THE EFFECTS OF EMULSIONS ON THE UPREGULATION OF SATIETY

HORMONES IN CELL CULTURE AND THE ACTIVATION OF THE ILEAL

BRAKE IN HUMAN SUBJECTS

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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August 2015

Major Subject: Food Science and Technology

ABSTRACT


To treat obesity, one approach is developing food products formulated to trigger the ileal brake, a gastric feedback mechanism that induces satiety, the prolonged feeling of fullness. PYY and GLP-1, biomarkers of the ileal brake, are secreted from the ileum upon macronutrient exposure. Circulating PYY and GLP-1 bind to receptors in the hypothalamus, suppressing appetite and food intake. In three research phases, the hypothesis that two orally consumed emulsions, composed of palm and fish oil, induce satiety and reduce energy intake, was tested.

In phase one, it was hypothesized that the emulsions promoted the secretion of PYY and GLP-1 from the murine intestinal cell line, STC-1. The STC-1 cells were treated with each emulsion and the hormone response was assayed. The results demonstrated that the emulsions promoted PYY and GLP-1 secretion from the STC-1 cultures. These findings suggest that the emulsions may have potential as satiety inducing agents in human volunteers.

In phase two, it was hypothesized that a formulated fruit and dairy smoothie-type beverage could serve as vehicle for the satiety inducing emulsions. Beverages with or without the emulsions constituted the treatments. To test this hypothesis, subjective and objective analyses were performed. Subjective analyses using human volunteers compared sensory aspects of each treatment. Objective analyses using appropriate instrumentation compared the viscosities, pH and color of each treatment. There were

no statistically significant differences in the subjective and objective results, confirming that the beverage is a suitable emulsion vehicle.

Phase three tested the hypothesis that the treatments induced satiety, reduced energy intake and induced a satiety hormone response in human subjects. 10 subjects consumed three treatments in a crossover study in which self-reported satiety, food intake, and satiety hormones were investigated. Blood sample analyses and completed satiety questionnaires were used to assess treatment effects over a 180 minute interval. Following the 180 minute interval, subjects consumed a satiety meal to achieve satiation, at which point food intake was measured. Consumption of each treatment did not demonstrate significant differences for any subject-reported satiety, biomarker analysis and food intake.

# ACKNOWLEDGEMENTS

## NOMENCLATURE

| | |
|---|---|
| GLP-1 | Glucagon-like-peptide 1 |
| PYY | Peptide YY |
| CCK | Cholecystokinin |
| GI | Gastrointestinal |
| TAG | Triacylglycerol |
| FFA | Free fatty acid |
| MCT | Medium chain triacylglycerol |
| LCT | Long chain triacylglycerol |
| DPP-4 | Dipeptidyl Peptidase-4 |
| POMC | Proopiomelanocortin |
| αMSH | Melanocyte-stimulating hormone |
| NPY | NeuropeptideY |
| AgRP | Agouti-related peptide |
| CNS | Central nervous system |
| ESNL | Exercise and Sports Nutrition Laboratory |
| SSS | Sensory specific satiety |
| OCCT | Orocecal transit time |
| DATEM | Diacetyl tartaric acid ester of mono-diglycerides |

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CHAPTER I

INTRODUCTION


**RESEARCH GOALS**

*Overall Goal*

The goal of this research project was to test the hypothesis that two orally consumed emulsions, composed of varying ratios of palm and fish oil, influenced satiety, the prolonged feeling of fullness and satiation following meal consumption. Specifically, it was hypothesized that the emulsions promoted satiety by triggering the ileal brake, a gastric feedback mechanism that promotes appetite suppression by delaying gastric emptying and inhibiting intestinal motility. This research project consisted of three phases briefly described below.


*Phase One –The Effects of Emulsions on Satiety Hormone Secretion in Cell Culture*

The goal for phase one was to determine if the two emulsions stimulated the secretion of the ileal brake biomarkers, PYY and GLP-1 from the murine intestinal cell line, STC-1. STC-1 cells secretes PYY and GLP-1 upon nutrient stimulation in a manner similar to human endocrine intestinal cells. The STC-1 cell cultures were treated with the two emulsions and their effects on the secretion of PYY and GLP-1 were assayed. The results of phase one determined if there was potential for the two emulsions to trigger the ileal brake when consumed by individuals.

*Phase Two – Analysis of a Beverage Formulated With Satiety Inducing Emulsions*

The goal of phase two was to formulate a fruit and dairy beverage to serve as a vehicle for the emulsions in human volunteers. For clarity, the beverage, when combined with either of the emulsions or control, was referred to as a treatment. For each treatment, subjective sensory tests and objective instrumental analysis were performed to ensure that there were no objective or subjective differences among treatments. For the subjective sensory tests, triangle tests and acceptance/affective tests were administered on individuals. The objective tests employed laboratory instrumentation to analyze and compare the viscosities, pH and Hunter L, a, b color values of each treatment. Any measurable differences between treatments could influence satiety measurements and food intake in human volunteers.

*Phase Three – Effects of Each Treatment on Satiety in Human Subjects*

In phase three, we investigated whether the three treatments formulated in phase two induced satiety, reduced food intake, and upregulated the expression of the satiety/hunger biomarkers, GLP-1, PYY, ghrelin and leptin in human volunteers to infer activation of the ileal brake. Ten subjects were recruited in a randomized, single-blind placebo-controlled, crossover study design. Each subject consumed each treatment separated by one week intervals. Over the 180 minute period post-treatment consumption, subjects reported satiety by completing VAS questionnaires. During that same timeframe, blood samples were collected and later assayed for ghrelin, leptin, PYY and GLP-1. To determine if the treatments reduced food intake, all subjects were

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provided a cheese pizza lunch following the 180 minute timeframe.  Subjects were

instructed to consume pizza until sufficiently satiated.  All of the aforementioned tests

were necessary to determine if the treatments affected satiety, energy intake and

hormone expression as a means to control appetite and counter obesity.


**BACKGROUND**

*Health Concerns Relating to Obesity*

Obesity has emerged as a serious threat to the world health population and has

continued on an upward trend since the mid-1980s.  Obesity is defined as abnormal or

excessive fat accumulation in individuals that may impair health.  It is caused by a

caloric surplus resulting from excessive food consumption combined with a lack of

sufficient physical activity.  Overweight and obese individuals are risk factors for

chronic diseases such as diabetes, cardiovascular diseases and cancer.

Body mass index (BMI) is the most common measurement of obesity, which is

calculated by dividing the individual's weight (in kilograms) by the square of their

height (in meters).  Table 1 displays the BMI classification chart.

| BMI range $(kg/m^2)$ | Classification |
|---|---|
| Less than 18.5 | underweight |
| 18.5 - 25 | normal weight |
| 25.1 – 29.9 | overweight |
| Greater than 30 | obese |

**Table 1 - BMI Classification Chart**

As of August 2014, the World Health Organization recently reported the following: worldwide obesity has nearly doubled since 1980; in 2008, more than 1.4 billion adults, 20 and older, were overweight, of which over 200 million men and nearly 300 million women were obese; 35% of adults aged 20 and over were overweight in 2008, and 11% were obese; 65% of the world's population live in countries where overweight and obesity kills more people than underweight; more than 40 million children under the age of 5 were overweight or obese in 2012 [1].

The World Health Organization recommends the following dietary guidelines to treat obesity: limit total fat intake, shift fat consumption away from saturated fats to unsaturated fats, eliminate foods high in trans-fatty acids, increase the consumption of fruits, legumes, whole grains, nuts and vegetables, limit the intake of free sugars, and limit sodium consumption [1]. In the USA, dietary guidelines are established jointly by the United States Department of Agriculture and the United States Department of Health and Human Services. Food product formulations are developed following those guidelines.

*Inflammatory Response in Obese Individuals*

In addition to adding excessive amounts of body fat, obesity also disrupts the normal metabolic process. One such disruption is the activation of immune response pathways leading to inflammation. For disease-free humans of normal BMI, inflammation represents an immune system response to a site of injury or infection. The response often leads to signs of redness, swelling, heat or pain. However, in obese

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individuals, as well as individuals suffering from Type 2 diabetes, the inflammatory response is strictly metabolic and caused by excessive nutrient consumption. It is characterized by abnormal cytokine production, and activation of a network of inflammatory signaling pathways in metabolic cells such as adipocytes [2, 3]. The exact methods that lead to stimulation however are unknown, but under normal conditions, nutrients will stimulate metabolic pathways, leaving the pathogen-sensing and immune response pathways inactive. In conditions of excess nutrient intake, the nutrients can overload the metabolic pathways stimulating the pathogen-sensing pathways and then after prolonged exposure can activate the immune response pathways. The latter pathways can prevent the drain of nutrient metabolism causing a backlog of nutrients in the system, blocking anabolic activity (Figure 1). Therefore anti-inflammatory ingredients/drugs are necessary to promote normal cell metabolism.
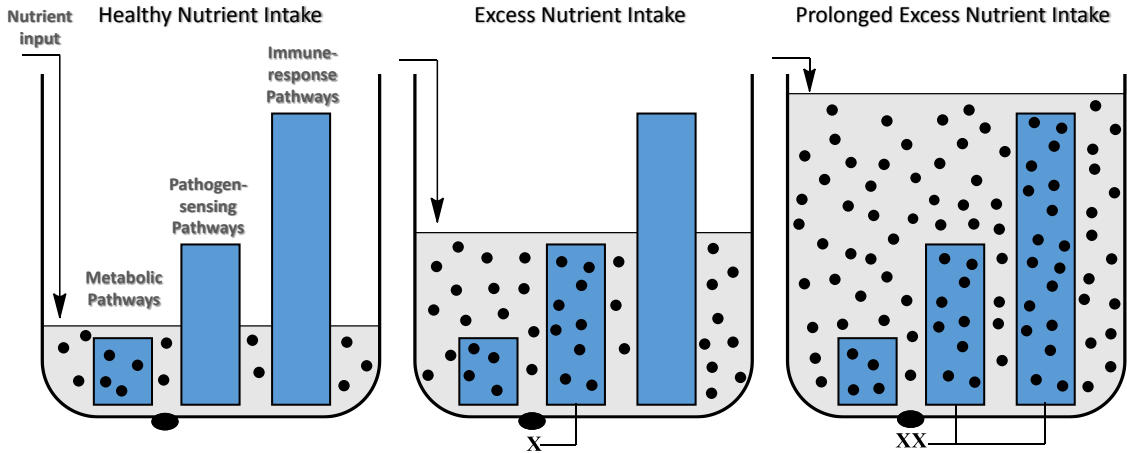


**Figure 1 - Prolonged Overconsumption of Nutrients Leads to Immune Response**
This figure illustrates how excessive nutrients affect the immune response in individuals. Adapted from Ref. [4]

*Satiety, Hunger and Appetite in Obesity*

Satiety, hunger, and appetite, directly or indirectly, are involved in energy balance and therefore are important consideration when considering obesity. Hunger is defined as the physiological need for food and is affected by factors such as an empty stomach, a decrease in blood glucose levels, and alterations in circulating hormones. Appetite is the psychological desire to eat, and is associated with the sensory aspects of food such as sight and smell. Hunger and appetite are not always mutually inclusive. During times of sickness individuals can experience hunger but not appetite. Conversely, the desire to eat a desert after consuming a nutritious meal influences appetite but not hunger. The latter instance plays a significant role in the development of obesity [5].

Satiation is the feeling of fullness that occurs during the onset of eating, while satiety is the physiological and psychological feelings of fullness and satiation that comes post meal. Individuals who are overweight or obese typically consume greater quantities of food to experience the necessary feelings of fullness and satiation after eating, leading to a surplus of stored energy [5].

Energy restriction is most commonly employed to counter obesity, but a more effective long term solution may lie in the manipulation of macronutrient composition of diets to fortify foods or beverages with macromolecules that can trigger the release of the hormones that regulate hunger, food intake and satiety to promote weight maintenance (Figure 2) [6].
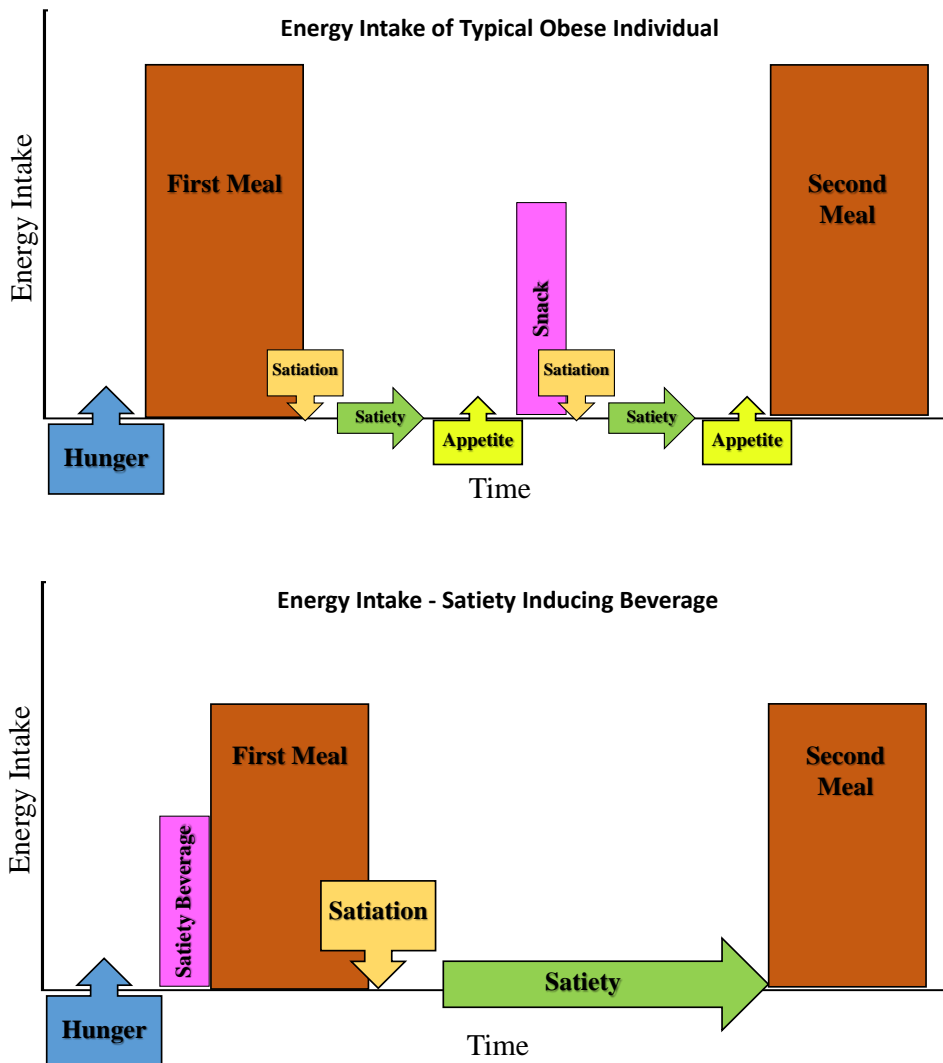
**Figure 2 - Potential Effect of Satiety Inducing Beverage on Energy Intake**
Shows how beverages supplemented with macromolecules that enhance feelings of satiety can potentially influence energy intake.

*Psychological Factors Affecting Satiety*

As will be discussed in greater detail in an upcoming section, the human body

possesses physiological mechanisms to control appetite.  However, it is important to take

into account not only such mechanisms but also external factors that may influence

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appetite or satiety. The factors that will be briefly discussed are palatability, variety, portion size, television viewing and social situations.

Palatability refers to the pleasurable experiences resulting from food consumption. Palatability of food may be altered by adding fat, sugar, protein, texture agents and color agents, etc. Typically, increasing the palpability by any of the aforementioned methods often leads to an increase in appetite, meal size, meal duration and eating rate [7, 8]. Several studies demonstrated that satiation and palatability have an inverse relationship, meaning that the most palatable energy dense foods tend to be least satiating while the least palatable foods tend to be most satiating [8-10]. De Graaf *et al.* argued that increasing palatability potentially affects satiation but not satiety [11]. While palatable energy dense foods have been demonstrated to reduce satiety, it was suggested that formulating less energy dense foods, that are both healthy and satiating should be a research focus [12].

Variety of food being offered also affects individual food consumption and satiety. It has been shown that offering a greater the variety of food, often leads to greater food consumption, partially due to a phenomenon known as sensory specific satiety (SSS). SSS refers to the individual desire to consume one food item that has already been consumed is reduced compared to another food item that has not been consumed [13]. Offering a wide of variety of foods seemingly minimizes the effect of SSS leading to higher overall food consumption and that restricting the variety of foods promotes satiety [13].

In the USA, portion sizes of foods (i.e. hamburger patties, fried foods) have increased over the past four decades, while portion sizes have remained relatively constant in Europe. The effect portion size has on satiety vary. Several studies found that satiety increased with larger portion sizes, while other studies found no differences in appetite ratings between larger and smaller portions sizes despite a significant increase in energy intake when consuming the larger portion size [14-19]. Generally, larger portion size appears to increase energy intake since consumption of a larger portion size is rarely followed by a subsequent energy reduction [7].

Television viewing has the potential to increase energy intake and food consumption in that it potentially desensitizes individuals to internal signals of satiation and satiety [20]. It has been suggested that television increases motivation to eat and that the attention needed to watch a continuous television programming is a distraction to an individual's normal eating patterns [21]. Television viewing increases sedentary time and increases opportunities to eat, promoting weight gain [22].

Peer influence in social settings potentially affects satiety since individuals have a tendency to consume greater food quantities in the presence of others than when alone [23]. Two studies demonstrated that individuals consumed greater food quantities in the presence of others in a social setting due to prolonged meal length, resulting in increased energy intake [24, 25]. Therefore, peer influence in social settings should also be taken into consideration as a factor affecting satiety.

In summary, palatability, variety, portion size, television viewing, and social interactions all have an effect on appetite and satiety and also highlight the inherit

problems in extrapolating the results of a satiety study performed in a clinic where all confounding variables are minimized and controlled.

*Satiety and its Role in Energy Homeostasis*

As previously stated, satiety is the physiological and psychological experience of fullness that comes post meal consumption. Satiety, however, is a much more complex process than such a definition would indicate. From a biological standpoint, satiety is very tightly regulated by various organ systems including the nervous, endocrine and digestive systems and they all must properly coordinate to maintain energy homeostasis.

In the nervous system, pro-hunger and pro-satiety neurons, located in the hypothalamic arcuate nucleus of the brain stem, coordinate to maintain energy homeostasis. The pro-hunger neurons are known as the neuropeptide Y/Agouti-related-peptide (NPY/AgRP) neurons and the pro-satiety neurons are known as the pro-opiomelanocortin/$\alpha$-melanocyte-stimulating-hormones (POMC/$\alpha$MSH) neurons. Both of which are responsible for relaying hunger or satiety stimuli to the central nervous system (CNS). See Figure 3.

The NPY/ArGP expressing neurons, known as the hunger center, are responsible for producing the two pro-hunger peptides NPY and AgRP, which are expressed in response to energy needs. Once expressed, NPY induces hunger as it binds to neurons located in the paraventricular nucleus, which will further relay hunger stimuli to the CNS. AgRP is simultaneously expressed with NPY and exerts its effects by inhibiting the action of the POMC/$\alpha$MSH neurons [26-28]. It is worth mentioning that in mice, the

deletion of the NPY gene unexpectedly promoted obesity, suggesting a disruption in the complex regulation system of bodyweight [29].

The POMC/αMSH expressing neurons, known as the satiety center, produce melanocortins (αMSH), which are cleaved from the POMC precursor molecule. αMSH exerts its effects upon binding to melanocortin receptors (MC4) located on neurons in the paraventricular nucleus that will further relay appetite suppressing stimuli to the CNS [30-32]. AgRP is released along with NPY and functions by preventing the binding of αMSH to the MC3 and MC4 receptors [33]. Mutations in the MC4 receptors have been linked to obesity [34].

Both the NPY/AgRP and POMC/αMSH neurons require specific intercellular interactions and extracellular stimuli in the form of neurotransmitters or hormones to maintain energy homeostasis and regulate satiety and hunger (Figure 1). The stomach, small intestine, pancreas, and adipose tissue all produce hormones that interact with both sets of neurons.

The main hormones responsible for maintaining energy homeostasis are ghrelin, leptin, cholecystokinin (CCK), glucagon-like-peptide 1 (GLP-1), and Peptide YY (PYY). The pro-hunger hormone ghrelin functions as an activator of the NPY/AgRP neurons. The pro-satiety hormones leptin and GLP-1 function as activators of the POMC/αMSH neurons. Leptin and PYY$_{3\text{-}36}$ function as inhibitors of NPY/AgRP neurons. Refer to Figure 3 and Figure 4 for a diagram illustrating their functions. The function of each of these hormones are briefly described in Table 2 and are elaborated on in further sections. Having an understanding of the biological mechanisms and

hormones that affect satiety and an intestinal feedback mechanism known as the ileal

brake play a significant role in development of food products to aid in the treatment of

obesity [35].
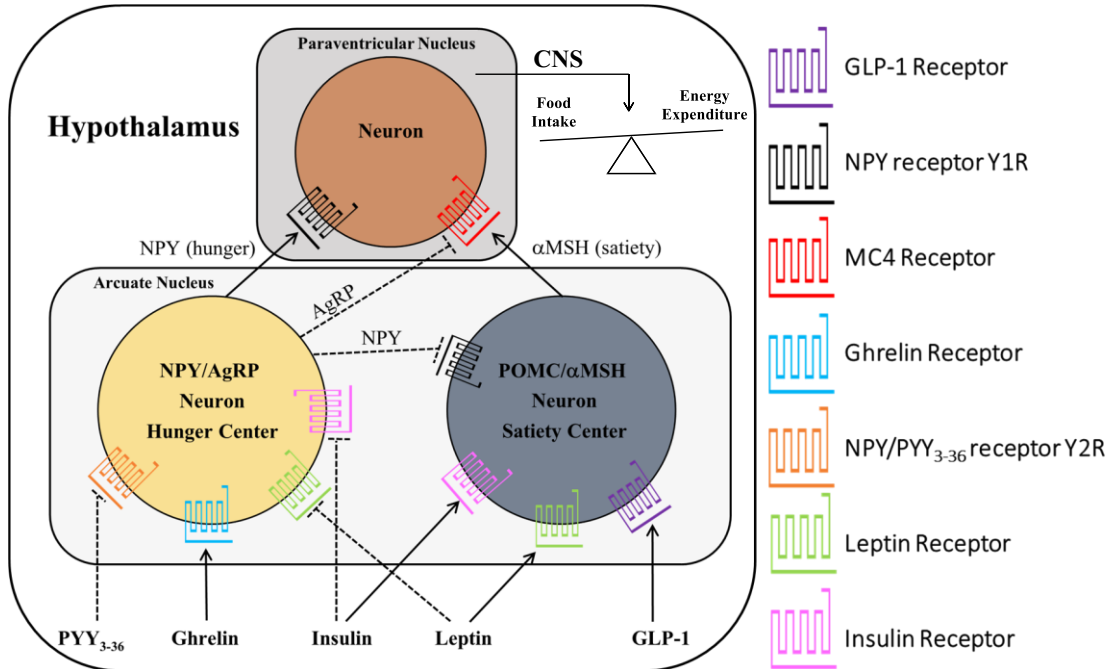


**Figure 3 - Diagram of Hormone Binding Sites in Hypothalamus**

Note: all solid lines indicate a neuronal stimulation effect, while dashed lines indicate a neuronal inhibitory effect. Ghrelin stimulates the Hunger Center to produce NPY and AgRP, while $PYY_{3-36}$, Leptin and insulin inhibit NPY production. Leptin, Insulin and GLP-1 activate the satiety center to produce αMSH. NPY inhibits the satiety center and AgRP prevents αMSH from binding to MC4 receptor.
Adapted from Ref. [36].
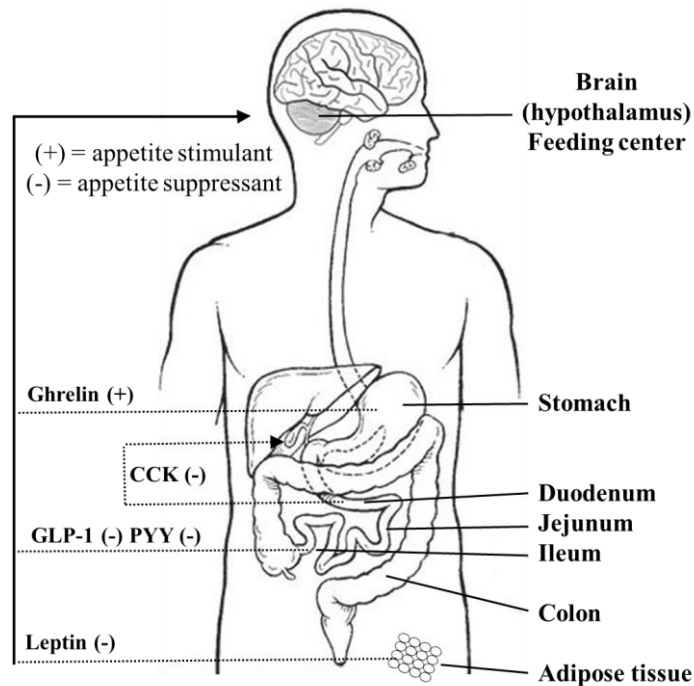
**Figure 4 - Organ Sources of Hunger and Satiety Hormones**
All satiety hormones exert their effects by binding to specific receptors in the hypothalamus (Ghrelin, GLP-1, PYY and Leptin) or the vagal afferents along the gut (CCK). Adapted from Ref. [37]

| Hormone | Function | Origin of Secretion | Brief Description of Effects |
|---|---|---|---|
| **Ghrelin** | Hunger | X/A-like cells of the stomach | Secreted into circulation in response to hunger. Will bind to ghrelin receptors in the brain to stimulate appetite. |
| **Leptin** | Satiety | Adipose tissue | Secreted from adipose tissue into circulation post meal consumption and will bind to leptin receptors in the hypothalamus to induce appetite suppression. |
| **GLP-1** | Satiety | L-cells of the ileal portion of the small intestine | Secreted from L-cells into the bloodstream post meal consumption. Will bind to receptors in the CNS and peripheral tissues to promote insulin secretion and inhibits glucagon release and delay gastric emptying. Degraded by DPP-4 once in circulation. |
| **PYY** | Satiety | L-cells of the ileal portion of the small intestine | Secreted from L-cells into circulation post meal consumption in full length form. It will be truncated by DPP-4 to the active 3-36 form. The 3-36 form binds to Y2 receptor to exert its effects on satiety. |
| **CCK** | Satiation | I-cells of the duodenal portion of the small intestine | Secreted by the I-cells into the bloodstream where it binds to CCK receptor and promotes the release of digestive enzymes and bile from the pancreas and gallbladder, and also delays gastric emptying. |

**Table 2- Description of Satiety and Hunger Hormones**

*GLP-1*

GLP-1 is one of several cleavage products of the proglucagon gene and is

predominantly produced in the intestinal L-cells located along the mucosa of the ileum

and the proximal colon (Figure 5) [38-41]. GLP-1 is an incretin that enhances glucose-

dependent insulin secretion, inhibits glucagon release from the pancreatic α-cells and

delays nutrient absorption through inhibition of gastric emptying and intestinal motility

[42-44]. Due to the distribution of GLP-1 receptors in the CNS and peripheral tissues,

GLP-1 exerts a wide range of effects [42-48]. GLP-1 exhibits rapid and prolonged

effects in response to nutrient digestion post-meal consumption. The rapid response of

GLP-1 is stimulated by the vagus nerve by the release of acetylcholine which binds to

M1 receptors on the L-cells promoting the secretion of GLP-1 [49-51]. Conversely, the

prolonged response of GLP-1 secretion involves nutrients that traverse the

gastrointestinal tract and directly interact with the L-cells upon binding to nutrient

specific G-protein receptors such as GRP40, GRP43, GRP119, GRP120, FATP4 and

TGR5 which activate a series of signal cascades leading to cleavage of proglucagon and

the secretion of GLP-1 into circulation [52-64]. Once in circulation, GLP-1 has a half-

life of ~1-2 minutes due to rapid degradation by dipeptidyl peptidase-4 (DPP-4) [65, 66].

