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EFFECT OF FOOD ACCEPTABILITY ON APPETITE HORMONES' RESPONSE IN NORMAL WEIGHT VS. OBESE MALE SUBJECTS

by
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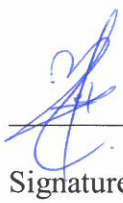

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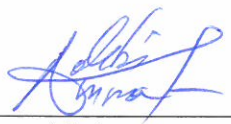

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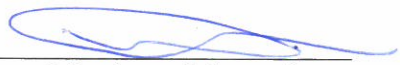

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AN ABSTRACT OF THE THESIS OF

Nehmat Albert El Helou for Master of Science
Major: Food Technology

Title: Effect of Food Acceptability on Appetite Hormones' Response in Normal Weight vs. Obese Male Subjects

The obesity prevalence is still increasing worldwide and extrapolations reveal that 20% of adults in the world or around 1 billion people, will be obese by 2030. Thus, understanding the complex phenomenon of appetite regulation.

The present study is the first to portray the effect of hedonic manipulation (high-acceptability vs. modified low-acceptability isocaloric food) on postprandial ghrelin, GLP-1, insulin levels and appetite scores in healthy male subjects. Thirty male subjects (15 normal-weight; BMI 18.5-24.9 kg/m² and 15 obese; BMI 30 -39.9 kg/m²) were recruited for a randomized, cross over design. Subjects were randomly assigned to one of the two meals, custard (HA) or custard with Acesulfame-K (LA, excessively sweet). Blood samples were withdrawn before the meal at time 0 and after finishing it at 15, 30, 60, 120,180 and 240 min and were analyzed for ghrelin, GLP-1, insulin and glucose. Appetite scores were also recorded at the same time points. Acceptability was measured after 2 spoons, when finishing the meal and after 240 min.

Ghrelin levels were significantly higher after the LA meal for both lean and obese subjects. GLP-1, insulin and glucose did not differ between the meals. Appetite scores varied from baseline levels with lower hunger and higher satiety and fullness after the HA meal at 240 min, but failed to reach a significant difference. Energy intake was close to being statistically significant between meals with higher intake after the LA meal. Moreover, lean and obese subjects were significantly different in prospective food consumption, ghrelin, GLP-1, insulin and glucose concentrations and in ad libitum with higher energy intake for obese participants.

These findings show that hedonic properties could affect food intake through stimulation or inhibition of postprandial appetite-related hormones. This offers a possibility to assess the acceptability of foods in formulating diets for a long term weight management solution.

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ABBREVIATIONS

%	Percent
/	Per
=	Equal
±	Plus Or Minus
×	By
≤	Less Than Or Equal To
≥	More Than Or Equal To
°C	Degree Celsius
AEBSF	4-(2-Aminoethyl)-Benzosulfonylfluorid-Hydrochloride
AUB	American University Of Beirut
BMI	Body Mass Index
Ca	Calcium
cm	Centimeter
dL	Deciliters
DPP IV	Dipeptidyl Peptidase Iv
EDTA	Ethylene-Diamine-Tetra-Acetate
et al.	Et Alii (And Others)
FAFS	Faculty Of Agricultural And Food Sciences
FC	Food Consumption
fMRI	Functional Magnetic Resonance Imaging.
g	Gram
GHSR	Growth Hormone Secretagogue Receptor
GI	Gastrointestinal Tract

GLP-1	Glucagon-Like Peptide-1
Goat	Ghrelin O-Acyltransferase
GPCRs	G Protein-Coupled Receptors
HA	High Acceptability
HOMA-IR	Homeostasis Model Assessment Of Insulin Resistance
i.e.	Id Est
K	Potassium
Kcal	Kilocalorie
kg	Kilogram
kJ	Kilojoules
LA	Low Acceptability
m	Meter
m^2	Meter Square
mg	Milligrams
min	Minute
ml	Milliliter
n	Sample Size
NY	New York
pg	Picograms
pM	Picomolar
REE	Resting Energy Expenditure
rpm	Rounds Per Minute
SE	Standard Error
STC1	Stanniocalcin-1
USA	United States Of America

vs.	Versus
WHO	World Health Organization
α	Alpha Significance Level
β	Beta
μU	Micro Unit

CHAPTER I

INTRODUCTION

Although "body image" is placed in high priority nowadays, obesity prevalence is still increasing worldwide. According to World Health Organization (WHO), nearly 600 million adults are obese (BMI ≥30 Kg/m²) (Di Angelantonio et al., 2016) and extrapolations reveal that 20% of adults in the world or around 1 billion people, will be obese by 2030. (Phillips, 2016). This obesity epidemic is due to a state in which energy intake exceeds energy expenditure over a long period of time. Currently, treatments are focusing on lifestyle modifications such as diet and exercise. However, in most cases, results are disappointing and the lost weight is regained within 5 years (De Silva & Bloom, 2012).

In fact, body weight is controlled by a complex phenomenon. Food intake and appetite are controlled by sensory, cognitive, hormonal and metabolic signals (Yin, Hewson, Linforth, Taylor, & Fisk, 2017). It is well known that sensory properties of a food item can direct our choices, preferences and portions, not only before and during, but also after an eating event (McCrickerd & Forde, 2016). Accordingly, understanding the effect of food hedonic properties on postprandial appetite-related hormonal response, offers a possibility of a long term weight management solution.

The principal objectives of the proposed work are to investigate whether the acceptability of a food item has a significant effect on postprandial appetite scores, on energy intake at the next meal and on appetite related hormones (GLP-1, ghrelin and insulin), and to assess whether these trends differ between lean and obese male subjects.

CHAPTER II

LITERATURE REVIEW

A. Food Acceptability

A food's sensory characteristics play a very important role in controlling energy intake within and across meals, and for the most part, palatability has been at the center of this (McCrickerd & Forde, 2016).

Palatability is defined as "the hedonic evaluation of oro-sensory food cues under standardized conditions". People use sensory characteristics such as taste, smell, appearance, texture, sound and trigeminal senses to assess the palatability of a certain food. Several studies have pointed to the primary role of taste in directing food selection or preference. (Anguah et al., 2017). Approximately, 85% of our energy intake comes from sweet or salty foods while less than 15% comes from bitter or sour foods. In fact, small changes in the food taste like adding sweetness, salt, herbs and spices can affect the appetite and food intake. (McCrickerd & Forde, 2016).

B. Taste

Taste is one of the sensory modalities that leads the organism to sense and consume nutrients while avoiding toxic compounds (Gregory C. Loney, 2012). Taste perception starts with the release of transmitters by taste cells to activate gustatory nerve fibers. Taste cells, also known as taste buds are classified into 4 types. Type I cells are the most abundant. Their role is to maintain the buds' structure and terminate synaptic transmissions. Type II, the receptor cells, convey many G protein-coupled receptors (GPCRs) which are in charge of the detection of sweet, bitter and umami tastes. Type III are responsible for expressing synaptic

proteins and generate depolarization-dependent Ca_{2+} transients. Type IV are non-polarized and undifferentiated basal cells (Cai, Maudsley, & Martin, 2014). Salt and sour tastes are detected because they activate ion channels (Cummings, 2015).

Long ago, it was believed that caloric density and macronutrient composition play a crucial role in the release of satiety hormones (Cummings & Overduin, 2007). However, in the past years, it was demonstrated that many metabolic hormones and their cognate receptors are present in the subsets of taste cells. Furthermore, taste receptors that are commonly found on the tongue are also found in the GI. It is also believed that the epithelium on the inner surface of the GI can sense chemical components of the luminal via the gustatory system (Cai et al., 2014; Geraedts, Troost, & Saris, 2011).

1. Receptors in taste cells

Ghrelin, GHSR (its cognate receptor) and GOAT (the enzyme that activates ghrelin) are expressed in type I, II, III and IV taste buds of mice (Figure 1). A study has shown that GHSR null mice show a significant reduced taste responsiveness to salty and sour tastants (Bartell et al., 2010).

In addition, GLP-1 is expressed in type II and type III cells (Figure 1). Its receptors knockout in mice showed a reduced taste response to sucrose and sucralose indicating that GLP-1 acts to maintain or enhance sweet taste sensitivity (Shin et al., 2008). Moreover, disruption of GLP-1 signaling in mice leads to an increase in the sensitivity to umami and sour tastants (Bartell et al., 2010).

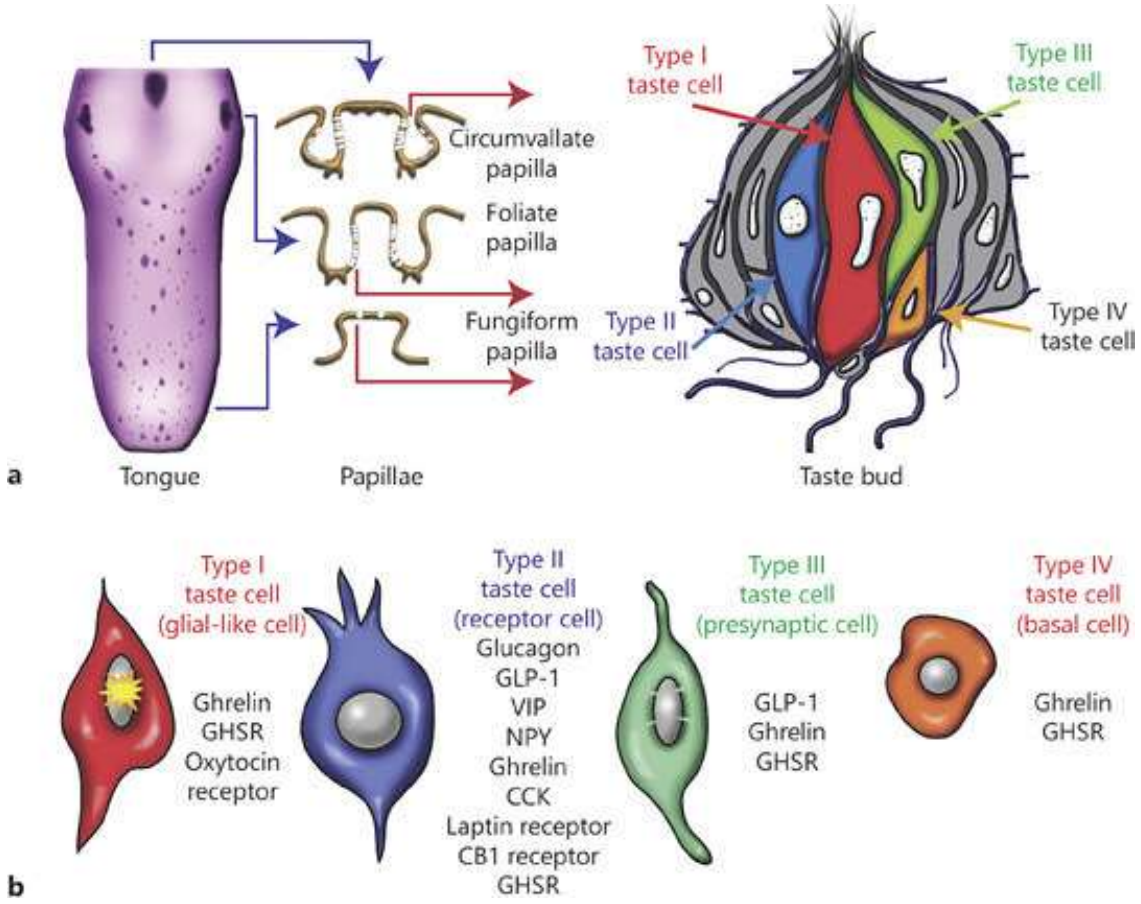


Figure 1. Location of metabolically-related hormones and their receptors within the gustatory system

2. Gut chemosensors

The discovery of taste receptors in the gut has led to intensive research on their functions. They are linked to digestive, metabolic and satiating effects, and they can influence nutrient utilization and inhibit appetite. Moreover, they can provide positive feedback signals, conditioning food preference and stimulate the appetite (Sclafani & Ackroff, 2012)

STC-1 were incubated with different concentrations of sweet, bitter, salty and umami tastants to measure GLP-1. GLP-1 increased depending on the dose and time of all tastants except for the acetic acid. Moreover, bitter tastants stimulated its release only during the first 15 minutes of exposure (Geraedts et al., 2011). (Janssen et al., 2011) showed that bitter taste receptor agonists stimulate the secretion of ghrelin through the gustatory G protein

To examine the effect of infused tastants on hunger, satiety and food intake in subsequent ad libitum meal, subjects received sweet, bitter, umami, all combined or water infusions. All of them being non-caloric, the umami taste and the combination of the 3 tastes increased the satiety and decreased the hunger and ad libitum intake. Neither sweet nor bitter taste exerted any effect. Moreover, these modifications were not accompanied by variations in the concentrations of GLP-1 (van Avesaat et al., 2015)

3. Taste sensitivity:

Individuals have different taste sensitivity that influences their dietary choice, resulting in the acceptance or avoidance of a number of foods (Gregory C. Loney, 2012). This fact played an important role in the survival of human beings by rejecting harmful and poisonous substances. Taste hedonics can be inferred by ingestive behaviors such as initiation or suppression of eating and drinking (Inui-Yamamoto, Furudono, & Yamamoto, 2009). For instance, sweet foods are preferred and are normally indicative of high caloric content whereas, bitter foods are avoided and considered to be indicative of toxicity, unripeness or spoilage (Inui-Yamamoto et al., 2009; Ishii, Blundell, Halford, & Rodgers, 2003).

C. Appetite Regulation:

Appetite suppression is a very common strategy for reducing energy intake (Avena, Murray, & Gold, 2013). Many studies have shown the effect of particular nutrients or ingredients on appetite control. But even foods of equal nutrient content act differently on appetite because many aspects other than the metabolic effects of nutrients are involved in appetite control. This complex interaction or the "satiety cascade" was described 30 years ago by(Blundell et al., 2010). A recent simplified version of this cascade (Figure 2), shows that food choice, satiation and satiety are influenced by cognitive, sensory, post-ingestive and

post-absorptive signals (L. Chambers, 2016). Satiation is the termination of eating within a meal which controls the meal size. It is also known as intra-meal satiety. Satiety is the process when further eating is inhibited, when hunger is declined and fullness is increased after a meal. It is also known as post-ingestive satiety or inter-meal satiety (Blundell et al., 2010).

Before food arrives in the gut, sensory and cognitive processes created by the sight, smell and the experience of food in the oral cavity affect not only the quantity eaten at the same meal, but also the period after consumption. (Lucy Chambers, McCrickerd, & Yeomans, 2015). When food enters the gastrointestinal system, post-ingestive information such as stomach distention, gastric emptying rate, gut peptide hormones, including ghrelin and GLP-1, have significant impact on appetite control.(L. Chambers, 2016). The post-absorptive phase is controlled by insulin, glucose and amino acids (Amin & Mercer, 2016).

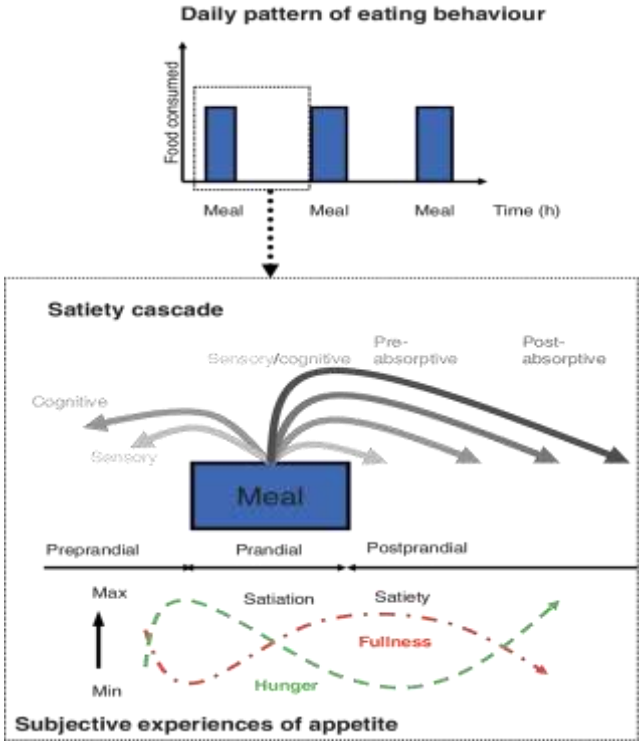


Figure 2. The satiety cascade (Halford & Harrold, 2012)

D. Role of Gut Hormones

1. Ghrelin:

Ghrelin is the only known orexigenic gut hormone, produced in the oxyntic glands of the gastric fundus. Described as a hunger hormone; its circulating levels increase during fasting to stimulate food consumption, and fall after a meal (Chen, 2016). Exogenous ghrelin infusions stimulate appetite and increase food intake (Halford & Harrold, 2012). Ghrelin exerts its orexigenic effect through receptors within the hypothalamus, where ghrelin receptors are concentrated (Hameed, Dhillo, & Bloom, 2009). Furthermore, it regulates body weight, adiposity and glucose metabolism and stimulates gut motility, gastric acid secretion, taste sensation and reward seeking behavior (Muller et al., 2015).

