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Dietary Strategies to Influence Appetite: Effects of Oat Beta-Glucan and Thylakoids from Spinach on Satiety and Reward-induced Eating Behavior

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DIETARY STRATEGIES TO INFLUENCE APPETITE: EFFECTS OF OAT BETA-GLUCAN AND THYLAKOIDS FROM SPINACH ON SATIETY AND REWARD-INDUCED EATING BEHAVIOR

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My education and work experience can best be described as eclectic. After studying law and doing a stint as an international flight attendant, I embarked on a career in nutrition and dietetics. It has all been a wonderful learning experience, and I am grateful to the people who have touched my life along the way.

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ABBREVIATIONS

5-HT	Serotonin
AACC	American Association of Cereal Chemists
BMI	Body Mass Index
CART	Cocaine-and-amphetamine-regulated transcript
CD36	Cluster of Differentiation 36
CKK	Cholecystokinin
CVD	Cardiovascular disease
FDA	United States Food and Drug Administration
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GPCR	G-protein Coupled Receptor
HDL-C	High Density Lipoprotein Cholesterol
hsCRP	High-sensitivity C-reactive protein
IO	Instant oatmeal
IOM	Institute of Medicine
LDL-C	Low Density Lipoprotein Cholesterol
NHANES	National Health and Nutrition Examination Survey
NSP	Non-starch polysaccharide
OEA	Oleoylethanolamine
PBRC	Pennington Biomedical Research Center
PYY	Peptide YY

RTEC	Ready-to-eat cereal
SO	Old fashioned oatmeal
TG	Triglycerides
US	United States
VAS	Visual Analog Scales

ABSTRACT

The objective of these studies was to examine the effects of oat-based cereals and a spinach extract, on eating behavior. The cereals compared included instant oatmeal (IO), old fashioned oatmeal (SO), and a ready to eat cereal (RTEC), each containing the soluble fiber β-glucan. The spinach extract containing thylakoids, the internal photosynthetic membrane of plants, was compared with a placebo.

The first study, a randomized crossover trial compared the effect of IO and SO on subjective ratings of satiety, with the RTEC. Subjects consumed isocaloric 150kcal servings of the cereals in random order. Visual analogue scale ratings evaluating satiety were completed before breakfast and throughout the morning. IO increased satiety compared to the RTEC ($p<0.05$); however, SO was not as effective. The second study using a similar design compared the effect of isocaloric 250kcal servings of IO and the RTEC, on subjective ratings of satiety and food intake. IO increased subjective satiety ($p<0.01$), and decreased energy intake at lunch compared to the RTEC ($p=0.012$). The content and physicochemical properties of β-glucan were determined in both studies. IO and SO had higher meal viscosities than the RTEC. IO also displayed higher initial meal viscosity than the RTEC.

In the third randomized cross-over trial, subjects consumed the spinach extract or placebo in random order. Subjective ratings of satiety, liking and wanting (reward components of eating behavior), and food intake were evaluated. Compared to the placebo, consumption of the spinach extract increased satiety over a two hour period ($p < 0.05$); however, there were no differences in measures of liking or wanting, and energy intake measured at four hours. Although not significant, males reduced their energy intake by 126 kcals ($p=0.08$) after consuming the spinach extract, compared to the placebo.

In conclusion, instant oatmeal in 150kcal and 250kcal portion sizes increases subjective satiety over four hours, compared to the RTEC; whereas, the 250kcal serving also reduces energy intake. Initial meal viscosity of oatmeal may be an important factor influencing satiety. While 5g of thylakoids increase subjective satiety over two hours compared to a placebo, the reward components, or energy intake are not different.

CHAPTER 1
INTRODUCTION

Eating in humans is a discontinuous process made up of meals and inter-meal intervals. Hunger is a conscious sensation that reflects a mental urge to eat whereas the process that brings an eating episode to an end is satiation leading to satiety, in which the perception of hunger is inhibited. Effectively, satiety prolongs the inter-meal interval. The nature and timing of physiologic events separately influence satiation and satiety.[1] The biological drive to eat is inextricably linked to the satiating and satiety enhancing power as well as the rewarding value of food. Obesity results from a chronic energy imbalance; hence, controlling the urge to eat assumes importance.

