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The Effects of an Acute Bout of Exercise on Hunger Hormones in Individuals at Risk for Type 2 Diabetes

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THE EFFECTS OF AN ACUTE BOUT OF EXERCISE ON HUNGER HORMONES IN
INDIVIDUALS AT RISK FOR TYPE 2 DIABETES

By

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Bachelor of Science – Nutrition Sciences
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2014

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science – Exercise Physiology

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Division of Health Sciences
The Graduate College

University of Nevada, Las Vegas
May 2017
This thesis prepared by

Sydney C. Spoon

entitled

The Effects of an Acute Bout of Exercise on Hunger Hormones in Individuals at Risk for Type 2 Diabetes

is approved in partial fulfillment of the requirements for the degree of

Master of Science – Exercise Physiology
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ABSTRACT

Background: Hunger hormone levels are typically dysregulated in obese and diabetic populations, however; postprandial exercise has been shown to influence hunger hormone levels.

Purpose: To determine if hunger hormones including acylated ghrelin and GLP-1 levels are altered in response to an acute bout (15 minutes) of walking in individuals at risk for type 2 diabetes mellitus (T2DM).

Methods: Ten subjects at risk for prediabetes (fasting blood glucose 100–125mg/dL) participated in this randomized crossover design trial. Subjects arrived at the laboratory following an overnight fast and underwent one of two conditions: 1) Test meal with no walking (CON) or 2) Test meal followed by a 15-minute treadmill walk at preferred walking speed (WALK). Blood samples were taken over two hours and assayed for acylated ghrelin and active GLP-1. A repeated measures ANOVA was used to compare mean differences for all outcome variables.

Results: There were no statistical differences in acylated ghrelin (F = 1.535, p = 0.247) or GLP-1 (F = 0.003, p = 0.955) concentrations between CON and WALK conditions at any time period. There was a main effect of time for ghrelin (F = 41.339; p < 0.001). Post hoc analysis indicated a significant difference between baseline and 60 minutes (p < 0.001) and between baseline and 120 minutes (p < 0.001) for acylated ghrelin concentrations. No difference was found between 60 minutes and 120 minutes (p = 0.834). There was a main effect of time for GLP-1 (F = 17.968; p < 0.001). Post hoc analysis indicated a significant difference between baseline and 60 minutes (p = 0.001) and between baseline and 120 minutes (p = 0.002) for GLP-1 concentrations. No difference was found between 60 minutes and 120 minutes (p = 0.665). There was no significant difference in the AUC for acylated ghrelin between the CON and WALK conditions (t = -1.257;
p = 0.240). There was no significant difference in the AUC for GLP-1 between the CON and WALK conditions (t = -0.107; p = 0.918). Correlations between perceived hunger and biological hunger were weak and nonsignificant (p > 0.05).

**Conclusion:** A 15-minute walk performed shortly after a meal does not have a significant impact on hunger hormones including acylated ghrelin and active GLP-1 concentrations in individuals at risk for T2DM.
ACKNOWLEDGEMENTS

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<tbody>
<tr>
<td>AgRP</td>
<td>Agouti-related peptide</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ARC</td>
<td>Arcuate nucleus</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CART</td>
<td>Cocaine and amphetamine-related transcript</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DPP-IV</td>
<td>Dipeptidyl-peptidase IV</td>
</tr>
<tr>
<td>GH</td>
<td>Growth hormone</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>GHS-R</td>
<td>Growth hormone-secretagogue receptor</td>
</tr>
<tr>
<td>GIP</td>
<td>Glucose-dependent insulinotropic polypeptide</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>GLP-1R</td>
<td>Glucagon-like peptide-1 receptor</td>
</tr>
<tr>
<td>GLP-2</td>
<td>Glucagon-like peptide-2</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>IFG</td>
<td>Impaired fasting glucose</td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired glucose tolerance</td>
</tr>
<tr>
<td>MPH</td>
<td>Miles per hour</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>OGGT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>PAR-Q</td>
<td>Physical activity readiness questionnaire</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
</tr>
<tr>
<td>PP</td>
<td>Pancreatic polypeptide</td>
</tr>
<tr>
<td>PYY</td>
<td>Peptide tyrosine-tyrosine</td>
</tr>
<tr>
<td>RPM</td>
<td>Repetitions per minute</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analog scale</td>
</tr>
<tr>
<td>VO_{2max}</td>
<td>Maximal oxygen uptake</td>
</tr>
<tr>
<td>SDA</td>
<td>Subdiaphragmatic vagal deafferentation</td>
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CHAPTER 1: INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) and impaired glucose regulation is increasing rapidly throughout the United States. Approximately, 415 million adults currently have diabetes and that number is estimated to rise to 642 million by 2040 [1]. T2DM is a major risk factor for end-stage renal disease, blindness, amputations, and coronary artery disease [2, 3]. In 2010, diabetes was the seventh leading cause of death in the United States with 69,071 death certificates listing it as the cause of death, plus a total of 234,051 death certificates listing diabetes as either an underlying or contributing cause of death [4]. A 2017 study suggests that diabetes prevalence may be higher than expected with 12% of American deaths related to diabetes, making it the third leading cause of death in 2010 [5]. The American Diabetes Association indicates that 1.4 million Americans are diagnosed with diabetes every year [4]. Consequently, the increased prevalence of T2DM has many direct and indirect financial burdens on healthcare costs. In 2012, the total costs of diagnosed diabetes in the United States was $245 billion [6], which will continue to increase as the prevalence of T2DM is expected to rapidly rise. Therefore, identifying effective prevention strategies is crucial in combating the burden of T2DM and its complications.

Prediabetes is defined as fasting blood glucose levels that are higher than normal (100–125 mg/dL), but not high enough to reach the diabetic range (≥126 mg/dL) [3]. There are currently three phenotypes of diabetes: impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or a combination of both (IFG + IGT) [7]. Individuals with prediabetes are at a greater risk for developing T2DM and/or cardiovascular disease (CVD) [7, 8]. In 2012, approximately 86 million Americans had prediabetes, which is particularly concerning due to the
elevated risk for T2DM and CVD [7]. Many risk factors both hereditary and lifestyle-related are involved in contributing to the development of prediabetes and T2DM. A few risk factors include: being overweight (the primary risk factor); physical inactivity due to cells becoming less sensitive to insulin; and waist circumference, which ultimately can indicate insulin resistance [9].

Hunger hormones are typically dysregulated in the diabetic population compared to healthy individuals [10]. The orexigenic hormone, ghrelin increases during periods of fasting and plays a role in the initiation of a meal [11]. Levels of ghrelin are suppressed after feeding, demonstrating its reaction to acute changes in nutritional state [12]. Simultaneously, anorexigenic hormones such as glucagon-like peptide-1 (GLP-1) play a role in satiety by being released into circulation following food ingestion [11]. Poykko et al. (2003) demonstrated that fasting plasma concentrations of total ghrelin were lower in subjects with T2DM compared to those without [13], while Adam et al. (2007) revealed that individuals with T2DM have blunted GLP-1 responses [14]. However, Shiiya et al. (2002) showed that there were no significant differences in plasma ghrelin concentrations between normal-weight healthy subjects and normal-weight T2DM subjects after a glucose injection [15]. There were no significant differences in plasma ghrelin concentrations between diabetic and nondiabetic groups in both lean and obese subjects. [15]. Attempts have been made to reverse the dysregulation of hunger hormones in the obese and diabetic populations. Therapies include infusion of satiety hormones [16, 17] or gastric procedures [12, 18] to mimic hormone levels of healthy nondiabetic individuals in overweight/obese adults.

A principal prevention strategy for the management of T2DM includes exercise, either alone or in combination with other lifestyle modifications. Indeed, the favorable effects of exercise on glucose levels and insulin sensitivity have been well established [19, 20]. Exercise
not only improves glucose levels, but it also has a positive impact on hormonal markers related to appetite [21]. Wasse et al. (2013) demonstrated vigorous running and cycling suppresses acylated ghrelin concentrations in a fasted state when compared to a sedentary group [22]. Similar results were found by Broom et al. (2007, 2009) with the suppression of acylated ghrelin during 60-minute treadmill running at ~ 68% of maximal oxygen uptake (VO\(_{2\text{max}}\)) and 72% VO\(_{2\text{max}}\) compared to the sedentary control group in a fasted state [23, 24]. Unfortunately, these studies did not look at the diabetic population. Most current studies have looked at a single bout of high intensity exercise > 30 minutes and its effects on hormone concentrations [22, 24]. Very few investigations have looked at an acute bout of postprandial exercise in the diabetic population or a short, moderate-intensity bout of exercise and its effects on the hunger hormones.

Deguchi et al. (2015) studied postprandial walking effects in type 2 diabetics. Natural walking, 10% fast walking, and 20% fast walking were compared. Only blood glucose measures were analyzed, which showed walking reduced postprandial blood glucose levels in an intensity-dependent manner [25]. Ueda et al. (2009a) studied healthy male subjects cycling at different exercise intensities including 50% VO\(_{2\text{max}}\) (moderate intensity) and 75% VO\(_{2\text{max}}\) (high intensity) or resting for 30 minutes. Levels of GLP-1 increased during exercise and were similar between the two exercise groups showing that GLP-1 was positively impacted by exercise regardless of intensity [26]. Ueda et al. (2009b) also found that a 60-minute cycle at 50% VO\(_{2\text{max}}\) resulted in a significant increase in GLP-1 concentrations in both healthy and obese subjects compared to the rest condition [27].

The purpose of this study is to determine if a short, moderate-intensity bout of exercise at a self-selected walking pace, will be sufficient in decreasing acylated ghrelin levels and increasing GLP-1 levels compared to a resting control group in individuals at risk for T2DM.
Research Question 1: Will an acute bout of exercise further decrease acylated ghrelin levels in individuals at risk for T2DM?

H₀: There will be a significant decrease in acylated ghrelin postprandially in the exercise group compared to the control group

H₁: There will not be a significant decrease in acylated ghrelin postprandially in the exercise group compared to the control group

Research Question 2: Will an acute bout of exercise further increase GLP-1 levels in individuals at risk for T2DM?

H₀: There will be a significant increase in GLP-1 postprandially in the exercise group compared to the control group

H₁: There will not be a significant increase in GLP-1 postprandially in the exercise group compared to the control group
Mechanisms in Regulating Hunger

Energy Regulation

Energy balance is a homeostatic system in which body weight is regulated by a complex system of peripheral and central factors. The main regions of the brain including the hypothalamus and brainstem, are responsible for the regulation of energy homeostasis through central factors. The peripheral factors, such as the gut, includes endocrine tissues that are also involved in the regulation of energy balance. Appetite regulation is controlled by anorexigenic and orexigenic gut hormones. These hormones interact with receptors located at various points along the ‘brain-gut axis’ to affect short-term and intermediate-term feelings of hunger and satiety. At the physiological and cellular level, the neuroendocrine system regulates both food intake and appetite [28].

The hypothalamus and brainstem receive neural and hormonal signals from the periphery to encode information regarding the acute nutritional state and adiposity [28]. Within the arcuate nucleus (ARC) of the hypothalamus, neuronal populations drive either orexigenic or anorexigenic responses. Neurons that express agouti-related peptide (AgRP) sense the metabolic needs of the body and orchestrate a state of hunger. Appropriate activation of these neuronal populations defines a regulatory system for energy balance. However, this system is influenced by multiple gut-derived peptides conveying temporary information regarding hunger and satiety throughout the transition from the fasted to the fed state. Leptin for example, is an adipose tissue-derived hormone that delivers essential information to the hypothalamus regarding long-term energy stores [29].
**Brain-Gut Axis**

The brain-gut axis refers to the lines of communication between the gastrointestinal tract (GIT) and the central nervous system (CNS). Both neural and endocrine signaling from the gut play important roles in the regulation of short-term appetite, which forms a crucial component in appetite regulation [30]. The ARC in the hypothalamus and the dorsal vagal complex, located in the brain stem are the main regions of the brain particularly involved in controlling hunger and satiety [31, 32]. Both of these structures have extensive reciprocal connections in order to receive neuronal feedback from the periphery, in particular; the brainstem-vagus nerve complex is significant in the control of feeding [30]. The GIT contains mechanoreceptors and chemoreceptors to provide signals to the brainstem through the vagal nerve. These neural signals are centrally integrated with the signals conducted by the numerous hormones secreted from the GIT. The gut hormones then stimulate the vagal pathways from the gut to the brainstem, or they act directly on neurons in the brain [28].

Within the ARC, there are two subsets of neurons that integrate signals and influence energy homeostasis. These neurons include the neuropeptide Y (NPY)/AgRP neurons and the pro-opiomelanocortin (POMC)/cocaine-and amphetamine-regulated transcript (CART) neurons [33]. Feeding behavior and energy expenditure are both affected by alterations in the release of these neuropeptides, which result in the maintenance of energy homeostasis [30]. NPY and AgRP neurons express orexigenic properties (stimulating hunger), while POMC and CART neurons express anorexigenic properties (suppressing hunger) [32, 33]. It is believed that ghrelin induces hunger by stimulating the NPY/AgRP neurons. Conversely, hormones such as insulin, leptin, GLP-1, and peptide tyrosine-tyrosine (PYY) are thought to decrease energy intake by inducing satiety [34].
Together, these hormones signals are assimilated within the ARC to influence energy expenditure, energy intake, and overall energy balance. The mechanisms in which hormones interact with CNS appetite centers are controversial. The proximity of the hypothalamus and brainstem to other structures with a comparative deficiency in the blood-brain barrier (BBB), could potentially allow circulating factors direct access to CNS neurons. Developing evidence suggests that the vagus nerve may be the primary site of action for some appetite-modulating hormones. Through targeting the interaction of appetite signals and their receptors within the vagal nerve, may offer the possible advantages for altering appetite at a site distant from the CNS [30].

