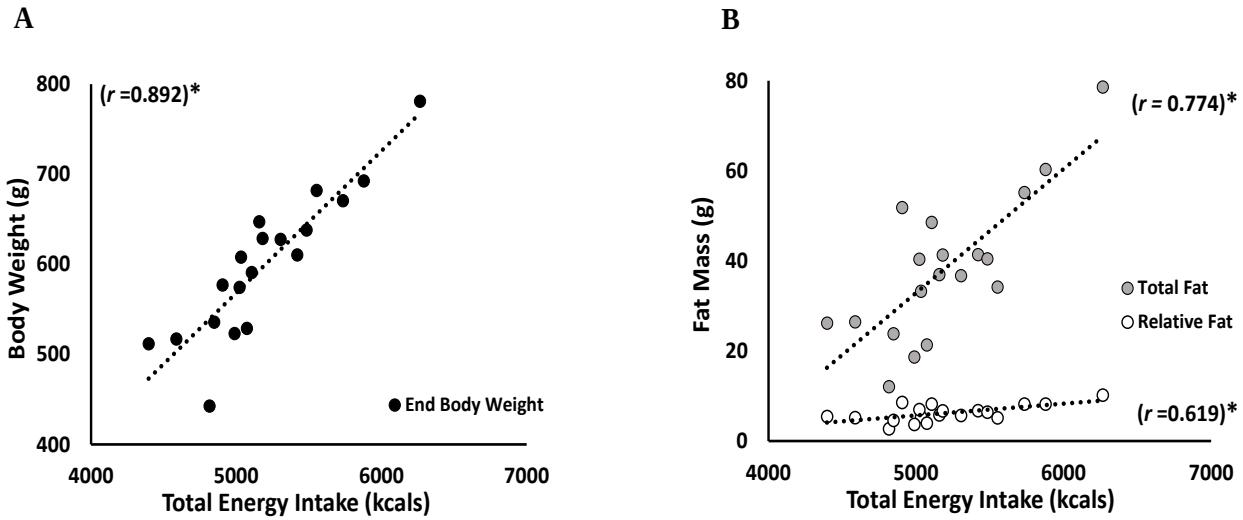


Figure 4.2 Correlation between total energy intake and *(A)* end body weight and *(B)* total fat and relative fat mass.

Values are means, n =9-10/gp. * Indicates significant correlation, P<0.05.

Table 4.2 Anthropometrics of SED, EX, and SED-WM rats after 8 weeks

	SED	EX	SED-WM
Final body weight (g)	628.1 ± 23.7	567.3 ± 25.3	582.0 ± 8.6
Total fat (g)	47.3 ± 4.8[a]	28.3 ± 3.4[b]	41.4 ± 3.2[a]
Relative fat mass (fat mass (g)/body weight(g))	7.4 ± 0.5[a]	4.9 ± 0.4[b]	7.1 ± 0.5[a]
Epididymal fat (g)	12.0 ± 1.0[a]	7.2 ± 1.1[b]	10.2 ± 0.7[a]
Brown fat (g)	0.3 ± 0.02	0.3 ± 0.03	0.3 ± 0.03
Retroperitoneal fat (g)	14.0 ± 1.5[a]	8.3 ± 1.2[b]	12.1 ± 1.0[a]
Peritoneal fat (g)	4.6 ± 0.4[a]	2.5 ± 0.4[b]	3.9 ± 0.3[a]
Inguinal fat (g)	16.4 ± 2.2[a]	10.1 ± 1.1[b]	14.8 ± 1.5[ab]

Values are means ± SEM, n = 9-10/gp. Superscripts indicate significant differences between groups. Labeled means without a common letter differ, $P<0.05$. EX, exercise; SED, sedentary; SED-WM, sedentary weight-matched.

4.3 Weekly Exercise Distance

One rat (Rat #45) from the EX-group was excluded from the study due to lack of exercise. A main effect of time was observed ($P<0.001$) (Table 4.1 & Figure 4.3). Exercise distance was higher in all weeks relative to week 1. Exercise distances increased from week 1 to week 2 ($P=0.005$) and week 2 to week 3 ($P=0.005$). Exercise peeked at week 3 ($M=4.77$ km) and stayed constant until week 6 when exercise decreased between week 6 to week 7 ($P=0.012$). Total exercise distance in EX was negatively correlated with final body weight ($r=-0.691$, $P=0.039$), total fat ($r=-0.792$, $P=0.011$), and relative fat ($r=-0.804$, $P=0.009$) (Figure 4.4).

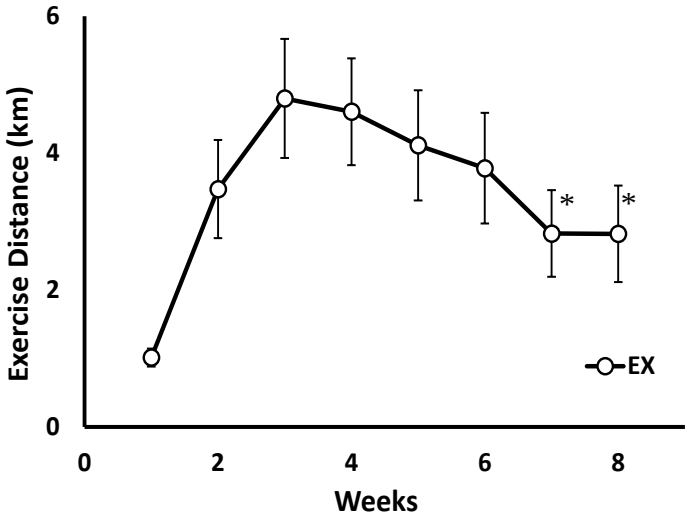


Figure 4.3 Exercise distance for EX over 8 weeks.
Values are means ± SEM, *n* = 9. *indicates significant difference from baseline, # indicates significant difference from timepoint before, *P*<0.05. EX, exercise.

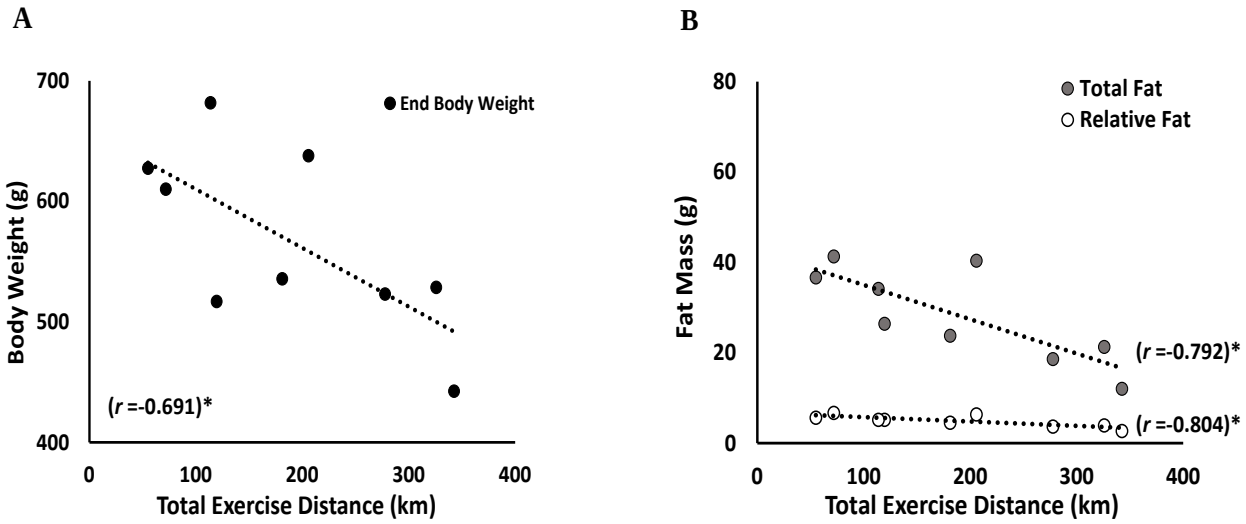


Figure 4.4 Correlation between total exercise distance and *(A)* end body weight and *(B)* total fat and relative fat mass.
Values are means, *n* =9. ***** Indicates significant correlation, *P*<0.05.

4.4 Glycemic and Appetite-Regulating Hormone Response

4.4.1 Glucose

There was a main effect of time (P<0.001) and a trend towards a time x group interaction effect for glycemic response to an OGTT(P=0.055). No differences were observed between groups (P=0.407) (Figure 4.5A(i)), AUC glucose (P=0.336) (Figure 4.5A(ii)) or portal glucose concentration (P=0.326) (Table 4.3).

4.4.2 Insulin

A main effect of time (P<0.001) and group (P=0.028) were observed. There was no interaction effect between time x group (P=0.346) (Figure 4.5 B(i)). A *post hoc* test identified a group difference between EX and SED (P=0.008), with EX having lower insulin relative to SED. A group difference in AUC insulin was observed (P=0.038), with SED having higher AUC insulin than EX (P=0.010) (Figure 4.5B(ii)). No difference between groups was observed for portal insulin concentration (P=0.138) (Table 4.3). Total exercise distance was negatively correlated with fasting insulin (r =-0.798, P=0.010). There was a positive Spearman's Correlation with fasting insulin and final body weight (ρ=0.486, P=0.007), total fat (ρ=0.662, P<0.001), and relative fat (ρ=0.652, P<0.001) and a positive Pearson's Correlation for total AUC insulin and final body weight (r =0.481, P=0.008), total fat (r =0.695, P<0.001), and relative fat (r =0.673, P<0.001).

4.4.3 Composite Insulin Sensitivity Index (CISI)

A main group effect was observed (SED: 2.89 ± 0.37; EX: 4.36 ± 0.40; and SED-WM: 2.69 ± 0.21, P=0.004). A *post hoc* test detected that EX had higher CISI than both SED (P=0.004) and SED-WM (P=0.003).

4.4.4 Leptin

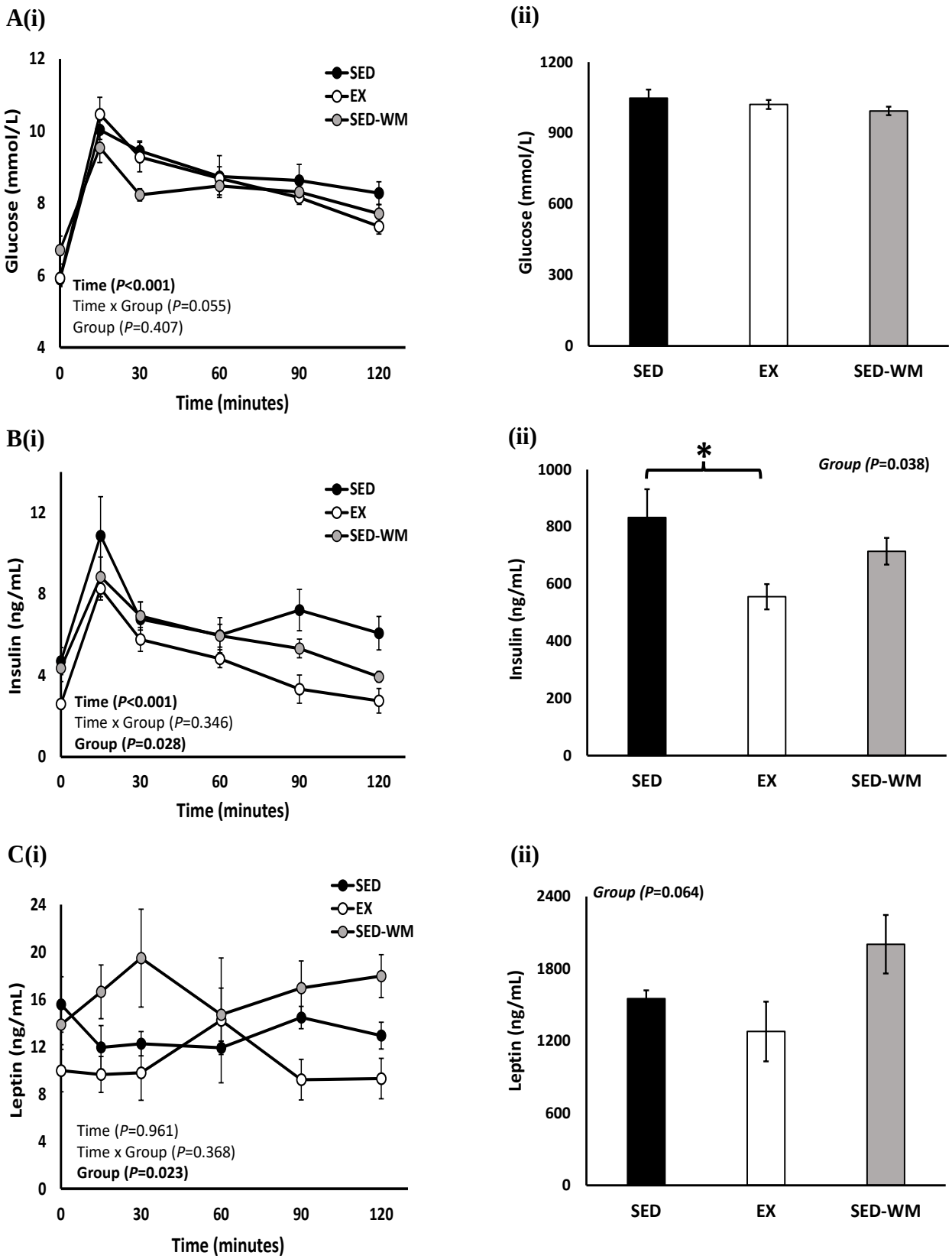
A main group effect was observed (P=0.023). No main effect of time (P=0.961) or interaction effect of time x group (P=0.368) were observed (Figure 4.5C(i)). A *post hoc* test identified a group difference between EX and SED-WM (P=0.006), EX had lower leptin relative to SED-WM. Group differences in AUC leptin trended towards significance (P=0.064) (Figure 4.5C(ii)). A main effect of group on portal leptin concentration was observed (P=0.009) (Table 4.3). A *post hoc* test showed a difference between EX and SED (P=0.022) and SED-WM (P=0.003), with SED-WM and SED having higher portal leptin concentrations relative to EX. There was a positive Pearson's Correlation between fasting OGTT and portal leptin and final body weight (r =0.571, P=0.001; r =0.440, P=0.017, respectively), total fat (r =0.696, P<0.001; r =0.736, P=<0.001, respectively) and relative fat (r =0.666, P<0.001; r =0.736, P<0.001, respectively). In EX, total exercise distance was negatively correlated with fasting leptin (r =-0.747, P=0.021) and portal leptin (r =-0.762, P=0.017).

4.4.5 Acylated Ghrelin

A main effect for time (P<0.001) and an interaction effect between time x group (P=0.048) were observed. No group effect was observed (P=0.377) (Figure 4.5D(i)). A follow up one-way ANOVA detected differences at time 30 (P=0.037), where SED-WM had reduced acylated ghrelin concentration relative to SED (P=0.025) and EX (P=0.027). No difference in AUC acylated ghrelin concentration (P=0.435) (Figure 4.5D(ii)) or portal acylated ghrelin concentration (P=0.247) (Table 4.3) were observed between groups. Total exercise distance positively correlated with fasting acylated ghrelin (r =0.862, P=0.003).

4.4.6 Glucagon-Like-Peptide-1 (GLP-1)

A main effect of time was observed (P=0.026). No time x group interaction (P=0.495) or group effect (P=0.889) were observed (Figure 4.5E(i)). No differences in AUC GLP-1(P=0.623) (Figure 4.45(ii)) or portal GLP-1 concentration (P=0.295) (Table 4.3) were observed between groups. Total exercise distance was negatively correlated with fasting GLP-1 (r =-0.671, P=0.048).



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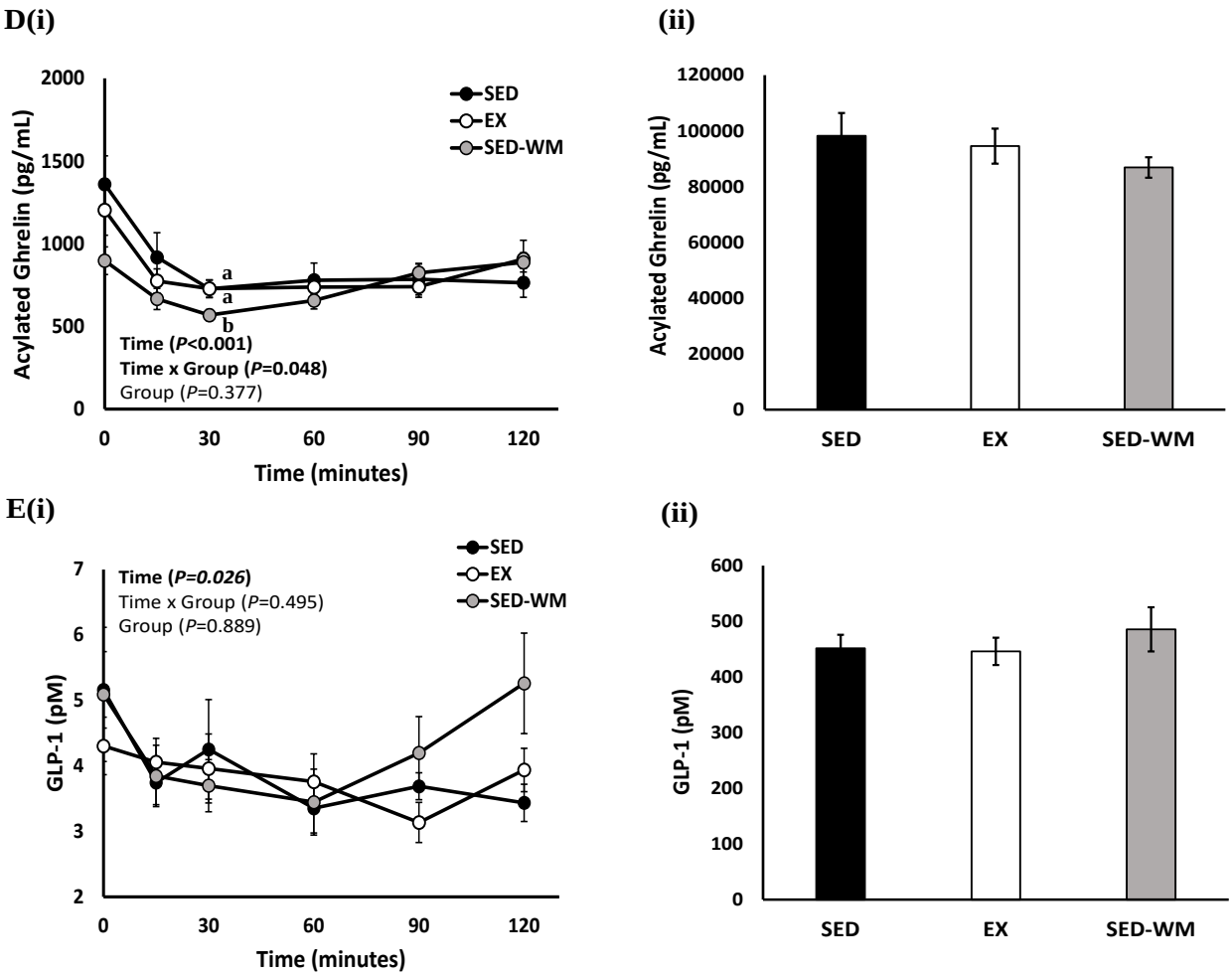


Figure 4.5 Measure of appetite-regulating hormones for SED, EX, and SED-WM during an OGTT. *(i)* Concentrations and *(ii)* total AUC measures of *(A)* glucose, *(B)* insulin, *(C)* leptin, (D) AG, and *(E)* GLP-1.