The circulating levels of ghrelin appear particularly sensitive to high-energetic, high osmotic loads. Dietary approaches affecting ghrelin levels such as manipulating the caloric content or the macronutrients composition of the ingested meal can decrease the desire to eat. In fact, a meal with higher energy content increases the lag period for recovery of ghrelin levels and decreases the intake at the next meal (Callahan et al., 2004). Another trial studied the effect of different nutrients on postprandial ghrelin. Lipids are the least effective and proteins are the most effective in lowering the levels of ghrelin. The largest initial drop resulted from the carbohydrates but induced a subsequent rebound to above pre-prandial levels. (Foster-Schubert et al., 2008)

2. GLP-1:

Glucagon like peptide-1 (GLP-1) is an amino acid peptide produced from the cleavage of preproglucagon and is mainly secreted from L-cells (Chaudhri, Small, & Bloom, 2006). GLP-1 is released into the circulation after eating, in proportion to the amount of food consumed (Dailey & Moran, 2013). Levels of circulating GLP-1 tend to rise after a meal and

decrease with fasting (Cappadona, 2016). In fact, GLP-1 shows a biphasic response after a meal. The first one starts within 10 to 15 minutes after meal initiation and is sustained for 30 minutes post-prandially. It involves a neuroendocrine loop where nutrients in the stomach (even before nutrients can access the L cells; lower in the intestine) stimulate the release of gastric inhibitory peptide and gastrin releasing peptide that act through vagal pathways to secrete GLP-1. Whereas the second phase is larger and is triggered by free fatty acids and starts at least one hour after a meal. (De Silva & Bloom, 2012; Shah & Vella, 2014).

Therefore, the secretion of GLP-1 is dependent on the concentration of nutrients. Studies have shown that glucose, triacylglycerol, fructose and some proteins affect directly the secretion of GLP-1. Carbohydrates have a rapid effect while fat, protein and mixed meals show a slower secretion but maintain the increased blood levels for several hours (Steinert, Beglinger, & Langhans, 2016; Wang, Liu, Chen, Li, & Qu, 2015). GLP-1 delays gastric emptying and intestinal motility by vagus mediated pathways. Circulating levels rise after a meal to promote satiety and decrease with fasting. Additionally, it acts by inhibiting glucagon secretion therefore inhibiting the production of endogenous glucose. GLP-1 also acts as an incretin hormone to enhance postprandial insulin synthesis and secretion (Cai et al., 2014; Donnelly, 2012).

3. Insulin:

Long term signals of feeding are regulated by leptin and insulin. Insulin is synthesized in the β cells of the pancreas and secreted in response to increased levels of metabolites after a meal. It increases the storage of glycogen, fat and protein, and has a hypoglycemic effect. Levels of circulating insulin are proportional to adipose tissue mass within the body (Suzuki, Jayasena, & Bloom, 2011).

Insulin is an anorexigenic hormone that regulates the hypothalamic control of food intake to achieve a long term stability of body weight and fat mass. It is the hormone of glucose homeostasis and low blood sugar level is a strong hunger signal. Insulin peaks quickly post ingestion; it is secreted into the blood in response to carbohydrates and protein, and less effectively to fat ingestion. (Chen, 2016; Munsters & Saris, 2014)**.**

E. Effect of Palatability on Appetite:

Effect of palatability on intake

Despite the fact that macronutrients composition influences palatability (foods higher in fat and sugar tend to have higher palatability). Controlled studies have shown that foods higher in palatability are consumed in higher amounts independent of macronutrients composition (Anguah et al., 2017; McCrory, Saltzman, Rolls, & Roberts, 2006). In fact, as palatability increases, the meal size and duration, and eating rate increase too (M. R. Yeomans, Gray, Mitchell, & True, 1997).

(Hill, Magson, & Blundell, 1984) found a difference in the desire to eat as soon as subjects saw the highly preferred or low preferred meal. Actually, an increase in the ratings was observed in response to the highly preferred meal and this increase persisted 2 hours after the test meal.

Sweeteners with different amounts were previously used to modify the palatability of yogurt (Monneuse, Bellisle, & Louis-Sylvestre, 1991; Pérez, Dalix, Guy-Grand, & Bellisle, 1994) and yogurt intake was higher with the optimal sweetener concentration compared to all other concentrations..

In addition, 54 French, subjects were asked to keep their diaries for 7 days along with the palatability ratings of each item eaten and that of the whole meal. The results revealed that higher palatability ratings were related to larger meal sizes (de Castro, Bellisle, & Dalix,

2000). A similar study conducted with 564 North Americans, where only global ratings of the palatability of the entire meal were recorded, showed that meals with higher ratings were 44% larger than the meals rated as having low palatability (de Castro, Bellisle, Dalix, & Pearcey, 2000).

Effect of palatability on hunger and subsequent intake

Hunger is a signal or a state that leads to the initiation of the eating process and ultimately results in the termination of eating. Commonly, these signals originate from the stomach where the vagus nerve transmits the state of emptiness or fullness. These indications are reinforced with the secretion of hormones (ghrelin, GLP-1) and also by metabolic signals such as blood glucose (hypoglycemia or hyperglycemia)(Amin & Mercer, 2016).

(Halliday et al., 2016) compared palatability, hunger and fullness in response to a low or high added sugar diet with adolescents. Subjects consumed equicaloric low added versus high added sugar diet for seven days. Results showed that both diets were reported to be equally palatable with similar hunger ratings. Only a greater fullness after the low added sugar diet was shown.

(Bobroff & Kissileff, 1986) gave subjects ad libitum banana colada frozen yogurt drink with or without adulteration with cumin on non-consecutive days. Subjects felt more satiated and less hungry after the palatable meal reflecting the larger amount eaten. Similar finding were reported by Yeomans et al. (1997) and Hill et al. (1984). Furthermore, another study conducted by (Warwick, Hall, Pappas, & Schiffman, 1993) showed that subjects were less hungry after they ate a palatable meal than after they ate a less palatable preload. However, the sensory properties of the preloads did not influence the total caloric intake at lunch served 5 h later.

On another hand, De Graaf et al. (1999) observed lower hunger ratings after the highly palatable preload. However, when the quantity of three preloads that only differ in palatability was fixed, there was no effect on hunger ratings and on ad libitum intake of the subsequent meal. It was concluded that the pleasantness of food had an effect on satiation but not on satiety (De Graaf, De Jong, & Lambers, 1999). (Rogers & Blundell, 1990) showed no difference in test meal intake, hunger ratings and desire to eat after preloads of different palatability levels. But during the three subsequent hours, a more rapid recovery of hunger feelings and desire to eat were observed after the palatable preload compared with no preload or less palatable preload. In addition, a 24 hour food recall in two studies reported a greater intake after ingestion of the preferred yogurt in men (Monneuse et al., 1991; Pérez et al., 1994).

F. Effect of palatability on appetite hormones:

When people see palatable foods in front of them, their hunger increases because of the activation of certain brain regions associated with ghrelin, mainly the amygdala (Cardinal, Parkinson, Hall, & Everitt, 2002; Holland, 2004).

(Erlanson-Albertsson, 2005) observed that ingested palatable foods (foods rich in fat and sugar) up regulate the expression of satiety signals and at the same time blunt the response to satiety signals. Moreover, it was demonstrated that a steeper decline in post-intake ghrelin was observed when subjects drank a milkshake higher in calories (palatable), compared to when they had the same milkshake but lower in calories on another day (Crum, Corbin, Brownell, & Salovey, 2011). When normal weight male and female subjects consumed Italian cakes with chocolate (pleasurable foods) on one-day and bread, milk, and butter (non-pleasurable foods) on another day, ghrelin levels and other appetite modulators differed. Ghrelin levels showed a statistically significant increase 120 min after consuming

the desirable meal than after the non-desirable one. Therefore, their increase, specifically ghrelin, caused the hunger and satiety scores to be higher after the palatable meals. Ghrelin not only acted as an orexigenic signal but also intervened in the adjustment of reward and driven behavior (Monteleone et al., 2012). Furthermore, a high palatable diet for 2 to 5 weeks for mice and rats lowered the serum ghrelin concentrations in both the fasted and postprandial states (Lindqvist, de la Cour, Stegmark, Hakanson, & Erlanson-Albertsson, 2005).

Few studies were conducted to assess the relationship between palatability and GLP-1. It was suggested that GLP-1 signaling within the taste buds plays an important role in modulating taste sensitivity. When mice were given sucrose, sucralose and citric acid, a reduced taste sensitivity was displayed to both sweeteners while hypersensitivity was shown after the consumption of citric acid which is the less palatable option (Shin et al., 2008). Thus, after consuming a palatable or non-palatable meal, GLP-1 levels would modify taste sensitivity altering the satiety of individuals. When a group of dogs was fed with a fat supplemented diet for 12 weeks compared to a normal diet, fasting plasma GLP-1 levels were 2.5 fold higher in fat fed dogs compared to controls (van Citters et al., 2002).

Administration of a high palatability food during 130 days induced insulin resistance and significantly higher blood glucose (Bock et al., 2015). (Stewart, Jacobs, Jerina, Duren, & Gordon, 2017) assessed the effect of palatability on glucose and insulin, in a recent experiment with horses. Two types of hay that differed in palatability, measured based on the eating rate, were given. The preferred type of hay which has a more consistent nutritional profile, resulted in more pronounced glucose and insulin responses. Furthermore, it was shown in rats that the amplitude of the insulin response is related to the palatability of the food stimulus(Lucas, Bellisle, & Di Maio, 1987).

Normal weight women were asked to choose foods that they found palatable or non-palatable. After an overnight fast on two separate days, the two types of foods were given

over a 2 min period. Subjects were asked to take a bite of the food, chew without swallowing for as long as the food felt comfortable in their mouth, and then expectorate it. Hunger and food palatability were monitored along with plasma insulin and glucose measurements. No significant difference in the magnitude of cephalic phase insulin release was found between the two days. The authors concluded that the actual ingestion of food enhances sensory stimulation and amplifies the changes caused by palatable versus unpalatable food (Teff & Engelman, 1996).

G. **Lean vs. Obese Subjects:**

The relationship between weight status and sweet, salty, sour or bitter tastes is still unclear (D. N. Cox, Hendrie, & Carty, 2016) but most of the literature indicates that obese subjects are less sensitive to sweet and bitter taste than normal weight subjects (Proserpio, Laureati, Bertoli, Battezzati, & Pagliarini, 2016). Moreover, obese people have delayed gastric emptying for both, solid meals or mixed liquid meals. They also have a higher caloric intake per minute when compared to normal weight subjects (Meyer-Gerspach et al., 2014). (Delgado-aros et al., 2004) showed that obese persons require around 225 kcal more than normal weight people to reach maximal satiation.

In general, obese people tend to have a specific preference for high-fat (palatable) foods and were shown to be more sensitive to the palatability of foods, they consume, more than lean controls (Spiegel, Shrager, & Stellar, 1989). Furthermore, the manipulation of palatability has a greater effect on obese subjects than the lean controls for high palatability foods whereas both lean and obese are likely to eat the same amount of a low palatability ice cream (Nisbett, 1968).

1. Ghrelin

Obese patients, with the Padder-Willi syndrome, who are hyperphagic, have very high circulating levels of ghrelin. In most cases, levels of ghrelin are low in obese subjects, especially obese binge eaters which demonstrates the fact that ghrelin is a result of overeating and not the cause (Muller et al., 2015). Moreover, ghrelin levels change with variations in body mass index (BMI), increasing after weight loss and falling with weight gain (Druce et al., 2005). In fact, ghrelin secretion is reduced in obesity states to reduce orexigenic stimulation (Nogueiras, Tschop, & Zigman, 2008). The hunger hormone ghrelin is down regulated in obesity which is a consequence of elevated leptin or insulin resistance since ghrelin levels are negatively linked to fasting levels of leptin and insulin. This down regulation is a physiological adaptation to the positive energy balance in obese people. Obese Pima Indians were found to have a 33% lower fasting plasma ghrelin than lean subjects (Tschöp et al., 2001). Furthermore, in comparison with normal weight controls, obese subjects showed a lower drop in plasma ghrelin after the meal ingestion, leading perhaps to a reduced feeling of satiety (Cappadona, 2016; Suzuki, Jayasena, & Bloom, 2012).

2. GLP-1

No differences were observed between lean and obese subjects regarding the levels and mechanism of GLP-1, when given several standard meals of different calories (Ahweyevu, Bhogal, & Le, 2008) or after fat and glucose infusion (Feinle, Chapman, Wishart, & Horowitz, 2002).

However, differences in GLP-1 concentrations were observed between lean and obese subjects. With an attenuation of the diurnal synthesis rhythm of GLP-1 in obese subjects (Muñoz, Rodríguez, & Morante, 2015). GLP-1 response to food consumption is often reduced in obese subjects compared to lean counterparts (Meyer-Gerspach et al., 2014;

Steinert et al., 2016). In addition, delays in post-prandial release of GLP-1 is also reported in obese individuals, resulting in reduced circulating levels of the peptide (Perry & Wang, 2012; Verdich et al., 2001).

3. Insulin

Fasting insulin and glucose concentrations are significantly higher in obese subjects (Meyer-Gerspach et al., 2016). In the obese population, insulin resistance is a concern where the subject requires more insulin to obtain the biological effect achieved by an inferior quantity of insulin in the normal state (Park, Park, & Sweeney, 2015). After meal consumption, the increase in plasma insulin is higher in obese people, even at a low glucose load. Additionally, a reduced clearance of insulin is observed in obese subjects, which is a factor that contributes to the pathogenesis of the hyperinsulinemia (Meyer-Gerspach et al., 2016).

H. Food compensation:

Food compensation is the adjustment of energy consumption provoked by previous intake of a given stimulus named a preload (Almiron-Roig et al., 2013). The standard technique to study the effects of food intake on short-term appetite is the manipulation of the preload. The test items can vary not only in energy density or macronutrients' composition, but also in taste. In this case, standardization is critical; changes should only affect the satiety-enhancing manipulations, and all other factors should be adequately controlled to be the same. This is very essential when adding a key functional ingredient that triggers distinct changes in sensory characteristics, cognitive impact or hormone release affecting satiety and satiation (Halford & Harrold, 2012).

In a recent study, with four experimental trials, low or high energy preloads were given before an ad libitum consumption of pasta-based (high palatable) or porridge-based (moderately palatable) meals. The consumption of the high energy preload decreased appetite and energy intake in both ad libitum meals. In addition, when given the pasta-based meal which is more palatable, energy intake was more strongly correlated with preceding changes in appetite (Deighton, Frampton, & Gonzalez, 2016).

(Tey, Chia, & Forde, 2016) gave preloads that varied in caloric content but had the same volume, sensory properties and palatability. Despite the big difference in energy content, ranging from 163 kcal to 1176 kcal, no significant difference was found in energy intake at subsequent meals.

CHAPTER III

MATERIALS AND METHODS

A. Subject Selection

Thirty male subjects (15 normal-weight; BMI 18.5-24.9 kg/m² and 15 obese; BMI 30 -39.9 kg/m²) completed the study based on the following criteria after having signed the consent form (Appendix I):

1. Inclusion Criteria

- Gender: Male
- Age: 18-50 years
- Body Mass Index (BMI): 18.5-24.9 kg/m^2 or 30 -39.9 kg/m^2
- Stable body weight for at least three months before the study with the absence of any form of dieting, food restriction or other abnormal eating behaviors (to minimize the effect of weight change on ghrelin and GLP-1 statuses)
- Agreement on the acceptability levels of the two versions (high and low acceptability) of custard

2. Exclusion Criteria

- Smoking
- Substance abuse such as alcohol or drugs
- Medical or psychological illness
- Previous gastrointestinal surgery
- History of weight fluctuation (weight loss of greater than 5% within the past 3 months)

All subjects were students from AUB and were recruited by direct approach. They were briefed about the study before their written informed consent was obtained. This study was approved by the Institutional Research Board of the American University of Beirut (AUB).

B. Experimental Design

The general design of the whole experiment is illustrated in Figure 3. The study was composed of 2 main phases.

During the first phase, subjects were screened using a screening questionnaire (Appendix II). Afterwards, they were asked to take an acceptability test where they had to taste two versions of custard and rate them using a 9-point hedonic scale (Peryam and Pilgrim, 1961) (Appendix III). If their scores fitted the low and high acceptability rating scores requirement (≤ 5 for LA and ≥ 7 for HA), subjects would be recruited. Anthropometric and resting energy expenditure measurements were recorded. Based on the results of phase 1, subjects were included in one of the two groups (normal weight/obese) of phase 2.

