The Role of Diet Composition and Fat-Free Mass on Appetite Regulation

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Dedication

This work is dedicated to my father, Joseph Graybeal, and my best friend, Titan, to take us across the finish line.

Chapter 1

Introduction

1.1 Appetite Regulation and Energy Balance

A foundational principle of metabolism is that weight change is associated with an imbalance between the amount of energy consumed through diet and the energy expended by the body to sustain life and perform physical labors[1]. This principle, also known as energy balance, is an extension of the first law of thermodynamics, which states that energy can neither be created nor destroyed but is instead transferred from one form to another. In humans this refers to the gain or loss of energy and demonstrates how body weight change ensues over time[2].

During energy balance, EI is equivalent to energy expenditure (EE) and body weight remains stable[3]. Energy intake (EI) refers to the energy consumed from fat, carbohydrate, protein, and alcohol. EE is due to resting metabolic rate (RMR), thermic effect of food (TEF), and physical activity[2]. RMR is the EE necessary for maintaining vital physiological functions and is the largest proportion of total daily EE[4]. RMR is also primarily driven by an individual's fat-free mass (FFM)[5]. TEF refers to the increase in EE above pre-prandial (before a meal) basal levels[6] that reflects the energy necessary to digest, absorb, transport, metabolize, and store food[7]. Approximately 5-15% of total daily EE is attributable to TEF but is dependent on the composition of the diet[6]. TEF is greatest for dietary protein and least for fat[8]. The most variable component of EE is physical activity[3], which refers to the energy expended from voluntary (i.e. exercise) and involuntary movement (i.e. shivering). Collectively, these components make up total daily EE and are determinants of energy balance.

As previously mentioned, energy imbalances lead to changes in body mass that are primarily attributable to changes in body fat; although, changes in FFM may occur simultaneously albeit to a lesser degree[9]. During positive energy balance, EI is greater than EE causing weight

gain. Over time, the constant state of positive energy balance gradually results in weight gain and eventually obesity. During negative energy balance, EE exceeds EI causing weight loss. A consistent state of negative energy balance may lead to states of physiological distress from low EI[10], especially in individuals with high levels of physical activity. It is a common misconception that EI and EE can be modified independently to achieve energy balance. For example, individuals may believe that they can increase their EE through exercise and that this will result in the negative energy balance necessary to induce weight loss. However, the relationship between EI and EE is regulated by both neural and peripheral physiological systems that detect energy imbalances and work together to maintain body mass[11]. As a result of this physiological regulation, EI and EE are dependent and one cannot change without compensatory changes in the other[2]. During positive energy balance, for example, the body responds by suppressing hunger and increasing EE. Conversely, the body responds to negative energy balance by stimulating hunger and suppressing EE[12]. Furthermore, the body's response to energy imbalance appears to be asymmetrical, suggesting that the anabolic components of energy balance are more powerful than the catabolic components[13]. This is particularly true in regard to appetite regulation, which may be a more prominent system than originally postulated.

Appetite, defined as the psychological drive to eat[14], is a multifaceted system that includes both central and peripheral elements involved in food intake and energy balance[15]. Branches of appetite regulation include perceptions of hunger and fullness and gut hormones that are either anorexigenic such as glucagon-like peptide 1 (GLP-1), cholecystokinin, or peptide YY (PYY), or orexigenic such as ghrelin[16]. Suppression of appetite may lead to several negative effects, specifically in athletes. Appetite suppression, combined with high EE from participation in sport, may lead to insufficient energy availability[17]. Energy availability is the amount of dietary energy available to sustain physiological functions after accounting for the energy expended during

physical activity and other metabolic processes[18]. Low energy availability is linked to several physiological impairments such as disturbances in reproductive health, bone metabolism, and endocrine function in both males and females[17]. A state of low energy availability in athletes that results in the above mentioned physiological dysregulation is known as relative energy deficiency in sport (RED-s) and has surged as a topic of interest since the inception of the definition in 2014 by the International Olympic Committee[10,19].

Appetite stimulation poses a difficult scenario for individuals attempting to sustain a constant state of negative energy balance or those with obesity. During negative energy balance, the human body responds by stimulating short-term orexigenic hormone production and suppressing satiety hormone circulation[20]. The alterations in gut hormone production influences neuropeptide release in the hypothalamus which stimulates appetite to increase EI. Even after energy balance is obtained, this process often continues and promotes continued EI despite sufficient body energy stores[21]. Over time, continual appetite stimulation and increases in EI may lead to obesity[22]. Furthermore, individuals with obesity develop disrupted appetite responses that make it difficult to lose weight. Specifically, individuals with obesity have impaired ghrelin response compared to leaner individuals, where postprandial ghrelin remains elevated despite consumption of a meal[23,24]. Moreover, fasting PYY levels are lower in individuals with obesity compared to individuals with normal weight[25]. Individuals with obesity are at greater risk for the development of several comorbidities such as type II diabetes mellitus, hypertension, dyslipidemia, many types of cancers, and cardiovascular disease[26].

Although it is well established that appetite has considerable influence on energy balance, research is needed to determine strategies that will effectively modulate this relationship. In the next few sections in this chapter we will examine the environmental, psychological, physiological, and dietary regulation of appetite.

1.2 Environmental and Psychological Regulation of Appetite

Appetite regulation is influenced at both the environmental and psychological levels[27]. Environmental changes, specifically the industrialization of our food systems[28,29], have shifted human appetite behavior considerably. The sharp increase in production of highly-palatable, ultraprocessed foods[30] in the modern food environment no longer compliment the primary purposes of eating such as replenishing depleted energy to maintain energy balance[31]. Instead, eating has taken a hedonic approach, where the modern food environment promotes appetite responses to induce reward rather than need[31]. Environmental factors that increase the hedonic approach to appetite are food availability, portion size, variety, and the continuous presence of food cues[32].

Food is now available at any time and in almost any location in Western countries[33]. Moreover, foods specifically designed to improve palatability and increase consumption are more available now than at any other point in human history[34]. Studies show that increased ability to obtain food leads to increases in *ad libitum* EI[35,36]. The increased palatability of foods in the current environment may also influence the amount of food consumed when sufficiently available[37]. This exposure to highly palatable foods has shown to increase hunger during the pre-prandial and early postprandial periods[38]. Moreover, studies report that appetite recovery is faster after consumption of a highly-palatable meal[39,40]. This is likely because both the availability and palatability of easily accessible foods alters homeostatic food intake and promotes continued consumption beyond what is physiologically necessary[33].

Portion-size also increases the risk of consuming excess food when available in surplus[41]. Under normal environmental conditions, hunger and satiety guide EI. However, when food is available in excess and portion sizes are large, a new eating norm is established that can overcome

signals of satiety to guide EI[35]. Furthermore, previous experience with foods may dictate portion size. The fullness experienced from a particular meal creates cognitive expectations about the degree of satiation and may determine future portion sizes[42,43].

The substantial variety of food in the modern environment has also been shown to be a potent stimulator of appetite[44]. It has been reported that consumption of a single food over time leads to decreased liking and desire to eat relative to other foods[45,46]. However, the presentation of new foods results in an increased desire to eat and EI despite being sufficiently satiated[47]. This is concerning given that an average grocery store sells more than 30,000 food items and over 12,000 new items are introduced into grocery stores annually[48].

All of the abovementioned factors are associated with food cue reactivity, which is defined as the degree to which an individual is susceptible to eating in the presence of food cues[49] such as the sight or smell of food[50]. Increased reactivity to food cues leads to overeating[51], especially in the modern food environment. Classical conditioning best describes how food-cues interact with appetite. In food cue conditioning, an initial association occurs between a cue unassociated with food and the actual consumption of food[52]. For example, original research from Ivan Pavlov reported increased salivation in canines after hearing a bell ring that preceded the offering of food[53]. After this association is established, the food cue gains the ability to promote EI by altering homeostatic pathways that increase hunger despite being in a state of satiation[54].

At the individual level, psychological factors that influence appetite regulation include both behavioral and emotional components. For example, a negative mood is associated with increased EI and an inability to delay reward[55]. Stress can also affect appetite regulation, resulting in either appetite suppression or stimulation depending on the psychological stressor[56]. For instance, a mild psychological stressor may increase EI whereas a severe stressor may decrease EI[57]. Factors largely responsible for variation in appetite responses also include duration of stress and baseline

hunger and satiety levels[56]. Stress is associated with emotional eating, which is the tendency to overeat during periods of emotional stress in an effort to alleviate negative mood states[58].

Similarly, depressive disorders are accompanied by changes in appetite with some exhibiting increased appetite while others experiencing appetite suppression as a result of increased depression[59]. Conversely, increased trait anxiety is related to increased EI[60]. Food cravings, another major psychological factor, is defined as the irresistible desire to consume a particular food[61]. Cravings are suggested to be a strong expression of hunger[62] that can override signals of satiety until consumption of the craved food and therefore places tremendous stress on the neural circuity involved in appetite regulation.

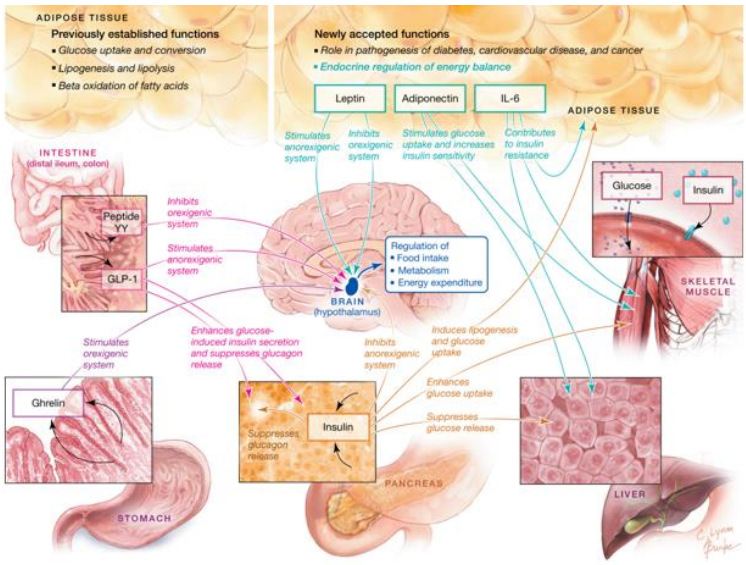
The study of restrained eating may provide insight to the psychological control of appetite. Restrained eating refers to the intentional restriction of EI and is associated with factors such as body dissatisfaction, cognitive appraisal, and social media pressure[63]. The ability to refrain from eating despite physiological fluctuations in hunger and satiety suggest that restrained eating is under psychological control rather than physiological control[64,65]. On the contrary, uncontrolled eating is demonstrated by the loss of control over food intake during eating periods[58]. Studies report that individuals with uncontrolled eating behaviors have greater EI and blunted appetite responses compared to controls[66]. The aforementioned psychological and environmental factors collectively plays a role in appetite control but represents only one aspect of appetite regulation.

1.3 Physiological Regulation of Appetite

Physiological regulation of appetite, also known as satiety[67], is dependent on several peripheral mechanisms that communicate with the brain. The gut-brain connection, also referred to as the gut-brain axis, is critical in the regulation of energy balance. The gut-brain axis is composed of both the central nervous system (CNS) and the gastrointestinal (GI) tract and together

both the neuroendocrine and the enteroendocrine systems work dependently to regulate energy balance (**Figure 1.1.**)[68] [1].

Figure 1.1



•Figure Legend:

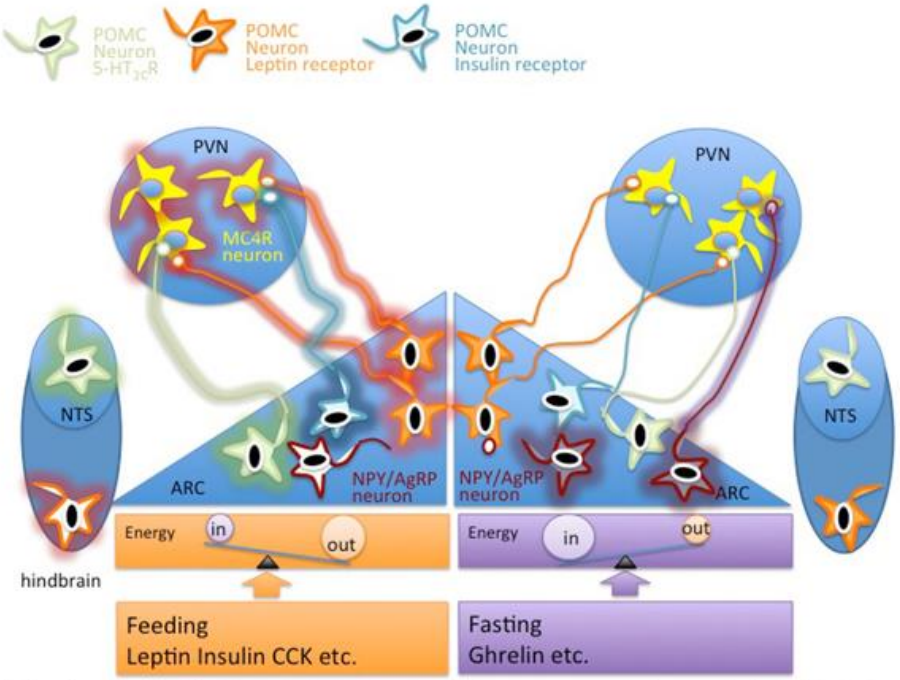
•Neuroendocrine and Endocrine Pathways of Obesity. Once a cell thought to be a simple, passive storehouse for lipids, the adipocyte is now known to be marvelously complex. It senses the body's energy state and sends signals to many organs, coordinating their function. The solution for the obesity epidemic might lie in better understanding adipocyte biology.

In the brain, energy balance is maintained through the actions of the neuroendocrine system which function to regulate EI[69]. The neuroendocrine system is composed of several hypothalamic regions and their corresponding neurons which produce either orexigenic or anorexigenic neuropeptides. Specifically, the arcuate nucleus (ARC) houses the hypothalamic-melanocortin system which is responsible for regulating EI through the stimulation or suppression of orexigenic

[1] Reproduced with permission from JAMA. 2012. 308(11): doi:10.1001/jama.2012.3209. Copyright©(2012) American Medical Association. All rights reserved.

or anorexigenic neurons. In the ARC (**Figure 1.2.**[70],[2]), the anorexigenic neuron pro-opiomelanocortin (POMC) releases the neuropeptides cocaine- and amphetamine-regulated transcript (CART) and α-melanocyte-stimulating hormone (α-MSH) to suppress food intake. Conversely, the orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons stimulate food intake[15].

Figure 2



POMC neurons in the melanocortin system. In the fed state, the signal to stop eating and to increase energy expenditure is conveyed by leptin and insulin released in the bloodstream by adipocytes and by the β-cells of the pancreas, respectively. These hormones cross the blood-brain barrier to reach the arcuate nucleus (ARC) of the hypothalamus and promote firing (indicated by glow around the cell perimeter) of distinct populations of POMC neurons expressing the LepR and insulin receptor. Other populations of POMC neurons in the arcuate nucleus and in the nucleus of the solitary tract (NTS) express the serotonin receptor 5-HT2CR. POMC neurons project to the paraventricular nucleus (PVN) to increase activity of MC4R neurons to decrease food intake and to increase energy expenditure. In the fasted state, POMC neurons in the arcuate nucleus are inhibited by decreased circulating leptin and insulin and by increased activation of AgRP/NPY neurons, which send inhibitory signals to reduce firing of POMC neurons and of MC4R neurons. References are in the main text.

In the paraventricular nucleus, melanocortin 4 receptor (MC4R) neurons are activated by α-MSH which provokes an anorexigenic effect that reduces food intake and increases EE[71]. Collectively, these hypothalamic neurons regulate meal initiation and termination based on their

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respective actions (orexigenic or anorexigenic)[72]. Furthermore, the neural circuitry involved in appetite regulation relies on the integration of several peripheral modulators[73].

The GI tract is primarily responsible for the peripheral modulation of appetite control through its endocrine activity[74]. The GI tract produces over 30 gut-hormones[74] that function to digest and absorb nutrients[75] but also secretes orexigenic and anorexigenic hormones that mediate hunger and satiety. In fact, these hormones are a crucial component of the gut-brain connection through their ability to signal hunger and satiety through circulation and by vagal afferent nerve stimulation and[76].

Circulating gut hormones secreted from the GI tract mediate hunger and satiety by signaling the stimulation or suppression of anorexigenic or orexigenic neuropeptides in the brain[77]. The gut hormones primarily responsible for this process include ghrelin, GLP-1, PYY, CCK[72]. Ghrelin, the only known peripheral orexigenic hormone, is secreted by the endocrine glands of the stomach during the pre-prandial period[78]. Ghrelin is found in three forms which include total, acylated, unacylated forms[79]. Overall, the primary action of ghrelin is to increase hunger and stimulate EI when food is unavailable and during fasting through stimulation of NPY and AgRP and suppression of POMC and MC4R[80]. There is growing interest in the actions of acylated ghrelin specifically due to its demonstrated susceptibility to dietary manipulations[81–83]. Ghrelin concentrations are typically suppressed after refeeding (consumption of food after prolonged fasting) and the delivery of nutrients to the GI tract[81].

The anorexigenic gut-hormones GLP-1 and PYY suppress food intake following meal ingestion by signaling to the POMC neurons to secrete and direct CART and α-MSH to bind to MC4R. GLP-1, an incretin hormone, is released from intestinal L cells following meal ingestion and functions to regulate insulin[84,85]. However, GLP-1 has shown satiation effects that are also mediated by vagal afferent nerve stimulation[86,87]. Although increases in GLP-1 typically occur

when nutrients reach the L cells of the intestine, rodent models show food anticipatory secretion occurs to regulate the consumption of a large meal[88,89]. Additional studies report peaks in GLP-1 10-15 minutes after a meal is initiated[90]. Conversely, inhibition of GLP-1 results in appetite increases and subsequent increases in food intake[91].

PYY, a member of the pancreatic polypeptide-fold family, occurs in the PYY_{1-36} and PYY_{3-36} form. PYY_{3-36} is generally associated with appetite control[83]. PYY is released from the L cells of the small intestine in response to food consumption and increases postprandial satiety. Circulating PYY rises within 15 minutes of food consumption, peaks at approximately 1-2 hours, and remains elevated for up to 6 hours relative to the both the caloric content and composition of a meal[78,92,93]. Like GLP-1, the time course of the increase in PYY suggest that initial postprandial releases are controlled neuronally since increases begin well before nutrients have reached the GI tract[94]. Further, a positive correlation has been shown between PYY concentrations and rate of gastric emptying which may also be a mechanism in which PYY increases satiety[95]. Additionally, inhibition of PYY leads to increased appetite and food intake[91,93].

Lastly, CCK is secreted throughout the small intestine and is the most established satiety hormone to date[96–98]. CCK is rapidly released into circulation following meal ingestion and serves as a postprandial satiety signal[99]. Unlike other gut hormones, CCK cannot cross the blood-brain barrier and therefore is suggested to work exclusively in peripheral tissues rather than centrally to inhibit food intake[100]. Instead, CCK modifies digestion to increase satiety by inhibiting gastric emptying and increasing intestinal motility[78]. Moreover, the rapid release of CCK simultaneous with gastric distention following meal ingestion helps reduce eating duration and the amount consumed within a given meal[101,102].

Together, the combination of the hypothalamic-melanocortin system and their associated gut hormones make up our mechanistic control of human appetite and energy balance. However,

physiological regulation of these systems depends on the nutritional state of the body which serves as a major moderator of appetite control. Specifically, variations in dietary composition may contribute to the regulation of appetite.

1.4 The Effect of Diet Composition on Appetite

Diet is an umbrella term simply defined as the energy and nutrients consumed from foods and beverages[103]. There are countless types of diets (e.g. high-protein diets, low-carbohydrate diets, low-energy diets, etc.), and each possess different subtypes. Many of these diets involve restricting one or more macronutrients. Restricting specific macronutrients has become increasingly popular due to the reported benefits of one composition relative to another. The diet industry itself plays a large role the surge of diet composition specifications[28] and often, suggests that selecting the appropriate diet composition can simply achieve the desirable outcomes such as weight loss, increased satiety, improved blood lipids, etc. Although the superiority of one dietary composition over another is rarely supported[104], there may be a reason to believe that this could be true from a physiological perspective. Manipulation of diet composition has the potential to alter an individual's internal environment, leading to changes in hormonal and metabolic states[28]. Specifically, abandonment or emphasis of a macronutrient in an individual's diet may shift energy balance by altering appetite.

The hierarchy of satiety responses for each macronutrient appears to be, in order from greatest to least: 1) protein, 2) dietary fat, and 3) carbohydrates. Dietary protein appears to be superior[105] based on the ability of this nutrient to both suppress hunger hormones and stimulate satiety hormones to a greater degree than its macronutrient counterparts[7,106,107]. Specifically, protein consumption results in a greater release of GLP-1, PYY, and CCK[108] alongside proportional increase in ratings of fullness and decrease in hunger[109]. Additionally, greater

suppression of ghrelin occurs following protein consumption[110]; although some experts claim that protein does not suppress appetite but rather, inhibits appetite stimulation[109,111].

The superiority of dietary protein on hunger and satiety is well established[107], but there remains contention as to whether fat or carbohydrates have a greater effect on appetite. Reports suggest that fats are more satiating than carbohydrates due to their ability to stimulate satiety hormone production to a greater degree rather than their ability to suppress hunger hormone production; although, this remains up for debate[112,113]. Appetite responses to both fat and carbohydrate may also be mediated by weight status. Lean individuals may be more sensitive to the appetite suppressive effects of carbohydrates when compared to individuals of higher weight status[113]. Further individuals with obesity appear to be more resistant to satiating effects of fat, noted by the smaller increases in GLP-1 relative to lean individuals following a high-fat meal[114,115]. However, the greatest increases in PYY and CCK are demonstrated after a meal high in fat, and more so when fat is less saturated[98].

Degree of saturation appears to have an important role in the satiation capacity of fat. The GI tract possesses a "fat sensing" ability that results in several endocrinal and mechanical changes in GI function[116]. Once the GI tract senses the presence of dietary fat, stimulation of GPL-1, PYY, and CCK occurs in conjunction with suppression of ghrelin[117] and slowed gastric emptying[118]. However, reports suggest that the capacity to satiate is dependent upon the physiochemical properties of the digested fat[119]. These properties include both fatty acid (FA) chain length and degree of saturation[120,121]. Specifically, monounsaturated and polyunsaturated FAs (MUFA, PUFA) appear to stimulate GLP-1 and PYY to a greater degree than saturated FAs (SFA)[122]. Despite the effect of decreased FA saturation on gut hormone stimulation, it remains unclear whether or not this results in decreased perceptions of appetite and food intake[123,124].