**Orexigenic Gut Hormones**

**Ghrelin**

Ghrelin is a 28-amino acid peptide hormone including the addition of an octanoyl group [28]. It was originally isolated from the stomach as an endogenous-ligand for the growth hormone-secretagogue receptor (GHS-R), but ghrelin protein has been identified in additional peripheral tissues including the GIT, pancreas, and adrenal cortex [35]. The expression of ghrelin in these tissues suggests that it has both paracrine and autocrine functions [36, 37]. Ghrelin peptide is the first gut hormone proven to have orexigenic properties through its ability to release growth hormone (GH) and induce appetite [38]. Currently, ghrelin is the only known orexigenic peripheral hormone that is predominantly secreted within the gastric mucosa [39]. About 65% of plasma ghrelin is produced by the X/A-like cells within the oxyntic glands of the stomach [40, 41], while a smaller amount (~30%) comes from the small intestine. Ghrelin not only plays a role in the stimulation of GH release from the pituitary, but also demonstrates a multitude of biological functions including appetite regulation and food intake; gastrointestinal motility;
gastric acid secretion; endocrine and exocrine pancreatic secretions; glucose and lipid metabolism; and cardiovascular and immunologic processes [42].

Ghrelin stimulates both gastric emptying [43] and gastrin release [44]. In addition, ghrelin may also promote appetite through the vagus nerve by facilitating the signal between the gut-brain axis [45, 46]. Arnold et al. (2006) studied subdiaphragmatic vagal deafferentation (SDA) and its effect on ghrelin-induced eating in rats. In this study, intraperitoneal injections of ghrelin encouraged eating as strongly in sham-operated control rats as it did with verified complete SDA rats. In rats with total subdiaphragmatic vagotomies, ghrelin also stimulated eating. This concluded that the acute eating-stimulatory effect of intraperitoneal ghrelin does not require vagal afferent signaling [46].

Two forms of ghrelin are present in the human body: acylated ghrelin and non-acylated ghrelin or desacyl ghrelin [47]. Both forms of ghrelin circulate into the bloodstream bound to lipoproteins [48]. The majority of circulating ghrelin originates from the stomach. Compared to the acylated form, non-acylated ghrelin circulates in larger amounts, but it does not dislocate ghrelin from its hypothalamic and pituitary binding sites [47]. Although acylated ghrelin only accounts for 10–20% of circulating ghrelin, it is the form believed to be responsible for appetite stimulation [38, 49]. The addition of an n-octanoyl group in acylated ghrelin allows it to cross the BBB and act on the CNS via a saturable transport system which consequently increases food intake [35]. By stimulating the release of GH, acylated ghrelin promotes appetite through its action on the brain [35]. It also influences feeding through GHS-R expressed on NPY neurons. Both acylated and desacyl ghrelin act on the GHS-R of the NPY/AgRP arcuate neurons, which stimulates the up-regulation of NPY and AgRP, ultimately increasing energy intake [50] and demonstrating that its effects are antagonistic to anorexigenic hormones [51]. Total ghrelin
measures do not provide an accurate representation of changes in the active form of ghrelin. Therefore, studying the acylated form of ghrelin is more informative for neuroendocrine energy intake regulation when investigating the effects of exercise on appetite stimulation [52]. Furthermore, acylated ghrelin appears to be more susceptible to acute manipulation of energy balance through energy deficits or macronutrient ingestion [42].

Levels of ghrelin differ throughout the day. High levels of ghrelin occur both before food intake and during the night, along with a reduction of ghrelin immediately following food ingestion. This shows that ghrelin may be an essential factor in meal initiation [15, 40, 53-55]. Additionally, ghrelin levels can change according to an acute or chronic nutritional status. Fasting will cause an elevation of ghrelin levels in which levels decrease immediately following food intake with postprandial levels decreased 60–120 minutes after ingestion [12, 40, 53, 55, 56]. Fasting also leads to an increased expression of ghrelin in the stomach with higher plasma concentrations, therefore; ghrelin induces both food intake and adiposity [57]. Ghrelin levels are elevated in a fasted state and decrease postprandially in proportion to the caloric load and circulating micronutrient signals of a meal, showing that resting ghrelin levels are inversely associated with adiposity.[12, 39, 40, 58].

The amount of ghrelin secreted by the stomach depends greatly on the nutritional state. The suppression of postprandial ghrelin is proportional to the ingested calorie load [58]. Callahan et al. (2004) observed an inverse correlation between ghrelin levels and calorie content when given meals of increasing calorie content, but with identical nutrient composition in humans [58]. They found that when the energy content of a meal is higher, the lag period for recovery of ghrelin levels is prolonged and ultimately results in lower caloric intake at the following meal [58]. The preprandial increase of ghrelin correlates with the hunger scores in healthy humans to
initiate meals voluntarily in the absence of food-related cues [59]. Compared to other macronutrients, carbohydrates exert a more suppressive effect on ghrelin secretion when consuming an isocaloric low-fat vs. low-carbohydrate diet, thus lowering fat content to total energy intake maintains a suppressive effect on ghrelin [29]. Along with acute nutritional status, ghrelin levels also represent chronic nutritional states [11], in which the hormone is suppressed in obese individuals, but increased in lean individuals [15].

Body weight gain and adiposity are induced by ghrelin [56] through stimulating food intake. It prefers fat ingestion, promoting fat storage, reducing energy expenditure and fat utilization, and consequently increasing carbohydrate utilization [42, 60, 61]. Weigle et al. (2003) found that low-fat diets have an inhibitory effect on ghrelin levels. This study reported a low-fat/high-carbohydrate diet resulted in weight loss without an increase in plasma ghrelin levels [29]. Similarly, Nedvidkova et al. (2003) revealed that healthy women who consumed a high-carbohydrate diet showed a larger drop in ghrelin levels compared to a high-fat diet [62]. Although ghrelin levels decreased in both groups, this shows that ghrelin is more responsive to carbohydrates than fat [63]. Furthermore, circulating ghrelin concentrations are negatively correlated with body mass index (BMI) [57]. Levels of ghrelin increase when obese individuals lose weight, and decrease when anorexia nervosa patients gain weight [64]. In addition, circulating ghrelin levels are higher in patients with anorexia nervosa and in animals after periods of fasting or in states of cachexia [65, 66] This suggests that ghrelin levels change in response to dieting for the maintenance of body weight [66, 67]. However, it is still unclear if these hormone alterations are the cause or the consequence of obesity.

Anorexigenic Gut Hormones

Glucagon-like peptide-1 (GLP-1)
GLP-1 is a 30-amino acid peptide derived from the glucagon precursor, proglucagon [68][69]. Proglucagon is produced within the enteroendocrine L cells of the intestines, primarily the ileum and colon, which serves as a precursor for the hormones glucagon, oxyntomodulin, GLP-1 and glucagon-like peptide-2 (GLP-2). GLP-2 is a 33-amino acid peptide that aids in energy homeostasis through acute and chronic effects on gut motility and nutrient ingestion and absorption [70]. Various stimuli aid in the release of GLP-1 including glucose, fat, proteins, amino acids, long-chain fatty acids, short-chain fatty acids, and bile acids [68]. However, the major stimulus for GLP-1 and GLP-2 secretion is the ingestion of nutrients, including glucose, fatty acids, and dietary fiber [71]. GLP-1 secretion is also regulated by the parasympathetic nervous system and circulating hormones such as cholecystokinin, glucose-dependent insulino tropic peptide (GIP) and leptin [68]. When nutrients are ingested, the release of GLP-1 and GLP-2 into circulation occurs in a biphasic manner, including of a quick (within 10–15 minutes) early phase followed by a prolonged (30–60 minutes) second phase [71].

Two different mechanisms are responsible for this biphasic pattern. First, the vagus nerve, the neurotransmitter gastrin-releasing peptide, and GIP all contribute to the rapid release of GLP-1 and GLP-2 into circulation when the L cells response to nutrient stimuli [71]. Secondly, the direct stimulation of the L cells by digested nutrients is responsible for the prolonged second phase. GLP-1 is secreted in two active forms, GLP-1_{7-37} and GLP-1_{7-36}, but its half-life is very short (less than 2 minutes) [72], whereas GLP-2 is more stable, with a half-life of approximately 5–7 minutes [73]. GLP-1_{7-36} is the active form that is found at the highest concentration in the plasma [69]. The enzyme responsible for this rapid degradation of GLP-1 and GLP-2 is the inhibitor dipeptidyl-peptidase IV (DPP-IV), which produces the inactive peptides GLP-1_{9-37} and GLP-2.
GLP-1 plays an important role in glucose and energy metabolism including glucose-stimulated insulin secretion, inhibition of glucagon secretion and gut motility, and the reduction of appetite [68]. GLP-1 regulates blood glucose levels through its combined actions on the stimulation of glucose-dependent insulin secretion and the inhibition of glucagon secretion, gastric emptying, and food intake. By inhibiting glucagon secretion and delaying gastric emptying [74], GLP-1 promotes satiety induction although that mechanism is not completely understood.

Once in circulation, GLP-1 acts by binding to the G-protein coupled GLP-1 receptor (GLP-1R). GLP-1R is expressed in pancreatic islets where GLP-1 functions as an incretin hormone, which allows for enhanced glucose-dependent insulin to be released postprandially [75]. GLP-1R is also present in many CNS areas associated with appetite control including the ARC and paraventricular nucleus in the hypothalamus and the area postrema in the brainstem [76]. GLP-1 receptors are widely distributed in the brain and peripheral organs such as the pancreatic islets and the GIT. In the brain, GLP-1 receptors are found in areas where the control of food intake and energy balance are implicated. As an incretin hormone, it stimulates glucose-induced insulin and inhibits glucagon release [64].

GLP-1 is released postprandially into circulation in proportion to the calories ingested [77]. Plasma GLP-1 levels in humans increase postprandially and reach a peak within one hour and then gradually decrease to basal levels [68]. GLP-1 inhibits food intake and promotes satiety in normal, obese, and diabetic humans [78]. A study by Adam and Wererterp-Plantenga (2007) analyzed levels of GLP-1 in a group of overweight/obese adults and found that preprandial levels were similar in overweight/obese adults compared with normal-weight controls. However, the
postprandial GLP-1 response in the overweight/obese adults was significantly blunted 30 minutes after the test meal compared to the control subjects [14].

The actual presence of nutrients in the gut lumen are responsible for the GLP-1 response [79]. Accordingly, a very rapid GLP-1 response is seen in humans after ileal instillations of lipids or carbohydrates in levels corresponding to the physiological malabsorption of these nutrients [80]. The meal response depends on the caloric size of the meal [81] and is strongly correlated to the gastric emptying rate [82]. In subjects with accelerated gastric emptying, such as gastrectomy or gastric bypass operations in obesity individuals, the secretion of GLP-1 may be exaggerated [82, 83]. In such patients, this potentially could be the cause of reactive hypoglycemia, due to an inappropriate hyperinsulinemia response [84].

Further important effects of GLP-1 include the inhibition of gastrointestinal secretion and motility [85, 86]. GLP-1 inhibits gastrin-induced acid secretion in humans [87], which subsequently revealed that GLP-1 also inhibits meal-induced secretion as well as gastric emptying and pancreatic secretion [86]. GLP-1 activates the ‘ileal brake’ by slowing down the rate of gastric emptying, which ultimately slows down nutrient absorption and contributes to the reduction of postprandial glycemia and enhanced satiety [88]. The hypothalamus-pituitary-adrenal axis demonstrates that when undigested nutrients reach the lower intestine, it causes an inhibition of gastric emptying, small intestinal transit, gastric acid secretion, pancreatic enzyme secretion, and bile acid secretion. This phenomenon in the GIT shows the negative feedback mechanism called the ileal brake [68].

**Leptin**

Leptin is a peptide hormone encoded by the obese gene and is primarily produced and secreted by mature white adipocytes, but it is also released from the GIT and has been shown to
inhibit food intake [89]. In smaller amounts, leptin is also produce in other human tissues including the stomach, mammary epithelium, placenta, and heart [90]. Leptin plays a role in regulating food intake, energy expenditure, and adiposity, as well as the immune and endocrine systems [91]. It acts within specific areas of the hypothalamus to help regulate body weight via the negative feedback loop [89]. Leptin influences food intake and energy expenditure by inhibiting the NPY/AgRP neurons and directly stimulating the POMC/CART neurons of the ARC [92]. It also stimulates energy expenditure and inhibits food intake through acting upon the hypothalamic leptin receptors.

The expression of leptin in adipocytes and its plasma concentration are both positively correlated with total adiposity [93]. Additionally, plasma leptin concentrations can be acutely modulated by a variety of physiological conditions (starvation-refeeding and cold exposure) and hormonal factors (insulin, catecholamines, glucocorticoids, thyroid hormones, gonadal steroids). Observations show how starvation can decrease plasma insulin and leptin levels. Obesity is also strongly associated with both hyperinsulinemia and hyperleptinemia [94, 95].