Values are means ± SEM, n = 9-10/gp. When a time x group effect was observed [abc] superscripts indicate significant group differences, labeled means at a time without a common letter differ, $P<0.05$. AG, acylated ghrelin, AUC, area under the curve; EX, exercise; GLP-1, glucagon like peptide-1; OGTT, oral glucose tolerance test; SED, sedentary; SED-WM, sedentary weight-matched.

Table 4.3 Fasting portal blood concentrations of glucose and appetite-regulating hormones in SED, EX, SED-WM rats after 8 weeks

	SED	EX	SED-WM
Glucose (mmol/L)	6.45 ± 0.23	6.12 ± 0.16	6.05 ± 0.19
Insulin (ng/mL)	8.46 ± 1.89	13.04 ± 1.63	9.19 ± 2.24
Leptin (ng/mL)	9.81 ± 0.90[a]	5.28 ± 1.22[b]	11.32 ± 1.65[a]
Acylated Ghrelin (ng/mL)	1.05 ± 0.14	1.22 ± 0.12	0.854 ± 0.082
GLP-1 (pM)	16.20 ± 1.96	17.30 ± 2.26	12.97± 1.77

Values are means ± SEM, n = 9-10/gp. Superscripts indicate significant differences between groups. Labeled means without a common letter differ, P<0.05.AG, acylated ghrelin; EX, exercise; GLP-1, glucagon like peptide-1; SED, sedentary; SED-WM, sedentary weight-matched.

4.4.7 Corticosterone

A main effect of time (P<0.001), time x group interaction (P=0.004), and group effect (P<0.001) were observed (Figure 4.6A). A *post hoc* comparison revealed that corticosterone decreased continuously over time during the OGTT. At baseline, corticosterone concentration was higher than at times 60min (P=0.009) and time 120 min (P<0.001). At time 60 min, corticosterone concentration was higher than at time 120 min (P<0.001). A follow up one way ANOVA was used to determine the interaction effect on time x group. Group differences were observed at time 0 min (P=0.041), time 60 min (P=0.004), and time 120 min (P<0.001). SED had higher corticosterone than EX (P=0.019) and SED-WM (P=0.049) at time 0 min. EX had lower corticosterone than SED (P=0.001) and SED-WM (P=0.021) at time 60 min. At time 120 minutes, EX had lower corticosterone than SED (P<0.001) and SED-WM (P<0.001). A *post hoc* test identified that SED had higher overall corticosterone than EX (P<0.001) and SED-WM (P=0.043), and SED-WM had higher corticosterone than EX (P=0.002). Additionally, a group difference in AUC corticosterone concentration was observed (P=0.009) (Figure 4.6B), with SED having higher AUC corticosterone relative to EX (P=0.001). No difference between groups was observed for portal corticosterone (P=0.064) (Figure 4.6C).

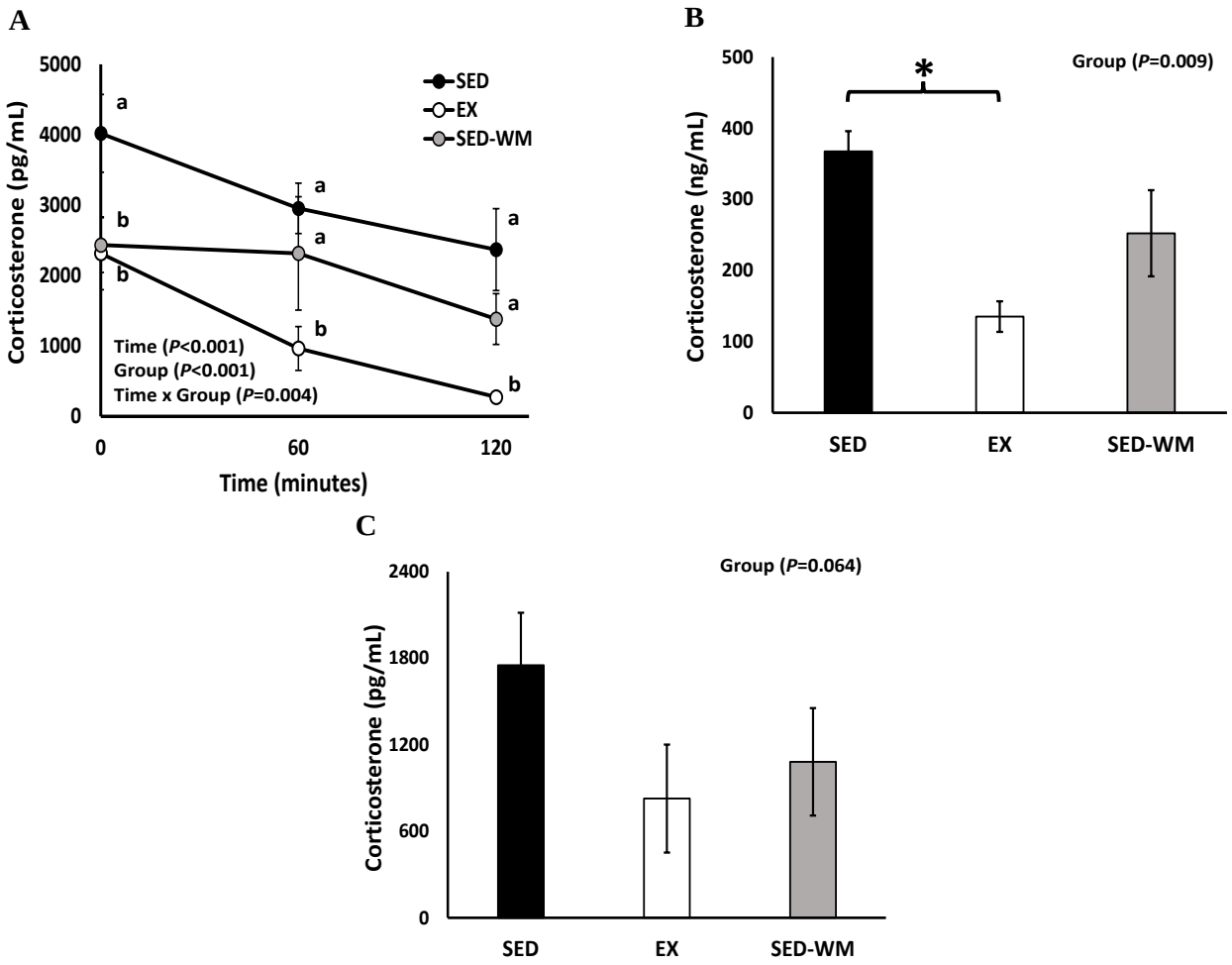


Figure 4.6 Measure of corticosterone for SED, EX, and SED-WM *(A)* Concentration at time 0-, 60-, and 120-minutes during OGTT; *(B)* total AUC; and *(C)* portal concentration. Values are means ± SEM, n = 9-10/gp. Superscripts or * indicate significant group differences. Labeled means at a time without a common letter differ, $P<0.05$. AUC, area under the curve; EX, exercise; OGTT, oral glucose tolerance test; SED, sedentary; SED-WM, sedentary weight-matched.

4.5 Fecal Gut Microbiota

4.5.1 Pre-Intervention

There were no group differences in *Bacteroides* (*P*=0.421), *Bifidobacterium* (*P*=0.193), *Akkermansia* (*P*=0.703), Enterobacteriaceae (*P*=0.161), *Methanobrevibacter* (*P*=0.064), *Lactobacillus* (*P*=0.365), *C. coccoides* (*P*=0.256), *C. leptum (P=0.990), C. cluster I (P=0.099), C. cluster XI (P*=0.397*), F. prausnitzii (P*=0.473), *Roseburia (P*=0.666*),* or Firmicutes *(P*=0.538) (Figure 4.7).

4.5.2 Time Effect

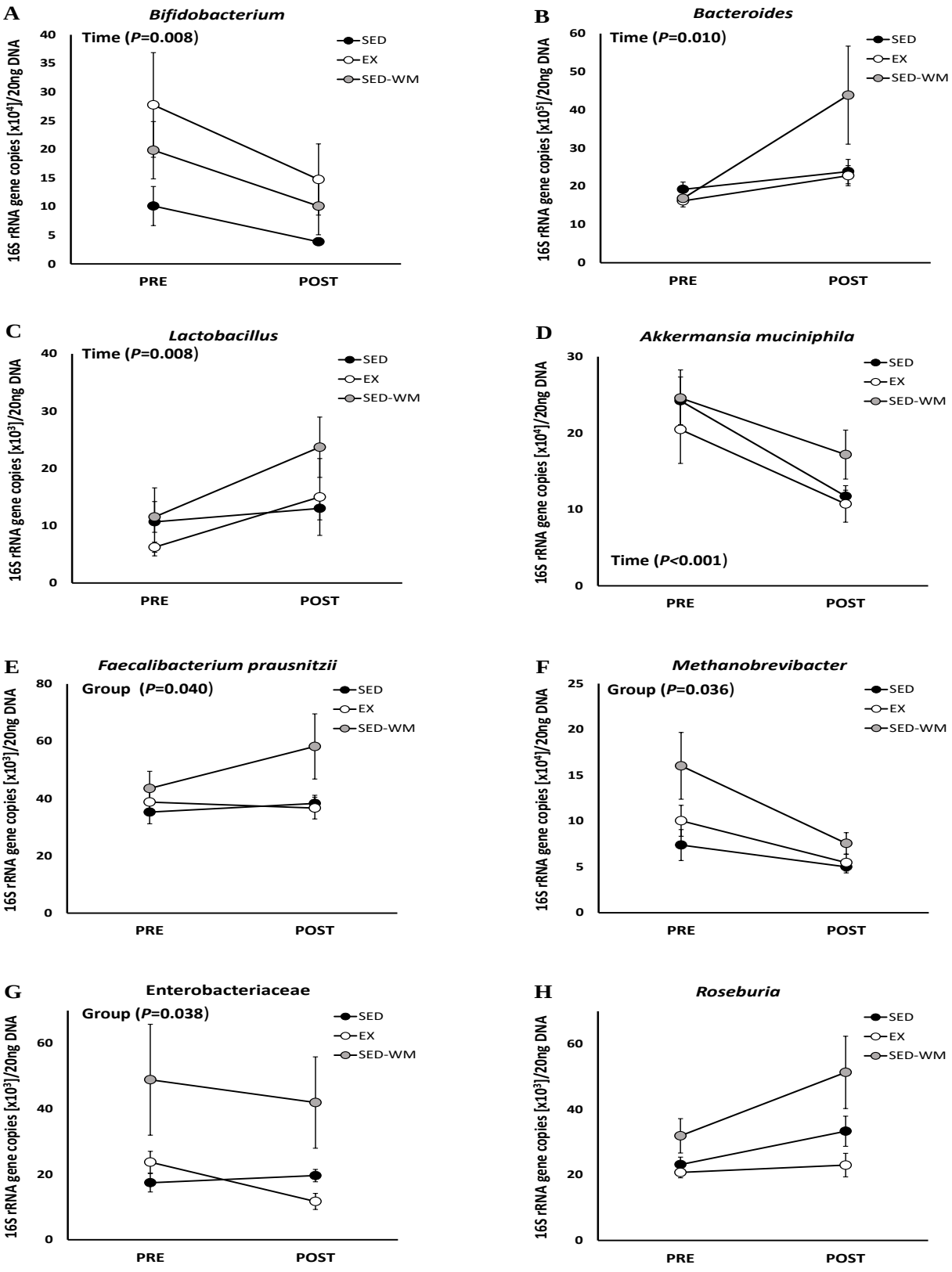
A main effect of time was observed for *Bacteroides* (*P*=0.010), *Lactobacillus* (*P*=0.008), *C. leptum (P=0.015), and C. cluster I (P=0.021),* with a higher concentration of each bacteria detected post intervention relative to pre-intervention. Additionally, a main effect of time was observed for *Bifidobacterium* (*P*=0.008), *Akkermansia* (*P*<0.001), *Methanobrevibacter* (*P*=0.001), and *C. cluster XI (P*<0.001*),* with lower concentrations of each bacteria detected post intervention relative to pre-intervention. No main effect of time was observed for Enterobacteriaceae (*P*=0.323), *C. coccoides* (*P*=0.767), *F. prausnitzii (P*=0.332), *Roseburia (P*=0.157*),* and Firmicutes *(P*=0.149) (Figure 4.7).

4.5.3 Interaction Effect

No time x group interactions were observed for *Bacteroides* (*P*=0.231), *Bifidobacterium* (*P*=0.712), *Akkermansia* (*P*=0.726), Enterobacteriaceae (*P*=0.580), *Methanobrevibacter* (*P*=0.210), *Lactobacillus* (*P*=0.815), *C. coccoides* (*P*=0.220), *C. leptum (P*=0.890), *C. cluster I (P*=0.292), *C. cluster XI (P*=0.707*), F. prausnitzii (P*=0.427), *Roseburia (P*= 0.063*), or* Firmicutes *(P*= 0.420) (Figure 4.7).

4.5.4 Group Effect

A main group effect was observed for Enterobacteriaceae (*P*=0.038), *Methanobrevibacter* (*P*=0.036), *C. coccoides* (*P*=0.013), *C. cluster XI* (*P*=0.013), *F. prausnitzii* (*P*=0.040). A *post hoc* test determined SED had lower overall *Methanobrevibacter* than SED-WM (P=0.013). SED-WM had higher overall Enterobacteriaceae, *C. coccoides*, *C. cluster XI*, and *F. prausnitzii* than SED (P=0.027, P=0.012, P=0.040, P=0.022, respectively) and EX (P=0.026, P=0.009, P=0.004, P=0.036, respectively). No group effect was observed for *Bacteroides* (*P*=0.307), *Bifidobacterium* (*P*=0.118), *Akkermansia* (*P*=0.239), *Lactobacillus* (*P*=0.137), *C. leptum (P=0.852), C. cluster I (P*=0.260*), Roseburia (P*= 0.277*),* or Firmicutes (*P*= 0.071) (Figure 4.7).



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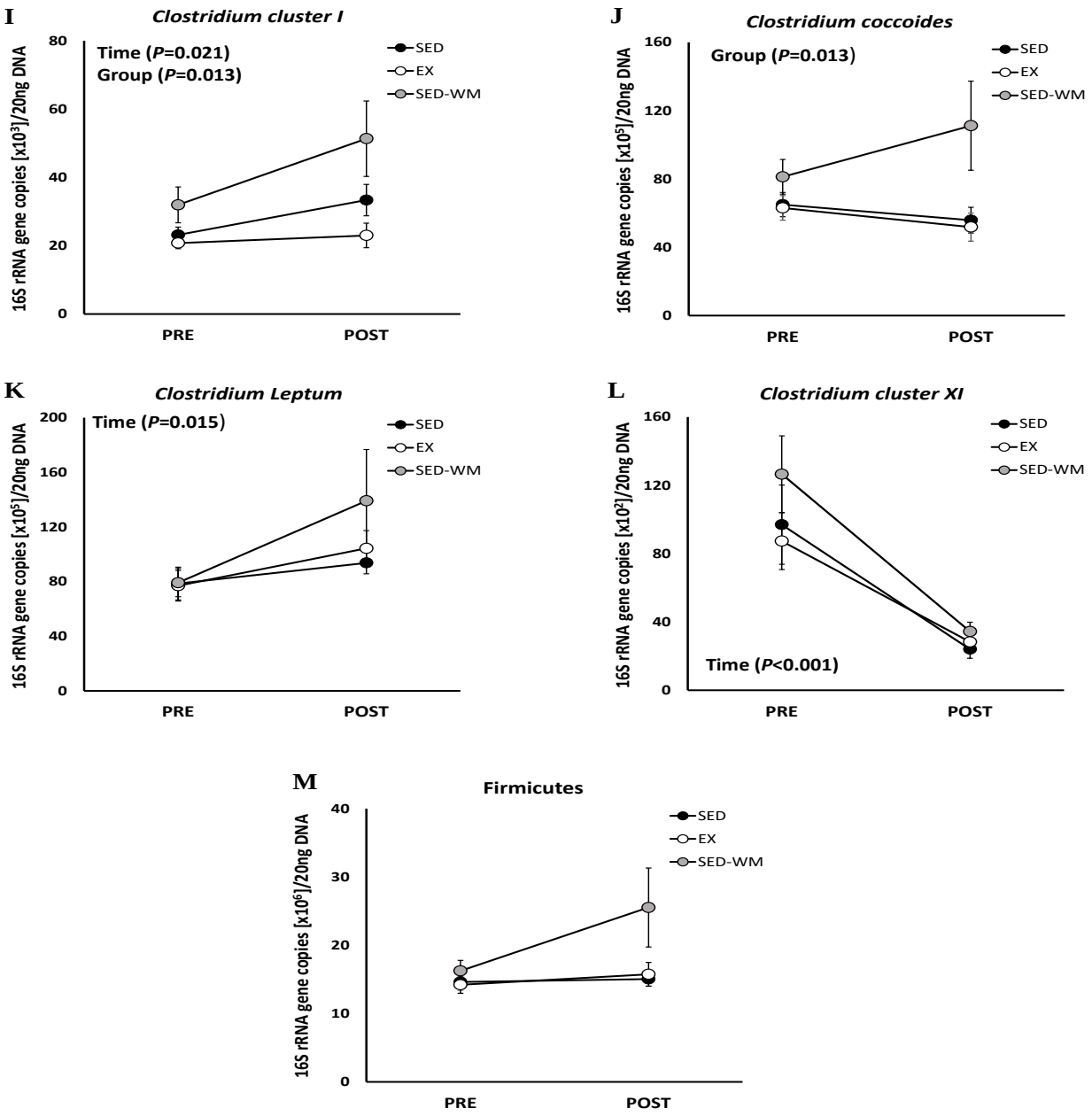
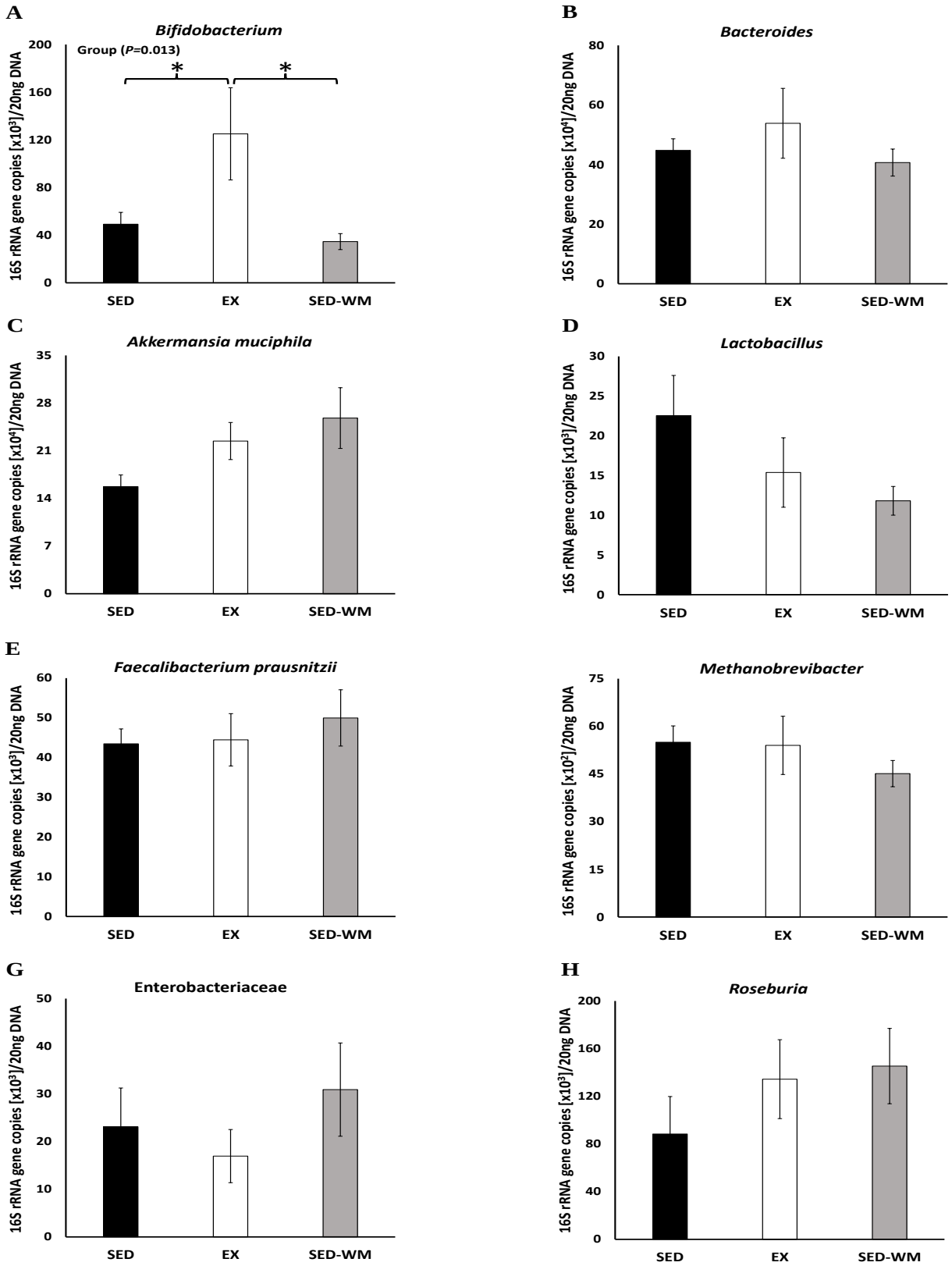