Certain dietary composition manipulations may also increase satiety through increased circulation of ketone bodies. Ketone bodies, produced in the liver, are used as a primary energy substrate during periods where glucose is unavailable[125]. The main ketone bodies include β-hydroxybutyrate (BHB) and acetoacetate which increase during long duration caloric deficits, metabolic distress (diabetic ketoacidosis), prolonged exercise, and planned restriction of carbohydrates[126]. An individual is considered to be in ketosis when the BHB concentration is $\geq$ 0.5 mmol/L[127]. Aside from the pathological state of diabetic ketoacidosis and completely fasting, nutritional ketosis is often the result of employing a very low-carbohydrate strategy[128], otherwise known as the ketogenic diet (KD).

The KD, defined as restricting carbohydrates to <10% of total daily energy or to 20-50 g/d[129], is a form of very low-carbohydrate diet used to induce nutritional ketosis. The KD has gained significant popularity in regard to appetite based on reports claiming appetite suppression despite a state of negative energy balance[130]. This is counterintuitive to the principles of energy balance but is thought to occur from the increased circulation of ketone bodies[131]. Specifically, increased ketone bodies during a KD are associated with decreased ghrelin[132,133], and increased satiety hormones[134] during negative energy balance states that would normally result in the opposite reactions. This is corroborated by studies finding that those in a ketogenic state experience alterations in circulating hunger and satiety hormones with proportional changes in perceptions of appetite[132–136]. However, many experts believe that the unintentional increase in proportions of protein and fat (by limiting carbohydrates) are responsible for the suppression of appetite during a KD. Moreover, most studies have examined this overweight an obese individuals. The question remains as to whether the appetite suppressive effects of varying dietary compositions possess the same effects across individuals with more optimal body compositions.

1.5 The Effect of Body Composition on Appetite

Human body composition is a multi-compartment system consisting of several molecular levels and embodies the lifetime accumulation of nutrients obtained from the environment[137]. Although there are several components that collectively make up human body composition, discussions of body composition typically reference two individual components: fat mass (FM) and FFM. FM refers to the collection of fat molecules that equates to the amount of stored fat in the body. FFM includes all non-fat molecules in the body (skeletal muscle, brain, liver, etc.) irrespective of where they occur. Although these two entities are distinct, each contributes to an individual's metabolic state. Specifically, FM and FFM possess considerable regulatory capacity over EE and therefore, have a role in appetite regulation based on principles of energy balance.

Both FM and FFM serve as secretory organs[138] and release hundreds of peptides that communicate with the brain and other organs. Leptin specifically, is an adipokine released from adipocytes and is regarded as having a primary role in appetite regulation[139]. Moreover, investigations of leptin are responsible for revealing the relationship between FM and appetite[76]. Acutely, leptin level increases in response to meal consumption and this signals to the POMC neurons to release α-MSH and CART which bind to MC4R and subsequently suppresses food intake[140]. During fasting, leptin decreases which stimulates NPY and AgRP, suppresses α-MSH and CART, and increases food intake[140,141]. Leptin works in similar fashion upon detection of sufficient FM and suppresses food intake as a result. However, the energy balance principles have called into question the role of FM in appetite regulation given that individuals with considerable FM (i.e. individuals with overweight and obesity) continue eating despite sufficient body energy.

This may, in part, be due to leptin resistance. Under normal internal environments, increased leptin should result in meal termination due to its anorexigenic response to the

accumulation of FM[142]. During excessive weight gain, leptin resistance occurs, rendering it unable to cross the blood-brain barrier and suppress the actions of NPY and AgRP resulting in hyperphagia[143]. Insulin, a satiety hormone secreted from pancreatic beta cells, is similarly subjected to insulin resistance during substantial weight gain. Increased FM may also result in dysfunctional ghrelin and PYY responses[25,144,145]. Specifically, individuals with excessive FM may have an impaired ghrelin response, where postprandial ghrelin remains elevated despite meal consumption[23,24]. Fasting PYY levels are also lower in individuals with obesity compared to individuals of normal weight[25]. Similarly, obesity may result in deficient GLP-1[146].

Contrary to the skepticism regarding the role of FM in satiety, there is considerable optimism regarding the role of FFM and its relationship with appetite regulation; although, this relationship is not yet fully understood[147]. The interaction between FFM and appetite control was manifested from original reports that suggested the positive relationship between EE and EI[148,149]. The energy demands of FFM contribute to an individual's EE to a greater degree than FM, and therefore, may better explain EI. Support for this theory comes from the relationship between RMR and FFM, where FFM is shown to be a primary determinant of RMR[4]. Because FFM drives RMR and RMR makes up the largest proportion of EE[5], it is possible that the relationship between EE and EI is mediated by the amount of FFM an individual possesses.

While there is limited evidence to suggest that FFM is directly related to peripheral satiety hormone production, there is sufficient theoretical support to suggest that this may occur indirectly. Skeletal muscle mass (SMM), which shares considerable overlap with FFM, secretes peptides known as myokines that communicate with organs such as the liver, brain, and pancreas[150,151]. Research suggest specific myokines secreted from SMM triggers the release of satiety hormones from various organs such as the small intestines and pancreas[150,152] and that circulation of these myokines are associated with suppression of appetite[153,154]. Several reports also demonstrate the

occurrence of muscle-specific satiety hormone production[155] and that the production is greater in individuals with a greater predisposition for SMM gain[156]. However, the exact mechanisms responsible for this potential interaction remain unclear and there are limited studies that have investigated this in a cause effect manner. This dissertation will present an in-depth review discussing the role of FFM in hunger and satiety hormone production.

1.6 Summary of the Aims

The aim of this dissertation was to examine and understand the effect of diet composition and body composition on appetite. In this dissertation, we examined the effects of varying amounts of dietary carbohydrate and fat and the different types of fatty acids on appetite. We also examined the role of FFM in appetite regulation.

In our first study, we examined the effects of three high-fat meals rich in either monounsaturated, polyunsaturated, or saturated fatty acids on subjective ratings of appetite and subsequent *ad libitum* lunch consumption in healthy premenopausal normal-weight women in a randomized cross-over single blind study.

In our second study, we examined the effects of a KD on fasting measures of appetite in highly-trained cyclists and triathletes. To conduct this study, participants consumed both a KD and a high-carbohydrate diet (HCD), for two weeks each, in a random order, after their habitual diet (HD). We also assessed postprandial appetite measures in response to a ketogenic meal after the KD, a high-carbohydrate meal after the HCD, and a standard American/Western meal after the HD.

In our third manuscript, we discussed in detail the potential relationship between FFM and hunger and satiety hormone production. Moreover, we addressed gaps in the current knowledge base and made recommendations for future research and practice.

This dissertation will conclude with a discussion of the findings from all three sections. In the conclusion, we discuss the contribution of this dissertation to the current knowledge and the implications for future research. Furthermore, we discuss the implications for endurance athletes and individuals with obesity.

Manipulation of fatty acid composition in a high-fat meal does not result in differential alterations in appetite or food intake in normal weight females: a single-blind randomized crossover study

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Abstract

A behavioral concept that generates a path to obesity is eating in the absence of hunger (EAH). One strategy that may be effective in preventing EAH is the manipulation of dietary fatty acid (FA) composition. However, it remains unclear which FA has the greatest impact on both appetite and EAH. Therefore, the purpose of this study was to examine the effect of different dietary FA compositions (monounsaturated, MUFA; polyunsaturated, PUFA; saturated, SFA) on subjective ratings of appetite and subsequent ad libitum eating after a 3h postprandial period. 16 apparently healthy normal weight females between ages 18-40 completed this randomized, single-blind, crossover study. Participants consumed a HF meal (65% energy from fat) rich in SFA, MUFA, and PUFA with an energy content corresponding to 35% of their measured resting metabolic rate on three separate occasions. Visual analog scales were collected while fasted and every 30min for 3h during a postprandial period to measure feelings of hunger, fullness, and desire to eat (DTE). Participants were provided an *ad libitum* buffet meal 3h after the HF meal. There were no statistically significant differences for ratings of hunger, fullness, or DTE across conditions. Further, there was no significant difference in energy intake during the *ad libitum* lunch. We conclude that the manipulation of FA composition in a HF meal does not differentially affect appetite sensations or subsequent energy intake.

Keywords: dietary fatty acids, appetite, hunger, satiety, fatty acid composition, high-fat

Abbreviations: EAH= eating in the absence of hunger; FA= fatty acids; SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; PCF= prospective consumption of food; HF= high-fat; PYY= peptide-YY; GLP-1= glucagon-like peptide-1; VAS= visual analog scale; BG= blood glucose; BP= blood pressure; EI= energy intake; DTE= desire to eat

1. Introduction

Body weight gain occurs through multiple mechanisms; however, it is most notably manifested through prolonged sedentary lifestyle and excessive energy intake (EI) above daily allowance[157]. Previous evidence suggests that degree of body weight gain may be positively correlated with the amount of dietary fat consumed in an individual's diet, and some experts propose that of all potential factors influencing obesity, high fat (HF) diets may elicit the strongest effect[158]. HF diets are defined as consuming >30% of daily energy requirements from dietary fat and it is estimated that most US adults have adopted this practice, theoretically contributing to the global obesity crisis. However, other studies suggest that eating more calories than necessary is more detrimental to weight gain than the macronutrient composition of one's diet[159]. Given that HF diets and excessive energy consumption are commonplace, but both contribute to the onset of obesity, it is of interest to determine how manipulation of these concepts can combat the obesity epidemic; specifically, in regard to eating behaviors.

Eating is a complex notion that is dependent on a variety of constructs including social influence, emotional regulation, economic motives, nutritional literacy, and appetite[160,161]. These constructs, among others, collectively guide eating and may determine the composition of one's food choices. One behavioral concept that generates a path to obesity is eating in the absence of hunger (EAH) [162]. EAH is a paradigm defined by hedonically eating more than needed when there is no physiological hunger present[163,164]. The EAH paradigm, originally conceptualized by Fisher and Birch[165], also captures several behavioral responses including increased appetite perceptions and increased EI in absence of physiological hunger or following a meal[166]. Food cues, which are bodily reactions that occur in preparation for the consumption of food, influence each of these facets within the EAH paradigm. Food cues themselves are conditioned responses that include both physiological (i.e. increased saliva production, gastric emptying, etc.) and psychological (i.e.

increased desire to eat) reactions[167]. In human studies, it has been suggested that just the sight and smell of food is enough to elicit increased reactivity to food cues such as desire to eat (DTE)[168,169]. When perceptions of appetite, such as DTE, increase in response to potential feeding periods, there becomes a greater risk for EAH. This increased risk is due the inability to resist highly palatable foods, such as HF foods, leading to a state of overeating, which has been previously described as a significant contributor to the obesity crisis. Therefore, strategies that can reduce perceptions of appetite, such as hunger and DTE, may also reduce the risk of EAH through decreased reactivity to food cues.

Dietary manipulations aimed at reducing both the DTE and hunger, while increasing satiety, are commonly used to promote weight loss and reduce overeating[170]. However, because the traditional American diet is both fat-rich and highly palatable there are few nutritional strategies that are effective for decreasing food cue reactivity and subsequent overeating[171]. One promising strategy that may be effective in reducing EAH through reduction of appetite perceptions is the manipulation of dietary fat composition, which emphasizes particular *types* of fat to increase satiety and reduce hunger, rather than eliminating them outright. Different types of fatty acids (FA) include monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), saturated fatty acids (SFAs) and trans fats. Only a few studies have examined acute appetite responses to meals enriched in different types of FA[121,122,172–178]. However, the available evidence shows that individual FAs have differential effects on peripheral markers of hunger and satiety[119] leading to varying states of physiological satiety. These markers include ghrelin, a hunger stimulating hormone, and the hunger suppressing hormones peptide-YY (PYY) and glucagon-like peptide-1 (GLP-1)[98]. Previous studies have shown physiological increases in markers of satiety in men following acute SFA consumption while similar increases were seen in women, with the addition of an increase in markers of satiety following acute PUFA consumption[122,175,179].

While studies have reported promise to the utility of FA manipulation as a strategy to reduce physiological hunger, its utility in reducing both appetite perceptions and eating when physiological hunger is not present is unknown. Studies examining *ad libitum* eating following a meal have typically measured consumption after a 5 h period. The 5 h period in which participants abstain from food is much longer than most US adults abstain from eating either another meal or a snack and therefore, this timeframe may not be a valid method for evaluating EAH[180]. Further, the studies that observe *ad libitum* eating standardize the energy content of the test meals rather than individualizing them across each participant[122]. This approach may lead to meals that are too restricted in energy content, resulting in meals that do not meet either psychological or physiological thresholds for satiety. In order to appropriately investigate the effect of FA manipulation on EAH, the amount of time following test meal consumption needs to be reduced to ensure a physiological absence of hunger while also administering a test meal with enough energy content to induce both psychological and physiological satiety.

Therefore, the purpose of this study was to examine the effect of different dietary FA compositions (MUFA, PUFA, SFA) across three different HF meals on subjective ratings of hunger and satiety and subsequent *ad libitum* eating after a 180 min abstention from food to determine the effect of FA manipulation on perceptions of appetite and EI. So while we are not collecting EAH specifically, understanding how manipulation of FA composition in a HF meal affects constructs within the EAH paradigm may provide strategies that ultimately reduce the practice of eating despite physiological satiety. We hypothesize that PUFA will elicit the greatest decline in ratings of hunger and DTE and the greatest increase in perceived fullness when compared to SFA and MUFA.

2. Methods and Materials

2.1 Participants

Sixteen healthy women completed this randomized, single-blind crossover study. The present study is part of a larger line of research conducted by this lab. The methods of this study are described elsewhere[122]; however, these methods were adapted to answer our specific research question and are described below. The main dependent variables present in this study share no relationship with any other works that stem from the larger line of research. The Texas Christian University institutional review board approved the study, and procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. Written informed consent was obtained prior to participation in the study. Inclusion criteria consisted of those ages 18-40 yr with a body mass index (BMI) between 18.5-24.9 kg/m^2. Exclusion criteria included having lost or gained more than 5% of total body weight in the three months prior to screening, those currently on a weight loss diet or exercise program, having any chronic disease such as diabetes, hypothyroidism, hyperthyroidism, cancer, or any cardiovascular disease, having undergone any surgeries that would affect swallowing or digestion, on a medically prescribed diet, possessing any food allergies, or taking any medications that may interfere with the results of the study.

2.2 Screening and lead-in diet procedures

Participants reported to the Obesity Prevention Laboratory (OPL) on four occasions which included a screening visit and three separate testing visits with a minimum of four days between each testing visit. Before each visit, including the screening visit, participants were instructed to abstain from food, supplements, medication, and exercise for $\geq$ 12 h. For the screening visit, participants reported to the OPL after an overnight fast of $\geq$ 12 h. Prior to metabolic measurements, measurements of height were collected using a stadiometer, weight using a calibrated scale, and waist and hip circumferences using a tape measure. Following anthropometric measures, resting

metabolic rate (RMR) was obtained. RMR was measured for 30 min during the fasted state using indirect calorimetry (TrueOne 2400 Canopy System, ParvoMedics, Sandy, UT, USA) under the recommended guidelines[181]. Measured RMR was used to develop each participant's HF meal recipe. At the screening visit, participants were also asked to record their personal rating of spaghetti which was the meal served during the *ad libitum* buffet at the end of each testing visit.

On the day prior to testing visits, participants were instructed to consume a breakfast meal before 1200 h. Breakfast the day prior to testing was self-selected, but participants were instructed to consume a meal corresponding to their habitual diet and required to eat that same meal before each subsequent visit. The breakfast meal was self-selected to allow participants to practice more habitual eating patterns so that prolonged abstention from "normal" foods or foods that they enjoy did not significantly impact perceptions of appetite the following day. The day preceding each visit, participants were provided all foods for lunch, dinner, and snacks from a set menu where all items were 55% carbohydrate, 15% protein, and 30% fat. Participants were required to consume the same meals, snacks, and drinks before each subsequent visit. All visits took place during the participants' follicular phase of the menstrual cycle (days 3-9). Because of this, the duration of the study for each participant was a minimum of two-to-three months since the washout period (four days) allowed for only two testing visits within the same follicular phase of the same menstrual cycle[182]. Participants were allowed to be on oral contraceptives so long as they had regular monthly menstrual cycles. Participants were instructed to continue normal dietary and physical activity practices for the entirety of the study.

2.3 High-fat meals and measurement of appetite ratings

For each study visit (visits 1-3), participants reported to the OPL at ~0700 h after an overnight fast of ≥ 12 h. Height, weight, body fat percentage (BF%), waist/hip circumference, and

RMR were measured at baseline. BF% was measured using air displacement plethysmography (BodPod, Cosmed, Chicago, IL). Following anthropometric and baseline metabolic measurements, participants completed a validated 100-mm visual analog scale (VAS) to assess subjective ratings of hunger, fullness, and DTE (Kral, Roe, & Rolls, 2004). The scale used displays a 100-mm line below each question with anchor words at each end of the line. Questions with anchor words in parentheses included: "How hungry are you right now" (Not at all hungry-Extremely hungry); "How full are you right now" (Not at all full-Extremely full); and "How much do you think you could eat right now" (Nothing at all-A large amount). Participants were instructed to make a mark on the 100-mm line that corresponds with their answer.

Participants were then provided their randomly assigned liquid HF meal (enriched in either MUFA, PUFA or SFA) and instructed to consume it in its entirety within 5 min. Each participant was provided a different HF enriched with a different FA than previously consumed at each subsequent visit. Each HF meal recipe was developed using a nutrient analysis software program to calculate specific amounts of nutrients (The Food Processor, ESHA Research, Salem, OR) and designed to provide the participants with 35% of their total daily energy needs. The only difference between each HF meal was the FA composition used during each of the three visits (**Table 2.1**). Each HF meal consisted of the same "base" ingredients and otherwise differed only in FA source. The "base" ingredients included a premade nutritional shake (Ensure®, Abbott Laboratories, Chicago, IL), a chocolate flavoring powder (Nesquik®, Nestlé, Vevey, Switzerland) and soy lecithin granules. The main ingredients added to the "base" for each HF meal were butter and palm oil for SFA; extra-virgin olive oil for MUFA; and sunflower and flaxseed oil for PUFA. All meals contained the same fluid volume (347.9±3.9 mL). Following the baseline VAS (t = 0) and complete consumption of the randomized HF meal, the participants were instructed to lay supine, and ratings of hunger, fullness, and DTE were collected at minutes 30, 60, 90, 120, 150, and 180. Participants

consumed 120mL of water at each hour mark to prevent dehydration (t = 60, 120, 180 min). Participants were also allowed to watch television during the postprandial period but were required to remain motionless and awake throughout.

Table 2.1. Liquid Test Meal Composition

	SFA		MUFA		PUFA	
	Mean	SE	Mean	SE	Mean	SE
Kilocalories	805.2	31.6	808.1	30.7	808.1	30.7
Kilocalories from fat	534.7	21.0	528.8	20.1	525.6	20.0
Protein (%)	10%		10%		10%	
Carbohydrates (%)	23.6%		24.5%		25%	
Dietary Fat (%)						
SFA	45.0%		6.9%		6.8%	
MUFA	15.9%		42.4%		15.9%	
PUFA	5.5%		16.2%		42.3%	
% of energy from fat	66.4%		65.5%		65.0%	
% energy from fat of interest	45.0%		42.4%		42.3%	

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SE, standard error.

2.4 Ad libitum lunch

At 180min following the consumption of the HF meal, participants were escorted to a feeding room and served an *ad libitum* lunch that included an excess (723.7±6.0 g) of spaghetti (Marie Callendars, Conagra Brands, Chicago, IL), and participants were provided with 120mL of

water, in addition to the 120mL provided at 180 min. The same amount of food and water were provided at all three study visits. All food and water were weighed discretely on a gram scale, to the nearest gram before the participant was served. Participants were instructed to eat until comfortably full. The weight of the leftover food and water was subtracted from the initial weight to determine intake (in grams). Energy consumed (kcal) was calculated using the nutritional formation provided by the manufacturer (Marie Callendars, Conagra Brands, Chicago, IL).

2.5 Statistical Analyses

IBM SPSS Statistics version 25 was used, and all values are expressed as mean ± standard error of the mean. All hypotheses and analytic plans were specified prior to data collection. Postprandial responses for VAS scores were calculated as the average of each time point except baseline. Differences between conditions for VAS scores were assessed using an ANOVA with repeated measures. Differences in EI for the ad libitum lunch were assessed using repeated measures. Bonferroni correction methods were used during simple main effects analyses following any significant findings. Area under the curve (AUC) was used to calculate total change from baseline between conditions for VAS scores. Differences in AUC between each HF meal was determined using repeated measures analysis. Bonferroni correction methods were used during simple effects analysis upon significant findings. Missing data was handled using multiple imputation. Missing data occurred on two of the 48 total study visits for anthropometric, VAS, and buffet data. All data was missing at random.

3. Results

3.1 Participant characteristics

Sixteen women of normal weight (BMI < 24.9kg/m^2) between the ages of 18 and 40 completed all study visits. Participant characteristics at screening are shown in **Table 2.2**. There were no significant differences in participant characteristics across all three study visits.

Table 2.2 Participant Characteristics at Screening

n = 16	Mean ± SE
Age (yr)	23.1 ± 0.7
Height (cm)	165.8 ± 0.0
Weight (kg)	60.7 ± 1.9
BMI (kg/m^2)	22.0 ± 0.5
BF%†	22.3 ± 0.9
RMR (kcals/d)	1443.1 ± 54.8

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SE, standard error; yr, years; cm, centimeters; kg, kilograms; BMI, body mass index; kg/m^2, kilograms per meter squared; RMR, resting metabolic rate; kcals/d, kilocalories per day.