Leptin is primarily involved in the long-term regulation of energy balance as it is released into circulation by the adipose tissue as a function of the energy stores [29]. Although deficiencies of leptin or the leptin receptor results in obesity, most human obesity is associated with elevated leptin levels [94, 96].

**Pancreatic Polypeptide (PP)**

Pancreatic polypeptide is part of the PP fold peptide family that is synthesized and released from the endocrine pancreas [28]. PP is secreted from pancreatic F cells in response to vagal sensing, cholecystokinin and ghrelin concentrations, and sympathetic nervous system activation. PP shows a preferential binding to the Y4 and Y5 receptors. It is secreted in
Proportion to food intake in which plasma levels can be elevated for up to six hours [97]. Reducing food intake through gastric emptying and motility by the inhibition of pancreatic secretions and gallbladder motility is its major physiological role. Individuals classified as morbidly obese or individuals with Prader-Willi syndrome show blunted PP responses postprandially [98]. In addition, individuals with Prader-Willi syndrome will decrease food intake with an administration of PP. Food ingestion is the main stimulus for PP release, although, other factors can alter circulating levels. When creating an energy deficit, exercise can produce an increase in PP levels [99].

**PYY**

Peptide YY is a 36-amino acid peptide belonging to the NPY family and is secreted primarily from the L cells within the intestinal mucosa of the ileum and colon in response to nutrient intake [100]. After nutrient ingestion, PYY can remain elevated for ~120 minutes after the meal [101]. PYY targets the Y2 receptor that is highly expressed in NPY neurons. The binding of PYY to the Y2 receptor leads to the inhibition of food intake [102]. PYY is also associated with energy homeostasis. PYY is part of a family of peptides including NPY and is known to alter food intake and prevent weight gain. PYY exists in two forms in human blood; PYY$_{1-36}$ and PYY$_{3-36}$, the latter of which is the predominant form and preferentially binds to the inhibitory presynaptic Y2 receptor subtypes. These receptors are expressed in the appetite regulatory center of the ARC within the hypothalamus. This hormone is similar to both NPY and PP, demonstrating the binding affinity for Y receptors. PYY$_{3-36}$ has a potent effect on feeding, however; the influence of PYY on regulation of food intake might not be exclusive to the hypothalamus [11].
PYY has been reported to be reduced or unaltered with obesity and increased with anorexia nervosa [103]. Infusion of PYY\textsubscript{3-36} in both lean and obese subjects, reduced food consumption, although; there are conflicting results suggesting that PYY infusion may need to be administered in a specific infusion pattern to exert its anorexigenic effects [99]. Infusing PYY\textsubscript{3-36} into both lean and obese humans resulted in a 30% reduction in calorie intake [102].

Alterations in Hunger Hormones

Diabetes/Obesity

Obesity, prediabetes, and T2DM are interrelated disorders within a disease continuum [104]. Generally, as the number of metabolic abnormalities increase, ghrelin levels decrease [105]. In both obese subjects [57] and subjects with T2DM, ghrelin levels are suppressed [15]. However, the degree of obesity drives the level of impairment of ghrelin [57]. For example, lean type 2 diabetic patients do not have decreased ghrelin levels [15]. Shiiya et al. (2002) studied plasma concentrations in patients with obesity, anorexia, and T2DM along with ghrelin responses to a glucose load and a test meal in normal and diabetic patients, respectively. The test meal given to the diabetic group consisted of a 450-calorie meal with half of the calories coming from carbohydrates. Blood was drawn at 0, 30, 60, and 120 minutes postprandially. Mean plasma ghrelin concentrations showed no significant difference between normal-weight healthy subjects and normal-weight diabetics. This study also showed no statistical difference in plasma ghrelin concentrations between diabetic and nondiabetic groups [15]. The meal tolerance test showed plasma ghrelin concentrations for diabetics decreased by 60 minutes after eating then increased thereafter.

Fasting ghrelin levels are lower in obese subjects than in normal weight subjects [15, 40, 57] and are negatively correlated to percent body fat [57]. However, ghrelin levels appear to
increase when obese subjects lose weight [67]. Hansen et al. (2002) studied eight obese women undergoing a six-month weight loss program. Plasma ghrelin levels were tested pre- and post-weight loss. With a 5% weight loss, fasting plasma ghrelin concentration was increased by 12% following weight loss, showing that the increase in ghrelin was positively correlated with weight reduction [67]. Weight loss induced by food restriction and by long-term exercise also increases ghrelin levels [106, 107]. Conversely, weight gain that results from overfeeding, pregnancy, olanzapine treatment, and a high-fat diet decreases ghrelin levels [12, 42, 108].

GLP-1 impairment and postprandial deficiency can also occur in obese individuals. However, findings regarding GLP-1 levels in obesity have been inconsistent. Few studies reported reduced GLP-1 levels in obese subjects compared to lean subjects [14, 78]. Both Muscelli et al. (2008) and Toft-Nielsen et al. (2001) found that postprandial GLP-1 secretion was found to be inversely related to BMI [109, 110]. Equally, other studies have not reported any differences in GLP-1 between lean and obese individuals [81, 111].

**Ghrelin and glucose homeostasis**

Plasma glucose and insulin have suppressive effects on ghrelin and are therefore important for ghrelin regulation [15, 112]. Tong et al. (2010) demonstrated that circulating ghrelin suppressed glucose-stimulated insulin secretion and deteriorated glucose tolerance in healthy subjects. The findings of this study raise the possibility that endogenous ghrelin could have a role in physiologic insulin secretion in which ghrelin antagonists could improve beta-cell function [113]. Compensatory hyperinsulinemia as a result of insulin resistance was associated with significantly reduced levels of ghrelin, which showed that fasting plasma concentration of total ghrelin was lower among subjects with T2DM compared to those without [13].
Subjects with insulin resistance such as T2DM show lower ghrelin levels. Also, insulin has been shown to inhibit ghrelin levels [114, 115]. Lucidi et al. (2002) further studied this concept by comparing plasma ghrelin concentrations after a short-term insulin infusion in healthy volunteers to either cause hypoglycemia or not (euglycemia). In both trials, plasma ghrelin concentrations significantly decreased (p < 0.01) after insulin infusion (hypoglycemia by 14%, euglycemia by 22%). A strong correlation (r = 0.91, p < 0.002) between insulin sensitivity and the percent suppression of ghrelin from baseline was found [114]. These data show that ghrelin is not required for the hormonal defenses against insulin-induced hypoglycemia and that insulin can suppress ghrelin levels in healthy humans. Moreover, these results demonstrate the possibility that postprandial hyperinsulinemia is responsible for the reduction of ghrelin that occurs during food intake.

Many human and animal studies have demonstrated that there is a negative correlation between circulating ghrelin levels and insulin secretion [112, 115, 116]. For example, Saad et al. (2002) studied eight non-diabetic subjects following a 10-hour overnight fast for infusion of insulin and glucose. Human insulin was infused for two hours while a variable infusion of 20% glucose was given to maintain plasma glucose at a fasting level. Blood samples were collected over the two hours to determine plasma insulin and ghrelin concentrations. Plasma ghrelin decreased by 90 minutes and continued to be suppressed for 15 minutes after the insulin infusion was discontinued. Consequently, plasma ghrelin levels rapidly increased to near-basal values within 60 minutes. The reciprocal relationship between insulin and ghrelin was observed consistently with the maximum insulin-induced suppression of ghrelin ranging from 19–64% and occurring 90–135 minutes after starting the insulin infusion [115]. The findings from this study
indicate that insulin is a physiological and dynamic modulator of plasma ghrelin. Also, insulinemia may possibly mediate the effects of nutritional status on its concentration.

In another study by Purnell et al. (2003), postprandial suppression of ghrelin correlated with a rise in insulin [117]. Sixty adult men and women with various ages and weights participated in this study. They were characterized in terms of body composition and hormone levels. Blood samples were taken over a 24-hour period ever 30 minutes from 8:00 am to 9:00 pm, then every hour until 8:00 am the next morning. Fasting ghrelin levels correlated positively with age and negatively with BMI. Fasting ghrelin levels correlated most strongly with insulin levels (p = 0.002). Also, meal induced ghrelin suppression correlated with the postprandial rise in insulin (p < 0.05) [117]. These data are consistent with the hypothesis that insulin may negatively regulate ghrelin.

Djurhuus et al. (2002) demonstrated that ghrelin and GLP-1 levels are negatively correlated after glucose intake [118]. They studied healthy lean women who came into the laboratory after a 10–12 hour fast for an oral glucose tolerance test (OGTT). Following a 30-minute rest, a basal blood sample was drawn and 75 g of glucose was ingested in a liquid form. Every 15 minutes for 300 minutes, blood was drawn to measure plasma glucose, insulin, ghrelin, and GLP-1. Plasma GLP-1 levels almost doubled after the OGTT. After the OGTT, circulating ghrelin levels were substantially suppressed. This novel observation of an inverse relationship between GLP-1 and ghrelin promotes the possibility in which ghrelin influences GLP-1 secretion or perhaps GLP-1 influences ghrelin secretion.

**The Incretin Effect**

The incretin effect refers to the augmentation of insulin secretion after an oral administration of glucose compared to an intravenous administration of glucose at matched
glucose levels. The incretin effect is mainly caused by the gut hormones GIP and GLP-1 and their action on the beta-cells of the pancreas. Success in using incretin therapy as a glucose-lowering strategy in T2DM has provoked a recent interest. In non-diabetic subjects, the incretin effect is responsible for 50–70% of insulin release during oral glucose administration while in T2DM patients, the incretin effect is impaired and contributes to only 20–35% of the insulin response to oral glucose [119].

Even though the reports in the literature are mixed, most studies including GIP and GLP-1 secretory responses to oral glucose or a mixed meal have shown fairly normal results in T2DM. In contrast, insulinotropic effects on both GIP and GLP-1 are impaired in diabetic individuals. A greater suppression of insulin secretion augmentation occurs with GIP compared to GLP-1 [119]. The cause of these defects suggests either a defective beta-cell receptor expression or a post-receptor defect. The defects are secondary to the diabetic environment or defective beta-cell function in general which results in a defective incretin effect and genetic factors that initiate incretin hormone resistance [120]. Identifying theses mechanisms in greater detail are important for understanding the strengths, weaknesses, and efficacy of incretin therapy in individuals to specifically target this glucose-lowering therapy.

**Sex Differences**

A few studies have investigated sex differences in appetite response to nutrient manipulations finding that women are more sensitive to overfeeding [121] and macronutrient changes [122]. This leads to greater changes in appetite sensation ratings and/or subsequent energy intake in women. However, it is difficult to generalize these results since almost all previous studies examined the acute effect of a single nutrient/food on appetite sensations. A
given nutrient or food may act differently on appetite sensations depending on interaction with other nutrients and foods present in the diet.

Hagobian et al. (2013) recently found that men and women do not differ in their hormonal or appetite responses to an exercise bout matched for relative energy expenditure (~30% of estimated daily energy expenditure). Both sexes had significant reductions in relative energy intake post-exercise and no noticeable changes in acylated ghrelin levels [123]. Regardless, it is generally accepted that women increase their energy intake during the luteal phase compared to the follicular phase of their menstrual cycle [124, 125]. For example, Campolier et al. (2016) investigated whether appetite responses vary after consuming the same meal in the different phases of the menstrual cycle. Results showed significant differences in ovarian and satiety hormone levels across the phases of the menstrual cycle [126]. Therefore, nutrition-related studies often avoid the participation of pre-menopausal women due to the potential effects of the menstrual cycle on appetite regulation.

**Acute Exercise on Appetite Regulation**

Understanding the impact of exercise on hormones involved in appetite regulation may provide insight to the mechanisms of energy balance regulation. Previously shown, exercise can increase satiety and suppress hunger [23, 24, 127-129]. At rest, acylated ghrelin is known to promote food intake while satiety hormones such as PYY, PP, and GLP-1 are known to inhibit food intake. Exercise has been shown to have various effects on these hormones in addition to the factors of exercise intensity, duration, and mode. Acute exercise appears to be intensity-dependent with increasing intensities resulting in a greater suppression of orexigenic signals and a greater stimulation of anorexigenic signals [130].
The effect of exercise mode is of particular interest because variety in exercise training is a potential factor in compliance. The effect of exercise on these hormones and energy intake has been studied extensively, although; no definitive consensus is present. There is evidence indicating that the concentration of acylated ghrelin is suppressed after vigorous endurance exercise while concentrations of the anorexigenic hormones (PYY, PP, and GLP-1) are increased [23, 24]. For example, Broom et al. (2009) examined resistance and aerobic exercises in 11 healthy males with a randomized crossover design. Each subject underwent three trials: 90-minute free weight lifting session (resistance training); 60-minute run (aerobic exercise); and control (rest). The results revealed a significance suppression of acylated ghrelin during both aerobic and resistance exercise along with an increased concentration of PYY during aerobic exercise [24] compared to the control trial.

Ghrelin

Burns et al. (2007) reported that the suppression of appetite during severe exercise was not related to the suppression of total ghrelin [127]. Total plasma ghrelin changes do not seem likely to occur, although, reductions of 9–54% have been observed in a few studies compared to pre-exercise or a sedentary control where exercise intensity did not seem to have an influence [127, 131-133]. On the other hand, acylated ghrelin is more relevant to moderate-intensity aerobic exercise (< 75% VO$_{2\text{max}}$, 30–90 minutes) and has demonstrated 14–60% reductions in concentration compared to pre-exercise or sedentary controls [24, 128, 132].