Figure 4.7 Fecal 16S rRNA gene copy/20ng of genomic DNA in SED, EX, and SED-WM rats over 8-weeks for *A) Bifidobacterium; B) Bacteroides/Prevotella; C) Akkermansia muciniphila; D) Lactobacillus; E) Faecalibacterium prausnitzii; F) Methanobrevibacter; G) Enterobacteriaceae; H) Roseburia; I) Clostridium cluster I; J) Clostridium coccoides (cluster XIV); K) Clostridium leptum (cluster IV); L) Clostridium cluster XI; M)* Firmicutes.
Values are means ± SEM, n = 9-10/gp. When an interaction effect was observed between time x group, superscripts indicate significant differences between groups. Labeled means at a time without a common letter differ, $P<0.05$. DNA, deoxyribonucleic acid; EX, exercise; rRNA, ribosomal ribonucleic acid; SED, sedentary; SED-WM, sedentary weight-matched.

4.6 Cecal Gut Bacteria

A group difference was observed in *Bifidobacterium* (P=0.013), EX had more *Bifidobacterium* than SED (P=0.036) and SED-WM (P=0.004). Additionally, a difference in *C. cluster I* was observed between groups (P<0.001). A *post hoc* test demonstrated that SED had more *C. cluster I* than EX (P=0.003) and SED-WM (P<0.001). The intervention did not affect *Bacteroides* (P=0.479), *Akkermansia* (P=0.097), Enterobacteriaceae (P=0.669), *Methanobrevibacter* (P=0.476), *Lactobacillus* (P=0.265), *C. coccoides* (P=0.171), *C. leptum* (P=0.106), *C. cluster XI* (P=0.926), *F. prausnitzii* (P=0.704), *Roseburia* (P=0.252), Firmicutes (P=0.136) (Figure 4.8).



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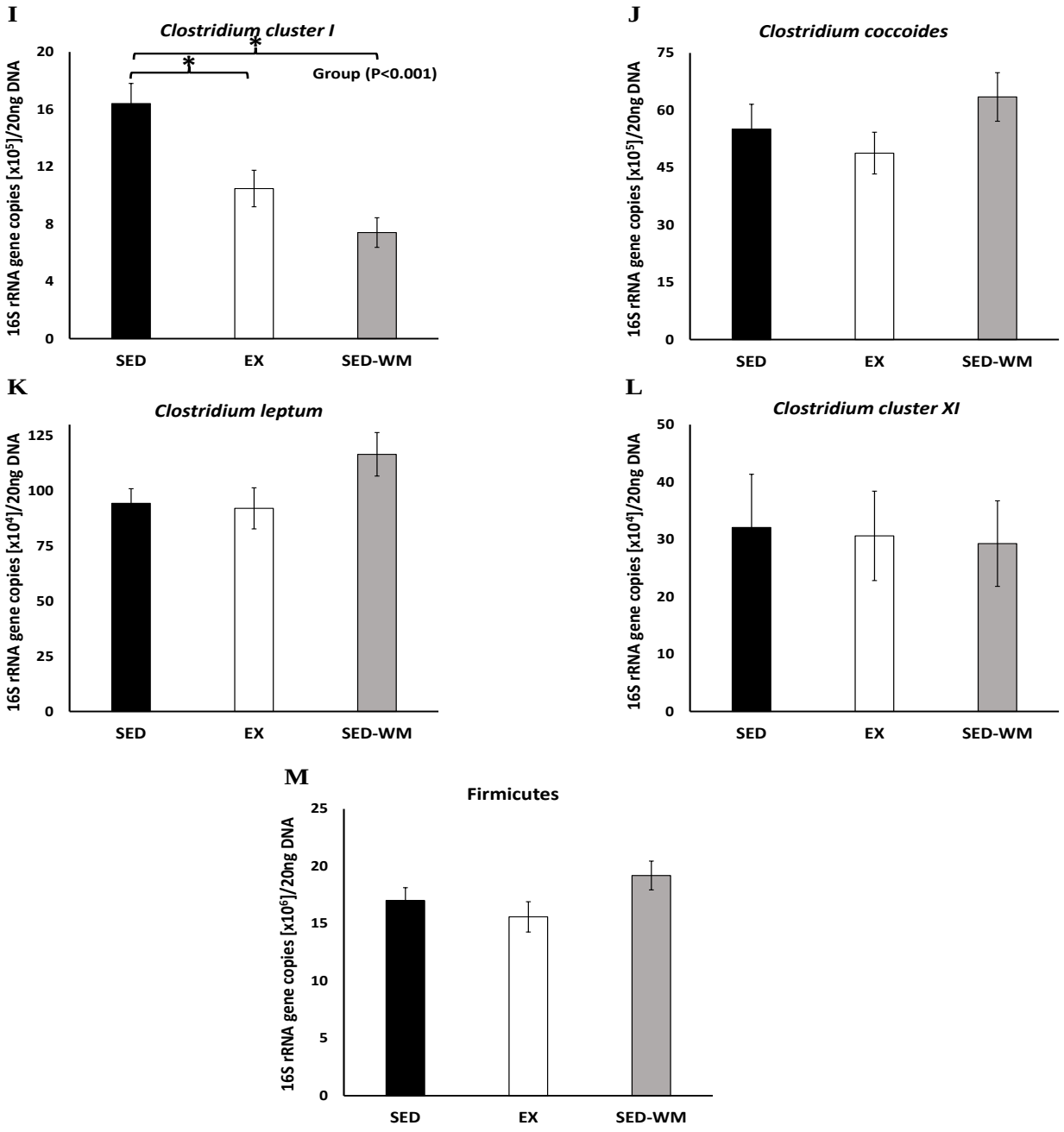

Figure 4.8 Cecal 16S rRNA gene copy/20ng of genomic DNA in SED, EX, and SED-WM rats over an 8-week period for *A) Bifidobacterium; B) Bacteroides/Prevotella; C) Akkermansia muciniphila; D) Lactobacillus; E) Faecalibacterium prausnitzii; F) Methanobrevibacter; G) Enterobacteriaceae; H) Roseburia: I) Clostridium cluster I; J) Clostridium coccoides (cluster XIV); K) Clostridium leptum (cluster IV); L) Clostridium cluster XI; M) Firmicutes.*

Values are means ± SEM, n = 9-10/gp. When a group effect was observed, * indicates significant group differences, $P<0.05$. DNA, deoxyribonucleic acid, EX, exercise; rRNA, ribosomal ribonucleic acid; SED, sedentary; SED-WM, sedentary weight-matched.

4.7 Hedonic System

4.7.1 Nucleus Accumbens Volume

No differences between groups and left NA volume (P=0.567) or right NA volume (P=0.225) were observed (Figure 4.9) There was a positive correlation between total EI and left NA volume (r =0.462, P=0.046). No correlation was observed between total EI and right NA (r =0.118, P=0.630) (Figure 4.10).

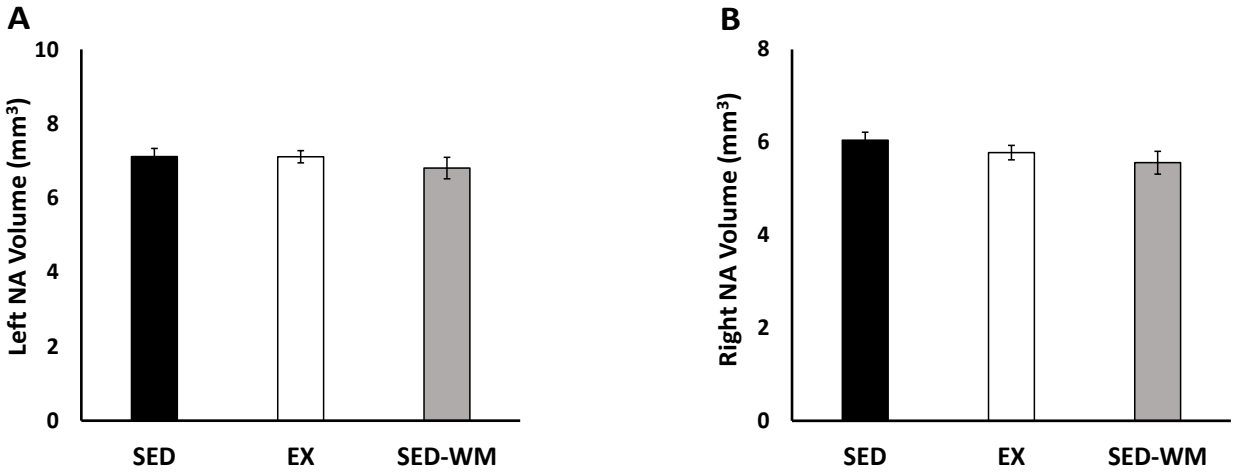

Figure 4.9 *(A)* left NA volume *and (B)* right NA volume for SED, EX, and SED-WM after 8 weeks.
Values are means ± SEM, n = 9-10/gp. When a group effect was observed * indicates significant group differences. EX, exercise; NA, nucleus accumbens; SED, sedentary; SED-WM, sedentary weight-matched,

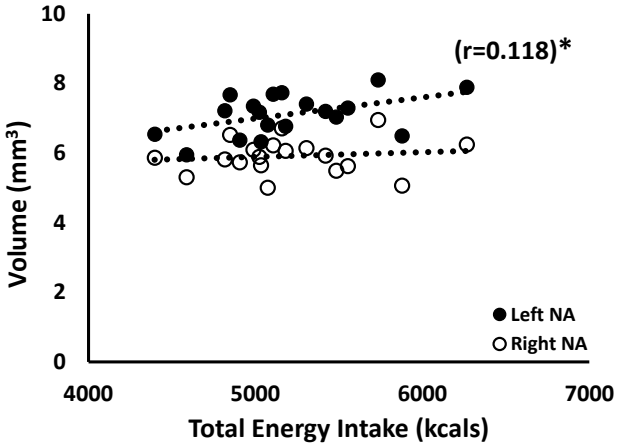

Figure 4.10 Correlation between total energy intake and left and right NA volume.
Values are means, n=9-10/group. Open circles display the right NA. Filled circles display left NA. ***** Indicates significant correlation, P<0.05. NA, nucleus accumbens.

4.7.2 Ventral Tegmental Area

Left VTA or right VTA at Bregma -4.80 were not different between groups (P=0.623; P=0.424, respectively) (Figure 4.11). There was no correlation between total EI and left VTA (r =-0.318, P=0.199) or right VTA (r =0.333, P=0.177) (Figure 4.12).

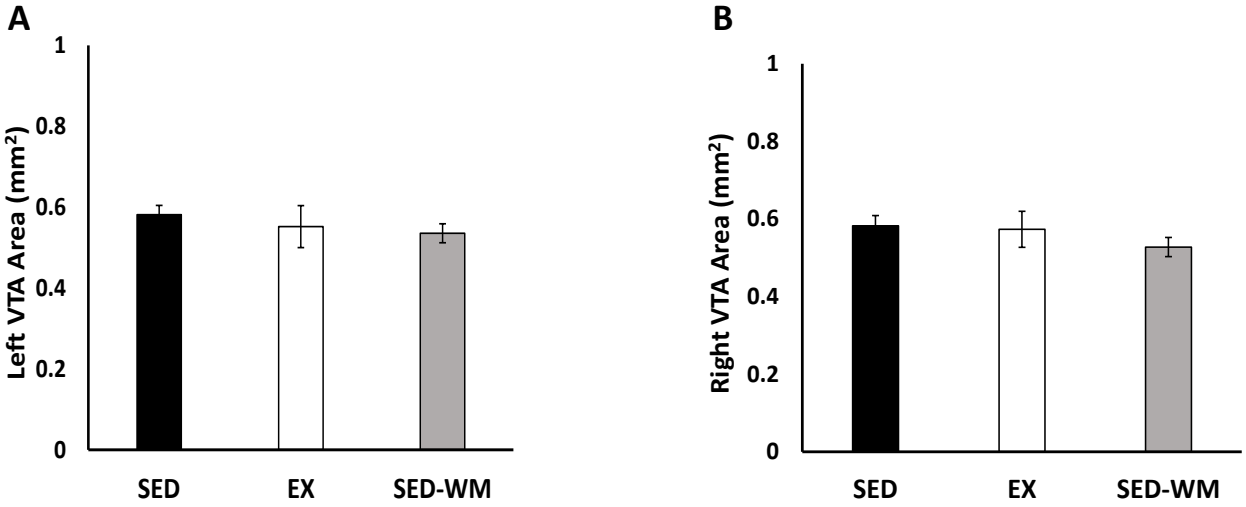

Figure 4.11 *(A)* left ventral tegmental area *and (B)* right ventral tegmental area for SED, EX, and SED-WM after 8 weeks.
Values are means ± SEM, n = 9-10/gp. EX, exercise; SED, sedentary; SED-WM, sedentary weight matched; VTA, ventral tegmental area.

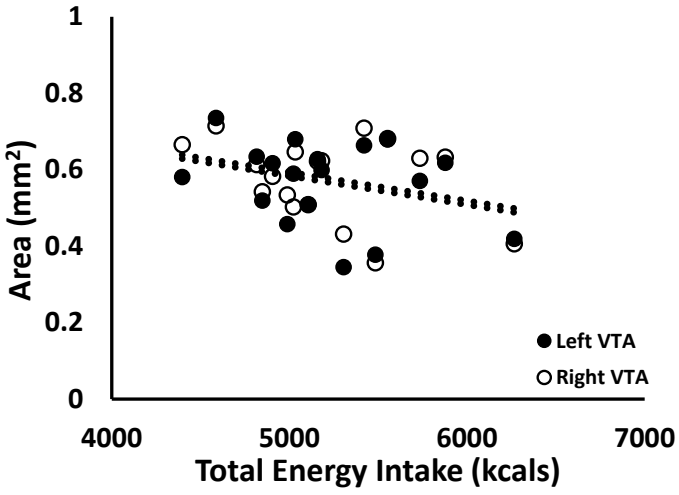

Figure 4.12 Correlation between total energy intake and left and right ventral tegmental area at Bregma -4.80mm.
Open circles display the area of the right VTA. Filled circles display the area of the left VTA. Values are means, n =8-10/gp. VTA, ventral tegmental area.

CHAPTER 5: DISCUSSION

To date, exercise has predominantly been used as a weight loss strategy; however, research has not yet demonstrated the long-term effectiveness of exercise on weight reduction. The mechanisms involved in exercise-induced energy compensation have yet to be elucidated. Using a rodent model, this study sought to understand the mechanisms driving the increase in energy intake after 8 weeks of voluntary wheel running, specifically looking at mechanisms involving appetite-regulating hormones, the gut microbiota, and the volume of the NA and an area of the VTA in male Sprague Dawley rats.

Results from our study indicated that body weight in each group varied over time. Body weight was initially reduced in EX, but no differences were present between SED and EX at the end of the study. A reduction was observed in relative fat mass in EX compared to both SED and SED-WM and in epididymal, retroperitoneal, peritoneal, and inguinal fat pads in EX compared to SED, which suggests that exercise may not have a weight reducing effect, but it may improve body composition. Energy intake was differentially affected by time, showing an early reduction in energy intake in EX relative to SED, followed by a steady increase. Using an OGTT to examine short-term appetite response, no differences in short-term appetite-regulating hormones, acylated ghrelin, or GLP-1 were observed. EX reduced portal leptin concentration compared to SED and SED-WM and reduced total insulin relative to SED. Although there was no difference in right or left NA volumes or area at Bregma -4.80mm for the VTA between groups, there was a positive correlation between left NA volume and energy intake as well as a trend toward an association between left NA and fasting leptin. Exercise had a mild impact on the gut microbiota, as the abundance of *Bifidobacterium* increased more in EX than in SED-WM and SED. Additionally, cecal *C. cluster I* was reduced in EX and SED-WM compared to SED.

5.1 Body Weight

Research has yet to fully establish the relationship between aerobic exercise on body weight and change in body weight. All groups in our study progressively increased body weight. EX body weight was initially reduced in week 3, 4, and 5 compared to SED; however, after 8 weeks, no difference in body weight between groups was observed, suggesting that exercise does not provide a protective effect on weight gain. This finding is consistent with previous research (109, 110, 160-164). A study conducted by de Carvalho et al. sought to understand the effects of 10 weeks of voluntary wheel running on body weight in mice (110). Mice were split into a control or high-fat diet group, with half of the mice from each group given access to a running wheel (110). Mice increased body weight regardless of exercise, and there were no differences in body weight between the exercise or sedentary groups (110). Similarly, a study conducted by Jung et al. aimed to understand the effects of 12 weeks of wheel running on body composition and body mass in male mice consuming either a high-fat or high carbohydrate diet (163). After two weeks of acclimatization, mice were randomly assigned to 1 of 5 groups: 1) high-fat sedentary (HF-S), 2) high-fat exercise (HF-E), 3) high carbohydrate sedentary (HCHO-S), 4) high carbohydrate exercise (HCHO-E), or 5) standard chow sedentary (CHOW-S) (163). After 12 weeks, body mass increased in all groups, however, there was no difference in body weight between HF-S and HF-E, or HCHO-S and HCHO-E (163). Additionally, previous research supports these findings, after 4 weeks (160, 161, 165), 8 weeks (164), and 12 weeks (109). Altogether, based on these studies, exercise does not prevent weight gain between exercise and sedentary animals.