In this second phase, blood withdrawals and appetite scores were conducted after the consumption of one of the two versions of custard (low or high acceptability). The experiment was a within subjects repeated measures design; with each subject serving as his own control. Subjects were randomly assigned to one of the two meal/food versions in the first session. After a week, in the second session, they were crossed over to ingest the other meal. Each session was done after a 3 day adaptation period. In addition, an acceptability test was conducted in each session; and at the end, subjects were given an ad lib access to cheese pizza to assess energy intake after the custard meal preload.

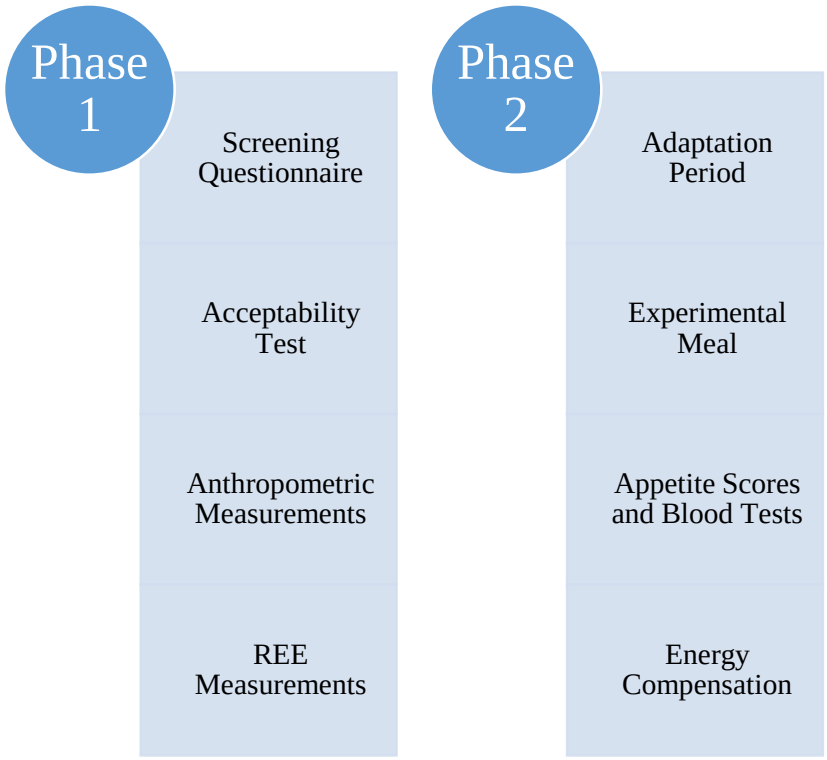


Figure 3. General study Design

C. Data Collection

1. Phase 1

a. Screening Questionnaire

AUB students and/or faculty members were randomly recruited by direct approach and after briefing them about the study. If they were interested, they were asked to read and sign the consent form. In addition, a preliminary screening questionnaire (Appendix II) had to be filled to further confirm the subjects' eligibility and to eliminate those who did not meet the aforementioned criteria.

Subjects were asked to fill the revised version of the Three Factor Eating Questionnaire R-18 (Appendix II) as a screening tool. Three subjects were excluded with high restraint, uncontrolled and emotional eating scores.

b. Acceptability test

Selected subjects were asked to undergo an acceptability test for vanilla custard. According to preliminary trials on several foods, vanilla custard was the best candidate food for modification to yield high (original recipe) and low (modified recipe) acceptability versions. The two versions only differed in palatability and were equicaloric (3.34 kcal/g measured using Parr, 6200 bomb calorimeter, TOWN). The high acceptability custard included 35 grams of custard (Royal, Al Ain, United Arab Emirates), 350 grams of liquid full-fat milk (Candia, Lebanon), 150 grams of cooking cream (Elle et Vire Excellence, France), 80 grams of sugar, 1 gram of locust bean gum (Sigma, St Louis, USA) and 15 drops of liquid vanilla (Foster Clarks products, Malta).

In the low acceptability custard, an additional 3.5 grams of acesulfame-K (HYET Sweet, France) was added to yield the low acceptability version of the meal/food due to its excessive sweetness and bitterness but without an alteration to the food's basic nutrient composition. Custard samples were prepared one day before the test and were stored overnight in a 5°C cooler. Samples were served in 30 ml plastic containers, purchased locally.

Subjects were asked to taste the two versions of custard and rate each of them using the 9-point hedonic scale (Appendix III). Only subjects who agreed on the acceptability levels of the two versions of custard, i.e. ≥ 7 for the high acceptability and ≤ 5 for the low acceptability, were selected to continue the next stages of the study, i.e. the anthropometric measurements and REE measurement, and therefore, ultimately take part in phase number 2 of the study. The rest of the participants were excluded from the study. The acceptability test took place in the Sensory Evaluation Laboratory of the Nutrition and Food Science Department (NFSC) at the American University of Beirut. Panelists were seated in booths with proper lighting and ventilation.

c. Anthropometric Measurements

The following standardized anthropometric measurements were recorded on the screening questionnaire (attached) in order to assess the subjects' BMIs were within the selected ranges for each group. Subjects were divided into two groups: normal weight vs. obese.

- Weight: subjects were weighed to the nearest 0.1kg in light clothes and bare feet using a Seca weight scale (Seca model 877, Germany).
- Height was measured to the nearest 0.5cm with the subject bare foot using a Seca stadiometer (Seca model 213, Germany).
- % body fat and lean body mass were measured using a bioelectrical impedance analysis (Inbody 230)
- Body Mass Index (BMI) was calculated using weight in kilograms divided by squared height in meters.

d. Resting Energy Expenditure (REE)

REE was measured at fasting state using the indirect calorimetry (Quark CPET, COSMED, Albano Laziale,Rome, Italy) to calculate each subject's caloric needs. Each subject was given a meal with 30% of the subject's REE.

REE and all other anthropometric measurements were conducted in room 520 at the Nutrition and Food Science Department at the American University of Beirut

2. Phase 2

a. Adaptation Period

Subjects were advised to follow a 3-day pre-experiment eucaloric diet consisting of 20% protein, 50% carbohydrate and 30% fat of the diet's caloric content.

Moreover, all subjects participating in the study were asked to avoid alcohol consumption or any strenuous exercise for the 24 hours prior to the start of the study but to maintain their normal activity level. Subjects were also asked to fast overnight (12 hours) before the study's experimental sessions, with water consumption allowed.

b. Experimental Diets

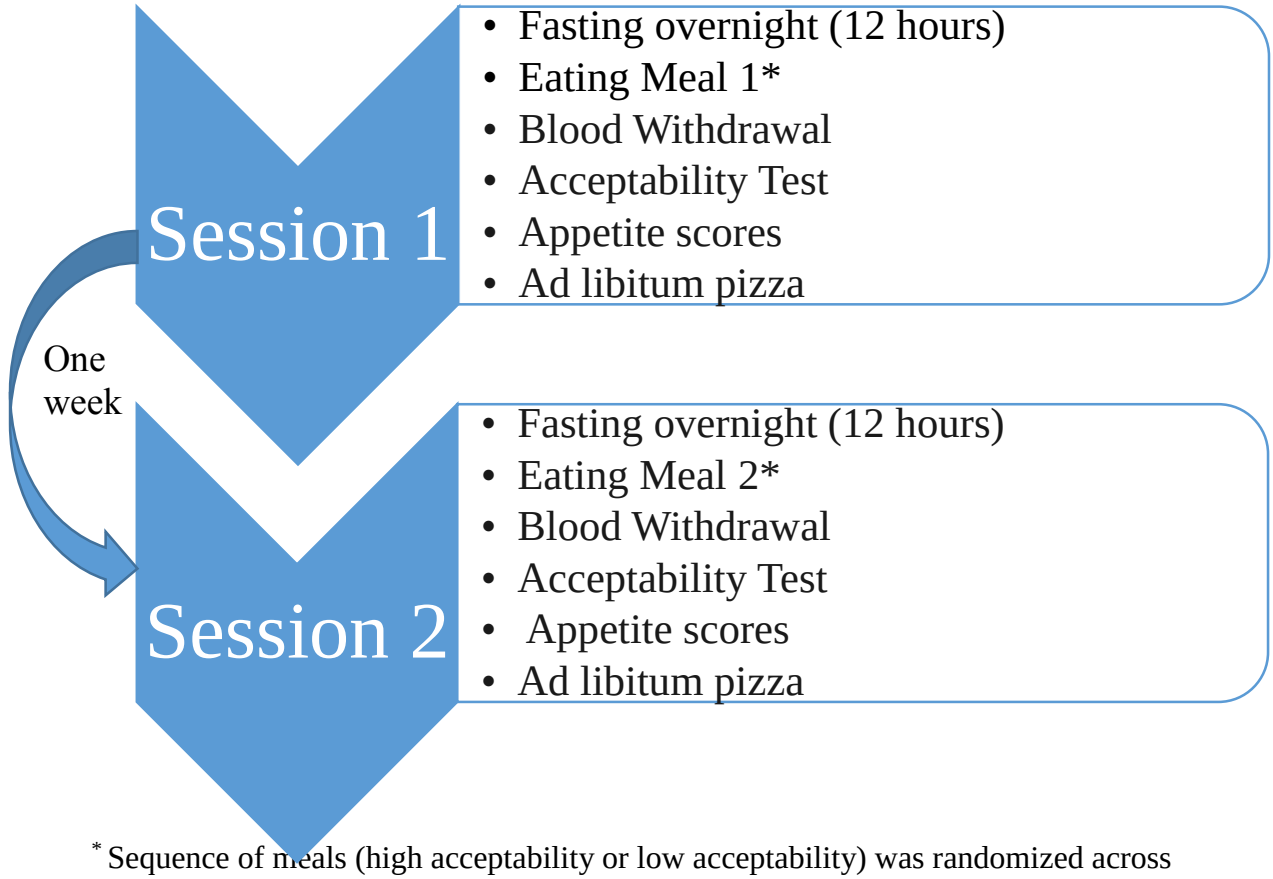
The custard meals provided a caloric content equivalent to 30% of each of the subjects' resting energy expenditure. All meals were prepared one day prior to the test day in the sensory evaluation laboratory of the Nutrition and Food Science Department at the American University of Beirut. The custard was fully consumed within a period of 10-15 minutes. The subjects abstained from any other food item for the 4 hours post-meal ingestion, only water consumption was allowed with a maximum amount of 500 mL.

c. Appetite Scores and Blood Tests

On the days of experimental sessions, subjects made it to the clinical research unit at the American University of Beirut Medical Center (AUBMC), early in the morning, fasting for a fixed 12 hour period. Blood samples were withdrawn, by a registered nurse, using an intravenous catheter that was inserted into an antecubital vein. Subjects kept the needle in their arm for four hours until all samples were collected. The catheter was closed with a heplock with no added heparin. This procedure also included the ingestion of any of the two custard versions of the food (low or high acceptability) within a period of 10-15 minutes.

Each session included an acceptability test, using the 9-point hedonic scale, on three instances: after sampling two spoonfuls, eating the whole portion and after 240 minutes post meal ingestion (Appendix IV). Blood samples and appetite scores were collected at fasting and after ingestion of meals at 15, 30, 60, 120, 180 and 240 minutes (Figures 4 and 5). The

visual analogue scale (Flint et al., 2000) was used for the assessment of appetite scores (Appendix IV). A saline solution was consistently infused following withdrawal in order to irrigate the site and keep the catheter sterile.



* Sequence of meals (high acceptability or low acceptability) was randomized across subjects

Figure 4. Blood Test Session

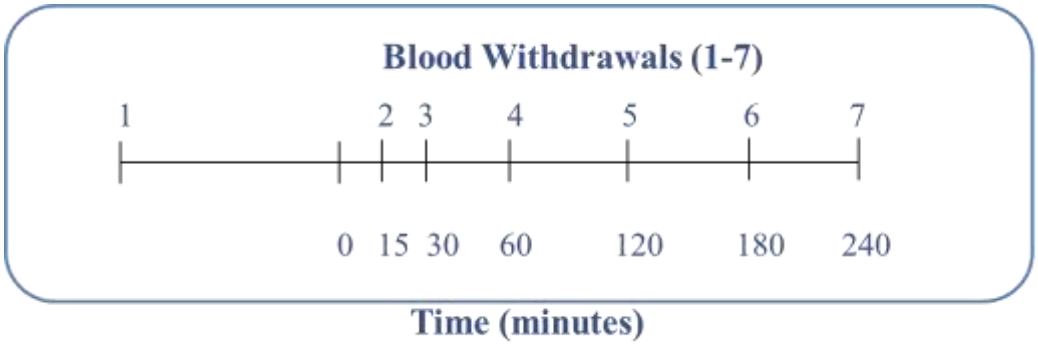


Figure 5. Blood Withdrawals and Appetite scores Timeline

i. Blood analysis

An amount of 5 mL of blood was collected at each withdrawal (a total of 35 mL per session). Blood was placed into 3 separate tubes: one ethylene-diamine-tetra-acetate (EDTA) tube (for GLP-1 determination), one ethylene-diamine-tetra-acetate (EDTA) tube (for ghrelin determination), and one serum separator tube with clot activator (for insulin determination).

For GLP-1 determination, DPP IV inhibitor was added immediately to the blood after withdrawing. Tubes were inverted for good mixing and placed on ice to be centrifuged within one hour. The blood samples were centrifuged at 1000 rpm for 10 minutes at a temperature of 3°C for the separation of plasma.

For ghrelin, insulin and glucose determinations, blood samples were kept on ice and processed within one hour to protect the ghrelin's octanoyl group from being cleaved off. Samples were centrifuged at 3500 rpm for 10 minutes at 3°C for the separation of plasma. After that, plasma samples were acidified with hydrochloric acid (HCl). Pefabloc SC (4-(2-Aminoethyl)-benzosulfonylfluorid-hydrochloride) was added as well in order to protect ghrelin's octanoyl group from being cleaved off.

For insulin determination, blood samples were centrifuged following the same procedure of serum separation.

All plasma and serum samples were stored at -20°C for 4 hours. The samples were then stored at -80°C until the time of analysis. Blood analysis for GLP-1, acylated ghrelin and insulin were performed at the Nutrition and Food Science Laboratory at FAFS, AUB using commercial ELISA Kits (EMD Millipore Corporation, St. Charles, Missouri, USA). Serum glucose levels were also determined by means of commercial enzymatic colorimetric tests on a Vitros analyzer (Ektachem DT60 II System; Johnson&Johnson Clinical Diagnostics, Rochester, N.Y., USA).

d. Energy Compensation

At the end of each session, subjects were given ad lib access to cheese pizza from AUB's cafeteria (due to the standard recipe used there) to assess the energy intake and compensation after the custard preloads. Participants consumed the ad libitum pizza in isolation to prevent any social influence affecting food intake. They were provided with plates containing one slice of pizza, and this was replaced before the participant had emptied the plate with minimal interaction. No time limit was set for eating, and participants were instructed to eat until "comfortably full". Food intake was determined as the weighted difference in food before and after eating.

D. Statistical analyses

A repeated measures analysis of variance was performed to assess the effect of the different predictor variables on the levels of the appetite hormones (ghrelin, GLP-1, insulin) and glucose. Each subject underwent all the treatments and served as his own control. A separate analysis of variance was performed for each hormone. The predictor variables are acceptability level (low and high acceptability), time elapsed (1 through 7 blood withdrawals) and weight status (obese or normal weight). Acceptability level, time elapsed and weight status were discrete variables in the statistical model.

The analysis of variance was performed using the SAS statistical software (SAS version 9.02, SAS Institute Inc., Cary, NC). In the statistical model, the response variable is the hormonal level. Each of the main effects and all two-way interactions were tested as well as the three-way interaction. Furthermore, the same analysis was conducted for the appetite scores, whereby each appetite score rating is the dependent variable. Comparisons were made

between lean and obese subjects, in addition to the interactions of these variables. Significance was pre-established at $\alpha < 0.05$.