Once GLP-1 crosses the blood brain barrier, it will bind to receptors on the

POMC/αMSH neurons hypothalamic arcuate nuclei to promote appetite suppression.

GLP-1 will also bind to receptors on the pancreatic β-cells to promote synthesis and

secretion of insulin [48, 67]. Lower circulating levels of GLP-1 have been reported in

obese individuals, normal levels can reportedly be restored with weight loss, further

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elaborating on the role of GLP-1 in weight management and energy homeostasis [68, 69].
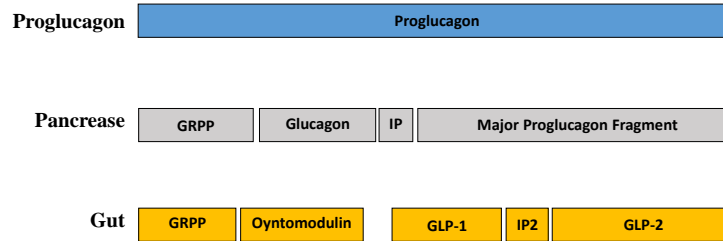


**Figure 5 - Organ Specific Proglucagon Cleavage Products**
Adapted from Ref. [41]

*PYY*

PYY, like GLP-1, is a satiety hormone that is secreted by intestinal L-cells and serves as an inhibitor of pancreatic exocrine secretion and intestinal motility [70]. PYY contains a tyrosine (Y) residue at the amino and carboxylic acid terminus. Unlike GLP-1, the specific receptors and signaling cascades that lead to activation of PYY gene expression has not been fully elucidated in humans. However in rats, it was reported that Insulin-like Growth Factor 1 functions as a positive regulator of PYY gene expression and that the stimulatory effect may be mediated by Sp1 proteins that bind to the proximal PYY promoter region [71]. Once in circulation PYY is degraded by DPP-4 into its active $PYY_{3-36}$ form. $PYY_{3-36}$ is able to exclusively bind to the Y2 receptor in the hypothalamic arcuate nucleus of NPY/AgRP-expressing neurons inhibiting the pro-hunger effects of NPY. DPP-4 mediated-cleavage of GLP-1 and PYY exert opposing effects on satiety. This occurrence suggests a complex layer of satiety regulation and may explain why the DPP-4 inhibitor, vildagliptin does not alter satiation or gastric

volume in people with type 2 diabetes, despite elevated GLP-1 circulation [72]. It was

previously shown that infusion of $PYY_{3-36}$ into human subjects, results in a 30% or

higher reduction in caloric consumption after two hours and a 33% reduction of food

intake over 24 hours [73, 74]. Obese individuals have been reported to have lower levels

of circulating $PYY_{3-36}$ corresponding to reduced satiety [72-75]. It is important to note

that in individuals who have undergone gastric bypass surgery, that circulating levels of

PYY and GLP-1 are significantly increased and has been suggested to be a significant

factor in weight loss post-surgery [76].


*Leptin*

Leptin, unlike CCK, GLP-1 and PYY, is not secreted from the intestines but

mainly from white adipose tissue in response to food consumption. Once in circulation,

leptin crosses the blood brain barrier where it will bind to leptin receptors on both the

POMC/αMSH and NPY/AgRP neurons enhancing the action of the former and

inhibiting the action of the latter. Taken together, this pathway provides information to

the brain regarding the amount of stored energy in adipose tissue in turn altering appetite

and affecting energy expenditure [77]. Leptin circulations levels correlate closely with

fat mass and decrease by as much as 50% after long periods of starvation [44, 77]. In

obese mice that were leptin deficient, it was demonstrated that leptin administration

reduced food intake and body mass [78]. However, leptin administration in obese

humans failed produce similar results since the majority of obese individuals are leptin

resistant [79]. Leptin resistance works in a method very similar to that of insulin in type

2 diabetes in that leptin is being produced but the appetite reducing effects are abrogated. The exact mechanism for leptin resistance is still under debate, but it has been proposed that accumulation of fat prevents leptins ability to cross the blood brain barrier and/or alterations in cellular leptin receptor signaling [80-82]. Leptin and/or leptin receptor mutations are linked to obesity due leading to excessive amounts of overeating, which can reversed by administration of leptin [83]. Leptin resistance and mutations is a significant factor in the development and treatment of obesity.

*Ghrelin*

Ghrelin is composed of 28 amino acids and is the only known appetite-stimulating hormone. It is produced in the $P/D_1$ cells in the stomach [84]. Circulating ghrelin levels rise after overnight fasting and decreases post meal consumption in normal weight individuals. In order for ghrelin to cross the blood barrier, the serine residue at position three must acylated by an octanoyl group. Once ghrelin passes the blood-brain barrier, it binds to ghrelin receptor on the NPY/AgRP neurons promoting the production of NPY, stimulating appetite [85, 86]. Lower ghrelin levels are observed in obese individuals compared to lean individuals, while high ghrelin levels are associated with anorexia [87, 88]. In addition, the suppression of ghrelin that occurs post-meal in lean subjects is not observed in obese people and it has been suggested that due to excess fat storage ghrelin levels have already been maximally suppressed [89]. It's worth nothing that dietary fat has been reported to suppress the release of ghrelin and therefore inhibit its pro-hunger effects [90].

*CCK*

CCK is considered a short-term, meal-reducing satiation hormone that is synthesized and secreted by the I-cells of the duodenum and was shown in rats and later in humans to suppress food intake [91-93]. Upon stimulation from nutrients, CCK is secreted into circulation and binds to receptors on several organs such as the gallbladder, pancreas and vagal afferents. Binding to the gallbladder will induce the secretion of bile to aid in digestion [94]. CCK exerts its inhibitory effects upon binding to the $CCK_1$ receptor on vagal afferents which relays information to the hypothalamus leading to increased gastric distension induced by slowing gastric emptying which contributes to the satiation response [95-97]. While the satiation actions of peripheral CCK are well characterized, the role for brain CCK has been more controversial. Given that CCK response occurs at the onset of meal consumption it is more appropriate to categorize CCK as a satiation hormone as opposed to a satiety hormone [7].


*Serotonin*

The role hormones play in satiety is well established and have become a target in obesity research and drug design, but the role of neurotransmitters, such as serotonin, play in satiety should not be ignored. Serotonin is derived from tryptophan and produced from enterochromaffin cells that line the intestinal epithelium. The conversion of tryptophan to serotonin is a two-step enzymatic process that involves hydroxylation at 5' position of the indole ring followed by decarboxylation of the alpha carbon. Once released into circulation, serotonin will cross the blood brain barrier and bind to 5-HT1B

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and 5-HT2C receptors located on the POMC/αMSH neurons to promote serotonin-induced satiety [98, 99].   Commercial drugs such as sibutramine, dexfenfluramine, fluoxetine and chlorophenylpiperazine are all known to be t5-HT2C receptor agonists, which have been used to treat obesity [100-103].  Findings indicate that consumption of tryptophan rich foods fails to increase serotonin production.  As such, formulated foods/diets rarely target serotonin-induced satiety, but instead target induction of the aforementioned satiety hormones.  [104].

*Effects of Macronutrients on Satiety*

Most studies have focused on the effects proteins, fats and carbohydrates have on satiety.  Studies suggest that protein has the greatest effect on satiety compared to equivalent quantities of fat and carbohydrates.  Most short and long term studies measuring the effects of protein on satiety involved making a comparison between higher and lower protein preloads or diets monitoring satiety, energy intake and bodyweight change [7, 105].  A review of studies on protein, satiety and weight loss by Halton and Hu examined the results of many short-term and longer-term studies on satiety, energy intake and bodyweight change.  They reported that eleven out of fourteen studies demonstrated that a higher protein preload significantly increased ratings of satiety, and eight out of fifteen studies demonstrated that the subsequent energy intake was significantly reduced in the higher protein condition than in the lower preload control.  They concluded that substituting refined carbohydrates with protein sources that are low in saturated fat may be beneficial to promote weightloss [105].  Studies

which compared high to low protein preloads provided insight into the effect protein quantity provides on satiety and energy intake. However those studies did not provide insight into the role protein plays on satiety compared to fats and carbohydrates until Weigle *et al.* examined the effects of an isocaloric high protein diet compared to fat [106]. In the study, subjects were provided a diet that consisted of either low-protein (15% energy from protein, 35% from fat, 50% from carbohydrate) or high protein (30% energy from protein, 20% from fat, 50% from carbohydrate). Subjects on the high protein diet reported higher feelings of satiety and a significant reduction of bodyweight based on being given *ad libitum* access to provided food after 12 weeks [106]. High protein diets are common when combined with very low amounts of carbohydrates. They are often known as ketogenic diets which saw a rise in popularity in the early-to-mid 2000s [107]. Such diets involve high fat, high protein and very low carbohydrate consumption to the push the body into a state of ketosis in which ketone bodies provide the main source of energy due to the lack of glucose from carbohydrates. One study examined the effects of a high-protein low carbohydrate ketogenic diet (LCKD) (30% energy from protein, 4% from carbohydrates, 66% from fat) and high-protein medium carbohydrate non-ketogenic diet (MCND) on food intake and satiety. Hunger and food intakes were found to be significantly lower for LCKD subjects compared to MCND subjects, while visual analogue scale (VAS) measurements of satiety were not found to be significantly different [108]. Evidence is consistent in that the energy from protein has a greater effect on satiety than equivalent energy amounts from fat or carbohydrates [7].

Carbohydrates are very diverse in that they include, mono-, di-, and polysaccharides all made from simple sugars (mainly glucose). The rate at which carbohydrates are digested is dependent on the chain length and the quantity of branch points. Although, a short glucose polymer (commercially known as Vitargo) was shown to be absorbed at a quicker rate compared to glucose, its effects on satiety were not tested. These findings suggest that other factors, in addition to chain length and branch point quantity, potentially affect digestion and absorption of carbohydrates [109]. Sucrose and high fructose corn syrup are two of the most common sweeteners present in beverages. Their effects on satiety have been examined in multiple studies. In all cases there was not a significant difference in satiety or energy intake between the two forms of sweeteners [110-112]. Much research focused on the effect the glycemic index has on satiety. The glycemic index is a measure of the capacity of carbohydrate-containing foods to raise blood glucose compared with glucose. Several studies examined the role and found that there was no significant difference in feelings of satiety comparing high-GI foods to low-GI foods [113-115]. Because of this it is very difficult to make definitive statements on the effect carbohydrates have on satiety.

The role fat plays in satiety is controversial and has been generally suggested that fat plays a less satiating role in satiety than proteins or carbohydrates [116, 117]. However, fat is significantly more energy dense (9 kilocalories per gram) than protein or carbohydrates (4 kilocalories per gram). One study compared the effects of the addition of either a carbohydrate or fat supplement to a standard breakfast in lean male subjects on subsequent energy intake. The carbohydrate supplement resulted in a short term

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reduction in energy intake, while the fat supplement produced no reduction in subsequent energy intake [118]. Another study compared the effects of high protein (HP), high carbohydrate (HC) or high fat (HF) breakfasts on appetite ratings and energy intake. They reported that the HP breakfast was most effective at suppressing appetite throughout the day, while the HF breakfast was least effective [119]. In the aforementioned studies factors such as chain length and degree of unsaturation were mostly not considered, but while it has been shown that chain length and degree of unsaturation may indeed influence feelings of satiety [120-122]. The form of fat and the method of delivery can have significant effects on satiety in activating a feedback mechanism known as the ileal brake, which will be discussed in the next section.

In summary, proteins, carbohydrates and fats all play a role in satiety/appetite and understanding the biological mechanisms in which they exert their effects is critical in the development of weightloss products designed to exploit these processes.


*Ileal Brake*

The ileal brake is the primary inhibitory feedback mechanism that controls the transit of food through the GI tract (Figure 6). Both GLP-1 and PYY serve as the most accepted biomarkers of the ileal brake. The term was first described by Read *et al*. who demonstrated that a test meal transit time down the GI tract was delayed upon lipid emulsion perfusion into the distal portion of small intestine while Spiller et al. demonstrated that infusion of fat into the ileum reduces jejunal motility [123, 124]. Ileal

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brake activation results in a delay in gastric emptying leading to an increase in small

intestine transit time for both solid and liquid food [70, 125, 126].

**Feedback Inhibition of Gastric Emptying**
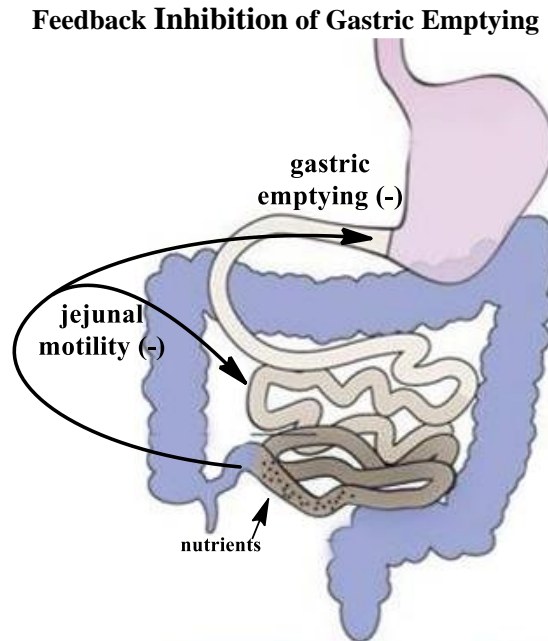


**Figure 6 - The lleal Brake Mechanism**
Adapted from Ref.[127]

Dietary fats predominantly in the TAG form that have been hydrolyzed into

FFAs are considered the most potent activators of the ileal brake, as confirmed by many

studies [70, 128-134]. Spiller *et al.* demonstrated that ileal infusion of FFA inhibited

jejunal motility to a far greater degree compared to TAG and glycerol [129]. This was

confirmed in several additional studies [135-137]. The possibility for FFAs having a

more potent effect is due to the relatively low lipase activity in the ileum compared to

the duodenum and jejunum. In regards to fatty acid chain length, jejunal infusion studies

demonstrated that FFAs with greater than ten carbons delay gastric emptying greater

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than FFAs of fewer than ten carbons [138, 139]. Ileal infusion of MCTs and LCTs were both shown to be activators of the ileal brake, which MCTs demonstrated a more rapid response [129]. The effect of degree of unsaturation of FFAs on ileal brake activation is still unclear, but some studies suggest that the satiating effects of unsaturated FFAs are greater than that of saturated FFAs [140-142].

As previously discussed, many studies demonstrated that protein and carbohydrate consumption affect appetite and energy intake. However, the studies did not determine if those effects were the result of ileal brake activation. A recent study investigated the effect of ileal infusion of sucrose and casein on food intake, release of CCK, GLP-1 and PYY, gastric emptying rate and small-bowel transit time. They reported that the ileal brake-satiating effect leading to a decrease in food intake is also obtained from protein and carbohydrates [143]. This study however, was conducted using only normal weight individuals as a proof of concept study.

Several earlier studies showed that carbohydrates, specifically maltose inhibit gastric emptying, reduce gastric acid secretion, decrease small intestine motility and decrease excretion of pancreatic enzymes which implies activation of the ileal brake, but those studies did not measure appetite or food intake [48, 144-146].

It is important to note that most of the studies examining whether certain macronutrients could activate the ileal brake, were conducted via ileal infusion, which is not practical as a consumer weight management solution. Ileal infusion of a macronutrient bypasses the extreme changes in pH and much of the mechanical and chemical digestion that orally consumed macronutrients would undergo. Therefore,

activating the ileal brake using dietary means poses a much greater challenge.  As such, there is great interest in fortifying food products with macronutrients/ingredients that promote slow release of nutrients such that they can reach the ileum intact and activate the ileal brake.  Since lipids appear to be the most potent activator of the ileal brake, it is important to have a rudimentary understanding of lipid digestion.

*Brief Review of Lipid Digestion*

Fatty acids in the diet are provided in the form of triacylglycerols and phospholipids (especially from lean meats) and are obtained from a variety of sources such as animal meat, milk fat, but primarily from plant oils.  The stomach and intestines serve as the main organs responsible for the digestion of fats, but digestion actually begins in the mouth via the secretion of lingual lipases.   Even though lingual lipases are secreted from the serous glands of the tongue their optimum pH of operation is at 4.5 – 5.5, which makes them most active in the stomach [147].  Once fat reaches the stomach further digestion continues via gastric lipase secreted from the gastric mucosa.  The churning of the stomach results in a physical reduction of fat particle size, which exposes the surface area for the lipases to act upon.  Gastric lipase cleaves at the sn-1 or sn-3 position of the triacylglycerol backbone leading to sn-2,3-diacylglycerols or sn-1,2 diacylglycerols and a free fatty acid.  The hydrolysis products of TAG help promote emulsion oil droplet formation.

While about 10-30% of fat digestion occurs in the stomach, the bulk of fat digestion occurs in the small intestine [147].   The pyloric sphincter opens and allows the

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chyme to pass into the duodenum where the stomach acids will be neutralized by sodium bicarbonate produced by the pancreas.  Once in the small intestine, fat will promote the release of CCK from the intestinal I-cells which will bind to receptors on the gallbladder to stimulate the release of bile salts and to receptors on pancreas to stimulate the release of pancreatic lipase and co-lipase.  In the duodenal lumen the bile salts will insert themselves into the emulsion oil droplet, which will expose the sn-1 or sn-3 fatty acids at the lipid-water interface.  Colipase activates pancreatic lipase, which will cleave two fatty acids at the sn-1 and sn-3 positions of the glycerol backbone leaving two FFAs and sn-2-monoacylglycerol.  Similarly pancreatic lipase also hydrolyzes phospholipids (mainly phosphatidyl-choline) into a diacylglycercol or lysophospholipid and an FFA. Cholesterol esters from animal fats also undergo hydrolysis by pancreatic lipase into cholesterol and FFA.

Bile salts promote micelle formation using the hydrolysis products of TAG, phospholipids and cholesterol esters.  In micelle form the fatty acids will be absorbed by the intestinal mucosa cells located primarily in the duodenum and jejunum.  Since most fatty acid absorption takes place in duodenum and the jejenum it remains a dietary and food design challenge to delay the absorption of fatty acids so that absorption takes place in the ileum.  Therefore it remains a challenge to design food systems that can modify lipid digestion such that the lipids are reaching the ileum intact.  Emulsions have been utilized as one such lipid delivery system with the potential to modify lipid digestion [148].

*Emulsions and Their Potential as a Lipid Delivery System*

An emulsion is a food system in which there is a mixture of two or more immiscible liquids in which one liquid is dispersed in the other, separated by a boundary known as the interface. The interface is stabilized by an amphipathic molecule known as an emulsifier and is a common component of food systems (i.e. mayonnaise, butter, cakes, milk). Examples of commercial emulsifiers include, lecithin, sodium stearoyl lactylate, and proteins such as casein due to their hydrophobic and hydrophilic properties. Several studies investigating the effects of orally consumed emulsions, demonstrated a reduction in appetite and subsequent energy intake [149-151].

To formulate an emulsion-based lipid delivery system, several factors have to be considered since any orally consumed emulsion will undergo many physical and chemical changes upon GI tract traversal. Factors such as pH, ionic composition, surface-active components, enzyme activity, and temperature are all considerations when designing an emulsion-based delivery system [152].

An important factor in controlling the rate of lipid digestion is the inhibition of pancreatic lipase, which prevents the hydrolysis of TAGs into FFAs and glycerol. Other factors to control the lipid digestion rate include, ingredient interactions such as dietary fiber-bile interactions, and mass transport barriers where lipid droplets are encapsulated within hydrogel beads, which decrease the rate of diffusion. Emulsions may influence lipid digestibility by several factors: droplet size, droplet lipid composition and the choice of emulsifier.

Droplet size potentially affects lipid digestion. Several studies demonstrated that the lipid digestion rate is increased with decreasing droplet size [153-156]. However, one study demonstrated that emulsions stabilized by β-lactoglobulin decreased the digestion rate with decreasing droplet size. It was suggested that the smaller β-lactoglobulin emulsions would be coated by a thicker layer of aggregated globular protein on smaller droplets. These findings demonstrated that the structure of the oil-water interface plays a role in lipid digestion [157].

Lipid composition of droplets is another consideration in formulating an emulsion-based delivery system. Examples of composition differences include digestible versus non-digestible oils, TAG oils versus essential oils, as well as fatty acid chain lengths and/or degree of unsaturation. Several studies demonstrated that long chain TAGs are known to digest at rate slower than medium chain TAGs since the long chain TAGs have a tendency to accumulate at the oil-water interface, while medium chain TAGs interact more favorably with the surrounding aqueous environment [158, 159]. Thus, lipid composition plays an important role in decreasing or increasing the rate of lipid digestion.

The choice of emulsifier is another significant consideration in formulating an emulsion-based delivery system. It was demonstrated in one study that emulsions formulated with phospholipid emulsifiers underwent more rapid digestion than emulsions formulated with proteins as emulsifiers [160]. Another study demonstrated that the rate of lipid digestion was lower for emulsion droplets initially coated by monoglycerides, than those coated by proteins or phospholipids [161]. Emulsions

formulated with galactolipids as emulsifiers from oat oil demonstrated a reduction in the rate of lipolysis by inhibition of the binding of pancreatic colipase and lipase at the oil-water interphase in the duodenum [148]. Another study demonstrated that galactolipids from an oat oil emulsion promoted the formation of needle-shaped fatty acid crystals that dissolved gradually while traversing the GI tract. The oat oil emulsion treatment yielded significantly greater quantities of fatty acids in the jejunum compared with a milk fat control treatment. The authors suggested that the fatty acids crystals formed from the oat oil emulsion could function as a "slow-release capsule" that could potentially increase fatty acid exposure to the ileum, triggering the ileal brake [133].

It should be noted that many food based emulsions are formulated with multiple emulsifiers. The primary emulsion is formed by combining an emulsifier such as lecithin (phospholipid) or mono/diacylglycerol with the oils and then combined with water. Once the primary emulsion droplets are formed, a secondary oppositely charged emulsifier (often casein) is added to the droplet to form the secondary emulsion, further increasing emulsion stability.

While droplet size, lipid composition and emulsifier choice aid in the formulation of an emulsion-based delivery systems, it is important to note that the interfacial composition of an emulsion changes as it traverses the GI tract. Individual genetic variation affects how emulsions might interact with bile salts, FFAs or phospholipids. All of which may affect the rate of lipid digestion. Nevertheless, it was demonstrated that emulsion-based lipid delivery systems have the potential to modify lipid digestion where FFA exposure to the ileum is increased, promoting ileal brake activation.

However, in order for emulsions to have potential as a satiety inducing ingredient, an appropriate food system for delivery must be formulated, followed by subjective and objective evaluation to ensure that the treatments are indistinguishable to human subjects.

*Subjective and Objective Analysis of Food Systems*

Subjective and objective analysis of a formulated food system are two key components in the formulation of food products that are combined with satiety inducing ingredients. Subjective analysis includes sensory evaluation of a food system using panelists, while objective evaluation of a food system employs laboratory instrumentation to measure the physical, visual and chemical parameters of a food system.