On the other hand, research by other groups has shown exercise to have a protective effect on weight gain. Previous research has found a reduction in body weight of exercising animals consuming a high-fat diet compared to the sedentary controls after 4 weeks (161), 6 weeks (124,

146), 8 weeks (166), 10 weeks (167), and 12 weeks (111). This effect has also been observed after 12 weeks in obese mice (109) and after 24 weeks of exercising in female mice (112). Similarly, in a long-term study (i.e., 12 months) body weight was reduced in both exercising male and female mice (113). Additionally, in a 4 week study employing a forced treadmill running and *ad libitum* feeding, rats completing exercise had a final body weight lower than the sedentary group (108).

The discrepancy between our study and previous literature on body weight after prolonged aerobic exercise may be due to methodological differences. Our study used healthy male Sprague Dawley rats and the AIN-93M purified diet that is lower in protein and fat and recommended for adult rodent weight maintenance. A diet effect has been observed in previous literature where there is an exercise-induced reduction in body weight when consuming a high-fat diet. A study conducted by Cordeira & Monahan sought to understand the effects of 5 weeks of 30 minutes of wheel running for 5 days/week on body weight in C57BL/6 male mice. The mice were split into 4 groups: 1) high-fat (HF) and standard chow (SC)-sedentary, 2) HF+SC-exercise, 3) SC-exercise, or 4) SC- sedentary. Body weight and fat mass were reduced in groups with access to HF+SC exercise compared to sedentary HF+SC group (168). However, body weight, weight change, and fat mass were not different between exercise-SC and sedentary-SC (168). While the mechanisms involved in body regulation in the presence of exercise and a high-fat diet have not been elucidated, it is speculated that the hedonic system may play a role in this protective effect of weight gain with exercise and the consumption of a high-fat diet. Both exercise and highly palatable diets stimulate the reward centers in the brain (168). It is purported that exercise may change the preference for highly palatable diets (168). Exercise may elicit similar reward effects as consuming a highly palatable diet, and therefore, instead of enhancing food reward, it replaces

food reward (135). This does not affect regular food intake, where exercising mice did not reduce standard chow intake (17). The two speculated mechanisms driving this reward replacement behaviour include increased leptin functioning in the VTA (169) and/or a change in the dopaminergic system (135). A study conducted by Scarpace et al. aimed to understand the effect of wheel running on leptin signalling after wheel running in rats (169). Rats were given access to both high-fat and chow food as well as a running wheel (169). After 2 days of wheel running, rats were administered leptin and euthanized 1 hour later (169). It was observed that wheel running increased leptin signalling in the VTA, which was associated with a reduction in preference for high-fat food compared to the sedentary rats (169). Additionally, it was observed that the dopaminergic areas in the brain were similar between rats with access to a running wheel and sedentary rats with access to a high-fat diet (135). Despite these findings, this diet effect on exercise is not always observed. Regardless of diet, no difference in body weight was observed after exercising for 6 weeks (170), 10 weeks (110), and 12 weeks (163) in animals. The difference between our study and previous studies remains unknown. Additional research is needed to understand and elucidate these mechanisms.

Similar to animal models, humans experience a considerable variation in response to exercise on weight loss. In a systematic review conducted by Riou et al., long-term exercise interventions in humans produce an ~ 84% exercise-induced energy compensation (10), suggesting that weight loss does not occur as predicted based on energy expenditure. Additionally, a study conducted by Myers et al. observed that after 12-weeks of supervised exercise in women with overweight or obesity, two-thirds of participants were considered non-responders, meaning that less than anticipated weight loss was observed. Non-responders had a weight reduction of ~22% of what was anticipated (15). Similar findings have been shown in previous literature after 24 weeks of

different doses of supervised exercise. Almost a quarter of the moderate dose (8kcals/kg/week) exercisers and half of the large dose (20 kcal/kg/week) exercisers did not lose any weight (99).

Overall, we observed that exercise does not protect against weight gain, however, previous evidence in human and animal studies shows a significant variation in response to exercise on weight loss. Furthermore, previous animal studies have shown that body weight may be influenced by the type of diet, potentially more than access to exercise. Based on this evidence, it may be necessary for future studies to investigate other strategies for weight loss, such as dietary interventions or a combination of exercise and diet rather than the independent use of exercise.

5.2 Body Composition

While there is still some ambiguity on the effects of exercise on body weight, the effect on body composition has been examined thoroughly. Previous research has shown that exercise effectively reduces body fat % and fat pad mass (20, 112, 113, 124, 146). In a study conducted by Levin et al., after 6 weeks of wheel running, exercising mice had an ~36% reduction of visceral fat pads compared to the sedentary controls (146). In agreement with the other studies, we found that total fat pad mass and relative fat mass were reduced in EX compared to SED by ~40% and ~33%, respectively. These effects have been seen in longer-term studies conducted by McMullan et al., where after 12 months of exercise, mice had reduced body fat by ~50% (113). However, one shorter-term study was unable to elicit the same effects. After 4 weeks of forced exercise training, Foright et al. observed only a trend toward improvement of body fat % in exercising male rats (108). This may be due to the length of the study, as it may not have been long enough to invoke these positive body composition benefits from exercise. However, in a study conducted by Gollisch et al., fat pad weight was reduced after 4 weeks of voluntary wheel exercising in mice (161). Therefore, it may be due to the total volume of exercise rather than

study duration. In the study conducted by Foright et al., the exercising distance, 900m/day (i.e., 15m/minute for 1 hour), may not have been sufficient to produce the same effects compared to Gollisch et al., where the female Sprague Dawley rats ran on average 8.8-9.3 km/day.

In our study, while epididymal, inguinal, retroperitoneal, and peritoneal fat pads were reduced in EX compared to SED, no differences in brown adipose mass were observed. Brown adipose tissue (BAT) functions to produce heat for the body through non-shivering thermogenesis (171). Heat production is mediated through uncoupling protein 1 (UCP-1) (172). BAT and browning of WAT are sensitive to exercise, and this is due to both sympathetic nervous system stimulation and non-SNS stimulation (172). After acute and chronic exercise, norepinephrine is released from the SNS and increases BAT activity by activating UCP-1 stimulation, gene transcriptions, mitochondrial biogenesis, and recruitment of WAT to BAT (172). Independent of the SNS activation, other mechanisms have been observed to directly affect BAT activity or influence browning of WAT, such as cardiac natriuretic peptides, irisin, interleukin 6, and fibroblast growth factor (172). Inconsistent results have been shown on the effects of exercise on BAT mass. Similar to our study, exercise has been observed to have no effect on BAT, after 12 (173) and 6 weeks (174) of wheel running in mice and rats, respectively. Exercise, however has shown to increase BAT mass after 11 weeks of swimming in 32°F water in rats (175) and after 6 weeks of treadmill exercise in female rats (176). Furthermore, BAT has also been observed to be reduced after 6 weeks (177) and 9 weeks (178) of treadmill running. In humans, evidence is still relatively new, however, a study conducted by Singhai et al. sought to understand the effects of cold exposure on BAT volume in young female athletes compared to non-athletes (179). BAT volume was observed to trend lower in female athletes compared to sedentary females (179). Additionally, the effect of aerobic exercise on browning of white adipose tissue (WAT) has

recently been investigated. Browning of WAT increases the energy burning capacity of WAT, thus, it is important to understand the effect of exercise on the browning of WAT (165). A recent review conducted by Townsend & Wright examined effects of exercise on browning of WAT (180). While there have been numerous studies in rodents that observe, after chronic exercise, browning of WAT, this may not be the case in humans, where exercise has not yet been able to elicit the same response (180). It was concluded that human interventions might need to be longer to see any browning effects in the WAT (180).

5.3 Energy Intake

Energy compensation is thought to result from an increase in energy intake and/or a reduction in NEAT. Our study observed a reduction in food intake in weeks 1 and 3 in the EX-group compared to the SED group. This suggests that an anorectic effect of exercise was occurring during the initial weeks of the study, which may explain the differences in body weight between EX and SED in week 3. This has also been seen in shorter duration studies (4-6 weeks). After 5 weeks of wheel running, Cordeira & Monahan identified a reduction in energy intake by 11% in HF+SC exercising mice compared to HF+SC sedentary mice (168). Foright et al. observed after 4 weeks of forced treadmill running, in male rats, exercise reduced food intake by ~5% (108). Similarly, after 5 weeks of resistance training, rats that completed exercise had an 11% reduction in food intake compared to their sedentary controls (123). The findings from these studies corroborate with what we observed in our study, where in the first three weeks of exercise, an ~10.6% (EX intake/SED intake) reduction in food intake was observed.

Throughout the remaining weeks of our study, EX continuously increased energy intake with a trend towards increased energy intake in week 7 in EX compared to SED. However, at the end of 8 weeks, there were no significant differences in weekly energy intake or overall energy

intake between SED and EX. Similar to our study, previous studies have demonstrated that exercise does not attenuate or potentiate overall energy intake compared to sedentary groups. This effect has been observed after 12 weeks regardless of diet (163), 12 weeks in lean mice (109), 6 weeks in male mice (146), and 8 weeks with consumption of a high-fat diet (181). Additionally, energy intake remained unchanged between 16-24 weeks in female mice (112). On the other hand, research has also shown that prolonged aerobic exercise increases energy intake, therefore, increasing energy compensation in humans (13) and in animals (110, 124, 164, 170). Energy intake has been observed to increase in exercised animals compared to sedentary counterparts by 22.6% after 8 weeks (164), between 10% and 23% after 8 weeks (170), 26% and 7% on a standard diet and 11% and 45% in a high-fat diet after 5 and 10 weeks, respectively (110), and 12% in chow-exercise and HFD-exercise after 6 weeks of exercise (124).

Our study, to our knowledge, is one of the few studies that measured energy intake daily. Most studies only report overall energy intake. As a result, understanding the time course effects of exercise on energy intake is often challenging. There are a few studies, however, that report weekly energy intake. Similar to our study, Chen et al. observed that after the first three weeks of wheel access, obese mice in the exercise group significantly reduced daily energy intake compared to the obese mice in the sedentary group. Interestingly, at the end of 8 weeks, energy intake between the two groups was similar (181). In another study, Levin et al. identified a slight reduction in food intake in the exercise *ad libitum* group after 1 week of wheel running and then an increase in weeks 3 and 4; however, at the end of 6 weeks, no difference was identified (146). In contrast to these findings, Cordeira & Monahan observed a reduction in weekly energy intake over 5 weeks between exercise and sedentary HF+SC diet groups (168). Counter to this finding, Takeshita et al. observed that between 0 and 16 weeks of exercise, exercising mice increased

energy intake consumption compared to sedentary mice, however, between 16 and 24 weeks, there was no difference in energy intake (112). Furthermore, Droste et al. observed no difference in weekly food intake over a four week wheel running intervention in exercising mice compared to sedentary mice (160). Overall, based on the available literature, there is contrasting data regarding the time-course impact of exercise on energy intake. The noted discrepancies in energy intake between studies may be due to the type of diet, the dose, intensity or frequency of exercise, the sex of the animal, or the study length.

Taken together, our findings show that exercise produces a short-term anorectic effect on energy intake in the first weeks of exercise, similar to what other short-term studies (4 to 6 weeks) have observed. Throughout the remainder of the study, energy intake increased in exercising rats and trended towards a significant increase in week 7, however, no difference was observed between groups overall or at the end of 8 weeks. While there are inconsistent findings between studies, study duration may be a major predictor of energy intake (10). A systematic review conducted by Riou et al. in humans suggests short-term studies can elicit an energy deficit however, as the duration of studies increase, so does energy intake and energy compensation (10). Therefore, the short-term studies showing an overall reduction in food intake may not have been long enough to provoke this exercise-induced energy compensation through an increase in energy intake that we saw at the end of our study. However, 8 weeks may have been too short of a duration to induce full energy compensation. It can be speculated that, in agreement with previous literature (10, 20, 109, 110, 124), the longer the duration of the intervention, the more energy compensation occurs. Furthermore, future research should consider reporting energy intake consistently by showing both overall and weekly energy intake. Reporting weekly energy intake may allow for further evaluation of trends in energy intake in

response to exercise as it could show when approximately energy compensation occurs. Additionally, it will make it easier to compare energy intake data between studies.

5.4 Chronic Exercise and Appetite-Regulating Hormones

Research to date has been unable to fully elucidate the mechanisms involved in exercise-induced energy compensation. Appetite-regulating hormones may be a predominant factor in the increase in energy intake, contributing to the less than anticipated weight reduction observed with exercise training. At the same time, there have been consistent findings after an acute bout of exercise, where little to no energy compensation is occurring, which can be attributed to transient suppression of appetite through a reduction in acylated ghrelin and an increase in satiety hormones. There has not been clear evidence of the effect of prolonged aerobic exercise on both episodic and tonic hormones and the extent to which these hormones contribute to energy compensation. This study did not identify any group differences in the episodic hormones GLP-1 and acylated ghrelin. These findings oppose our original hypothesis, in which we speculated that exercise would increase satiety hormones and reduce hunger hormones, contributing to a reduction in energy intake. This hypothesis was based on previous research showing that in humans, chronic exercise increased both PYY (97, 117) and late postprandial release of GLP-1 (118, 119) and suppressed postprandial secretion of acylated ghrelin (14, 119). After a 12 weeks intervention, Gibbons et al. identified that only individuals who responded to exercise with reduced body weight had increased GLP-1 and PYY and reduced acylated ghrelin, suggesting that body weight changes were highly associated with the change in appetite-regulating hormones (14).

The results from our study show that after 8 weeks of prolonged wheel running, there was no difference in acylated ghrelin concentrations between groups. Similar to our study, Haghshenas

et al. conducted an 8-week randomized control study to investigate the effects of endurance training on appetite-regulating hormones in rats fed a high-fat diet (182). After 8 weeks of running, no differences existed between groups and acylated ghrelin (182). This finding, however, is not always reported in the literature. Previous evidence from both human and animal studies has shown inconsistent findings. In contrast, acylated ghrelin has been observed to increase after 6 days of wheel running (132). Acylated ghrelin has been observed to be reduced after 12 weeks of voluntary wheel running (109) and 5 weeks of resistance training (123) in rodents. A systematic review conducted by Ouerghi et al. assessed ten human studies, and identified that after chronic exercise, acylated ghrelin was either increased, reduced, or unchanged (183). Larson-Meyer et al. suggest that these inconsistencies in the literature could result from using different intensities, doses, volumes, and modalities of exercise or could be attributed to the sex of the participants (70). In many human studies that observed increased acylated ghrelin after exercise, female participants were recruited (70, 96, 119, 184). Intensity and dose may also contribute to these differences. An increase in acylated ghrelin concentrations has been observed in habitual runners compared to walkers (70). Acylated ghrelin was observed to be reduced after moderate dose exercise but remained unchanged after low dose aerobic exercise (185). While many factors may influence acylated ghrelin concentration, the extent to which prolonged aerobic exercise affects the secretion of acylated ghrelin remains relatively unclear.

Similar to acylated ghrelin, research has found inconsistent findings on the effect of chronic exercise on GLP-1 concentration. We found exercise to have no impact on OGTT GLP-1, total GLP-1, or portal GLP-1 concentrations. Previous human research has shown no change in total GLP-1 concentration after 12 weeks of exercise training in individuals with overweight or

obesity, however, there was a trend towards a delayed increase in total GLP-1 in the last 90 (90-180 minutes) and 60 minutes (120-180 minutes) posttest meal (119). Additionally, after 5 days of exercise in males who were overweight and normal weight, total GLP-1 concentration did not differ compared to pre-exercise (118). On the other hand, chronic exercise has been observed to increase GLP-1 concentration, which was only observed in individuals who lost weight from exercise (14). The lack of weight loss in our exercising rats may be one of the reasons why there was no increase in GLP-1. In previous studies, fasting GLP-1 was highly correlated with a reduction in body weight (184), and only exercise responders increased GLP-1 concentration compared to non-responders who showed no change in GLP-1 concentration (14).

Another important finding in this study was the association between exercise distance and GLP-1 and acylated ghrelin. While we did not observe a difference between groups and GLP-1 or acylated ghrelin concentrations, we identified a negative association between exercise distance and GLP-1 and a positive association between exercise distance and acylated ghrelin. This suggests that the more exercise volume, the lower the fasting GLP-1 concentration and the higher fasting acylated ghrelin concentration. This finding contradicts both what has been established after short bouts of aerobic exercise, where satiety increases and hunger decreases (62, 186), as well as the increase in GLP-1 concentration in responders (i.e., individuals that lose weight) observed after long-term exercise studies (14). This association demonstrates that the body may be physiologically defending against an exercise-induced energy deficit by increasing hunger hormones and reducing satiety hormones, potentially leading to increased energy intake. It may be important to understand the physiological difference between responders and non-responders as it potentially could drive this difference in GLP-1 observed. More research is required to reconcile this difference observed after long-term exercise studies.

The tonic satiety hormones insulin and leptin may play a more prominent role in the increased energy intake. The relationship between exercise, fat mass, and leptin concentration have been well established. Our results show that exercise reduced portal leptin compared to SED and SED-WM. These results are consistent with the review conducted by Fedewa et al., where a reduction in leptin was observed after chronic exercise, which was predominantly influenced by the improvement of body composition, specifically, reducing body fat% (121). Additionally, our study found a negative correlation between exercise distance and fat mass, relative fat mass, end body weight, and leptin concentration and a positive correlation between relative fat mass, total fat mass, body weight, and leptin concentration. An increase in exercise improves body composition and reduces leptin concentration, which can be supported by previous animal research that identified exercise reduces leptin concentration (112, 122, 124, 132, 146) and reduces overall fat mass (112, 122, 124, 146).

In addition to the reduction in leptin observed after prolonged exercise, we also observed a negative correlation between exercise distance and leptin concentration. This would suggest that more exercise leads to a greater hunger drive and an increase energy intake, resulting in a less than anticipated weight reduction from exercise. In agreement with our findings, previous studies have shown a reduction in leptin increases energy intake, positive energy balance, and body weight gain (187). Additionally, after 12 weeks of aerobic training in individuals with overweight or obesity, a reduction of leptin after exercise has also been observed to increase the hedonic drive of wanting and liking, potentially driving food reward after exercise (188). On the other hand, as mentioned above, exercise has been observed to increase leptin signalling in the VTA and reduce palatable food intake (169). Taken together, there is clear evidence suggesting that with an increase in exercise, a reduction in fat pads, total fat, and leptin occurs. This

reduction in leptin may lead to a long-term reduction in satiety, contributing to an increase in energy intake. More research, however, is needed to understand the effects of prolonged exercise on leptin secretion, and how it may influence food reward behaviours.