Obesity is generally defined as excess body fat; however, the level of adiposity that constitutes obesity lacks consensus.[2] The measurement of body fat using historical methods such as underwater weighing is cumbersome and expensive, and few epidemiologic studies have included direct measures of body fat.[3] Thus, obesity is often defined as excess body weight rather than excess body fat and a surrogate measure known as body mass index (BMI) or weight in kilograms (kg) per square of height in meters (m) is commonly used to estimate adiposity. It is a convenient measure to assess the adiposity status of a population over time; however, it does not distinguish between fat and muscle mass. Moreover, height and weight are subject to demographic shifts making them highly empirical; hence, BMI may not be the most reliable indicator of individual adiposity status.[4] Nevertheless, the United States (US) and international standard definition of obesity is a BMI of 30 kg/m^2 or above, and overweight is defined as a BMI of 25 to 29.9 kg/m^2;[5, 6] although, national organizations in some Asian countries have adopted lower BMI cut-off points to define overweight and obesity.[7]

The 2013 Global Burden of Disease study showed that worldwide, the number of overweight and obese individuals increased by 857 million in 1980 to 2.1 billion in 2013, an increase of 27.5% among adults and 47.1% among children. Although over the past eight years developed countries have witnessed an apparent slowing of the rise in the prevalence of obesity, no country has reported significant decreases for three decades. In 2013, the US accounted for 13% of the 671 million obese people worldwide.[8] National Health and Nutrition Examination Survey (NHANES) data aggregated from 2007 to 2012 showed that in the US, approximately 35% of males and 36% of females 25 years of age or older were obese.[9]

Obesity is a multidimensional problem and its complex contributing factors, include behavioral,[4] psychological,[10] environmental,[11] economic,[12] genetic,[13] reproductive,[14] chronobiological,[15] cultural,[16] and social issues[17] in addition to prescription medication use[18] and factors influencing gut microbiota.[19] Moreover, weight loss induces neuroendocrine changes that impinge on what is called 'willpower.' In the short term a motivated individual will lose weight; but, weight maintenance is hampered by a biological resistance to weight loss and a predisposition to weight gain prompted by counter-regulatory mechanisms that provoke a powerful unconscious impulse to eat.[20] The need to exercise a constant control over eating behavior in the face of physiologic pressures to regain weight can be trying for even the most motivated individuals who have achieved weight loss.

The biology of human appetite control must be considered in the context of the hedonic and homeostatic expression of appetite. The biological need to maintain energy stores drives the homeostatic control of appetite by increasing the motivation to eat. Negative feedback signals terminate eating once the need is met. Reward based pathways rather than a biological need drive the hedonic control of appetite. The psychological components of reward include learning about

relationships among stimuli and the outcomes of consequent actions, liking or the orosensory stimulation of food coupled with the associated pleasure, and wanting or the motivation to engage in eating.[21] The homeostatic and hedonic controls interact to determine eating behavior, and this interaction defends well against an energy deficit; however, in an environment that is beset with energy intake triggers, it lacks potency. The result is a regulatory system that is not nearly as sensitive to over-consumption as it is to under-consumption.[22] Therefore, the importance of foods or food components that help individuals cope with hunger or the rewarding value of food stimuli cannot be overstated.

Dietary Fiber

Dietary fiber refers to carbohydrate polymers or oligomers that escape digestion in the small intestine and are partially or fully fermented on reaching the large intestine.[23] The role of dietary fiber in promoting satiation and satiety has been investigated as previously reviewed,[24 25] and there is evidence to indicate that consumption of fiber-rich foods has a modest long-term effect on weight loss.[26, 27] Based on its solubility in water and buffer solutions, dietary fiber may be soluble or insoluble.[28] Most fibers are not exclusively soluble or insoluble, but rather are a mixture of both types. Soluble fibers may or may not be viscous. When mixed with liquids, soluble fibers are expected to absorb water. When hydrated, viscous soluble fibers may induce thickening, and gel forming fibers may start forming a gel. Viscosity refers to resistance to flow. Gelling refers to the three-dimensional networks of molecules that can entrap liquids and behave like solids. Viscous fibers include many soluble dietary fibers such as gums, pectins, and β-glucans.[29]