3.2 Hunger

Importantly, baseline ratings of hunger (SFA=50.9±5.7 mm; MUFA=51.2±6.6 mm; PUFA=45.5±5.9 mm) were not different between conditions (p=0.626). For postprandial hunger ratings, there were significant main effects of time (p<0.001; η^2=0.667) but no significant main effects of condition (p=0.873; η^2=0.009) or condition x time (p=.578; η^2=0.04). The time course for hunger responses are presented in **Figure 2.1a,** and average postprandial appetite ratings are reported in **Table 2.3**. Although lower, hunger was no longer significantly different from baseline at 150 min (SFA=32.9±5.1 mm; MUFA=32.9±5.2 mm; PUFA=32.8±4.2 mm; p=0.100) and 180

min (SFA=39.9±6.1 mm; MUFA=43.69±5.8 mm; PUFA=42.7±4.8 mm; p=1.000) following ingestion of the HF meal. Change in hunger ratings are presented in **Table 2.3**. AUC for the total hunger response was also not significantly different between conditions (p=0.458; η^2=0.50; **Table 2.3; Figure 2.2a**).

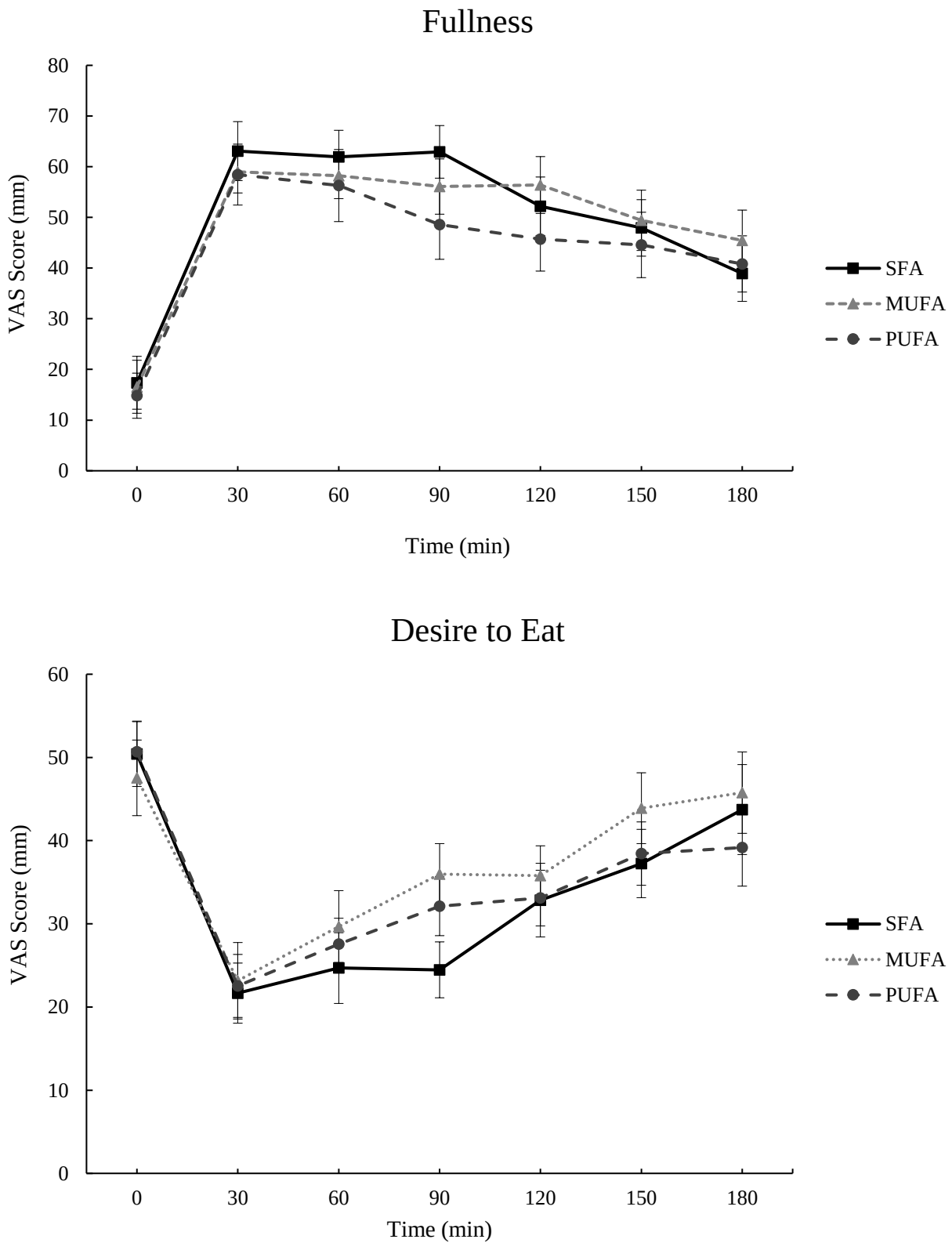


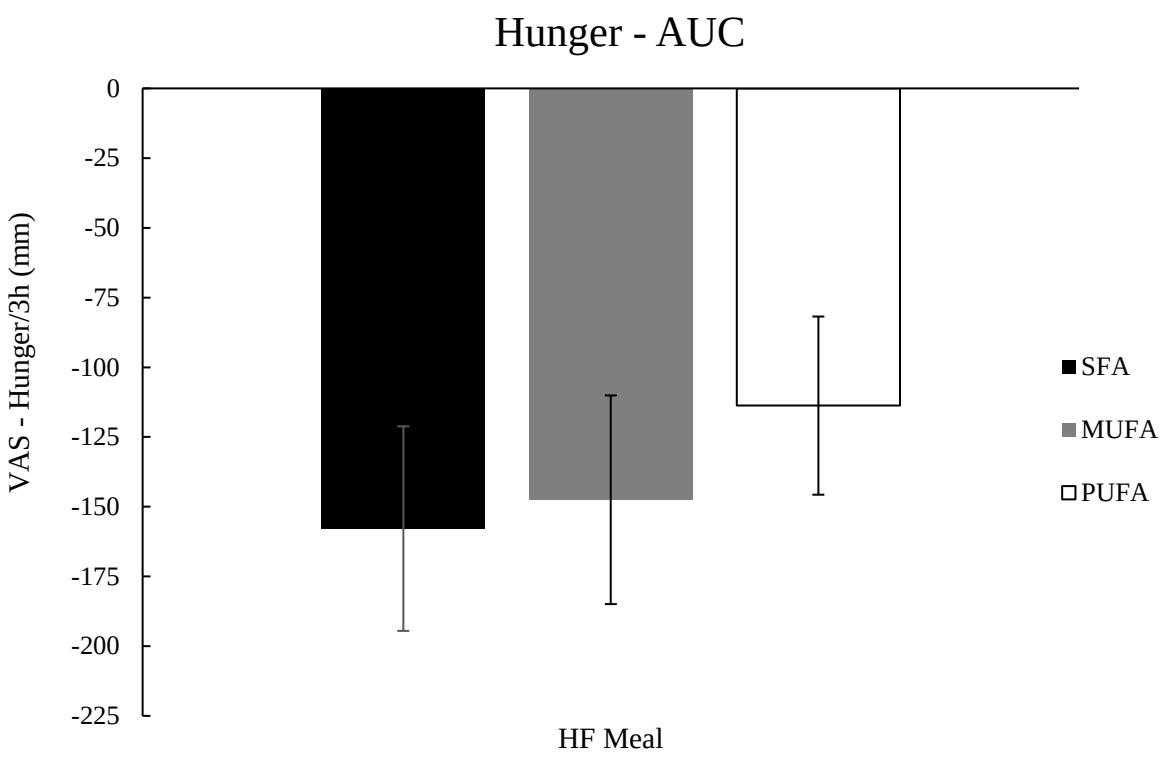
Figure 2.1a-c: Ratings of Hunger, Fullness, and Desire to Eat during a 3 h Postprandial Period

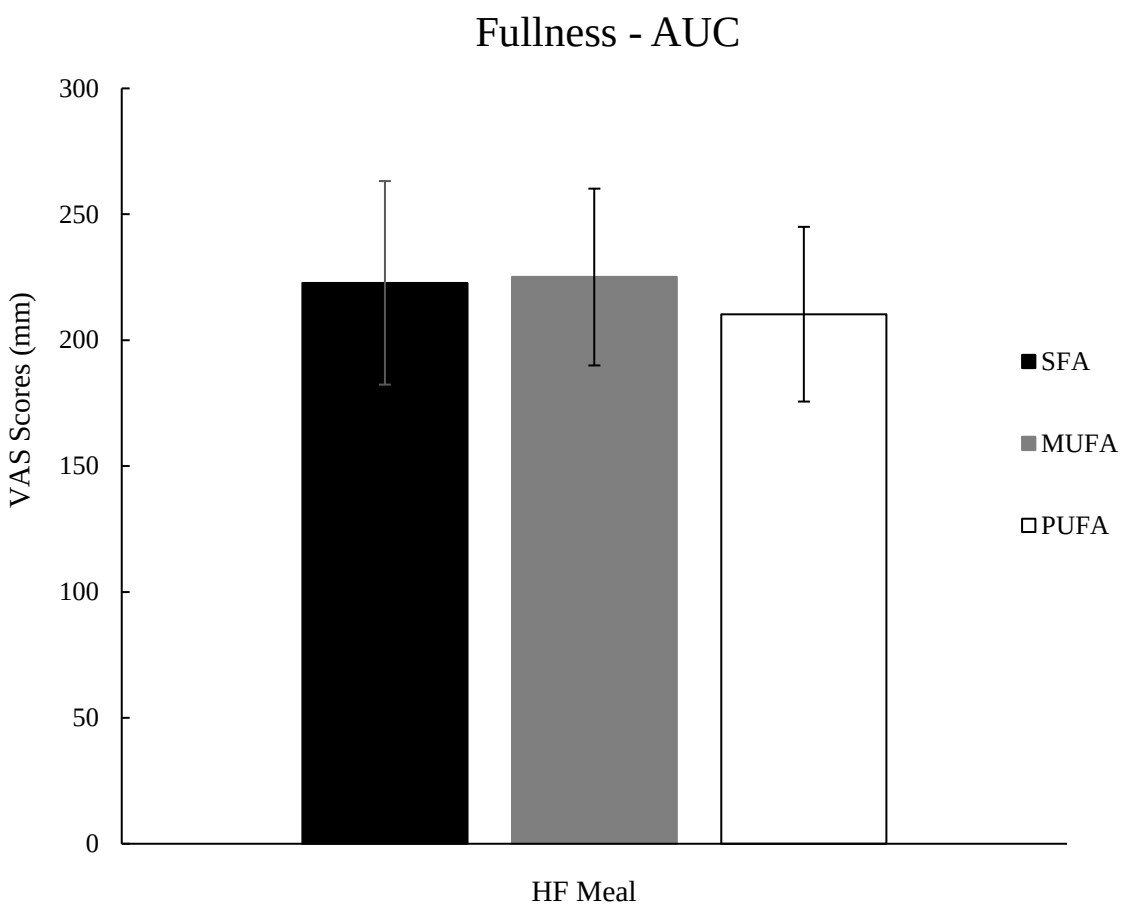
(a) Average hunger at baseline and average hunger of all time points from 30 to 180 min after test meal consumption. Results of a repeated measures ANOVA revealed significant effects of time (p=<0.001), but no significant differences were observed between FAs. **(b)** Average fullness at baseline and average fullness of all time points from 30 to 180 min after test meal consumption. Results of a repeated measures ANOVA revealed significant effects of time (p=<0.001), but no significant differences were observed between FAs. **(c)** Average DTE at baseline and average DTE of all time points from 30 to 180 min after test meal consumption. Results of a repeated measures ANOVA revealed significant effects of time (p=<0.001), but no significant differences were observed between FAs. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; VAS, visual analog scale; mm, millimeters; min, minutes; expenditure; AUC, area under the curve; h, hours; HF, high fat, DTE, desire to eat.

Table 2.3. VAS Postprandial Appetite Ratings (mm)

	SFA	MUFA	PUFA
n = 16	Mean ± SE	Mean ± SE	Mean ± SE
Hunger	24.59 ± 3.90	26.63 ± 4.47	26.41 ± 3.18
Hunger – Change	-26.31 ± 6.11	-25.51 ± 6.17	-18.87 ± 5.14
Fullness	54.50 ± 5.08	54.09 ± 4.69	48.64 ± 5.73
Fullness – Change	37.13 ± 6.74	39.55 ± 5.49	34.58 ± 5.63
DTE	30.78 ± 15.41	35.71 ± 3.59	32.15 ± 2.91
DTE – Change	-19.63 ± 4.51	-12.74 ± 4.86	-18.24 ± 3.25
Hunger AUC	-157.85 ± 36.68	-147.51 ± 37.41	-113.75 ± 31.94
Fullness AUC	222.78 ± 40.42	225.08 ± 35.11	210.32 ± 34.69
Desire AUC	-117.75 ± 27.07	-71.04 ± 29.65	-112.45 ± 19.80

VAS, visual analog scale; mm, millimeters; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SE, standard error; DTE, desire to eat; AUC, area under the curve.


Hunger - AUC
0
-25
-50
-75
-100
-125
-150
-175
-200
-225
VAS - Hunger/3h (mm)
HF Meal
SFA
MUFA
PUFA


Fullness - AUC
300
250
200
150
100
50
0
VAS Scores (mm)
SFA
MUFA
PUFA
HF Meal

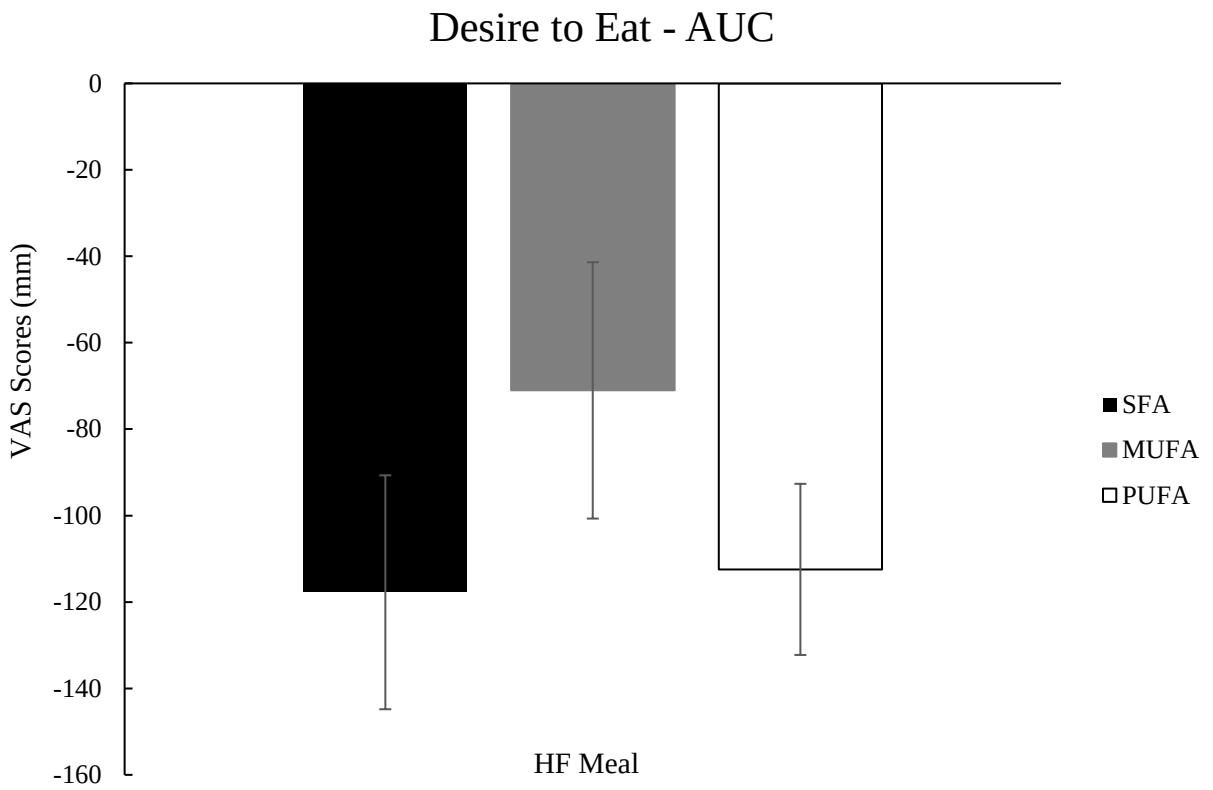


Figure 2.2a-c: Area Under the Curve for Ratings of Hunger, Fullness, and Desire to Eat

(a) Hunger AUC for each FA composition. Results of a repeated measures ANOVA revealed no significant differences between FA compositions (p=0.458). **(b)** Fullness AUC for each FA composition. Results of a repeated measures ANOVA revealed no significant differences between FA compositions (p=0.862). **(c)** DTE AUC for each FA composition. Results of a repeated measures ANOVA revealed no significant differences between FA compositions (p=0.293). DTE, desire to eat; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; VAS, visual analog scale; mm, millimeters; min, minutes; expenditure; AUC, area under the curve; h, hours; HF, high fat.

3.3 Fullness

Baseline ratings of fullness (SFA=17.4±5.2 mm; MUFA=16.6±5.2 mm; PUFA=14.8±4.5 mm) were not different between conditions (p=0.859). For postprandial fullness, there were significant main effects of time (p<.001; η^2=.730), but no significant effect of condition (p=0.270; η^2=0.084; **Table 2.3**) or condition x time (p=0.328; η^2=0.071). The time course for fullness responses are presented in **Figure 2.1b**. Fullness remained significantly higher from baseline up to and at 180 min (SFA=38.9±5.5 mm; MUFA=45.5±5.9 mm; PUFA=40.8±5.6 mm; p=0.007) following ingestion of the HF meal. Change in ratings of fullness are presented in **Table 2.3.** AUC for total fullness was also not significantly different between conditions (p=0.862; η^2=0.010; **Table 2.3; Figure 2.2b**).

3.4 Desire to eat

Baseline ratings for DTE (SFA=50.4±3.9 mm; MUFA=47.6±4.6 mm; PUFA=50.7±3.7 mm; p=0.007) were not different between conditions (p=.964). For postprandial DTE, there were significant main effects of time (p<.001; η^2=.632) but no significant main effects of condition (p=0.179; η^2=0.109; **Table 2.3**) or condition x time (p=.253; η^2=0.080). The time course for DTE is presented in **Figure 2.1c**. Although it remained lower, DTE was no longer significantly different from baseline at 150 min (SFA=37.3±4.1 mm; MUFA=43.9±4.3 mm; PUFA=38.4±3.8 mm; p=0.093) and 180 min (SFA=43.7±5.4 mm; MUFA=45.8±4.9 mm; PUFA=39.2±4.6 mm; p=1.000) following ingestion of the HF meal. Change in DTE is presented in **Table 2.3.** AUC for total postprandial DTE was also not significantly different between conditions (p=0.293; η^2=0.078; **Table 2.3; Figure 2.2c**).

3.5 Energy Intake

The macronutrient breakdown of the *ad libitum* lunch is presented in **Figure 2.3**. There was no difference in the composition of the meals across conditions. There were no significant differences in postprandial EI during the *ad libitum* lunch between conditions (SFA=291.4±43.1 g; MUFA=320.8±44.9 g; PUFA=317.3±49.8 g; p =0.531; η^2=0.034).

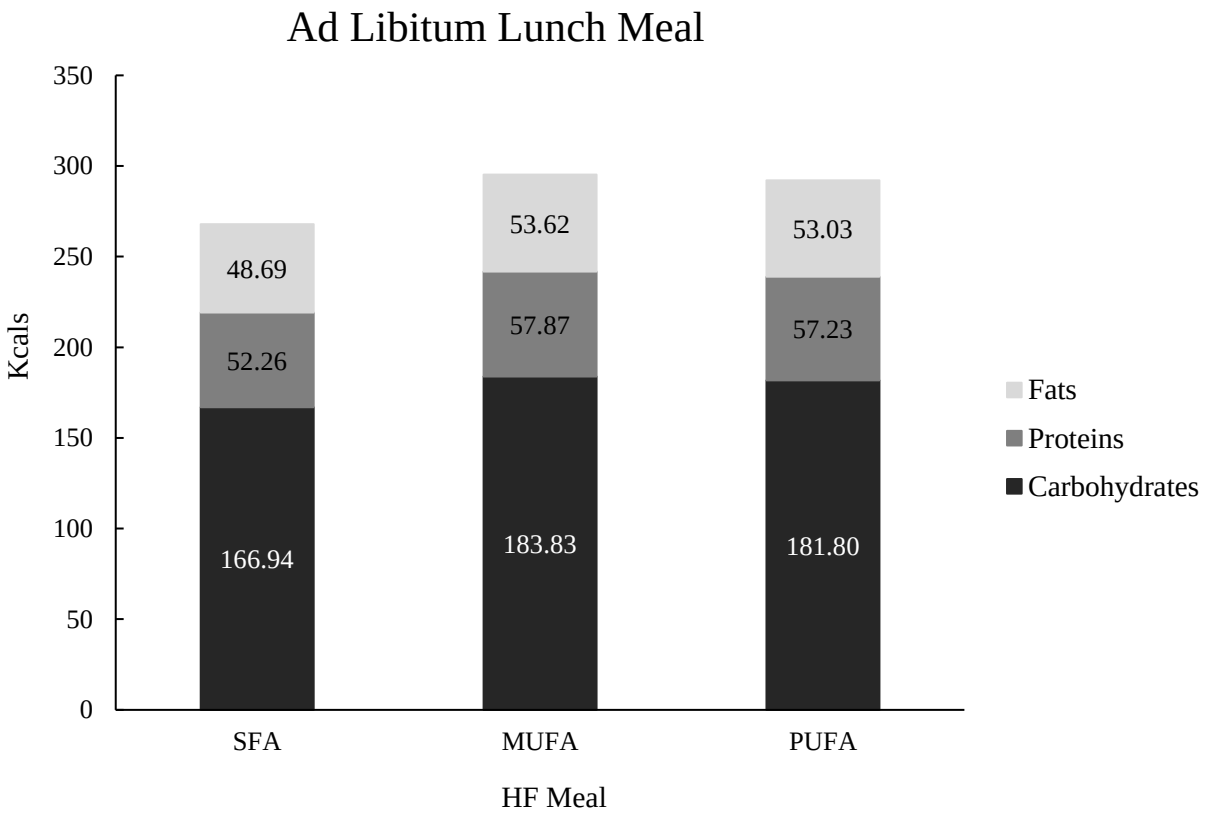


Figure 2.3: Consumption and Composition of an *Ad Libitum* Lunch

Average calories consumed at an *ad libitum* lunch 3 h after consuming a high fat test meal rich in different fatty acids. There were no differences in fat, carbohydrates, or protein for each *ad libitum* lunch meal. Results of a repeated measures ANOVA revealed no significant difference in amount consumed following consumption of a high fat test meal rich in varying FA compositions (p=0.531). Kcals, kilocalories; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HF, high fat.