Subjective tests are sensory evaluations of a food product that require recruitment of panelists to assess the quality and characteristics. Sensory testing may involve describing food color, texture, flavor, aftertaste, astringency or aroma utilizing the five senses of touch, sight, odor, taste and even sound. The three classes of sensory testing most commonly administered are affective, discrimination and descriptive tests. Each test serves a different purpose in product quality assessment.

Affective tests quantify the degree of liking or disliking of one product. They are administered using untrained panelists who are recruited to represent the typical consumer of the product of interest. Examples of affective tests, include the consumer acceptance test which provides a rating to individual attributes of a food system that

consumers rank on a scorecard. Examples of sensory attributes that can be ranked are the pleasantness, visual appeal, the smell/odor and the taste on a 1-10 scale in accordance to previously published methodology [162-164].

Discrimination tests are administered using untrained panelists to determine if any difference exists between two products. The two products being tested usually possess minor ingredient differences and/or formulations. One such discrimination test is the triangle test. During a triangle test, panelists are presented with one different and two identical samples on a tray. The panelists are instructed to identify the different sample. Each sample is labelled with a random 3-digit code to minimize bias. The triangle test is useful in determining if differences in formulations of a food system may produce an overall difference but no specific flavor, color or texture attributes were identified as being affected [165].

Descriptive tests require highly trained panelists and seek to describe specific product attributes related to, but not limited to, flavor, texture, astringency or viscosity. Panelists rate the intensities of specific sensory characteristics to define flavor and texture profiles of a food system [165]. Descriptive analysis is the most comprehensive and information-specific means of providing sensory feedback on a food system.

Objective evaluation of a food system employ laboratory instrumentation to analyze physical, chemical and visual attributes such as viscosity, pH and Hunter L, a, b color. Viscosity is a measure of a food system's resistance to gradual deformation by shear stress. The viscosity is calculated by dividing the shear stress, the force acting in the plane of a fluid, by the shear rate, the velocity experienced by the fluid between

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moving planes.   It is an important characteristic of a food system that affects texture, appearance and mouthfeel.  The viscosity of a food system is typically classified as being either Newtonian or Non-Newtonian.  The viscosity of a Newtonian food system remains constant at a given temperature and shear stress regardless of shear rate.  Water and honey are examples of Newtonian food systems.  The viscosity of a Non-Newtonian food system fluctuates depending on the shear rate.  Examples of non-Newtonian foods may include ketchup, mayonnaise and yogurt.

pH is the measure on a 1-14 logarithmic scale of a solution's acidity or alkalinity (basicity).  pH is calculated as the negative log of the hydrogen ion concentration.  Any solution with a pH below 7 is considered acidic and any solution with a pH above 7 is considered alkaline or basic.  Formulating and processing to stable pH is a key quality parameter of a food system that is necessary to preserve flavor, texture and color.  If the pH decreases, a sour flavor may form due to protonation of weak acids, affecting the mouthfeel and the texture.  The pH may impact the color of the food system due to the chemical alteration of pigment molecules.  Therefore, ensuring that the pH is consistent between treatments and remains stable over a period of time is critical in the development of a food system.

The color of a food system is a critical visual indicator and is closely related to the consumer perception of quality.  Food color is determined by the presence of specific pigment molecules in the food system, and may be affected by changes in pH, texture or microbial growth.  The Hunter L, a, b, scale measures the specific color values and is often used to provide an analysis of color and can detect changes in color over time.  The

"L" scale measures light vs. dark where a low number (0-50) is toward dark, 0 being black, and a high number (51-100) indicates light, 100 being white. The "a" scale measures red vs. green where a positive number indicates "redness" and a negative number indicates "greenness" in a product. The "b" scale measures yellow vs. blue where a positive number indicates "yellowness" and a negative number indicates "blueness." Ensuring that color is consistent and remains stable over a period of time is critical in the quality assessment of a food system.

In a blind placebo satiety study, the food system should be formulated such that there are no statistically significant objective or subjective differences among the treatments that may otherwise influence satiety or subsequent energy intake.

*Overview of Relevant Satiety Studies*

There have been many satiety studies over the past several decades that have tested the effects of various weight loss supplements/satiety agents on human subjects. This section will emphasize the satiety studies that are most relevant to the research in this dissertation.

Several studies by Burns *et al.* investigated the effects of a yogurt supplemented with Olibra™, a fat emulsion composed of a 95:5 palm:oat oil ratio. Much of the research detailing how Olibra™ was formulated and the rationale for the choice of oils and composition remain unpublished. In their first study, Burns *et al.* investigated the short term effects of Olibra™ on energy and macronutrient intake in only non-obese subjects (BMI < 30) [150]. The study involved two double-blind, placebo-controlled,

within-subject crossover phases that were conducted three months apart. Twenty-nine (15 female, 14 male) subjects participated in the first phase and thirty (16 female, 14 male) subjects participated in the second phase. In each phase, subjects were given in random order, seven days apart, either a treatment was composed of 200g yogurt + 6g of Olibra™ or the control treatment composed of 200g yogurt + 6g of milk fat at 1:00pm on the test day. Four hours post-consumption subjects were given *ad libitum* access to a range of foods. The amounts of food consumed by subjects was determined by pre- and post-covert weighing of individual serving dishes. The results demonstrated that mean energy intakes were significantly lower after consumption of the Olibra™ treatment compared with the control treatment for both phases. The corresponding fat, protein and carbohydrates in both phases were significantly reduced for the Olibra™ compared to the control treatment. They concluded that the possibility exists that the physicochemical characteristics of small amounts of fat can potentially affect short-term satiety [150].

For their second study, Burns *et al.* investigated the effects of Olibra™ on energy and macronutrient intakes in non-overweight, overweight, and obese subjects at four and eight hours post-consumption. They conducted a randomized double-blind, placebo-controlled, within-subject crossover study comparing an emulsion treatment and a control treatment. The emulsion treatment was composed of 200g yogurt + 6g of Olibra™, while the control treatment was 200g yogurt + 6g of milk fat. Twenty (10 male, 10 female) non-overweight (BMI 20-24.9 kg/m$^2$), twenty (10 male, 10 female) overweight (BMI 25-29.9 kg/m$^2$) and twenty (7 male, 13 female) obese (BMI > 30

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kg/m$^2$) subjects for a total of 60 subjects participated in the study. Subjects were given

in random order, seven days apart, either the emulsion treatment or control treatment

starting at 9:00am on the day of the study. At four and eight hours post-consumption

subjects were given *ad libitum* access to a range of foods. Amounts of food consumed

were determined by pre and post-covert weighing of individual serving dishes. Over the

following 24 hours, subjects weighed and recorded all food intakes. The results showed

that mean energy intakes were significantly lower for the emulsion treatment compared

with the control in non-overweight and overweight subjects, four hours post-

consumption and in all subjects eight hour post-consumption. Macronutrient intakes

were also significantly reduced in non-overweight and overweight subjects at four hours

post-consumption and in all subjects eight hours post-consumption. They concluded that

Olibra™ has the potential to reduce mean energy intakes up to eight hours post-

consumption and concluded that the Olibra™ was likely exerting its satiety effects via

activation of the ileal brake [149].

For their third study, Burns *et al.* investigated if the energy and macronutrient

intake responses to Olibra™ were dose-dependent and if that effect could be maintained

for up to 36 hours in non-overweight subjects (BMI 20-24.9 kg/m$^2$) using single-blind,

placebo-controlled, within-subject cross-over design [151]. Fifty subjects, consisting of

thirty female and twenty males, participated in the study. Each subject was studied on

four occasions, on the same day of the week in a randomized treatment order in one-

week intervals. Subjects were provided a 200 gram portion of yogurt containing either

0, 2, 4, or 6 gram quantity of Olibra™ at 9:00am on each study day. The 0 g quantity

served as the control treatment. At 1:00pm subjects were given *ad libitum* access to a range of foods. Amounts of food consumed were measured by covert pre- and post-consumption weighing of individual serving dishes. For the remainder of the day and the following 24 hours, subjects weighed and recorded all food intakes. Compared to the control yogurt, mean energy intakes were progressively reduced with increasing doses of Olibra^TM fat. Energy and macronutrient intakes for the remainder of each study day and over the following 24 hours were significantly lower after all dose levels compared to the control. Their results suggest that Olibra^TM reduced the effect of overeating during an *ad libitum* lunch meal and subsequent food intake up to 36 hours post-consumption [151].

Logan *et al.* investigated the effect of Olibra on medium-term food intake and appetite in twenty-eight (14 male, 14 female) non-obese subjects (BMI $> 30$ kg/m$^2$) using a double-blind, placebo-controlled, within-subject crossover [166]. The emulsion treatment was composed of 200g yogurt + 5g of Olibra™, while the control treatment was 200g yogurt + 5g of milk fat. Subjects were randomly assigned to receive either the treatment or control for breakfast for two three week study phases, separated by a three week interval. On days 1, 8 and 22 of the study phases, food intake was assessed by pre- and post-covert weighing at an *ad libitum* buffet-style test lunch, four hours post-consumption of treatments. Throughout each of these study days, appetite was assessed using visual analogue scales at regular intervals. For the remainder of the study days, and the following 24 hours, subjects reported their food intake using dietary records. The results showed that consumption of the Olibra^TM treatment had no significant effect

on mean energy, macronutrient or amounts of food consumed at the lunch four hours post-consumption. Self-reported food intakes indicated that there was no significant reduction of food intakes for the Olibra™ treatment. There was no consistent effect of the Olibra™ treatment on appetite ratings. In contrast to results demonstrated by Burns *et al*, there was no evidence of a short- or medium-term effect of the Olibra™ treatment on food intake or appetite. Logan *et al.* suggested that their results could be owing to numerous confounding factors that influence eating behavior.

K. Diepvens *et al.* assessed the effects of Olibra™ on weight maintenance after weight loss including effects on body composition, resting energy expenditure (REE), fat oxidation, hunger feelings and satiety hormones using a randomized, placebo-controlled, double-blind, parallel design in fifty overweight women (BMI 25-32 kg/m$^2$) [167]. The study involved a six week weight loss period that was followed by eighteen weeks of weight maintenance. The emulsion treatment was composed of 200g yogurt + 5g of Olibra™, while the control treatment was 200g yogurt + 5g of milk fat. In weeks 1, 7 and 25, VAS satiety questionnaires were administered and blood samples were collected for analysis of satiety hormones. In weeks 2, 8 and 26, REE, body weight and BMI were recorded. The results demonstrated that during the weight maintenance phase after significant body weight reduction, there was no significant increase in body weight for the Olibra™ treatment group, while the control treatment group gained weight. Compared to the control treatment group, the Olibra™ treatment group demonstrated significantly less hunger four hours post-consumption in week 25 and showed significantly increased GLP-1 values 180 minutes post-consumption. They reported that

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measured REE as a function of fat-free mass (FFM) was significantly higher than predicted REE in week 26 for the Olibra$^{TM}$ treatment group, but not for the control treatment group. Fat mass was significantly more decreased in the Olibra$^{TM}$ treatment group compared to the control treatment group. They also reported that consumption of the Olibra$^{TM}$ treatment improved weight maintenance compared to placebo, which can be explained by the relatively higher REE as a function of FFM, relatively higher decrease in FM and the relatively lower increase in hunger. Overall, the authors concluded that the Olibra™ treatment improved weight maintenance compared to the control treatment [167]. These results were not consistent with the later work of Olsson et al. who reported that the addition of Olibra$^{TM}$ treatment to a meal-replacement diet plan resulted in 0.9% decrease in BMI, but there was not a significant difference in bodyweight change for the Olibra$^{TM}$ treatment compared to the milkfat treatment [168].

K. Diepvens *et al.* also conducted a study examining the short term effects of Olibra™ on satiety and energy intake using a randomized, double-blind, placebo-controlled, crossover design. The study consisted of forty one total subjects divided into two weight categories: twenty one normal weight (~BMI 22.0 kg/m$^2$) and twenty overweight (~BMI 27.7 kg/m$^2$). The Olibra$^{TM}$ treatment was composed of 200g yogurt + 5g of Olibra™, while the control treatment was 200g yogurt + 5g of milk fat. VAS satiety questionnaires were administered on each visit with a one week interval between treatments. They reported that in the normal weight subjects, consumption of Olibra$^{TM}$ treatment reduced hunger and desire to eat during the morning. No significant differences in appetite scores between the Olibra$^{TM}$ treatment and the control treatment

were seen for the overweight subjects. No effect on energy intake was seen in the total group, in the junior-normal weight and senior-overweight subjects. The authors concluded that the Olibra$^{TM}$ treatment exerted a suppressive effect on appetite ratings for over three hours in normal weight women aged and that consumption of Olibra$^{TM}$ treatment did not affect subsequent energy intake in either group [169].

A study by Haenni *et al*. is one of the few studies that specifically examined whether examined Olibra$^{TM}$ was exerting its effects on satiety and energy intake via activation of the ileal brake. They examined whether consumption of the Olibra$^{TM}$ treatment had an effect on orocecal transit time (OCTT) in healthy men using a controlled, double-blind, cross-over-designed study in fifteen healthy men (BMI 22-28). Subjects were provided both the Olibra$^{TM}$ treatment that was composed of 200g yogurt + 5g of Olibra$^{TM}$, or the control treatment was 200g yogurt + 5g of milk fat in randomized order separated by one week intervals. OCTT was determined by following blood sulfapyridine levels, a metabolite of salazopyrine in the colon. Orally consumed salazopyrine serves as a marker for OCTT since it is poorly absorbed in the gastrointestinal tract, but is rapidly absorbed in the colon upon being hydrolyzed by microflora. The results showed that there was a statistically significant delay in the emergence of sulfapyridine in serum after consumption of the Olibra$^{TM}$ treatment versus control treatment, demonstratung a greater OCTT due to fat emulsion consumption. The authors concluded that their study provided the first evidence to suggest that Olibra$^{TM}$ may affect the ileal brake mechanism by delaying the gastrointestinal transit time [170]. Similar findings were reported by Knutson *et al*. who hypothesized that the effects

observed could be due to the presence of fat crystals in the jejunum that occurred for subjects who consumed the Olibra[TM] treatment [133].

Chan et al. examined the effect of Olibra[TM] on satiety and short-term food intake under a range of dietary conditions in contrast with previous studies where Olibra[TM] was combined with yogurt to serve as the test treatment [171]. This was controlled, double-blind, cross-over-designed study that involved eighteen lean men (BMI 18–25 kg/m$^2$) who received six treatments in a randomized order: (i) lipid emulsion, LE (15 g Olibra[TM], containing 4.2g lipid, 0.2 MJ)+water, (ii) lipid control, LC (15 g non-emulsified lipid/water, containing 4.2g lipid, 0.2 MJ)+water, (iii) lipid emulsion+yoghurt, LE+Y (1.2 MJ), (iv) lipid control+yoghurt, LC+Y (1.2 MJ), (v) lipid emulsion+muffin, LE+M (1.2 MJ), (vi) lipid control+muffin, LC+M (1.2 MJ), each given as a test breakfast at 8.30 am. They administered the emulsion along with 185 mL water, stirred into a semi-liquid dairy yoghurt, and co-presented with a solid food breakfast muffin to determine whether Olibra[TM] enhances satiety and suppressed short-term food intake under different dietary conditions. Subjects completed VAS questionnaires, and *ad libitum* energy intake was measured at a lunch meal 3.5 hours post-consumption. Olibra[TM] increased satiety compared to iso-caloric lipid control but only when administered with yogurt. There were no effects on satiety ratings when co-presented with water or with the solid food muffin. Energy and macronutrient intake were not significantly decreased by any of the emulsion treatments. They demonstrated that though the effects were small, the format in which Olibra[TM] is consumed influences satiety, and there is no evidence that Olibra[TM] alters eating behavior at the subsequent

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meal. The authors concluded that yogurt may play a synergistic role when consumed with Olibra™ in suppressing appetite [171]. The authors do not speculate as to why such may be the case, but yogurt is highly viscous, contains proteins that may aid in emulsion stabilization and has been shown to decrease appetite [172].

In the most recent study examining the effects on satiety and energy intake Rebello et al. determined if Olibra™ in conjunction with a healthy diet resulted in weight reduction that is associated with energy intake reduction. The study was conducted over 12 weeks as randomized, placebo-controlled, double-blind, parallel trial that measured the effects of Olibra™. The Olibra™ treatment was composed of 200g yogurt + 2.1g of Olibra™, while the control treatment was 200g yogurt + 1.95g of milk fat. The Olibra™ treatment or milk fat control treatment were administered twice daily, and the effects on food intake, appetite, satiety, weight, and body composition. Eighty-two subjects (number of males and females were not reported) were recruited (BMI 25-40 kg/m$^2$). Subjects were required six total visitations to the clinic: day -7 (visit 1), day 0 (visit 2), day 14 (visit 3), day 28 (visit 4), day 56 (visit 5) and day 84 (visit 6). On days -7, 0, and 28, Olibra™ or milkfat treatment served as breakfast and lunch. Food intake, appetite, and satiety were assessed after lunch and dinner. Body weight was measured on all visits. After 12 weeks, differential group effects were not significant for body fat, waist-hip ratio, food intake, appetite, and satiety. The authors concluded that at the administered dose, Olibra™ did not exert a consistent effect on food intake, appetite regulation, body weight, or body composition [173].

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There is sufficient evidence in the literature demonstrating the effects that emulsions, specifically Olibra[TM], exert on satiety, energy reduction and ileal brake activation [149-151, 166-171, 173]. Although the findings from other studies are not in complete agreement [168, 169]. At this time there have been no reported satiety studies which examined the effects of fish oil emulsions on satiety or ileal brake activation. Fish oil has several properties suggesting it could serve as a potential activator of the ileal brake. Fish oil is comprised of a high amount of long chain (80%) and unsaturated fatty acids (55%), both of which have been demonstrated to have a positive effect on satiety and ileal brake activation [138-142]. In addition to the potential effect on satiety, the omega-3 (n-3) fatty acids are considered anti-inflammatory nutrients, which could potentially alleviate the inflammatory response of obesity [174]. Therefore, there are multiple health-related benefits in determining if an emulsion containing fish oil can induce satiety, a reduction in energy intake and trigger ileal brake activation.

*Cell Culture Studies Measuring the Response of Satiety Hormones*

Given the expense and manpower required to conduct human clinical trials, cell culture studies utilizing isolated intestinal cells are employed as an alternative to testing the effects of nutrients on hormone secretion, specifically PYY and GLP-1. While isolated human intestinal cells provide the best screening model to test effects of nutrients on hormone secretions, their isolation is a lengthy process and produces fairly low yields. Because of such difficulties, the STC-1 cell line, derived from an intestinal endocrine tumor in a double-transgenic mouse, has been utilized for such studies [175].

Cordier Busset *et al.* demonstrated that addition of protein hydrolysates to the STC-1 cell medium stimulated GLP-1 secretion and the upregulation of the proglucagon gene [176, 177]. Wang *et al.* demonstrated that luminal CCK-releasing factor promoted CCK secretion in STC-1 cells and was confirmed that in a later study using protein hydrolysates [177-179]. Saris *et al.* demonstrated that supplementing the STC-1 cell culture medium with FFAs of various length stimulated the secretion of PYY [180]. Such studies suggest that the STC-1 cell line remains a viable alternative in analyzing the response of CCK, GLP-1 and PYY secretion to nutrient supplementation. Despite the advantages in cost and time, the STC-1 model only determines if a specific ingredient or satiety agent can promote PYY and GLP-1 secretion and does not determine whether such an ingredient/satiety agent can promote satiety in human volunteers.

CHAPTER II

EMULSIONS COMPOSED OF FISH OIL AND PALM OIL PROMOTE PEPTIDE YY

AND GLP-1 SECRETION FROM STC-1 CELLS

**BACKGROUND AND RATIONALE**

The goal of phase one was to test the hypothesis that two emulsions composed of

varying palm oil to fish oil ratios (referred to as fish oil emulsions for simplicity)

induced glucagon-like-peptide 1 (GLP-1) and peptide-YY (PYY) secretion from STC-1

cells, a murine enteroendocrine cell line that secretes hormones in a manner that

resembles intestinal L-cells in humans.

GLP-1 is an incretin produced and secreted by the intestinal L-cells of the ileum

and proximal colon [38-41]. GLP-1 enhances glucose-dependent insulin secretion,

inhibits glucagon release from the pancreatic $\alpha$-cells and delays nutrient absorption

through inhibition of gastric emptying and intestinal motility [42-44]. Macronutrient

binding to receptors on the intestinal L-cells stimulates production and secretion of GLP-

1 into the bloodstream [52-64]. From there GLP-1 will cross the blood brain barrier and

bind to receptors in the hypothalamic arcuate nuclei to suppress appetite and induce

satiety [48, 67]. GLP-1 is rapidly degraded by DPP-4 and therefore a sufficient amount

needs to be produced to exert its effects [65, 66].

PYY is produced and secreted by intestinal L-cells and exhibits a similar

inhibitory effect on gastric emptying and jejunal motility as GLP-1 [70]. The exact

intercellular mechanisms leading to PYY production are still unknown, but upon nutrient

stimulation PYY is produced and secreted from the L-cells into the bloodstream where PYY is degraded by DPP-4 into its active $PYY_{3-36}$ form. $PYY_{3-36}$ exclusively binds to the Y2 receptors expressed in the hypothalamic arcuate nucleus to promote appetite suppression and satiety [70-75].

Given their role in delaying gastric emptying and inhibiting jejunal motility, GLP-1 and PYY are considered biomarkers of the ileal brake, a gastric inhibitory feedback mechanism that controls the transit of food through the gastrointestinal tract that has been shown to reduce food intake and increase satiety [121-132]. As a result the ileal brake has become an attractive target for appetite control.

STC-1 cells have been shown to secrete GLP-1 and PYY upon ingredient supplementation to the cell culture medium in a manner similar to intestinal L-cells [176, 177, 180]. At the time of writing, there has been no reported study that has examined whether an emulsion can stimulate secretion of PYY or GLP-1 in STC-1 cells.

An emulsion is a mixture of two immiscible liquids in which one liquid is dispersed (dispersed phase) in the other (continuous phase). This mixture is stabilized by an emulsifier that contains a hydrophilic head to interact with the water phase and the hydrophobic tail to interact with the oil phase. Example of commercially used emulsifiers consist of lecithin, diacetyl tartaric acid ester of mono- and diglycerides (DATEM) and casein. The simplest emulsions are classified as oil in water (O/W) or water in oil (W/O).

The commercial product Olibra$^{TM}$ (Lipid Technologies Provider AB (LTPAB), Karishamn, Sweden) is an emulsion composed of a 95:5 ratio of palm oil to oat oil in

which the hydrophilic galactolipids derived from oat oil serve as the emulsifier. Olibra[TM] when consumed with yogurt has been previously shown to suppress appetite and reduce food intake in clinical studies [149-151]. Much of the research detailing how Olibra[TM] was designed and the justification for using palm oil as the main constituent of the emulsion remains unpublished. However, it is known that in addition to being readily available palm oil does possesses high amounts of palmitic acid (~44%) and oleic acid (~37%) both of which had been shown to have potential in activation of the ileal brake [138, 139].

For this specific study two oil in water emulsions that contained varying ratios of palm oil to fish oil were prepared and provided by Omega Protein (known previously as Omega Pure) a publicly traded company located in Houston, Texas that specializes in developing ingredients such as protein, omega-3 fatty acids, and antioxidants that can be added to functional foods to improve their nutritional value. Omega Protein had an interest in developing their own emulsion using fish oil extracted from menhaden fish instead of oat oil to serve as an alternative to Olibra[TM]. Fish oil has several properties suggesting it could serve as a potential activator of the ileal brake. First, over 80% of fish oil is comprised of long chain fatty acids, which have been demonstrated to delay gastric emptying [138, 139]. Second, fish oil also contains a high amount of unsaturated fatty acids (55%) which some studies suggest that a higher degree of unsaturation corresponds to a greater effect on satiety, although findings are inconclusive [140-142]. In addition to the potential effect on satiety, the omega-3 (n-3) fatty acids specifically docosahexaenoic acid (DHA-22:6n-3) and eicosapentaenoic acid (EPA-20:5n-3) are

considered anti-inflammatory nutrients and have been often been used a dietary supplement for inflammatory diseases such as rheumatoid arthritis and cardiovascular disease [174]. Therefore, there are multiple health-related benefits to knowing if a fish oil emulsion can induce the secretion of GLP-1 and PYY in STC-1 cells.

If the fish oil emulsions were shown to stimulate GLP-1 and PYY secretion from STC-1 cells it would be reasonable to investigate whether oral consumption of fish oil emulsions could induce satiety in human volunteers.