CHAPTER IV

RESULTS

A. Subjects Characteristics

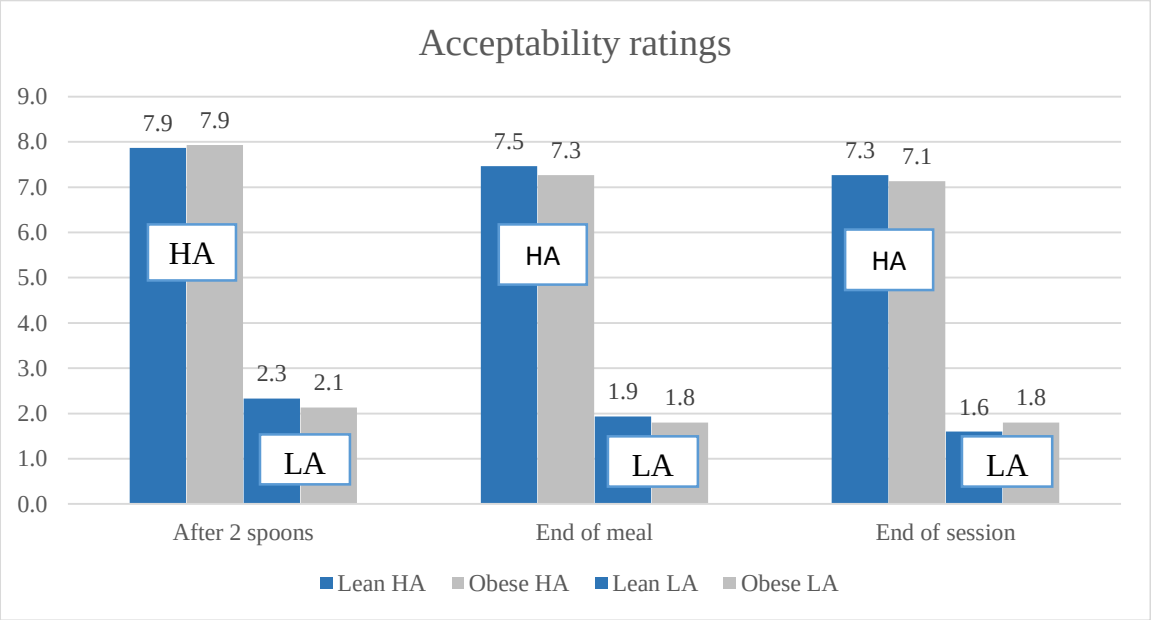
99 participants were recruited for the first acceptability test and 83% responded with a score ≥7 for the HA custard while 67% responded with a score of ≤5 for the LA custard. When both scores were combined, 56 participants (56%) fit the above criteria. Three subjects were excluded with high restraint, uncontrolled and emotional eating scores. The 23 remaining participants did not continue the experiment due to personal reasons unrelated to study activities. Thirty participants were able to proceed with the experiment based on the above.

The subjects' characteristics are summarized in Table 1. All subjects had a BMI within the normal ranges for both lean and obese participants. They were given and thus consumed 30% of their energy requirement in terms of caloric content per subject and meal.

Table 1. Subjects Characteristics; mean ± SE

	Lean (n = 15)	Obese (n = 15)	P value
Age (years)	20.1 ± 0.4	21.7 ± 0.9	0.110
Height (m)	1.79 ± 0.02	1.79± 0.02	0.000
Weight (kg)	70.9 ± 2.8	113.2± 4.3	0.978
BMI (kg/m^2)	22.0 ± 0.5	35.1 ± 1.0	0.000
Fasting ghrelin	243.4 ± 36.3	161.3 ± 9.4	0.037
Fasting GLP-1	11.4 ± 5.2	13.1423 ± 6.1	0.828
Fasting Insulin	24.8 ± 6.0	53.0 ± 12.2	0.047
Fasting Glucose	81.6 ± 1.3	82.8 ± 2.2	0.653

B. Acceptability ratings



Graph 1: Acceptability ratings for both high and low acceptability meals in lean and obese subjects

The two meals were well- tolerated by all subjects. None of the subjects experienced any particular discomfort after eating both meals. The acceptability ratings of the HA and LA meals are illustrated in Graph 1. The two meals were significantly different in acceptability ratings ($P<0.001$). Acceptability ratings of palatability showed that the manipulation of preference was effective. Ratings of the HA custard/meal were significantly higher than those of the LA one at all-time points.

However, there was no significant effect for lean- obese by type of meal (HA, LA) interaction at all-time points. It is essential to note that the mean acceptability ratings for lean and obese were similar at all stages for the two meals.

Moreover, there was no significant time effect on both HA and LA meals given that the time× meal interaction was not statistically significant ($P>0.05$), despite a slight decreasing trend between 0 and 15 and 240 minutes.

C. Appetite scores

The different appetite scores for different time points are summarized in Table 2.

Table 2. Appetite scores (hunger, satiety, fullness, prospective, sweet, salty, savory and fatty food consumption (FC) for high acceptab1ility (HA) and low acceptability (LA) meals at different time points for lean and obese subjects

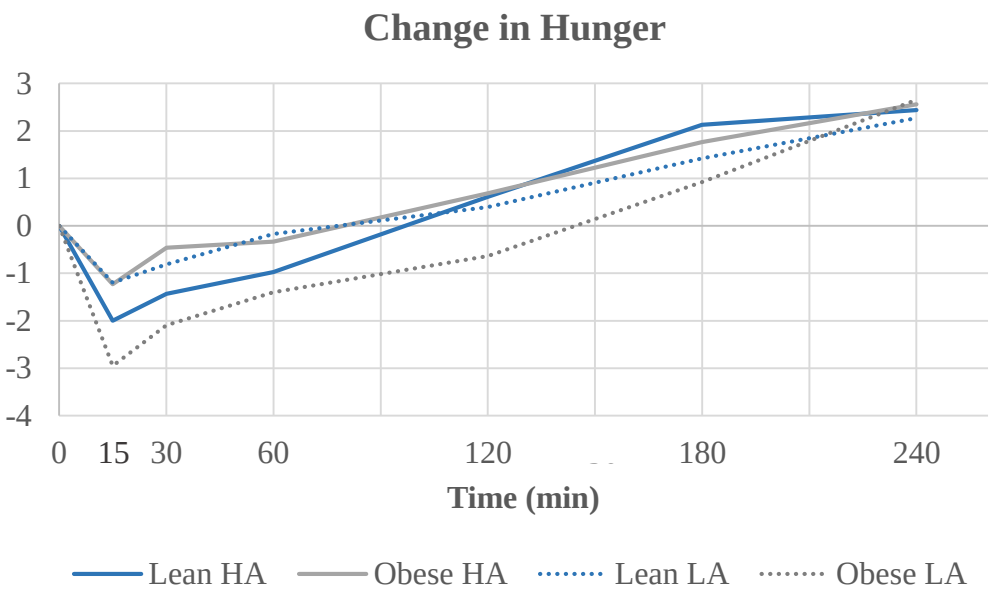
Variable	Meal	0[1]	15	30	60	120	180	240
Hunger								
Lean	HA	5.2 ± 0.5	3.2 ± 0.5	3.4 ± 0.4	4.2 ± 0.4	5.8 ± 0.5	7.4 ± 0.3	7.6 ± 0.4
	LA	5.5 ± 0.6	4.3 ± 0.6	4.7 ± 0.6	5.3 ± 0.6	5.8 ± 0.6	6.9 ± 0.4	7.8 ± 0.4
Obese	HA	4.9 ± 0.7	3.7 ± 0.6	4.4 ± 0.5	4.5 ± 0.6	5.6 ± 0.6	6.6 ± 0.7	7.4 ± 0.5
	LA	6.0 ± 0.6	3.0 ± 0.5	3.9 ± 0.6	4.6 ± 0.5	5.3 ± 0.5	6.7 ± 0.4	7.9 ± 0.3
Satiety								
Lean	HA	3.0 ± 0.6	5.6 ± 0.5	5.5 ± 0.5	5.0 ± 0.5	3.6 ± 0.3	2.6 ± 0.4	2.1 ± 0.4
	LA	2.7 ± 0.5	4.8 ± 0.6	5.0 ± 0.5	3.7 ± 0.5	3.6 ± 0.4	2.3 ± 0.3	1.5 ± 0.2
Obese	HA	3.0 ± 0.7	4.9 ± 0.8	4.7 ± 0.6	4.3 ± 0.7	3.9 ± 0.6	2.5 ± 0.6	2.7 ± 0.6
	LA	3.2 ± 0.7	5.6 ± 0.5	4.4 ± 0.5	4.5 ± 0.6	4.1 ± 0.6	2.5 ± 0.4	2.0 ± 0.3
Fullness								
Lean	HA	3.1 ± 0.6	5.4 ± 0.5	5.1 ± 0.5	4.6 ± 0.4	3.5 ± 0.2	2.3 ± 0.4	1.9 ± 0.4
	LA	2.7 ± 0.5	4.3 ± 0.6	4.9 ± 0.5	4.0 ± 0.4	3.8 ± 0.4	2.7 ± 0.3	1.7 ± 0.2
Obese	HA	3.0 ± 0.7	4.6 ± 0.7	4.5 ± 0.6	4.4 ± 0.6	3.4 ± 0.5	2.3 ± 0.5	2.6 ± 0.6
	LA	3.1 ± 0.7	5.5 ± 0.5	4.4 ± 0.5	4.6 ± 0.6	4.2 ± 0.5	3.0 ± 0.4	2.2 ± 0.4
Prospective FC								
Lean	HA	6.0 ± 0.6	4.5 ± 0.5	4.8 ± 0.5	4.8 ± 0.5	6.6 ± 0.4	7.7 ± 0.3	7.7 ± 0.4
	LA	6.2 ± 0.5	5.1 ± 0.4	5.3 ± 0.4	5.7 ± 0.4	6.2 ± 0.5	7.2 ± 0.4	7.9 ± 0.4
Obese	HA	6.6 ± 0.5	5.3 ± 0.6	5.2 ± 0.6	5.4 ± 0.6	6.7 ± 0.5	7.3 ± 0.6	7.7 ± 0.5
	LA	6.4 ± 0.5	4.0 ± 0.5	4.2 ± 0.5	5.1 ± 0.6	6.0 ± 0.5	6.9 ± 0.4	7.5 ± 0.3
Sweet FC								

Lean	HA	3.6 ± 0.6	5.4 ± 0.6	5.4 ± 0.6	5.3 ± 0.5	5.9 ± 0.5	5.6 ± 0.7	6.1 ± 0.6
	LA	3.7 ± 0.6	6.0 ± 0.8	5.4 ± 0.6	5.9 ± 0.6	5.7 ± 0.6	5.8 ± 0.7	6.2 ± 0.7
Obese	HA	3.5 ± 0.6	6.1 ± 0.6	5.7 ± 0.7	5.6 ± 0.6	5.5 ± 0.6	5.3 ± 0.7	5.7 ± 0.7
	LA	3.8 ± 0.6	6.9 ± 0.7	6.3 ± 0.7	6.1 ± 0.6	6.0 ± 0.6	6.2 ± 0.7	5.5 ± 0.6
Salty FC								
Lean	HA	4.3 ± 0.6	4.3 ± 0.5	4.5 ± 0.5	4.3 ± 0.5	3.5 ± 0.6	2.9 ± 0.6	3.0 ± 0.6
	LA	4.4 ± 0.6	4.5 ± 0.6	4.0 ± 0.6	4.5 ± 0.5	4.1 ± 0.6	3.4 ± 0.7	3.4 ± 0.7
Obese	HA	3.4 ± 0.5	3.8 ± 0.6	3.8 ± 0.6	3.9 ± 0.6	3.9 ± 0.7	3.6 ± 0.7	2.8 ± 0.4
	LA	3.5 ± 0.5	3.9 ± 0.4	4.3 ± 0.4	4.2 ± 0.6	3.8 ± 0.6	3.5 ± 0.6	2.9 ± 0.5
Savory FC								
Lean	HA	5.2 ± 0.6	4.8 ± 0.3	4.7 ± 0.5	5.0 ± 0.4	4.0 ± 0.5	3.8 ± 0.4	4.2 ± 0.6
	LA	4.7 ± 0.6	4.8 ± 0.6	5.0 ± 0.5	5.1 ± 0.5	4.8 ± 0.5	4.4 ± 0.6	3.9 ± 0.6
Obese	HA	3.6 ± 0.6	4.3 ± 0.6	4.7 ± 0.7	4.6 ± 0.7	4.4 ± 0.7	3.8 ±0.8	3.1 ± 0.5
	LA	4.0 ± 0.7	4.7 ± 0.7	4.3 ± 0.7	4.7 ± 0.9	4.5 ± 0.8	3.8 ± 0.8	3.6 ± 0.7
Fatty FC								
Lean	HA	6.1 ± 0.6	6.3 ± 0.6	6.5 ± 0.6	6.1 ± 0.6	5.1 ± 0.6	4.1 ± 0.7	3.9 ± 0.8
	LA	6.8 ± 0.5	6.9 ± 0.6	6.5 ± 0.6	6.3 ± 0.6	5.5 ± 0.7	4.7 ± 0.7	3.9 ± 0.8
Obese	HA	5.0 ± 0.7	6.3 ± 0.8	6.2 ± 0.8	6.4 ± 0.7	5.5 ± 0.8	4.9 ± 0.8	4.6 ± 0.8
	LA	5.0 ± 0.7	5.6 ± 0.7	5.7 ± 0.7	5.5 ± 0.7	5.1 ± 0.8	4.9 ± 0.8	4.4 ± 0.8

[1]Time in minutes

1. Hunger

Hunger ratings showed that there was no significant difference between meals, and between lean and obese subjects. However, there was a significant time effect ($P<0.001$). Changes in hunger ratings are illustrated in Graph 2.

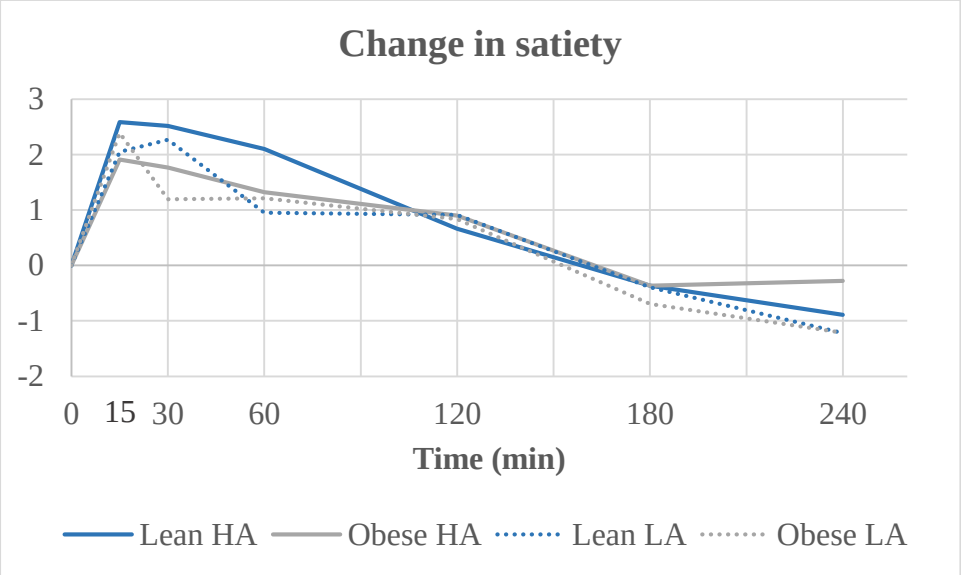


Graph 2: Changes in hunger ratings from baseline for the HA and LA meals in lean and obese subjects at different time points

Similarly, when comparing the changes from baseline, there was a significant time effect ($P<0.001$). In fact, there is a clear decrease in ratings for both meals in lean and obese males at 15 min. This fall is more pronounced after consuming the LA meal in obese subjects. Whereas, hunger rating decreased more after the HA meal in lean subjects. All ratings increased from 15 min onwards to reach their highest levels at 240 min with no significant difference between meals at the above time point.

2. *Satiety*

There was no significant difference between meals, and between lean and obese subjects for satiety. However, there was a significant time effect (P<0.001).

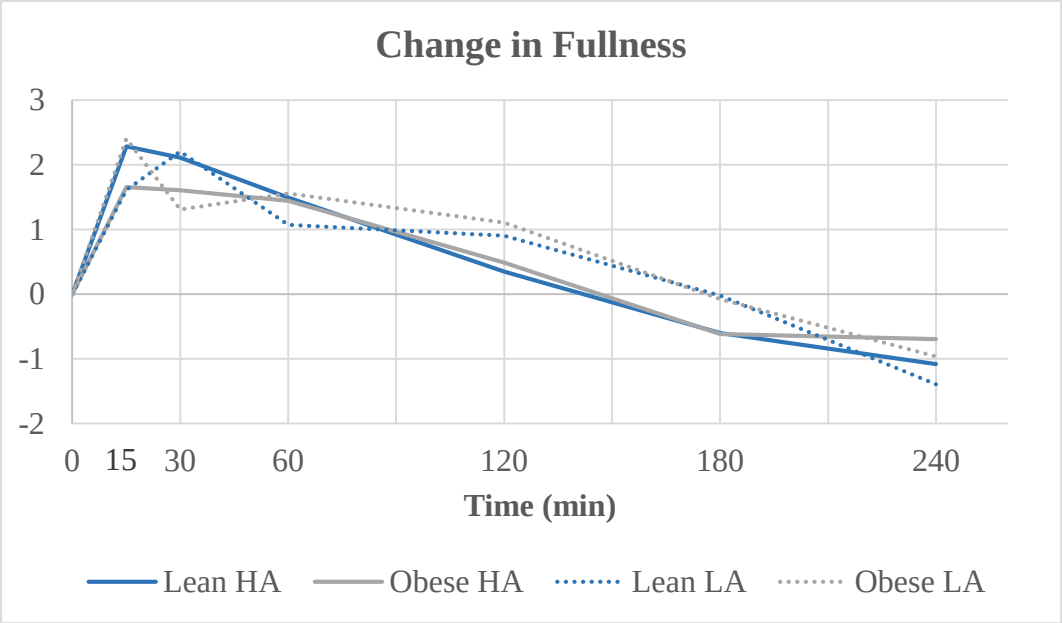


Graph 3: Changes in satiety ratings from baseline for the HA and LA meals in lean and obese subjects at different time points

Changes in hunger ratings are illustrated in Graph 3. Ratings increased sharply from 0 to 15 min in all cases. In lean subjects, ratings remain steady between 15 and 60 minutes after the HA meal, while they dropped at 30 min for the LA meal. All ratings increased sharply for obese subjects between 0 and 15 min. Afterwards, ratings with the HA meal decreased slowly till 120 min and sharply till 180 min followed by a steady level till 240 min. On the other hand, levels decreased sharply after the LA meal, specifically after 15 min onwards to reach a lower level at 240 when compared to the HA meal, once more without reaching a significant difference.