Although, there is some evidence that shows exercises at higher intensities suppress hunger, long duration exercise can also suppress acylated ghrelin and appetite [23, 24]. Previous studies have demonstrated appetite suppression during and briefly after intense bout of exercise (> 60% of VO$_{2\text{max}}$) [23, 134]. Most studies have the bouts of exercise conducted at intensities of
< 75% VO_{2\text{max}}. Deighton et al. (2013) shows evidence that supramaximal exercise may be more potent for suppressing acylated than vigorous endurance exercise (~ 68% VO_{2\text{max}}) [132]. However, the supramaximal sprint exercise led to higher hunger levels later on in the observation, albeit no changes in energy intake [132]. It has been suggested that studies involving exercises that produce a greater metabolic and mechanical demand, are more likely to suppress levels of hunger and acylated ghrelin [23, 128]. In one study, 12 weeks of training led to a greater change and suppression of acylated ghrelin postprandially while levels of GLP-1 tended towards greater increases (1.5–2 hours) postprandially [135].

Interestingly, exercise-induced satiety appears in the presence of decreased circulating levels of ghrelin independent of changes in body weight [23, 24, 128]. The effects of an acute bout of brisk walking on appetite, energy intake, and plasma acylated ghrelin were measured by King 2010a [136]. Fourteen young males completed two 8-hour trials (one brisk walking for 60 minutes and one control). During the main trials, ad libitum buffet meals were offered in which appetite was assessed at 30-minute intervals throughout. Results show that the acute bout of exercise did not significantly influence appetite, energy intake, or acylated ghrelin during or after exercise [136]. Although, participants did not compensate for the energy expended during exercise showing an energy deficit occurred.

Broom et al. (2007) studied nine male subjects who participated in two, 9-hour trials (exercise + rest). The exercise trial consisted of a 60-minute run at 72% VO_{2\text{max}}, rested for eight hours followed by a test meal. AUC values for plasma acylated ghrelin concentrations were lower over the first three hours compared to the control trial [23]. Using the visual analog scale (VAS), values for hunger were lower over the first three hours of the exercise trial compared to
the control trial. The results from this study demonstrate how plasma acylated ghrelin and hunger levels are both suppressed during and following a high-intensity exercise.

Adiposity alters acylated ghrelin levels, but adiposity can potentially alter the effect of exercise and feeding on acylated ghrelin responses. Heden et al. (2013) wanted to determine whether adiposity influences the effect of exercise and feeding on acylated ghrelin, hunger, and fullness. Fourteen normal weight and 14 obese individuals completed two trials including exercise (1-hour of treadmill walking at 55–60% peak O$_2$ uptake) and no exercise trial. Blood samples were taken to assess ghrelin while VAS questionnaires were used to assess perceived hunger and fullness. Exercise reduced fasting acylated ghrelin concentrations by 18% in the normal weight individuals [137]. Also, in response to feeding, the change in acylated ghrelin was attenuated by 39%. However, perceived hunger and fullness were not altered. In obese individuals, there were no changes in fasting or postprandial acylated ghrelin concentrations with the exercise trial. Also, postprandial fullness was attenuated by 46% compared to the no exercise trial. They concluded that exercise preformed the night before a meal suppresses acylated ghrelin in normal weight individuals despite unaltered perceived hunger or fullness. Although obese individuals saw no change in acylated ghrelin levels, the exercise trial reduced the fullness response to the test meal [137].

**GLP-1**

Moderate-intensity aerobic exercise (50–70% VO$_{2max}$, 30–60 minutes) increases active GLP-1 concentrations by 11–50% [26, 27] and total GLP-1 by 16–1477% [129, 138] compared to non-exercise controls. For example, Ueda et al. (2009b) examined whether gut hormone levels differ between obese males and normal weight subjects. They found that 60 minutes of cycling at 50% VO$_{2max}$ (moderate-intensity) resulted in a significant increase in GLP-1 concentrations after
exercise in both obese subjects and normal weight subjects compared to the rest condition [27]. Only a few studies have examined the effects of high exercise intensity on GLP-1 with ambiguous results. Beaulieu et al. (2015) demonstrated that sprint interval training resulted in no change in active GLP-1[139]. Alternatively, Chanoine et al. (2008) investigated the effects of exercise on GLP-1 concentrations and appetite markers. Normal weight and at risk of overweight male individuals participated in this study. Five consecutive days of 1-hour aerobic exercises were completed. The exercise seemed to cause an increase in the acute GLP-1 response to the test meal, which was similar in both groups. Although, there was no significant correlation between markers of GLP-1 concentrations and appetite [140].

Diabetes

The functional relevance of the disruption of ghrelin levels in obesity and T2DM is not clear [10]. Several reports have found that insulin and glucose had no direct effect on acylated ghrelin concentrations [23, 128, 141], nor did insulin sensitivity have any relationship with acylated ghrelin [142]. However, King et al. (2015) measured hormone levels the day after prolonged exercise, which may lead to conflicting results. Importantly, the elevations of plasma glucose following a high-intensity exercise bout may contribute to increased levels of GLP-1 given its established role as an incretin hormone, which could stimulate insulin secretion in response to plasma glucose [120].

Acute effects of fast walking have shown opposing results [25]. Deguchi et al. (2016) used fast walking as the intervention for subjects with T2DM. They believed it was important for patients to independently determine the appropriate amount of exercise to obtain maximum benefit with the lowest risk in their daily life. Walking was the intervention with comparisons between natural walking and fast walking. Because T2DM patients are typically sedentary in
their daily life, exercise intensity was set at a relatively low level [143]. A randomized crossover clinical trial was used to determine the immediate effects of walking on postprandial blood glucose in patients with T2DM. Fourteen participants were randomly allocated to three walking programs (natural walking, 10% fast walking, 20% fast walking) without changing diet or medications. Each walking program consisted of treadmill walking for 30 minutes, one hour after lunch. Fast walking speeds were calculated from the subjects’ self-selected walking speed at the screening visit. The primary outcome showed fast walking reduced postprandial blood glucose levels in an intensity-dependent manner. During 15 and 30 minutes of 10% and 20% fast walking, postprandial blood glucose levels decreased acutely. However, the difference was not significant, showing that patients with T2DM may also experience the same immediate effects on postprandial blood glucose levels as a results of 20% fast walking for 30 minutes as they can with 10% fast walking for 30 minutes [25]. As a result of this study, T2DM patients could easily use this method of walking to determine their exercise intensity, making it possible to adapt to an exercise program in their daily life.

Knudsen et al. (2013) compared exercise-induced postprandial satiety and ghrelin responses in overweight subjects with T2DM compared to healthy controls. Baseline levels of total and acylated ghrelin were significantly lower in subjects with T2DM compared to healthy subjects [10]. Because fasting levels of ghrelin were lower in the overweight T2DM group compared to the overweight healthy controls, this suggests that the diabetic state could be a contributing factor in the impairment of ghrelin levels. Although plasma ghrelin levels were unaffected by exercise, there was an increase in postprandial fullness in the T2DM group. These data suggest that the presence of T2DM suppresses ghrelin levels and drives poor appetite
regulation. However, a single bout of exercise is sufficient enough to restore the oral glucose-induced fullness independently of ghrelin [10].

A study conducted by Hordern et al. (2011) demonstrated the effects of short-term exercise training in patients with T2DM. Exercise training included a four-week regimen of two 1-hour sessions and one 30-minute session per week for four weeks. Over the exercise training period, there were significant decreases in resting heart rate, systolic blood pressure, and fat mass [144]. There was a significant time effect in the blood glucose decrease pre- to post exercise session during the exercise sessions over the four-week period. Also, there was a significant (p < 0.05) difference between the percent decrease observed in week one compared to week four [144]. The main finding of this study shows that the acute blood glucose lowering effect of a single bout of exercise is augmented over a four-week period in patients with T2DM.

**Perceived Hunger**

A VAS is a measurement instrument that helps to measure a characteristic or attitude that is believed to range across a continuous line of values that cannot be directly measured easily [145]. A horizontal line, 100 mm in length is anchored with word descriptions at either end in which the subject would mark a line at the point where they feel best represents the perception of their current state. The score is determined by measuring in millimeters from the left hand end of the line to the point that the subject marks [145].

A VAS has been used to assess subjective ratings of hunger, satisfaction, fullness, and prospective food consumption [146]. Subjects were asked: how hungry do you feel, how satisfied do you feel, how full do you feel, and how much do you think you can eat. Hunger assessment at one end (0 mm) of the scale had the descriptor “not at all hungry” and the other extreme (100 mm) was the descriptor “totally hungry”. Hagopian et al. (2013) utilized this
perceived hunger scale and showed no significant difference was seen between sexes in any appetite rating (hunger, fullness, satisfaction) when using the VAS [123].

Vatansever-Ozen et al. (2011) demonstrated the relationship between perceived hunger (VAS) and biological hunger (acylated ghrelin) with the variable of exercise. Over 240 minutes, the exercise group had a significantly higher perceived hunger rating at 120 and 180 minutes ($p < 0.05$) compared to the rest (control) group. Baseline fasting plasma acylated ghrelin concentrations did not differ significantly between trials. However, plasma acylated ghrelin was significantly lower ($p < 0.05$) at 120 and 240 minutes in the exercise group compared to the rest group [133].

Prado et al. (2014) performed a study with obese girls investigating the effects of exercise at ventilatory threshold on hunger scores. The control group (rest) at 150 minutes had significantly higher hunger scores compared to the exercise group. The magnitude of change in percentage increase in hunger scores in the control condition was almost 100% and 275% from baseline to 30 minutes and 150 minutes, respectively. Interestingly, the magnitude of change for increased hunger scores in the exercise condition was only 13% from baseline to 30 minutes (nonsignificant; $p > 0.05$), but it increased by 139% from baseline to 150 minutes ($p = 0.01$) [147].

Alternative Therapies

Infusion of Satiety Hormones

One therapy studied for the treatment of obesity includes intravenous infusion of satiety hormones to replicate the physiology of the fed state in humans. Infusion of GLP-1$_{7-36}$ in humans showed a great increase in insulin secretion, glucagon suppression, and lower glucose levels prompting the consideration of using the hormone as a pharmacological incretin in the
management of T2DM [75]. Short-term administration of PYY$_{3-36}$ and GLP-1 separately leads to a reduction in energy intake by mechanisms involving changes in central regulation of appetite [76]. Flint et al. (1998) examined the effect of GLP-1 infusion in young healthy men. Infusion of GLP-1 mixed with saline (treatment) or saline alone (control) was infused at the start of the test meal for breakfast. Blood samples were obtained along with the completion of VAS scores. The GLP-1 infusion satiety and fullness compared with the placebo. Also, spontaneous energy intake at the ad libitum meal, was reduced significantly by 12% in the treatment group compared to the control group [148].

Schmidt et al. (2014) conducted a study to test the outcomes of PYY$_{3-36}$ and GLP-1 administration alone and in combination. Compared with the saline placebo, PYY$_{3-36}$ and GLP-1 alone had no effect on energy intake, however; the combination of both hormones resulted in a significant reduction in ad libitum energy intake [17]. Although, increased subjective ratings of nausea was present with the infusion.

**Gastric Sleeve**

Another treatment for obesity and diabetes management is the possible role of the sleeve gastrectomy. Insulin secretion and peripheral insulin sensitivity were studied by Basso et al. (2011) utilizing the intravenous glucose tolerance test. Obese type 2 diabetics and nondiabetic obese patients were evaluated prior to and after their sleeve gastrectomy. The first phase of insulin secretion promptly improved following surgery for the diabetic patients. The second phase of insulin secretion significantly decreased for all groups following the sleeve gastrectomy showing an improvement in insulin peripheral sensitivity. Intravenous glucose stimulation determined a decrease in ghrelin levels and an increase in GLP-1 and PYY values for both pre-
and postoperative groups. Basal and intravenous stimulated GLP-1 and PYY postoperative values were higher than preoperative values in all groups [18].

Due to ghrelin being produced primarily in the stomach, weight loss after gastric bypass surgery may be accompanied by impaired ghrelin secretion. Cummings et al. (2002) studied hormone levels in obese subjects before and after a dietary weight loss program and compared it to obese individuals who had undergone gastric bypass surgery with normal weight controls [12]. They found that weight loss induced by gastric bypass surgery produce contradictory results in ghrelin levels: an increase [149, 150], a decrease [12, 151] or no change at all [152].
CHAPTER 3:
METHODOLOGY

Subjects

Subjects were recruited from the community of Las Vegas through fliers, e-mails, word-of-mouth, and online announcements (Appendix A). All subjects were healthy men and women between the ages of 45–69 years, BMI 25–40 kg/m², nonsmoking, and inactive (fewer than two 20-minute bouts of exercise per week during the previous three months). Volunteers were asked to complete an online survey addressing the inclusion criteria to screen for initial eligibility. Individuals completed a physical activity readiness questionnaire (Par-Q) to determine if they were healthy enough to exercise (Appendix B). Anyone who answered “yes” to any of the questions on the Par-Q was excluded for safety reasons. All subjects were free of unsolved medical conditions, not diagnosed with a chronic disease including diabetes, and weight stable within the past three months (±/− 3 kg). If female, they no longer had a menstrual cycle. In addition, all subjects were willing to follow the study protocol and were able to walk on a treadmill at a moderate pace of ~ 2.5 miles per hour (mph).