The second tonic hormone investigated was insulin. Our study identified that chronic exercise reduced total insulin concentration compared to SED rats. These results were consistent with previous research (109, 123, 124). In a randomized control trial, Bradley et al. aimed to understand the impacts of voluntary exercise on insulin sensitivity in diet-induced obesity mice (124). C57NL/6 mice were randomly divided into two groups: 1) chow diet and 2) high-fat diet for 4 weeks. After 4 weeks, the mice were further divided into a sedentary and voluntary exercise group for 6 weeks (124). It was identified that exercise, regardless of diet, reduced fasting insulin concentration compared to the sedentary control groups (124). The findings with insulin, however, are not always as consistent as findings observed with leptin. Some research shows that aerobic exercise has no impact on insulin concentration (108, 112, 182). This discrepancy in research findings could have been due to the length of the study, with four weeks being too short to produce a long-term reduction in fasting insulin (108). A systematic review conducted by Marson et al. identified that aerobic exercise interventions lasting longer than 8 weeks were seen to reduce fasting insulin concentration in humans (189).

Our study demonstrated a positive association between fasting insulin and total and relative fat mass. Blundell et al. suggest that fat and fat-free mass may influence insulin secretion and overall appetite control (186). Fat mass may inhibit energy intake in individuals of normal weight, but not in individuals with overweight or obesity, which may be due to an increase in insulin resistance (186). It was speculated that appetite is harder to control in individuals with obesity or overweight due to increased insulin resistance, leading to overconsumption of food

and increased fat mass (186). In our study we observed a negative association between exercise distance and fasting insulin levels. This finding is similar to previous research where exercise improves body composition and insulin sensitivity and reduces insulin secretion (186). Taken together, this data suggests that individuals who exercise have improved appetite control, have a stronger ability to match their energy expenditure and energy intake, leading to an overall increase in energy intake and compensation (186). Exercise may prevent overconsumption of food due to improved insulin sensitivity and improved body composition, however, exercise may also increase appetite, which may potentiate feeding to the level of energy expenditure, therefore, preventing weight loss from occurring.

Finally, while studies have looked at the effects of caloric restriction and exercise, this study is one of the first to examine how chronic exercise impacts insulin and leptin independent of weight. By restricting the SED-WM food intake to control for weight changes in the exercise group, we were able to observe that, independent of weight, exercise reduced leptin to a greater extent than caloric restriction alone in our SED-WM group. In the absence of any observable changes in the short-term appetite-regulating hormones GLP-1 and acylated ghrelin in our study, these findings suggest that this exercise-induced reduction in leptin and insulin potentially drive this increased energy intake.

5.5 Volume of Nucleus Accumbens and Ventral Tegmental Area

The meso-corticolimbic dopamine system comprises the VTA, NA, prefrontal cortex, amygdala, and hippocampus (50). The VTA functions in reward, motivation, cognition, and aversion behaviours. 65% of the neurons in the VTA belong to dopaminergic neurons (190). The NA is the area of the brain that regulates motivation and action, specifically regarding feeding, sexual reward, stress, and addiction behaviours (191). The prefrontal cortex is involved in higher

cognitive function such as processing, integrating, and adapting information (192). The amygdala is involved in processing emotions, cognition, and memory (193). Finally, the hippocampus plays a large role in learning, memory (194), and decision-making (195). While these regions of the brain function separately, they are not independent from each other. They are all activated in the presence of highly palatable foods and function to regulate eating behaviours, specifically hedonic eating (195). Hedonic drive involves both wanting and liking. Wanting is controlled by neural inputs from the VTA to the NA, hippocampus, amygdala, and prefrontal cortex (50). Additionally, the VTA sends dopamine neuron signals to the NA. These signals within the NA are involved in the appetite and food intake phase of eating and eventually the decision-making process of eating (50). Previous research has observed that NA is one of the primary mediators of unhealthy eating behaviours, food rewards, and addictions (196, 197). Previous literature has identified a correlation between the volume of the NA and BMI (144), energy intake (136), and leptin concentration (198). No study, to our knowledge, has looked at the impact of chronic exercise on the volume of the NA and whether any potential changes are associated with energy intake. In this study, it was determined that no differences existed between the groups and the NA volume or area of the VTA. Additionally, no correlation was established between body weight and the volume of the NA. Our research did identify a positive association between left NA volume and energy intake. Interestingly, previous literature also identified this association between increased volume of the NA and energy intake in children prone to obesity (199) and individuals with binge eating disorders (139). Additionally, we observed a trend towards a positive association between left NA and leptin concentration. Previous research has identified a positive association between leptin and the grey matter volume of the NA and a positive correlation between grey matter volume in the NA and BMI (198).

Leptin acts centrally on the brain to control energy balance but has also been shown to influence food reward as leptin receptors are present in the NA, VTA, and hippocampus (200). While the mechanisms have not been completely elucidated, it has been shown that leptin may respond appropriately when in a state of starvation, however, it is not able to prevent obesity (200). In the presence of highly palatable food, the body may naturally become leptin resistant, allowing individuals to overconsume beyond homeostatic regulation (200). As mentioned previously, exercise may counteract this drive to consume highly palatable foods. While our research was exploratory in nature and research in this area is still in its infancy, the effects of how exercise influences brain structures and whether it impacts energy intake are still largely unknown. Future studies should seek to understand the effects of exercise on the hedonic system and whether it drives energy intake.

5.6 Gut Microbiota

The examination of aerobic exercise and its impact on the gut microbiota and effects on energy intake is a relatively new area of research. Emerging evidence suggests that exercise may have a mild to moderate influence on the gut microbiota, independent of diet (109), and increase the production of bacteria-produced SCFAs acetate, propionate, and butyrate. In our study, after 8 weeks of aerobic exercise training, EX had a significantly higher concentration of cecal *Bifidobacterium* than SED-WM and SED. *Bifidobacterium* is a prominent health-promoting bacteria found in the gut. It is one of the first bacteria to inoculate the GI tract after birth and makes up the majority of bacteria in the gut of a newborn (201). *Bifidobacterium* is a main acetate producing bacteria that aids in preventing infection from pathogenic bacteria (202) and protects against some gastrointestinal diseases and cancers (203). Consistent with our findings, previous research suggests that prolonged aerobic exercise increases the representation of

Bifidobacterium within the gut microbiota (132, 204, 205). In contrast, Evan et al. conducted a 12-week wheel running intervention to assess how exercise affects the gut microbiota in mice (111). Mice were fed either a low-fat diet or high-fat diet (111). It was observed that after 12 weeks, the low-fat exercising mice had reduced *Bifidobacterium* relative to sedentary low fat, while exercising mice fed a high-fat diet had no change in *Bifidobacterium* (111). Unfortunately, it is challenging to draw a definite conclusion from previous research regarding the precise impact of exercise on representation of *Bifidobacterium* due to methodological differences in DNA extraction protocols and primers being used. Taken together, with our results and other research, exercise may elicit beneficial effects in gut microbiota by increasing *Bifidobacterium* abundance.

In contrast to health-promoting bacteria within the gut microbiota, many organisms represented within the gut microbiota are considered to be pathogenic. *C. cluster I* is one group of bacteria within the microbiota that contains potential pathogens, including, *Clostridium tetani*, *Clostridium chauvoei*, *Clostridium botulinum* and *Clostridium perfringens* (206). While many pathogenic bacteria exist in this group, it is also important to note that this group also contains non-pathogenic bacteria which can contribute to the diversity and health of the gut (206). *C. cluster I* is the main contributor to food-borne illnesses (207). We observed a reduction in *C. cluster I* in both EX and SED-WM relative to SED. Currently, there is limited research assessing the impact of exercise on *C. cluster I.* One study conducted by Lambert et al. found that 6 weeks of aerobic exercise increased *C. cluster I* levels in mice (204). In another study, *C. cluster I* was found to be reduced in individuals with obesity (208). With very few studies looking at *C. cluster I*, this may be due to only looking at specific classes, orders, or genera and not at the species level. Unfortunately, while limited studies look specifically at the effects of exercise on *C.*

cluster I, it is challenging to make a definite conclusion. Future studies may want to consider using deep sequencing analysis to gain a more comprehensive understanding of the effects of exercise on *C. cluster I.*

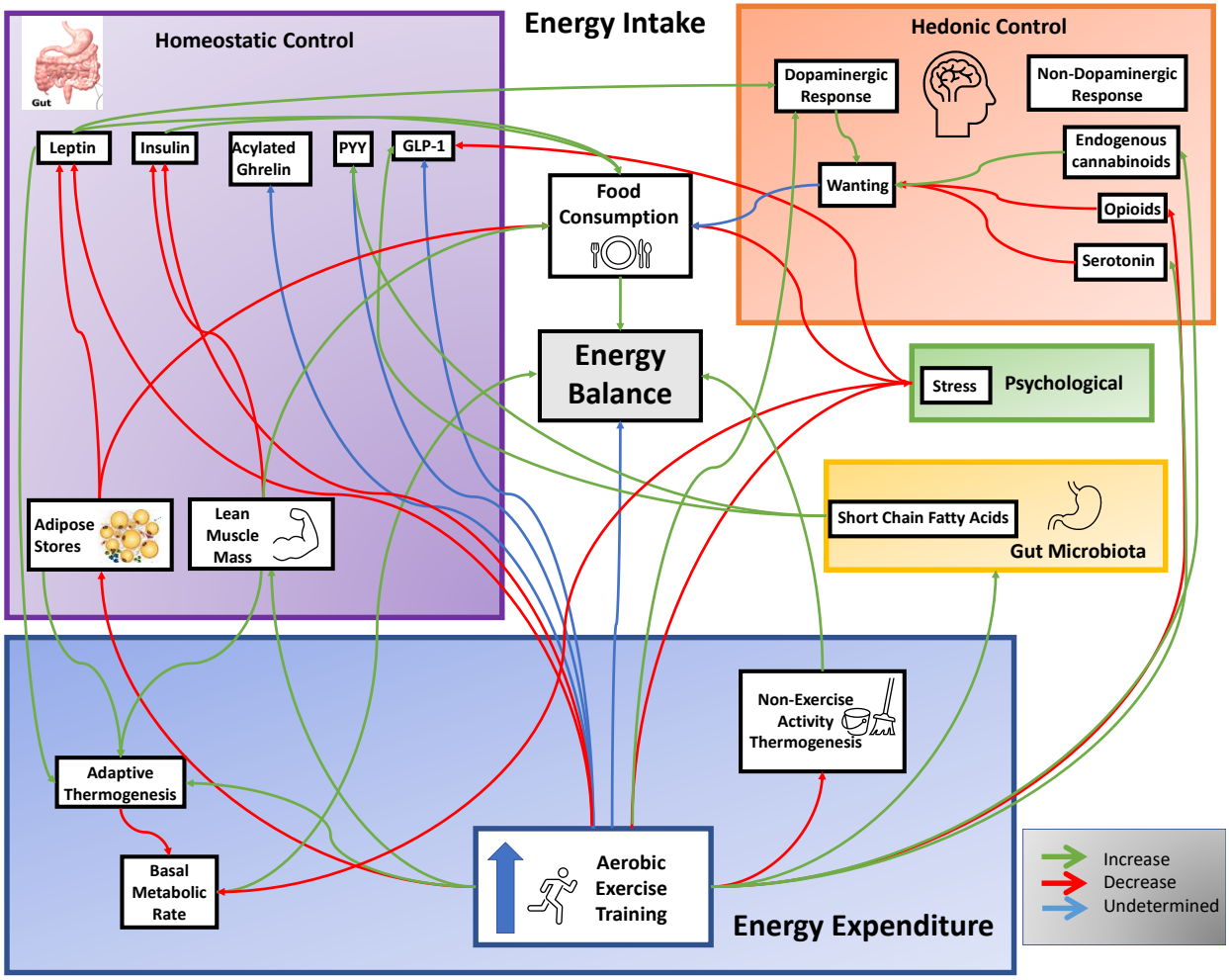

Figure 5.1 Working model of how exercise training influences energy balance and body weight regulation through physiological factors.

5.7 Strengths & Limitations

5.7.1 Study design

In relation to human studies that have attempted to examine the long-term impacts of exercise on appetite, one notable strength of this study was the incorporation of an animal model. Human energy compensation studies are usually confounded by many factors such as measurement of energy intake. Additionally, long-term adherence and the use of standardizing energy intake makes participant recruitment and retainment more challenging leading to lower sample sizes and less power. Animal models enhance the ability to control for factors such as energy intake and body weight over an extended period. We used a standardized AIN-93M purified diet that conforms to the American Institute of Nutrition recommendations. This diet has uniform energy density, is a purified diet lower in protein and fat, and is recommended for adult rodent weight maintenance. Using this diet increases the reproducibility of this study as it is a commonly recommended experimental rat food that is easily accessible. Measuring energy intake daily increases the accuracy of energy intake measures, which human studies struggle to do during long-term studies.

For our study, we utilized a randomized control trial (RCT). RCT can develop a cause-effect relationship between outcome variables and control for biases and confounding factors (209). To ensure high internal validity, we were able to control for the starting weights. Having a SED-WM group also allowed us to investigate the weight-independent impacts of aerobic exercise on appetite-regulating hormones, which is not possible with a study conducted in humans

One limitation of our study was intervention duration. While our study was 8 weeks long, it may not have been long enough to provoke a full exercise-induced energy compensation response. At the end of our study, we did observe a trend towards an increase in energy intake. Therefore, based on previous research (10, 20, 109, 110, 124), it may have been beneficial to

have an additional 4 weeks, as many studies 12 weeks or longer have observed a rise in energy intake compared to the sedentary group, suggesting a greater degree of energy compensation.

A notable limitation to all animal studies is external validity, as animal studies lack real-life conditions and cannot, therefore, be generalizable between all populations. Animal experiments are highly controlled, as ours was for diet and exercise, and thus cannot mimic appropriately human conditions. However, rodent models have been used in research for a long time as they are anatomical, physiological, and genetically similar to humans (210). Additionally, previous evidence has shown that rats are an effective model to reflect the human response to exercise through blood analysis (211).

Additionally, our study only used male rats; therefore, our results cannot be generalized to females. Previous studies have observed exercise to impact energy intake differently in females compared to males. In a short, 4-week forced treadmill intervention, Foright et al. observed a reduction in overall energy intake in male exercise rats and an increase in female exercise rats (108). This has also been observed in human studies where two studies with identical study designs were completed in both males and females. It was observed that after 7 days of exercise, females had a partial energy compensation (16), whereas males had no energy compensation (98). This differential response to exercise may result from sex differences in appetite-regulating hormones. Hagobian et al. identified that after 4 days of exercising, females increased acylated ghrelin and reduced insulin concentration compared to males (105). As well, after an acute bout of exercise, females increased GLP-1, whereas males increased PYY secretion (84). Another factor to consider is the female menstrual cycle. A study conducted by Giles et al. observed a change in energy balance due to fluctuating energy intake throughout the female rat's cycle (212). It was observed that female rats ate the most in the diestrus phase and the lowest in the

estrous phase (212). This was also observed in another human study showing that energy intake and energy expenditure increased during the luteal phase, where there were higher food cravings compared to the follicular phase (213). Previous studies support that males and females respond differently to exercise-induced energy intake. Therefore, because of the differences in physiology, specifically sex-related hormone differences and the impact of the menstrual cycle on energy intake, we cannot generalize our results to females.

5.7.2 Body Composition Measures

There are multiple non-invasive ways to measure body composition including magnetic resonance spectroscopy and dual-energy X-Ray absorptiometry (DXA). Unfortunately, these techniques are costly and not widely available. Like many studies in the past, our study used an end-point body composition measure (110, 146, 168), where post-euthanasia, fat pads were excised and weighed for an overall estimate of body composition. Dissecting and weighing fat pads can be very accurate, however, a big limitation of this type of measurement is that it cannot provide longitudinal changes in fat mass. On the other hand, DXA has become one of the most valid and reliable ways to measure body composition in humans and in rodents (214). Using a DXA or MRI to measure body composition would have provided longitudinal data (215), allowing us to assess both pre-intervention fat mass and post-intervention fat mass, therefore, providing a better understanding of the effect of exercise on body composition.

5.7.3 Appetite-Regulating Hormone Analysis

Blood collection during the oral glucose tolerance test assessed appetite-regulating hormones and glycemic concentration. This analysis was used to identify the effects of prolonged exercise on appetite-regulatory hormones. One limitation to our study design was that we did not analyze

pre-intervention OGTT to assess differences in starting appetite-regulating hormone levels. This would have been helpful to confirm that baseline appetite-regulating hormone concentrations were similar. Furthermore, with the serial blood collections during the OGTT, we were not able to collect a sufficient blood volume to run our samples in duplicate for appetite-regulating ELISA hormone analysis. Running samples in single may have affected the validity of the analysis. Furthermore, another major limitation was the stress induced during the tail-vein catheterization. Given the challenges with this procedure, multiple catheter placement attempts were often required. As our results confirm, this led to an increase in corticosterone release. The elevated stress response likely modified the appetite-regulating hormones response. In particular, the GLP-1 response, with GLP-1 levels failing to rise after the dextrose gavage, was likely impacted by the stress response induced through the catheterization. Utilization of an animal model with an implanted catheter prior to initiating the OGTT may have helped mitigate this effect.

5.7.4 Gut Microbiota Analysis

In this study, we used a qPCR to understand the abundance of bacteria. While this method accurately assesses the abundance, it does not provide a comprehensive analysis of what is happening with all the gut microbiota as it uses specific primers that limit the number of taxa that can be analyzed. In future studies, it would be beneficial to use 16Sr RNA sequencing or next-generation sequencing methods. Sequencing allows for rapid analysis of the whole community, as well as it can determine the abundance of known and unknown bacteria, and provides a more detailed characterization of the various bacteria in the gut (216). In addition, sequencing provides a comprehensive analysis of alpha and beta diversity of bacteria which is an indicator of the health-promoting capacity of the gut microbiota (216). Sequencing can also provide valuable

information regarding the functional and metabolic capacity of the gut microbiome, thus providing a better understanding of the mechanisms by which different profiles of gut microbiota influence host health.

5.7.5 Non-Exercise Activity Thermogenesis

Previous research has identified a reduction of NEAT after aerobic exercise (18, 20, 110, 145, 147). A significant limitation of this study was that we were unable to measure NEAT. In our study it remains unknown how exercise may affect NEAT and its contribution to energy compensation. It can be speculated that since body weight and energy intake were not significantly different between EX and SED, a reduction in NEAT could be a contributing factor to why EX rats did not have a reduction in body weight. Furthermore, in our study, exercise was not associated with energy consumed, suggesting that exercise-induced energy compensation may not be solely from an increase in energy intake. Future research should consider using a metabolic cage or light beams for rodents to assess the effect of exercise on NEAT, as it could potentially elucidate another mechanism underlying this increase in body weight observed in the exercising group.