3. Fullness

Means of fullness ratings showed that there was no significant difference between meals, and between lean and obese subjects. However, there was a significant time effect (P<0.001). Satiety and fullness results mirrored each other as shown in Graph 4.

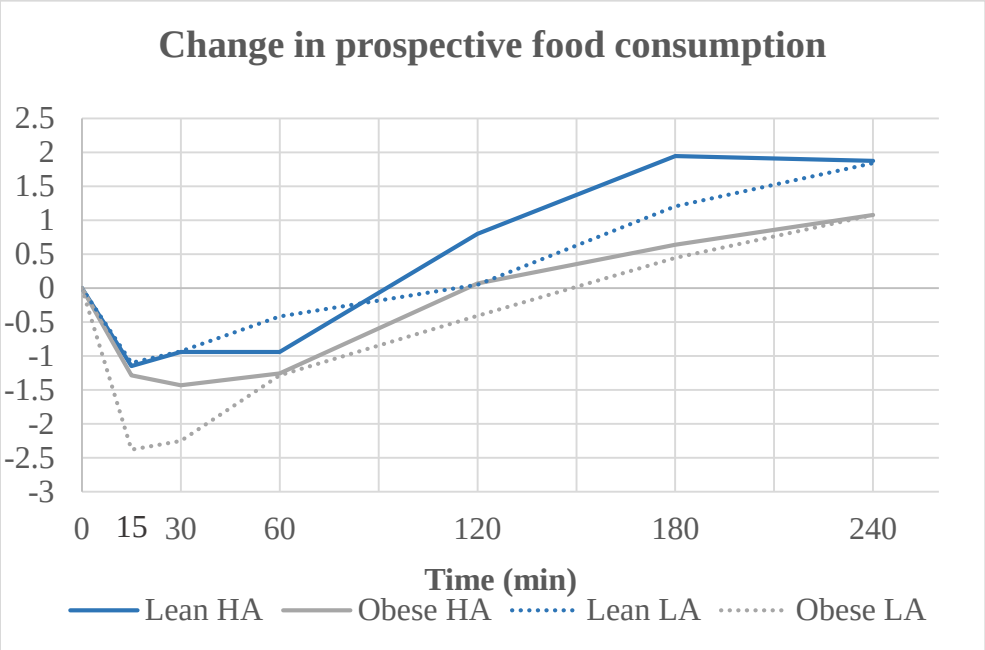


Graph 4: Changes in fullness ratings from baseline for the HA and LA meals in lean and obese subjects at different time points

In lean subjects, ratings increased from 0 to 15 min sharply, then they remained steady between 15 and 60 minutes after the HA meal. As for the LA meal, ratings increased from 0 to 30 min before they dropped from that time onwards. All ratings increased sharply between 0 and 15 min for obese subjects. Afterwards, ratings for the HA meal remained steady from 15 till 60 min, then decreased till 180 min followed by a steady level till 240 min. After consuming the LA meal, ratings decreased after 15 min onwards and they were lower than ratings after the HA meal at 240 min with no significant difference.

4. Prospective food consumption

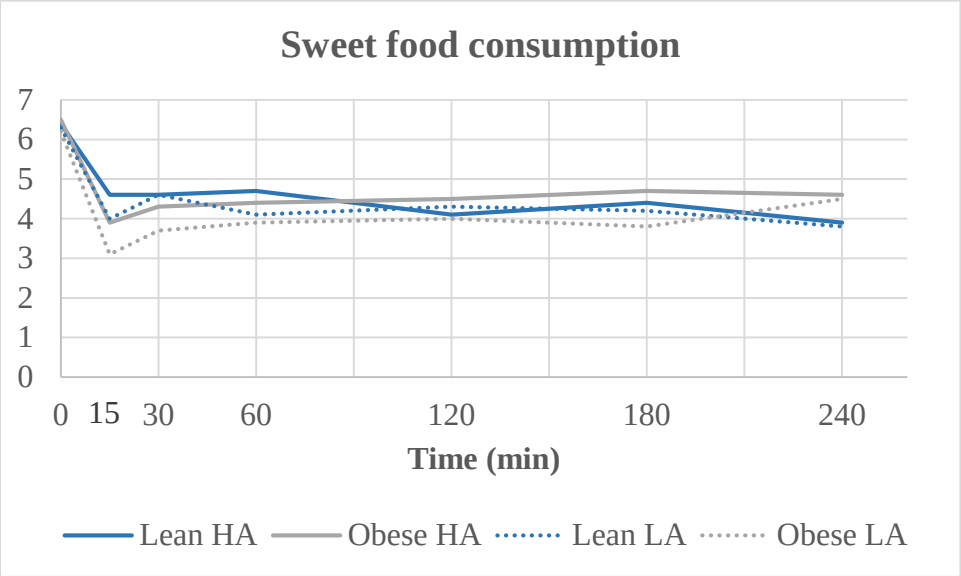
Means of prospective food consumption revealed that there was no significant difference between meals, and between lean and obese subjects. However, there was a significant time effect ($P<0.001$). Moreover, the interaction between lean-obese × time was close to significance ($P=0.059$), meaning that the response of lean and obese significantly differed over time. Change in prospective food consumption from baseline is illustrated in Graph 5. A significant difference between time ($P<0.001$) and between lean and obese ($P<0.05$) was shown. In fact, in lean subjects, the prospective food consumption decrease from 0 to 15 min for both meals. After the HA meal it remains steady for 60 min, but it starts increasing at 15 min after the LA meal. At 240 min, they both reach approximately the same value. In obese subjects, after the HA meal, levels decrease at 15 min, remain stable from 15 to 60 min and then increase to reach their maximal levels at 240 min. After the LA meal, levels decrease sharply at 15 min, remain steady for 30 min and then increase to reach approximately the same value as the HA meal at 240 min.



Graph 5: Changes in prospective food consumption ratings from baseline for the HA and LA meals in lean and obese subjects at different time points

5. Sweet food consumption

Means of sweet food consumption show a significant time effect ($P<0.001$) with no significant difference between meals and between lean and obsess subjects. Sweet food consumption ratings are illustrated in Graph 6.

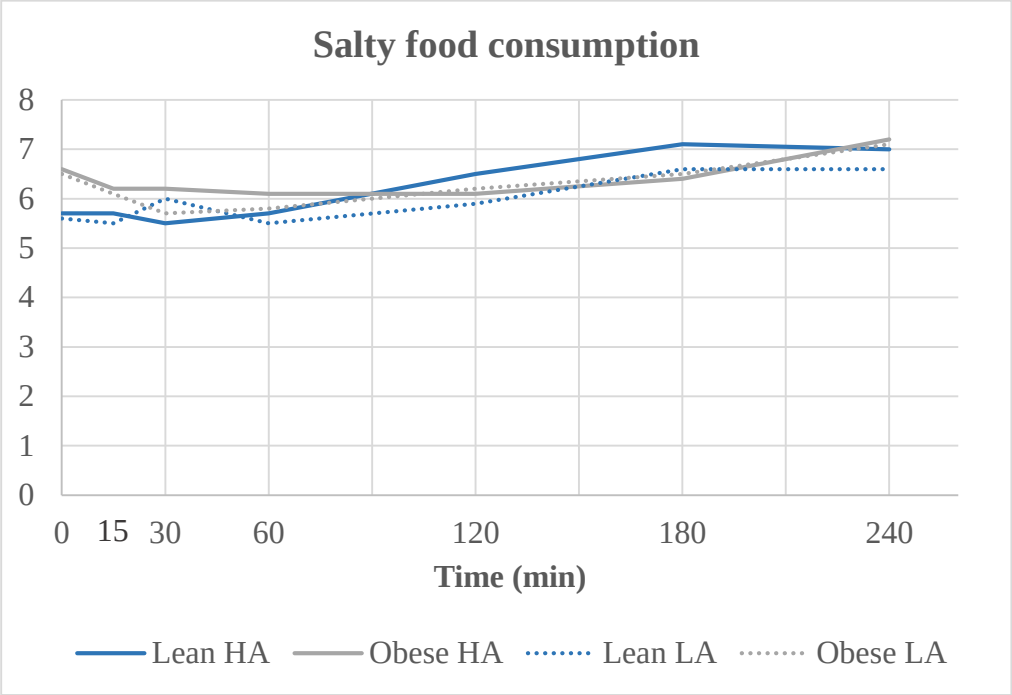


Graph 6: Sweet food consumption ratings for both HA and LA meals in lean and obese subjects at different time points

In fact, the desire for sweet food consumption decreased sharply after eating both meals, due to their sweet nature. The decrease is more pronounced after the LA meal for lean and obese subjects. Afterwards, all levels slightly increase to remain steady till the end, without significant difference between meals and subjects.

6. Salty food consumption

There was a significant time effect among the mean values ($P<0.05$). Ratings are illustrated in Graph 7.

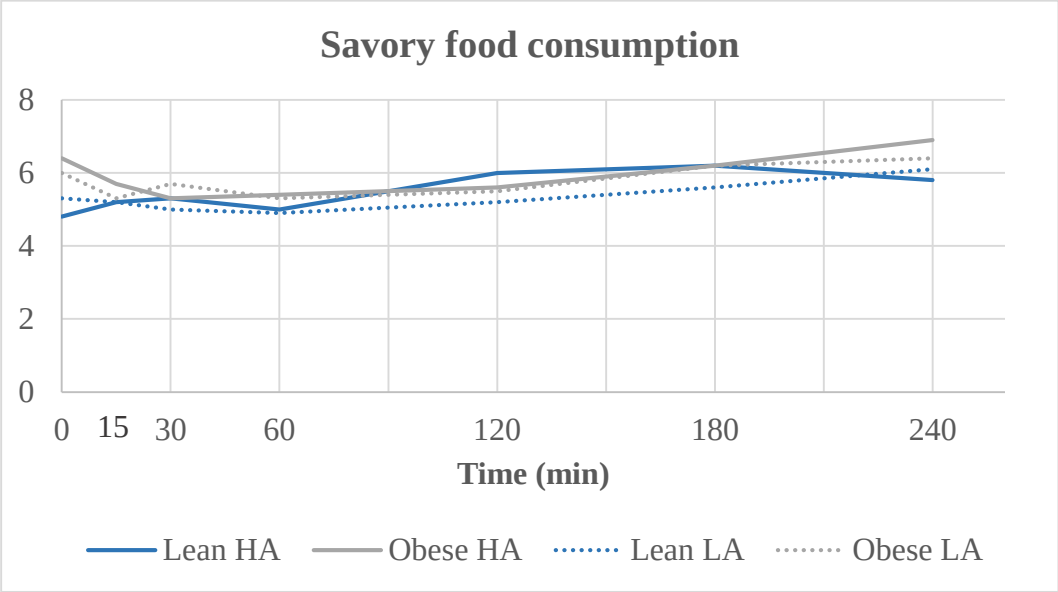


Graph 7: Salty food consumption ratings for both HA and LA meals in lean and obese subjects at different time points

In lean subjects ratings remain steady till 60 min and then increased slightly to become steady after 180 min. at 240 min, ratings were higher after the HA meal without reaching a significant difference. In obese subjects, ratings slightly decreased at 15 min to remain steady till 120 min. they increased after that to reach approximately the same value for both meals.

7. Savory food consumption

No significant difference was shown with the ratings of savory food consumption (Graph 8).

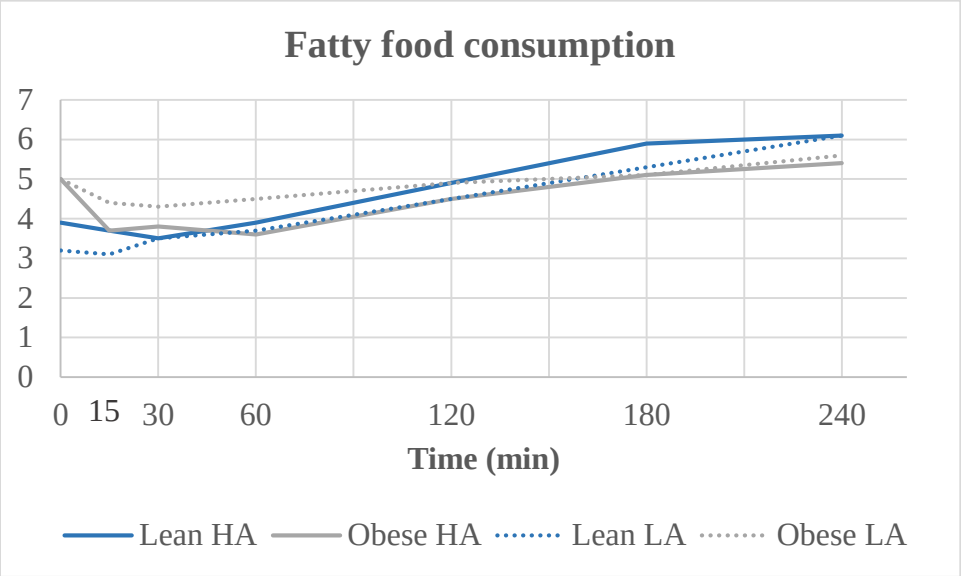


Graph 8: Savory food consumption ratings for both HA and LA meals in lean and obese subjects at different time points

All ratings slightly decreased after consumption and then increased from 60 min onwards except in lean subjects after the HA meal. In this case, ratings increased till 30 min, then fluctuate to reach their maximal level at 180, followed by a slight decrease thereafter.

8. Fatty food consumption

Fatty food consumption ratings show that there was no significant difference between meals, and between lean and obese subjects (Graph 9). However, there was a significant time effect ($P<0.001$).



Graph 9: Fatty food consumption ratings for both HA and LA meals in lean and obese subjects at different time points

In lean subjects, the desire to eat something fatty decreased after the HA meal till 30 min and then increased to reach the maximal value at 240 min. After the LA meal, the ratings remain steady for the first 15 min and then increased to reach approximately the same value at 240 compared to the HA meal. In obese subjects, the desire to eat something fatty after the LA meal decreased at 15 min and then increased slightly to reach the highest value at 240 min. After the HA meal, the decrease at 15 min was lower when compared to the LA meal. Levels then remain steady and then increased from 60 min onwards. The desire to eat something fatty was higher at 240 min after the LA meal without a significant difference.

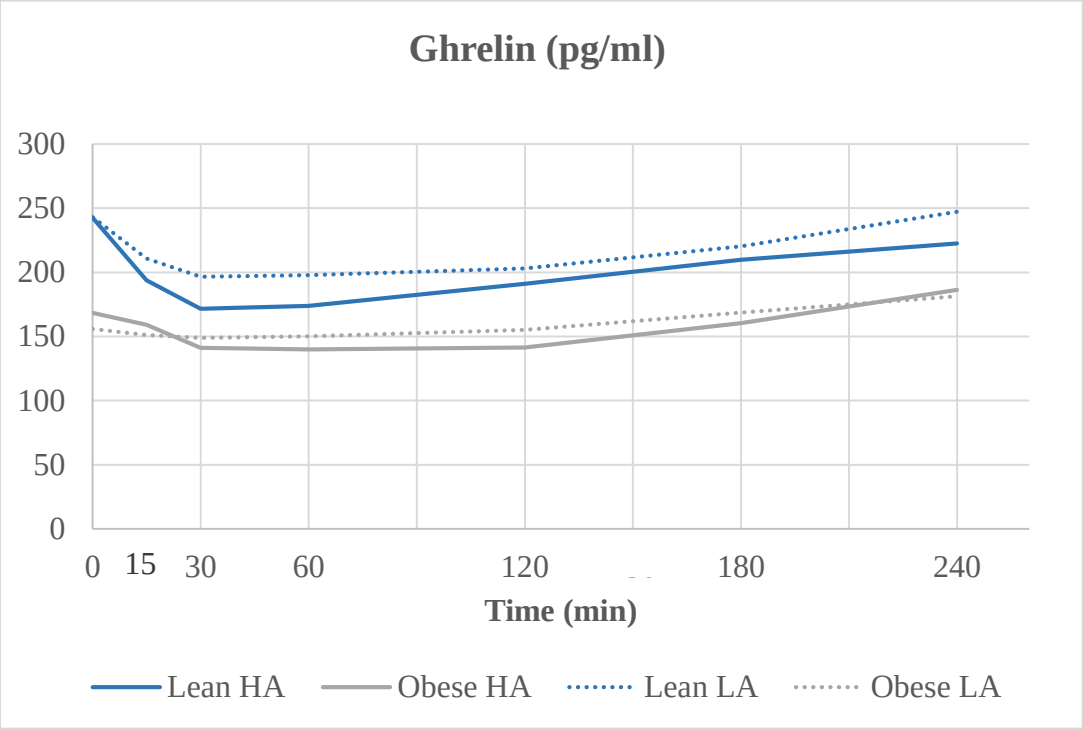
D. Hormones

1. Ghrelin

The active ghrelin concentrations are illustrated in Graph 10. There was no significant difference between meals (P= 0.115). Moreover, there was a significant difference between lean and obese (P<0.001), and a significant time effect (P<0.05). Furthermore, there was no significant difference regarding meal× time, lean-obese ×meal- lean and obese ×time interactions.