An a priori power analysis was performed to determine the sample size necessary to detect significant changes in the hunger hormones acylated ghrelin and GLP-1. From previous available data [27, 128], it was determined that for a within-subjects repeated measures design, to detect significance at a 0.05% significance level and power > 0.80 with an expected 20% dropout rate, 4–5 subjects would need to be recruited. For changes in postprandial GLP-1 after a single acute bout of exercise, one previous study achieved statistical power for detecting differences in two-hour AUC with 14 subjects [27]. Another study demonstrated a significant change in acylated ghrelin over a 90-minute exercise period with nine subjects [128]. The
University of Nevada, Las Vegas Institutional Review Board (IRB) approved this study and all volunteers provided written informed consent prior to participation (see Appendix C for IRB approval and informed consent).

**Experimental Design**

A randomized crossover design with two different test days, one control (rest) and one treatment (exercise) was used in this study. All trials were separated by approximately one week. Subjects received compensation on a graded scale for each of the study visits.

![Study design diagram](image)

Figure 1. Study design from recruitment to trial implementation. Subjects completed two trials in a randomized order spaced one week apart.
Screening Visit

Participants who met the inclusion criteria obtained from the online survey were scheduled to attend an initial screening visit. All subjects were given a written consent form to fill out and sign. Potential subjects were included if their fasting capillary blood sugar (obtained by fingerstick) was within the prediabetic range (100–125 mg/dL). Individuals who meet all preliminary inclusion criteria were then further assessed.

Subjects completed a medical history questionnaire to verify the absence of any contraindications to the exercise and dietary protocols. Height was measured on a stadiometer and recorded in centimeters. Weight and body composition were measured using a scale with bioelectrical impedance analysis (InBody™ 770, Seoul, South Korea). Waist circumference was measured per the American College of Sports Medicine guidelines and recorded in centimeters [153].

During this visit, the preferred walking speed of each subject was obtained by using a validated 10-meter walk test [154]. Briefly, each subject walked at a comfortable, typical walking pace across a 10-meter measured area four different times. The first time was not recorded, as it was a practice trial. For the following three trials, only the middle six-meters were measured to eliminate the acceleration and deceleration effects. Start and stop time was recorded when the toes of the leading foot crossed the two-meter and the eight-meter mark, respectively. The time taken to complete each of the last three walks was measured and used to calculate preferred walking speed in mph.

Diet

Standardized meals were used for a 24-hour period the day prior to each of the two testing days. Meals were prepared in the kitchen housed in the College of Hotel Administration
at the University of Nevada, Las Vegas by registered dietitians and trained nutrition students. Meals were frozen and given to subjects prior to each testing visit. Two days before each visit, subjects arrived at the laboratory to pick up their meals. On the day prior to each experimental day, the subjects consumed the standardized meal plan starting with breakfast. No food was consumed after the standardized dinner meal and participants fasted overnight with the exception of water consumption until they arrived in the research laboratory the following morning. Meal composition was specifically designed to mirror a typical American diet [155]. Carbohydrates made up 50% of the total calories with protein at 15%, and fat at 35%. The total calories were ~2000 for each day.

At each visit, subjects consumed identical test meals comprised of a common breakfast meal including a bagel with butter (20 g), apple juice (8 oz.) and added sugar (6 tsp.). The meal was a total of 670 calories with the macronutrient composition of 70% carbohydrate, 23% fat, and 7% protein.

**Study Trials**

Two additional visits (~2 hours each) were scheduled after the baseline visit. Each visit occurred approximately one week apart and subjects were asked to arrive at the laboratory at the same time of day for each visit. The order of the two conditions was randomly determined for each individual. Subjects began testing between 6:00 am and 7:00 am following at least a 10-hour overnight fast. Subjects were instructed to remain sedentary the previous day and the morning of testing.

Fasting venous blood samples were collected by a trained phlebotomist. All blood draws were processed and frozen in the lab until analysis. After the fasting blood draw, subjects were given ten minutes to consume the test meal (bagel + juice). The two-hour clock started following
the first bite of the meal. Acylated ghrelin and GLP-1 blood samples were collected at minutes 0, 60, and 120. A VAS (Appendix B) was used to measure subjective hunger and satiety levels at 0, 60, and 120 minutes. Subjects were not allowed to consume any food or beverage (with the exception of water and the test meal) during the visit. A snack (High Protein Ensure) was provided when testing was over.

1. CON: After consuming the test meal, subjects remained in the lab for blood testing with minimal activity for the remainder of the two hours.

2. WALK: After consuming the test meal, subjects were given five minutes to transition to the treadmill in the exercise physiology lab. At minute 15, subjects began walking on a motorized treadmill at their calculated preferred walking speed (mean = 2.49 ± 0.31 mph). Heart rate was continuously monitored during exercise using a heart rate monitor (Polar Electro Oy, Kempele, Finland). Venous blood samples were measured immediately following the walk at minute 30. Subjects then rested for the remaining 1.5 hours and further blood samples were taken.

Blood Collection

A fasting venous blood sample was collected by a trained phlebotomist to assess for active GLP-1 and acylated ghrelin. Post-meal blood samples were collected for two hours by the trained phlebotomist. This included a total of three blood samples taken (0, 60, and 120 minutes) to be assayed using ELISA (Enzyme-linked immunosorbent assay) kits. The concentrations of acylated ghrelin and active GLP-1 were determined using Human Ghrelin Active ELISA kit (EMD Millipore, Billerica, MA, USA) and High Sensitivity GLP-1 Active Chemiluminescent ELISA kit (EMD Millipore, Billerica, MA, USA) following the manufacturer’s instructions. The sensitivity of the assays was 8 pg/mL (20 µL sample size) for acylated ghrelin and 0.14 pM (20
μL sample size) for GLP-1. All samples were assayed in duplicate and samples from one participant session were analyzed in the same assay to minimize the effects of inter-assay variation.

Before assays were run, all blood samples were collected directly into EDTA (ethylenediaminetetraacetic acid) tubes. Immediately after collection (within 30 seconds), DPP-IV inhibitor was added to the GLP-1 tubes (10 μL/mL blood) then inverted to mix. For acylated ghrelin, blood was collected in EDTA tubes and pefabloc inhibitor was immediately added to the blood sample (10 μL/mL blood). All samples were then centrifuged at 3000 repetitions per minute (rpm) for 15 minutes at 4°C, using a temperature-controlled centrifuge. Hydrochloric acid (HCl) was then added for acylated ghrelin to acidify samples. The acylated ghrelin samples went back into the refrigerated centrifuge to be spun for 15 minutes at 3000 rpm. The plasma was then aliquoted into Eppendorf tubes and then stored at -20°C for acylated ghrelin and -70°C for GLP-1 until assayed.

**Biochemical Analysis**

**Ghrelin**

For the Human Ghrelin Active ELISA assay procedure, samples were thawed and 10X concentrated wash buffer was used to dilute the samples 10-fold by mixing both buffers with 900 mL de-ionized water. Strips from the microtiter assay plate were assembled in the empty plate holder then each well was filled with 300 μL diluted wash buffer. The wash buffer was decanted and residual was removed by inverting the plate and tapping it onto absorbent towels. The assay plate was washed using this procedure two additional times. Twenty μL matrix solution was then added to the blank, standards, and quality control wells. Thirty μL and 10 μL assay buffer was added to each of the blank sample wells and the standard/quality control wells, respectively.
Duplicate 20 µL ghrelin standards were added in the order of ascending concentrations to the appropriate wells. Then, 20 µL quality control one and 20 µL quality control two were added to the appropriate wells. In the remaining wells, 20 µL of the samples were added in duplicate.

Next, 50 µL of the antibody solution mixture was added to each well. The plate was then covered and incubated at room temperature for two hours on an orbital microtiter plate shaker at 500 rpm. After, solutions from the plate were decanted, making sure all residual solutions were removed. Wells were washed three times with diluted wash buffer (300 µL per well per wash). Then, 100 µL enzyme solution was added to each well before incubation for 30 minute (at room temperature on the microtiter plate shaker). Afterwards, wells were washed six times with diluted wash buffer and tapped after each wash ensuring all residual buffer was removed. The substrate solution (100 µL) was added to each well then incubated on the plate shaker for 20 minutes. A blue color developed in the ghrelin standard wells with intensity proportional to increasing concentrations of ghrelin. After the sealer was removed, 100 µL of stop solution was added to each well and the plate was tapped by hand to complete the mixing of solution. Within five minutes, absorbance was read at 450 nm and 590 nm in a plate reader.

GLP-1

For the high sensitivity GLP-1 active ELISA assay, all reagents and samples were thawed to room temperature and 300 µL of diluted wash buffer was added to each well of the plate. Wash buffer was decanted to remove residual volume by inverting the plate and tapping it onto absorbent towels then repeated two additional times. Next, 50 µL of matrix solution was added to blank, standards, and quality control wells. Then, 50 µL assay buffer was added to each of the blank and sample wells, 50 µL of standards, and 50 µL of samples were added to the appropriate wells. GLP-1 capture antibody (20 µL) was then added to each well. The plate was covered and
incubated at room temperature for two hours on an orbital microtiter plate shaker set to 500 rpm. Reagents were decanted from the plate and tapped to remove any residual volume. Wells were washed three times with 300 µL per well per wash. Detection antibody (100 µL) was added to each well before re-covering plate and incubating for one hour at room temperature on the plate shaker. The plate was removed, decanted, and washed three times with the wash buffer. Then 100 µL enzyme solution was added to each well, covered, and incubated with moderate shaking at room temperature for 30 minutes. Reagents from the plate were decanted and wells were washed six times. Lastly, 100 µL of working substrate solution was added to each well and placed on plate shaker for one minute. The assay plate was read by a luminometer plate reader within five minutes after adding the substrate and measured at 425 nm.

Statistics

All analyses were conducted using the Statistical Package for Social Sciences (SPSS Version 24.0, Armonk, NY, USA). Data were reported as mean ± standard deviation (SD) unless otherwise noted. All outcome variables were tested for normality using the Shapiro-Wilk test, homogeneity using Levine’s test, and sphericity using Mauchly’s test of sphericity, and transformed when necessary. Descriptive statistics were used to describe subject characteristics. Two-way repeated measures ANOVA (analysis of variance) were used to determine the interactive and main effects between the two conditions (exercise + rest). Significance was accepted at p < 0.05.
CHAPTER 4:

RESULTS

Subject Characteristics

Seventy-eight volunteers completed the initial online eligibility survey. Of these individuals, 34 qualified and came into the laboratory for the screening visit. Eighteen individuals were excluded at the screening visit due to normal fasting blood glucose concentrations, as measured by capillary blood (< 100 mg/dL). One subject declined to participate due to denied consent while another declined due to scheduling conflicts. Two subjects dropped out after completing their first trial: one for digestive issues and the other for an unknown reason. Consequently, 10 subjects (3 males, 7 females) were enrolled and completed all trials of this study.

Table 1. Physical characteristics of subjects at baseline.

<table>
<thead>
<tr>
<th></th>
<th>All Subjects (N = 10)</th>
<th>Male (N = 3)</th>
<th>Female (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>59.7 ± 6.8</td>
<td>62.0 ± 7.2</td>
<td>58.7 ± 6.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.9 ± 7.0</td>
<td>173.3 ± 9.2</td>
<td>168.4 ± 6.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>87.9 ± 19.9</td>
<td>89.3 ± 24.0</td>
<td>87.3 ± 20.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.7 ± 4.7</td>
<td>29.7 ± 4.6</td>
<td>31.1 ± 5.0</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>100.6 ± 23.3</td>
<td>88.7 ± 40.5</td>
<td>105.7 ± 12.9</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>39.4 ± 7.1</td>
<td>31.2 ± 4.2</td>
<td>42.8 ± 4.7</td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>53.2 ± 11.4</td>
<td>61.5 ± 12.9</td>
<td>49.5 ± 9.3</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>108.5 ± 6.5</td>
<td>104.3 ± 4.2</td>
<td>110.3 ± 6.7</td>
</tr>
<tr>
<td>Walk Speed (mph)*</td>
<td>2.5 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>Average HR (bpm)</td>
<td>97 ± 13.6</td>
<td>83 ± 13.7</td>
<td>104 ± 8.2</td>
</tr>
</tbody>
</table>

Values represent mean ± SD.
*Calculated using the 10-meter walk test. Average of three trials to determine preferred walking speed of each subject.
Postprandial Ghrelin and GLP-1

There were no statistical differences in acylated ghrelin \((F = 1.535, p = 0.247)\) or GLP-1 \((F = 0.003, p = 0.9559)\) concentration between CON and WALK conditions at any time period (Table 2). Figure 2 shows the pattern of ghrelin concentrations over the two-hour postprandial period. There were no statistical differences in ghrelin concentrations (range: 419.73–512.44 pg/mL; \(p = 0.204\)) at baseline. There was a main effect of time for ghrelin \((F = 41.339; p < 0.001)\). Post hoc analysis indicated a significant difference between baseline and 60 minutes \((p < 0.001)\) and between baseline and 120 minutes \((p < 0.001)\) for acylated ghrelin concentrations. No difference was found between 60 minutes and 120 minutes \((p = 0.834)\).