5.8 Conclusion and Future Consideration

5.8.1 Future Considerations

While exercise is a common strategy for weight loss, our results show a degree of energy compensation occurring, potentially through an increase in energy intake, leading to no difference in body weight between groups. We explored three mechanisms involved in the exercise-induced increase in energy intake: 1) appetite-regulating hormones, 2) volume of the NA and area of the VTA, and 3) gut microbiota. While we were unable to elucidate the mechanisms behind this increase in energy intake, it may be imperative to consider a few factors

in future research. Factors such as intensity and modality of exercise may impact appetite-regulating hormones, the gut microbiota, and the hedonic system on energy intake. Previous studies have observed that exercise intensity may influence energy compensation through an increase in preference for highly palatable foods (103). Using a forced treadmill may provide valuable information on the intensity and duration of exercise. This would allow researchers to control how long and fast the animals are running, providing a better understanding of whether these variables affect energy intake. Resistance training has become a popular choice of exercise for many individuals, and evidence has shown a reduction in mean energy intake after resistance training compared to aerobic exercise (60), a reduction in ghrelin (123), and a reduction in the hedonic drive to eat highly palatable food (217). Therefore, with the popularity and initial evidence on the effects of resistance training on appetite and appetite-regulating hormones, it may be valuable for future studies to assess how resistance training affects appetite in animal models using wire netting and progressively adding load to the tail. Taken together, an increase in the duration of the study, measuring the intensity, frequency, and dose of the exercise, as well as exploring different modalities of exercise, may provide helpful information on the mechanisms involved in energy compensation and the effect of exercise on weight loss and weight maintenance and therefore guide future exercise-induced weight loss strategies.

Previous research has identified the mild to moderate effects of exercise on the gut microbiota and associated gut microbiota-released metabolites, including SCFAs. In this study, we did not measure SCFA concentrations in stool. In future studies looking at the impact of exercise on appetite, it would be valuable to measure SCFA concentrations using gas-chromatography mass spectrometry (218). SCFA production has been causally implicated in the release of GLP-1 and PYY (38, 219, 220). Understanding the effects of chronic aerobic exercise on the production of

SCFA and whether there is an association between SCFA production and GLP-1 and PYY secretion and energy intake may provide some understanding of how the gut microbiota influences exercise-induced energy compensation.

While still exploratory, this study did not determine an exercise-induced effect on the volume of the NA or area of the VTA. This was one of the first studies to assess the effects of exercises on the NA volume and area of the VTA. We identified that the volume of the NA was associated with total energy intake. This finding shows a potential link between the hedonic system and energy intake. As such, a more intensive investigation is warranted. One such study could involve looking at the microstructures in the NA. A study conducted by Samara et al. sought to understand the effects of the basal ganglia regions of the brain and their role in reward behaviours in adults with and without obesity (221). It was observed that individuals with obesity had larger NA volumes (221). Additionally, increased reward eating behaviours were associated with lower axonal density (221). This implicates the brain structures and reward eating, therefore, it may be beneficial to see whether exercise impacts axonal density in the NA rather than just assessing the impact of exercise on the volume. Another avenue to explore is whether exercise influences gene expression of tyrosine, u-opioid receptors, dopamine active transporter, and dopamine receptors. While the mechanism is still unknown, research has shown that the dopamine system is affected by physical activity (222). The dopamine system may also influence the amount of physical activity undertaken (222). The dopaminergic system influences the reward behaviours of wanting highly palatable foods and appetite-regulating hormones, which work together to increase or decrease energy intake and food reward behaviours (197). It has been observed that acylated ghrelin increases the turnover of dopamine, causing an increase in food reward, and on the other hand, leptin inhibits the dopamine system and reduces food

intake (223). Therefore, future studies should seek to understand the link between physical activity, the brain structure, gene expression of the hedonic system, and its role on energy intake as it may provide greater insight into mechanisms involved in exercise-induced energy compensation.

Since many exercise studies have identified the ineffectiveness of exercise on weight loss, it may be more effective to look at other approaches to weight loss. GLP-1-based therapies such as liraglutide, semaglutide, tripeptide, and endogenous GLP-1 have become of interest, as studies have shown them to elicit satiation and increase weight loss (224). A previous study observed that after 12 weeks of semaglutide treatment, energy intake, body weight, and food cravings decreased (225). In another study, participants with type 2 diabetes were assigned to a GLP-1 or saline group (226). Over a six-weeks of either saline or GLP-1 administration, it was identified that fasting glucose, body weight, and appetite were reduced (226). Sustained weight loss from GLP-1 agonist therapy has been observed over an extended period as long as the therapies are continued (224). Unfortunately, once the therapies are stopped, weight regain has been observed to occur (224). While GLP-1 agonist therapy remains a successful weight loss strategy alone (224), a GLP-1 agonist in conjunction with exercises shows enhanced benefit. A study conducted by Lundgren et al. sought to understand the effects of exercise, liraglutide, or a combination of both on weight loss in individuals with obesity (227). Individuals with obesity were initially on a low caloric diet. Individuals who successfully lost 5% of their body weight were then randomized into a year-long intervention (227). The three groups included: 1)exercise + placebo, 2)liraglutide + normal activity, or 3) a combination of liraglutide and exercise (227). After 1 year, in all exercise groups, body weight and body fat percentage were reduced, however, in the

combined treatment, body weight and fat were reduced nearly two times compared to a single treatment alone (227).

Another way to induce a caloric deficit is through a nutritional intervention. Typical diets include hypocaloric, low-fat, high-fat, low carbohydrate, high protein, the Mediterranean, and intermittent diets (228). Most diets alone will show positive effects on body weight, such as intermittent fasting which has been observed to be safe and has shown promise in the treatment of obesity (229). Diets rich in proteins have also been identified to elicit moderate benefits on body weight management (230). Research has also observed that strict hypocaloric diets may induce weight loss however this is usually at the expense of muscle mass (231). Together, exercise and nutritional interventions have been shown to improve weight loss and either maintain or improve muscle mass. A study conducted by Kotarsky et al. looked at time restricted eating in conjunction with supervised aerobic and resistance training on body composition and weight loss (232). Individuals who were physically inactive and overweight or obese were randomly split into a time restricted feeding group, eating between the hours from 12:00 pm to 8:00 pm, or a normal feeding group (232). Each group completed 3 similar resistance training programs each week in combination with 50 to 60 minutes a day of moderate or 150 minutes of vigorous physical activity per week for 8 weeks (232). Both conditions induced a significant energy deficit, however, greater weight reductions were seen in the time restricted feeding compared to normal eating, while exercise also increased lean mass compared to physically inactive individuals (232). Similarly, Galbreath et al. aimed to understand the effects of resistance training in combination with a high protein diet compared to a hypocaloric high carbohydrate diet on body composition and body weight change in 54 older females who were sedentary and overweight (233). The female participants completed 3 sessions/week of 30

minutes supervised circuit style resistance training (233). Following a high protein diet and resistance training, participants showed a significant reduction in body weight and body fat percentage while maintaining lean mass compared to the high carbohydrate diet and exercise group (233). Evidence shows that in conjunction with exercise, using a nutritional intervention may help improve satiety, improve long-term adherence, sustain or improve lean mass, and increase the energy deficit produced by exercise alone, leading to greater weight reduction.

Additionally, a new exercise-related, metabolic target for weight loss has recently been identified that may help to potentiate the beneficial effects of exercise on body weight management. A study conducted by Li et al. observed that exercise produces N-lactoyl-phenylalanine (Lac-Phe), a metabolite that suppresses food intake and obesity (234). In the human intervention, lac-Phe concentrations were measured after a bout of resistance, endurance, and sprint training (234). An increase in Lac-Phe was observed after all training bouts, with the sprint training showing the largest increase, with levels staying elevated over 3 hours post-exercise (234). Lac-Phe was also highly correlated with lactate, another suspected mechanism involved in energy compensation (234). The animal studies identified that chronic administration of Lac-Phe reduced energy intake and adiposity and improved glucose tolerance in DIO mice (234). Furthermore, it was also identified that chronic exercise and ablation of Lac-Phe led to increased energy intake and body weight, however, these results were not observed in sedentary knockout mice (234). While still relatively new, future research needs to continue investigating this metabolite and its effect after prolonged exercise on energy intake in humans and overall weight management.

5.8.2 Conclusion and Significance

The mechanisms controlling energy compensation in response to exercise training are still largely unknown and require investigation. While our study and many other studies have shown exercise to be ineffective for reducing body weight, exercise has many beneficial effects on body composition and metabolic health, such as improved insulin sensitivity and reduced risk of diabetes, cardiovascular disease, non-alcohol fatty liver disease, and some cancers. Therefore, it may be important to make a clear distinction between the difference in weight loss and fat loss. While exercise may not produce weight loss, exercise reduces fat mass, and increases lean body tissue. This increase in lean body mass may mediate some weight-independent, metabolic health improvements associated with exercise. Exercise should still be encouraged as a strategy for improving health rather than weight loss. While many human studies have assessed the effect of chronic exercise on the homeostatic system, these studies are usually confounded by weight loss. Our study is one of the first studies to assess how, independent of weight, 8 weeks of aerobic training impacts energy intake in male Sprague Dawley rats. We sought to understand the potential mechanisms mediating this exercise-induced increase in energy intake observed after prolonged aerobic exercise, specifically looking at appetite-regulating hormones, the gut microbiota, and the volume of the NA and an area of the VTA. There were three important findings relating to the mechanisms involved in energy compensation. Firstly, exercise did not protect against weight gain but improved body composition. Secondly, while exercise reduced energy intake in the first few weeks of the study, exercise was associated with a continuous increase in energy intake throughout the study. This may be partly due to a reduction in both tonic hormones, insulin and leptin, leading to a reduction in satiety and increase in hunger. Additionally, we observed that independent of weight loss, exercise improves body composition

and reduces leptin concentration to a greater degree than caloric restriction alone. Finally, aerobic exercise-induced positive effects on gut microbiota, increasing cecal *Bifidobacterium* and reducing *C. cluster I.* Taken together, our study suggests that these exercise-induced improvements in body composition may counteract weight loss by reducing insulin and leptin, leading to reduced satiety, and possibly leading to increased energy intake, and increased energy compensation. Ultimately, evidence has pointed to the importance of exercise for health-related outcomes, however, exercise in conjunction with other modalities (i.e., diet, GLP-1 therapies) may enhance weight loss efforts. It may also be beneficial for governing bodies such as the Canadian Society of Exercise Physiology or Health Canada to acknowledge the general findings that exercise is not always effective for weight reduction and change their guidelines, to shift the focus from using exercise as a tool for weight loss to using exercise as a means to improve health. This shift in focus may improve long-term adherence and motivation, therefore leading to overall improved health for Canadians.

References

1. Dishman RK, Berthoud H, Booth FW, Cotman CW, Edgerton VR, Fleshner MR, et al. Neurobiology of exercise. Obesity. 2006;14(3):345-56.
2. WHO. Physical Activity. 2018 [Available from: https://www.who.int/news-room/fact-sheets/detail/physical-activity.
3. Levine J. Non-exercise activity thermogenesis (NEAT). Best Pract Res Clin Endocrinol Metab. 2002;16(4):679-702.
4. Skov-Ettrup LS, Petersen CB, Curtis T, Lykke M, Christensen AI, Tolstrup JS. Why do people exercise? A cross-sectional study of motives to exercise among danish adults. Public Health (London). 2014;128(5):482-4.
5. Warburton DER. Health benefits of physical activity: the evidence. Canadian Medical Association Journal (CMAJ). 2006;174(6):801-9.
6. Poirel E. Psychological benefits of physical activity for optimal mental health. Santé Mentale au Québec. 2017;42(1):147.
7. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, PT K. Effect of physical inactivity on major non-communicable diseases worldwide: An analysis of burden of disease and life expectancy. The Lancet (British Edition). 2012;380(9838):219-29.
8. Foright RM, Presby DM, Sherk VD, Kahn D, Checkley LA, Giles ED, et al. Is regular exercise an effective strategy for weight loss maintenance? Physiology & Behavior. 2018;188:86-93.
9. Arzu D, Tuzun EH, Eker L. Perceived barriers to physical activity in university students. J Sports Sci Med. 2006;15(5):615-20.
10. Riou MÈ, Jomphe-Tremblay S, Lamothe G, Stacey D, Szczotka A, Doucet É. Predictors of energy compensation during exercise interventions: A systematic review. Nutrients. 2015;7(5):3677-704.
11. King NA, Hopkins M, Caudwell P, Stubbs RJ, Blundell JE. Beneficial effects of exercise: shifting the focus from body weight to other markers of health. British Journal of Sports Medicine. 2009;43(12):924.
12. Hall KD, Heymsfield SB, Kemnitz JW, Klein S, Schoeller DA, Speakman JR. Energy balance and its components: implications for body weight regulation. Am J Clin Nutr. 2012;95(4):989-94.
13. Thomas DM, Bouchard C, Church T, Slentz C, Kraus WE, Redman LM, et al. Why do individuals not lose more weight from an exercise intervention at a defined dose? An energy balance analysis. Obesity Reviews. 2012;13(10):835-47.
14. Gibbons C, Blundell JE, Caudwell P, Webb D-L, Hellström PM, Näslund E, et al. The role of episodic postprandial peptides in exercise-induced compensatory eating. The Journal of Clinical Endocrinology & Metabolism. 2017;102(11):4051-9.
15. Myers A, Dalton M, Gibbons C, Finlayson G, Blundell J. Structured, aerobic exercise reduces fat mass and is partially compensated through energy intake but not energy expenditure in women. Physiology & Behavior. 2019;199:56-65.
16. Stubbs RJ, Sepp A, Hughes DA, Johnstone AM, King N, Horgan G, et al. The effect of graded levels of exercise on energy intake and balance in free-living women. International Journal of Obesity. 2002;26(6):866-9.
17. Whybrow S, Hughes DA, Ritz P, Johnstone AM, Horgan GW, King N, et al. The effect of an incremental increase in exercise on appetite, eating behaviour and energy balance in lean men and women feeding ad libitum. British Journal of Nutrition. 2008;100(5):1109-15.

18. Riou M-È, Jomphe-Tremblay S, Lamothe G, Finlayson GS, Blundell JE, Décarie-Spain L, et al. Energy compensation following a supervised exercise intervention in women living with overweight/obesity is accompanied by an early and sustained decrease in non-structured physical activity. Frontiers in Physiology. 2019;10:1048.
19. Ridgers ND, Timperio A, Cerin E, Salmon J. Compensation of physical activity and sedentary time in primary school children. Med Sci Sports Exerc. 2014;46(8):1564-9.
20. Copes LE, Schutz H, Dlugosz EM, Acosta W, Chappell MA, Garland T. Effects of voluntary exercise on spontaneous physical activity and food consumption in mice: Results from an artificial selection experiment. Physiology & Behavior. 2015;149:86-94.
21. Lutter M, Nestler EJ. Homeostatic and hedonic signals interact in the regulation of food intake. The Journal of Nutrition. 2009;139(3):629-32.
22. Saper CB, Chou TC, Elmquist JK. The need to feed: Homeostatic and hedonic control of eating. Neuron. 2002;36(Generic):199-211.
23. Finlayson G, Dalton M. Hedonics of food consumption: Are food 'liking' and 'wanting' viable targets for appetite control in obese? Current Obesity Reports. 2012;1(1):42-9.
24. Benelam B. Satiation, satiety and their effects on eating behaviour. Nutrition Bulletin. 2009;34(2):126-73.
25. Janssen P, Vanden Berghe P, Verschueren S, Lehmann A, Depoortere I, Tack J. Review article: The role of gastric motility in the control of food intake. Alimentary Pharmacology & Therapeutics. 2011;33(8):880-94.
26. Bauer PV, Hamr SC, Duca FA. Regulation of energy balance by a gut–brain axis and involvement of the gut microbiota. CMLS. 2015;73(4):737-55.
27. Kadouh HC, Acosta A. Current paradigms in the etiology of obesity. Techniques in Gastrointestinal Endoscopy. 2017;19(1):2-11.
28. Berthoud H-R, Münzberg H, Morrison CD. Blaming the brain for obesity: Integration of hedonic and homeostatic mechanisms. Gastroenterology (New York, NY 1943). 2017;152(7):1728-38.
29. Murphy KG, Bloom SR. Gut hormones and the regulation of energy homeostasis. Nature (London). 2006;444(7121):854-9.
30. Stensel D. Exercise, appetite and appetite-regulating hormones: Implications for food intake and weight control. Ann Nutr Metab. 2010;57:36-42.
31. Karra E, Batterham RL. The role of gut hormones in the regulation of body weight and energy homeostasis. Molecular and Cellular Endocrinology. 2010;316(2):120-8.
32. Mackelvie KJ, Meneilly GS, Elahi D, Wong ACK, Barr SI, Chanoine J-P. Regulation of appetite in lean and obese adolescents after exercise: Role of acylated and desacyl ghrelin. The Journal of Clinical Endocrinology and Metabolism. 2007;92(2):648-54.
33. Schubert MM, Sabapathy S, Leveritt M, Desbrow B. Acute exercise and hormones related to appetite regulation: A meta-analysis. Sports Medicine. 2014;44(3):387-403.
34. Mani BK, Castorena CM, Osborne-Lawrence S, Vijayaraghavan P, Metzger NP, Elmquist JK, et al. Ghrelin mediates exercise endurance and the feeding response post-exercise. Molecular Metabolism (Germany). 2018;9:114-30.
35. Nauck MA, Meier JJ. Incretin hormones: Their role in health and disease. Diabetes, Obesity & Metabolism. 2018;20:5-21.
36. Ohlsson L, Kohan AB, Tso P, Ahren B. GLP-1 released to the mesenteric lymph duct in mice: Effects of glucose and fat. Regulatory Peptides. 2014;189:40-5.
37. Drucker DJ. The biology of incretin hormones. Cell Metabolism. 2006;3(3):152-65.

38. Tolhurst G, Heffron H, Lam Y, Parker H, Habib A, Diakogiannaki E, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-Protein—Coupled receptor FFAR2. Diabetes (New York, NY). 2012;61(2):364-71.
39. Bomhof M, editor Anaerobic: Impact of exercise on gut microbiomes. Canadian Society of Exercise Physiology: FOR THE HEALTH OF IT; 2019; Kelowna, BC, Canada.
40. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano G, Gasbarrini A, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms (Basel). 2019;7(1):14.
41. Parada Venegas D, De la Fuente MK, Landskron G, Julieta Gonzalez M, Quera R, Dijkstra G, et al. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Frontiers in Immunology. 2019;10:277-.
42. Le Poul E, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, et al. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. The Journal of Biological Chemistry. 2003;278(28):25481-9.
43. Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature. 2002;415:339-43.
44. Minokoshi Y, Haque MS, Shimazu T. Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. Diabetes. 1999;48(2):287-91.
45. Suzuki K, Simpson KA, Minnion JS, Shillito JC, Bloom SR. The role of gut hormones and the hypothalamus in appetite regulation. Endocrine Journal. 2010;57(5):359-72.
46. Chambers ES, Morrison DJ, Frost G. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? Nutrition Society. 2015;74(3):328-36.
47. Lowe MR, Butryn M. Hedonic hunger: A new dimension of appetite? Physiology & Behavior. 2007;91(4):432-9.
48. Blundell JE, Finlayson G. Is susceptibility to weight gain characterized by homeostatic or hedonic risk factors for overconsumption? Physiology & Behavior. 2004;82(1):21-5.
49. Morris MJ, Beilharz JE, Maniam J, Reichelt AC, Westbrook RF. Why is obesity such a problem in the 21st century? The intersection of palatable food, cues, and reward pathways, stress, and cognition. Neuroscience and Biobehavioral Reviews. 2015;58:36-45.
50. Berthoud H-R. The neurobiology of food intake in an obesogenic environment. Proceedings of the Nutrition Society. 2012;71(4):478-87.
51. Cameron JD, Goldfield GS, Finlayson G, Blundell JE, Doucet É. Fasting for 24 hours heightens reward from food and food-related cues. PloS one. 2014;9(1):e85970-e.
52. Beaulieu K, Hopkins M, Blundell J, Finlayson G. Homeostatic and non-homeostatic appetite control along the spectrum of physical activity levels: An updated perspective. Physiology & Behavior. 2018;192:23-9.
53. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature. 2006;443(21):289-95.
54. Münzberg H, Flier JS, Bjørbæk C. Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. Endocrinology. 2004;145(11):4880-9.
55. Vincent RP, Ashrafian H, Le Roux CW. Mechanism of disease: The role of gastrointestinal hormones in appetite and obesity. Nature. 2008;5:268-77.
56. Sellami M, Gasmi M, Denham J, Hayes LD, Straaton D, Padulo J, et al. Effects of acute and chronic exercise on immunological parameters in the elderly aged: Can physical activity counteract the effects of aging? Frontiers in Immunology. 2018;9(2187):1-17.