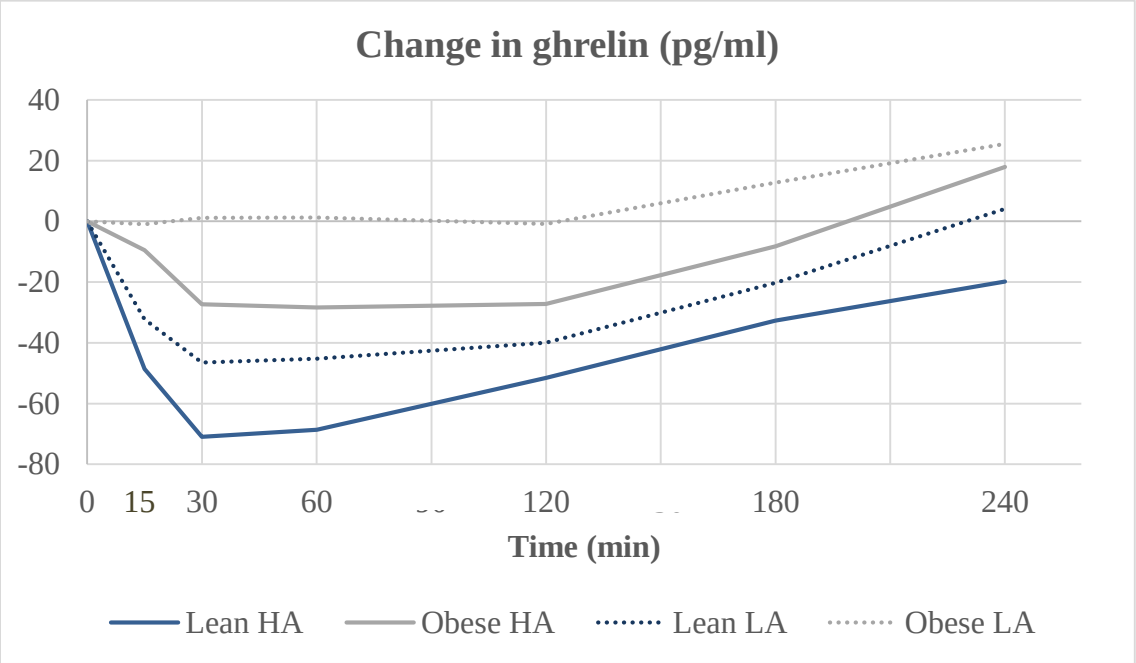
For lean subjects, comparing postprandial ghrelin levels to fasting ghrelin showed a clear decrease from 242.5 ± 41.8 pg/ml to 173.9 ± 18.8 pg/ml at 60 minutes after the HA meal. Levels also decreased from 242.9 ± 39.4 pg/ml to a lower level, 197.7 ± 26.6 pg/ml at 60 minutes after the LA meal. Afterwards, levels increased at 60 minutes for both meals reaching a higher value at 240 min for the LA meal (247.1 ± 30.9 pg/ml) compared to the HA meal at 240 min (222.6 ± 26.5 pg/ml). It failed to reach statistical significance due to the related large standard error.

For obese subjects, comparing postprandial ghrelin levels to fasting ghrelin, results showed a clear decrease from 168.4 ± 14.5 pg/ml to 141.1 ± 11.8 pg/ml at 30 minutes after the HA meal. Ghrelin levels then remained steady and then increased at 120 minutes to reach their maximal levels (186.3 ± 14.3 pg/ml) at 240 min. With the LA meal, ghrelin levels slightly decreased from 155.89 ± 6.56 pg/ml to 151.2 ± 15 pg/ml at 15 min, remain stable then increased at 120 min to reach 181.4 ± 11.6 pg/ml at 240 min.



Graph 10: Ghrelin levels for HA and LA meal in lean and obese subjects at different time points

Due to the large variation in the fasting ghrelin concentrations both among subjects, as well as within subjects prior to the consumption of each of the two meals, the postprandial ghrelin changes from baseline for each meal were also assessed and the results are illustrated in Graph 11.



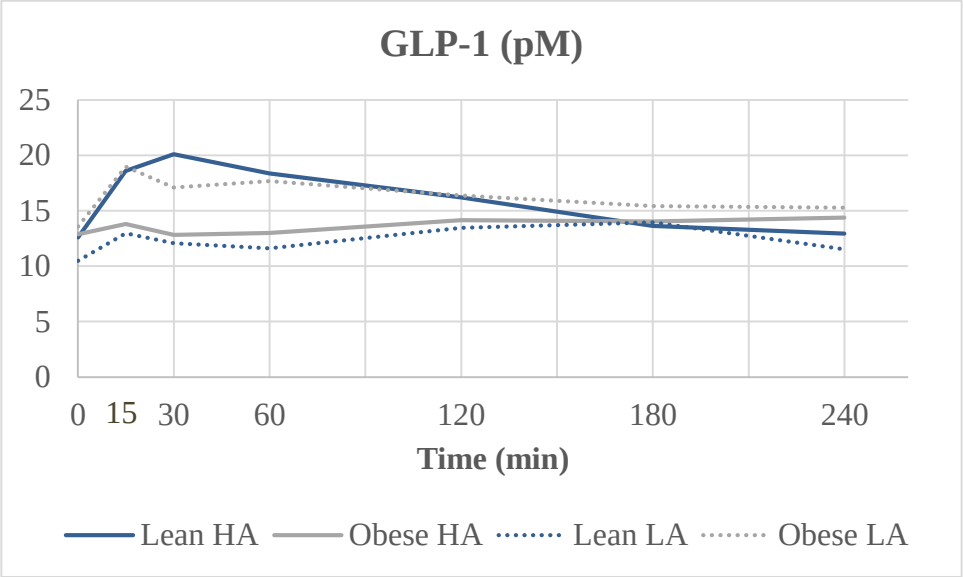
Graph 11: Ghrelin changes from baseline for the HA and LA meals in lean and obese subjects at different time points

Postprandial ghrelin change from baseline is statistically significant between the low and high acceptability meals ($P<0.05$) and between lean and obese subjects ($P<0.001$). There was also a significant time effect ($P< 0.001$).

In lean subjects, a postprandial ghrelin suppression up until 30 minutes was clearly evident. The decrease was higher after the HA meal. Ghrelin levels remained steady and then increased after 60 minutes for the HA and after 120 minutes for the LA meal. However, at 240 minutes, ghrelin levels are higher than the baseline after the LA meal and remained 20 units lower than the baseline after the HA meal.

Ghrelin levels decreased for obese subjects after the HA meal at 30 min, remained constant and then increased at 120 min. However, the concentrations after the LA meal were steady and then increased at 120 minutes. Levels of ghrelin at 240 min were higher after the LA meal when compared to the HA meal.

2. GLP-1



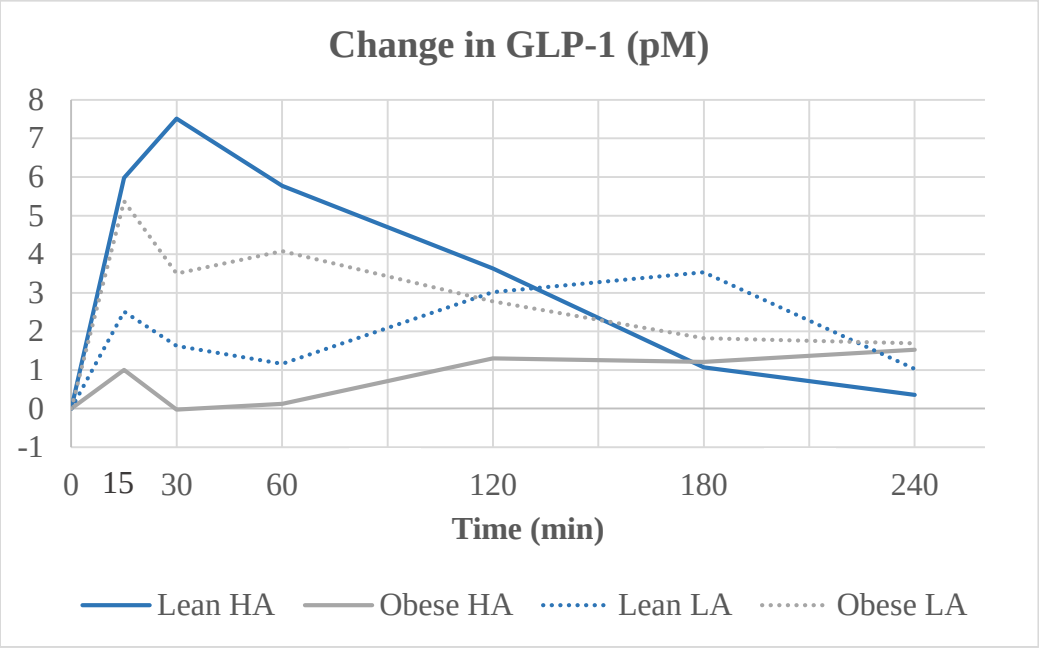
Graph 12: GLP-1 levels for both HA and LA meals in lean and obese subjects at different time points

The active GLP-1 concentrations are illustrated in Graph 12.

Results did not demonstrate any significant difference between meals (P = 0.954), or between lean and obese (P= 0.857) subjects and that there was no time effect (P= 0.993).

GLP-1's response to the HA and LA meal in lean and obese subjects tended to follow dissimilar patterns. In fact, in lean subjects, after the HA meal, GLP-1 levels rose at 15 min and then at 30 min before a sharp drop till 240 min. After the LA meal, GLP-1 increased at 15 min and then at 180 min. This implies that GLP-1 response differed over the sampling time between the meals but it failed to reach statistical significance. For obese males, the response seemed to be steady after the HA meal, with a small increase at 15 and 120 minutes. Whereas after the LA meal, levels increased at 15 and 60 min.

The postprandial GLP-1 changes from baseline were illustrated in Graph 13.



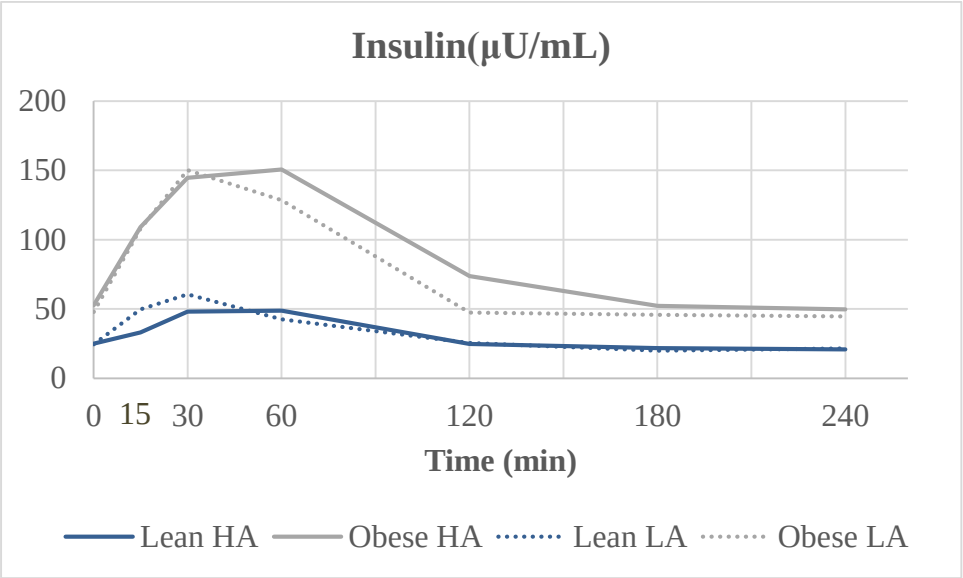
Graph 13: GLP-1 changes from baseline for the HA and LA meals in lean and obese subjects at different time points

Likewise, the postprandial GLP-1 change from baseline failed to reach statistical significance, except for the interaction between lean and obese ×time ($P<0.05$), which indicated a significant difference among GLP-1 levels measured at different time between lean and obese participants. Despite a sharp increase from baseline after the HA meal in lean subjects, there was no significant difference between meals. It is obvious that both lean and obese have a biphasic increase of GLP-1 after the two meals.

3. Insulin

The mean insulin concentrations are illustrated in Graph 14. There was a non-significant difference among the HA and LA meals ($P= 0.503$). However, there was a significant difference between lean and obese subjects ($P< 0.001$) and a significant time effect ($P<0.001$). Moreover, the interaction between lean-obese × time indicated a

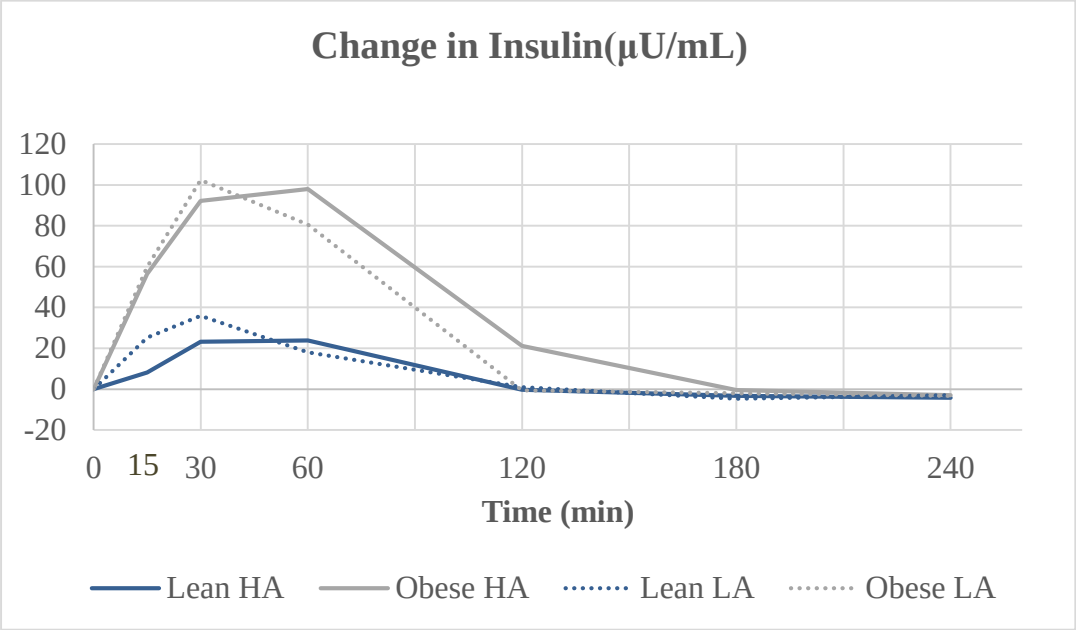
significant difference among the mean insulin levels measured at time 0, 30, 60, and 180.



Graph 14: Insulin levels for both HA and LA meals in lean and obese subjects at different time points

In lean subjects, insulin increased at 15 min in both meals to reach its maximum level at 60 min for the HA meal, and at 30 min for the LA meal. Afterwards, insulin levels decreased to remain steady until 240 min. Insulin promptly increased in obese subjects. Similarly, it reached its highest level at 60 min for the HA meal and at 30 min for the LA meal without a significant difference between the two. Subsequently, insulin levels fell to remain stable until 240 min.

In comparing to the mean levels of insulin, the postprandial insulin changes from baseline revealed the same significant differences between lean and obese, time and their interaction, in the absence of significant differences between the two meals. This is illustrated in Graph 15.