Table 2. Postprandial acylated ghrelin and GLP-1 concentrations and AUC for each condition.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Walk</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acylated Ghrelin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minute 0</td>
<td>419.73 ± 168.95</td>
<td>512.44 ± 275.03</td>
<td>0.204</td>
</tr>
<tr>
<td>Minute 60</td>
<td>221.19 ± 135.29</td>
<td>295.65 ± 202.89</td>
<td>0.239</td>
</tr>
<tr>
<td>Minute 120</td>
<td>238.86 ± 145.38</td>
<td>269.75 ± 172.41</td>
<td>0.533</td>
</tr>
<tr>
<td>AUC</td>
<td>550.48 ± 272.94</td>
<td>686.75 ± 415.52</td>
<td>0.240</td>
</tr>
<tr>
<td><strong>GLP-1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minute 0</td>
<td>2.67 ± 2.90</td>
<td>2.13 ± 1.67</td>
<td>0.616</td>
</tr>
<tr>
<td>Minute 60</td>
<td>5.37 ± 2.47</td>
<td>6.20 ± 3.89</td>
<td>0.465</td>
</tr>
<tr>
<td>Minute 120</td>
<td>6.14 ± 3.31</td>
<td>5.13 ± 2.40</td>
<td>0.409</td>
</tr>
<tr>
<td>AUC</td>
<td>10.01 ± 2.97</td>
<td>10.14 ± 5.24</td>
<td>0.918</td>
</tr>
</tbody>
</table>

Values represent mean ± SD.

\(P\) values calculated using paired t-tests for each time point and AUC \((p < 0.05)\).
Figure 2. Change in postprandial acylated ghrelin concentrations at baseline and after meal consumption. Error bars represent ± standard error of the mean (SEM). CON = control, WALK = walking.

Figure 3 shows the pattern of GLP-1 concentrations over the two-hour postprandial period. There were no statistical differences in ghrelin concentrations at baseline (range: 2.67–2.13 pM; p = 0.616). There was a main effect of time for GLP-1 (F = 17.968; p < 0.001). Post hoc analysis indicated a significant difference between baseline and 60 minutes (p = 0.001) and between baseline and 120 minutes (p = 0.002) for GLP-1 concentrations. No difference was found between 60 minutes and 120 minutes (p = 0.665).

The AUC for both acylated ghrelin and GLP-1 are shown in Figure 3. There was no significant difference in the AUC for acylated ghrelin between the CON and WALK conditions (t = -1.257; p = 0.240). Furthermore, there was no significant difference in the AUC for GLP-1 between the control and walk conditions (t = -0.107; p = 0.918).
Figure 3. Change in postprandial GLP-1 concentrations at baseline and after meal consumption. Error bars represent ± SEM. CON = control, WALK = walking.

Figure 4. Comparison of area under the curve for acylated ghrelin and GLP-1 between trials. Graph A (p = 0.240; repeated measures ANOVA). Graph B (p = 0.918; repeated measures ANOVA). Error bars represent ± SEM. CON = control, WALK = walking.
Hunger Hormones and VAS

Correlations between perceived hunger and biological hunger were weak and nonsignificant \( (p > 0.05) \) between any measure. No significant correlations were found between VAS question one and control \( (p = 0.260, r = 0.212) \) or walk \( (p = 0.401; r = 0.162) \) for acylated ghrelin. No significant correlations were found between VAS question two and control \( (p = 0.712; r = 0.072) \) or walk \( (p = 0.844; r = -0.038) \) for GLP-1. No significant correlations were found between VAS question three and control \( (p = 0.339; r = -0.184) \) or walk \( (p = 0.872; r = -0.031) \) for GLP-1.

Table 3. Perceived hunger scores for each condition.

<table>
<thead>
<tr>
<th>Question</th>
<th>Control</th>
<th>Walk</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minute 0</td>
<td>4.3 ± 2.6</td>
<td>5.9 ± 1.4</td>
<td>0.119</td>
</tr>
<tr>
<td>Minute 60</td>
<td>2.3 ± 2.2</td>
<td>2.2 ± 1.5</td>
<td>0.833</td>
</tr>
<tr>
<td>Minute 120</td>
<td>3.2 ± 2.5</td>
<td>2.8 ± 2.1</td>
<td>0.487</td>
</tr>
<tr>
<td>Question 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minute 0</td>
<td>3.2 ± 1.5</td>
<td>4.4 ± 1.9</td>
<td>0.200</td>
</tr>
<tr>
<td>Minute 60</td>
<td>2.5 ± 2.0</td>
<td>2.4 ± 1.7</td>
<td>0.878</td>
</tr>
<tr>
<td>Minute 120</td>
<td>3.7 ± 2.1</td>
<td>2.9 ± 1.7</td>
<td>0.275</td>
</tr>
<tr>
<td>Question 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minute 0</td>
<td>6.1 ± 2.3</td>
<td>4.8 ± 2.4</td>
<td>0.363</td>
</tr>
<tr>
<td>Minute 60</td>
<td>2.8 ± 2.0</td>
<td>3.0 ± 2.2</td>
<td>0.803</td>
</tr>
<tr>
<td>Minute 120</td>
<td>3.8 ± 2.5</td>
<td>3.3 ± 2.1</td>
<td>0.458</td>
</tr>
</tbody>
</table>

Values represent mean ± SD. 
\( P \) values calculated using paired t-tests for each time point \( (p < 0.05) \). 
The VAS questionnaire was used to quantify perceived hunger ratings. See Appendix B for VAS questionnaire.

There were no statistical differences in VAS question one \( (F = 0.493, p = 0.5) \), VAS question two \( (F = 0.037, p = 0.852) \) or VAS question three \( (F = 0.562, p = 0.473) \) between CON
and WALK conditions at any time period (Table 3). There was a main effect of time for VAS question one (F = 9.292; p = 0.007). Post hoc analysis indicated a significant difference between baseline and 60 minutes (p = 0.005) and between baseline and 120 minutes (p = 0.036) for VAS question one. No difference was found between 60 minutes and 120 minutes (p = 0.051). There was a main effect of time for VAS question two (F = 4.871; p = 0.02). Post hoc analysis indicated a significant difference between baseline and 60 minutes (p = 0.025) and between 60 minutes and 120 minutes (p = 0.010) for VAS question two. No difference was found between baseline and 120 minutes (p = 0.362). There was a main effect of time for VAS question three (F = 12.557; p = 0.004). Post hoc analysis indicated a significant difference between baseline and 60 minutes (p = 0.002), between 60 minutes and 120 minutes (p = 0.021), and baseline and 120 minutes (p = 0.020) for VAS question three.
CHAPTER 5: DISCUSSION

The increased prevalence of T2DM in the United States prompted this study’s objective to evaluate the effect of an acute bout of exercise compared to no exercise in individuals at risk for developing T2DM. Our major finding was that there was no significant difference between the two conditions (exercise + rest) for both acylated ghrelin and GLP-1 concentrations. Additionally, there was no significant difference in AUC for both acylated ghrelin and GLP-1. An acute bout of aerobic exercise has only been shown to decrease levels of acylated ghrelin [23, 24] without affecting total ghrelin [127, 129]. With this in mind, we measured acylated ghrelin compared to total ghrelin. However, it is likely that we did not see a difference due to the short duration of either the exercise bout or its frequency.

Martins et al. (2010) studied the effects of a 12-week exercise program on hunger hormones, which resulted in a greater change and suppression of 1.5–2 hours postprandial acylated ghrelin compared to the control [135]. Broom et al. (2007) demonstrated that a 60-minute bout of exercise had lower acylated ghrelin concentrations over three hours after exercise compared to the control trial [23]. These findings are similar to a subsequent study, which showed two trials, including 90-minutes of resistance training and a 60-minute run, both revealed decreased acylated ghrelin compared to the control [24]. Because these previous studies saw a suppression of acylated ghrelin during and after exercise, we expected to see an attenuation of acylated ghrelin after 15 minutes of walking. However, the acute bout of exercise in the present study may have been too short of duration to elicit a change in hormone levels.

Perhaps, we did not see a significant change because the acute bout of exercise was at too low of an intensity to greatly affect hunger hormones. The acute bout of exercise of a 15-minute
self-selected walking pace was selected for easy adherence for sedentary individuals. However, this intensity was lower than those observed in previous studies that saw a change in hunger hormones. Deighton et al. (2013) showed evidence that supramaximal (~ 68% VO2max) exercise might elicit a more potent suppression of acylated ghrelin compared to vigorous endurance exercise [132]. Alternatively, other studies have shown that higher intensities have a greater affect [128]. King et al. (2010b) showed decreased acylated ghrelin levels after 90 minutes of exercise at subject’s 70% VO2max [128]. However, it is unlikely that obese or sedentary individuals would complete an exercise for the duration of 90 minutes or that they could maintain that high of an intensity. The self-selected walking pace was specifically chosen to stimulate adherence with minimal effort. Based on the average heart rates of individuals during our walking trial, the intensity was considered moderate (~ 61% age-predicted maximal heart rate).

Moderate-intensity aerobic exercises has shown an increase in active GLP-1 concentrations by 11–50% [26, 27] compared to the non-exercise controls. For example, Ueda et al. (2009a) determined that 30 minutes of aerobic exercise at 50% VO2max resulted in a significant increase in GLP-1 concentrations after exercise compared to the non-exercise control [26]. Similarly, Martins et al. (2007) revealed GLP-1 levels rose postprandially and mean values were significantly higher after a 60 minute bout of exercise at 65% maximal heart rate, compared to the non-exercise group [129]. Considering these findings, we wanted to determine if a single shorter bout of moderate-intensity exercise could elicit the same effect. Although, Chanoine et al. (2008) found that 1-hour of aerobic exercise seemed to have an increase in the acute response of GLP-1 to the test meal, we found contrasting results. One explanation for this result is the exercise frequency. In the study by Chanoine and colleagues, exercise trials included five
consecutive days of aerobic exercise, whereas the present study, included a single bout of exercise. Perhaps, the exercise consistency could have elicited a greater hormone response in the obese subjects compared to a single exercise bout. Also, their exercise trial lasted one hour compared to the 15-minute bout of exercise in our study, leading us to believe the duration had the highest impact.

The demographic population used in this study may have influenced hunger hormones due to T2DM and obesity playing a role in blunted hormone levels. Previous research has shown an impairment in hunger hormones in the obese and diabetic populations [10]. For example, ghrelin levels are lower in obese subjects than in normal weight subjects [15]. Since obese subjects already have lower ghrelin levels, there is a chance we could not see a significant decrease in this population. It is possible that we may have seen an effect in a healthy population or with normal weight subjects like Vantansever-Ozen et al. (2011) demonstrated. They found that healthy men had significantly lower acylated ghrelin concentrations in the exercise trial compared to the control trial [133]. Similarly, Heden et al. (2013) found supporting evidence that normal weight subjects significantly reduced acylated ghrelin levels in the exercise trial compared to the control trial [137]. These studies revealed healthy subjects show greater hormone responses after a bout of exercise. However, we continued with the obese population because individuals at risk for metabolic diseases including T2DM, need an intervention to prevent, or at least prolong chronic disease.

For time, there was a significant difference between 0 and 60 minutes in acylated ghrelin. In all subjects, acylated ghrelin decreased significantly from 0 to 60 minutes. This is most likely the result of eating a meal at the beginning of the two-hour time period, as ghrelin is known to increase before a meal and decrease postprandially following a normal diurnal variation [15, 40].
Fasting causes an elevation of ghrelin levels in which levels decrease immediately following food intake with postprandial levels suppressed for up to 60–120 minutes after ingestion [53]. Therefore, we cannot conclude that the decrease in ghrelin was a result of exercise only.

Composition of a meal has a strong influence on concentrations of ghrelin [42]. Previous research has shown that ingested lipids suppress ghrelin less effectively than carbohydrates or proteins [156]. It is possible that if our test meal was high in fat to elicit a lipid response, we may not have seen a decline in ghrelin levels. Therefore, since our test meal was high in carbohydrates, it was adequately designed to assess ghrelin, although we did not see a difference between trials. For example, Nedvidkova et al. (2003) found that women who consumed a high-carbohydrate diet showed a larger drop in ghrelin levels than women who consumed a high-fat diet [62]. Considering our test meal was composed of 70% carbohydrates, this could have had a suppressing effect on acylated ghrelin from baseline to 60 minutes.

Although the mechanism of GLP-1 as a satiety hormone is not completely understood, we know that it promotes satiety induction through nutrient sensing in the lower intestine. GLP-1 is released postprandially into circulation in proportion to the calories ingested [77]. GLP-1 is released by various stimuli including glucose, fats, and proteins. In humans, GLP-1 levels increase postprandially and generally peak within one hour then gradually decrease to basal levels [68]. We found a significant difference in GLP-1 concentrations between 0 and 60 minutes. In all subjects, GLP-1 increased significantly from 0 to 60 minutes. This is likely the cause of satiety increasing postprandially due to the normal diurnal pattern. A study by Adam and Wererterp-Plantenga (2007) analyzed levels of GLP-1 in a group of overweight/obese adults and found that preprandial levels were similar in overweight/obese adults compared with normal-weight controls. However, the postprandial GLP-1 response was significantly blunted 30 minutes
after a test meal compared to the control subjects [14]. Because of this, it is possible that our subjects had a blunted GLP-1 response that we missed, prompting a lower concentration of GLP-1 postprandially. Again, because hunger hormones are altered postprandially, we cannot conclude that GLP-1 concentrations responded only to exercise.