**EXPERIMENTAL DESIGN**

*Emulsion Composition and Preparation*

The emulsions for the beverage were prepared by Omega Pure (Houston, Texas). Two emulsions referred to as A and B were tested. The emulsions differed in their palm oil to fish oil ratios, being either 2:1 (A) or 1:1 (B). Table 3 shows the quantities of each ingredient used in preparing a 200g emulsion sample. Table 4 presents a description of the components used to prepare emulsion A and emulsion B. Figure 7 shows the treatment preparation diagram.

| Emulsion A | | | | Emulsion B | | |
|---|---|---|---|---|---|---|
| 2:1 palm oil: fish oil ratio | | | | 1:1 palm oil: fish oil ratio | | |
| Ingredient | % | Amt / 200 g | | Ingredient | % | Amt / 200 g |
| Water | 66.58% | 133.16 g | | Water | 66.58% | 133.16 g |
| SansTrans 39 | 19.58% | 39.16 g | | SansTrans 39 | 14.69% | 29.38 g |
| OP HSN | 9.79% | 19.58 g | | OP HSN | 14.69% | 29.38 g |
| Na-caseinate | 1.96% | 3.92 g | | Na-caseinate | 1.96% | 3.92 g |
| Gum Arabic | 1.37% | 2.74 g | | Gum Arabic | 1.37% | 2.74 g |
| K-sorbate | 0.49% | 0.98 g | | K-sorbate | 0.49% | 0.98 g |
| Panodan | 0.23% | 0.45 g | | Panodan | 0.23% | 0.45 g |

**Table 3 - Quantity of Ingredients Present in Each Emulsion**

| Ingredient | Function |
|---|---|
| **Gum arabic** | A complex mixture of glycoproteins and polysaccharides that is used primarily in the food industry as a stabilizer for the purpose of reducing the surface tension of emulsions. |
| **Panadan** | A diacetyl tartaric acid ester of mono-diglycerides (DATEM) made from edible, refined soybean oil. Served as the emulsifier to make primary emulsion. |
| **Na-caseinate** | Proteins derived from milk that are commonly used as food stabilizers. Used as coating to the primary emulsion to form the secondary emulsion. |
| **K-sorbate** | Served as a preservative against molds and yeasts. |
| **SansTrans™ 39** | Served as the palm oil portion of the palm oil phase. Developed by Lokers Croklaan™ and sold commercially as multi-bakery shortening based on palm oil and fractions of palm oil. It has been designed as an alternative to shortening due to the fact that it is non-hydrogenated and contains no trans-fatty acids. Melting point is reported to be between 37-41°C and is composed of 49.9% of saturated fatty acids, 40.1% mono-unsaturated, and 9.6% polyunsaturated according to manufacturer spec sheet. |
| **Omega Pure (OP) HSN** | Served as the fish oil portion of the fish oil phase. Derived from menhaden fish. Composed of 32% unsaturated fatty acids, 24% mono-unsaturated, 36.74% polyunsaturated (35.66% omega-3) and 7.12% that is not defined on the sheet. EPA + DHA composed 26.12% of total fatty acid composition. Also contains tocopherols to serve as antioxidants. |

**Table 4 - Ingredients Used in Emulsions**

All of the above ingredients were necessary to prepare a stable emulsion. Emulsions A and B differed only in the amount of SanTrans™ 39 and OP HSN
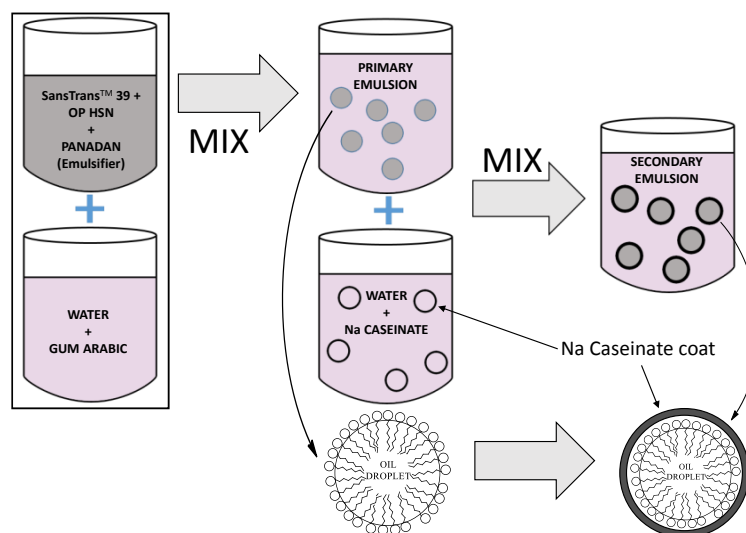


**Figure 7 - Emulsion Preparation Diagram**

Emulsion A and B both differed in the amount of SansTrans™ 39 (palm oil) and OP HSN (fish oil) present. Both oils were mixed together plus the DATEM emulsifier to make the oil phase. The oil phase was mixed with the aqueous gum arabic solution to create the primary emulsion. The primary emulsion was then mixed with the aqueous sodium caseinate resulting in the final (secondary) emulsion.

To prepare the oil phase, both solid SansTrans$^{TM}$ 39 (palm oil source) and liquid

OP HSN (fish oil source) were heated in separate beakers on the same heating plate

(Corning PC-420D) to 44°C.  The temperature of the oil mixture was monitored using a

standard thermometer.  A temperature of 44°C was sufficient to completely melt the

SansTrans$^{TM}$ 39 and ensured that mixing of both oils would be uniform.  Both oils were

combined with a proprietary quantity of rosemary antioxidant and Panodan emulsifier to

create the oil phase.  Due to confidentiality agreements with Omega Pure$^{TM}$, the exact

quantity of rosemary extract is unknown.  Panodan, in liquid form, was added to the

mixture using a 5mL pipette.  The solution was mixed with a stir bar at a speed of 500

rpm.

To prepare the water phase, two equivalent water solutions were prepared: one

that contained dissolved gum arabic and one that contained dissolved sodium caseinate.

The gum arabic and sodium caseinate were dissolved in distilled water via stir bar

mixing at a speed of 500 rpm in separate beakers and mixing plates at room temperature.

Using an Ultra Turrax T 50 Basic shearing mixer (IKA Werke$^{TM}$ Wilmington,

NC) the oil phase solution was slowly added to the gum arabic water solution.  The

shearing speed was gradually increased to maximum as the dispersion became more

evenly mixed.  After this step, the range of the size of the oil droplets was approximately

1-10 μm. Once the dispersion was sufficiently mixed it was then passed through a

homogenizer (Niro Soavi$^{TM}$ NS10011 2K Bedford, NH) at 50 Bar to break up larger oil

droplets and then passed again at 400 Bar to further decrease the oil droplet size down to

0.2–2 μm with some flocculation. The resulting dispersion was referred to as the

primary emulsion as shown in Figure 7.

Using the Turrax mixer the primary emulsion was gradually added to the sodium

caseinate solution where the negatively charged caseinate functioned as an emulsion

stabilizer. Sodium caseinate can stabilize emulsions by lowering the interfacial tension

due to its adsorption at the interface. Similar to the first step the shearing speed of the

Turrax mixer was gradually increased as the dispersion became more uniform. The

resulting dispersion was then passed through a homogenizer at 400 Bar to ensure a

consistent oil droplet size range of 0.2–2 μm with minimal flocculation. The emulsions

were examined under a microscope to ensure minimal flocculation and consistent droplet

size. The emulsions were sealed and refrigerated at $4^0C$ until it was time to for them to

be added to the cell culture.


*Cell Culture*

The STC-1 hormone secretion assay was performed using a protocol established

by Geraedts et al [180]. The STC-1 cell line was obtained from ATCC with permission

granted by Dr. Hanahan from the University of California at San Francisco. The cells

were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with high

glucose (1.5g/L), 1mM sodium pyruvate (Sigma), 1X non-essential amino acids

(Sigma), 10% fetal bovine serum with penicillin (100units/mL) and streptomycin (100

ug/ml) in an incubator (Thermo™ Forma Series II) at 37ºC in 5% $CO_2$. Cells were

passed until there was a sufficient number of cells to seed at least six 24-well plates at

~$10^5$ cells/well.  Cell counts prior to seeding were measured using Muse™ Cell

Analyzer made by EMD-Millipore.


*STC-1 Hormone Secretion Assay and Analysis*

After 72 hours of incubation in the DMEM medium, the wells were washed three

times with phosphate buffer saline (PBS) and then incubated ($37^{\circ}C$ in 5% $CO_2$) with the

six treatments, all diluted to 1% using Hanks Balanced Salt Solution (HBSS), described

in Table 5.


| Treatment | Description | Function |
|---|---|---|
| **HBSS** | Hanks Balanced Salt Solution.  Sample diluent. | Negative control. [180]. |
| **1% Emulsifier** | Solution containing all the emulsion components except for the oil phase. | Comparison control. |
| **1% Palm** | Emulsion composed of 100% palm oil for oil phase | Sample with 0.29mg palm oil |
| **1% Fish** | Emulsion composed of 100% fish oil for oil phase | Sample with 0.29mg fish oil |
| **1% Emulsion A** | Emulsion composed of 2:1 palm:fish ratio for oil phase | Sample with 0.195mg palm oil, 0.095g fish oil |
| **1% Emulsion B** | Emulsion composed of 1:1 palm:fish ratio for oil phase | Sample of with 0.145mg palm oil, 0.145mg fish oil |

**Table 5 – Treatment Description**


HBSS served as a negative control as previously described [180].   The

emulsifier was used as a comparison control to eliminate the possibility that the

ingredients in the emulsion other than the palm oil or the fish oil could stimulate GLP-1

or PYY secretion.  Emulsions composed of 100% palm oil and 100% fish oil were also

utilized to examine their individual effects on GLP-1 or PYY secretion.  1mL of 1%

treatment solution was added to each well bringing the total lipid concentration to

~290μg/mL per non-control well.

Each treatment was arranged in triplicate in a 24 well plate as represented in

Figure 8.  Each well contained ~$10^5$ STC-1 cells upon seeding.
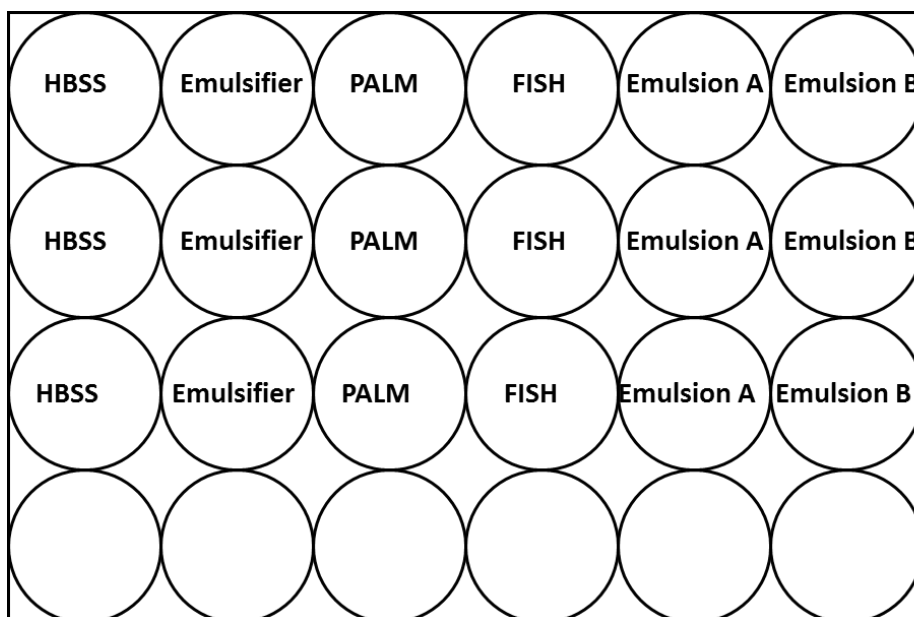


**Figure 8 - Treatment Arrangement Diagram for 24-well Plate**
Each treatment was vertically arranged in triplicates.  Each well contained ~$10^5$ STC-1 cells.

Once the corresponding treatment samples were pipetted into each well, the

plates were incubated at 37°C in 5% $CO_2$.  There were four total plates with each plate

representing specific timepoints of 30, 60, 90 and 120 minutes for which the supernatant

from the wells were collected.  Upon removal, the supernatants from each well were

pipetted into labelled test tubes and frozen at -20°C until ready for analysis.

For analysis the treatment samples were thawed on ice and analyzed for GLP-1

and total PYY using the Metabolism Multiplex Assay kit (EMD-Millipore cat.

MMHMAG-44K) on the Luminex 200 Multiplexin Instrument according to manufacturer's instructions. Total PYY ($PYY_{1-36}$ + $PYY_{3-36}$) is what is measured since DPP-4's found in bloodstream and is not known to be produced by L-cells or STC-1 cells nor can the hormone kit discriminate between the two forms.

*Statistical Analysis*

Differences in the potency of the emulsions to increase PYY and GLP-1 secretion in STC-1 cells were analyzed using a two-way analysis of variance (ANOVA). Differences were considered to be significant at a p-value $< 0.05$. Data are presented as the mean ± SEM in Figure 9 and Figure 10. All experiments were performed in triplicate.

## RESULTS

*Emulsion Induced GLP-1 Secretion by STC-1 Cells*

There was not a significant GLP-1 induction difference between the emulsifier and the HBSS control at any timepoint and therefore the emulsifier can be considered non-inducing for GLP-1 secretion (Figure 9). Palm oil showed the greatest induction of GLP-1 secretion at every timepoint with the 60 minute point being the greatest compared to the HBSS control (Figure 9). The fish oil emulsion also showed an increase in GLP-1 secretion compared to the HBSS control at all timepoints, but was far less pronounced than the palm oil emulsion (Figure 9). For emulsion A (2:1 palm:oil) at all timepoints there a significantly greater induction of GLP-1 secretion compared to control with the

120 minute timepoint showing the greatest difference (Figure 9).  For emulsion B there

was a significant increase in GLP-1 secretion at all timepoints compared to the HBSS

control (Figure 9).  When comparing Emulsion A to Emulsion B, there was not a

significant difference at the 30 and 120 minute timepoints, but Emulsion B induced

significantly greater GLP-1 secretion at the 60 and 90 minute timepoints.  It is important

to note that the palm and fish oil emulsions showed the greatest induction of GLP-1

secretion at the 30 minute timepoint (Figure 9).
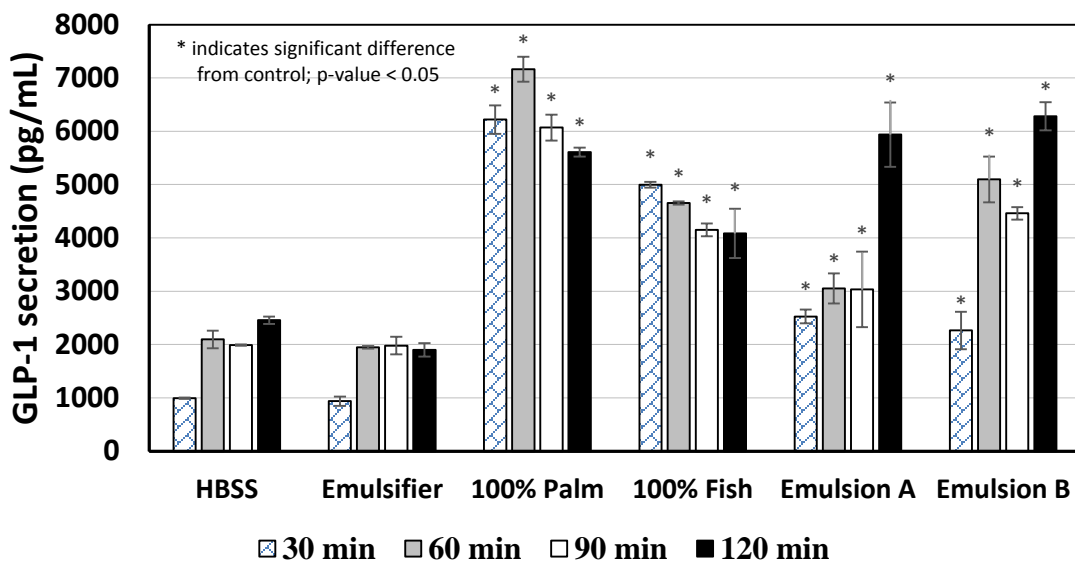


**Figure 9 - GLP-1 Secretion from STC-1 Cells**
The stimulatory effect of each treatment on GLP-1 secretion was investigated after an incubation period of 30, 60, 90
and 120 min.  The results are reported as the mean ± SEM (n=3).  Refer to Table 5 for treatment description.

*Emulsion Induced PYY Secretion by STC-1 Cells*

Similar to GLP-1 secretion there was not a significant difference between the

emulsifier and the HBSS control at any timepoint and therefore the emulsifier can

likewise be considered non-inducing for PYY secretion (Figure 10). The results for the

HBSS control show a consistent secretion of PYY at ~11pg/mL at all timepoints which

is consistent with the previously reported ~16pg/mL [180]. In complete contrast to

GLP-1 secretion, the palm oil emulsion induced the least amount of PYY secretion with

the 90 minute timepoint showing the only significant difference compared to control

(Figure 10). The fish oil emulsion, as well as emulsion A and emulsion B, all induced a

10-fold level increase of PYY secretion compared to the HBSS control, which suggests

that the fish oil present in the emulsions could play a significant role in inducing PYY

secretion (Figure 10).
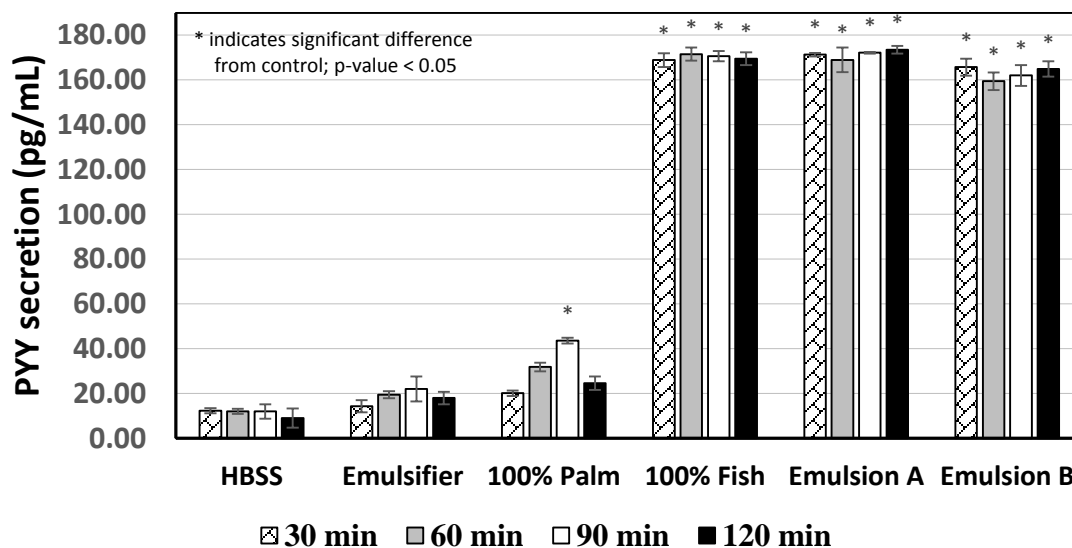


**Figure 10 - PYY Secretion from STC-1 Cells**
The stimulatory effect of each treatment on PYY secretion was investigated after an incubation period of 30, 60, 90 and 120 min. The results are reported as the mean ± SEM (n=3). Refer to Table 5 for treatment description

**DISCUSSION**

Research is ongoing in the food and drug industry as well as academia to design food systems that target the ileal brake as a means to control appetite and counter obesity. Olibra$^{TM}$ is one such emulsion that when combined with yogurt has been used for this exact purpose [149-151]. STC-1 hormone secretion assays have been utilized as a preliminary model to determine if certain ingredients or components of a food system can stimulate GLP-1 and PYY secretion [176-180].

It has now been demonstrated in our study that emulsions A and B stimulate the secretion of GLP-1 and PYY from STC-1 cells. The underlying mechanisms that both emulsions utilize to induce PYY and GLP-1 secretion is not clear. Based on the results and given that the emulsifier treatment fails to induce a significant amount of PYY and GLP-1 secretion it is a distinct possibility that the oils themselves are directly responsible, but the possibility that the oils work synergistically with the emulsifier also exists. The palm oil emulsion induced the greatest level of GLP-1 secretion, while inducing the lowest level of PYY secretion. Conversely, emulsions A and B as well as the 100% fish oil emulsion were able to stimulate both GLP-1 and PYY. Such a result indicates that there are different mechanisms involved in ingredient-induced GLP-1 and PYY secretion in STC-1 cells. Nutrient-stimulated secretion of GLP-1 occurs upon nutrient interaction with G-protein receptors (GRP40, GRP43, GRP119, GRP120, FATP4 and TGR5) of the intestinal L-cells [52-64]. Specifically, it was demonstrated that oleic acid interaction with the FATP4 receptor stimulates GLP-1 secretion [64]. 100% palm oil contains a greater composition of oleic acid (~38%) than emulsions A

and B (~28 and ~24%) and it is possible that the palm oil-induced effect is being mediated through the FATP4 receptor.  At the time of writing, the mechanism of nutrient-stimulated release of PYY has not been elucidated, although our data demonstrates that PYY secretion is stimulated by unsaturated fatty acids or even omega-3 fatty acids to greater effect compared to saturated fatty acids.  It is worth noting that it was previously reported that saturated fatty acids of chain length of 4, 12, 14, 16 and 18 carbons dispersed in HBSS stimulate PYY secretion upon addition to STC-1 cells at concentrations of about 30-40 pg/mL compared to the ~160-170 pg/mL range that was shown in our study for emulsions A and B [180].  This further supports the possibility of unsaturated fatty acids being more potent inducers of PYY secretion.  Examining individual unsaturated fatty acids following the previously reported method could help clarify the situation regarding this effect [180].  Emulsions A and B were able to significantly increase secretion of GLP-1 and PYY in far greater amounts compared to control, which suggests that there is potential for the emulsions to serve as activators of the ileal brake when consumed.

CHAPTER III

SENSORY AND RHEOLOGICAL CHARACTERISTICS OF A PREPARED

BEVERAGE TO SERVE AS VEHICLE FOR SATIETY INDUCING EMULSIONS


**BACKGROUND AND RATIONALE**

In the first phase of this research it was shown that two emulsions referred to as emulsion A and emulsion B composed of different ratios of palm and fish oil induced the secretion of the hormones GLP-1 and PYY from STC-1 cells, a murine enteroendocrine cell line capable of secreting hormones in a manner similar to humans.  In humans, GLP-1 and PYY are considered biomarkers for the ileal brake.  The ileal brake is a gastric inhibitory feedback mechanism that controls the transit of food through the gastrointestinal tract that has been shown to reduce food intake and increase satiety, making it an attractive target for appetite control [121-132].  Since both emulsion A and emulsion B were shown to induce the secretion of PYY and GLP-1 in STC-1 cells, a distinct possibility exists that both emulsions have the potential to serve as satiety inducing agents when orally consumed.

In recent years, beverages blended with a combination of fruit and dairy products known as smoothies have been promoted as a healthy beverage option that can serve as a meal replacement or as a between meal snack.  These have become a popular choice among consumers [181].  Smoothies are perceived as having less calories than solid foods because of their fluid texture, making them an attractive option for individuals concerned with weight management [182-184].

The beverage developed in this study is a functional food, and was formulated using skim milk, bananas, Adams vanilla extract, Jell-O Brand vanilla flavored sugar and fat free powdered pudding, Hershey's chocolate syrup, fresh strawberries, McCormick cinnamon, and Horizon Fat Free Organic Yogurt, as shown in Table 8. This formulation is an enrichment of the yogurt that served as the vehicle for Olibra[TM] from previous studies [149-151]. All ingredients were fat-free to ensure that the emulsions and the milkfat control represented the only source of fat. Yogurt was chosen since it is has been shown to serve as an effective vehicle in previous studies that involved using emulsions as an appetite suppressing ingredient [149-151]. Skim milk was chosen to disperse the ingredients. Milk also contains casein that could potentially aid in emulsification and contribute to mouthfeel [185]. Jell-O pudding, in addition to flavor and texture, contains the emulsifier sodium stearoyl lactylate (SSL) and sodium alginate which is a hydrocolloid that functions as an emulsifier and increases viscosity. In combination, these may improve overall emulsion stability [186, 187]. Cinnamon and vanilla extract were added to enhance the beverage flavor. Hershey's chocolate syrup provide flavor and was the primary contributor to the color of the beverage. Hershey's chocolate syrup contains high fructose corn syrup (HFCS) with an unspecified ratio of glucose to fructose. It has been reported that hunger, satiety, or short-term energy intakes are not significantly different when comparing beverages sweetened with either sucrose or HFCS [112]. Banana and strawberries, the fruit component of the beverage, contributed to flavor, nutrients, texture and viscosity.

In the second research phase, which is the focus of this report, a smoothie beverage, containing emulsions to deliver them to the digestive tract to induce PYY and GLP-1 secretion, was prepared using locally purchased fresh ingredients shown in Table 7. Each prepared beverage was supplemented with one of the two emulsions or milkfat control. The beverage combined with the milkfat control was referred to as the control treatment, while the beverage combined with emulsion A was referred to as treatment A and treatment B for emulsion B. The beverages were formulated with the goal of eliminating subjective and objective variations among treatments. Figure 11 outlines the objective and comparison tests performed for each treatment.
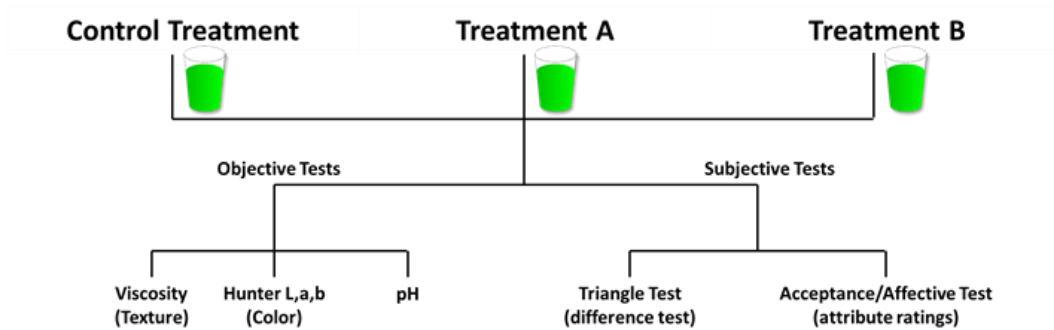


**Figure 11 - Outline of Emulsion Treatment Comparison Tests**
Control treatment refers to the beverage supplemented with the milkfat control, while treatments A and B refer to the beverage supplemented with emulsions A and B. Emulsions A and B differ in their ratios of palm and fish oil. Emulsion A has a 2:1 palm:fish oil ratio, while emulsion B has a 1:1 palm:fish oil ratio. Objective and Subjective tests determined if there were any differences between treatments.