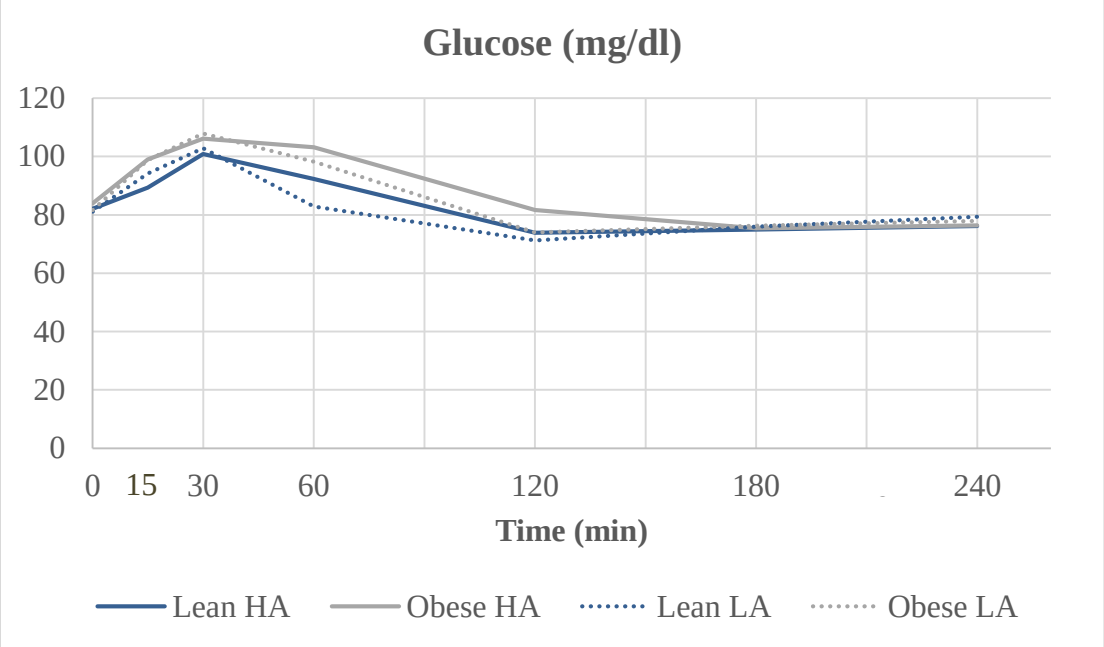
Graph 15: Insulin changes from baseline for the HA and LA meals in lean and obese subjects at different time points

Levels of insulin increased from baseline for both meals with a higher response in obese subjects. In fact, insulin resistance, measured by HOMA-IR is significantly different between lean and obese ($P < 0.05$) with higher values observed among obese (10.24 ± 1.57) than among lean males (5.53 ± 0.897). Levels were highly similar at 180 and 240 min. Insulin results for lean subjects returned to baseline at 120 min for both meals. Levels returned to the baseline at 180 min after the HA meal in obese subjects and after 120 min when consuming the LA meal, without any statistical difference between the meals for obese subjects.

4. Glucose

The results of glucose concentrations are illustrated in Graph 16. There was no meal effect ($P = 0.494$). The absence of a significant meal × time interaction translated the absence of significant differences between the two meals at the same time points.

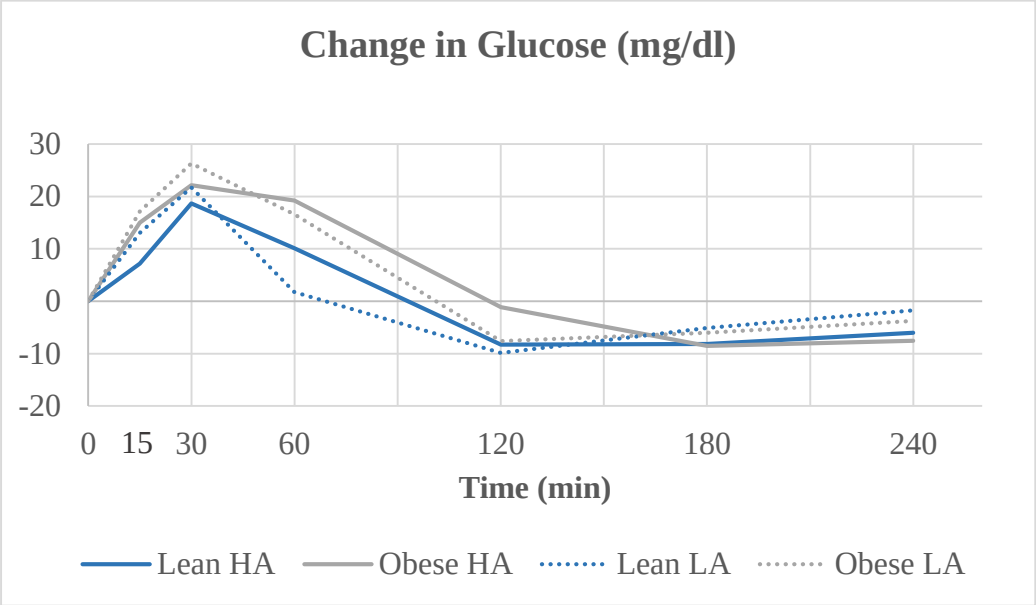
As expected, there was a significant difference between lean and obese males (P< 0.01). In addition there was a significant time effect (P<0.001).



Graph 16: Glucose levels for both HA and LA meals in lean and obese subjects at different time points

All glucose levels maximally increased at 30 minutes. The interaction between lean and obese × time showed a significant difference at 60 min (P< 0.05).

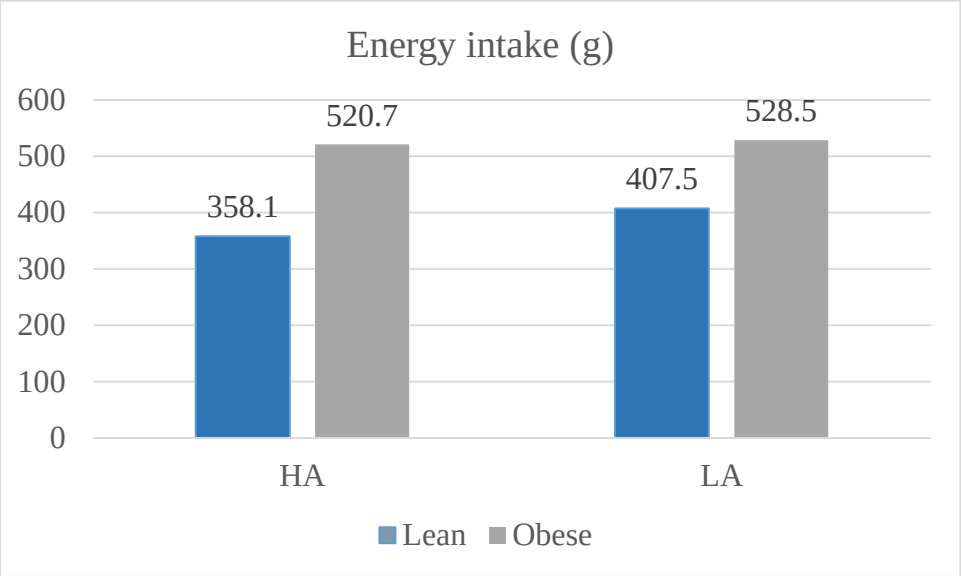
The postprandial glucose changes from baseline revealed the same significant differences between lean and obese and the time effect. Furthermore, it revealed a significant interaction between lean- obese × time (P<0.05). As demonstrated in Graph 17, glucose levels increased to a maximum value at 30 min for both lean and obese subjects after the HA and LA meal. After that, glucose levels dropped to become lower than the baseline from 120 min onwards, followed by a modest increase thereafter.



Graph 17: Glucose changes from baseline for the HA and LA meals in lean and obese subjects at different time points

E. Energy compensation

Add libitum intake of cheese pizza after the HA and LA preloads are illustrated in Graph 18. The difference between preloads was close to being statistically different (P=0.062). In fact, ad libitum intake in terms of quantity was clearly higher after the LA meal for both lean and obese. Moreover, there was a significant difference between lean and obese male subjects (P< 0.001), as would be expected.



Graph 18: Ad libitum intake of cheese pizza after the HA and LA preloads in lean and obese subjects

CHAPTER V

DISCUSSION

This study was designed to have a better understanding of the effect of food hedonic properties on postprandial appetite-related hormonal response to meals in lean and obese males.

The major findings were: 1) in healthy lean and obese male subjects, food acceptability/palatability affects ghrelin and 2) although not significant, LA and HA meals affect postprandial appetite scores and 3) both lean and obese subjects eat a larger quantity in ad libitum cheese pizza after 240 min of the LA preload 4) lean and obese subjects were statistically different in prospective food consumption, ghrelin, GLP-1, insulin and glucose concentrations and in ad libitum energy intake.

In the present study, food type, energy density and food composition are constant for the HA and LA meals. Moreover, the food was consumed within 10 min of the meal onset. The meals only substantially differed in their taste. It must be noted that the quantity of meal was 30% of the REE of each subject to be representative of a somewhat typical breakfast.

A. Acceptability

In this study, the acceptability ratings between tasting the first two spoonfuls of the meal and the end of the session (after 240 min of eating the whole portion) decreased approximately by 0.6 points for lean and by 0.8 for obese subjects after the HA meal. Also, ratings decreased by around 0.6 points for lean and by 0.3 for obese subjects after the LA meal.

According to previous studies, acceptability ratings decrease during the course of a meal (Hill et al., 1984; M. Yeomans, 2000; Zhu & Hollis, 2014). Furthermore, (Bobroff & Kissileff, 1986) showed that after two versions of frozen yogurt that differ in palatability, the post-meal ratings were one unit lower that the taste test ratings on the 9 point hedonic scale.

Moreover, there was no significant difference between lean and obese subjects. In fact, studies have shown that lean and obese do not differ in the selection of foods with apparent tastes and hedonic attributes (D. Cox, Perry, Moore, Vallis, & Mela, 1999; Nasser, 2001).(Mattes & Considine, 2013) showed a non-significant difference in hedonic and sensory responses between lean and obese subjects.

B. Appetite scores

The literature provides different perspectives on whether hedonics affect appetite or not. Subjects were hungrier after a preferred meal when compared to a less preferred one (Bobroff & Kissileff, 1986; Hill et al., 1984; Rogers & Schutz, 1992) and a greater intake was observed after ingestion of the preferred preload (Monneuse et al., 1991; Pérez et al., 1994).On another hand, other studies found that subjects were less hungry after palatable foods(Warwick et al., 1993). Other studies showed no significant differences in appetite scores between the two meals (De Graaf et al., 1999; Johnson & Vickers, 1992; Rogers & Blundell, 1990; M. R. Yeomans, 1996).

In our study, both meals had the same composition, volume, quantity, and energy density to avoid any effect, other than the taste, on appetite scores (Merrill, Cardello, Kramer, Lesher, & Schutz, 2004; Robinson, Gray, Yeomans, & French, 2005). Although no significant effect was found on appetite scores between the two

meal versions, both lean and obese were more satiated and less full after the LA meal at 240 min.

Furthermore, appetite scores showed no significant differences between lean and obese subjects in a similar manner to other studies (Mattes & Considine, 2013; Mourao, Bressan, Campbell, & Mattes, 2007)

In our study, the only significant difference between lean and obese is in prospective food consumption at 240 min. Ratings of obese where lower due to a tendency to underestimate the quantity they can eat (Chandon & Wansink, 2007; Lansky & Brownell, 1982).

The desire for sweet, savory and fatty food followed the same pattern and decreased immediately after consuming the meals but the desire for salty food was unaffected.

Given the sweet nature of both meals, the desire for sweet food consumption declined and remained lower than the baseline. Although not significant, the LA meal resulted in less desire to eat sweet food in both lean and obese subjects. This is to be expected, given the excessive sweet nature of the LA meal, and has been previously shown (Griffioen-Roose, Hogenkamp, Mars, Finlayson, & de Graaf, 2012; Montelius et al., 2014). The desire for eating salty, savory and fatty foods started recovering between 60 and 120 min approximately, with the increase of hunger and decrease of satiety and fullness.

C. Hormones

1. Ghrelin

In this study, ghrelin levels increased to a greater extent, at the end of the experiment, for the LA meal than the HA one for both lean and obese subjects (Graph 11), suggesting that the acceptability of a meal could increase satiety and delay the initiation of the next meal.

Due to the variations in fasting ghrelin, the difference was clearer after the change from baseline. Differences in fasting ghrelin levels may be due to subject characteristics. It may be probable that individuals who regularly consume breakfast have higher fasting levels than those who usually skip it. Moreover, it is possible that the duration of an over-night fast affects ghrelin. In our study, subjects did not fast for a fixed number of hours, but instead fasted for a minimum of 12 hours. Hence, it is probable that ghrelin might have been slightly affected by this factor given that the duration of the fast at the two visits of testing increased or decreased, respectively.

All ghrelin concentrations were lower in obese subjects when compared with lean ones. Fasting ghrelin levels are known to correlate negatively with BMI (Churm, Davies, Stephens, & Prior, 2017; Muller et al., 2015; Tschöp et al., 2001).

In line with previous studies, present findings showed that circulating ghrelin considerably fall after the ingestion of both meals (Merkestein, Dickson, & Adan, 2017; Nedvídková et al., 2003; Tschöp et al., 2001). However, the magnitude and duration of postprandial ghrelin suppression differ across the studies.

In lean subjects, the response was lower, in this work, after the HA meal where the concentrations started increasing at 60 min without reaching the baseline at 240 min. After the LA meal, levels were higher that then baseline at 240 min. These findings

align with a previous study demonstrating a steeper decline in ghrelin post intake after a high palatable milkshake compared to when they had the low palatable milkshake (Crum et al., 2011). Our results for both subjects group were also consistent with a study performed on mice and rats which revealed that a high palatablity diet for 2 to 5 weeks lowers the serum ghrelin concentration in both fasted and postprandial state (Lindqvist et al., 2005).

When comparing lean and obese subjects, obese subjects showed a lower drop in plasma ghrelin after meal ingestion, leading to a reduced feeling of satiety. This was proved by several studies (Cappadona, 2016; Suzuki et al., 2012). Moreover, it is interesting that ghrelin levels in obese subjects, after the LA meal, decreased slightly to remain steady till 120 min and then increased afterwards. Ghrelin levels were not significantly altered after the LA meal. Furthermore, it was clearly apparent that in obese subjects, ghrelin levels became higher than the baseline after 120 min and 240 min for LA and HA meals, respectively, explaining their higher hunger ratings at this time.

Both meals differed only in palatability, meaning that the difference in ghrelin response is related to palatability. Both HA and LA meals had the same macronutrients composition to avoid any composition related effect on ghrelin (Al Awar, Obeid, Hwalla, & Azar, 2005; Tannous dit El Khoury, Obeid, Azar, & Hwalla, 2006). On another note, a study found that ghrelin is likely to be involved in hedonic hunger. Plasma ghrelin increased after a palatable meal more than after the non-palatable one, indicating that highly palatable food might stimulate the secretion of ghrelin even when no calories were needed (Monteleone et al., 2012). But, this latter study was conducted

on 8 subjects, five females and 3 males, and the two meals where of different composition and quantity.

2. GLP-1

Few studies have been conducted to assess the relationship between palatability and GLP-1. In the present study, the biphasic response of GLP-1 is clear in both groups and meals. In lean subjects, although the difference between meals failed to reach statistical significance, GLP-1's response was the highest after the HA meal. These results explained the lower ratings for hunger and higher levels of satiety of fullness for the HA meal.

There was a difference over time between GLP-1's concentrations in lean and obese subjects. Other studies found a reduced GLP-1 response in obese subjects compared to lean counterparts. (Meyer-Gerspach et al., 2014; Muñoz et al., 2015; Nauck, Vardarli, Deacon, Holst, & Meier, 2011; Steinert et al., 2016). In addition, delays in post-prandial release of GLP-1 is also reported in obese individuals, resulting in reduced circulating levels of the peptide (Perry & Wang, 2012; Verdich et al., 2001).

Comparing the aforementioned studies with the present findings reveals different results. Obese subjects' response was attenuated only after the HA meal. The difference is that we measured active GLP-1 unlike all the other experiments where total GLP-1 was measured. In our study, although there was no significant difference between meals, GLP-1 levels were higher after the LA meal, at 240 min, explaining their lower hunger ratings and higher ratings in satiety and fullness when compared to the HA meal.

It is interesting how lean and obese subjects responded differently in GLP-1 concentrations after the two meals. The response might vary depending on their inherent sensitivity to GLP-1. Moreover, there is a wide range of GLP-1 sensitivity and number of receptors within people (Aulinger, Vahl, Wilson-Pérez, Prigeon, & D'Alessio, 2015).

3. Insulin and glucose

Glucose and insulin responses across the meals tended to follow similar patterns, in terms of change over time, indicating similar rates of gastric emptying. Results showed lack of any significant differences between the meals, which confirms the initial plan of having two meals than only differed in palatability with the exact same macronutrients composition and energy density.