57. CSEP. Training for Health & Performance. Canadian Society for Exercise Physiologist - Physical Activity Training for Health (CSEP-PATH)2013.
58. Vatansever-Ozen S, Tiryaki-Sonmez G, Bugdayci G, Ozen G. The effects of exercise on food intake and hunger: relationship with acylated ghrelin and leptin. Journal of Sports Science & Medicine. 2011;10(2):283-91.
59. Dorling J, Broom D, Burns S, Clayton D, Deighton K, James L, et al. Acute and chronic effects of exercise on appetite, energy intake, and appetite-related hormones: The modulating effect of adiposity, sex, and habitual physical activity. Nutrients. 2018;10(9):1140.
60. Laan DJ, Lim E, Leidy HJ, Campbell WW. Effects and reproducibility of aerobic and resistance exercise on appetite and energy intake in young, physically active adults. Applied Physiology, Nutrition, and Metabolism. 2010;35(6):842-7.
61. Martins C, Morgan LM, Bloom SR, Robertson MD. Effects of exercise on gut peptides, energy intake and appetite. Journal of Endocrinology. 2007;193(2):251.
62. King NA, Burley VJ, Blundell JE. Exercise-induced suppression of appetite: Effects on food intake and implications for energy balance. European Journal of Clinical Nutrition. 1994;48:715-24.
63. King JA, Garnham JO, Jackson AP, Kelly BM, Xenophontos S, Nimmo MA. Appetite-regulatory hormone response on the day following a prolonged bout of moderate intensity exercise. Physiology & Behavior. 2015;141:23-31.
64. Maraki M, Tsofliou F, Pitsiladis YP, Malkova D, Mutrie N, Higgins S. Acute effects of a single exercise class on appetite, energy intake and mood. Is there a time of day effect? Appetite. 2005;45(3):272-8.
65. Pomerleau M, Imbeault P, Parker T, Doucet E. Effects of exercise intensity on food intake and appetite in women. The American Journal of Clinical Nutrition. 2004;80(5):1230-6.
66. King JA, Wasse LK, Broom DR, Stensel DJ. Influence of brisk walking on appetite, energy intake, and plasma acylated ghrelin. Medicine and Science in Sports and Exercise. 2010;42(3):485-92.
67. Ueda S, Yoshikawa T, Katsura Y, Usui T, Nakao H, Fujimoto S. Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. Journal of Endocrinology. 2009;201(1):151-9.
68. Ueda S, Yoshikawa T, Katsura Y, Usui T, Fujimoto S. Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. Journal of Endocrinology. 2009;203(3):357-64.
69. Jokisch E, Coletta A, Raynor HA. Acute energy compensation and macronutrient intake following exercise in active and inactive males who are normal weight. Appetite. 2012;58(2):722-9.
70. Larson-Meyer D, Palm S, Bansal A, Austin K, Hart A, Alexander B. Influence of running and walking on hormonal regulators of appetite in women. Journal of Obesity. 2012;2012:1-15.
71. Hopkins M, Blundell JE, King NA. Individual variability in compensatory eating following acute exercise in overweight and obese women. British Journal of Sports Medicine. 2014;48(20):1472.
72. Burns SF, Broom DR, Miyashita M, Mundy C, Stensel DJ. A single session of treadmill running has no effect on plasma total ghrelin concentrations. J Sports Sci. 2007;25:635-42.
73. Dall R, Kanaley J, Hansen TK, Møller N, Christiansen JS, Hosoda H, et al. Plasma ghrelin levels during exercise in healthy subjects and in growth hormone-deficient patients. Eur J Endocrinol. 2002;147:65-70.

74. Jurimae J, Hofmann P, Jurimae T, Palm R, Maestu J, Purge P, et al. Plasma ghrelin responses to acute sculling exercises in elite male rowers. Eur J Appl Physiol. 2007;99:467-74.
75. Kallio J, Pesonen U, Karvonen MK. Enhanced exercise-induced GH secretion in subjects with Pro7 substitution in the prepro-NPY. The Journal of Clinical Endocrinology & Metabolism. 2001;86:5348-52.
76. Kraemer RR, Durand RJ, Acevedo EO, Johnson LG, Kraemer GR, Hebert EP, et al. Rigorous running increases growth hormone and insulin like growth factor-I without altering ghrelin. Experimental Biology and Medicine (Maywood, NJ). 2004;229(3):240-6.
77. Schmidt A, Maier C, Schaller G. Acute exercise has no effect on ghrelin plasma concentrations. Hormone and Metabolic Research. 2004;36(3):174-7.
78. Christ ER, Zehnder M, Boesch C, Trepp R, Mullis PE, Diem P, et al. The effect of increased lipid intake on hormonal responses during aerobic exercise in endurance-trained men. European Journal of Endocrinology. 2006;154(3):397-403.
79. Vestergaard ET, Dall R, Lange KHW, Kjaer M, Christiansen JS, Jorgensen JOL. The ghrelin response to exercise before and after growth hormone administration. The Journal of Clinical Endocrinology and Metabolism. 2007;92(1):297-303.
80. Broom DR, Stensel DJ, Bishop NC, Burns SF, Miyashita M. Exercise-induced suppression of acylated ghrelin in humans. Journal of Applied Physiology. 2007;102(6):2165-71.
81. Douglas JA, Deighton K, Atkinson JM, Sari-Sarraf V, Stensel DJ, Atkinson G. Acute exercise and appetite-regulating hormones in overweight and obese individuals: A meta-analysis. Journal of Obesity. 2016;2016:2643625-8.
82. King JA, Deighton K, Broom DR, Wasse LK, Douglas JA, Burns SF, et al. Individual variation in hunger, energy intake, and ghrelin responses to acute exercise. Medicine and Science in Sports and Exercise. 2017;49(6):1219-28.
83. Drenowatz C, Greier MdC, Greier K. Association of exercise with control of eating and energy intake. Current Addiction Reports. 2019;6(3):210-7.
84. Hazell TJ, Townsend LK, Hallworth JR, Doan J, Copeland JL. Sex differences in the response of total PYY and GLP-1 to moderate-intensity continuous and sprint interval cycling exercise. European Journal of Applied Physiology. 2017;117(3):431-40.
85. He Z, Gao Y, Alhadeff AL, Castorena CM, Huang Y, Lieu L, et al. Cellular and synaptic reorganization of arcuate NPY/AgRP and POMC neurons after exercise. Molecular Metabolism (Germany). 2018;18:107-19.
86. Jiaxu C, Weiyi Y. Influence of acute and chronic treadmill exercise on rat brain POMC gene expression. Medicine and Science in Sports and Exercise. 2000;32(5):954-7.
87. Olive JL, Miller GD. Differential effects of maximal- and moderate-intensity runs on plasma leptin in healthy trained subjects. Nutrition (Burbank, Los Angeles County, Calif). 2001;15(5):365-9.
88. Hazell TJ, Islam H, Townsend LK, Schmale MS, Copeland JL. Effects of exercise intensity on plasma concentrations of appetite- regulating hormones: potential mechanisms. Appetite. 2016;90:80-8.
89. Horner KM, Schubert MM, Desbrow B, Byrne NM, King N. Acute exercise and gastric emptying: A meta-analysis and implications for appetite control. Sports Medicine (Auckland). 2014;45(5):659-78.
90. Alajmi N, Deighton K, King JA, Reischak-Oliveira A, Wasse LL, Jones J, et al. Appetite and energy intake responses to acute energy deficits in females versus males. Medicine and Science in Sports and Exercise. 2016;48(3):412-20.

91. Douglas JA, King JA, Clayton DJ, Jackson AP, Sargeant JA, Thackray AE, et al. Acute effects of exercise on appetite, ad libitum energy intake and appetite-regulatory hormones in lean and overweight/obese men and women. International Journal of Obesity (2005). 2017;41(12):1737.
92. Bachman JL, Deitrick RW, Hillman AR. Exercising in the fasted state reduced 24-hour energy intake in active male adults. Journal of Nutrition and Metabolism. 2016;2016:1984198-7.
93. Deighton K, Zahra J, Stensel D. Appetite, energy intake and resting metabolic responses to 60 min treadmill running performed in a fasted versus a postprandial state. Appetite. 2012;58(3):946-54.
94. King NA, Hopkins M, Caudwell P, Stubbs RJ, Blundell JE. Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss. International Journal of Obesity (2005). 2007;32(1):177-84.
95. Caudwell P, Gibbons C, Hopkins M, King N, Finlayson G, Blundell J. No sex difference in body fat in response to supervised and measured exercise. Medicine and Science in Sports and Exercise. 2013;45(2):351-8.
96. Koshki MH, Mollanovruzi A, Lamir AR. Effect of chronic high-intensity exercise on hunger and satiation and levels of acylated ghrelin and leptin in women. Biomedical Human Kinetics. 2018;10(1):67-75.
97. Rosenkilde M, Reichkendler MH, Auerbach P, Toräng S, Gram AS, Ploug T, et al. Appetite regulation in overweight, sedentary men after different amounts of endurance exercise: a randomized controlled trial. Journal of Applied Physiology. 2013;115(11):1599-609.
98. Stubbs RJ, Sepp A, Hughes DA, Johnstone AM, Horgan GW, King N, et al. The effect of graded levels of exercise on energy intake and balance in free-living men, consuming their normal diet. European Journal of Clinical Nutrition. 2002;56(2):129-40.
99. Martin CK, Johnson WD, Myers CA, Apolzan JW, Earnest CP, Thomas DM, et al. Effect of different doses of supervised exercise on food intake, metabolism, and non-exercise physical activity: The E-MECHANIC randomized controlled trial. The American Journal of Clinical Nutrition. 2019;110(3):583-92.
100. Flack K, Hays H, Moreland J, Long D. Exercise for weight loss: Further evaluating energy compensation with exercise. Medicine & Science in Sports & Exercise. 2020;52(11):2466-75.
101. Hough J, Esh C, Mackie P, Stensel DJ, Zakrzewski-Fruer JK. Daily running exercise may induce incomplete energy intake compensation: a 7-day crossover trial. Applied Physiology, Nutrition & Metabolism. 2020;45(4):446-9.
102. Höchsmann C, Dorling JL, Apolzan JW, Johannsen NM, Hsia DS, Martin CK. Baseline habitual physical activity predicts weight loss, weight compensation, and energy intake during aerobic exercise. Obesity (Silver Spring, Md). 2020;28(5):882-92.
103. Alkahtani SA, Byrne NM, Hills AP, King NA. Interval training intensity affects energy intake compensation in obese men. International Journal of Sport Nutrition and Exercise Metabolism. 2014;24(6):595-604.
104. Martins C, Aschehoug I, Ludviksen M, Holst J, Finlayson G, Wisloff U, et al. High-intensity interval training, appetite, and reward value of food in the obese. Medicine and Science in Sports and Exercise. 2017;49(9):1851-8.
105.Hagobian TA, Sharoff CG, Stephens BR, Wade GN, Silva JE, Chipkin SR, et al. Effects of exercise on energy-regulating hormones and appetite in men and women. American Journal of Physiology Regulatory, Integrative and Comparative Physiology. 2009;296(2):R233.

106. Doucet É, McInis K, Mahmoodianfard S. Compensation in response to energy deficits induced by exercise or diet. Obesity Reviews. 2018;19(S1):36-46.
107. Evaluation. NRCUSoCfD. Errors in nutrient intake measurements. 1986. In: Nutrient adequacy: Assessment using food consumption survey [Internet]. Washington National Academy Press.
108. Foright RM, Johnson GC, Kahn D, Charleston CA, Presby DM, Bouchet CA, et al. Compensatory eating behaviors in male and female rats in response to exercise training. Am J Physiol Regul Integr Comp Physiol. 2020;319(2):R171-R83.
109. Campbell SC, Wisniewski PJ, Noji M, McGuinness LR, Häggblom MM, Lightfoot SA, et al. The effect of diet and exercise on intestinal integrity and microbial diversity in mice. PLoS One. 2016;11(3).
110. de Carvalho FP, Benfato ID, Moretto TL, Barthichoto M, de Oliveira CAM. Voluntary running decreases nonexercise activity in lean and diet-induced obese mice. Physiology & Behavior. 2016;165:249-56.
111. Evans CC, LePard KJ, Kwak JW, Stancukas MC, Laskowski S, Dougherty J, et al. Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. PLOS ONE. 2014;9(3):e92193.
112. Takeshita H, Horiuchi M, Izumo K, Kawaguchi H, Arimura E, Aoyama K, et al. Long-term voluntary exercise, representing habitual exercise, lowers visceral fat and alters plasma amino acid levels in mice. Environmental Health and Preventive Medicine. 2011;17(4):275-84.
113. McMullan RC, Kelly SA, Hua K, Buckley BK, Faber JE, Pardo-Manuel de Villena F, et al. Long-term exercise in mice has sex-dependent benefits on body composition and metabolism during aging. Physiological Reports. 2016;4(21):e13011-n/a.
114. Beaulieu K, Hopkins M, Blundell J, Finlayson G. Does habitual physical activity increase the sensitivity of the appetite control system? A systematic review. Sports medicine (Auckland). 2016;46(12):1897-919.
115. Tehrani AB, Nezami BG, Gewirtz A, Srinivasan S. Obesity and its associated disease: a role for microbiota? Neurogastroenterology and Motility. 2012;24:305-11.
116. Allen JM, Mailing LJ, Niemiro GM, Moore R, Cook MD, White BA, et al. Exercise alters gut microbiota composition and function in lean and obese humans. Medicine and Science in Sports and Exercise. 2018;50(4):747-57.
117. Jones TE, Basilio JL, Brophy PM, McCammon MR, Hickner RC. Long-term exercise training in overweight adolescents improves plasma peptide YY and resistin. Obesity. 2009;17(6):1189-95.
118. Chanoine JP, Mackelvie KJ, Barr SI, Wong ACK, Meneilly GS, Elahi DH. GLP-1 and appetite responses to a meal in lean and overweight adolescents following exercise. Obesity. 2008;16(1):202-4.
119. Martins C, Kulseng B, King NA, Holst JJ, Blundell JE. The effects of exercise-induced weight loss on appetite-related peptides and motivation to eat. The Journal of Clinical Endocrinology and Metabolism. 2010;95(4):1609-16.
120. Martins C, Kulseng B, Rehfeld JF, King NA, Blundell JE. Effect of chronic exercise on appetite control in overweight and obese individuals. Medicine and Science in Sports and Exercise. 2013;45(5):805-12.
121. Fedewa M, Hathaway E, Ward-Ritaco C, William T, Dobbs W. The effects of chronic exercise training on leptin: A systematic review and meta-analysis of randomized controlled trials. Sports Medicine. 2018;48(6):1437.

122. Bi S, Scott KA, Hyun J, Ladenheim EE, Moran TH. Running wheel activity prevents hyperphagia and obesity in otsuka long-evans tokushima fatty rats: Role of hypothalamic signaling. Endocrinology. 2005;146(4):1676-85.
123. Ebal E, Cavalie H, Michaux O, Lac G. Effect of moderate exercise on the regulatory hormones of food intake in rats. Appetite. 2007;49(2):521-4.
124. Bradley RL, Jeon JY, Liu F, Maratos-Flier E. Voluntary exercise improves insulin sensitivity and adipose tissue inflammation in diet-induced obese mice. American Journal of Physiology: Endocrinology and Metabolism. 2008;295(3):E586.
125. Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. Annual Review of Medicine. 1998;49(1):235-65.
126. Lund MT, Taudorf L, Hartmann B, Helge JW, Holst JJ, Dela F. Meal induced gut hormone secretion is altered in aerobically trained compared to sedentary young healthy males. European Journal of Applied Physiology. 2013;113(11):2737-47.
127. Allen JM, Berg Miller M, Pence BD, Whitlock K, Nehra V, Gaskins HR, et al. Voluntary and forced exercise differentially alters the gut microbiome in C57BL/6J mice. Journal of Applied Physiology (1985). 2015;118(8):1059-66.
128. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology - Human gut microbes associated with obesity. Nature. 2006;444(7122):1022-3.
129. Mahdieh M, Maryam J, Bita B, Neda F, Motahare M, Mahboobeh B, et al. A pilot study on the relationship between lactobacillus, bifidibacterium counts and inflammatory factors following exercise training. Archives of Physiology and Biochemistry. 2021:1-10.
130. Resende A, Leite G, Lancha Junior A. Changes in the gut bacteria composition of healthy men with the same nutritional profile undergoing 10-week aerobic exercise training: A randomized controlled trial. Nutrients. 2021;13(8):2839.
131. Barton W, Penney N, Cronin O, Garcia-Perez I, Molloy M, Holmes E, et al. The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level. Gut. 2018;2017;67(4):625-33.
132. Queipo-Ortuño MI, Seoane LM, Murri M, Pardo M, Gomez-Zumaquero JM, Cardona F, et al. Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. PloS One. 2013;8(5):e65465.
133. Finlayson G, Caudwell P, Gibbons C, Hopkins M, King N, Blundell J. Low fat loss response after medium-term supervised exercise in obese is associated with exercise-induced increase in food reward. Journal of Obesity. 2011;2011:1-8.
134. Moody L, Liang J, Choi PP, Moran TH, Liang N. Wheel running decreases palatable diet preference in Sprague–Dawley rats. Physiology & Behavior. 2015;150:53-63.
135. Liang N, Bello NT, Moran TH. Wheel running reduces high-fat diet intake, preference and mu-opioid agonist stimulated intake. Behavioural Brain Research. 2015;284:1.
136. Demos KE, Heatherton TF, Kelley WM. Individual differences in nucleus accumbens activity to food and sexual images predict weight gain and sexual behavior. J Neurosci. 2012;32:5549-52.
137. Rapuano KM, Zieselman AL, Kelley WM, Sargent JD, Heatherton TF, Gilbert-Diamond D. Genetic risk for obesity predicts nucleus accumbens size and responsivity to real-world food cues. Proc Natl Acad Sci. 2017;114(1):160-5.
138. Samara A, Li Z, Rutlin J, Raji CA, Sun P, Song SK, et al. Nucleus accumbens microstructure mediates the relationship between obesity and eating behavior in adults. Obesity. 2021;29(8):1328-37.