Perceived hunger determined using the VAS questionnaire had no correlation to the biological markers, acylated ghrelin and GLP-1. Multiple studies have used VAS questionnaires to assess perceived hunger. Vantansever-Ozen et al. (2011) assessed hunger on one end (0 mm) of the line with the descriptor “not at all hungry” while on the other extreme (100 mm) was the descriptor “totally hungry”. Although we used the same question and descriptors in this study, Vantansever-Ozen found a significant correlation between acylated ghrelin and hunger after 120 minutes of exercise, while we did not. Perhaps we did not see a correlation due to the exercise protocol and duration. The intensity of exercise in the previous study included 50–70% VO₂max running, which could have produced greater suppression on ghrelin levels. If ghrelin levels are further suppressed, perceived hunger should be suppressed as well. Since we determined 15 minutes of moderate intensity walking is an insufficient duration to lower acylated ghrelin levels, we should expect that perceived hunger would also be unaffected. Hagobian et al. (2013) found hunger was significantly lower and fullness was significantly higher after their buffet meal in both exercise and rest conditions [123]. This is most likely due to the self-selection of food consumed at a buffet meal. Unlike a buffet meal, our test meal was predetermined. Since our subjects could not select their own meal, it is difficult to say they were fully satisfied by the food given. This could result in an alteration on their hunger and fullness perception. Similar to our findings, Hagobian et al. (2013) found that appetite hormones and appetite rating were weak and nonsignificant. Additionally, Heden et al. (2013) also found that fasting perceived hunger was
not significant between trials [137]. Although, there was no correlations in our study, we found a main effect for time, which has also been seen in previous research. Broom et al. (2007) found a main effect for time in hunger, showing a decrease in hunger after the test meal for three hours [23]. Prado et al. (2014) also demonstrated that hunger scores increased greatly in both exercise and conditions from baseline to 150 minutes [147].

**Strengths**

This study has many strengths. First, a repeated measures crossover design where each subject served as his or her own control for each treatment was utilized. This specific design prompted not only a valid and reliable study, but reduced the risk of error. Secondly, subject meals were standardized 24 hours prior to each visit, eliminating any significant differences in dietary composition between conditions. This decreased the impact that external factors might have on our findings. Lastly, this is the only study to our knowledge that has looked at a short bout of exercise on hunger hormones in a prediabetic population. Since this population is at a higher risk for T2DM and cardiovascular disease, identifying the impact of preventive strategies on regulating hunger hormones is important yet understudied.

**Limitations**

This study has some limitations. Physical activity prior to testing visits was not measured which could have a potential effect on hormone levels. As found in Heden et al. (2013), exercise the night before a meal suppresses acylated ghrelin concentrations. However, we specifically chose a sedentary group of individuals and asked subjects to refrain from any form of exercise at least 24 hours prior to visits to eliminate variations in activity. Secondly, many studies have measured hormone levels more frequently [127, 137, 142]. However, due to cost, we could not have blood draws as frequently as desired. We decided to drop the 30-minute measure since
GLP-1 peaks from 1–2 hours and postprandial acylated ghrelin levels are elevated 60–120
minutes after ingestion [53]. Finally, samples according to the assay procedures should have
been immediately placed in a refrigerated centrifuge. This was problematic because the wet lab
and exercise physiology lab are located in two separate buildings, therefore; the proximity could
have affected the integrity of the samples. However, all blood samples were kept in a cooler to
maintain temperature during transit and every effort was made to get the samples into the
refrigerated centrifuge as quickly as possible.

**Future Directions**

Future studies should utilize different exercise intensities and durations within a similar
protocol in the prediabetic population to assess the effects of various exercise modalities on
hunger regulation. It would be beneficial to look at an exercise bout 60-120 minutes
postprandially to see if exercise can influence hormone levels when the normal diurnal pattern
shows ghrelin typically starts to raise again in preparation for the next meal. Also, with a similar
protocol, future studies should aim to collect blood draws more frequently to see changes we
could have missed. Finally, future research should consider including multiple populations
within the same protocol. For example, include healthy individuals, prediabetics, and T2DM
subjects to see how postprandial exercise affects each of these populations differently. From
there, researchers could get a better idea of what prevention strategies are best to target each
population.

**Conclusion**

This study showed that a 15-minute walk performed shortly after a meal does not have a
significant impact on hunger hormones including acylated ghrelin and active GLP-1
concentrations in individuals at risk for T2DM.
APPENDIX A:

RECRUITMENT FLIERS
The Nutrition Program at UNLV is recruiting non-smoking, sedentary adults (45-69 years of age) who are free of chronic disease.

The purpose of this research study is to examine whether a postmeal walk can influence the metabolic impact of a typical American diet.

**Participation will include:**

- An initial screening visit and 2 testing visits at the UNLV exercise physiology lab
- 4 days of prepared meals before and after 2 testing visits
- Providing blood samples
- Body composition testing
- Personalized Nutrition Assessment
- Incentives of $100 dollars

INTERESTED? PLEASE FILL OUT A SHORT SURVEY AND WE’LL CONTACT YOU!

https://www.surveymonkey.com/r/NQGN3RT
Qualifications:

- Non-smoking, sedentary
- Ages 45-69 years old
- Must be free of chronic disease, weight stable in past 3 months, not pregnant or planning on becoming pregnant

Participation will include:

- An initial screening visit and 2 testing visits at the UNLV exercise physiology lab
- Body composition testing
- 4 days of prepared meals before and after 2 testing visits
- Willingness to follow study protocol: providing blood samples, wearing glucose meter, eating test meals, walking on treadmill and staying on test site for max of 3 hours each visit
- Personalized Nutrition Assessment
- Incentives of $100 dollars

INTERESTED?? Please visit our recruitment site:

https://www.surveymonkey.com/r/NQGN3RT
Adults Needed for UNLV Mealtime Walking Study

The UNLV Nutrition Program is recruiting non-smoking, sedentary adults for a research trial. This 2-week research study is to examine whether a postmeal walk can influence the metabolic impact of a typical American diet. Your commitment will consist of an initial screening visit and two testing days. Incentives will be provided during the study, totaling $100 dollars, as well as access to individual lab results following completion of the study, including an explanation of the findings.

For more information or to apply for the study, please visit our recruitment site:

https://www.surveymonkey.com/r/NQGN3RT
APPENDIX B:

SUBJECT QUESTIONNAIRES
By completing this survey, you are consenting to provide us with these data as part of a research study. The purpose of this study is to evaluate the effect of postmeal walking on 24-hour glucose control in individuals at risk for diabetes. Consent for this study is a 2-step process. The initial consent is a series of preliminary questions contained within this survey. If eligible, you will then be asked to further participate in the study and come to the Kinesiology laboratory at UNLV to complete the final consent. Participation in this survey is voluntary and you may exit the survey at any time.

1. Please provide your e-mail address:
2. What is your height and approximate weight?
3. Are you between the ages of 45 and 69 years old?
4. Are you healthy and free of chronic disease?
5. Are you able and willing to walk on a treadmill at a brisk pace (~3mph) for 15 minutes?
6. Do you have any food allergies?
7. Would you be willing and able to visit the UNLV campus for 3 study visits, spaced 1 week apart for this study? (The first visit will take ~60 min and 2 & 3 will take ~2.5 hours).
8. Are you ok providing blood sample via a fingerstick?
9. Are you willing to eat the provided standardized meals on test days?
10. If female, do you still have a menstrual cycle?
HEALTH HISTORY QUESTIONNAIRE

ID#___________________

1. Gender: M  F

2. Age: _________

3. Have you lost or gained more than 10 lbs in the last 12 months? Yes  No
   If yes, how much lost or gained? _________  How long ago? _________

4. Ethnicity: (please circle) Native American  African-American  Caucasian  Hispanic
   Asian  Other

5. Do you smoke? No, never _________
   Yes ________  # Cigarettes per day = _________
   I used to, but I quit _________ months/years (circle) ago

6. Have you ever been pregnant? __________________
   If yes, date of last pregnancy? ___________

7. What were the dates of your last menstrual cycle? ________________________

8. Do you take any medications regularly? Yes  No  If yes, list type and frequency:

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dosage</th>
<th>Frequency</th>
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<tbody>
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</table>

9. Do you currently take supplements (vitamins, minerals, herbs, etc.)? Yes  No  If yes, list type and frequency:

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Dosage</th>
<th>Frequency</th>
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<tbody>
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10. Have you ever been hospitalized? Yes  No  If yes, for what?

_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

58
11. Please ANSWER (YES/NO) if you currently have or if you have ever been clinically diagnosed with any of the following diseases or symptoms:

<table>
<thead>
<tr>
<th>Condition</th>
<th>YES</th>
<th>NO</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary Heart Disease</td>
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<tr>
<td>High Blood Pressure</td>
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<td>Heart Murmur</td>
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<td>Rheumatic Fever</td>
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<td>Irregular Heart Beat</td>
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<td>Varicose Veins</td>
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<td>Stroke</td>
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<td>Diabetes</td>
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<td>Low Blood Sugar</td>
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<td>Bronchial Asthma</td>
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<td>Hay Fever</td>
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<td>Leg or Ankle Swelling</td>
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<td>Eating Disorder</td>
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<td>Chest Pain</td>
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<tr>
<td>Shortness of Breath</td>
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<td>Heart Palpitations</td>
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<tr>
<td>Any Heart Problems</td>
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<tr>
<td>Coughing of Blood</td>
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<tr>
<td>Feeling Faint or Dizzy</td>
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<td>Lung Disease</td>
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<td>Liver Disease</td>
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<td>Kidney Disease</td>
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<td>Thyroid Disease</td>
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<td>Anemia</td>
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<td>Hormone Imbalances</td>
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<td>Depression</td>
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Please elaborate on any condition listed above.

________________________________________________________________________________________

12. How would you rate your lifestyle?

Not active ___________ Active ___________

Somewhat active ___________ Very Active ___________

13. Please circle the total time you spend in each category for an average week.

**Light activities** such as:
Slow walking, golf, slow cycling, doubles tennis, easy swimming, gardening
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

**Moderate activities** such as:
Mod. Moderate walking, cycling, singles tennis, moderate swimming, weight lifting
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

**Vigorous activities** such as:
Fast walking/jogging, fast cycling, court sports, fast swimming, heavy/intense weight lifting
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

14. How much alcohol do you drink? (average drinks per week) ___________
15. Do you have any food allergies?  Yes  No  If yes, explain:_____________________________________

16. The test shake contains dairy and gluten. Is this a problem?  Yes  No  If yes, explain:_____________________________________

Physical Activity Readiness Questionnaire (PAR-Q) and You

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly:

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<th>YES</th>
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**YES to one or more questions**

Talk to your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want – as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

**NO to all questions**

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- Start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- Take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

**Delay becoming much more active:**

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.
Visual Analog Scale (VAS)

Please mark a vertical line at the point on the lines below where you feel best represents your current state of hunger.

1. How hungry do you feel?

______________________________________________________________________________

Not at all hungry                                      Totally hungry

2. How satisfied do you feel?

______________________________________________________________________________

Completely satisfied                                      Not at all satisfied

3. How full do you feel?

______________________________________________________________________________

I can’t eat another bite                                      Empty
APPENDIX C:

INFORMED CONSENT, IRB APPROVAL
INFORMED CONSENT
Department of Kinesiology and Nutrition Sciences

TITLE OF STUDY: Effects of Postmeal Walking on 24-hour Glucose Control

INVESTIGATOR(S): Jessica Knurick, PhD

For questions or concerns about the study, you may contact Jessica Knurick at Jessica.knurick@unlv.edu or 702-895-1453.

For questions regarding the rights of research subjects, any complaints or comments regarding the manner in which the study is being conducted, contact the UNLV Office of Research Integrity – Human Subjects at 702-895-2794, toll free at 877-895-2794 or via email at IRB@unlv.edu.

Purpose of the Study
You are invited to participate in a research study. The purpose of this study is to evaluate the effect of postmeal walking on 24 hour glucose control in individuals at risk for diabetes.

Participants
You are being asked to participate in the study because you fit these criteria: you have indicated to us that you are 45-69 years of age, generally sedentary, and do not smoke. If female, you have no possibility of being pregnant and no longer have a menstrual cycle. You have not been diagnosed with a chronic disease, including diabetes and you are free from unresolved medical conditions. You have not been weight stable (+/- 3kg) in the past 3 months and you are able to walk on a treadmill at a moderate pace (~3.5 mph). Finally, you are willing to follow the study protocol including: eating test meals, providing blood, wearing the 24-hour glucose meter for ~60 hours, staying at the test site for 2.5 hours each visit, and completing short surveys.

Procedures
If you volunteer to participate in this study, you will be asked to do the following:

This study will initially involve the completion of brief demographic and health history questionnaires to demonstrate the absence of conditions that may affect health and blood assessments. This research entails that you visit our test facility on two additional occasions. At each of these occasions you will consume a test meal (bagel and apple juice), and provide venous blood samples.

At the first lab visit, you will be asked to complete a questionnaire about your general health. A fasting pre-screen blood sample from a finger prick will be obtained to determine inclusion in the study. Your height, bodyweight, waist circumference, and blood pressure will be measured. In addition, body composition will be measured using an InBody scale. This scale uses a
‘bioelectric impedance’ approach – it sends a small electronic current through you. You will not feel anything and this takes only about 3-minutes. Lastly, you will be asked to walk across a short measured area to assess preferred walking speed. This visit will last ~45 minutes. Two additional visits will be scheduled based on availability.