Subjective sensory tests and objective instrumental analysis were performed on each treatment and the results were statistically analyzed using an ANOVA. For the subjective sensory tests, triangle tests and acceptance/affective tests were administered using untrained consumers. The triangle test was utilized to determine if there was a

60

sensory difference between each emulsion treatment compared to milkfat control. The triangle test is useful when treatment effects of a food system may produce an overall difference but no specific flavor, color or texture attributes can be identified as having been affected [165]. While the triangle test is useful in determining an overall difference, the acceptance/affective tests rank or otherwise determine if there are individual attributes of a food system that are different using a scorecard. In our study, subjects ranked the overall pleasantness, visual appeal, the smell/odor and the taste on a 1-10 scale in accordance to previously published methodology [162-164].

The objective tests employed laboratory instrumentation to analyze and compare the viscosities, pH and Hunter L, a, b color values of each treatment. Viscosity is a measure of a food system's resistance to gradual deformation by shear stress and is an important characteristic of a food system that affects texture, appearance and mouthfeel. In addition, research has shown that foods of higher viscosity may positively influence satiety [188-190].

Formulating and processing to stable pH is a key quality parameter of a food system that is necessary to preserve flavor, texture and color. If the pH decreases, a sour flavor can form due to protonation of weak acids. If the pH decreases below 4.6 (the pI of casein) the casein from the milk and the emulsion in the beverage will coagulate and precipitate. This affects the mouthfeel and the texture. The pH also potentially impacts pigment molecules, hence the color of the food system. Therefore ensuring that the pH is consistent between treatments and remains stable over a period of time is critical in the development of a food system.

The color of a food system is a critical visual indicator and is closely related to the consumer perception of quality. Food color is due to presence of specific pigment molecules in the food system, and may be affected by changes in pH, texture or microbial growth. Ensuring that color is consistent and remains stable over a period of time is critical in the quality assessment of a food system. The Hunter L, a, b, scale measures the specific color values and is often used to provide an analysis of color and can detect changes in color over time. The "L" scale measures light vs. dark where a low number (0-50) is toward dark, 0 being black, and a high number (51-100) indicates light, 100 being white. The "a" scale measures red vs. green where a positive number indicates "redness" and a negative number indicates "greenness" in a product. The "b" scale measures yellow vs. blue where a positive number indicates "yellowness" and a negative number indicates "blueness."

All of the aforementioned subjective sensory tests and objective instrumental analyses are necessary to test the hypothesis that the formulated fruit and dairy smoothie-type beverage could serve as vehicle for the satiety emulsions in human volunteers.

**METHODS**

*Treatment Preparation*

Beverage ingredients excluding the emulsions were purchased from an H.E.B. supermarket located in College Station, Texas on a weekly basis in order to ensure consistency and freshness for each experiment as much as possible. The treatments were

freshly prepared prior to each subjective and objective comparison test.  Milkfat from

heavy whipping cream served as the control in accordance to previous studies [149-151].

| | | Carbohydrates (g) | Sugar (g) | Fiber (g) | Starch (g) | Fat (g) | Protein (g) | Kilocalories |
|---|---|---|---|---|---|---|---|---|
| (a) | Banana | 14 | 8.74 | 1.38 | 3.68 | 0 | 0.46 | 50.6 |
| | Vanilla | 0 | 0.23 | 0 | 0 | 0 | 0 | 5.52 |
| | Fat Free Vanilla Pudding | 1.2 | 0 | 0 | 1.15 | 0 | 0 | 4.6 |
| | Hersheys Syrup | 11 | 9.2 | 0.46 | 1.38 | 0 | 0.46 | 46 |
| | Strawberries | 1.3 | 0.81 | 0.32 | 0.16 | 0 | 0.161 | 5.15 |
| | Skim milk | 2.8 | 2.76 | 0 | 0 | 0 | 1.84 | 19.78 |
| | Yogurt | 3.5 | 3.45 | 0.23 | 0 | 0 | 2.53 | 25.3 |
| | **Total (pre-treatment)** | **33** | **25.2** | **2.4** | **6.4** | **0** | **5.5** | **157.0** |
| (b) | Milkfat (control) (6g) | 0 | 0 | 0 | 0 | 1.8 | 0.1 | 16.6 |
| | Emulsion A (6g) | 0 | 0 | 0 | 0 | 1.8 | 0.1 | 16.6 |
| | Emulsion B (6g) | 0 | 0 | 0 | 0 | 1.8 | 0.1 | 16.6 |
| | **Total with treatment** | **33** | **25.2** | **2.4** | **6.4** | **1.8** | **5.5** | **173.2** |

**Table 6 - Macronutrient Composition of Treatments**

Macronutrient composition was estimated using food nutrition labels.  (a) refers to the beverage only and (b) refers to
the emulsions.  The Strawberry and banana composition was estimated using Dole's nutrition website.  The control is
composed of milkfat from heavy whipping cream.

All of the ingredients were combined into a blender (Oster 14 Speed, Rye, NY)

and set on the lowest speed (setting "stir") for one and a half minutes.  The resulting

treatments had a brown appearance and viscous texture (Figure 12).

**Figure 12 – Visual Representation of Each Treatment**

The above picture was taken after treatment preparation and is intended to provide a visual representation of the color and viscosity.

*Emulsion Composition and Preparation*

The emulsions for the beverage were prepared by Omega Pure (Houston, Texas). Two emulsions which were referred to as A and B were tested. The emulsions differed in their palm oil to fish oil ratios, being either 2:1 (A) or 1:1 (B). Table 7 shows the quantities in grams of each ingredient present in a six gram sample emulsion, the amount added per 200g of beverage. Table 8 presents a description of the components used to prepare emulsion A and emulsion B. Figure 13 shows the treatment preparation diagram.

| Emulsion A | | | | Emulsion B | | |
| 2:1 palm oil: fish oil ratio | | | | 1:1 palm oil: fish oil ratio | | |
| Ingredient | % | Amt / 6g | | Ingredient | % | Amt / 6g |
| --- | --- | --- | --- | --- | --- | --- |
| Water | 66.58% | 4g | | Water | 66.58% | 4g |
| SansTrans 39 | 19.58% | 1.2g | | SansTrans 39 | 14.69% | 0.9g |
| OP HSN | 9.79% | 0.6g | | OP HSN | 14.69% | 0.9g |
| Na-caseinate | 1.96% | 0.1g | | Na-caseinate | 1.96% | 0.1g |
| Gum Arabic | 1.37% | 0.1g | | Gum Arabic | 1.37% | 0.1g |
| K-sorbate | 0.49% | ~0g | | K-sorbate | 0.49% | ~0g |
| Panodan | 0.23% | ~0g | | Panodan | 0.23% | ~0g |

**Table 7 - Quantity of Emulsion Ingredients Present in Each Treatment**

| Ingredient | Function |
| --- | --- |
| **Gum arabic** | A complex mixture of glycoproteins and polysaccharides that is used primarily in the food industry as a stabilizer for the purpose of reducing the surface tension of emulsions. |
| **Panadan** | A diacetyl tartaric acid ester of mono-diglycerides (DATEM) made from edible, refined soybean oil. Served as the emulsifier to make primary emulsion. |
| **Na-caseinate** | Proteins derived from milk that are commonly used as food stabilizers. Used as coating to the primary emulsion to form the secondary emulsion. |
| **K-sorbate** | Served as a preservative against molds and yeasts. |
| **SansTrans™ 39** | Served as the palm oil portion of the palm oil phase. Developed by Lokers Croklaan™ and sold commercially as multi-bakery shortening based on palm oil and fractions of palm oil. It has been designed as an alternative to shortening due to the fact that it is non-hydrogenated and contains no trans-fatty acids. Melting point is reported to be between 37-41°C and is composed of 49.9% of saturated fatty acids, 40.1% mono-unsaturated, and 9.6% polyunsaturated according to manufacturer spec sheet. |
| **Omega Pure (OP) HSN** | Served as the fish oil portion of the fish oil phase. Derived from menhaden fish. Composed of 32% unsaturated fatty acids, 24% mono-unsaturated, 36.74% polyunsaturated (35.66% omega-3) and 7.12% that is not defined on the sheet. EPA + DHA composed 26.12% of total fatty acid composition. Also contains tocopherols to serve as antioxidants. |

**Table 8 - Ingredients For Emulsion Formulations**

All of the above ingredients were necessary to prepare a stable emulsion. Emulsions A and B differed only in the amount of SanTrans™ 39 and OP HSN.
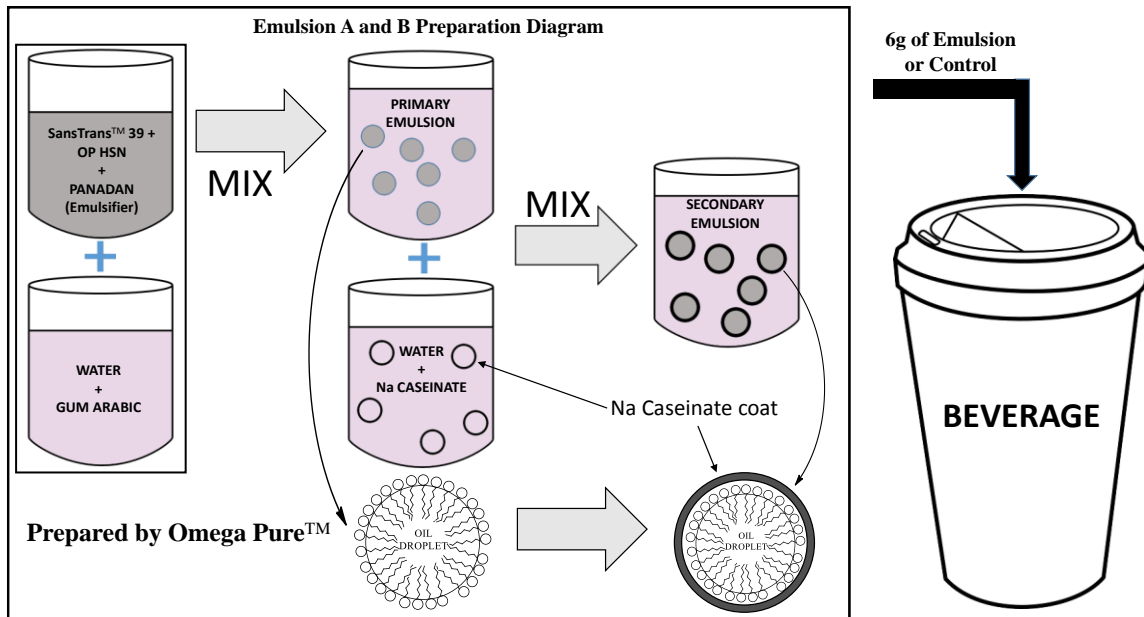
**Figure 13 – Treatment Preparation Diagram**
Emulsion A and B both differed in the amount of SansTrans™ 39 (palm oil) and OP HSN (fish oil) present. Both oils were mixed together plus the DATEM emulsifier to make the oil phase. The oil phase was mixed with the aqueous gum arabic solution to create the primary emulsion. The primary emulsion was then mixed with the aqueous sodium caseinate resulting in the final (secondary) emulsion. 6g of the secondary emulsion or control was added to the beverage.

To prepare the oil phase, both solid SansTrans™ 39 (palm oil source) and liquid OP HSN (fish oil source) were heated in separate beakers on the same heating plate (Corning PC-420D) to 44°C. The temperature of the oil mixture was monitored using a standard thermometer. A temperature of 44°C was sufficient to completely melt the SansTrans™ 39 and ensured that mixing of both oils would be uniform. Both oils were combined with a proprietary quantity of rosemary antioxidant and Panodan emulsifier to create the oil phase. Due to confidentiality agreements with Omega Pure™, the exact quantity of rosemary extract is unknown. Panodan, in liquid form, was added to the mixture using a 5mL pipette. The solution was mixed with a stir bar at a speed of 500 rpm.

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To prepare the water phase, two equivalent water solutions were prepared: one that contained dissolved gum arabic and one that contained dissolved sodium caseinate. The gum arabic and sodium caseinate were dissolved in distilled water via stir bar mixing at a speed of 500 rpm in separate beakers and mixing plates at room temperature.

Using an Ultra Turrax T 50 Basic shearing mixer (IKA Werke$^{TM}$ Wilmington, NC) the oil phase solution was slowly added to the gum arabic water solution. The shearing speed was gradually increased to maximum as the dispersion became more evenly mixed. After this step, the range of the size of the oil droplets was approximately 1-10 μm. Once the dispersion was sufficiently mixed it was then passed through a homogenizer (Niro Soavi$^{TM}$ NS10011 2K Bedford, NH) at 50 Bar to break up larger oil droplets and then passed again at 400 Bar to further decrease the oil droplet size down to 0.2–2 μm with some flocculation. The resulting dispersion was referred to as the primary emulsion as shown in Figure 13.

Using the Turrax mixer the primary emulsion was gradually added to the sodium caseinate solution where the negatively charged caseinate functioned as an emulsion stabilizer. Sodium caseinate can stabilize emulsions by lowering the interfacial tension due to its adsorption at the interface. Similar to the first step the shearing speed of the Turrax mixer was gradually increased as the dispersion became more uniform. The resulting dispersion was then passed through a homogenizer at 400 Bar to ensure a consistent oil droplet size range of 0.2–2 μm with minimal flocculation. The emulsions were examined under a microscope to ensure minimal flocculation and consistent droplet

size.  The emulsions were sealed and refrigerated at $4^0$C until it was time to for them to be supplemented with the beverage as shown in Figure 13.

*Subjective Test - Triangle Test*

To perform the triangle test, three different groups of subjects were recruited from Texas A&M University using mass emails sent out to the Department of Nutrition and Food Science.  All subjects were college students of ages 18-25.  Group one had 22 subjects and compared treatment A to the milkfat control treatment.  Group two also had 22 subjects and compared treatment B to the control treatment, while group three had 19 subjects and compared the treatment A to treatment B.  The triangle test was administered in the sensory lab located in the Centeq building at Texas A&M University.  The sensory lab contained four booths with each booth being separated by a divider to minimize subject interaction.  The treatment samples were prepared in a kitchen adjoining the sensory lab.  Each subject was assigned a booth and was instructed to not interact with any other subjects to avoid influencing the results.  Each subject was provided three treatment samples in a randomized order on a plastic food tray.  Each treatment sample was identified with random three digit numbers to minimize selection bias.  The different treatment sample was randomized for each subject.  The treatment samples were delivered to each subject via flip door pass-through located in the wall that separates the kitchen and sensory lab.  Two beverage samples supplemented with the milk fat control and one with either emulsion A or emulsion B were arranged on the food tray as shown in Figure 14.

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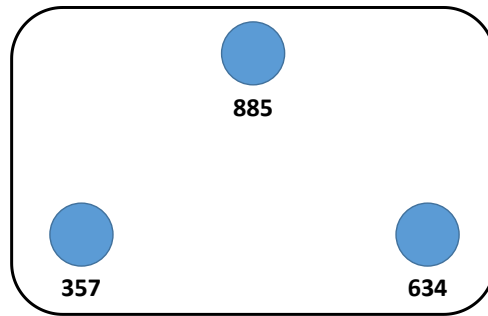
**Figure 14 - Layout of Triangle Test Samples on a Food Tray**

Subjects were instructed to consume samples on the food tray from left to right and then asked to identify which one of the three treatment samples was different. The numbers shown above are examples of randomly generated numbers.

The subjects were instructed to taste each sample identified with a randomly generated number left to right and then asked to identify which sample was different. An example scorecard is shown in Figure 15.

| INSTRUCTIONS: Taste the samples from LEFT to RIGHT. Two are identical. Determine the odd sample. If no difference is apparent you MUST guess. | | |
|---|---|---|
| Part 1 | 730 | 953 | 677 |
| Part 2 | 885 | 357 | 634 |

**Figure 15 – Example Scorecard for Triangle Test**

Subjects were asked to circle the different sample on the scorecard. All subjects were screened before each test. Part 1 refers to the screen and Part 2 refers to the test between treatments

The entire test consisted of two separate parts: a screen referred to as Part 1 and the actual treatment comparison referred to as Part 2.  The screening consisted of performing a triangle test by giving the subjects two HEB brand lowfat milk samples and one HEB brand skim milk sample supplemented with fish oil and asking the subjects to identify which sample was different.  After the screen, subjects were given saltine crackers and water to cleanse the palate for five minutes before proceeding to perform

the treatment triangle test. Subjects that failed the screen were excluded from participating in the treatment triangle test. The test involved three different groups of subjects. Level of significance was set at 0.05.

*Subjective Test – Affective/Acceptance Tests*

The method for the affective test was adapted from a previously described method [162]. Ten different subjects were recruited for the affective/acceptance test from Texas A&M University using mass emails sent out to the Department of Nutrition and Food Science and The Department of Health and Kinesiology. All subjects were college students ages 18-30. Each subject was screened using the same triangle test in the previous section. The test took place in the Exercise and Sports Nutrition Laboratory (ESNL) at Texas A&M University and required three separate visits from each subject. Subjects were assigned a specific study day and were required to arrive on that same day for each treatment for three weeks. All subjects were provided each treatment, which consisted of 200 grams of the beverage mixed with six grams of one of the emulsions or control. All three treatments were consumed by each subject in a randomized order. Post-treatment consumption, subjects were asked to assess the sensory attributes of each treatment's "pleasantness," "smell," "visual appeal," and "taste." The pleasantness attribute refers to the overall desirability of each treatment. The smell attribute refers to how desirable the subjects found the smell of each treatment. The visual appeal refers to how desirable the subjects found the appearance of each treatment. The taste attribute refers to how desirable the subjects found the taste/flavor of each treatment. 10cm lines

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were anchored left to right by the statements in a left to right order "Not pleasant/Pleasant," "Visually appealing/Visually unappealing," "Not pleasant/Pleasant," and "Horrible/Fantastic". See Figure 16 for a visual representation of the scorecard.

## BEVERAGE SCORECARD

1. Pleasantness

Not pleasant | 1 2 3 4 5 6 7 8 9 10 | Very pleasant

2. Visual Appeal

Visually unappealing | 1 2 3 4 5 6 7 8 9 10 | Visually appealing

3. Smell

Not pleasant | 1 2 3 4 5 6 7 8 9 10 | Pleasant

4. Taste

Horrible | 1 2 3 4 5 6 7 8 9 10 | Fantastic
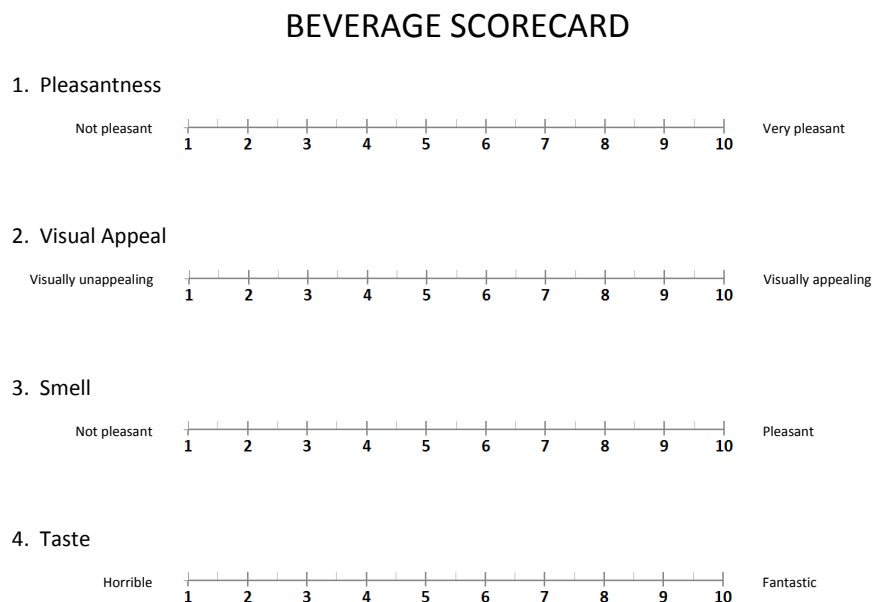
**Figure 16 - Scorecard for Rating Sensory Attributes of Treatments**
Subjects (n=10) were required to fill out the beverage scorecard immediately post-beverage consumption. Subjects marked their rating on each line. Subjects marked their rating of each attribute on any part of the line.

*Treatment Storage for the Objective Tests*

Prior to each of the objective analyses, the treatments were stored at 4°C and each sample was allowed to reach a temperature of 15°C before each experiment to ensure consistency. Three samples of each treatment were prepared and three readings were recorded for each sample for a total of nine readings for each experiment per day. For measuring the viscosity, nine readings were taken at each rotational speed.

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*Objective Test – Viscosity Analysis of Treatments*

The viscosities of each treatment were measured using a rotational viscometer (Cole-Parmer 98936-00 Vernon Hills, IL), which measures the torque required to rotate a rod in a fluid at a known speed (rpm). The torque required to turn an object in a fluid is a function of the viscosity of that fluid. Viscosity was measured in centipoise (cp) as a function of rotational speed. The rotation speeds were set at 1.5 rpm, 3 rpm, 6 rpm, 12 rpm, 30 rpm, and 60 rpm. Treatment samples were shaken for one minute prior to reading to ensure sample uniformity.

*Objective Test -pH Analysis of Treatments*

The pH of each treatment was analyzed using a pH meter (Accumet Waltham, MA). The pH meter was calibrated each day before reading the first sample. Each treatment was measured daily over a four day period. The pH meter measuring probe was placed in each treatment sample and then the pH was displayed.

*Objective Test - Hunter L, a, b Color Analysis*

The Hunter L, a, b color of each treatment was measured using a colorimeter (Hunter D25 NC Reston, VA). Prior to Hunter L, a, b color measurements each treatment sample was allowed to reach a temperature of 15ºC to ensure consistency. Each treatment sample was analyzed and the L, a, b, values were recorded.

*Statistical Analysis*

The objective (pH, color and viscosity) and affective/acceptance tests results were analyzed using a one way analysis of variance (ANOVA). For the triangle test, the null hypothesis of "no difference between samples" was rejected if at least eleven subjects correctly selected the different treatment using the students t-chart for reference. The level of significance was set at 0.05 for all tests.

## RESULTS

*Subjective Test – Triangle Test Results*

The triangle test demonstrated that there was no detectable sensory difference between each emulsion treatment compared to milkfat control (Table 9). In each triangle test less than eleven subjects correctly identified the different treatment meaning that there was a failure to reject the null hypothesis of no difference.

|  | Treatments Compared | Number of Subjects | Number Correct | Reject $H_0$ = No Difference ($\alpha$=0.05) |
|---|---|---|---|---|
| Group One | **Control Treatment A** | 22 | 5 | No |
| Group Two | **Control Treatment B** | 22 | 8 | No |
| Group Three | **Treatment A Treatment B** | 19 | 4 | No |

**Table 9 – Treatment Triangle Test Results**
When comparing the treatments the second treatment listed was the different sample

*Subjective Test – Affective/Acceptance Tests Results*

For each treatment, subjects reported no significant rating differences among pleasantness, visual appeal, smell and taste attributes. Each attribute was rated on a 1-10

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scale.  Any attribute rating greater than 5 was considered favorable [162].  The mean

scorecard rating of each attribute for each treatment is summarized in Table 10.

| Mean Scorecard Ratings ± SD (n=10) | | | | |
|---|---|---|---|---|
| | *Pleasantness* | *Visual Appeal* | *Smell* | *Taste* |
| **Control** | 7.0 ± 2.3 | 6.7 ± 2.5 | 7.9 ± 1.4 | 6.9 ± 2.8 |
| **Treatment A** | 6.4 ± 2.4 | 5.9 ± 2.5 | 7.3 ± 1.5 | 6.1 ± 2.9 |
| **Treatment C** | 6.9 ± 2.4 | 6.0 ± 2.7 | 7.9 ± 1.4 | 6.8 ± 3.1 |

**Table 10 - Mean Treatment Sensory Attribute Ratings**
On a scorecard, ten subjects rated each sensory attribute on a 1-10 scale post-treatment consumption.  Any rating greater than 5 was considered favorable.

There was not a significant difference in the mean ratings of each sensory

attribute for each treatment.  Subjects tended to rate the pleasantness at 7.0 for the

control treatment, 6.4 for treatment A and 6.9 for treatment B indicating that the subjects

found each treatment to be more pleasant than not pleasant.  The mean visual appeal was

rated at 6.7 for the control, 5.9 for treatment A and 6.0 for treatment B indicating that the

subjects found each treatment to be visually appealing.  The mean smell was rated at 7.9

for the control treatment, 7.3 for treatment A and 7.9 for treatment B indicating that the

subjects found each treatment to have a more pleasant than an unpleasant smell. The

mean taste was rated at 7.9 for the control treatment, 7.3 for treatment A and 7.9 for

treatment B indicating that the subjects found each treatment to have a more "fantastic"

taste (Figure 17).   It should be noted that Treatment A had the lowest mean rating for

each sensory attribute although the difference was not significant.
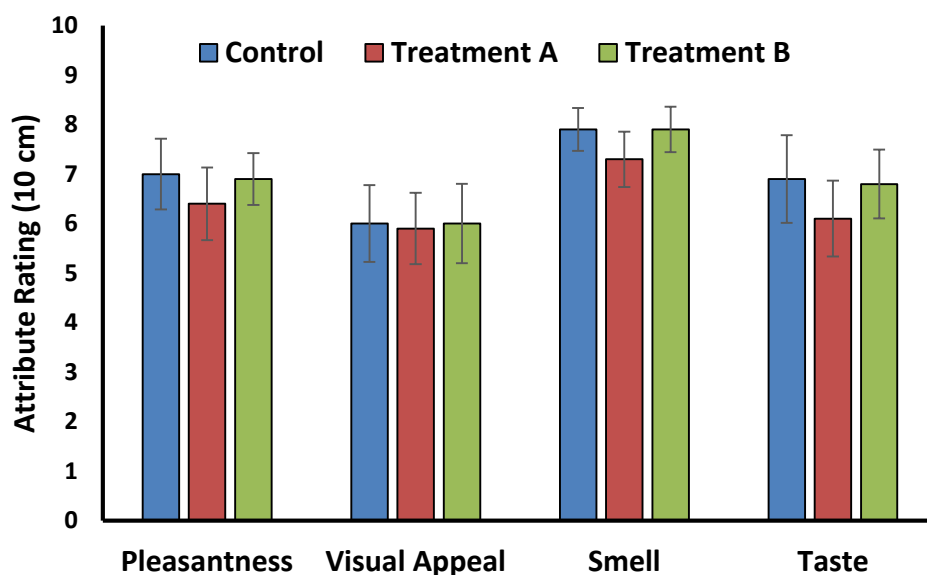
**Figure 17 – Treatment Sensory Attribute Means Across Treatments**
There were no significant differences compared to control for either treatment according to subjects (n=10)
The error bars represent the standard deviation.