4. Discussion

The objective of the present study was to investigate the effect of subjective appetite measures and postprandial EI following consumption of a HF meal rich in either SFAs, MUFAs, or PUFAs. To address methodological concerns from previous studies, we intentionally designed our test meals to be 35% of each participants total daily energy requirements[122]. The large energy content of each test meal allowed us to ensure a physiological satiety response and measure perceptions of appetite during a physiologically satiated state. It is also not uncommon to consume a third of total daily energy in a single meal (eating three meals a day). We also provided participants with higher amounts of both FA of interest and total energy from fat than prior investigations[122,172,173,179]. This allowed us to elicit a sufficient physiological response to each individual FA. Additionally, it is not uncommon for Americans to consume an acute HF meal with this proportion of total fat. Therefore, this allowed us to determine if manipulation of FA composition in HF meal had favorable effects on perceptions of appetite. Further, we deliberately made our postprandial period much shorter than previous studies to examine the effects of our HF meals rich in varying FAs during a more typical interprandial period[183–185]. Our study found that manipulation of FA composition in a HF meal had no differential effects on postprandial hunger, fullness, or DTE. Moreover, we found that ratings for hunger and DTE were no longer significantly different from baseline at 2.5 h following ingestion of the HF meal across conditions; although, fullness remained elevated above baseline for the entire postprandial period. Our study also showed that manipulation of FA composition in a HF meal did not differentially affect subsequent EI at the next meal. Together, our results show that manipulation of FA composition in a HF meal does not differentially affect appetite sensations or EI in premenopausal normal weight women.

Our study found no difference in ratings of hunger, fullness, DTE, or EI across conditions. However, several acute studies have reported physiological differences in hunger and satiety hormones between HF meals of varying FA compositions[119,122,175,179,186–189]. Despite these reports, however, results remain inconclusive. Nevertheless, it appears that consumption of a HF meal rich in either MUFAs or PUFAs results in a favorable, suppressive effect on postprandial ghrelin compared to a HF meal rich in SFAs[122]. However, Cooper et al.,[179] reported in opposition. Similar to ghrelin, some studies report that the appetite suppressive hormone GLP-1 is stimulated to a greater degree by HF meals rich in MUFAs and PUFAs compared to SFAs[186,189], while others report no differences[122,188]. For the appetite suppressive hormone PYY, the effect of each individual FA remains unclear[119,122,175,179,187].

Although hunger and satiety hormones were not presented in the current study, we assume that our test meals enacted sufficient physiological hunger and satiety hormone responses relative to the FA of interest, given the elevated FA content of our test meals. Additionally, our study had both higher energy content in our test meals and a shorter postprandial period than prior studies, which theoretically, should induce suppressed hunger and increased satiety hormone concentration for the duration of our postprandial period. However, despite the effect that each individual FA composition may have had on our participants' markers of hunger and satiety, this did not result in different appetite ratings or different EI at the next meal. These findings are in agreement with numerous reports which also found no difference in EI or VAS scores for hunger, fullness, or DTE between HF meals rich in MUFAs, PUFAs, or SFAs[121,122,172–178,187,190]. The eating behaviors in aforementioned studies are indicative of EAH, where despite clear differences in hunger and satiety hormone concentrations between HF meals of differing FA, differences were absent in regard to perception of appetite or subsequent EI. EAH was apparent in our study, where even after consuming a HF meal containing 35% of our participants' total daily energy requirements,

VAS ratings for both hunger and DTE neared baseline at 2.5 h of the postprandial period across FA compositions. Regardless of the FA composition of the meal, however, physiological hunger should remain suppressed and satiety elevated relative to baseline for longer than 2.5 hours following the ingestion of a meal amounting to 35% of one's daily energy needs. Therefore, the question remains, what is the discord between physiological markers of hunger and satiety and actual perceptions of hunger?

There are several theories that may explain the apparent discordance between hunger and satiety hormones and both perception of appetite and food intake. Food intake may be the most susceptible to this disconnect, where the modern food environment provides incredible access to highly palatable foods, and food cues are presented in endless continuity[191]. In our study specifically, all of our participants rated their liking for our *ad libitum* lunch (spaghetti) as either agree or strongly agree prior to inclusion. In essence, one could assume that our participants had previously generated a strong association between our lunch meal and consumption of food, which would ultimately initiate cognitive reward[192], and cephalic phase responses[49,193] when presented with the meal. It is possible that the association with the food cue presented in our study resulted in interrupted homeostatic circuitry which increased the amount our participants felt they could eat, despite each individual FAs effect on physiological satiety and their nutrient state[49,193,194]. However, since ratings of hunger and DTE approached baseline well before the lunch meal was presented across all HF meals, the lack of difference in EI for our study is most likely due to the fact that our participants felt as hungry as they did upon arrival (after a minimum 12 h fast) 30 min before the cessation of the postprandial period across conditions.

The increased ratings of hunger well before cessation of the postprandial period is particularly of interest and speaks to the power of executive function in appetite. Although our test meals were high in energy content and most likely resulted in different physiological hunger and

satiety responses between FA compositions, it is possible that the liquid composition of our test meals were both untraditional and unsatisfying relative to a solid meal. Studies report an inverse relationship between meal viscosity and hunger[195,196], which may be why hunger and DTE approached baseline before the end of our postprandial period across FA compositions. The taste of our test meals may have also had an effect on our participants' hunger ratings. It is possible that our test meals were not palatable enough to meet our participants "reward threshold"[197,198], which caused our participants to maintain a sense of hunger, despite the potential variation in physiological satiation induced by our HF meals. Further, the biological sex of our participants may have mediated their reward mechanism. Studies show that estrogen plays a role in eating behaviors and altered reward during feeding[199–201]. It is also possible that appetite regulation is not unidirectional, and that hunger and satiety hormones are unable to overcome the power of executive function in eating behaviors. In a review by Kaviani and Cooper[123], nine of the 13 qualifying studies found no difference in VAS ratings across FA compositions. Further, of the three acute studies that found significantly different hunger and satiety hormone concentrations between either MUFAs, PUFAs, or SFAs[122,123,175,187], all three found no differences in ratings of hunger or prospective consumption of food. A fourth study that measured both appetite ratings and hunger and satiety hormones found that hunger was lower for MUFAs and PUFAs than SFAs but did not find any difference in hormonal concentrations[119]. Based on our findings and the findings of previous studies, it appears that the postprandial hunger and satiety hormone fluctuations that occur, relative to the FA composition of a HF meal, may have a smaller role in the regulation of appetite than previously thought. Finally, it could be suggested that appetite ratings are more effective in the prediction of EI than hunger and satiety hormones when examining the FA composition of a HF meal. Measurements of hunger and satiety hormones come at an expensive cost to the researcher but has yet to provide sufficient evidence in regard to subsequent eating

behaviors following consumption of a HF meal rich in varying FAs. While the effect of hormonal concentrations on EI remains unclear, several studies that report no differences in VAS appetite ratings also found no differences in EI[122,172,176,178,190].

The current study does possess some limitations. First, our study did not report hunger and satiety hormone concentrations, which would have provided more evidence to the disconnect between hunger and satiety hormones and appetite sensations. However, appetite ratings may be more intuitive for eating behaviors than physiological markers based on previous studies. As previously stated, the liquid form of our test meals may have impacted our results and led to increased hunger during the postprandial period. However, we employed a liquid meal to standardize time to complete ingestion of the meal and to limit the variation in time between each participants first and last 'bite'. Our *ad libitum* lunch was also not a typical eating environment, which may have impacted our results for EI. Further, several study visits occurred on weekend days. It is well known that eating patterns differ on weekend days compared to weekdays, and it is possible that participants ate less during the lunch meal after a weekend visit if they had plans to eat more palatable foods afterwards.

Conclusion

In conclusion, manipulation of FA composition in an acute HF meal does not differentially alter postprandial appetite perceptions or EI at the following meal in premenopausal normal weight females. Increased physiological satiation that occurs from differing FA compositions may not have the power to overcome executive function or EAH. Although the reasons for the discord between perception and reality regarding appetite is unclear, the disconnect between both avenues of appetite regulation is consistent. Therefore, we urge researchers investigate the disconnect between physiological satiety and eating behaviors and evaluate this in a cause-effect manner.

Further, we suggest that future studies place a greater emphasis on external factors and prior associations with food. The results of the current study should urge clinicians and health professionals to consider the power of EAH and employ appetite reducing strategies that rely less on physiological markers of hunger and satiety.

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The effects of a ketogenic diet compared to high-carbohydrate and habitual diets on fasting and postprandial measures of hunger and satiety in highly-trained endurance athletes: a pilot study

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Abstract

Endurance athletes may implement rigid dietary strategies, such as the ketogenic diet (KD), to improve performance. The effect of the KD on appetite remains unclear in endurance athletes. This study set out to analyze the effects of a KD, a high-carbohydrate diet (HCD), and habitual diet (HD) on objective and subjective measures of appetite in trained cyclists and triathletes.

Six participants consumed the KD and HCD for two weeks each, in a random order, following their HD. Fasting appetite measures were collected after two weeks on each diet. Postprandial appetite measures were collected following consumption of a ketogenic meal after the KD, high-carbohydrate meal after the HCD, and standard American/Western meal after the HD. Fasting total ghrelin (GHR) concentration was lower and glucagon-like peptide-1 (GLP-1) was higher following the KD versus HD and HCD ($p<0.05$). Postprandial GHR was lower and GLP-1 was higher following the ketogenic versus the standard and high-carbohydrate meals ($p<0.05$). Fasting hunger perception was higher with the KD versus HD and HCD ($p<0.05$). Postprandial perceptions of appetite were not different across test meals.

Fasting and postprandial GHR were lower and GLP-1 higher following the KD versus HCD and HD in cyclists and triathletes. Subjective measures of appetite did not correspond with objective measures of appetite and require further study.

Keywords: ketogenic diet; high-carbohydrate diet; appetite; ghrelin; glucagon-like peptide 1; endurance athletes

INTRODUCTION

Recently, the ketogenic diet (KD) has surged in popularity, becoming the most searched diet on Google in 2018[202]. The KD involves restricting carbohydrate (CHO) intake to 20-50 g/d or to 5-10% of total daily energy, respectively[129]. Historically, the KD has been used to lose weight and improve co-morbidities associated with obesity[203]. However, endurance athletes are currently employing the KD to promote "fat adaptation", which may allow for greater utilization of fat during exercise intensities that would typically require glycogen as the primary energy source[204]. This strategy, however, contradicts the guidelines set forth by several professional organizations[205] which state that CHO availability, via consuming a high-carbohydrate diet (HCD), improves exercise performance in endurance-based events. Nevertheless, endurance athletes continue to utilize the KD in an attempt to gain a competitive advantage despite studies showing inconsistent results on performance[206–209].

In addition to the ambiguity for performance outcomes, employing the KD may have a negative effect on appetite. Sufficient energy intake (EI) is necessary for athlete health and performance. Intensive and progressive training requires close dietary monitoring to avoid physiological distress from insufficient EI, also known as relative energy deficiency in sport (RED-s)[210,211]. However, most non-elite endurance athletes do not track their EI or energy expenditure[212,213]. Athletes typically rely on their perceptions of appetite such as hunger, fullness, and desire to eat (DTE) for regulation of daily EI. This may be a cause for concern, as recent evidence suggests that acute endurance exercise results in diminished appetite[214]. Over time, low EI may lead to impaired bone health, immunity, and reproductive function associated with RED-s[10]. Moreover, diet-induced ketosis may also cause a significant reduction in appetite relative to other restrictive diets[131]. However, there are no studies that have investigated the effects of a KD

on objective and subjective measures of appetite in highly-trained endurance athletes, specifically when compared to a habitual diet (HD) or the more traditionally recommended HCD.

The purpose of this study was to evaluate the fasting and postprandial effects of a KD compared to a HCD and HD on objective and subjective measures of appetite in highly trained cyclists and triathletes. We hypothesized that the KD versus the HCD and HD will result in lower fasting levels of hunger, DTE, prospective consumption of food (PCF), and total ghrelin (GHR), and higher fasting levels of fullness and glucagon-like peptide-1 (GLP-1). Moreover, we hypothesized that a ketogenic test meal compared to a high-carbohydrate or habitual test meal will result in lower postprandial hunger, DTE, PCF, and GHR and higher fullness and GLP-1 levels.

MATERIALS AND METHODS

Participants

Six highly-trained cyclists and triathletes completed the study. To be eligible for the study, participants had to be highly-trained cyclists or triathletes (cycling ≥100 km/wk for the past year and maximal oxygen consumption ($\dot{V}O_{2max}$) ≥80th percentile for their age and sex) and between the ages 18-75 yr. Exclusion criteria included dieting or using medications or supplements to lose weight, following a ketogenic (<10% total EI from carbohydrate) or high-carbohydrate (>65% of total EI from carbohydrate) diet, using tobacco, drinking heavily (>14 drinks/wk for males; >7 drinks/wk for females), having food allergies, diabetes, heart disease, stroke, liver disease, kidney disease, untreated thyroid disease, anemia, eating disorders, or uncontrolled hypertension, or undergone surgeries that affect swallowing or digestion.

The university Institutional Review Board approved the study. Written informed consent was obtained from participants before entering the study. The study was conducted in accordance with the Helsinki principles for research with human subjects.

Experimental Design

Participants began the study maintaining their HD and were then randomly assigned to either a 14-day KD or HCD, after which they crossed over to the other diet. Randomization was counterbalanced using block randomization. Fasting and postprandial measures of appetite (objective and subjective) were taken immediately following the HD, KD, and HCD. The meal administered for postprandial measurements was ketogenic after the KD, high-carbohydrate after the HCD, and a standard meal (similar in composition to the typical American intake[215]) after the HD. The fasting measures (time point = 0 mins) were taken after a 12-hour overnight fast, and the postprandial measures were taken at 30, 60, 120, and 180 mins after meal consumption.

Diet Intervention and Compliance

For the KD, the participants were instructed to consume <10% of total daily energy from CHO, 75-85% from fat, and 15% from protein. For the HCD, participants were instructed to consume >65% from CHO, <20% from fat, and 15% from protein. Sample menus and recipes (7-days each) for both the KD and HCD were provided to the participants.

Compliance on the KD and HCD was monitored by instructing the participants to send daily food logs using an online app (NutritIO, Bucharest, Romania) to the investigators. A registered dietitian (RD; PR) reviewed the logs daily and provided daily feedback to the participants based on the data received. Participants were also instructed to complete a 3-day diet

record (two weekdays and one weekend day) during the second week of the KD and HCD for assessing compliance to these diets. Three-day diet records were also collected on the HD.

In addition to the dietary records, compliance on the KD was assessed by instructing the participants to check their daily urinary ketone levels using urinary ketone strips (VALI, CA), and send a photo of the test strip to the investigators each day. Ketosis was indicated when the color on the urinary strip corresponded with a β-hydroxybutyrate (BHB) concentration ≥0.5 mmols/L. Capillary blood was also collected to assess BHB (KETO-MOJO, Auburn, CA), in duplicate, at day 7 and at the end of each diet. Compliance to the KD was interpreted as having an average capillary BHB blood concentration of ≥0.5 mmols/L.

Participants were asked to keep their physical activity level (PAL) consistent for the duration of the study. PAL was self-recorded in daily exercise logs and contained information on type and duration of exercise, distance covered, and rating of perceived exertion (RPE) for each exercise session (sRPE).

Body weight was monitored daily and participants were given instructions to modify their EI to address deviations in body weight. A weight change of ≥5% from their initial weight was considered a basis of exclusion from the study to limit the effect of weight change on appetite responses.

Test Meals

The test meals were liquid and contained 60% of measured resting metabolic rate (RMR) for each participant. The ketogenic meal contained 9.5% energy from CHO, 75.1% fat, and 15.4% protein. The corresponding percentages were 69.1%, 15.7%, and 15.2%, respectively for the high-carbohydrate meal and 53.4%, 31.4%, and 15.2%, respectively for the standard meal. The ketogenic meal was made using pecan butter, heavy whipping cream, unsweetened cashew milk,

whole milk Greek yogurt, avocado, medium-chain triglyceride (MCT) oil powder, whey protein powder, and water. The high-carbohydrate meal contained honey, dates, whole milk, plain whole-milk yogurt, frozen bananas, whey protein, Nesquik® strawberry powder, and water. The standard meal contained honey, honey-flavored yogurt, whole milk, frozen strawberries, frozen bananas, and MCT oil powder. The meal volume, fiber content, and energy content were kept constant across the three test meals within each participants.

Measures

Anthropometry

Height was measured, without shoes, using a stadiometer (SECA®, Hamburg, Germany). Weight was measured, in light clothing and without shoes, using a calibrated scale (SECA®, Hamburg, Germany).

VO_{2max} Testing

At the first screening visit, participants underwent a $\dot{V}O_{2max}$ test using their personal bike which was mounted to a cycle ergometer (CompuTrainer, RacerMate, Seattle, WA). Participants warmed up at a self-selected pace for 10 min and then began the $\dot{V}O_{2max}$ test at a load of 50-100 watts (W). Intensity was increased by 25 W/min until volitional failure. Oxygen uptake was monitored using a metabolic cart (TrueOne 2400, Parvo Medics, Sandy, UT, USA). $\dot{V}O_{2max}$ was defined as the highest 30-second $\dot{V}O_2$ value achieved. To validate the observed $\dot{V}O_{2max}$ participants then performed a validation test as described by Poole & Jones[216]. A 5% adjustment for cycle ergometry values was used to compare to ACSM treadmill norms[217].

Air Displacement Plethysmography

Participants were asked to undergo a ≥12-hr overnight fast from food, medications, and supplements and a ≥12-hr abstention from exercise prior to this test. Body composition was assessed by air displacement plethysmography (ADP) with measured thoracic lung volume (BodPod™, Life Measurement Inc., Concord, CA)[218].

Resting Metabolic Rate (RMR)

RMR was measured, after ≥12-hr overnight fast, using the TrueOne® 2400 (ParvoMedics, Sandy, UT, USA) indirect calorimetry system with a ventilated hood following the recommended guidelines[181]. Respiratory gases were collected for 20 min in a climate-controlled room, but only the final 15 min were used for calculation of RMR.

Visual Analog Scales

Participants were asked to provide subjective ratings of appetite in the fasting and postprandial states using a validated iPad-based visual analog scale (VAS)[219]. The scale displayed a 100-mm line below each question with anchor words at each end of the line. An example question with anchor words in parentheses included: "*How hungry are you right now*" (Not at all hungry; Hungrier than I've ever been)[220]. Participants were instructed to slide the marker to a point on the digital 100-mm line that corresponded with their answer.

Dietary Intake

Participants were instructed to complete a 3-day diet record, a validated measure[221] for assessing dietary intake. This involved recording and photographing all meals on three days (two weekdays and one weekend day) during each diet. Total daily EI and macronutrient composition

were analyzed from the dietary records using a nutrition analysis software (Food Processor, ESHA, Salem, OR).

Bioassays

Serum GHR and GLP-1 were measured, in duplicate, using ELISA (abcam, Cambridge, UK). The intra- and inter-assay coefficients of variations for GHR were 6.4% and 9.5%, respectively. The corresponding values for GLP-1 were 7.9% and 9.6%, respectively.

Procedure

Each participant reported to the lab for two screening visits, three data collection visits, and two compliance visits. At the first screening visit, participants completed demographic, behavioral, and health questionnaires, and underwent the $\dot{V}O_{2max}$ testing. Anthropometric, RMR, and body composition data were collected at the second screening visit.

The three data collection visits occurred after screening while the participants were on their HD and immediately following the completion of the KD and HCD. Participants were instructed to fast for ≥12-hr from food, medication, and supplements prior to each data collection visit. They were also instructed to abstain from any exercise for 24 hr and engage in only light to moderate intensity exercise from 24-48 hr before each trial. Upon arrival to the lab, height, weight and capillary blood for BHB concentrations were collected. Following this, an IV catheter was placed in the participant's antecubital vein for collection of blood draws to assess circulating GHR and GLP-1. Following this, a liquid test meal corresponding with each diet was provided. Participants were given 10 min to consume the meal. Time to consume the test meal was kept the same for all three meals and was based on the time taken to consume the first test meal. Blood samples were collected during fasting (time point 0 = mins) and in the postprandial state at 30, 60, 120, and 180

min after meal consumption. Subjective measures of appetite were assessed using VAS at the same time points. To maintain adequate hydration, four ounces of water was provided every hour. Participants were allowed to watch movies during the postprandial period from a pre-determined list.

Statistical Analysis

All outcome variables were normally distributed as assessed by Shapiro-Wilk and visual inspection of Q-Q plots. The effects of diet condition on fasting hunger, DTE, PCF, fullness, GHR, and GLP-1 were assessed by repeated measures analysis. Meal condition, time, and the interaction between condition and time effects on postprandial hunger, DTE, PCF, fullness, GHR, and GLP-1 levels were assessed using mixed-effects model repeated measures analysis. Area under the curve (AUC) was calculated for GHR, GLP-1, and all VAS ratings using the trapezoidal rule. The effect of meal condition on AUC was assessed using repeated measures analysis. Post hoc analyses with Tukey LSD were conducted only following any significant findings. Missing data occurred for a single VAS time point for one participant and was handled using mean imputation. There was no influence of order on any diet condition effects in the present study ($p>0.05$).

Body weight, EI, and percent energy from macronutrient intake were compared by diet condition using repeated measures analysis. Post hoc analyses with Tukey LSD were conducted only following any significant findings. PAL and sRPE were compared between the KD and HCD using a paired t-test.

IBM SPSS Statistics version 26 (Armonk, New York) was used for statistical analysis. Statistical significance was set at $p<0.05$.

RESULTS

Participants

Six highly-trained endurance athletes (male=2, female=4) between the ages of 18-56 y completed the study in its entirety. Participant characteristics at screening are shown in **Table 3.1**.