For the remaining 2 visits (~2.5 hours/visit) you will be asked to fast (no food or drink with the exception of water in the preceding 12 hours). You will also be instructed not to exercise the day prior to each visit and to consume standardized meals the day before and of testing, which will be prepared by our nutrition staff. The test meal will be consumed within 15 minutes of arrival. The two visits will be spaced approximately one week apart. At these visits you will consume the test meal with or without a postmeal treadmill walk. During the treadmill walk, we will record heart rate data from a monitor placed around the upper abdomen. For each session, a fasting blood sample will be obtained for determination of basal concentrations of blood glucose. You will then be given 15 minutes to eat a standardized meal (bagel and apple juice). Venous blood samples will be collected prior to meal ingestion and at 15, 30, 60, and 180 minutes post meal.

Approximately 36 hours prior to testing, you will be asked to wear a continuous glucose monitor, which will measure your blood sugar for the subsequent 60 hours. The glucometer will be placed on your lower abdomen and will require a single needle insertion. After this time period, you will be asked to return to the lab, so that we are able to remove the glucometer.

**Benefits of Participation**
There will not be direct benefits to you as a participant in this study. However, you will be provided with all your health marker test results if desired including your body fat composition, and fasting blood glucose concentrations. You will have the opportunity to attend a free healthy living strategies class after study completion offered by a registered dietitian.

**Risks of Participation**
There are risks involved in all research studies. This study may include only minimal risks. A trained researcher will perform glucose draws via fingerstick under standard and sterile conditions, but temporary bruising of the skin or a feeling of faintness is possible at the time of the blood draw. For the blood pressure tests, a squeezing pressure will be applied to the upper arm for a short time. The test meal is a bagel and juice and contains gluten, which is unsuitable for individuals who have gluten intolerance. Treadmill walking will be at low-moderate intensity (2.5-3.0 mph) pace under the supervision of a trained exercise physiologist. We will ask that you bring appropriate walking shoes to this visit. You are asked to tell investigators to stop any testing at any time if desired.

**Cost /Compensation**
There may be financial cost to you to participate in this study related to time and travel. The study will take approximately 6 total hours of your time. Participants will receive cash incentives $50 at lab visit 2 and $50 at lab visit 3 to offset some of these costs.

**Confidentiality**
All information gathered in this study will be kept as confidential as possible. No reference will be made in written or oral materials that could link you to this study. All deidentified records will be stored in a locked facility at UNLV for up to 10 years after completion of the study.
Blood samples will be identified only by the subject number and date and stored in freezers in the Nutrition labs at the BHS building for up to 5 years. Only the study investigators and the laboratory staff will access these samples. After the storage time the information gathered will be destroyed.

**Voluntary Participation**
Your participation in this study is voluntary. You may refuse to participate in this study or in any part of this study. You may withdraw at any time without prejudice to your relations with UNLV. You are encouraged to ask questions about this study at the beginning or any time during the research study.

**Participant Consent:**
I have read the above information and agree to participate in this study. I have been able to ask questions about the research study. I am at least 18 years of age. A copy of this form has been given to me.

________________________  ______________
Signature of Participant     Date

________________________
Participant Name (Please Print)
UNLV Biomedical IRB - Expedited Review
Approval Notice

DATE: October 19, 2016
TO: Jessica Knurick, PhD
FROM: UNLV Biomedical IRB

PROTOCOL TITLE: [869576-3] Effects of Postmeal Walking on 24-hour Glucose Control
SUBMISSION TYPE: Revision

ACTION: APPROVED
APPROVAL DATE: October 19, 2016
EXPIRATION DATE: October 18, 2017
REVIEW TYPE: Expedited Review

Thank you for submission of Revision materials for this protocol. The UNLV Biomedical IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a protocol design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

PLEASE NOTE:
Upon approval, the research team is responsible for conducting the research as stated in the protocol most recently reviewed and approved by the IRB, which shall include using the most recently submitted Informed Consent/Assent forms and recruitment materials. The official versions of these forms are indicated by footer which contains approval and expiration dates. If your project involves paying research participants, it is recommended to contact Carisa Shaffer, ORI Program Coordinator at (702) 895-2794 to ensure compliance with subject payment policy.

Should there be any change to the protocol, it will be necessary to submit a Modification Form through ORI - Human Subjects. No changes may be made to the existing protocol until modifications have been approved.

ALL UNANTICIPATED PROBLEMS involving risk to subjects or others and SERIOUS and UNEXPECTED adverse events must be reported promptly to this office. Please use the appropriate reporting forms for this procedure. All FDA and sponsor reporting requirements should also be followed.

All NONCOMPLIANCE issues or COMPLAINTS regarding this protocol must be reported promptly to this office.

This protocol has been determined to be a Minimal Risk protocol. Based on the risks, this protocol requires continuing review by this committee on an annual basis. Submission of the Continuing Review Request Form must be received with sufficient time for review and continued approval before the expiration date of October 18, 2017.

If you have questions, please contact the Office of Research Integrity - Human Subjects at IRB@unlv.edu or call 702-895-2794. Please include your protocol title and IRBNet ID in all correspondence.

Office of Research Integrity - Human Subjects
4505 Maryland Parkway . Box 451047 . Las Vegas, Nevada 89154-1047
(702) 895-2794 . FAX: (702) 895-0805 . IRB@unlv.edu
Modification Request Form

Instructions:
1. Complete all sections of this form.
2. Submit all previously submitted documents that contain information affected by the modification(s).

Note:
1. Handwritten and hand delivered forms will not be accepted.
2. INCOMPLETE FORMS WILL BE RETURNED.
3. Modification may not be implemented until you have received notification of IRB approval.
4. For your records, it is important that you keep a copy of this completed form.

General Information

<table>
<thead>
<tr>
<th>Submittal Date: 10/24/2016</th>
<th>Principal Investigator Name: Jessica Knurick</th>
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<tbody>
<tr>
<td>Protocol Title: Effects of Postmeal Walking on 24-hour Glucose Control</td>
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</tr>
<tr>
<td>Protocol Number: 689576-4</td>
<td>Last Approval Date: 10/24/2016</td>
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<td>Prior Approval: [ ] Expedited Review [ ] Full Board Review [ ] Exempt</td>
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</table>

Description of Modification

Type of Modification (check all that apply):
- [ ] Currently approved procedure
- [ ] Informed Consent
- [ ] Number of subjects
- [ ] Survey/Questionnaire
- [ ] Research Team**
- [ ] Title
- [ ] Other (e.g., advertisement, flyer, etc.)

Modification Summary
Briefly describe the modification.

1. Added Sydney Spoon as a co-investigator to the protocol proposal form (Section 3).
2. Changed the method of body composition from the BodPod to the InBody. The BodPod in our lab is no longer working and the InBody has been shown to be sufficiently adequate to assess body composition. Changes have been made to both the proposal form (section 7.3) and the informed consent (Below Procedures).
3. The amount of time wearing the continuous glucose monitor is being changed from 1 day to 2 days. Continuous glucose monitors are traditionally worn for a period of 7 days, so we do not anticipate any additional risk to the subject. The reason for this change is because after meeting with the company, we were told that the first day of wear is usually inaccurate and accuracy significantly goes up on day 2. For this reason, we will provide the continuous glucose monitor at the same time period when the subject comes to the lab to pick up their meals (approximately 36 hours prior to testing. These changes can be found on the PPF 7.3 and informed consent (highlighted).
4. Added FOA Grant.

**Note: Addition of research team must include name(s) and role(s). Change in PI must be submitted and signed by the original PI on the protocol. Include the reason for the change in the modification summary.

Reanalysis of Risk (check one)

- [x] This modification does not increase risk to participants enrolled in this study.
- [ ] This modification does increase risk to participants enrolled in this study.
Signatures of Assurance

A. Investigator’s Assurance:
I certify that the information provided in this application is complete and accurate. As Principal Investigator, I have ultimate responsibility for the conduct of this study, the ethical performance of the project, the protection of the rights and welfare of human subjects and strict adherence to any stipulations designated by the IRB. I agree to comply with all UNLV policies and procedures, as well as with all applicable Federal, State and local laws regarding the protection of human subjects in research including, but not limited to the following:

- Performing the project by qualified personnel according to the approved protocol.
- Not changing the approved protocol or consent form without prior IRB approval (except in an emergency, if necessary, to safeguard the well-being of human subjects).
- Obtaining proper informed consent from human subjects or their legally responsible representative, using only the currently approved, stamped consent form.
- Promptly reporting adverse events to the ORI – Human Subjects in writing according to IRB guidelines.
- Arranging for a co-investigator to assume direct responsibility, if the PI will be unavailable to direct this research personally, as when on sabbatical leave or vacation.

***FACULTY ADVISOR (IF APPLICABLE): By my signature as Principal Investigator on this research application, I certify that the student/fellow investigator is knowledgeable about the regulations and policies governing research with human subjects and has sufficient training and experience to conduct this particular study in accordance with the approved protocol. In addition:

- I agree to act as the liaison between the IRB and the student/fellow investigator with all written and verbal communications.
- I agree to meet with the student/fellow investigator on a regular basis to monitor the progress of the study.
- I agree to be available and to personally supervise the student/fellow investigator in solving problems, as they arise.
- I assure that the student/fellow investigator will promptly report adverse events to the ORI – Human Subjects according to IRB guidelines.
- I will arrange for an alternate faculty advisor to assume responsibility if I become unavailable, as when on sabbatical leave or vacation.
- I assure that the student/fellow investigator will follow through with the storage and destruction of data as outlined in the protocol.

By submitting this form electronically, I agree to the assurance as stated above.
REFERENCES


139. Beaulieu, K., et al., Energy intake over 2 days is unaffected by acute sprint interval exercise despite increased appetite and energy expenditure. Appl Physiol Nutr Metab, 2015. 40(1).


CURRICULUM VITAE

Sydney C. Spoon, RDN, LD
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EDUCATION

Master of Science: Kinesiology – Exercise Physiology
University of Nevada, Las Vegas, Las Vegas, NV
Expected May 2017

Certified Registered Dietitian/Nutritionist
Commission on Dietetic Registration, Chicago, IL
Sept 2015

Bachelor of Science: Nutrition Sciences – Dietetics/Sports
University of Nevada, Las Vegas, Las Vegas, NV
Dec 2014

PROFESSIONAL EXPERIENCE

Weight Loss Dietitian Premier Physicians Weight Loss & Wellness
May 2016 – Present
• Provide various patient educations regarding healthy eating, portion sizes, mindful eating, exercise is medicine, and diabetes management
• Create patient handouts and meal plans

Graduate Assistant University of Nevada, Las Vegas
Aug 2014 – Present
Coordinator and Instructor UNLV Nutrition Center
• Develop, edit, and teach nutrition presentations regarding:
  o Weight Management
  o Cardiovascular Health
  o Diabetes Management
  o Sports Nutrition
  o Healthy Eating on a Budget
  o Nutrition Workshops
• Manage scheduling of events and classes for the Nutrition Center
• Presented cooking demonstrations with the Children’s Heart Center of Nevada
• Created various undergraduate and dietetic intern independent study projects, presentations, and workshops
• Guest lecture for undergraduate kinesiology and nutrition classes
• Coordinate grant-funded grocery store tours
  o Trained instructors (dietetic interns and undergraduates)
  o Manage publicity – newspaper advertisements and flyers
  o Scheduled tours at various locations
• Provide nutrition education presentations to UNLV sports teams and physical education classes
• Manage and organized demographic data sheets for grant maintenance
• Provide one-on-one nutrition consultations for the Las Vegas Community members

**Dietetic Intern** *University of Nevada, Las Vegas*  
Jan 2015 – Aug 2015

- Learned skills necessary to earn a position as a dietitian including nutrition assessment, patient educations, food-service management, and community nutrition assessment
- Gathered practical experience using evidenced-based practice that challenged the application of didactic knowledge

**GRANTS/SCHOLARSHIPS**

2016  
Graduate and Professional Student Association Research Grant at University of Nevada, Las Vegas to research The Effects of an Acute Bout of Exercise on Hunger Hormones in Individuals at Risk for Type 2 Diabetes ($1000 funded)

2017  
Department of Kinesiology and Nutrition Sciences at University of Nevada, Las Vegas grant to research The Effects of an Acute Bout of Exercise on Hunger Hormones in Individuals at Risk for Type 2 Diabetes ($1500 funded)

**RESEARCH**


**CERTIFICATIONS/TRAINING**

2015  
Nevada Department of Health and Human Services: Licensed Dietitian

2015  
Collaborative Institutional Training Initiative:
  - Biosafety training
  - Blood borne Pathogens training
  - Chemical Hygiene training
  - Hazard Communications training
  - HIPAA training
  - Personal Protective Equipment training
  - Radiation Safety training
  - Unsealed Sources training

2014  
American Heart Association: CPR and AED certified

2014  
Food Handler Safety Training – Southern Nevada Health District
MEMBERSHIPS

Academy of Nutrition and Dietetics
  Sports, Cardiovascular, and Wellness Nutrition (SCAN) DPG
  Weight Management DPG
  Women’s Health DPG
  Behavioral Health Nutrition DPG
Southern Nevada Dietetic Association
Nevada Dietetic Association
American College of Sports Medicine – Southwest Chapter
Phi Kappa Phi – National Honors Society

REFERENCES

Jessica Knurick, PhD, RDN, LD
Assistant Professor
UNLV Nutrition Sciences
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Laura Kruskall, PhD, RDN, LD, CSSD, FACSM, FAND
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Program Director
UNLV Nutrition Sciences
Director of Dietetic Internship
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John Young, PhD, FACSM
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UNLV Kinesiology and Nutrition Sciences
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