*Objective Test – Treatment Viscosity Analysis Results*

Viscosity was measured in centipoise (cp) as a function of rotational speed. The rotational speeds on the viscometer were set at 1.5 rpm, 3 rpm, 6 rpm, 12 rpm, 30 rpm, and 60 rpm in order to characterize rheological characteristics. Readings were taken each day over a four day period. For each treatment, the viscosity was not constant and depended on the rotational rate of the spindle rod, which is a characteristic consistent with a non-Newtonian pseudoplastic. The treatments were very viscous at 1.5 rpm (~6211cp) and became less viscous as the rotational rate increased. On days 1, 2, 3 and 4 the viscosities for each treatment did not differ significantly at any rotational speed. For all treatments, the viscosity decreased as the rotational rate of the spindle rod increased. Bar graphs of the results are shown in Figure 18. It should be noted that in

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most scientific literature viscosity (y-axis) is graphed as a function of shear rate or

rotational speed (x-axis).  The bar graph was chosen to provide a clearer visual

distinction between the viscosities of each treatment.
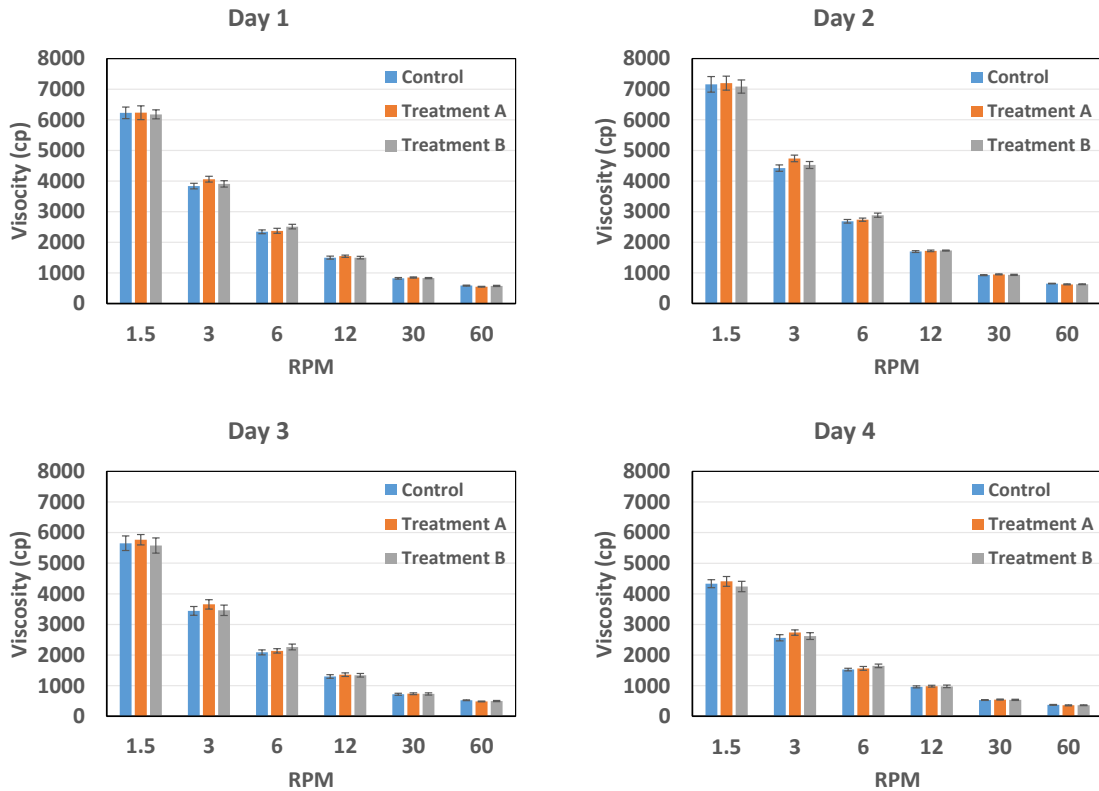


**Figure 18 - Treatment Effect on Viscosity Over a Four Day Period**
The viscosities of each treatment did not significantly differ at each rotational speed during any of the four days of the four day period.

*Objective Test - pH Analysis of Treatments Results*

       The pH of each treatment was not significantly different nor did the pH of any

treatment significantly change over the four day period.  The pH of each treatment

remained at approximately 4.75, which indicates an acidic food system (Figure 19).
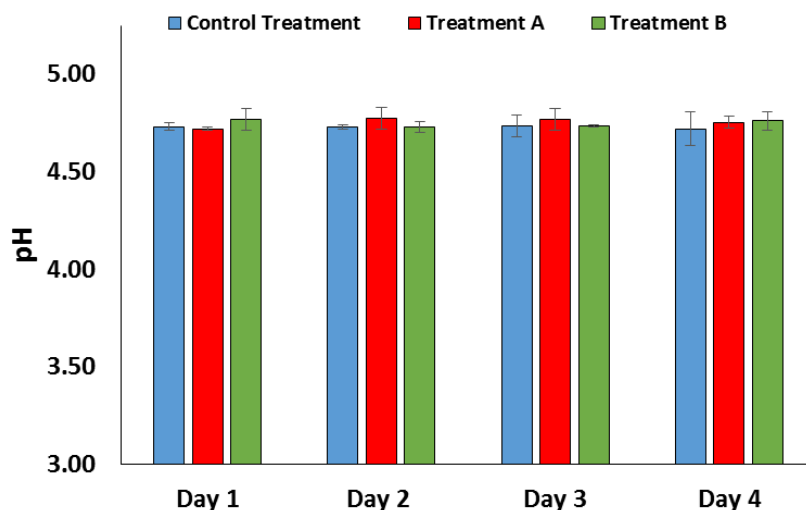
**Figure 19 - pH Analysis of Treatments**
pH of each treatment was measured over a four day period.

*Objective Test – Comparison of Hunter L, a, b Values*

The Hunter L, a, b, color space values of the treatments did not significantly differ when compared on the same day. However, each treatment demonstrated a statistically significant color space change each day over a four day period (Table 11). The mean L value for each treatment did decrease slightly over four days which indicates the sample became darker. The mean a value increased over four days which indicates that the intensity of the red color increased for each treatment. The mean b value of each treatment also significantly increased over four days indicating that the intensity of blue increased.

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|  |  | Mean Hunter L, a, b Color Values ± SD (n=9) | | |
|---|---|---|---|---|
|  |  | L | a | b |
| **Day 1** | Control | $50.010^a \pm 0.690$ | $9.069^b \pm 0.109$ | $15.423^c \pm 0.071$ |
|  | Treatment A | $50.005^a \pm 0.713$ | $9.066^b \pm 0.117$ | $15.395^c \pm 0.082$ |
|  | Treatment B | $49.960^a \pm 0.710$ | $9.076^b \pm 0.117$ | $15.395^c \pm 0.093$ |
| **Day 2** | Control | $49.707^d \pm 0.378$ | $9.172^e \pm 0.042$ | $15.884^f \pm 0.052$ |
|  | Treatment A | $49.687^d \pm 0.428$ | $9.169^e \pm 0.055$ | $15.863^f \pm 0.066$ |
|  | Treatment B | $49.673^d \pm 0.510$ | $9.172^e \pm 0.052$ | $15.841^f \pm 0.052$ |
| **Day 3** | Control | $48.811^g \pm 0.197$ | $9.420^h \pm 0.065$ | $16.189^i \pm 0.075$ |
|  | Treatment A | $48.743^g \pm 0.268$ | $9.409^h \pm 0.059$ | $16.162^i \pm 0.084$ |
|  | Treatment B | $48.782^g \pm 0.309$ | $9.411^h \pm 0.050$ | $16.159^i \pm 0.082$ |
| **Day 4** | Control | $49.142^j \pm 0.159$ | $9.319^k \pm 0.023$ | $16.110^l \pm 0.044$ |
|  | Treatment A | $49.073^j \pm 0.189$ | $9.321^k \pm 0.034$ | $16.116^l \pm 0.071$ |
|  | Treatment B | $48.995^j \pm 0.236$ | $9.309^k \pm 0.045$ | $16.124^l \pm 0.062$ |

**Table 11 - Hunter L, a, b Values Measured Over Four Days**

"L" values measures light vs. dark where 0-50 indicates dark and a 51-100 indicates light. The "a" values indicate red vs. green where a positive number indicates red and a negative number indicates green. The "b" values indicate yellow vs. blue where a positive number indicates yellow and a negative number indicates blue. Values with different letters are considered significantly different (p-value > 0.05).

## DISCUSSION

In phase one of the study it was shown that emulsions A and B stimulated the secretion of the hormones PYY and GLP-1 in STC-1 cells indicating the possibility for the emulsions to serve as satiety inducing ingredients via activation of the of the intestinal feedback mechanism known as the ileal brake of which PYY and GLP-1 are considered biomarkers for. Since the emulsions exhibit a stimulatory effect on PYY and GLP-1 secretion in STC-1 cells, there is great interest in determining if the emulsions when orally consumed can similarly affect ileal brake activation in human subjects.

The purpose of phase two was to develop a food system beverage to serve as a vehicle for the emulsions. The beverage was supplemented with either milkfat control or emulsion A or Emulsion B and were referred to as the control treatment, treatment A and treatment B. The results demonstrated that our beverage is able to mask any sensory, flavor, color, pH and rheological differences of each treatment with the only ingredient being different are the emulsions. Therefore the beverage has the potential to serve as vehicle for the emulsions in a satiety study with humans. This is important because any significant sensory differences between treatments could potentially influence satiety in human volunteers.

For the triangle test, 22 subjects were asked to choose between treatment A and two control treatments. The subjects correctly selected treatment A only five times. When comparing treatment B to control only five subjects correctly selected treatment B. When comparing treatment A to treatment B, only four out of nineteen subjects correctly chose treatment B which served as the different sample. The results indicate that each treatment does not significantly exhibit an overall sensory difference.

For the affective/acceptance tests, subjects were asked to rate the sensory attributes of pleasantness, visual appeal, smell and taste for each treatment. Each subject consumed all three treatments one week apart in a randomized treatment order. Subjects did not report a significant difference between pleasantness, visual appeal, smell and taste for each treatment. Subjects rated the mean pleasantness of the beverage at 6.8, while the mean visual appeal was rated at 6.2, the mean smell was rated at 7.7, and mean the taste was rated at 6.6 indicating that overall the subjects found each attribute to be

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more desirable than undesirable since the mean ratings of each attribute did not fall place 5 indicating that all three treatments were equally liked.

All of the objective test results showed that there was no significant difference among treatments, which was consistent with the subjective test results. Treatment viscosities did not significantly differ at the 1.5 rpm, 3 rpm, 6 rpm, 12 rpm, 30 rpm, and 60 rpm rotational speeds on any of the four test days. However, the viscosities of each treatment did significantly change over the four day period. The treatments display rheopectic, followed by thixotropic characteristics since the viscosity increases and then decreases over time at a constant shear rate. From day 1 to day 2 each treatment exhibited rheopectic characteristics since there was a significant increase in viscosity at all rotational speeds. From day 2 to day 3 and day 3 to day 4 each treatment exhibited thixotropic characteristics where the viscosities decreased at all rotational speeds. On each day, the treatment viscosities non-linearly decreased as the rotational speed of the spindle increased, a process known as shear thinning. Shear thinning is a defining characteristic of a non-Newtonian pseudoplastic beverage, which is consistent with previous studies using smoothies [181, 182]. These changes in viscosity are likely caused by inactivation of the fruit enzymes as well as other components that influence viscosity, which can affect the shelf life. It was previously shown that satiety was higher in subjects that consumed a high viscosity (29500 cp) beverage described as "barely pourable" compared to a low viscosity (60cp) beverage described as "watery" [190]. Another study that examined the effect of viscosity on satiety and reported that a fruit smoothie that contained fresh mango, peach and papaya fruit juice with tara gum that

had an estimated viscosity range of 800 cp – 3800 cp increased satiety [189]. The viscosity of each treatment in this study falls within this range, although the main components of the beverage that contributed to the texture were the yogurt, milk, bananas and strawberries.

Unlike color or viscosity, the pH for each treatment did not significantly change over the course of four days and remained at approximately 4.75. The main ingredients contributing to the pH were likely the yogurt and the strawberries. Yogurt and strawberry have acidic pHs between 3.0 - 4.0, while milk has a pH at around 6.6. Maintaining a stable pH is an important characteristic of a stable food system since an unstable pH can affect the flavor, color and texture. An acidic pH can lead to a sour flavor due to protonation of weak acids. pH can also affect the texture and viscosity since the proteins such as casein will precipitate and coagulate if the pH decreases below the isoelectric point. The pH can also affect pigment molecules leading to color changes. All of the aforementioned qualities can negatively affect food system quality. However in all treatments, pH variation or fluctuation was not an issue.

Hunter L, a, b color values for each treatment did not significantly differ on any of the four test days. However, the Hunter L, a, b colors of each treatment did significantly change over the four day period. The brownish dominant color of each treatment was still present on Day 1 and Day 4. On Day 4 the beverage had undergone noticeable water separation as shown in the right picture of Figure 20.

Day 1                                    Day 5

**Figure 20 – Treatment Texture Changes Over a Five Day Period**

On Day 1, the beverage had a uniform appearance. By Day 4, the beverage had undergone noticeable water separation. There was not a noticeable difference in separation between treatments. It is important to note that the photographs inadvertently altered the color appearance due to lighting variations on different days. The actual color difference of the beverages was not pronounced, both being chocolate brown. The photographs were not intended to show color changes, but rather to emphasize the beverage emulsion stability change over time.

In this phase of the research it was demonstrated that the treatments are objectively and subjectively indistinguishable from one another. In addition to the emulsions, the beverage also contains several other ingredients that can potentially influence satiety or emulsion stability.

However, the beverage has some limitations in regards to shelf life. Appearance, texture and color all significantly changed over the course of four days. The treatments displayed water separation after five days and a statistically significant color change over the same time frame, potentially affecting palatability and the beverage's ability to properly mask the emulsion differences. Due to lack of time and funding, trained panelists could not be recruited to define a flavor profile for the beverage and determine how those flavors might be affected by the addition of the emulsions or how the flavors would change over the course of several days. Several researchers observed that the banana and strawberry flavors were the dominate flavors on day 1, but on day 2, the

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chocolate flavor became more dominant and by day 3 the banana and strawberry flavors were not present. Despite these limitations, it has been demonstrated that the treatments are objectively and subjectively indistinguishable from one another and are therefore viable to test the effects of the treatments on satiety in human subjects.

To reiterate, in phase one it was shown that emulsions A and B stimulated the secretion of the ileal brake biomarkers, GLP- and PYY in STC-1 cells. In this phase it was demonstrated that the formulated beverage was capable of serving as vehicle for the emulsions. Therefore, the next phase of the study is to determine if the emulsion treatments could influence feelings of satiety, energy intake and the expression of ileal brake hormones, GLP-1, PYY, ghrelin and leptin in human subjects. All of which were necessary to determine whether the emulsion treatments had the potential to serve as an option for individuals concerned with weight management.

CHAPTER IV

FISH OIL EMULSIONS AND THEIR EFFECTS ON SATIETY ON HUMAN

SUBJECTS

**BACKGROUND AND RATIONALE**

In phase one of this research it was demonstrated that two emulsions referred to

as emulsion A and emulsion B composed of different ratios of palm and fish oil induced

the secretion of the hormones GLP-1 and PYY from STC-1 cells.  In the second phase, a

fruit and dairy blended beverage was formulated to serve as a vehicle for the emulsions.

The beverage was formulated such that there were no statistically significant objective or

subjective differences between the beverage supplemented with emulsion A or emulsion

B compared to control.  The objective tests compared the viscosities, pH and color of

each treatment.  For the subjective sensory tests, triangle tests and affective tests were

administered and demonstrated no significant difference between treatments.  Therefore

there is an interest in determining if the emulsion treatments may exhibit effects on

satiety and energy intake individuals.

Obesity, which is defined as abnormal or excessive fat accumulation that may

impair health has emerged as a very serious threat to the world health population and has

continued on an upward trend since the mid-1980s.  As of August 2014, the World

Health Organization recently reported the following: worldwide obesity has nearly

doubled since 1980; in 2008, more than 1.4 billion adults, 20 and older, were

overweight, of which over 200 million men and nearly 300 million women were obese;

35% of adults aged 20 and over were overweight in 2008, and 11% were obese; 65% of the world's population live in countries where overweight and obesity kills more people than underweight; more than 40 million children under the age of 5 were overweight or obese in 2012 [1]. Energy restriction is most commonly used to counter obesity, but a better solution may lie in the manipulation of macronutrient composition of diets to fortify foods or beverages with macromolecules, such as protein, fats or emulsions that exploit various biological mechanisms that regulate energy intake. One such biological feedback mechanism is the ileal brake.

The ileal brake is the primary inhibitory feedback mechanism that controls the transit of food through the GI tract. Ileal brake activation results in a delay in gastric emptying leading to an increase in small intestine transit time for both solid and liquid food [70, 125, 126]. Dietary fats that have been hydrolyzed into FFA form are considered the most potent activators of the ileal brake and have been confirmed by many studies [70, 128-134]. However, a recent proof-of-concept study involving normal weight individuals investigated the effect of ileal infusion of sucrose and casein on food intake, release of CCK, GLP-1 and PYY, gastric emptying rate and small-bowel transit time. They demonstrated that the ileal brake-satiating effect leading to a significant decrease in food intake is also demonstrated by proteins and carbohydrates [143]. The hormones, GLP-1 and Peptide-YY (PYY) are secreted from the intestinal L-calls into circulation upon nutrient stimulation and are considered biomarkers of the ileal brake and satiety.

GLP-1, an incretin, is one of several cleavage products of the proglucagon gene. It is produced in the intestinal L-cells located along the mucosa of the ileum and the proximal colon that is secreted into circulation upon nutrient stimulation. GLP-1 enhances glucose-dependent insulin secretion, inhibits glucagon release from the pancreatic $\alpha$-cells and delays nutrient absorption through inhibition of gastric emptying and intestinal motility [38-41]. GLP-1 exhibits rapid and prolonged effects in response to nutrient digestion post-meal consumption [49-51]. Once in circulation, GLP-1 has a short half-life of ~1-2 minutes due to rapid degradation by the DPP-4 [52-66]. Once GLP-1 crosses the blood brain barrier, it will bind to receptors in the hypothalamic arcuate nuclei, promoting appetite suppression [48, 67].

PYY, like GLP-1, is a satiety hormone that is secreted by intestinal L-cells and serves as an inhibitor of pancreatic exocrine secretion and intestinal motility [70]. Once in circulation, PYY is degraded by DPP-4 into its active $PYY_{3-36}$ form. $PYY_{3-36}$ is able to exclusively bind to the Y2 receptor in the hypothalamic arcuate nucleus. Once bound to the Y2 receptor $PYY_{3-36}$ suppresses the expression of the prohunger hormone neuropeptide Y (NPY) [72-75]. It is important to note that in a study examining individuals who underwent gastric bypass surgery, that PYY and GLP-1 plasma levels were significantly elevated and has been suggested that this is a possible factor in weightloss post-surgery [76].

Leptin is a satiety hormone that is secreted from white adipose tissue in response to food consumption, but is not considered to be a biomarker for the ileal brake. Once in

the blood stream leptin crosses the blood brain barrier where it will bind to leptin

receptors in hypothalamic arcuate nucleus to promote appetite suppression [77-82].

Unlike GLP-1, PYY, and leptin, ghrelin is an appetite-stimulating hormone that

is produced by the $P/D_1$ cells that line the fundus of the stomach [84]. Ghrelin plasma

levels elevate during fasting and decreases post meal consumption in normal individuals.

Once ghrelin crosses the blood-brain barrier it binds to ghrelin receptors in hypothalamic

arcuate nucleus inducing the expression of NPY increasing appetite [85, 86].

It is important to note that most of the studies examining whether certain

macronutrients could activate the ileal brake were performed via ileal infusion, which is

not practical for common weight management [70, 128-134]. As such, dietary activation

of the ileal brake poses a greater challenge. Therefore, there is great interest in fortifying

food products with lipid delivery systems, such as emulsions, which inhibit lipid

digestion such that lipid exposure to the ileum is increased, potentially activating the

ileal brake.

An emulsion is a mixture of two immiscible liquids in which one liquid is

dispersed (dispersed phase) in the other (continuous phase). This mixture is stabilized

by an emulsifier, a molecule containing a hydrophilic group that interacts with the water

phase, and the hydrophobic group that interacts with the oil phase. The simplest

emulsions are classified as oil in water (O/W) or water in oil (W/O).

The commercial product Olibra[TM] (Lipid Technologies Provider AB (LTPAB),

Karishamn, Sweden) is an emulsion composed of a 95:5 ratio of palm oil to oat oil in

which the galactolipids derived from oat oil serve as the emulsifier. Olibra[TM] when

consumed with yogurt has been previously shown to suppress appetite and reduce food intake in human subjects [149-151].

There have been numerous studies that have examined the effects of Olibra$^{TM}$ on appetite suppression, energy intake and potential ileal brake activation. The earliest known study examined the effects of Olibra$^{TM}$ added to yogurt (Olibra$^{TM}$ treatment) on energy and macronutrient intake in only non-obese subjects (BMI < 30). It was found that mean energy intakes were significantly lower after consumption of the Olibra$^{TM}$ treatment compared with the milkfat control treatment [150]. These results were consistent with a later study that also included non-overweight (BMI 20-24.9 kg/m$^2$), overweight (BMI 25-29.9 kg/m$^2$) and obese (BMI > 30 kg/m$^2$) subjects and found that mean energy intakes were significantly lower for the Olibra$^{TM}$ treatment compared with the control in non-overweight and overweight subjects, but not obese subjects [149]. Another study by the same group examined whether there effects of Olibra$^{TM}$ were dose-dependent using non-overweight subjects (BMI 20-24.9 kg/m$^2$) and reported that mean energy intakes were progressively reduced with increasing doses of Olibra$^{TM}$ treatment. The authors suggested that Olibra$^{TM}$ exerted it's effects via the ileal brake [151]. Another study examined whether consumption of the Olibra$^{TM}$ treatment had an effect on orocecal transit time (OCTT) by following blood sulfapyridine levels, a metabolite of salazopyrine in the colon. Orally consumed salazopyrine serves as a marker for OCTT since it is poorly absorbed in the gastrointestinal tract, but is rapidly absorbed in the colon upon being hydrolyzed by microflora. The results showed that there was a statistically significant delay in the emergence of sulfapyridine in serum after

consumption of the Olibra™ treatment versus milkfat control treatment. This resulted in a greater OCTT due to fat emulsion consumption and provided the strongest evidence that Olibra™ was exerting its effects via ileal brake activation [170]. The most comprehensive study on the Olibra™ treatment assessed its effects on weight maintenance after weight loss including effects on body composition, resting energy expenditure, fat oxidation, reported appetite, and satiety hormones analysis. Their findings indicated that consumption of the Olibra™ treatment resulted in a significant decrease in body composition, significantly greater resting energy expenditure, significantly greater fat oxidation, significantly decreased appetite and greater satiety hormone expression results compared to the milkfat control treatment [167]. However, the findings of later studies were not consistent with these [168, 169]. It is important to note that Olibra™ reportedly only exhibits its effects when consumed with yogurt [171].

A few studies have investigate the effects of other emulsion treatments. One such case involves a Korean pine nut oil emulsion (Pinnothin™) which exhibited similar effects on satiety and energy intake as Olibra™ [191-193]. Taken together there is sufficient evidence that demonstrates the potential of utilizing emulsions as satiety inducing agents.

For our specific study, two oil in water emulsions that differed in their palm oil to fish oil ratios, being either 2:1 (A) or 1:1 (B) were prepared by Omega Protein (Houston Texas). Omega Protein had an interest in developing their own emulsion to serve as a marketable alternative to Olibra™. Palm oil does possesses high amounts of palmitic acid (~44%) and oleic acid (~37%) both of which had been shown to have

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potential in activation of the ileal brake [138, 139]. In addition to the benefits provided

by palm oil, fish oil has several properties that suggest it could serve as a potential

activator of the ileal brake. First, over 80% of fish oil is comprised of long chain fatty

acids, which have been demonstrated to delay gastric emptying [138, 139]. Second, fish

oil also contains a high amount of unsaturated fatty acids (55%) which some studies

suggest that a higher degree of unsaturation corresponds to a greater effect on satiety,

although it is not conclusive [140-142]. In addition to the potential effect on satiety, the

omega-3 (n-3) fatty acids specifically docosahexaenoic acid (DHA-22:6n-3) and

eicosapentaenoic acid (EPA-20:5n-3) are considered anti-inflammatory nutrients and

have been often been used a dietary supplement for inflammatory diseases such as

rheumatoid arthritis and cardiovascular disease [174]. Figure 21 illustrates the

emulsions potential mechanism of action. Phase three tested the hypothesis that the

treatments induced satiety, reduced energy intake and induced a satiety hormone

response in human subjects.