Table 3.1. Participants Characteristics at Screening

	Total (n=6)	Males (n=2)	Females (n=4)
	Mean ± SD	Mean ± SD	Mean ± SD
Age (y)	37.2 ± 12.2	41.5 ± 20.5	35.0 ± 9.5
Height (cm)	172.3 ± 10.0	183.5 ± 1.0	166.8 ± 5.0
Weight (kg)	68.5 ± 17.5	89.1 ± 7.1	58.2 ± 8.3
BMI (kg/m^2)	22.7 ± 3.4	26.5 ± 2.3	20.9 ± 2.0
Body Fat (%)	21.3 ± 4.6	21.1 ± 7.2	21.4 ± 4.2
Fat-free mass (kg)	53.8 ± 13.2	70.1 ± 0.8	45.6 ± 5.0
Fat mass (kg)	14.7 ± 5.9	19.07 ± 7.9	12.6 ± 4.2
$\dot{V}O_{2max}$ (ml/kg/min)	46.6 ± 6.7	47.3 ± 6.7	46.3 ± 7.8
RMR (kcals/d)	1617.3 ± 314.7	1999.5 ± 68.6	1426.3 ± 132.0

SD, standard deviation; BMI, body mass index; $\dot{V}O_{2max}$, maximal volume of oxygen; RMR, resting metabolic rate

Compliance

Total daily kilocalories and percent total energy consumed from protein, CHO, and fat by diet condition are presented in **Table 3.2**. There was no difference in EI between diet conditions ($p=0.34$). There was a significant diet effect for percent energy from CHO ($p<0.001$), fat ($p<0.001$), and protein ($p<0.001$). Percent energy intake from CHO was significantly lower on the KD versus the HD ($p<0.001$) and HCD ($p<0.001$) and higher on the HCD versus the HD ($p=0.006$). Percent energy from fat was higher on the KD versus HD ($p<0.001$) and HCD ($p<0.001$) and on the HD versus HCD ($p=0.011$). Percent energy from protein was significantly higher on the KD versus HD ($p<0.001$) and HCD ($p<0.001$).

BHB was ≥0.5 mmols/L at day 7 (0.95±0.30 mmols/L) and the end (0.99±0.25 mmols/L) of the KD. BHB was <0.5 mmols/L on day 7 (0.08±0.05 mmols/L) and end (0.10±0.07 mmols/L) of the HCD. The concentration was <0.5mmols/L for the HD (0.28±0.06 mmols/L). BHB concentration was ≥0.5 mmols/L for all but one participant during the KD; however, the participant met the requirements of the diet and was therefore included in the final analysis. Additionally, urinary ketone measures indicated compliance to the KD for all participants (1.82±0.52 mmols).

Total distance of exercise recorded on the participants exercise logs was not different between the KD or HCD (KD: 211±42 miles/2wk; HCD: 227±48 miles/2wk; $p=0.591$). Additionally, sRPE during that time period was not different between the KD or HCD (KD: 482±107; HCD: 579±92; $p=0.347$).

Weight was significantly different by diet condition (HD: 68.7±7.1 kg; KD: 66.5±6.9 kg; HCD: 68.6±7.1 kg; $p<0.001$). It was lower during the KD versus HD ($p<0.001$) and HCD ($p<0.001$). However, none of the participants lost more than our previously described threshold of 5% initial weight; all six participants were therefore included in the analysis.

Table 3.2. Energy Intake and Diet Composition during the HD, KD, and HCD

	HD	KD	HCD	
	Mean ± SE	Mean ± SE	Mean ± SE	P Value[a]
Kilocalories (kcals/d)	2140.0 ± 226.7	2447.2 ± 195.8	2418.4 ± 266.3	0.340
Protein (% energy)	16.5 ± 1.7[d]	26.0 ± 1.2	14.4 ± 1.3[d]	<0.001
Carbohydrate (% energy)	45.8 ± 2.8[d]	8.7 ± 1.2	63.3 ± 3.6[c,d]	<0.001
Fat (% energy)	38.2 ± 3.2[d]	64.1 ± 2.2	20.8 ± 3.1[b,d]	<0.001

HD, habitual diet; KD, ketogenic diet; HCD, high-carbohydrate diet; SE, standard error

[a] Differences by diet condition were determined by repeated measures analysis. [b] $p<0.05$ compared with HD, [c] $p<0.01$ compared with HD, [d] $p<0.001$ compared with the KD

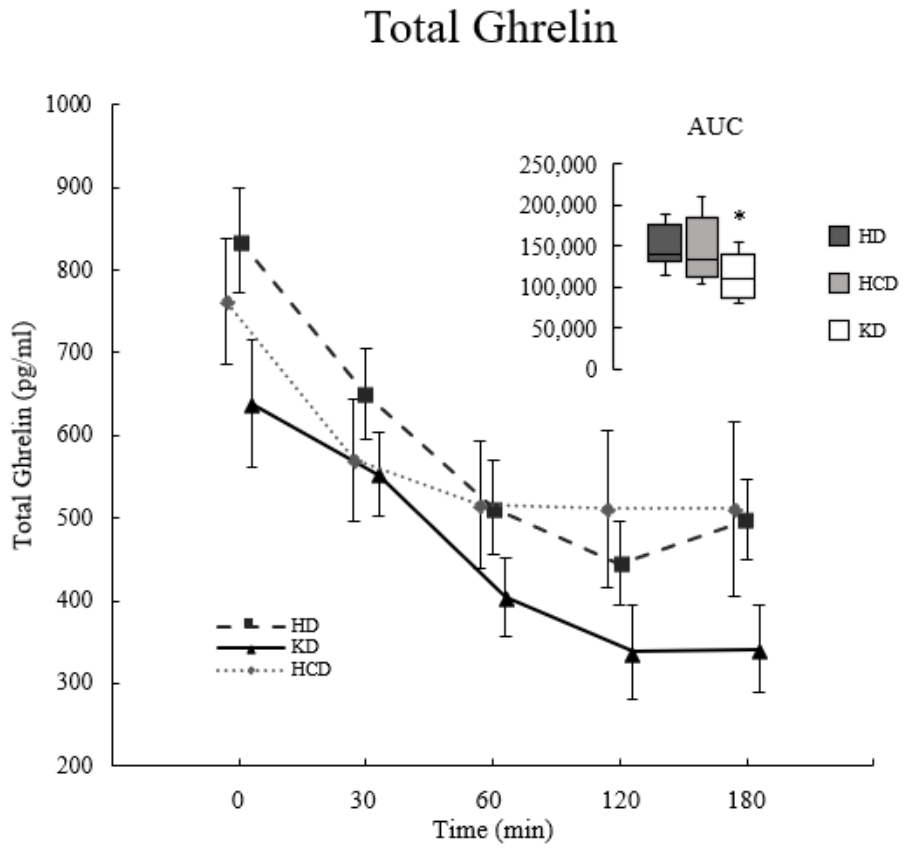
Objective Appetite Responses

Fasting GHR and GLP-1 concentrations are presented in **Table 3.3**. Postprandial and AUC concentrations for GHR and GLP-1 are presented in **Figures 3.1a-b**.

Total Ghrelin

Fasting GHR was significantly different by diet condition (p=0.001). It was significantly lower for the KD versus HD (p=0.001) and HCD (p=0.031). For postprandial GHR, there were significant effects of meal condition (p=0.010) and time (p<0.001), but not for meal condition x time (p=0.23). Postprandial GHR was significantly lower following the ketogenic versus standard (p=0.007) and high-carbohydrate (p=0.031) meals. Peak postprandial GHR was also significantly different by meal condition (p=0.016), and it was significantly lower on the ketogenic versus standard (p=0.025) and high-carbohydrate (p=0.044) meals. Postprandial AUC for GHR was significantly different by meal condition (p=0.025). AUC was significantly lower for the ketogenic versus high-carbohydrate (p=0.045) and standard (p=0.016) meals.

A


Total Ghrelin
Total Ghrelin (pg/ml)
1000
900
800
700
600
500
400
300
200
0
30
60
120
180
Time (min)
HD
KD
HCD
AUC
250,000
200,000
150,000
100,000
50,000
0
*
HD
HCD
KD

B

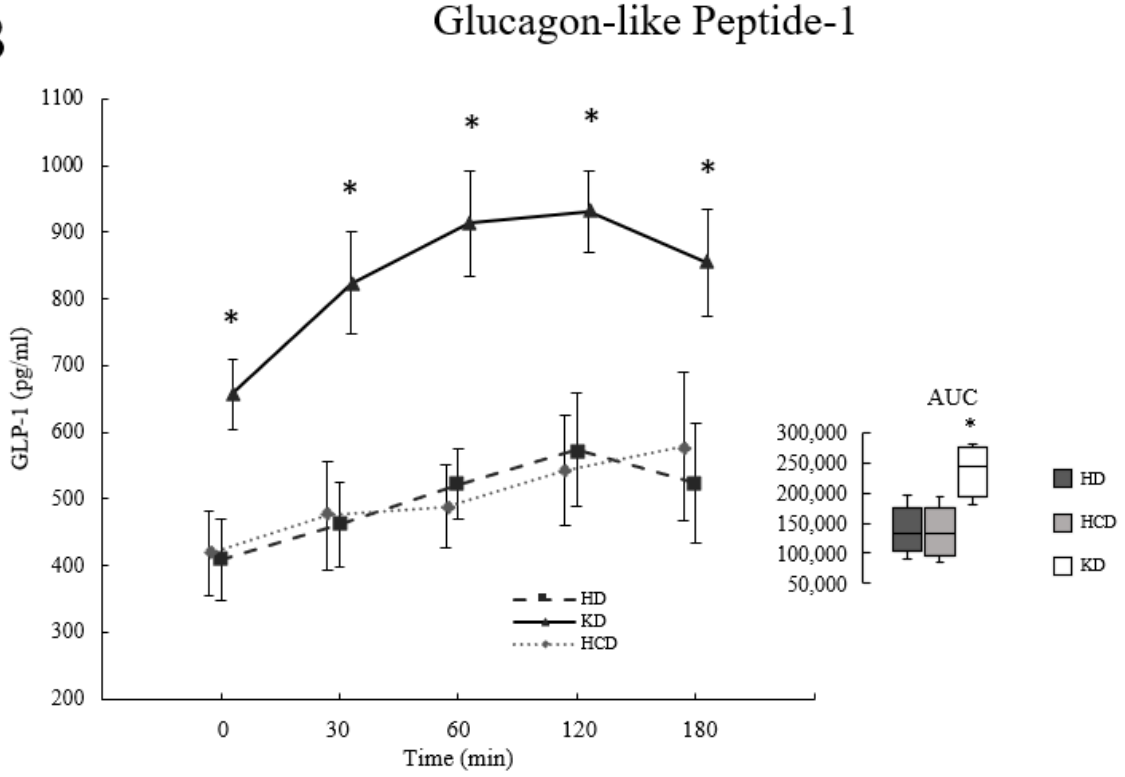

Glucagon-like Peptide-1
GLP-1 (pg/ml)
1100
1000
900
800
700
600
500
400
300
200
0
30
60
120
180
Time (min)
*
*
*
*
*
HD
KD
HCD
AUC
300,000
250,000
200,000
150,000
100,000
50,000
*
HD
HCD
KD

Figure 1: Postprandial total ghrelin (GHR) (**A**) and glucagon-like peptide-1 (GLP-1) (**B**) responses to the ketogenic meal (KM), high-carbohydrate meal (HCM), and standard meal (SM). The line graphs show means and standard errors. The box plots represent area under the curve (AUC) and show the median (line within the box), 25th and 75th percentiles (lower and upper limits of the box), and 10th and 90th percentiles (error bars). Mixed-effects model repeated measures analysis showed significant time and condition effects for GHR ($p<0.001$ and $p<0.05$, respectively) and condition, time, and interaction effects for GLP-1 ($p<0.05$, respectively). Both GHR and GLP-1 were significantly lower ($p<0.05$) on the KM compared to the HCM and SM. GLP-1 was significantly higher at all timepoint on the KM compared to the HCM and SM. AUC for GHR was significantly lower ($p<0.05$) and significantly higher for GLP-1 ($p<0.05$) on the KM compared to the HCM and SM.

Table 3.3. Fasting Appetite Measures

	HD	KD	HCD	
	Mean (95%CI)	Mean (95%CI)	Mean (95%CI)	P Value[a]
GHR (pg/ml)	836[c] (659, 1014)	638 (420, 857)	761[b] (547, 975)	0.001
GLP-1 (pg/ml)	408.7[b] (234, 583)	657 (510, 804)	419[b] (240, 598)	0.027
Hunger (mm)	57.3[b] (39.5, 75.2)	76.3 (62.9, 90.0)	60.2[c] (46.1, 74.2)	0.013

Fullness (mm)	10.7	3.5	11.3	0.202
	(-1.1, -22.5)	(-2.9, 9.9)	(0.02, 22.7)	
DTE (mm)	55.2	88.3	74.2	0.127
	(17.1, 93.3)	(75.6, 101.0)	(45.0, 103.4)	
PCF (mm)	41.8[b]	66.5	63.2	0.021
	(23.6, 60.0)	(48.6, 84.4)	(42.7, 83.6)	

HD, habitual diet; KD, ketogenic diet; HCD, high-carbohydrate diet; CI, confidence intervals; GHR, total ghrelin; GLP-1, glucagon-like peptide-1; DTE, desire to eat; PCF, prospective consumption of food. [a] Differences by diet condition were determined by repeated measures analysis. [b] $p<0.05$ compared with KD. [c] $p<0.01$ compared with the KD.

Glucagon-like peptide-1

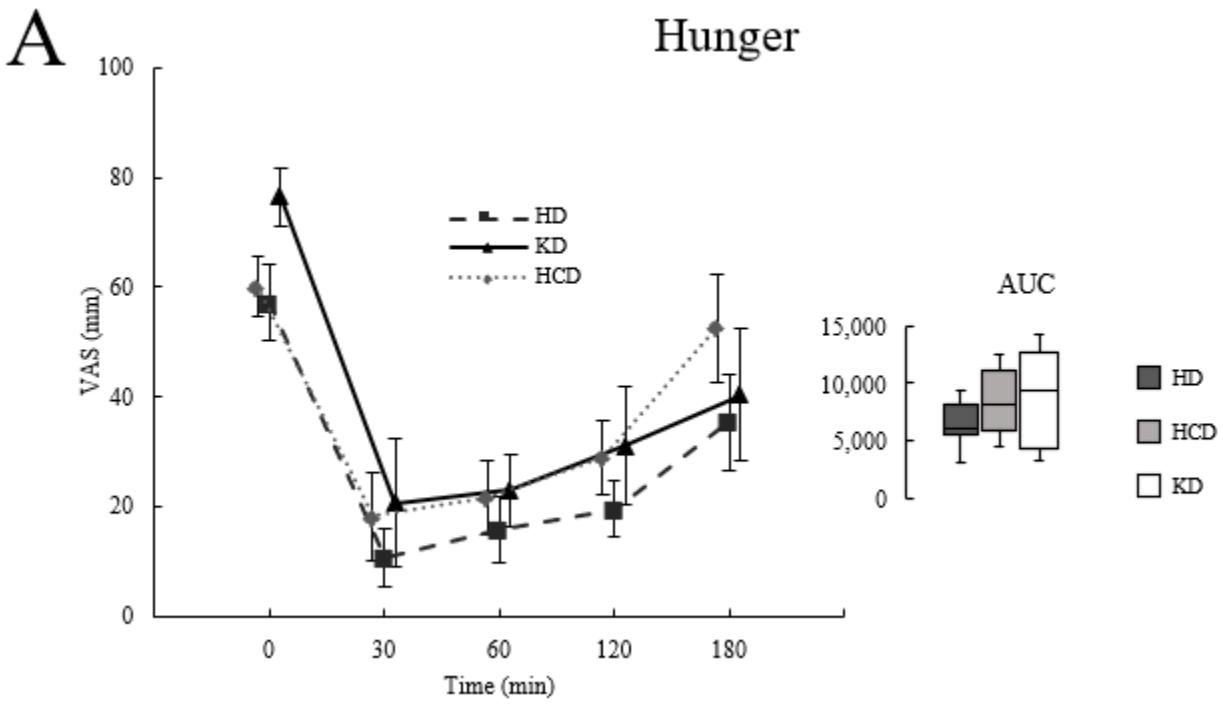
Fasting GLP-1 was significantly different by diet condition (p=0.027). Fasting GLP-1 was significantly higher for the KD versus HD (p=0.041) and HCD (p=0.033). For postprandial GLP-1, there were significant effects for meal condition (p=0.001), time (p<0.001), and condition x time (p=0.047). Postprandial GLP-1 was significantly higher for the ketogenic versus standard (p=0.006) and high-carbohydrate (p=0.003) meals at all timepoints (p<0.05). Postprandial AUC for GLP-1 was statistically significant by meal condition (p=0.001). GLP-1 AUC was higher for the ketogenic versus standard (p=0.004) and high-carbohydrate (p=0.002) meals.

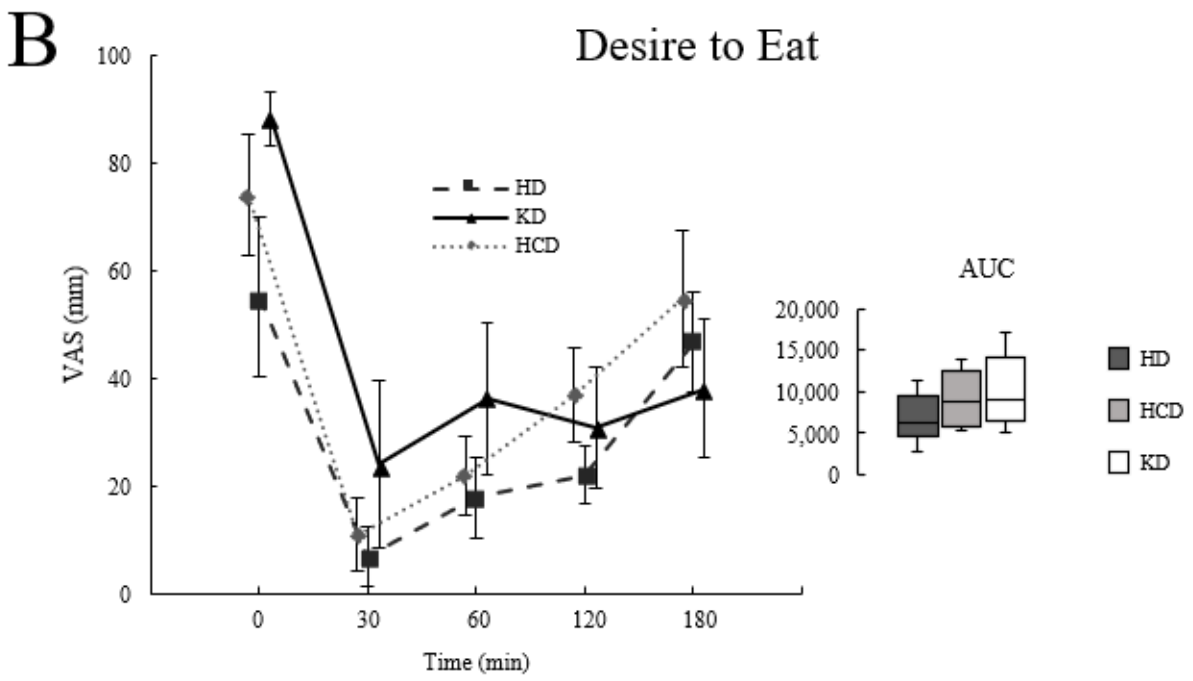
Subjective Appetite Responses

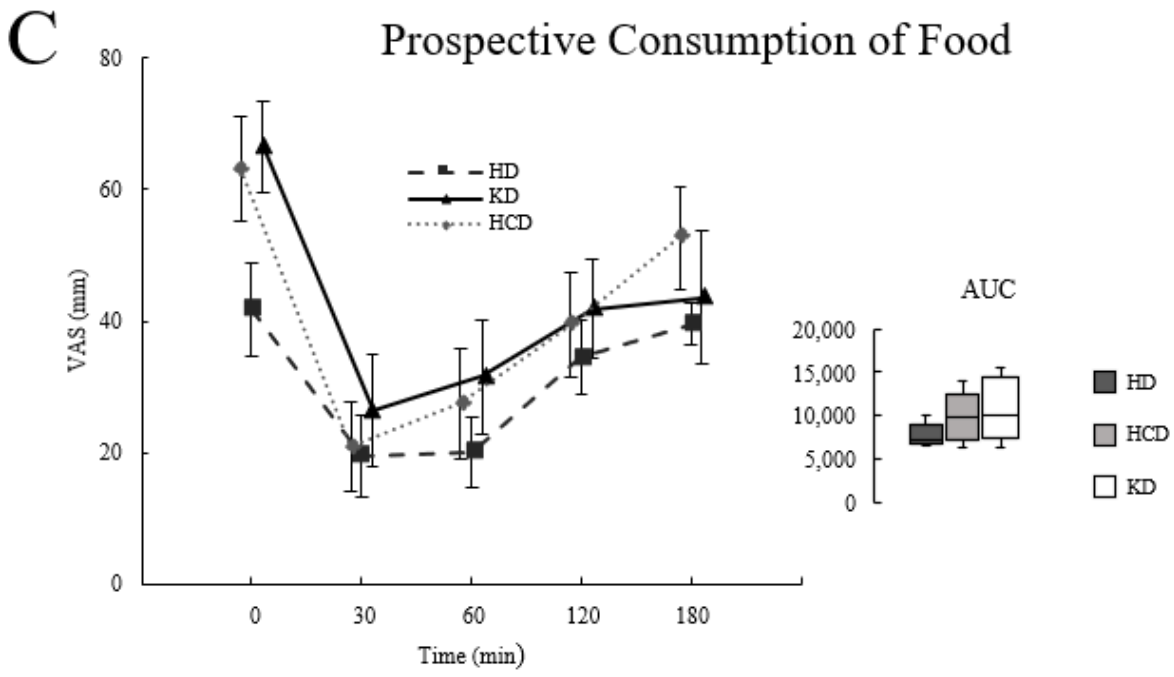
Fasting hunger, fullness, DTE, and PCF are presented in **Table 3.3**. Postprandial and AUC values for these measures are presented in **Figures 3.2a-d**.

Hunger

A significant diet condition effect was observed for fasting hunger (p=0.013). Fasting hunger was significantly higher for the KD than the HD (p=0.042) and HCD (p=0.004). For postprandial hunger ratings, there was a significant effect of time (p<0.001) but not meal condition (p=0.17) or meal condition x time (p=0.22). Postprandial AUC for hunger response was also not significantly different by meal condition (p=0.22).