139. Abdo N, Boyd E, Baboumian S, Pantazatos SP, Geliebter A. Relationship between binge eating and associated eating behaviors with subcortical brain volumes and cortical thickness. J Affect Disord. 2020;274:1201-5.
140. Beyer F, García-García I, Heinrich M, Schroeter ML, Sacher J, Luck T, et al. Neuroanatomical correlates of food addiction symptoms and body mass index in the general population. Hum Brain Mapp. 2019;40(9):2747-58.
141. Horstmann A, Busse FP, Mathar D, Müller K, Lepsien J, Schlögl H, et al. Obesity-Related differences between women and men in brain structure and goal-directed behavior. Front Hum Neurosci. 2011;5(58).
142. Dekkers IA, Jansen PR, Lamb HJ. Obesity, brain volume, and white matter microstructure at MRI: a cross-sectional UK Biobank study. Radiology. 2019;291(3):763-71.
143. García-García I, Michaud A, Dadar M, Zeighami Y, Neseliler S, Collins DL, et al. Neuroanatomical differences in obesity: meta-analytic findings and their validation in an independent dataset. Int J Obes (Lond). 2019;43(5):943-51.
144. García-García I, Morys F, Dagher A. Nucleus accumbens volume is related to obesity measures in an age-dependent fashion. Journal of Neuroendocrinology. 2020;32(12).
145. O'Neal TJ, Friend DM, Guo J, Hall KD, Kravitz AV. Increases in physical activity result in diminishing increments in daily energy expenditure in mice. Current Biology. 2017;27(3):423-30.
146. Levin BE, Dunn-Meynell AA. Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats. Am J Physiol Regul Integr Comp Physiol. 2004;286(4):R771.
147. Lark D, Kwan J, McClatchey P, James MN, James FD, Lighton JRB, et al. Reduced nonexercise activity attenuates negative energy balance in mice engaged in voluntary exercise. Diabetes (New York, NY). 2018;67(5):831-40.
148. Melanson EL, Keadle SK, Donnelly JE, Braun B, King NA. Resistance to exercise-induced weight loss: Compensatory behavioral adaptations. Medicine and Science in Sports and Exercise. 2013;45(8):1600-9.
149. Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: Final report of the american institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. The Journal of Nutrition. 1993;123(11):1939-51.
150. Welly RJ, Liu TW, Zidon TM, Rowles JL 3rd, Park YM, Smith TN, et al. Comparison of diet versus exercise on metabolic function and gut microbiota in obese rats. Med Sci Sports Exerc. 2016;48(9):1688-98.
151. Benedé-Ubieto R, Estévez-Vázquez O, Ramadori P, Javier Cubero F, Nevzorova YA. Guidelines and considerations for metabolic tolerance tests in mice. Diabetes Metab Syndr Obes. 2020;13:439-350.
152. Lee G, Goosen K. Sampling blood from the lateral tail vein of the rat. J Vis Exp (JoVE). 2015;99:e52766.
153. Grover G, Koetzner L, Wicks J, Gahler R, Lyon M, Reimer R, et al. Effects of the soluble fiber complex PolyGlycopleX® (PGX®) on glycemic control, insulin secretion, and GLP-1 levels in Zucker diabetic rats. Life Sci. 2011;88:392-9.
154. Soueid J, Nokkari A, Makoukji J. Chapter 15: Techniques and methods of animal brain surgery: Perfusion, brain removal, and histological techniques. Kobeissy FH, editor. Boca Raton (FL): CRC Press/Taylor & Francis; 2015.

155. Bomhof MR, Saha DC, Reid DT, Paul HA, Reimer RA. Combined effects of oligofructose and Bifidobacterium animalis on gut microbiota and glycemia in obese rats. Obesity (Silver Spring). 2014;22:763-71.
156. Parnell JA, Reimer RA. Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR: LA-cp rats
Br J Nutr. 2012;107:601-13.
157. Paxinos G, Watson C. The rat brain in stereotaxic coordinates: hard cover edition. Access Online via Elsevier,2006 [Available from: http://labs.gaidi.ca/rat-brain-atlas/.
158. Rasband WS. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA,1997-2018 [Available from: https://ui.adsabs.harvard.edu/abs/2012ascl.soft06013R/abstract.
159. NC3Rs. Temporary Cannula (non-surgical). National Center for the Replacement, Refinement and Reduction of Animals in Research.2022 [Available from: https://www.nc3rs.org.uk/3rs-resources/blood-sampling/blood-sampling-rat.
160. Droste SK, Gesing A, Ulbricht S, Müller MB, Linthorst ACE, Reul JMHM. Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis. Endocrinology. 2003;144(7):3012-23.
161. Gollisch KSC, Brandauer J, Jessen N, Toyoda T, Nayer A, Hirshman MF, et al. Effects of exercise training on subcutaneous and visceral adipose tissue in normal- and high-fat diet-fed rats. Am J Physiol Endocrinol Metab. 2009;297(2):E495-504.
162. Harri M, Lindblom J, Malinen H, Hyttinen M, Lapvetelainen T, Eskola S, et al. Effect of access to a running wheel on behavior of C57BL/6 J mice. Laboratory Animal Science. 1999;49(4):401-5.
163. Jung AP, Luthin DR. Wheel access does not attenuate weight gain in mice fed high-fat or high-CHO diets. Med Sci Sports Exerc. 2010;42(2):355-60.
164. Swallow JG, Koteja P, Carter PA, Garland T. Food consumption and body composition in mice selected for high wheel-running activity. Journal of Comparative Physiology, Biochemical, Systemic, and Environmental Physiology. 2001;171(8):651-9.
165. Tanimura R, Kobayashi L, Shirai T, Takemasa T. Effects of exercise intensity on white adipose tissue browning and its regulatory signals in mice. Physiological Reports. 2022;10(5):e15205.
166. Huang P, Li S, Shao M, Qi Q, Zhao F, You J, et al. Calorie restriction and endurance exercise share potent anti-inflammatory function in adipose tissues in ameliorating diet-induced obesity and insulin resistance in mice. Nutr Metab (Lond). 2010;7(59).
167. Brown JD, Naples SP, Booth FW. Effects of voluntary running on oxygen consumption, RQ, and energy expenditure during primary prevention of diet-induced obesity in C57BL/6N mice. J Appl Physiol. 2012;113(3):473-8.
168. Cordeira J, Monahan D. Voluntary wheel running reduces weight gain in mice by decreasing high-fat food consumption. Physiol Behav. 2019;207:1-6.
169. Scarpace PJ, Matheny M, Zhang Y. Wheel running eliminates high-fat preference and enhances leptin signaling in the ventral tegmental area. Physiol Behav. 2010;100(2):173-9.
170. Bell RR, Spencer MJ, Sherriff JL. Voluntary exercise and monounsaturated canola oil reduce fat gain in mice fed diets high in fat. The Journal of Nutrition. 1997;127(10):2006-10.
171. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev. 2004;84(1):277-359.

172. Sanchez-delgado G, Martinez-tellez B, Olza J, Aguilera CM, Gil Á, Ruiz JR. Role of exercise in the activation of brown adipose tissue. Annals of Nutrition & Metabolism. 2015;67(1):21-32.
173. Knuth CM, Peppler WT, Townsend LK, Miotto PM, Gudiksen A, Wright DC. Prior exercise training improves cold tolerance independent of indices associated with non-shivering thermogenesis. J Physiol. 2018;596:4375-91.
174. Wu MV, Bikopoulos G, Hung S, Ceddia RB. Thermogenic capacity is antagonistically regulated in classical brown and white subcutaneous fat depots by high fat diet and endurance training in rats: impact on whole-body energy expenditure. J Biol Chem. 2014;289:34129-40.
175. Hirata K. Blood flow to brown adipose tissue and norepinephrine- induced calorigenesis in physically trained rats. Jpn J Physiol. 1982;32:279-91.
176. Yoshioka K, Yoshida T, Wakabayashi Y, Nishioka H, M. K. Effects of exercise training on brown adipose tissue thermogenesis in ovariectomized obese rats. Endocrinol Jpn. 1989;36:403-8.
177. Richard D, Arnold J, Leblanc J. Energy balance in exercise-trained rats acclimated at two environmental temperatures. J Appl Physiol. 1986;60:1054-9.
178. Scarpace PJ, Yenice S, Tumer N. Influence of exercise training and age on uncoupling protein mRNA expression in brown adipose tissue. Pharmacol Biochem Behav. 1994;49:1057-9.
179. Singhal V, Maffazioli GD, Ackerman KE, Lee H, Elia EF, Woolley R, et al. Effect of chronic athletic activity on brown fat in young women. PLOS ONE. 2016;11(8):e0160129.
180. Townsend LK, Wright DC. Looking on the “brite” side exercise-induced browning of white adipose tissue. Pflügers Archiv. 2019;471(3):455-65.
181. Chen W, Wang HJ, Shang NN, Liu J, Li J, Tang DH, et al. Moderate intensity treadmill exercise alters food preference via dopaminergic plasticity of ventral tegmental area-nucleus accumbens in obese mice. Neurosci Lett. 2017;641:56-61.
182. Haghshenas R, Jafari M, Ravasi A, Kordi M, Gilani N, Shariatzadeh M, et al. The effect of eight week endurance training and high-fat diet on appetite-regulating hormones in rat plasma. Iranian Journal of Basic Medical Science. 2014;17(4):237-43.
183. Ouerghi N, Feki M, Bragazzi NL, Knechtle B, Hill L, Nikolaidis PT, et al. Ghrelin response to acute and chronic exercise: Insights and implications from a systematic review of the literature. Sports Med. 2021;51(11):2389-410.
184. Ueda SY, Miyamoto T, Nakahara H, Shishido T, Usui T, Katsura Y, et al. Effects of exercise training on gut hormone levels after a single bout of exercise in middle-aged Japanese women. Springerplus. 2013;2(1).
185. Bowyer KP, Carson JA, Davis JM, Wang X. The influence of exercise training dose on fasting acylated ghrelin concentration in older women. J Behav Med. 2019;62(2):235-43.
186. Blundell JE, Gibbons C, Caudwell P, Finlayson G, Hopkins M. Appetite control and energy balance: impact of exercise. Obesity Reviews. 2015;16(S1):67-76.
187. Guyenet SJ, Schwartz MW. Regulation of food intake, energy balance, and body fat mass: Implications for the pathogenesis and treatment of obesity. The Journal of Clinical Endocrinology & Metabolism. 2012;97(3):745-55.
188. Hopkins M, Gibbons C, Caudwell P, Webb DL, Hellström PM, Näslund E, et al. Fasting leptin is a metabolic determinant of food reward in overweight and obese individuals during chronic aerobic exercise training. Int J Endocrinol. 2014;2014:323728.

189. Marson EC, Delevatti RS, Prado AK, Netto N, Kruel LF. Effects of aerobic, resistance, and combined exercise training on insulin resistance markers in overweight or obese children and adolescents: A systematic review and meta-analysis. Prev Med. 2016;93:211-8.
190. Bouarab C, Thompson B, Polter AM. VTA GABA neurons at the interface of stress and reward. Front Neural Circuits. 2019;13(78).
191. Fernández-Espejo E. Cómo funciona el nucleus accumbens? [How does the nucleus accumbens function?]. Rev Neurol. 2000;30(9):845-9.
192. Hathaway WR, Newton BW. Neuroanatomy, Prefrontal Cortex. Treasure Island (FL): StatPearls Publishing; 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499919/.
193. AbuHasan Q, Reddy V SW. Neuroanatomy, Amygdala. 2021. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537102/.
194. Anand KS, Dhikav V. Hippocampus in health and disease: An overview. Ann Indian Acad Neurol. 2012;15(4):239-46.
195. de Macedo IC, de Freitas JS, da Silva Torres IL. The influence of palatable diets in reward system activation: A mini review. Adv Pharmacol Sci. 2016;2016:7238679.
196. Berthoud HR, Morrison C. The brain, appetite, and obesity. Annu Rev Psychol. 2008;59:55-92.
197. Volkow ND, Wang GJ, Tomasi D, Baler RD. Obesity and addiction: neurobiological overlaps. Obes Rev. 2013;14(1):2-18.
198. Horstmann A, Busse FP, Mathar D, Müller K, Lepsien J, Schlögl H, et al. Obesity-related differences between women and men in brain structure and goal-directed behavior. Frontiers in Human Neuroscience. 2011;5(58).
199. Rapuano KM, Zieselman AL, Kelley WM, Sargent JD, Heatherton TF, Gilbert-Diamond D. Genetic risk for obesity predicts nucleus accumbens size and responsivity to real-world food cues. Proc Natl Acad Sci U S A. 2017;114(1):160-5.
200. Berthoud HR. Interactions between the 'cognitive' and 'metabolic' brain in the control of food intake. Physiol Behav. 2007;91:486-98.
201. Makino H. Bifidobacterial strains in the intestines of newborns originate from their mothers. Biosci Microbiota Food Health. 2018;37(4):79-85.
202. Lim HJ, Shin HS. Antimicrobial and immunomodulatory effects of Bifidobacterium strains: A review. J Microbiol Biotechnol. 2020;30(12):1793-800.
203. O'Callaghan A, van Sinderen D. Bifidobacteria and their role as members of the human gut microbiota. Front Microbiol. 2016;7.
204. Lambert JE, Myslicki JP, Bomhof MR, Belke DD, Shearer J, Reimer RA. Exercise training modifies gut microbiota in normal and diabetic mice. Appl Physiol Nutr Metab. 2015;40(7):749-52.
205. Munukka E, Ahtiainen JP, Puigbó P, Jalkanen S, Pahkala K, Keskitalo A, et al. Six-week endurance exercise alters gut metagenome that is not reflected in systemic metabolism in over-weight women. Front Microbiol. 2018;9:2323.
206. Dohrmann AB, Walz M, Löwen A, Tebbe CC. Clostridium cluster I and their pathogenic members in a full-scale operating biogas plant. Appl Microbiol Biotechnol. 2015;99(8):3585-98.
207. Bintsis T. Foodborne pathogens. AIMS Microbiol. 2017;3(3):529-63.
208. Zuo HJ, Xie ZM, Zhang WW, Li YR, Wang W, Ding XB, et al. Gut bacteria alteration in obese people and its relationship with gene polymorphism. World J Gastroenterol. 2011;17(8):1076-981.

209. Spieth PM, Kubasch AS, Penzlin AI, Illigens BM, Barlinn K, Siepmann T. Randomized controlled trials - a matter of design. Neuropsychiatric Disease and Treatment. 2016;12:1341-9.
210. Bryda EC. The mighty mouse: the impact of rodents on advances in biomedical research. Missouri Medicine. 2013;110(3):207-11.
211. Goutianos G, Tzioura A, Kyparos A, Paschalis V, Margaritelis NV, Veskoukis AS, et al. The rat adequately reflects human responses to exercise in blood biochemical profile: a comparative study. Physiological Reports. 2015;3(2):e12293.
212. Giles ED, Jackman MR, Johnson GC, Schedin PJ, Houser JL, MacLean PS. Effect of the estrous cycle and surgical ovariectomy on energy balance, fuel utilization, and physical activity in lean and obese female rats. American Journal of Physiology Regulatory, Integrative and Comparative Physiology. 2010;299(6):R1634.
213. Davidsen L, Vistisen B, Astrup A. Impact of the menstrual cycle on determinants of energy balance: a putative role in weight loss attempts. Int J Obes (Lond). 2007;31(12):1777-85.
214. Brommage R. Validation and calibration of DEXA body composition in mice. Am J Physiol Endocrinol Metab. 2003;285(3):E454-9.
215. Gerbaix M, Metz L, Ringot E, Courteix D. Visceral fat mass determination in rodent: validation of dual-energy x-ray absorptiometry and anthropometric techniques in fat and lean rats. Lipids Health Dis. 2010;9:140.
216. Cani PD. Gut microbiota and obesity: lessons from the microbiome. Briefings in Functional Genomics. 2013;12(4):381-7.
217. McNeil J, Cadieux S, Finlayson G, Blundell JE, Doucet E. The effects of a single bout of aerobic or resistance exercise on food reward. Appetite. 2015;84:262-70.
218. Panasevich MR, Allen JM, Wallig MA, Woods JA, Dilger RN. Moderately fermentable potato fiber attenuates signs and inflammation associated with experimental colitis in mice. J Nutr. 2015;145(12):2781-8.
219. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nature Reviews Endocrinology. 2015;11(10):577-91.
220. Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes. 2016;7(3):189-200.
221. Samara A, Li Z, Rutlin J, Raji CA, Sun P, Song SK, et al. Nucleus accumbens microstructure mediates the relationship between obesity and eating behavior in adults. Obesity (Silver Spring). 2021;29(8):1328-37.
222. Knab AM, Lightfoot JT. Does the difference between physically active and couch potato lie in the dopamine system? Int J Biol Sci. 2010;6(2):133-50.
223. Lenard NR, Berthoud H. Central and peripheral regulation of food intake and physical activity: Pathways and genes. Obesity (Silver Spring). 2012;16:S11-S22.
224. Shah M, Vella A. Effects of GLP-1 on appetite and weight. Rev Endocr Metab Disord. 2014;15(3):181-7.
225. Blundell J, Finlayson G, Axelsen M, Flint A, Gibbons C, Kvist T, et al. Effects of once-weekly semaglutide on appetite, energy intake, control of eating, food preference and body weight in subjects with obesity. Diabetes, Obesity & Metabolism. 2017;19(9):1242-51.
226. Zander M, Madsbad S, Madsen JL, Holst J. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet. 2002;359(9309):824-30.

227. Lundgren JR, Janus C, Jensen SBK, Juhl CR, Olsen LM, Christensen RM, et al. Healthy weight loss maintenance with exercise, liraglutide, or both combined. N Engl J Med. 2021;384(18):1719-30.
228. Koliaki C, Spinos T, Spinou M, Brinia ME, Mitsopoulou D, Katsilambros N. Defining the optimal dietary approach for safe, effective and sustainable weight loss in overweight and obese adults. Healthcare (Basel). 2018;6(3):73.
229. Welton S, Minty R, O'Driscoll T, Willms H, Poirier D, Madden S, et al. Intermittent fasting and weight loss: Systematic review. Can Fam Physician. 2020;66(2):117-25.
230. Hansen TT, Astrup A, Sjödin A. Are dietary proteins the key to successful body weight management? A systematic review and meta-analysis of studies assessing body weight outcomes after interventions with increased dietary protein. Nutrients [Internet]. 2021;13(9).
231. Wilborn C, Beckham J, Campbell B, Harvey T, Galbreath M, La Bounty P, et al. Obesity: Prevalence, theories, medical consequences, management, and research directions. J Int Soc Sports Nutr. 2005;2:4-21.
232. Kotarsky CJ, Johnson NR, Mahoney SJ, Mitchell SL, Schimek RL, Stastny SN, et al. Time-restricted eating and concurrent exercise training reduces fat mass and increases lean mass in overweight and obese adults. Physiol Rep. 2021;9(10):e14868.
233. Galbreath M, Campbell B, LaBounty P, Bunn J, Dove J, Harvey T, et al. Effects of adherence to a higher protein diet on weight loss, markers of health, and functional capacity in older women participating in a resistance-based exercise program. Nutrients. 2018;10(8):1070.
234. Li VL, He Y, Contrepois K, Liu H, Kim JT, Wiggenhorn AL, et al. An exercise-inducible metabolite that suppresses feeding and obesity. Nature. 2022;606(7915):785-90.