The impact of palatability on insulin and glucose in humans showed a higher insulin response after the high palatablity meal (France Bellisle, Drewnowski, Anderson, Westerterp-Plantenga, & Martin, 2012; F Bellisle, Louis-Sylvestre, Demozay, Blazy, & Le Magnen, 1985) and higher serum glucose after the more palatable meal (Sawaya et al., 2001). However, all of these similar studies had different textures for the two meal versions.

Both fasting insulin and glucose concentrations were significantly higher in obese subjects. Moreover, insulin resistance is a concern with a HOMA-IR =10.24 ± 1.57 in obese subjects. Thus, insulin response in obese was significantly higher than that in lean subjects. This is consistent with other studies (Meyer-Gerspach et al., 2016; Park et al., 2015; Thota, Perez-Lopez, Benites-Zapata, Pasupuleti, & Hernandez, 2017).

D. Energy compensation

Although there was no significant effect for appetite scores between the two meal versions, both lean and obese were more satiated and less full after the LA meal. This explained the results in the ad libitum pizza intake, where the quantity eaten after the LA meal was higher in all subjects and was close to being statistically significant (P=0.062)**.** *Moreover, there was a significant difference between lean and obese subjects with a higher intake in obese males, as expected* (Brede et al., 2017; Delgado-aros et al., 2004; Meyer-Gerspach et al., 2014).

Diet products and weight loss diets are branded by the absence of taste enhancing ingredients such as fat and sugar. But, results so far have been mostly disappointing due in part to low compliance. One of the main causes of poor adherence is the acceptability or palatability of meals (MacLean et al., 2015). The value of this work thus lies in indicating the importance of a good taste in satisfying one's desire to eat.

This is the first study, to our knowledge, where the impact of aversive taste has been shown in acceptability, appetite scores and appetite hormones along with the impact of taste on energy intake at the next meal. It can be distinguished from all of the aforementioned studies, since it is the only one where the preloads only differed in taste with the same macronutrients composition, quantity and energy density. Moreover, the present study compared the response of lean and obese subjects. The major limitation of the present study is that it assessed the response of acceptability manipulation in males only and thus, any gender differences may have been missed. Secondly, a larger number participants would have provided more strength to the results.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

In conclusion, the present findings demonstrate that food acceptability/palatability affects the appetite hormones: ghrelin, GLP-1 and insulin in healthy lean and obese male subjects. This study also indicates that a manipulation of the diet based on palatability of preloads might affect the energy intake at the next meal, whereby eating a palatable food may satisfy one's desire and reduce intake at the following meal. Thus, understanding the effect of food hedonic properties can offer a potential long term solution in weight management and could assist in curbing obesity.

A larger number of each BMI group would have provided more strength to the results, particularly in appetite scores and energy compensation, where some trends did not reach significance, probably due to the number of subjects. Moreover, it is highly recommended that future experiments measure the total GLP-1, which is more stable and has a substantially longer plasma half-life than the active form. Furthermore, this study paves the way for further studies on other appetite hormones such as leptin and PYY. Another suggestion is to fix the fasting over-night duration to minimize the within subjects variability in fasting hormones levels.

Future work should consider coupling the measurement of appetite hormones with fMRI data to show the brain reward region activity after the manipulation of palatability. It is also interesting to study the difference in brain activity between lean and obese individuals. Moreover, the same study should be conducted on females to account for possible gender differences.

APPENDIX I

CONSENT FORM

Effect of Food Acceptability on Appetite Hormones' Response in Normal Weight vs. Obese Male Subjects

Principal Investigator:	Ammar Olabi
Co-Investigator:	Nahla Hwalla
Research Team:	Nehmat Helou, Hamza Daroub
Address:	American University of Beirut Bliss Street Beirut, Lebanon
Phone:	01-350000, Extension: 4500, 5445

A hard copy of this consent form will be given to you.

You are being asked to participate in a research study entitled "**Effect of Food Acceptability on Appetite Hormones' Response in Normal Weight vs. Obese Male Subjects**" conducted at the American University of Beirut. Please, take your time to read the following information carefully before you decide whether you want to participate in this study or not. Take all your time to consider whether you want to participate in the study or not. Please, keep in mind that participation in this study is voluntary and that refusal to participate will involve no loss in benefit. In addition, if you participate in the study, you may discontinue the study at any time without loss of benefits. Also, your participation may be ended by the study investigators. Feel free to ask your doctor if you need more information or clarification about what is stated in this form and the study as a whole.

Purpose of the Research Study:

Ghrelin and Glucagon like peptide (GLP-1) are gut hormones which were found to be part of the appetite regulating cycle. The properties of ghrelin include increase of appetite and increase of gastric motility and acid secretions. The properties of GLP-1 include increase of satiety and decrease of gastric motility and acid secretions.

The objective of this study is to investigate the effect of food preferences on GLP-1, ghrelin and insulin levels.

Project/Procedures Description

A sample size of around 30 male subjects (20 normal-weight; BMI 18.5-24.9 and 20 obese; BMI 30 -39.9 kg/m2) will be recruited for this study. Recruitment will be done by direct approach. You will be recruited randomly from AUB campus (AUB students or Faculty members aging above 18 years).After you sign the informed consent form, you will need to come for a screening visit, which will take about one hour of your time.

You will undergo an acceptability test on two versions of custard. Only subjects who agree on the acceptability level (around 22 subjects; 11 participants from each group) will be selected to continue.
A screening questionnaire will be filled. Your height, weight and % body fat will be measured. In addition, the amount of calories that you require per day will be also determined through another procedure that involves resting on a bed for a period of 20-30 minutes to be able to determine your oxygen consumption, thus your energy expenditure at rest.

The acceptability test and anthropometric measurements will take place in the sensory evaluation laboratory and room 520 at FAFS, AUB respectively.

If you possess any of the exclusion criteria, you will not be eligible to continue the study.

You will be asked to participate in the study if you fit the following criteria:

- Gender: Male
- Age: 18-50 years
- Body Mass Index (BMI): 18.5-24.9 kg/m2 or 30 -39.9 kg/m2
- Stable body weight for at least three months before the study with absence of any form of dieting, food restriction or other abnormal eating behaviors (to minimize the effect of weight change on GLP-1 and ghrelin status)
- Agree on the acceptability of the two versions (high and low acceptability) of custard

You will not be recruited if you fit at least one of the following criteria:

- Smoking
- Substance abuse such as alcohol or drugs
- Medical or psychological illness
- Use of medications
- Previous gastrointestinal surgery
- History of weight fluctuation (weight loss of greater than 5% within the past three months)

Duration

Following that, you will be asked to return on two separate visits separated from each other by one week washout period. At each visit, you will be asked to consume one of two different custard meals (high and low acceptability): Meal 1 or Meal 2 according the meal serving sequence assigned to you. One of the two different meal serving sequences will be randomly distributed to each subject. Each test will take approximately 4 – 5 hours, and you need to be present in the Clinical Research Unit at AUBMC for all the required time.

Prior to each visit, you will be advised to follow a 3-day diet consisting of 20% protein, 50% carbohydrate and 30% fat of energy. On the day before your visit, you will be asked to fast for 10-12 hours overnight during which you may drink only water. You will be asked not to exercise or drink alcohol for the 24 hours before your test.

At each visit, a thin plastic tube will be inserted into your hand's vein by a registered nurse. This is done in order to obtain blood samples. Upon arrival in the fasting state, 5*mL* of blood will be withdrawn. Following this, you will be given one of the two meals to ingest. After 15 minutes of meal consumption, blood will be withdrawn. In the following four hours, 5*mL* of blood will be collected at times 30, 60, 120, 180 and 240 minutes after meal consumption. A total of 35*mL* of blood will be withdrawn. You cannot consume any other food item or beverage for the 4-hour duration after the test meal is ingested with the exception of mineral water (maximum amount allowed 500 mL). Each visit will include as well an acceptability test of the consumed meal and a hunger rating questionnaire. At the end of each session, you will be given ad lib access to cheese pizza.

You may leave the study at any time. If you decide to stop participating, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with AUB.

Risks, Discomforts and Benefits

Your participation in this study does not involve any physical risk or emotional risk to you beyond the risks of daily life. There are no serious risks anticipated in this study. The only risks that may be related to the study include dizziness, pain and bruising from the needle stick when blood is withdrawn.

By participating in this study, you will be contributing to science. Blood tests and questionnaires will be performed without any charge. The results of this study may help us determine whether special changes in the composition of the diet are necessary for optimal management of blood ghrelin and GLP-1 levels, and thus for appetite control and weight management. All new findings will be conveyed to you by the end of the study.

Confidentiality

The investigators are committed to preserve your privacy, to keep the results confidential and to give them only to the participant involved.

- If you agree to participate in this research study, the information will be kept confidential. Unless required by law, only the study investigators and designees, the ethics committee and inspectors from governmental agencies will have direct access to your medical records.

- You may stop the study at any time or refuse to participate. This will not change your treatment plan and will involve no losses. In case of any adverse event as a result of the study, there will be no compensation to cover such expenses in case it is not covered by a third party or governmental insurance.

There may be circumstances where your confidential information must be released. For example, personal information regarding your participation may be disclosed if required by the AUB IRB, the U.S. Office of Human Research Protections or other federal or international regulatory agencies, or the sponsor of the study, if any, or agency supporting the study.

Compensation/Incentive

You will not be paid for participating in the study. However, a reimbursement sum of 15,000 L.L. (Fifteen Thousand Lebanese Lira) after each blood withdrawal session will be given to you to cover the cost of transportation and parking. You will be paid for every session you complete.

There are no anticipated expenses for you to pay if you participate in the study.

Contact Information and Questions

1) If you have any questions or concerns about the research you may contact Dr. Ammar Olabi at 01-350000, extension 4500, or any of his designees involved in the study.

2) If you have any questions, concerns or complaints about your rights as a participant in this research, you can contact Dr. Fuad Ziyadeh in the Institutional Review Board for human rights at 01-350000, extension 5445.

Participant Rights

Participation in this study is voluntary. You are free to leave the study at any time without penalty. Your decision not to participate is no way influences your relationship with AUB.

Please, indicate whether you are interested to be contacted for other research studies

___ Yes, I would like to

___ No, thank you

Investigator's Statement:

I have reviewed, in detail, the informed consent document for this research study with _____, the purpose of the study and its risks and benefits. I have answered all the patient's questions clearly. I will inform the participant in case of any changes to the research study.

_____

Name of Principal Investigator

_____

Signature of Principal Investigator

_____

Date

_____

Time

Subject's Participation:

I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily agree to be a part of this research study and I know that I can contact Dr. Ammar Olabi at 01-350000, extension 4500, or any of his designees involved in the study in case of any questions. If I felt that my questions have not been answered, I can contact Dr. Fuad Ziyadeh in the Institutional Review Board for human rights at 01-350000, extension 5445. I understand that I am free to withdraw this consent and discontinue participation in this project at any time, even after signing this form, and it will not affect my care. I know that I will receive a copy of this signed informed consent.

_____

Name of Subject

_____

Signature of Subject

_____

Date

_____

Time

APPENDIX II
Screening Questionnaire

American University of Beirut

Faculty of Agricultural and Food Sciences

Department of Nutrition and Food Science

Effect of Food Acceptability on Appetite Hormones' Response in Normal Weight vs. Obese Male Subjects

Name: _____

Date: _____

Part 1

1. **Age:** _____
2. **Weight:** _____
3. **Height**: _____

4. **Smoker**: □ Yes □ No □ Occasional

5. **Are you on any medication?** □ Yes □ No

6. **Have you ever had GI surgery?** □ Yes □ No

7. **Are you following any diet?** □ Yes □ No

If yes, describe that diet.

_____
_____
_____

8. **Have you ever been on a diet before?** □ Yes □ No

9. **Have you had any weight fluctuation over the past 3 months?** □ Yes □ No

10. **Do you exercise/go to the gym?** □ Yes □ No

If yes, how many times per week? _____

11. **Have you had any recent surgeries?** □ Yes □ No

12. **Are you allergic to certain foods?** □ Yes □ No

If yes, list those foods.

_____
_____
_____

13. **Do you eat 3 meals a day?**

□ Breakfast □ Lunch □ Dinner

14. **What does your dietary intake mostly consist of?**

□ Carbohydrate □ Protein □ Fat

15. **When you see a visual representation (commercial or actual food product), does it increase your appetite?** □ Yes □ No

16. **Do you imagine your favorite meal when you are hungry?** □ Yes □ No

17. Do you suffer from? □ Fatigue □ Stress

18. Does obesity run in your family? □ Yes □ No

Part 2

1. **When I smell a sizzling steak or juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.**

□Definitely true □Mostly true
□Mostly false □Definitely false

2. **I deliberately take small helpings as a means of controlling my weight.**

□Definitely true □Mostly true
□Mostly false □Definitely false

3. **When I feel anxious, I find myself eating.**

□Definitely true □Mostly true
□Mostly false □Definitely false

4. **Sometimes when I start eating, I just can't seem to stop.**

□Definitely true □Mostly true
□Mostly false □Definitely false

5. **Being with someone who is eating often makes me hungry enough to eat also.**

□Definitely true □Mostly true
□Mostly false □Definitely false

6. **When I feel blue, I often overeat.**

□Definitely true □Mostly true
□Mostly false □Definitely false

7. **When I see a real delicacy, I often get so hungry that I have to eat right away.**

□Definitely true □Mostly true
□Mostly false □Definitely false

8. **I get so hungry that my stomach often seems like a bottomless pit.**

□Definitely true □Mostly true
□Mostly false □definitely false

9. **I am always hungry so it is hard for me to stop eating before I finish the food on my plate.**

□Definitely true □Mostly true

□Mostly false □Definitely false

10. When I feel lonely, I console myself by eating.

□Definitely true □Mostly true
□Mostly false □Definitely false

11. I consciously hold back at meals in order not to weight gain.

□Definitely true □Mostly true
□Mostly false □Definitely false

12. I do not eat some foods because they make me fat.

□Definitely true □Mostly true
□Mostly false □Definitely false

13. I am always hungry enough to eat at any time.

□Definitely true □Mostly true
□Mostly false □Definitely false

14. How often do you feel hungry?

□Only at meal times □Sometimes between meals
□Often between meals □Almost always

15. How frequently do you avoid "stocking up" on tempting foods?

□Almost never □Seldom
□Usually □Almost always

16. How likely are you to consciously eat less than you want?

□Unlikely □Slightly likely
□Moderately likely □Very likely

17. Do you go on eating binges though you are not hungry?

□Never □Rarely
□Sometimes □At least once a week

18. On a scale of 1 to 8, where 1 means no restraint in eating (eating whatever you want, whenever you want it) and 8 means total restraint (constantly limiting food intake and never "giving in"), what number would you give yourself?

_____

APPENDIX III

Food Acceptability Questionnaire

American University of Beirut
Faculty of Agricultural and Food Sciences
Department of Nutrition and Food Science

Effect of Food Acceptability on Appetite Hormones' Response in Normal Weight vs. Obese Male Subjects

Name: _____

Date: _____

Kindly, taste each of the following samples, in the order indicated, and then answer the question.

Please rinse your mouth with water before tasting the first sample, and between samples.

All things being considered, which statement below describes how you feel about the product?

Sample Number: _____

1	2	3	4	5	6	7	8	9
□	□	□	□	□	□	□	□	□
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

All things being considered, which statement below describes how you feel about the product?

Sample Number: _____

1	2	3	4	5	6	7	8	9
□	□	□	□	□	□	□	□	□
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Thank you

APPENDIX IV

Hunger Ratings and Food Acceptability Questionnaire

American University of Beirut
Faculty of Agricultural and Food Sciences
Department of Nutrition and Food Science

Effect of Food Preferences on Appetite Hormones' Response in Normal Weight Male Subjects

Name: _____

Date: _____

Visit: _____

Time: _____ **Sequence:** _1-7__

Kindly, answer the following questions: *(Please put a slash (↓) mark somewhere on the line below)*

Question 1: How hungry do you feel?

I am not hungry at all _____ I have never been hungrier

Question 2: How satisfied do you feel?

I am completely empty _____ I cannot eat another bite

Question 3: How full do you feel?

Not at all full _____ Totally full

Question 4: How much do you think you can eat?

Nothing at all _____ A lot

Question 5: Would you like to eat something sweet?

Yes, very much _____ No, not at all

Question 6: Would you like to eat something salty?

Yes, very much _____ No, not at all

Question 7: Would you like to eat something savoury?

Yes, very much _____ No, not at all

Question 8: would you like to eat something fatty?

Yes, very much ——————————————————— No, not at all

Question 9: All things being considered, which statement below describes how you feel about the product?

1	2	3	4	5	6	7	8	9
□	□	□	□	□	□	□	□	□
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Question 10: Kindly, indicate below whether you have any additional comments on the product.

_____
_____
_____
_____

Questions 1-2-3-4-5-6-7 and 8 are repeated at 0, 15, 30, 60, 120, 180 and 240 min

Questions 9 and 10 are repeated after sampling a spoonful, after eating the whole portion and at 240 min.

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