B
Desire to Eat
VAS (mm)
100
80
60
40
20
0
HD
KD
HCD
0
30
60
120
180
Time (min)
AUC
20,000
15,000
10,000
5,000
0
HD
HCD
KD


C
Prospective Consumption of Food
VAS (mm)
80
60
40
20
0
HD
KD
HCD
0
30
60
120
180
Time (min)
AUC
20,000
15,000
10,000
5,000
0
HD
HCD
KD

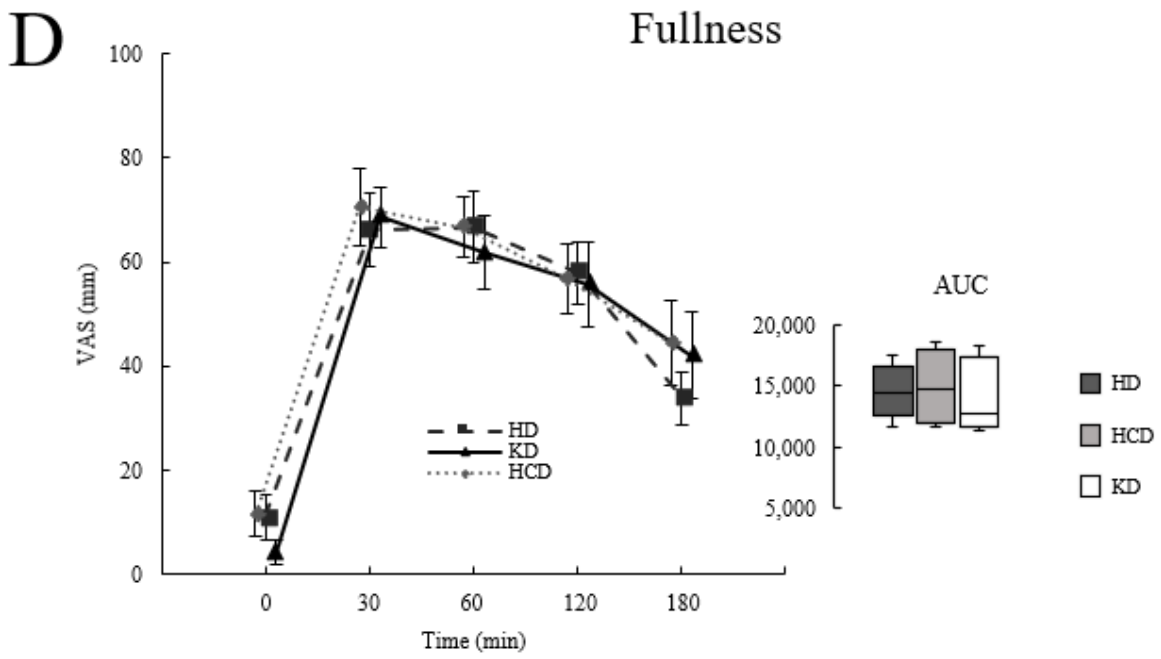


Figure 3.2: Postprandial hunger (**A**), desire to eat (DTE) (**B**), prospective consumption of food (PCF) (**C**), and fullness (**D**) responses to the ketogenic meal (KM), high-carbohydrate meal (HCM), and standard meal (SM). The line graphs show means and standard errors. The box plots represent incremental area under the curve (AUC) and show the median (line within the box), 25th and 75th percentiles (lower and upper limits of the box), and 10th and 90th percentiles (error bars). Mixed-effects model repeated measures analysis showed significant ($p<0.05$) postprandial time but not meal condition or meal condition by time effects for hunger, DTE, PCF, and fullness. AUC for hunger, DTE, PCF, and fullness were not significant by meal condition ($p>0.05$).

Desire to Eat

Fasting DTE was not different by diet ($p=0.13$). For postprandial DTE, there was a significant main effect of time ($p<0.001$) but not meal condition ($p=0.38$) or meal condition x time ($p=0.14$). Postprandial AUC for DTE was not significantly different by meal condition ($p=0.40$).

Prospective Consumption of Food

Fasting PCF were significantly different by diet (p=0.021). Fasting PCF was higher for the KD versus HD (p=0.020) but not HCD (p=0.69). Fasting PCF was higher for the HCD versus HD (p=0.050). For postprandial PCF, there was a significant main effect of time (p<0.001) but not meal condition (p=0.18) or meal condition x time (p=0.38). Postprandial AUC for PCF was also not different by meal condition (p=0.33).

Fullness

Fasting fullness was not different by diet (p=0.202). For postprandial fullness, there was a significant main effect of time (p<0.001), but not meal condition (p=0.71) or meal condition x time (p=0.89). Postprandial AUC for fullness was not significantly different by meal condition (p=0.80).

DISCUSSION

The present study was the first to examine the effects of a KD compared to a HCD and HD on fasting and postprandial objective and subjective measures of hunger and satiety in highly-trained endurance athletes. Concentration of fasting GHR was lower and GLP-1 was higher on the KD versus the HCD and HD. Postprandial concentration of GHR was lower and GLP was higher after the ketogenic versus the high-carbohydrate and standard meals. These objective differences in fasting and postprandial hormone levels were not matched by the subjective measures of appetite.

Several studies have shown that nutritional ketosis reduces GHR and increases GLP-1 concentrations[132,133,222]. Moreover, acute hyperketonemia from exogenous ketone supplementation

has also led to similar changes in GHR and GLP-1[134,223]. The above studies corroborate the findings from the present study on lower GHR and higher GLP-1 with a KD. Nutritional ketosis may contribute to lower GHR by increasing BHB[224]. Because the brain cannot utilize FFAs for energy, it relies on BHB[225]. BHB, as well as GHR, signal to the hypothalamus via vagal afferent pathways[225,226]. While the mechanism in which BHB inhibits GHR is unidentified, rodent studies show that GHR deficient mice have higher BHB concentrations[227] while other studies suggest that BHB binds to enteroendocrine cells to alter gut hormone secretion[134,228]. The potential relationship between BHB and GHR secretion is substantiated by studies showing increased GHR after returning to a non-ketogenic state[132,133,222]. In addition, increased BHB results in FFA mobilization, and increased FFAs have been reported to reduce GHR[229,230]. The increase in GLP-1 with the KD may also be due to increased BHB. Short-chain fatty acid butyrate has the ability to affect levels of GLP-1[231]. Butyrate promotes the release of GLP-1 from intestinal L cells in cell culture models[232], and rodent models show that this occurs through the use of butyrate as a primary energy source for colonocytes[233]. Another explanation for our findings may be the relationship between insulin and GLP-1 and GHR. In the pancreas, GLP-1 enhances insulin secretion[234]. During fasting conditions (similar to the fasting measures in our study), insulin and GHR have been shown to be inversely related[235]. Therefore, GHR may have been lower following the KD due to the insulinotropic effect of increased GLP-1. The heightened consumption of dietary fat during the KD may also have caused the increases in GLP-1[113], where increased dietary fat consumption has shown to increase GLP-1 in lean individuals.

Despite decreases in GHR and increases in GLP-1 during the KD, we found that fasting hunger and PCF were significantly higher during the KD which is in contrast to that observed by other studies[131–135,222,236]. The hypothalamus is well known as the “feeding center” of the brain and is primarily responsible for the regulation of food intake via orexigenic neuropeptide

stimulation[237]. During instances of both caloric restriction and increased ketone circulation, hypothalamic adenosine monophosphate-activated protein kinase (AMPK) is increased in the arcuate nucleus[15], stimulating hunger and food intake[238]. However, when BHB levels meet the threshold for utilization in the brain (i.e., 4 mmol/L)[239], BHB inhibits the phosphorylation of hypothalamic AMPK[225,240,241] resulting in appetite suppression. Thus, it is possible that although our participants experienced ketosis during the KD, they did not reach the 4 mmol/L threshold necessary for utilization in the brain leading to increased hunger and PCF. Multiple studies corroborate these findings[133,242,243]. Additionally, acute hyperketonemia from the ingestion of ketone esters has shown to reduce hunger and DTE[134] independent of CHO intake. Thus, it is possible that the concentration threshold for BHB utilization in the brain must be met in order to experience suppression of appetite during the KD.

Neuropsychological factors may also be responsible for the increased fasting hunger on the KD. It is possible that the restrictive nature of the KD made participants feel more hungry since they were unable to consume foods and beverages that they enjoy[62] or use to fuel themselves for training[244]. The removal of foods that an individual repeatedly eats when hungry (in this case carbohydrates) results in increased cravings for the removed food[62]. Therefore, the KD may result in dysfunctional appetite responses due to the power of executive function that can override physiological signals of hunger and satiety.

A limitation of the study is that the COVID-19 pandemic required us to terminate our study resulting in a smaller sample size than originally anticipated. Nonetheless, our study was able to detect significant differences for several primary outcomes. However, the results should be interpreted with caution and future studies should include a larger sample size. Another limitation of the study is that the KD and HCD were consumed for only two weeks. However, this period was within the 3-5 days it takes to reach ketosis with a KD[245]. Moreover, our participants met the

required BHB threshold for nutritional ketosis[127]. Participants were unable to meet the protein requirements during the KD despite daily feedback from an RD. Results of a sensitivity analysis showed that GHR, but not GLP-1 was robust to the differences in percent protein intake. However, the results should be interpreted from a real-world perspective given that endurance athletes employing a KD likely do not control protein intake to such a degree. Providing all the food during a KD may help to better manage the protein intake in future studies. Weight loss occurred during the KD despite similar EI between diets and regular monitoring by the RD to maintain body weight. Hall et al.,[246] has shown that initial weight loss on a low-fat diet is mostly likely due to changes in total body water and glycogen stores rather than deceased fat mass. Moreover, sensitivity analysis showed that both GHR and GLP-1 were robust to changes in body weight. Our study had several strengths including daily dietary feedback from an RD. The test meals provided in our study were individualized to each participants RMR. Our study also collected capillary and daily urinary BHB to verify ketosis. The training status of our participants was also confirmed by measured $\dot{V}O_{2max,}$ and exercise distance and perceived exertion were similar across diets.

In conclusion, the KD resulted in lower fasting GHR and higher GLP-1 concentrations compared to the HCD and HD. Postprandial concentrations of GHR were lower and GLP-1 were higher on the ketogenic versus high-carbohydrate and standard meals. Subjective ratings of appetite did not correspond with the hormone measures, however. These results need to be confirmed by a larger study with longer diet duration.

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Declaration of interest: none

Body composition and appetite regulation: emerging evidence of the relationship between fat-free mass and physiological signals of appetite

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Abstract

Historically, fat mass has been considered to have significant influence on human body energy homeostasis through its indirect relationship with appetite control. However, over the last decade there has been a surge of evidence supporting the potential role of fat-free mass (FFM) in appetite control, and thus the regulatory involvement of body composition in appetite-related measures. Yet the underlying physiological mechanisms that explain the role of FFM in appetite regulation remain unclear. Available evidence points towards a potential relationship between FFM and appetite-related hormones; however, these relationships may be both appetite hormone specific and population dependent. Therefore, the purpose of this narrative review is to make aware to other researchers and clinicians that there may be a relationship between FFM and appetite hormones, and that further research is needed to elucidate potential cause-effect. Overall, available evidence suggests that the appetite stimulating hormone ghrelin has an inverse relationship with FFM, but the relationship between FFM and the appetite suppression hormones peptide tyrosine-tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) has been relatively unexplored. The information presented in this review should encourage researchers, clinicians, and health professionals to consider FFM preservation as a suitable strategy during weight loss for successful long-term weight management through potentially decreased appetite.

Keywords: appetite; body composition; fat-free mass; FFM; ghrelin; GLP-1; PYY; satiety

Abbreviations: FM= fat mass; EE= energy expenditure; FFM= fat-free mass; POMC= pro-opiomelanocortin; NPY= neuropeptide Y; AGRP= agouti-related protein; CCK= cholecystokinin; α-MSH= α-melanocyte-stimulating hormone; CART= cocaine-amphetamine-related transcript; MC4R= melanocortin 4 receptor; EI= energy intake; GLP-1= glucagon-like peptide-1; PYY= peptide-YY or peptide tyrosine-tyrosine; SMM= skeletal muscle mass; RMR= resting metabolic rate; IL-6= interleukin-6; WAT= white adipose tissue; AMPK= AMP-activated protein kinase;

BDNF= brain-derived neurotrophic factor; UAG= unacylated ghrelin; AG= acylated ghrelin; TG= total ghrelin; RTT= Rett's Syndrome; GH= growth hormone; GHRH= growth hormone releasing hormone; BMI= body mass index; VAT= visceral adipose tissue; T2D= type II diabetes; GLP-1RA= glucagon-like peptide-1 receptor agonist

Introduction

The World Health Organization defines obesity as the excess accumulation of fat mass (FM) which may lead to significant health risks[247]. There are still few or no effective non-surgical long-term treatments for obesity despite the fact that since 1980, obesity rates have doubled in more than 70 countries[248] with an anticipated global prevalence of overweight and obesity of 57.8% by 2030[249]. Caloric restriction (CR), commonly used for weight loss, is often not effective in the long-term due to the increased hunger and decreased energy expenditure (EE) that promotes weight regain[141,250,251]. Therefore, treatments for obesity require more sophisticated approaches to combat the high failure rates of traditional lifestyle approaches[252,253], and strategies that emphasize appetite regulation may provide long-term utility.

The mechanisms of appetite control have been studied for almost 70 years and much has changed since the initial 'dual center' hypothesis proposed by Anand and Brobeck[254] due in part to the significant technological advances made over that same time span[76,255]. The original hypotheses proposed by Kennedy and Parks[256], Miller et al.[257], and Anand and Brobeck[254] 70 years ago were the first to establish the hypothalamic action of appetite control and until very recently, much of the research has focused on hypothalamic activity and its relation to food intake[32,255]; although, other brain regions such as the prefrontal cortex and ventral tegmental area have also been studied given their activation in the presence of palatable foods[258,259]. More modern appetite regulation models include actions from both the central nervous system and gastrointestinal tract due to the well demonstrated interplay between the two systems[260]. Several peripheral signals in the form of orexigenic and anorexigenic gut peptides have proven to be critical drivers of hunger and satiety through their ability to inform the brain of the body's short-term nutritional status[108,261–263]. Through the 'gut-brain axis', the brain processes the encoded nutritional information sent from various endocrine organs and together, both the neuroendocrine and the enteroendocrine systems

work dependently to regulate energy balance[264,265]. The combination of hypothalamic control of food intake, hedonic eating, and peripheral drivers of appetite now make up our current mechanistic understanding of human appetite regulation; however, this model does not adequately account for the contributions of other physiological factors[266] and is limited in this regard. Thus, previous independent appetite regulation models should be integrated to reflect the complex interdisciplinary nature of appetite, while also recognizing that some potential effect modifiers are not yet understood[27]. Given the relative lack of emphasis on body composition and its relationship to appetite in prior research syntheses, it is critical to discuss the contribution of body composition, particularly fat-free mass (FFM), on long-term appetite regulation. Therefore, the purpose of this narrative review is to make aware to other researchers and clinicians of the relationship between FFM and appetite hormones, and that further research is needed to elucidate potential cause-effect.

1. Adipose tissue as a physiological regulator of food intake

Energy homeostasis, which is the homeostatic maintenance of body energy stores, is attributed to a network of physiological contributors that detect and regulate the amount of stored energy in the body[255]. The hypothalamic-melanocortin system, located in the arcuate nucleus, is responsible for communicating the body's nutritional state in two ways: (i) stimulation of the pro-opiomelanocortin (POMC) neurons following food intake or when sufficient body energy stores are present or (ii) stimulation of neuropeptide Y (NPY) neurons and agouti-related protein (AGRP) in the absence of food or when insufficient body energy stores are present[237]. Following the ingestion of food, short-term increases in peripheral appetite hormones such as leptin, cholecystokinin (CCK), and insulin signal the POMC neurons to release the anorexigenic hormones α-melanocyte-stimulating hormone (α-MSH) and cocaine-amphetamine-related transcript (CART) to bind to melanocortin 4 receptor (MC4R), resulting in decreased food

intake[267,268]. In the absence of food, the stimulation of NPY and AGRP from the hormone ghrelin inhibits the actions of the POMC on the MC4R thus increasing food intake and decreasing EE[268,269]. Together, the hypothalamic-melanocortin system and its associated hormones work to maintain body energy stores. Investigations of the hormone leptin deserve much of the credit for the energy homeostasis theory[32,76,261,270,271]. Collectively, this research demonstrated the secretion of leptin by adipocytes in response to both acute ingestion of food and adequate energy stores[139,272,273]. Moreover, the relationship between leptin and energy homeostasis was further demonstrated by the fall in leptin levels after prolonged fasting, resulting in overfeeding and decreased EE[13,270,272].

Following the original characterization of leptin, a compelling narrative was made for the role of adipose tissue as a central regulator of appetite due to leptin's apparent capacity to satiate and subsequently regulate body weight through the reduction of food intake[261]. The idea of adipose tissue as a central regulator was further established by reports that show the remarkable adaptability of adipocytes relative to other biological tissues[274,275]. There appears to be a greater tendency to regulate adipose tissue[276] due to the strain placed on the adipocytes during the remodeling process[274], which may result in a strong biological drive to eat as a way to relieve the strain imposed on the adipocyte during weight loss[275,277]. Many experts have suggested, therefore, that the role of adipocyte-derived hormones serve to maintain sufficient energy stores rather than promote satiety[13,278–280], an evolutionary adaptation which equipped us to defend our adiposity by driving food intake during periods of famine and prolonged energy deficits[281,282]. Despite disagreement over the precise role of adipose tissue in the homeostatic maintenance of body energy stores, it is well established that adipose tissue does possess some level of regulatory responsibility[273]. However, as Speakman[283] stated, "If adipose tissue is under physiological regulation (i.e. peripheral appetite signals), how is there an obesity epidemic?". Moreover, if we

accept that: (i) obesity is a product of continuous positive energy balance and (ii) adipose tissue down regulates food intake as a product of sufficient energy stores[276], then how do individuals develop obesity through the overconsumption of food?

Several ideas provide potential counterarguments against the central role of adiposity in appetite regulation, some of which functionally override energy homeostasis[255]. One of the theories that counters the pivotal role of adiposity on energy intake (EI) and subsequent obesity development is the concept of eating in the absence of hunger. The modern obesogenic food environment promotes weight gain through multiple pathways including the increased availability of highly-palatable, energy-dense foods[191,284]. This environment overrides the inherent ability to self-regulate food intake, leading to abnormal eating behaviors that increase body energy stores despite the physiological down regulation of feeding signals[285]. Eating despite physiological satiety[286,287] is a behavior that may have the power to override the physiological responses associated with sufficient adipose tissue stores[288], suggesting that there are important hedonic influences on appetite regulation that at a minimum work alongside or interact with peripheral signals. Early research may have undermined the role of executive function and hedonic behaviors, but it is now accepted that the modern eating environment places tremendous pressure on the brain systems involved in eating[289]. As a result of the continuous food cues displayed in our modern food environment, the neuronal circuitry involved in appetite control is disrupted, and peripheral satiety signaling becomes impaired, resulting in strong behaviors of passive overconsumption[290].

While the modern food environment is only one strand in the complex web of obesity, the obesity epidemic does not support the regulatory ability of FM. If the theory were true that fat stores strongly regulate food intake during energy balance, then a reduction in EI would be expected before the onset of obesity[255]. However, it appears that not only does FM exert little regulatory capacity over homeostatic food intake, but it is also unable to resist continued growth

despite sufficient or excessive body energy stores[291]. Interestingly, overfeeding studies refute this claim, showing that during overfeeding and rapid weight gain the body resists continued growth through a hypophagic drive to return to its initial weight[12]. However, one could argue that most individuals do not develop obesity overnight and that the pathway to obesity is one of long continuous growth over time that occurs despite increased body energy in the form of FM. Moreover, reports suggest that FM is not associated with whole-day EI and as FM increases, its regulatory effect on acute EI fades[292]. One may also consider body fat percent as potential regulator of EI due to its reported moderation on energy expenditure[292]; however, this was reported in females who have inherently more FM[293], percent body fat[294], less FFM, and fat distribution that is protective of metabolic consequences compared to their male counterparts. It is therefore difficult to consider that FM is unequivocally responsible for appetite regulation given that increases in FM do not appear to decrease appetite and resist growth. This may be partially explained by the finding that, as an individual gains weight in excess, leptin and insulin resistance occur, thereby reducing the ability of these hormones to promote satiety[142,295,296]. In normal physiology, increases in both leptin and insulin concentrations would be expected to initiate an anorexigenic response. In obesity, however, leptin and insulin concentrations remain elevated without the anorexigenic response, suggesting the development of resistance to these hormones through defective signaling[297]. For leptin specifically, resistance is characterized by its inability to cross the blood-brain barrier, a necessary process for inhibition of the orexigenic neuropeptides located in the arcuate nucleus[143,272]. Increased levels of adiposity, such as those observed in obesity, impair a number of physiological regulators of appetite. English et al.,[144] reported no ghrelin suppression following consumption of a mixed meal in participants with obesity. Other studies, however, suggest that higher protein content in meals effectively suppresses postprandial ghrelin concentration and increases glucagon-like peptide-1 (GLP-1) but not leptin response in

individuals with obesity[107,113,115,298]. Individuals with obesity also have impaired peptide tyrosine-tyrosine (PYY) responses, where basal and postprandial levels are lower in comparison to their leaner counterparts[25,145]. Reports suggest, however, that PYY levels can be restored with weight loss[299]. Lastly, GLP-1 plays a role in satiety responses, but whether its responses are related to excessive adiposity is unresolved[85,300–302]. It does appear that body energy homeostasis is an unbalanced process, where the net anabolic components of body energy homeostasis (i.e., stimulation of food intake) have much stronger effects than the net catabolic components (suppression of food intake)[13,280]. When obesity results in hormonal resistance, negative feedback is impaired. This results in inhibition of the satiety promoting effect of anorexigenic hormones and may promote overeating in the modern food environment[147].

Overall, the role of FM in appetite regulation has been characterized as weak and susceptible to disruption[255]. Therefore, given that the modern food environment is far from harmonic to our biological design, we must consider that the trivial regulatory role of FM on long-term appetite regulation is no longer complementary to our current environment. As such, interventions that examine other body composition components, namely FFM, may elicit a better understanding of whether body composition truly influences feeding behavior.