**Fatty Acid Profile of Olibra™ and Emulsion Treatments**

| Fatty Acid | | Olibra™ | Emulsion A | Emulsion B |
|---|---|---|---|---|
| Myristic | 14:0 | 0.95 | 3.79 | 5.10 |
| Palmitic | 16:0 | 42.23 | 35.24 | 31.35 |
| Palmitoleic | 16:1 | 0.00 | 4.28 | 6.30 |
| Stearic | 18:0 | 4.19 | 3.99 | 3.85 |
| Oleic | 18:1 | 36.57 | 28.17 | 24.20 |
| Linoleic | 18:2 | 10.70 | 6.65 | 5.50 |
| Linolenic | 18:3 | 0.10 | 0.51 | 0.75 |
| Arachidonic | 20:4 | 0.00 | 0.54 | 0.80 |
| EPA | 20:5 | 0.00 | 4.93 | 7.25 |
| DHA | 22:6 | 0.00 | 4.01 | 5.90 |
| Other | -- | 5.28 | 7.88 | 9.00 |
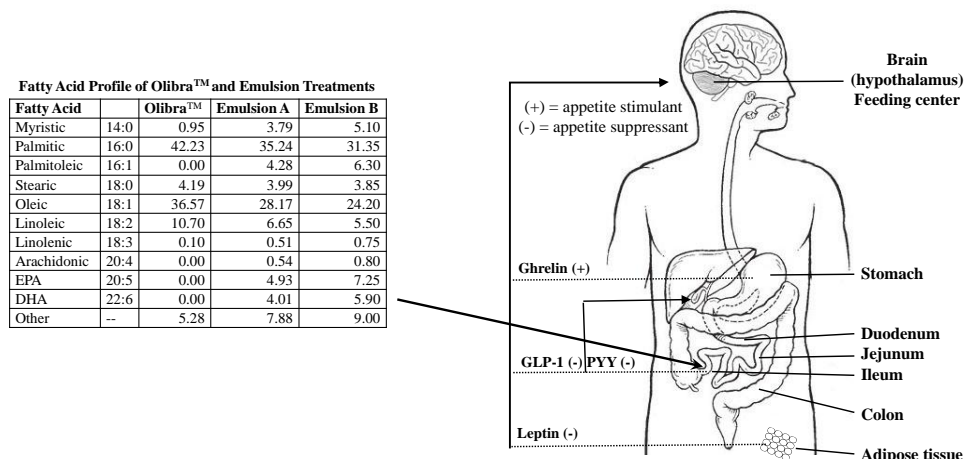
**Figure 21 - Proposed Mechanism of Action of Emulsions**

Olibra™ and the emulsions have different fatty acid profiles. Fatty acids that reach the ileum intact have the potential
to activate the ileal brake. This will induce the release of satiety hormones into circulation.

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## SUBJECTS AND METHODS

*Subject Recruitment*

Ten subjects, consisting of eight females and two males were recruited for the study using posted advertisements and mass emails sent out to the Department of Nutrition and Food Science and The Department of Health and Kinesiology at Texas A&M University.  The subjects had a mean age of 21.8 years, a mean height of 165.1 cm, a mean weight of 73kg, and a mean BMI of 26.8.  Subjects were non-smoking, non-dieting, not pregnant, and not taking any prescribed medication.  All subjects indicated a preference for cheese pizza as the satiety lunch.  All subjects signed consent forms before participating in the study.  The study was approved by the Texas A&M University IRB.

*Study Design*

The study was conducted in the Exercise and Sports Nutrition Laboratory (ESNL) at Texas A&M University.  The study was a randomized placebo controlled single-blinded crossover trial that involved testing the effects of three treatments on satiety and energy consumption.  Each subject picked a day of the week, excluding Saturday and Sunday, as their day of study and were required to arrive on that same day for each treatment for three consecutive weeks.  The order that each subject received each of the three treatments was randomized.  For each study day, subjects were instructed to arrive fasted for 12 hours, arrive at 8:45am to have the baseline blood sample collected and complete a baseline VAS satiety scorecard.  After the baseline

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blood collection at 9:00am, subjects were instructed to consume the treatment beverage

within five minutes.  Additional blood samples were collected at 30, 90 and 180 minutes.

Subjects were also instructed to complete VAS satiety scorecards every 30 minutes until

noon.  From arrival at the laboratory until noon, subjects were instructed not to engage in

any strenuous physical activity nor, were they permitted to consume anything other than

water.  At noon, subjects were provided a whole cheese pizza purchased from Little

Caesars Pizza and instructed to eat until full.  The amount of pizza consumed by each

subject was recorded and weighed.  Figure 22 displays the schedule for each study day.
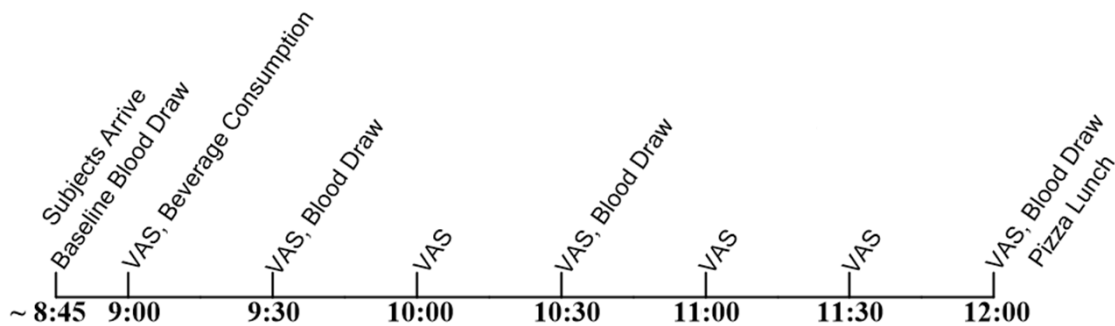


**Figure 22 - Study Day Schedule**

Subjects (n=10) followed the above schedule for each treatment for three consecutive weeks.  Each subject received a
different treatment each week.  The order of treatment consumption was randomized for each subject.

*Treatment Preparation*

Beverage ingredients excluding the emulsions were purchased from an H.E.B.

supermarket located in College Station, Texas on a weekly basis in order to ensure

consistency and freshness for each experiment.  The treatments were freshly prepared

prior to each subjective and objective comparison test.  Milkfat from heavy whipping

cream served as the control in accordance to previous studies [149-151].  Table 12

contains an approximate macronutrient composition from a 200 gram sample of

beverage containing 6 grams of each emulsion or the milkfat control.

|  | | Carbohydrates (g) | Sugar (g) | Fiber (g) | Starch (g) | Fat (g) | Protein (g) | Kilocalories |
|---|---|---|---|---|---|---|---|---|
| (a) | Banana | 14 | 8.74 | 1.38 | 3.68 | 0 | 0.46 | 50.6 |
|  | Vanilla | 0 | 0.23 | 0 | 0 | 0 | 0 | 5.52 |
|  | Fat Free Vanilla Pudding | 1.2 | 0 | 0 | 1.15 | 0 | 0 | 4.6 |
|  | Hersheys Syrup | 11 | 9.2 | 0.46 | 1.38 | 0 | 0.46 | 46 |
|  | Strawberries | 1.3 | 0.81 | 0.32 | 0.16 | 0 | 0.161 | 5.15 |
|  | Skim milk | 2.8 | 2.76 | 0 | 0 | 0 | 1.84 | 19.78 |
|  | Yogurt | 3.5 | 3.45 | 0.23 | 0 | 0 | 2.53 | 25.3 |
|  | **Total (pre-treatment)** | **33** | **25.2** | **2.4** | **6.4** | **0** | **5.5** | **157.0** |
| (b) | Milkfat (control) (6g) | 0 | 0 | 0 | 0 | 1.8 | 0.1 | 16.6 |
|  | Emulsion A (6g) | 0 | 0 | 0 | 0 | 1.8 | 0.1 | 16.6 |
|  | Emulsion B (6g) | 0 | 0 | 0 | 0 | 1.8 | 0.1 | 16.6 |
|  | **Total with treatment** | **33** | **25.2** | **2.4** | **6.4** | **1.8** | **5.5** | **173.2** |

**Table 12 - Macronutrient Composition of Beverage and Emulsions**
Macronutrient composition was estimated using food nutrition labels. (a) refers to the beverage only and (b) refers to the emulsions. The Strawberry and banana composition was estimated using Dole's nutrition website. The control is composed of milkfat from heavy whipping cream.

All of the ingredients were combined into a blender (Oster 14 Speed, Rye, NY)

and set on the lowest speed (setting "stir") for one and a half minutes. The resulting

treatments had a chocolate brown appearance and viscous texture (Figure 23).
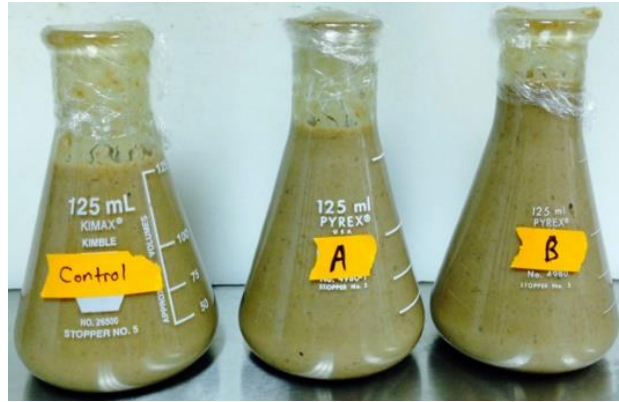
**Figure 23 – Visual Representation of Each Treatment**
The above picture was taken following beverage preparation and is intended to provide a visual representation of the color and viscosity.

*Emulsion Composition and Preparation*

The emulsions for the beverage were prepared by Omega Pure (Houston, Texas). Two emulsions which were referred to as A and B were tested. The emulsions differed in their palm oil to fish oil ratios, being either 2:1 (A) or 1:1 (B). Table 13 shows the quantities in grams of each ingredient present in a six gram sample emulsion, the amount added per 200g of beverage. Table 14 presents a description of the components used to prepare emulsion A and emulsion B. Figure 24 shows the treatment preparation diagram.

| **Emulsion A** | | | | **Emulsion B** | | |
| 2:1 palm oil: fish oil ratio | | | | 1:1 palm oil: fish oil ratio | | |
| Ingredient | % | Amt / 6g | | Ingredient | % | Amt / 6g |
|---|---|---|---|---|---|---|
| Water | 66.58% | 4g | | Water | 66.58% | 4g |
| SansTrans 39 | 19.58% | 1.2g | | SansTrans 39 | 14.69% | 0.9g |
| OP HSN | 9.79% | 0.6g | | OP HSN | 14.69% | 0.9g |
| Na-caseinate | 1.96% | 0.1g | | Na-caseinate | 1.96% | 0.1g |
| Gum Arabic | 1.37% | 0.1g | | Gum Arabic | 1.37% | 0.1g |
| K-sorbate | 0.49% | ~0g | | K-sorbate | 0.49% | ~0g |
| Panodan | 0.23% | ~0g | | Panodan | 0.23% | ~0g |

**Table 13 - Quantity of Emulsion Ingredients Present in Each Treatment**

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| Ingredient | Function |
|---|---|
| **Gum arabic** | A complex mixture of glycoproteins and polysaccharides that is used primarily in the food industry as a stabilizer for the purpose of reducing the surface tension of emulsions. |
| **Panadan** | A diacetyl tartaric acid ester of mono-diglycerides (DATEM) made from edible, refined soybean oil. Served as the emulsifier to make primary emulsion. |
| **Na-caseinate** | Proteins derived from milk that are commonly used as food stabilizers. Used as coating to the primary emulsion to form the secondary emulsion. |
| **K-sorbate** | Served as a preservative against molds and yeasts. |
| **SansTrans™ 39** | Served as the palm oil portion of the palm oil phase. Developed by Lokers Croklaan™ and sold commercially as multi-bakery shortening based on palm oil and fractions of palm oil. It has been designed as an alternative to shortening due to the fact that it is non-hydrogenated and contains no trans-fatty acids. Melting point is reported to be between 37-41°C and is composed of 49.9% of saturated fatty acids, 40.1% mono-unsaturated, and 9.6% polyunsaturated according to manufacturer spec sheet. |
| **Omega Pure (OP) HSN** | Served as the fish oil portion of the fish oil phase. Derived from menhaden fish. Composed of 32% unsaturated fatty acids, 24% mono-unsaturated, 36.74% polyunsaturated (35.66% omega-3) and 7.12% that is not defined on the sheet. EPA + DHA composed 26.12% of total fatty acid composition. Also contains tocopherols to serve as antioxidants. |

### Table 14 - Ingredients For Emulsion Formulations

All of the above ingredients were necessary to prepare a stable emulsion. Emulsions A and B differed only in the amount of SanTrans™ 39 and OP HSN.
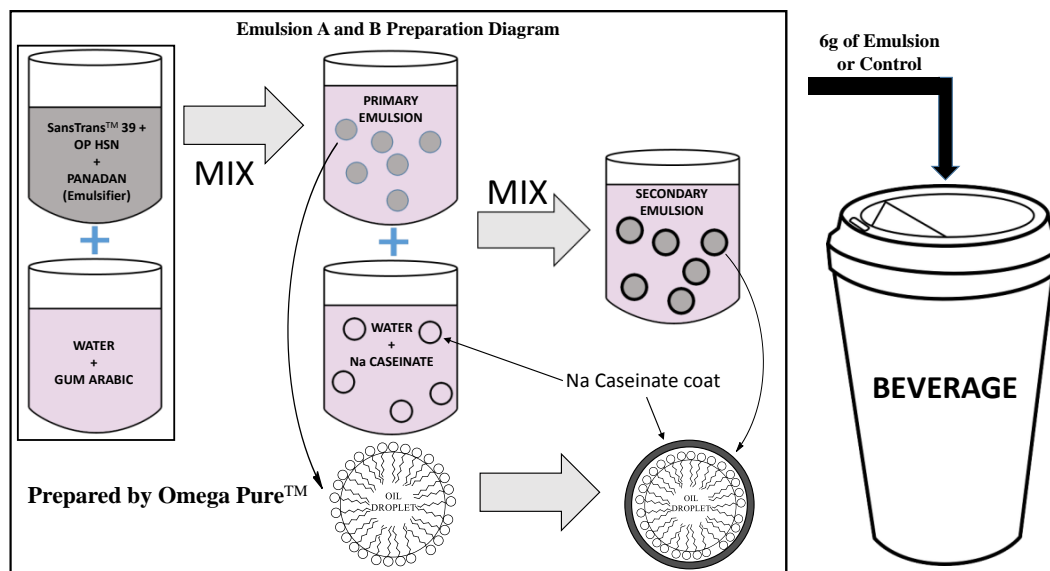


### Figure 24 – Treatment Preparation Diagram

Emulsion A and B both differed in the amount of SansTrans™ 39 (palm oil) and OP HSN (fish oil) present. Both oils were mixed together plus the DATEM emulsifier to make the oil phase. The oil phase was mixed with the aqueous gum arabic solution to create the primary emulsion. The primary emulsion was then mixed with the aqueous sodium caseinate resulting in the final (secondary) emulsion. 6g of the secondary emulsion or control was added to the beverage.

To prepare the oil phase, both solid SansTrans™ 39 (palm oil source) and liquid

OP HSN (fish oil source) were heated in separate beakers on the same heating plate

(Corning PC-420D) to 44°C. The temperature of the oil mixture was monitored using a

standard thermometer. A temperature of 44°C was sufficient to completely melt the

SansTrans™ 39 and ensured that mixing of both oils would be uniform. Both oils were

combined with a proprietary quantity of rosemary antioxidant and Panodan emulsifier to

create the oil phase. Due to confidentiality agreements with Omega Pure™, the exact

quantity of rosemary extract is unknown. Panodan, in liquid form, was added to the

mixture using a 5mL pipette. The solution was mixed with a stir bar at a speed of 500

rpm.

To prepare the water phase, two equivalent water solutions were prepared: one

that contained dissolved gum arabic and one that contained dissolved sodium caseinate.

The gum arabic and sodium caseinate were dissolved in distilled water via stir bar

mixing at a speed of 500 rpm in separate beakers and mixing plates at room temperature.

Using an Ultra Turrax T 50 Basic shearing mixer (IKA Werke™ Wilmington,

NC) the oil phase solution was slowly added to the gum arabic water solution. The

shearing speed was gradually increased to maximum as the dispersion became more

evenly mixed. After this step, the range of the size of the oil droplets was approximately

1-10 μm. Once the dispersion was sufficiently mixed it was then passed through a

homogenizer (Niro Soavi™ NS10011 2K Bedford, NH) at 50 Bar to break up larger oil

droplets and then passed again at 400 Bar to further decrease the oil droplet size down to

0.2–2 μm with some flocculation.  The resulting dispersion was referred to as the primary emulsion as shown in Figure 23.

Using the Turrax mixer the primary emulsion was gradually added to the sodium caseinate solution where the negatively charged caseinate functioned as an emulsion stabilizer.  Sodium caseinate can stabilize emulsions by lowering the interfacial tension due to its adsorption at the interface.  Similar to the first step the shearing speed of the Turrax mixer was gradually increased as the dispersion became more uniform.  The resulting dispersion was then passed through a homogenizer at 400 Bar to ensure a consistent oil droplet size range of 0.2–2 μm with minimal flocculation.  The emulsions were examined under a microscope to ensure minimal flocculation and consistent droplet size.  The emulsions were sealed and refrigerated at $4^0C$ until it was time to for them to be supplemented with the beverage as shown in Figure 23.

*Visual Analogue Scales to Measure Satiety*

Upon arrival and prior to treatment consumption and blood collection, subjects rated the satiety attributes of Hunger, Satisfaction, Fullness, and Thoughts of Food (TOF) using VAS scorecards to obtain the baseline appetite ratings following previously established protocols [162-164].  Subjects completed the same scorecard at 30, 60, 90, 120, 150, and 180 minutes.  The subjects marked an "X" on the line to indicate their feelings at the timed intervals above for each of the four attributes on different 10cm lines anchored by statements in a left to right order "I am not hungry at all/Very hungry," "I am not full at all/Very full," "I am completely empty/I can't eat another

bite," and "Nothing at all/A large amount," as shown in Figure 25.  In reporting satiety

via VAS, the post-baseline ratings at each timepoint were subtracted from the baseline

and graphed as the change from baseline [162-164].

## SATIETY

1. How **hungry** do you feel?

I am not hungry at all |—1—2—3—4—5—6—7—8—9—10| Very hungry

2. How **full** do you feel?

I am not full at all |—1—2—3—4—5—6—7—8—9—10| Very full

3. How **satisfied** do you feel?

I am completely empty |—1—2—3—4—5—6—7—8—9—10| I can't eat another bite

4. How **much** do you think you can **eat** now?

Nothing at all |—1—2—3—4—5—6—7—8—9—10| A large amount
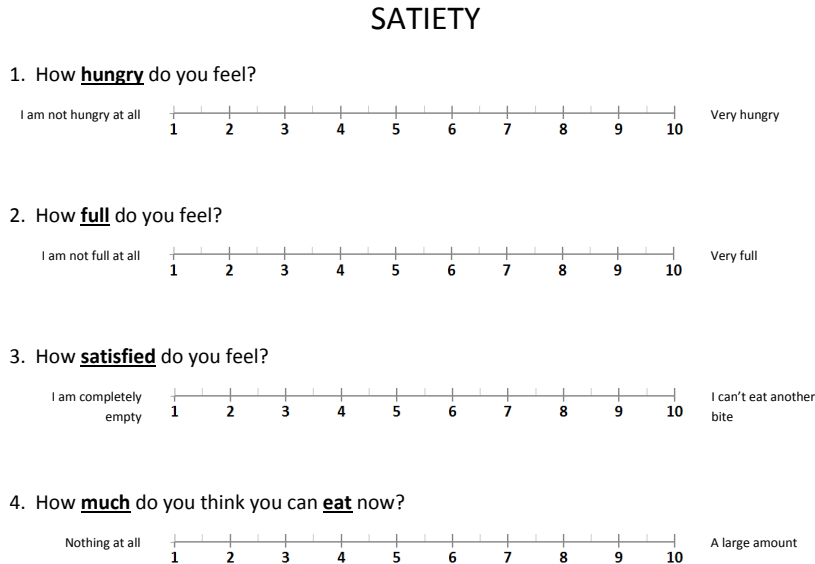
**Figure 25 - Beverage Sensory Scorecard and VAS Appetite Scorecard**
Subjects were required to complete satiety scorecard pre-beverage consumption for the baseline and every 30 minutes
during the 180 minutes post-beverage consumption prior to pizza lunch.

*Satiety Meal Measuring Kilocaloric Intake*

Cheese pizza was chosen as the single satiety meal food item. The pizzas were

purchased from Little Caesars Pizza in College Station, TX.  Little Caesar's pizza was

chosen because of cost, convenience and was predetermined through subject/researcher

interactions to be a preferred satiety lunch offering as a reliable method to estimate

kilocarolic intake 180 minutes post-treatment consumption.  This is consistent with a

previously reported study that used cheese pizza as their satiety lunch [163].  The pizzas

were approximately 35.6 cm in diameter with eight total slices.  According to the

nutrition facts provided by the Little Caesars website, each slice of pizza weighed approximately 117g and provided 250 kilocalories.  Pizzas were weighed and inspected prior to purchase to confirm that the totals provided were accurate.

Following the 180 minute blood collection, subjects were instructed to eat whole slices of pizza until satiation.  They were instructed to not leave any crust in order to accurately estimate kilocalorie consumption per treatment.  In the event that the subject did not feel they could eat a whole slice, the slice was cut in half and kilocalories were estimated based on the weight.

*Blood Sample Collection*

Subjects arrived at the study laboratory before 9am and a baseline blood sample was collected into EDTA suction tubes using a syringe by a registered nurse or a certified staff member in an enclosed area of the laboratory.  Subjects were monitored by the ESNL staff to ensure subject comfort.  Immediately after the baseline blood collection, subjects were returned to the lounge area of the laboratory where they consumed their assigned beverage emulsion treatment for that day.  Additional blood samples were collected at 30, 90, and 180 minutes post-treatment consumption. Immediately following collection, all blood samples were centrifuged and the plasma was collected and then stored in a -20ºC freezer in the ESNL until analysis.

*Analysis of GLP-1, PYY, Ghrelin and Leptin Expression*

For analysis, plasma samples were thawed on ice and dipeptidyl peptidase IV inhibitor was added to the test tubes to prevent degradation of GLP-1$_{7-36}$ into the inactive form. GLP-1 concentration was analyzed in its active form which consists of only amino acid residues 7-36. Leptin, ghrelin, total PYY and GLP-1 were analyzed using the Human Metabolic Hormone Magnetic Bead Panel - Metabolism Multiplex Assay kit provided by EMD-Millipore (Billerica, MA) on a Luminex 200 according to manufacturer's instructions.

*Statistical Analysis*

For each time point, mean VAS scorecard ratings and the mean hormone levels present in the blood samples for each treatment were compared and analyzed using a one way analysis of variance (ANOVA). The level of significance was set at a p-value < 0.05 for all experiments.

**RESULTS**

*Analysis of VAS Scorecards Satiety Ratings*

Using VAS scorecards, each subject rated their Hunger, Satisfaction, Fullness, and Thoughts of Food (TOF), prior to the consumption of each treatment to serve as the baseline. VAS scorecards were subsequently filled out at 30, 60, 90, 120, 150 and 180 minutes post-treatment consumption. The mean ratings of each satiety attribute for each treatment were plotted as a function of time (Figure 26). There was a decrease in hunger

rating for all treatments at 30 minutes with the control demonstrating the greatest

decrease, but was not significantly different from treatment A or B (Figure 26A).  For

satisfaction rating there was not a significant difference between the treatments, but

treatment B demonstrated the greatest satisfaction rating up to 120 minutes post-

treatment consumption (Figure 26B).  For fullness rating, there was not any significant

nor any noteworthy differences between treatments (Figure 26C).  For TOF rating, there

were no significant differences among treatments, although treatment B demonstrated

the smallest decrease at 60 and 90 minutes, but treatment B also demonstrated the

greatest TOF rating increase after 90 minutes, which persisted until 180 minutes (Figure

26D).