2. The relationship between FFM and appetite

FFM is a molecular-level body composition compartment that includes all non-fat molecules in the body, irrespective of where they occur. While commonly equated with skeletal muscle mass (SMM), these entities are distinct. Nonetheless, there is meaningful overlap between the molecules comprising FFM and SMM, where SMM is one of multiple organs-tissues (e.g. liver, heart, brain, etc.) comprising FMM . FFM is reported to be a strong predictor of EE, which

may have implications on appetite control[303]. However, the relationship between FFM and appetite is not yet fully understood[147].

Original findings that support the role of FFM in appetite control contrast with the initial view of FM as the primary body composition component involved in appetite control[255]. Lissner et al.,[304] determined that FFM, not FM, was positively associated with energy requirement which may be due to the higher resting energy requirements of FFM. Caudwell et al.,[305] reported that changes in body composition components were a much greater predictor of EI than weight alone. Blundell et al.,[4] found that FFM, not FM or BMI, was positively associated with both EI and meal size independent of biological sex. Interestingly, Weise et al.,[306] reported that FFM index was positively correlated with EI while FM index was negatively correlated and that EI was predicted by FFM index to a greater degree than FM index. Vainik et al.,[307] also reported a positive relationship between FFM and EI in adolescent males. The findings by Blundell et al.,[4] spearheaded the theory that the relationship between FFM and EI is due to the energy demands of FFM and that the amount of FFM determines the minimal amount of EI.

The energy demands of FFM are manifested in an individual's resting metabolic rate (RMR)[308]. Several studies have found FFM to be a significant predictor of RMR, citing that FFM-based prediction equations provide the best accuracy for the estimation of RMR in comparison to FM and/or bodyweight[309–312]. The energy balance principle, which describes the relationship between EE and EI[148,149], could influence appetite control. Therefore, if FFM is the primary driver of RMR, and RMR is the largest component of EE[5], it is possible that FFM influences eating given the relationship between EE and EI[147]. Support for this theory comes from the reported positive relationships between RMR and meal size, EI, eating patterns, and hunger[303,313–316], as well as studies suggesting that FFM is positively associated with hunger in men and negatively associated with satiety in adolescents[317,318]. Other evidence suggests that the effect of FFM on appetite

depends on the state of energy balance, where at energy balance and during positive energy balance FFM increases drive to eat through increased RMR. Conversely, while at negative energy balance FFM and appetite are negatively correlated due to simultaneous decreases in RMR[319]. However, it is well known that positive and negative energy balance affect FM to a greater degree than FFM under certain circumstances such as with or without exercise and during aging[320] suggesting that energy balance only loosely explains the relationship between FFM and appetite regulation. Further, several studies propose that the relationship between FFM and EI is mediated by EE[315,321]. However, modulation of EE also occurs in several brain region associated with feeding behaviors and appetite-related hormones[322] and therefore, it is possible that FFM may have some physiological input on appetite hormone circulation that interacts with areas of the brain associated with EE. Nevertheless, it remains unclear whether or not the role of FFM in appetite control is due to its influence on RMR or if it possesses another level of physiological input such as an influence on hunger and satiety hormone circulation.

2.2 Relationship between FFM and hunger and satiety hormones

Considering the aforementioned investigations, a positive relationship may exist between FFM and EI; however, the underlying mechanisms explaining this relationship are less clear. FFM may be associated with molecules that act on the peripheral components of appetite control (**Figure 4.1.**). It is well known that SMM acts as a secretory organ[138]. SMM secretes hundreds of identified and unidentified peptides, known as myokines, which communicate with other organs such as the brain, liver, adipose tissue, and pancreas[150,151,323]. For example, SMM secretes the myokine interleukin-6 (IL-6) in response to exercise[324], which has been shown to mediate communication between intestinal L cells and pancreatic islets[138,150]. Through this mechanism, muscle-specific IL-6 has been reported to increase GLP-1 secretion in the small intestine[152] which

indirectly suppresses appetite. Further, acute administration of exogenous IL-6 results in appetite suppression[154], while IL-6 deficiency results in weight gain[325,326]. Studies also report the lipolytic effects of IL-6[327] and suggest that the lipolytic effect occurs in SMM[328]. The lipolytic effects of IL-6 increases free fatty acid circulation, resulting in decreased ghrelin concentrations[230,329,330]. The myokine irisin is also delivered in response to exercise from SMM and promotes the browning of white adipose tissue (WAT) in rodents[331,332]. Although inconclusive, reports suggest that irisin may also induce the browning of WAT in humans[333]. The browning of WAT by irisin has been shown to increase EE in rodents without changes in movement or food intake,[334] suggesting it may be caused by the increased EE that occurs during satiety signaling in the POMC. In humans, expression of irisin has also been observed in the hypothalamus, specifically, the paraventricular nucleus (PVN)[335]. The PVN receives input from NPY and AGRP[336] and is involved in reward and motivation[337]. The PVN also expresses GLP-1 receptors, and activation of these receptors in the PVN of rodents results in decreased food intake and food-seeking behaviors[337]. However, it is unknown if and how irisin affects the actions of the PVN in regard to satiety signaling. Other reports also suggest that irisin is regulated by leptin[338], where leptin increases result in irisin decreases, thus inhibiting the browning of WAT in individuals with morbid obesity. However, other studies have reported different associations between leptin and irisin in children[339,340]. Nonetheless, it is possible that the myokine irisin plays a role in appetite regulation given its association with both central and peripheral signals of appetite. AMP-activated protein kinase (AMPK) pathways are another mechanism in which FFM may regulate food intake. AMPK functions to detect available energy in SMM and increases fatty acid oxidation when energy stores are in excess[341]. Due to its ability to detect and signal energy needs, AMPK is thought to be associated with central and peripheral regulation of body energy homeostasis[153,342]. In particular, the gut-derived anorexigenic hormone CCK has been shown to increase metabolic rate in rodent

SMM through its increase of AMPK[343]. Similarly, leptin has also shown to increase fatty acid oxidation in rodent SMM from increased phosphorylation of AMPK[344]. AMPK is also expressed in the hypothalamus[342,345,346], and hypothalamic AMPK has shown to increase food intake through its influence on hypothalamic neuropeptide expression[153,347,348]. Lastly, reports suggest that the anorexigenic responses of both leptin and CCK are initiated through brain-derived neurotrophic factor (BDNF)[349]. BDNF is a neurotrophic factor that elicits neuroprotective effects under conditions such as hypoglycemia and is found in several brain regions including the hypothalamus[350]. Studies report that lower expression of BDNF results in increased feeding and lower satiety signaling[351]. Interestingly, BDNF is also expressed in both SMM and skeletal muscle satellite cells[352,353], and BDNF mRNA expression is positively associated with AMPK[354]. It is possible that secretion of BDNF from SMM is responsible for mediating the actions of both CCK and leptin. However, despite the conceivable mechanisms in which FFM may influence peripheral appetite hormones and subsequent EI, studies that investigate the relationship between FFM and the above-mentioned hormones are scarce.

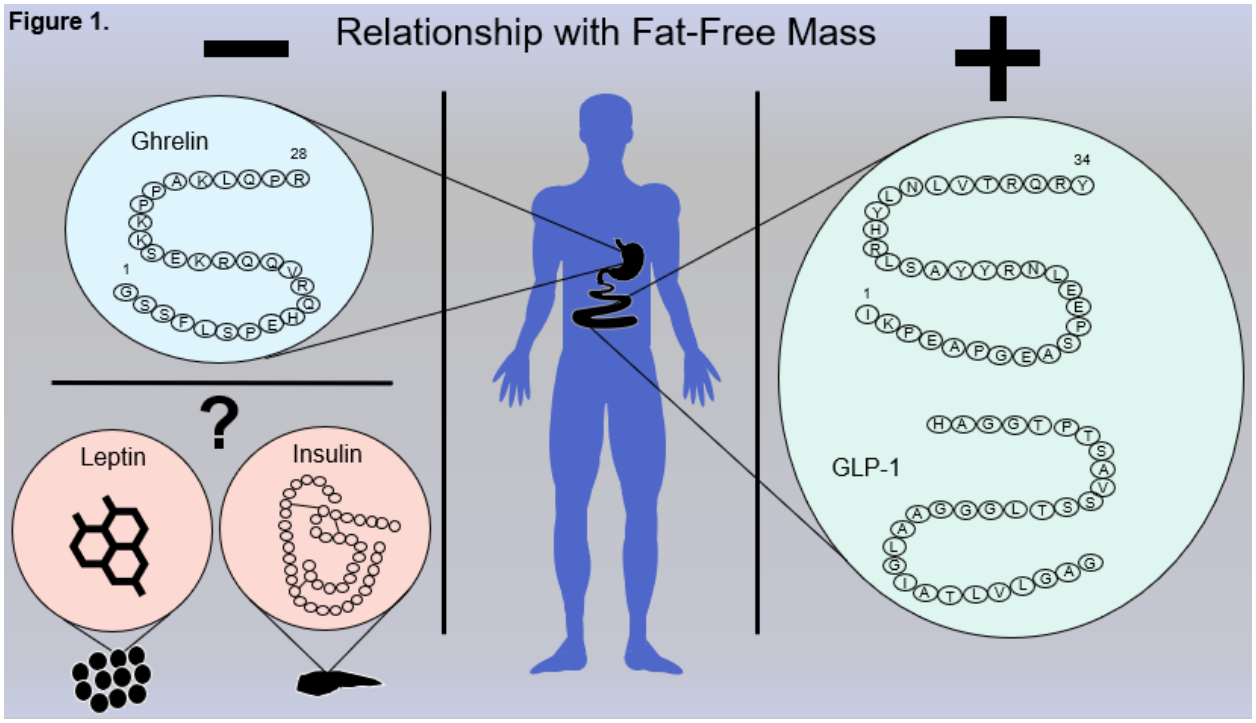

Figure 4.1. Overview of the relationships between fat-free mass and appetite hormones

The orexigenic gut hormone ghrelin is released from the stomach and comes in both the acylated and unacylated form. While studies report mixed results, available evidence suggests that total ghrelin and fat-free mass (FFM) are inversely related. There is limited available evidence evaluating the relationship between acylated ghrelin and FFM. Peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), two anorexigenic gut hormones, are released from intestinal L cells following consumption of a meal. While there are limited studies examining the relationship between FFM and PYY and GLP-1, the available evidence points towards a positive relationship between FFM and both hormones. In addition to being delivered by the intestine, it appears that PYY is also released from the skeletal muscle in the absence of a meal, further highlighting the potential relationship. The anorexigenic hormones leptin and insulin are released from adipocytes and pancreas, respectively, however whether they exert a relationship with FFM is unknown at this point.

2.3 FFM & Ghrelin

There are several brain-derived orexigenic hormones that function to stimulate hunger in the absence of food including: NPY, AGRP, orexin, and melanin-concentrating hormone (MCH)[355]. Ghrelin, however, is the only orexigenic gut-derived hormone and functions to stimulate food intake through hypothalamic receptors[80,356,357] by (i) homeostatic feeding fueled by metabolic need, and (ii) centrally by modifying reward and motivated feeding behavior[358,359]. Produced in the gastrin-producing cells of the stomach[360] in the instance of low blood glucose, ghrelin stimulates the lateral hypothalamic nuclei and both NPY and AGRP to increase food intake during fasting or weight loss[79,80,361]. Interestingly, ghrelin dysfunction is seen in individuals with obesity; particularly, the postprandial suppression of ghrelin is decreased when compared to normal weight individuals[23,24]. This creates a scenario in which, despite the consumption of a meal, individuals with obesity still maintain a sense of hunger which can result in dysfunctional feeding behaviors[24]. Substantial increases in circulating ghrelin have been observed during weight loss, which is one reason why dietary interventions have such high failure rates[362–364]. However, it remains unclear if the observed increases in ghrelin during weight loss are due to unintentional losses in FFM.

Ghrelin exists in both the unacylated (UAG) and acylated form, and combined, make up total ghrelin (TG) concentration[365]. Acylated ghrelin (AG) is considered the functional form of ghrelin given its ability to cross the blood-brain barrier and act on the arcuate nucleus[365,366]. Several studies have reported an inverse relationship between varying forms of ghrelin and FFM[367–381], however, other studies report either a positive association[382] or no association with TG[383–385]. There are several potential mechanisms that may explain the different findings. Most notably may be the action of ghrelin as a survival hormone, where during periods of starvation or cachexia ghrelin

acts to increase blood glucose concentrations and prolong life[386]. This may be why higher ghrelin concentrations are sometimes observed concurrent with lower FFM during prolonged hypocaloric diets. While a complete discussion of ghrelin's mechanisms of action are beyond the scope of the present review, interested readers are directed to the work of Mani & Zigman[386].

It is known that androgens are positively associated with FFM and inversely associated with FM[387], and that chronic energy deficits may result in decreased testosterone[388,389]. Weight loss studies show that energy restriction without testosterone supplementation leads to losses in FFM and FM concomitantly, whereas testosterone supplementation causes only FM to be lost[390]. Additionally, energy restricted diets also lead to increased ghrelin concentrations[391,392]. Recently, Karl et al.,[393] reported that supplementation with testosterone during an energy deficit attenuated increases in AG and UAG in non-obese men whereas AG and UAG increased in those receiving a placebo. Moreover, this study reported an inverse relationship between changes in free testosterone and changes in AG[393], which is supported by other investigations that observe this relationship between TG and testosterone[373,374]. The association between testosterone and ghrelin may provide insight to an interplay between the reproductive and enteroendocrine system. It is possible that the anabolic signaling of increased testosterone concentrations mimics the effect of satisfied energy stores and decreases ghrelin secretion.

Several studies examining adolescent boys report negative relationships between FFM and TG[373,374,377]. It is possible that increases in testosterone during the pubertal transition in young boys result in both increased FFM and decreased ghrelin. This theory is supported by the findings of Jürimäe et al.,[374] which found that prior to the onset of puberty, FFM and TG were not related but were inversely related after the onset of puberty. This may also explain the findings from Caffarelli et al.,[368] who observed this relationship between FFM and TG in girls with Rett Syndrome (RTT). Multiple reports show precocious puberty and delayed menarche in patients with RTT[394], where

patients report a pubertal development of Tanner stage 3 as early as 6 years old[395] or plasma testosterone concentrations similar to postpubertal men[396]. It is possible that increased testosterone in this population explains the observed inverse relationship between FFM and ghrelin. Interestingly, Devesa et al.,[396] also observed undetectable plasma estradiol in a patient with RTT that also exhibited extremely high testosterone. Multiple studies report positive relationships between estradiol, and TG and AG[397,398]. It has been shown that obesity results in increased conversion of testosterone to estradiol via increased aromatase activity in adipose tissue[399]. This is supported by the results of Schulte et al.,[399] who found that weight loss from caloric restriction resulted in increased testosterone and decreased estradiol conversion in men with obesity. Although this contradicts the results seen for caloric restriction and testosterone in normal weight men, experimental weight loss studies that find an inverse relationship between ghrelin and FFM in overweight men or men with obesity[375,378] may observe this because estradiol conversion rates decrease during weight loss resulting in increased testosterone and increased FFM. Estradiol concentrations may also explain the positive relationship between FFM and TG in young females reported by Serra-Prat et al.,[382]. Reports for postmenopausal women suggest an estradiol range that is associated with optimal body composition (i.e. lowest FM, highest FFM)[400]. It is possible that this range exists in young females and results in both higher FFM and TG simultaneously. Changes in sex hormones may also provide insight into the negative relationship between FFM and ghrelin in postmenopausal women. Foster-Schubert et al.,[371] reported an inverse relationship between FFM and TG at baseline in postmenopausal women, but not after a 12-month aerobic training program. It has been reported that exercise decreases both estradiol and testosterone in postmenopausal women[401]. It could be that exercise was able to maintain FFM despite lower testosterone concentrations, but ghrelin changes still occurred due to its relationship with both sex hormones. Conversely, Jürimäe et al.,[372] investigated the relationship between FFM and ghrelin in

postmenopausal women in a 12-month prospective study and found that both baseline measures and changes in FFM were inversely related to TG. Without exercise, natural aging during menopause results in lower estradiol and unchanged testosterone leading to higher testosterone to estradiol ratios[402]. It could be that higher testosterone to estradiol ratios result in lower ghrelin in this population. However, the potential relationship between testosterone and ghrelin in postmenopausal women does not explain the mixed results observed in elderly individuals.

It is well established that appetite and food intake decline during aging simultaneous with a marked decrease in SMM and increase in FM[403]. However, along with these body composition changes in elderly individuals are lower ghrelin concentrations[404,405]. Therefore, it is possible that studies that fail to observe a relationship between FFM and ghrelin in older adults do so because of the natural declines in both ghrelin and FFM that occur during aging[382,385]. This, however, does not explain studies that do observe an inverse relationship between ghrelin and FFM in older adults.

Growth hormone (GH) is another hormone known to affect body composition. Similar to testosterone, GH is positively associated with FFM and negatively associated with FM[370,406–408]. Ghrelin's action in the arcuate nucleus releases growth hormone releasing hormone (GHRH) and has been reported as a potential therapy for GH deficient individuals[409]. However, those with GH deficiency that undergo GH therapy seem to display an inverse relationship between GH and TG possibly due to negative feedback[370]. Although studies in humans are scarce, Engström et al.,[370] reported that exogenous GH treatment in GH deficient patients results in decreased TG concentrations and that decreases in TG are inversely related with increases in FFM. The negative feedback initiated by GH on ghrelin could explain the inverse relationship between FFM and ghrelin observed in elderly populations[367,371]. It is possible that during aging ghrelin resistance occurs. Decreased signaling of ghrelin to the arcuate nucleus reported during aging[410] would

theoretically result in increased circulating ghrelin, decreased GHRH secretion, and decreased FFM.

Body composition alone may also provide some explanation. When comparing ghrelin in lean adolescents and adolescents with obesity, an inverse relationship between FFM and UAG was seen in lean adolescents, but not adolescents with obesity[376]. Conversely, Rigamonti et al.,[379] reported an inverse relationship between FFM and ghrelin in adolescents with severe obesity. It could be because at extremes of obesity FFM content is higher than lean individuals, resulting in decreased ghrelin regardless of body mass index (BMI). This is supported by Tai et al.,[381] who reported that despite an inverse relationship between BMI and fasting TG, SMM was the only significant predictor of fasting TG in healthy adults. It is possible that greater amounts of more metabolically active tissue signals sufficient body energy stores and reduces ghrelin, although this unclear. Visceral adipose tissue (VAT) may also provide an explanation. Similar to FFM, studies report an inverse relationship between VAT and TG[330,411]. VAT displays increased lipolytic activity resulting in increased circulation of free fatty acids[329,330] and free fatty acids have shown to decrease TG[230]. Given the positive relationship between VAT and BMI[412] it is possible that individuals with obesity have both increased VAT and FFM, but that VAT better explains ghrelin responses in this instance. Given the increased difficulty in accurately measuring VAT[413] relative to measuring FFM, studies that collect measurements of both tissues in relation to ghrelin are lacking.

Although it appears that FFM is inversely related to TG at baseline[369,371,372,375,379] and to TG[367,368,373,377,381] and AG[376] in cross-sectional studies, those that examine changes in FFM and TG[371,375,378–380,384,385] or UAG[369] over time during exercise or dietary interventions have mixed results. Stimulation of muscle protein synthesis due to amino acid ingestion or exercise may provide a putative explanation for the association that is sometimes observed. Several studies

report the suppressive effects of protein consumption, relative to other macronutrients, on circulating ghrelin[110,115,414,415]. Low-protein diets may cause unwanted reductions in SMM alongside increased ghrelin concentrations, thus, increasing the risk of weight regain following weight loss. This may also be a potential explanation for the finding that energy-restricted diets alone have had limited success in weight loss maintenance[251] but energy-restricted diets that are higher in protein[416] and that add exercise interventions have demonstrated better outcomes[417,418]. During energy-restricted diets, although not deliberate, it is common to have losses in both FM and FFM[419]. It is possible that the success of diets that prescribe simultaneous energy restriction, high protein intake, and exercise is partially attributed to the increased muscle protein synthesis that assists in SMM maintenance. This increased protein consumption and higher SMM would theoretically result in lower ghrelin concentrations. This theory is supported by findings from Scheid et al.,[380] who examined the effect of exercise and weight loss on ghrelin and PYY concentrations. This group found that during exercise in the absence of weight loss and with significant increases in physical fitness, ghrelin was unchanged; however, they found that when exercise was combined with weight loss, ghrelin was significantly higher[380]. Likewise, ghrelin was inversely correlated with changes in weight and FFM in this study, but not with changes in FM[380]. Similarly, both Guelfi et al.,[420] and Pil-Byung et al.,[421] also reported a significant increase in FFM following a resistance training program concurrent with decreased AG concentrations. Moran et al.,[375] found that a 16wk energy restricted diet with either high-protein or standard-protein consumption resulted in an inverse relationship between FFM and TG at baseline and 16wk. Interestingly, despite significant reductions in weight and FM, only the small decreases in FFM were inversely related to TG at study conclusion[375]. It could be that adequate consumption of protein alone is not enough to overcome the effect of energy restriction on FFM and that the inclusion of exercise is required to sufficiently preserve FFM and avert ghrelin increases. Overall,

these findings suggest that weight reduction which results in FFM loss leads to higher ghrelin concentration, whereas weight reduction that does not involve changes on FFM results in unchanged ghrelin[379]. The implications of this potential relationship could be of great importance, particularly if maintaining FFM during weight loss could reduce the potential for weight regain through lower ghrelin concentration. Further research is needed to elucidate the underlying mechanisms of this relationship. Specifically, information as to whether the associations between FFM and ghrelin lead to changes in appetite or EI is lacking. Future research should investigate the effect of FFM-induced changes in ghrelin on appetite perceptions and EI. Further, future research should determine whether associations occur with stomach or muscle derived ghrelin.

2.4 FFM & Peptide-YY

PYY, known as both peptide tyrosine-tyrosine and peptide YY (PYY), is an anorexigenic gut hormone derived from intestinal L cells that belongs to the pancreatic polypeptide-fold family[83]. PYY exist in two forms: PYY_{1-36} and PYY_{3-36}, where PYY_{3-36} is the form generally associated with appetite control[83]. PYY is controlled partially by neural mechanisms due to its observed increase (15 min postprandially) well before nutrients reach the small intestine[94]. PYY increases are also observed following caloric intake and promote postprandial satiety and decreased EI in a similar fashion to leptin[422,423]. Additionally, PYY increases may also reduce the rate of gastric emptying[95], another avenue in which PYY promotes satiety. It is still unknown, however, if there is a relationship between PYY and FFM. To our knowledge, Pereira et al.,[424] have conducted the only study that reports a positive relationship between FFM and postprandial PYY secretion, although the mechanism is not yet understood. Generally, PYY is released from the small intestine or pancreas following a meal; however, recent investigations have reported the secretion of PYY in human SMM[155]. Thalacker-Mercer et al.,[156] provided the first evidence for

this, finding that baseline PYY gene expression in SMM was larger in individuals who displayed greater hypertrophic responses to resistance training compared to those who showed little to no increases in muscular hypertrophy during resistance training. This was later confirmed by the work from Gheller et al.,[155], who found that PYY is secreted from both human SMM and satellite cells, the stem cells that are responsible for SMM repair[155]. It has been established that greater expression of SMM satellite cells exists in those that show greater hypertrophic responses to exercise[425]. It is possible that those with a larger number of satellite cells and those with increased SMM have a greater ability to produce PYY, which would theoretically increase satiety following exercise.

Several studies have demonstrated PYY increases following acute exercise which is supported by a review from Zouhal et al.,[426] and a meta-analysis by Schubert et al.,[83]. Interestingly, while most studies report an increase in PYY during or following exercise, it appears that higher intensity training types increase PYY to a greater degree than moderate intensity continuous exercise or steady state exercise[427–430]. Additionally, this is supported by studies that report decreased appetite ratings in both moderate and high intensity training[431,432] while other studies report larger decreases in appetite ratings following trainings of higher intensities[428–430,433]. Some studies also report higher PYY concentrations in males than in females following exercise[430,434]. It is possible that the sex differences observed in PYY are related to the larger FFM content in males. Although, studies report increased PYY during and following exercise, most studies observe this when examining lean participants. While studies are limited, acute studies that investigate individuals with overweight or obesity show no increase in PYY following exercise[435,436]. Several studies report decreased postprandial PYY responses in individuals with overweight or obesity[437–439]; however, it is unclear how PYY responses are attenuated in the absence of a meal following exercise. In reference to FFM, Scheid et al.,[380] reported no changes in PYY following exercise or exercise combined with weight loss and no relationship between

PYY and FFM in healthy premenopausal females. To date, there are no other studies that examine the direct relationship between PYY and FFM.

In sum, it could be possible that those with greater predispositions for muscle gain would inherently produce more PYY, resulting in increased physiological satiety through detection of increased body energy stores. However, this alone does not explain satiety in its entirety. It is also possible that muscle-specific IL-6 plays a role in PYY production as evidence has been shown in rodent models that suggests IL-6 injection increases PYY mRNA expression[152,440]. Given the evidence that increased FFM is positively associated with EI, it is possible that neural mechanisms associated with appetite override peripheral satiety signaling. The relationship between FFM and PYY requires further investigation as evidence is conflicting[299,441–444].

2.5 FFM & GLP-1

GLP-1, an incretin hormone released from the small intestine (L cells) in response to meals[84,85], has demonstrated satiation effects mediated by vagal afferent nerve activation[86,87]. Although increases in GLP-1 are generally nutrient driven (i.e. when nutrients reach the L cells of the intestine), rodent models suggest that there are food anticipatory signals that help regulate the consumption of a large meal[88,89]. Additional studies report peaks in GLP-1 10-15 minutes after a meal is initiated[90]. Therefore, it seems that GLP-1 has two peaks that function to signal satiety: 1) after meal ingestion and 2) after gastric emptying[88]. Conversely, inhibition of GLP-1 increases appetite and food intake[91]. Further, it has been suggested that obesity results in deficient GLP-1 responses[445,446].

It is still unknown, however, if FFM is related to GLP-1 production. Pereira et al.,[424] reported the first positive relationship between postprandial GLP-1 and FFM five years after gastric bypass surgery. Because of the relationship GLP-1 shares with insulin, therapies that can

exploit this relationship are appealing to those with excessive weight gain and/or type 2 diabetes (T2D) given the importance of FFM in glycemic control [447]. GLP-1 receptor agonists (GLP-1RA) are a commonly used secondary treatment for weight loss in those with obesity and T2D. Ideally, reductions in FM and remission from T2D are the desired outcomes of this therapy; however, it seems that the anorexigenic effects GLP-1RA act on both FM and FFM simultaneously. A meta-analysis by Sargeant et al.[448] revealed this potential relationship, reporting that in 11 of 17 qualifying studies that employed GLP-1RA treatments for weight loss, 22%-65.2% of the weight lost came from decreases in FFM. Interestingly, this large range is consistent with those observed following bariatric surgery[448–450]. Bariatric surgery patients specifically, suffer from significant losses in FFM due to their very low-calorie diets concurrent with increased endogenous GLP-1 which promotes satiety[451]. Therefore, more research is needed as current information is conflicting.

Conclusions

In conclusion, it is clear that FFM is associated with appetite-related hormones and the reason for this relationship may be both hormone specific and population dependent. For ghrelin, there is a strong case for an inverse relationship with FFM that is independent of FM, although the underlying explanation for this relationship remains unclear. Factors related to FFM such as sex hormones, GH, age, and weight status may indirectly affect ghrelin; however, further investigation is warranted. Based on the available literature, clinicians and dietary professionals should emphasize strategies that focus on the maintenance or increase of FFM. Given that both decreased EE and increased hunger are associated with weight regain following weight loss, FFM maintenance strategies may have desirable outcomes for long-term weight management. For PYY and GLP-1, a clear relationship with FFM has yet to be confirmed. PYY appears to be released from SMM during and following exercise in the absence of a meal, but it is unclear if the amount

of FFM an individual possesses results in greater PYY release from SMM. Further research examining relationship across varying levels of FFM is warranted. To date, limited research exists for the relationship between GLP-1 and FFM, and it is unclear if and how this relationship occurs. Future experimental studies evaluating cause-effect should investigate this. Although there is no clear direction of the relationship between FFM and appetite-related hormones, there is a consistent relationship. The information presented in this review should encourage researchers, clinicians, and health professionals to consider FFM preservation as a suitable strategy during weight loss for more successful long-term weight management through potentially decreased appetite.

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Chapter 5

Discussion

5.1 Summary

In this dissertation, we examined the effects of varying amounts of dietary carbohydrate and fat, and the different types of fatty acids (FA) on appetite. We also examined the role of FFM in appetite regulation.

In our first study, we found that the manipulation of FA composition (monounsaturated, polyunsaturated, or saturated) in a high-fat meal did not result in different postprandial ratings of hunger, fullness, or desire to eat in premenopausal normal-weight females. There was also no difference in energy intake (EI) during the *ad libitum* lunch across meal conditions after the postprandial period.

In our second study, we showed that a two-week ketogenic diet (KD), consumed under free-living conditions, led to significantly higher fasting GLP-1 and lower ghrelin concentrations compared to a high-carbohydrate diet (HCD) and a habitual diet (HD) in highly-trained cyclists and triathletes. We also found that consumption of a ketogenic meal following a two-week ketogenic diet led to significantly higher postprandial GLP-1 and lower ghrelin levels compared to the high-carbohydrate and standard American meals consumed after the high-carbohydrate and habitual diets, respectively. However, this study revealed that appetite perceptions did not match the abovementioned hormonal changes and requires further evaluation.

In our final manuscript, we discussed in detail the potential relationship between FFM and hunger and satiety hormone production. From this paper, we concluded that there is an association between FFM and appetite-related hormones. Specifically, higher FFM may be linked to decreases in the orexigenic hormone, ghrelin.

5.2 Contribution to knowledge base

All studies presented in this dissertation examined appetite regulation collectively. However, each research project investigated components of appetite regulation in a unique way. Our outcomes on the objectives measures of appetite in highly-trained cyclists and triathletes appear to be in agreement with the hierarchical effects of diet composition on satiety in other populations (protein > fat > carbohydrate)[266].

Similar to previous studies, we found that nutritional ketosis from a KD reduces ghrelin and increases GLP-1[132,133,222]. This is also known to occur following acute ingestion of exogenous ketone supplements[134,223]. Our study was the first to show this relationship in a population of highly-trained male and female cyclists and triathletes, whereas prior studies have mostly investigated individuals with overweight and obesity that are likely to suffer from physiological dysfunctions. However, given that our findings were similar to prior studies in regard to the effect of ketosis on appetite hormone production, it appears that a KD results in greater satiety promoting alterations in appetite hormones compared to a HCD or a HD regardless of weight status.

Our study had dissimilar results for subjective appetite responses compared to previous investigations[131–135,222,236]. We found greater fasting hunger and prospective consumption of food (PCF) during the KD than the HCD or HD despite having the lowest fasting ghrelin and highest fasting GLP-1 with the former diet. Moreover, we did not find any significant differences in appetite ratings after consumption of an acute ketogenic test meal compared to a high-carbohydrate test meal or a standard test meal. Studies that employ longer diet durations have shown that a KD results in decreased subjective appetite compared to diets higher in carbohydrate[131–135,222,236]. Studies that investigate appetite after consumption of exogenous ketones also report greater subjective appetite suppression[134,223].

Therefore, it appears that degree of ketosis may be responsible for subjective appetite alterations and that extended diet durations or acute hyperketonemia are necessary to reach the threshold required to shift appetite behaviors on the KD. This is supported by Nymo et al.,[133] who showed that subjective hunger increases on a KD for up to three weeks and decreases thereafter. Therefore, our study contributes to the existing literature by showing that significant ghrelin decreases, and GLP-1 increases can occur during a short term KD compared to a HCD or HD in a group of highly-trained male and female cyclists and triathletes but that this is not matched by changes in subjective appetite responses possibly because the study needed to be performed for a minimum of three weeks. Studies with longer diet durations are required to further support our findings.

Our FA meal study showed that there are no differences in subjective appetite measures between FA compositions which is consistent with most previous studies. Specifically, nine of 13 studies that investigated subjective appetite responses to varying FA compositions found no differences, and eight of 10 studies found no difference in future EI[123,124]. However, studies that have assessed FA composition in relation to objective measures of appetite have shown that unsaturated FA suppress appetite to a greater degree than saturated FA (SFA)[121–123,187,189,452]. Given the findings of previous studies and our own, there appears to be a discord between objective and subjective measures of appetite following consumption of a high-fat meal rich in varying amounts of FA. The reason for disagreement between these measures has yet to be determined and future research should focus on factors that modulate the interaction between objective and subjective measures of appetite.

Our FA meal study contributes to the knowledge base by addressing several limitations observed in previous studies that found no effect of FA composition on subjective measures of appetite and future EI. A limitation of the previous studies is the use of a fixed energy content in

test meals regardless of individual energy needs[122]. Therefore, we fed our participants a test meal with an energy content equal to 35% of their total daily energy needs, which we believe enacted a sufficient physiological satiety response. We also employed a greater proportion of total energy from the FA of interest in our test meals than previous studies, and ensured that the amount of each FA of interest was matched by amount across test meals[124]. According to a review study, uneven proportions of energy from the FA of interest in acute test meals may be why there have been difficulties detecting differences between FA compositions[123]. Most studies also investigate FA composition and appetite regulation in individuals with overweight and obesity. Individuals with overweight and obesity have shown to have defective fat metabolism[453] and impaired appetite hormone response[23,24] to a meal. This may diminish any significant differences between FA type. Further, age-related changes in appetite hormone production and EI are common in older adults[405,454]. Finally, most previous studies have examined the postprandial response to a high-fat meal enriched in varying FAs for longer durations than the 3 h postprandial period in our study[124]. Because Americans rarely go longer than three hours without consuming a meal or snack, longer postprandial durations may result in normal increases in hunger that washout the individual effects of each FA type and have limited external validity. Therefore, we investigated healthy young premenopausal normal-weight women which allowed us to limit any discrepancies in appetite response. Furthermore, our study investigated ad-libitum eating after a 3 h postprandial period to improve external validity. Although our study addressed numerous limitations in the existing literature, we came to conclusions similar to previous investigations. Therefore, we suggest that selection of FA in a high-fat meal does not differentially influence subjective appetite measures or future food intake and that there appears to be discordance between hormonal markers of appetite and perceptions of appetite.

Our review paper is the first to examine the relationship between FFM and physiological appetite alterations. Although there have been several reviews that have investigated the role of FFM in energy balance[147,255], the role of skeletal muscle loss in appetite regulation during weight loss[455], and the role of FFM in appetite regulation at extremes of obesity[303], no manuscript has conducted an in-depth review of the relationship between FFM and appetite hormone regulation. Because the position of FM in the hierarchy of appetite regulation has been called into question, there is now a growing interest in the effect of FFM on appetite regulation. Based on our review of studies on FFM and appetite regulation,, we concluded that FFM is related to several appetite hormones. Specifically, there appears to be an inverse relationship between FFM and ghrelin which has not to our knowledge been previously reviewed or discussed. There are several possible mechanisms that may explain the inverse relationship reported between FFM and ghrelin, but more research is needed to determine this in cause-effect manner. The satiety inducing relationships between FFM and PYY and GLP-1 have also been reported, but only in a single study making it difficult to determine a clear relationship[424].

5.3 Discussion of knowledge gaps and implications for future research

The papers in this manuscript clarify many pre-existing knowledge gaps in the current literature, but several questions remain unanswered with implications for future research.

There are a number of remaining questions regarding the effects of a KD on appetite regulation. First, future studies that investigate the effect of a KD on appetite regulation should employ longer duration interventions, specifically in endurance athlete populations. Because the length of our study was short in duration, it is unclear whether a longer duration KD would have resulted in subjective as well as objective appetite suppression similar to other studies. Although participants in this study reached the threshold for nutritional ketosis[127], the increase in ketosis was modest[103].

Future studies should investigate whether higher BHB concentrations results in greater appetite suppression and determine this in a cause effect manner. It is also well known that long-term adherence to the KD is poor[456]. Because individuals that practice the KD in the real world may experience a transient ketogenic state, studies that determine how fluctuations in ketosis influence appetite are clinically relevant. Longer duration studies that investigate the KD and appetite regulation should therefore consider an intervention with fixed periods of normal carbohydrate intake. There are also unanswered questions regarding the composition of the KD, specifically protein consumption. Future studies should examine whether a modified KD that replaces a proportion of total fat intake with protein results in increased satiety and adherence. This may be more clinically relevant due to the difficult nature of controlling protein to such a degree on the KD outside of a laboratory setting. There are also only a couple of studies that have investigated the effects of ketone ester supplementation on appetite regulation[134,223]. Given the low adherence rates for the KD and the simplicity of ketone supplementation, it is important to determine if appetite responses similar to a KD can be achieved with acute ketone supplementation. Finally, it is unknown how athletes of different sports would respond to a KD. Future studies should investigate the effect of a KD in athletes of varying sports.

There are also several studies that have examined acute meal responses similar to our FA meal study. While this provides insight to short-term satiety responses to high-fat meals enriched in varying FA, a longer duration diet may be more revealing for long-term satiety and be more practical than acute meal studies. There are few long-term diet studies and all are limited by their short duration and test meal design[174,457,458], leaving questions regarding the optimal length of interventions and amount of FA of interest to employ. Furthermore, there are limited studies that investigate long-term satiety signals (i.e. leptin, insulin) after a long-term diet enriched in varying FA[457,458]. Our study also measured EI in a laboratory setting which is much different than normal

eating environments. By giving participants a predetermined meal, EI can only be adjusted by consuming more or less of the provided meal[459]. Because the *ad libitum* lunch/buffet meals across acute meal studies are not common and do not offer the same food available in traditional diets[123], EI following consumption of an acute high-fat meal in a laboratory setting may be a reflection of temporary diet changes rather than the effect of FA composition on EI[123,460]. There are few studies that have measured free-living EI following a high-fat meal enriched in varying FA, but future studies should consider this method as it may be a better reflection of real world eating behaviors. Studies examining the effect of FA composition on EI following a high-fat meal should be interpreted with caution.

Regarding FFM and appetite, we suggest conducting randomized trials that further investigate the relationship between varying degrees of FFM and peripheral signals of appetite. While our review found consistent inverse relationships between FFM and ghrelin, there was limited existing evidence for a relationship between FFM and several satiety hormones such as GLP-1, PYY, and CCK, although there is some theoretical backing to support potential relationships. Future studies should consider interventions that increase FFM to determine if this affects objective and subjective measures of appetite. In our review, we only examined the relationship between FFM and short-term appetite regulators, but less is known about the relationship between FFM and long-term appetite regulators such as leptin and insulin. Future studies should investigate if there is a relationship between FFM and other long-term signals of satiety.

5.4 Implications for appetite regulation in athletes and obese individuals

Our studies offer several implications for future practice in athletes and individuals undergoing weight management. We show that diet composition, specifically the amount of

carbohydrate and fat, have a significant effect on appetite regulation. However, the type of fat does not influence subjective appetite measures or future EI. Further we show that exercise may have a significant effect on appetite regulation through increases in FFM.

It is common for endurance athletes to implement short-term dietary restrictions, such as the KD to improve performance during competition or to achieve a desirable body composition. Endurance athletes that employ varying forms of dietary restriction should do so with caution given that certain diets as well as their training and higher FFM may result in appetite suppression. Given the effects of a KD on objective and subjective markers of appetite, athletes employing a longer duration KD should be cautious of the potential for appetite suppression that may result in decreased EI and eventually relative energy deficiency in sport (REDs). Because athletes are at a greater risk for appetite suppression based on their FFM and exercise habits, long-term dietary changes that are outside of their habitual intake should be avoided or closely monitored to reduce the risk of insufficient EI and subsequent health concerns. Coaches and dietitians should be aware of the effects of a KD as it pertains to appetite and prescribe this strategy safely with close monitoring of EI and weight changes. Further, coaches and dietitians should prescribe this to athletes without a history of unhealthy eating behaviors or eating disorders.

Acute consumption of a high-fat meal is a common practice in the US. For individuals undergoing weight management or individuals with obesity, specific selection of FA in a high-fat meal does not favorably influence appetite regulation. Moreover, we do not recommend increased consumption of SFAs given their well-known association with increased risk for cardiovascular disease. Further, food high in SFAs are often more palatable than their unsaturated counterparts and are easily overconsumed. Therefore, we recommend replacing SFAs with unsaturated FA based on reported health benefits not measured in our studies.

A better method for individuals undergoing weight management or individuals with obesity may be the utilization of strategies that increase or maintain FFM. Based on the reported relationships reviewed in our manuscript, increased or maintained FFM from exercise or increased consumption of protein during weight loss may have a beneficial effect on appetite regulation that favors decreased hunger. Registered dietitians and other health professionals should recommend dietary interventions and exercise regimens that support FFM maintenance to attenuate the increased expression of hunger that often leads to strategy abandonment and weight regain.

Conclusions

In conclusion, a very low-carbohydrate high-fat diet versus diets with more carbohydrate and less fat led to more anorexigenic alterations in the objective measures of appetite. These changes were not matched by the subjective measures of appetite. Due to the changes in objective measures of appetite during a KD, athletes, coaches, and dietitians should utilize this strategy with caution and monitor appetite behaviors and weight regularly. However, more research is needed to determine the reason behind the observed differences between objective and subjective appetite measure in our study. The type of fat in a high-fat meal in our study did not affect perception of appetite or future energy consumption even though unsaturated FA has been found to suppress appetite to a greater degree than saturated FA. More research is necessary to understand the disparate findings between the objective and subjective measures of appetite following a high-fat meal with varying amounts of FA. Finally, FFM may have a role in appetite regulation but requires further study. FFM does appear to have an inverse association with ghrelin; however, the physiological mechanism behind this association remains to be further elucidated. Studies are also needed to further determine the relationship between FFM and other appetite hormones.

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Abstract

Appetite is regulated by perceptions of hunger and fullness and gut hormones that are either anorexigenic such as glucagon-like peptide 1 (GLP-1), cholecystokinin, or peptide YY, or orexigenic such as ghrelin. Appetite suppression may lead to a number of negative effects including relative energy deficiency which impairs health and performance in athletes. Appetite stimulation, on the other hand, may lead to a positive energy balance and weight gain which are associated with many chronic diseases. A number of factors affect appetite regulation. In this dissertation, we examined the effects of varying amounts of dietary carbohydrate and fat and the different types of fatty acids on appetite. We also examined the role of fat-free body mass in appetite regulation.

It is unclear whether different fatty acids vary in their effects on appetite and eating in the absence of hunger. In our first study, we examined the effects of three high-fat meals rich in either monounsaturated, polyunsaturated, or saturated fatty acids on subjective ratings of appetite and subsequent *ad libitum* lunch consumption in healthy premenopausal normal-weight women in a randomized cross-over single blind study. We found that postprandial ratings of hunger, fullness, or desire to eat did not vary across meal conditions. There was also no difference in energy intake during the *ad libitum* lunch across meal conditions.

Many endurance athletes follow a ketogenic diet (KD) (very low in carbohydrate and high in fat) to improve performance. The effect of a KD on appetite remains unclear in this population. In our second study, we examined the effects of a KD on fasting measures of appetite in highly-trained cyclists and triathletes. The participants consumed both a KD and a high-carbohydrate diet (HCD), for two weeks each, in a random order, after their habitual diet (HD). We also assessed postprandial appetite measures in response to a ketogenic meal after the KD, a high-carbohydrate meal after the HCD, and a standard American/Western meal after the HD. The results showed that

the KD led to significantly higher fasting GLP-1 and lower ghrelin concentrations compared to the HCD and HD. The ketogenic meal also led to significantly higher postprandial GLP-1 and lower ghrelin levels compared to the high-carbohydrate and standard meals. Results on appetite perceptions did not match the hormonal changes and needs further evaluation.

Energy homeostasis is defined as the maintenance of body energy stores over time. To maintain these energy stores, appetite responds to changes in body mass by stimulating or suppressing food intake. It is well known that fat mass possesses a level of physiological regulation over appetite but the influence of fat-free mass in physiological appetite regulation is unclear. In our final manuscript, we discussed in detail the potential relationship between fat-free mass and hunger and satiety hormone production. From this paper, we concluded that there is an association between fat-free mass (FFM) and appetite-related hormones. Specifically, higher FFM may be linked to decreases in the orexigenic hormone, ghrelin. Clinicians and dietary professionals should emphasize strategies that focus on the maintenance or increase of FFM to regulate appetite.

In conclusion, a very low-carbohydrate high-fat diet versus diets with more carbohydrate and less fat led to more anorexigenic alterations in the objective measures of appetite. These changes were not matched by the subjective measures of appetite. More research is necessary to determine the reasons for these differences. The type of fat in a high-fat meal did not affect perception of appetite or future energy consumption. Fat-free mass may have a role in appetite regulation but requires further study.