**How hungry do you feel?**

**(a)**

**How satisfied are you?**

**(b)**

**How full do you feel?**

**(c)**

**How much can you can eat now?**

**(d)**
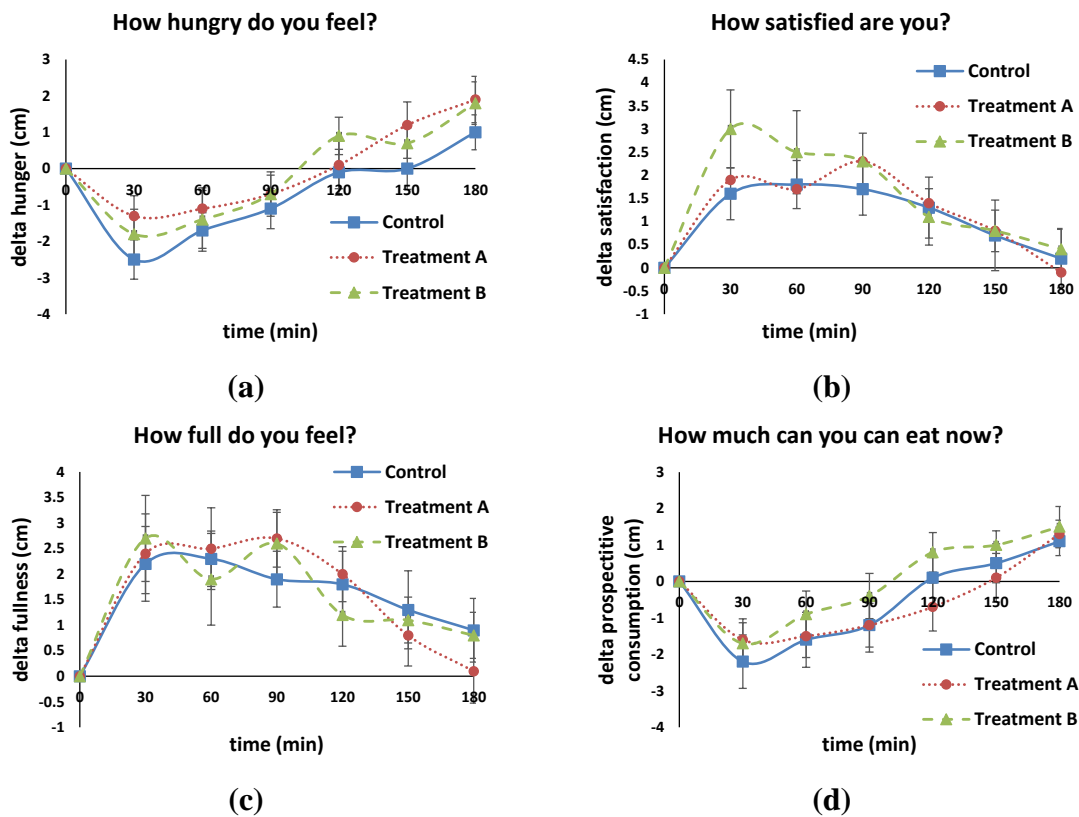
**Figure 26 - Mean Satiety VAS Ratings for Treatments**

The effect on each satiety attribute response from the VAS scorecards of each treatment presented as change from baseline scores were graphed as function of time. Data at each time point is means ± SEM (n = 10). The title of each graph corresponds to the question presented to each subject on the VAS scorecard shown in Figure 25.

*Kilocaloric Intake of Satiety Lunch*

At minute 180 and completion of their blood draw, subjects were provided one whole cheese pizza and were instructed to eat at a normal pace on each treatment day. In all instances, subjects reached satiation within 30 minutes and there was not a single instance of a subject eating more or wanting to eat more than one whole pizza. Subjects who were provided the control treatment consumed between 3 - 8 slices of cheese pizza, 2 - 6.66 slices for treatment A and 1.5 - 6 slices for treatment B. The average number of

slices consumed for each treatment was as follows: control treatment, 4.20 ± 0.53 slices; treatment A, 4.16 ± 0.47 slices; and treatment B, 3.85 ± 0.41 slices.  However, there was not a significant difference in the amount of kilocalories consumed per treatment, eventhough subjects who ingested treatment B consumed, on average, 100 calories less cheese pizza (Figure 27).
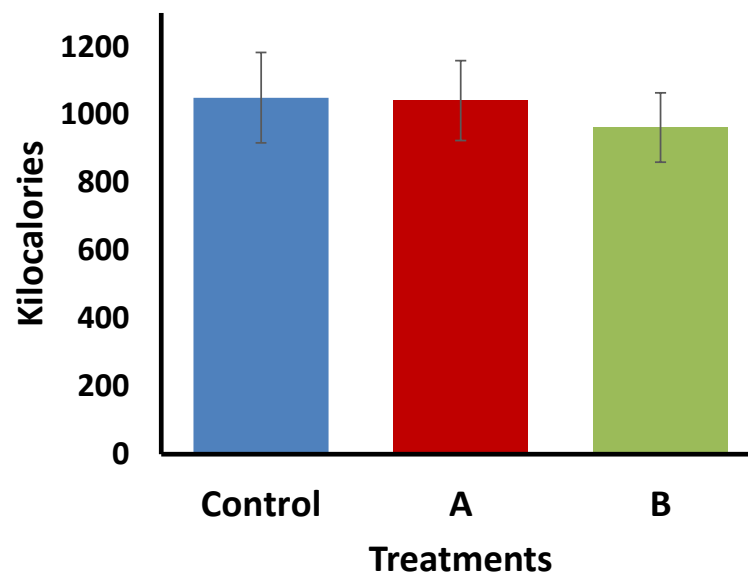


**Figure 27 - Kilocalories Consumed Post-Treatment Consumption**
Subjects (n=10) were provided a cheese pizza 180 minutes post-treatment consumption.  The kilocalorie consumption per treatment were graphed as the mean ± SEM.

*Analysis of PYY, GLP-1, Leptin and Ghrelin Plasma Expression*

Blood samples were collected from subjects at 0 (baseline), 30, 90, and 180 minute time points and were graphed as the change in mean plasma expression from baseline.  The change in plasma expression of the following hormones were analyzed: GLP-1, ghrelin, leptin and total PYY.  Compared to the control, treatment B

demonstrated the greatest GLP-1$_{7-36}$ expression at the 30 and 90 minute timepoints, but in neither case were the differences significant.  At the 180 minute timepoint, both treatments demonstrated greater GLP-1$_{7-36}$ expression compared to control.  (Figure 28a).

The total level of PYY, which includes PYY$_{1-36}$ and PYY$_{3-36}$ forms of the peptide, were also analyzed.  While none of the total PYY expression differences among treatments were significant it is worth noting that at the 30 and 90 minute timepoints only the control treatment demonstrated an increase in total PYY concentration, while treatment B demonstrated a decrease compared to baseline.  At the 180 minute timepoint, treatment A demonstrated the greatest total PYY expression, while treatment B demonstrated the smallest decrease (Figure 28b).

Leptin expression was also analyzed and compared.  There were not any significant differences between the three treatments for leptin expression at any of the timepoints (Figure 28c).

Unlike the previously analyzed hormones, ghrelin is considered a hunger hormone.  Ghrelin expression among treatments was not significantly different at any timepoint, but it should be noted that the control treatment demonstrated the smallest ghrelin expression decrease at 30 minutes and the greatest increase at 90, and 180 minutes compared to baseline and that both treatments demonstrated a smaller level of ghrelin expression (Figure 28d).

**GLP-1 ACTIVE**

(a)

**TOTAL PYY**

(b)

**LEPTIN**

(c)

**GHRELIN**

(d)
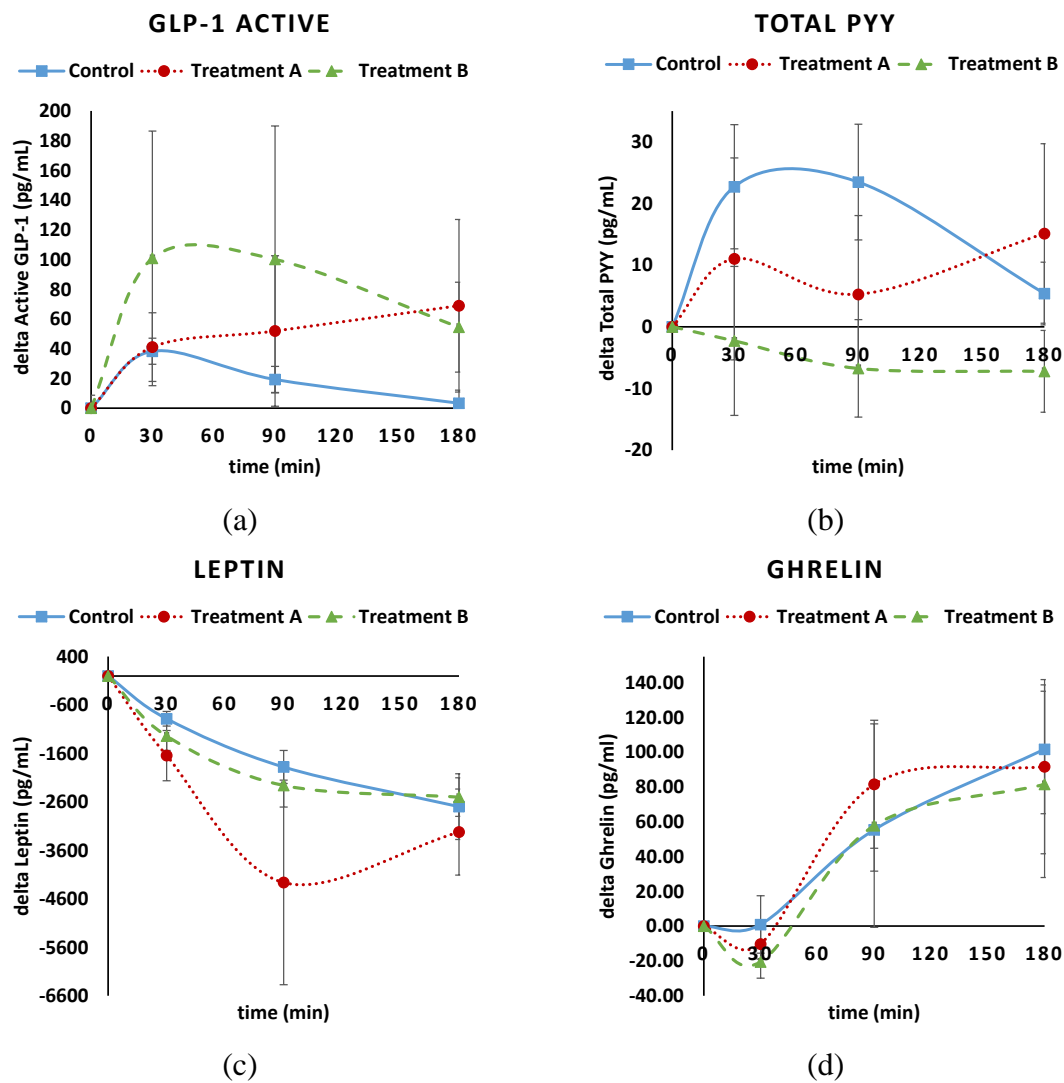
**Figure 28 – Plasma Hormone Sample Analysis of Treatments.**
The effect of each treatment on the plasma expression of GLP-1, PYY, leptin and ghrelin. For each treatment, change from baseline scores were graphed as function of time. Data at each time point is means ± SEM (n = 10).

## DISCUSSION

The goal of phase three was to determine if the emulsion treatments could

enhance satiety via activation of the ileal brake and to see if there was a correlation with

the results from phase one. Due to budgetary, location and time constraints this study

was limited to 10 subjects and the length of each study day had to be shortened compared to previous studies that often examined the effects for up to eight hours post-consumption.  Taking those restrictions into consideration, there only appears to be little evidence with statistical significance that the emulsion treatments induce satiety, despite the fact the emulsions demonstrated a significant increase in PYY and GLP-1 secretion in STC-1 cells in phase one.  As demonstrated in phase two, our developed beverage was able to mask any sensory, flavor, color, pH and rheological differences of each treatment with the only ingredient being different are the emulsions.   Therefore, it is unlikely that the treatment differences could have influenced the scorecard ratings or satiety.  There were a few instances in the satiety, energy intake and satiety hormone profile tests where the results might be worth investigating further to determine if there is a statistically significant difference using a larger sample size.  Subjects who consumed Treatment B consumed 100 kilocalories less pizza compared to control or treatment A.  Any satiety agent that can potentially reduce kilocalorie intake in subsequent meals is an important consideration for individuals interested in weight management.  Both treatments A and B demonstrated greater expression levels of GLP-1 compared to control.  Analysis and interpretation of PYY data is difficult since the analysis kit is unable to distinguish between full length PYY and $PYY_{3-36}$, the truncated form known to specifically exert the effects on satiety.  After 30 minutes, treatment B demonstrated the greatest decrease of ghrelin expression levels, an effect which persisted throughout the entire 180 minute treatment period.  Unlike leptin, GLP-1, and PYY, ghrelin is a hunger hormone produced by the stomach and given that treatment B demonstrated the greatest decrease

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in ghrelin expression could be worth investigating further. The results showed that the expression of leptin significantly decreased was an unanticipated result. The expectation was that leptin expression levels would increase following the consumption of the treatments.

There are several potential factors that could potentially explain the emulsion treatments inability to exhibit significant effects on satiety, energy intake and satiety hormone circulation. In the previous studies that examined Olibra$^{TM}$, the emulsion was always consumed in the presence of only yogurt [149-151, 166-171, 173]. Without yogurt, the effect on satiety and energy intake was abrogated [171]. Yogurt was previously shown to have higher satiety ratings following consumption relative to fruit drink or dairy fruit drink, possibly due to higher viscosity [172]. Although, our beverage formulated in phase two contained yogurt, the other ingredients such as skim milk decreased viscosity, which could have negatively influenced satiety. One way to determine if there was a beverage formulation flaw, would be to follow the same study design in phase three and add an additional treatment containing Olibra$^{TM}$. Olibra$^{TM}$, emulsion A and emulsion B have a similar fatty acid profile in that they are all comprised of over 50% palmitic and oleic acid. However, the oat oil component was not present in emulsion A or B. The galactolipids present in oat oil have been suggested to prevent pancreatic lipase from binding to the emulsion interface in the duodenum and thus delay lipid digestion [148]. Therefore adding an oat oil component to emulsions A or B is also a future consideration to refining the emulsion formulation.

Despite the lack of evidence and statistical significance in this pilot study, the only way to truly assess the effectiveness of emulsions A & B as satiety inducing agents is to recruit more subjects, extend the length of the study day and add Olibra$^{TM}$ as an additional treatment. If the treatments were shown to be effective in appetite suppression and reduce energy intake they may provide a cost-effective alternative over pharmacological drugs in facilitating weight loss in individuals interested in weight management and overall health.

CHAPTER V

SUMMARY AND FUTURE CONSIDERATIONS


**SUMMARY OF RESULTS**

*Overall Goal*

The goal of this research project was to investigate whether two orally consumed emulsions that differed in their palm oil to fish oil ratios, being either 2:1 (A) or 1:1 (B), influenced satiety via activation of the ileal brake, a gastric feedback mechanism that induces satiety by delaying gastric emptying and inhibiting intestinal motility. Biomarkers of the ileal brake and satiety are PYY and GLP-1. This research project consisted of three phases that are summarized below.


*Phase One Summary*

Phase one examined whether emulsions A and B stimulated the secretion of PYY and GLP-1 from the murine intestinal cell line, STC-1 *in vitro*. STC-1 cells secrete PYY and GLP-1 upon nutrient stimulation in a manner similar to human L-cells. In humans, PYY and GLP-1 are produced and secreted from the intestinal L-cells of the ileum upon nutrient exposure. Once in circulation, PYY and GLP-1 cross the blood brain barrier and bind to their respective receptors in the hypothalamus inducing satiety and reducing food intake. Emulsions A and B served as treatments for the STC-1 cell cultures. Their effect on the secretion of PYY and GLP-1 was analyzed at timepoints of 30, 60, 90 and 120 minutes. The results were compared and statistically analyzed using an ANOVA.

The results demonstrated that both emulsion treatments promoted GLP-1 and PYY secretion from STC-1 cell cultures. Although, there was not a significant difference between emulsion A and emulsion B, indicating that the palm and fish oil ratio were not responsible for the difference. These findings indicated that there may be potential for the emulsions to serve as activators of the ileal brake *in vivo*.

*Phase Two Summary*

In phase two, a fruit and dairy beverage was formulated to serve as a vehicle for the emulsions in human volunteers. Each beverage was prepared with either a milkfat control, or emulsion A, or emulsion B. For clarity of terms, each beverage preparation was referred to as the control treatment, treatment A and treatment B. Any subjective (sensory) or objective (instrumental analyses) differences in treatments could influence the observed results in satiety in human volunteers. The beverages were formulated with the goal of eliminating variations among treatments so that the only differences were in reported satiety. Subjective sensory tests and objective instrumental analyses were performed on each treatment and the results were statistically analyzed using an ANOVA. For the subjective sensory tests, triangle tests and acceptance/affective tests were administered using untrained subjects. The objective tests employed laboratory instrumentation to analyze and compare the viscosities, pH and Hunter L, a, b color values of each treatment. The results demonstrated that the formulated beverage masked any sensory, flavor, color, pH and rheological differences of each treatment with the only ingredient being different are the emulsions.

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*Phase Three Summary*

In phase three, the treatments formulated in phase two were investigated to determine if they induced satiety, reduced energy intake, and upregulated the expression of satiety biomarkers in human volunteers. Ten subjects were recruited in a randomized, single blind placebo-controlled, crossover study design. Subjects were instructed to complete VAS questionnaires as a baseline (time 0) for the subjective satiety rating prior to treatment consumption. Blood samples were also collected prior to treatment consumption as a baseline (time 0). Over the course of 180 minutes post-treatment consumption, VAS questionnaires were completed at 30 minutes intervals. Additional blood samples were collected at 30, 90 and 180 minutes post-treatment consumption. Following the 180 minutes of data collection, subjects were provided an eight-slice, 2000 kilocalorie, cheese pizza satiety lunch for which they were instructed to consume until sufficiently satiated. Upon satiation, the kilocaloric intake from the satiety meal was calculated. The collected blood samples were analyzed for the circulating plasma levels of ghrelin, leptin, PYY and GLP-1. Neither the VAS results, nor biomarker results demonstrated significant differences among treatments. However, although not significant, subjects provided treatment B (1:1 palm:fish oil composition), on average consumed 100 fewer kilocalories of cheese pizza.

In order to fully elucidate the role that emulsions play in inferring the activation of the ileal brake via upregulation of PYY and GLP-1, a more comprehensive study should be considered with the following suggested revisions: larger more representative

sample size, more frequent blood collection, have subjects keep record of subsequent food intake for up to 24-hours and a satiety lunch that offers a buffet.

## FUTURE CONSIDERATIONS

*Satiety Study Considerations*

In order to evaluate whether the emulsion beverage treatments promote satiety via activation of the ileal brake, a more comprehensive study is necessary which would include several refinements of protocol. The first refinement to the experimental design of the study would be to recruit additional subjects. Statisticians typically recommend between 20-30 subjects in order to minimize the effect of outliers and provide adequate power to the study. A much more established method would be to follow Burns *et al.* protocol, whose study involved sixty subjects, with twenty subjects separated into BMI categories of normal, overweight and obese. Each BMI weight category would require approximately ten males and ten females and with a more diverse recruitment pool [149]. The second refinement would be to expand the recruitment pool, since the satiety study in phase three was limited to students at Texas A&M University. To address this, the recruitment pool could be expanded to include any adults, aged 18-60, currently residing in the community as per other studies. The third refinement is to collect additional blood samples at the 15, 60, 75, 105, 120, 135, 150 minute timepoints and another collection following consumption of the satiety meal to demonstrate the anticipated hormone response. It should be considered however, that additional blood

collection intervals may lead to subject discomfort, which could influence satiety/appetite. The fourth refinement would be to include Olibra$^{TM}$ as a treatment to serve as a basis for comparison to the results in phase three. Such a revision could clarify if there was a limitation due to beverage or emulsion formulation. Despite conflicting reports, the effects of Olibra$^{TM}$ on satiety are well established [149-151, 166-171, 173]. The fifth refinement would be to require the subjects to complete records of food consumption for the 24 hour period prior to and the 24 hour post-treatment consumption. The sixth refinement would be to provide the subjects an *ad libitum* lunch that consists of a restricted buffet style meal instead of a cheese pizza that has been utilized in many studies [149-151, 166-171, 173]. The advantage of an *ad libitum* buffet style lunch is that additional food choices provide subjects access to foods that may suite their personal preferences. As a reminder, the cheese pizza satiety lunch in phase three was predetermined by the subjects to be suitable, however a buffet style revision might enable the subjects to more accurately mimic normal eating habits and potentially provide a more accurate measurement of kilocalorie intake. An additional refinement to consider is the location of the study, specifically the location of the satiety meal. In the study in phase three, subjects consumed their satiety lunch at a large conference table located in an open area that prevented privacy. Although, it can be argued that such a setting is more appropriate in mimicking real world eating situations.

Despite the lack of statistical significance in the satiety study, a more comprehensive way to assess the satiety effects of both emulsions is to implement the refinements to the study design. If either of the emulsions are demonstrated to promote

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satiety, they may provide cost-effective alternative over pharmacological drugs in facilitating weight loss in subjects who desire to improve their weight and overall health.

*Beverage Formulation and Product Development Considerations*

There is a possibility that the emulsion delivery method and/or formulations were a factor in the phase three results. This section will address the considerations in the beverage formulated in phase two. In order to fully characterize the treatment sensory parameters, descriptive analysis could be administered using trained panelists. Unlike the affective and triangle tests where untrained panelists provided quantitative assessments of quality, descriptive analysis provides qualitative descriptions of the sensory attributes of a food. Such a test would further clarify the beverage sensory parameters to aid in product development.

While the beverage was successful in masking the flavor of the emulsions, several formulation adjustments could be considered for future studies. The critical beverage ingredient is the fat free yogurt since it was combined with Olibra$^{TM}$ in all previous studies [149-151]. Most importantly, Olibra$^{TM}$ was shown to be ineffective in inducing satiety without yogurt [171]. Consumption of yogurt alone was demonstrated to promote satiety compared to consumption of a fruit drink or dairy fruit smoothie, possibly due to higher viscosity [172]. The skim milk dispersed the ingredients and contributed to mouthfeel, but lowered the viscosity, which may have decreased satiety. Although, the Jell-O pudding component possessed ingredients that function as emulsifiers and thickening agents, the final quantity of said ingredients in the treatments

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was unlikely to have an effect on viscosity. To further increase beverage viscosity, a thickening agent such as low-methoxy pectin may be included in the formulation. Unlike high methoxy pectin, low-methoxy pectin does not require significant amounts of sugar to gel properly, although the addition of calcium may be necessary. As formulated, the beverage pH was measured at 4.75, which is within the pH tolerance range (2.5-5.5) for pectin gelation. The cinnamon, vanilla extract contributed to flavor, while the chocolate syrup contributed to the flavor, color and texture. Chocolate syrup provided the treatments their light brown color. It is unlikely that the chocolate syrup, cinnamon or vanilla extract negatively impacted satiety.

While shelf life is a minor factor in evaluating the treatments effects on satiety, it is a significant factor when considering commercial viability. As such, improving beverage shelf life should be considered a priority. One modification in the beverage formulation that may improve the shelf life, would be the removal of the fruit component. While fresh strawberries and bananas provided flavor, they undergo spoilage more rapidly than the other ingredients. The removal of the milk component is a consideration, since it may also contribute to rapid spoilage. Potential fat free replacements include almond milk, coconut milk, soy milk, rice milk or hemp milk. Pasteurization and the addition of antimicrobial agents such as sodium benzoate are also considerations that may prolong the shelf life. Any reformulation of the beverage treatments would require repeating the subjective and objective tests from phase two.

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*Emulsion Delivery and Formulation Considerations*

There is a possibility that the emulsion delivery method and/or formulations were a factor in the phase three results.  If the beverage formulated in phase two was determined to be ineffective as an emulsion delivery system, emulsion encapsulation or emulsion component reformulation may be considered for refinements in experimental design.  Emulsion encapsulation by alginate beads is a consideration, since it has been shown to delay lipid digestion, potentially increasing lipid exposure to the ileum [194, 195].  Reformulation factors which address specific components of the emulsion, including emulsifier charge or refinement of the oil phase, are discussed below.

It was demonstrated that emulsifiers with charged functional groups promote a high-surfactant-to-bile ratio, which can inhibit lipolysis by preventing mixed micelle formation [196].  Inhibition of lipolysis increases the emulsion intestinal transit time and therefore increases the possibility of free fatty acids, liberated from the oil component of the emulsions, reaching the ileum intact.  Both emulsions examined in the satiety study were prepared by Omega Pure with diacetyl tartaric acid ester of mono-diglycerides (DATEM) serving as the primary emulsifier.  Aside from a single carboxylic acid, DATEM does not possess any charged functional groups which may have affected emulsion stability.  However, since casein served as the secondary emulsifier to provide an additional layer of stability, such an effect was likely minimized, but is still a consideration.

Another formulation consideration would be to incorporate oat oil into the emulsion formulation since it is a critical component of Olibra$^{TM}$.  Oat oil contains

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galactolipids, which due to their bulk size prevent pancreatic lipases from binding to the emulsion interface in the duodenum, delaying lipid digestion [148]. Therefore, reformulating emulsions A and B to include an oat oil component is a consideration.

In summary, factors such as encapsulation, emulsifier charge and oil composition are all considerations in reformulating the emulsion and/or delivery method to promote delayed lipid digestion that may increase lipid exposure to the ileum.

*Summary*

The experimental results in phase three did not support the hypothesis that emulsions induce satiety through inferred activation the ileal brake in human subjects. However, the study was limited in the number of subjects, area of recruitment, number of blood collection intervals and satiety meal offering. As such, experimental design refinements could be implemented into future satiety studies that would further clarify if such was the case. Changes to implement in the satiety study include, the recruitment of additional subjects, offering a greater variety in food selection for the satiety lunch, expanding the recruitment pool and the collection of blood samples at additional timepoints. Beverage formulation considerations include subjective tests with trained panelists, increasing beverage viscosity and prolonging the shelf life. Emulsion formulation may be enhanced by the addition of an oat oil fraction, altering the choice of emulsifier or encapsulation of the emulsions to serve as an alternative vehicle for delivery to the ileum.

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