There are multiple mechanisms by which dietary fibers influence satiety. A reduction in the metabolizable energy may directly lead to a reduction in food intake or indirectly contribute

to increasing perceptions of satiety by lowering the energy density or amount of energy per unit weight of a food or beverage.[30] Fiber increases mastication; and, it requires time and effort to eat the fiber-containing food. The prolonged presence of food in the oral cavity and increased exposure to sensory receptors allows the induction of signals that suppress appetite.[31, 32] Viscous soluble fibers are associated with beneficial physiologic responses that mediate appetite regulation such as delayed gastric emptying and increased stomach distension. The increased viscosity of intestinal contents prolongs transit time and the absorption rate of nutrients allowing a greater interaction with cells that release hormones associated with stimulating satiety.[33] Lastly, dietary fiber is fermented by gut microbiota to short chain fatty acids which have a beneficial effect on appetite regulation and energy homeostasis acting through several proposed mechanisms.[34]

Oats

Oats have served as a component of the diet for centuries and the cultivation of oats dates back to the Bronze age.[35] Cereal grains are designed to be chemically, physically, and biologically inactive until the appropriate conditions allow the seed to germinate into a new plant. This evolutionary adaptation confers on cereal grains the capacity to accumulate micronutrients and macronutrients important in the human diet.[36] Oats are usually processed as a whole grain, and contain a host of nutrients particularly, thiamin, folic acid, biotin, and pantothenic acid. Oats are also a source of magnesium, manganese, selenium, iron, calcium, zinc, and copper. Additionally, along with barley, oats are the predominant source of the viscous soluble fiber β-glucan, in the diet.[35] The effect of oat β-glucan on satiety has been actively investigated and the results suggest that eating oats has the potential to increase satiety.[37-45]

Dietary Fat

Dietary fats provide nine kilocalories (kcal) per gram as opposed to four kcal per gram provided by carbohydrates and proteins making dietary fat the most energy dense macronutrient. Thus, fat is a concentrated source of energy that can contribute to increased energy intake. In addition to the metabolic need, other factors influencing food intake include the rewarding value of a food and temporal factors such as the time of day. The neural control circuitry and intracellular signaling pathways involved in energy balance comprise numerous nuclei and reciprocal connections among many brain structures that rapidly remedy small variations in the energy status of the body. This tight regulation notwithstanding, the control of energy balance is vulnerable to environmental manipulations especially from foods designed to provoke the senses and cause people to succumb to eating, often in the absence of nutritional need.

Foods high in fat especially those also containing added sugars are generally hard to resist.[46] The non-homeostatic and metabolic consequences of eating foods high in fat contribute to their reward value, and the selection of these foods over other choices must result from an integration of the oral and post-oral signals they provoke.[47] The neural circuits that drive the hedonic control of eating also mediate the rewarding response to drugs of abuse which only serves to highlight how easily an individual may be drawn to over-consume these foods.[48] Nevertheless, the presence of fats in the gastrointestinal (GI) tract reduces the drive for food.[49] Given this paradoxical nature of fat, it is not inconceivable to devise interventions directed at enhancing the effect of fat on the regulation of appetite as a means of helping consumers for whom the rewards of indulging in high energy dense foods are hard to overcome. Delaying fat digestion is important for eliciting the desired signals.

Thylakoids

Thylakoids are the internal membranes in the chloroplasts of green plants that are the location for the light reactions of photosynthesis. Lipids, proteins, and chlorophylls, make up more than 98% of the mass of thylakoids. However, lipids make up approximately 30% of the membrane surface, whereas the vast majority of the thylakoid surface is occupied by protein complexes which comprise approximately 70% of total thylakoid membrane area. Thylakoids inhibit the activity of pancreatic lipase to delay fat digestion.[50-54] The effect has been attributed largely to the protein component but the membrane galactolipids may also have a role.[54, 55] Although resistant to the action of gastric and pancreatic enzymes, thylakoids are ultimately digested.[56] Thus, lipid digestion is delayed, but not inhibited. Therefore, any unpleasant side effects such as oily stools are avoided. Thylakoid supplementation has been shown to increase satiety.[57, 58] A decrease in the urge for rewarding stimuli such as sweet foods and chocolate has also been demonstrated among women.[59, 60]

Justification

Current obesity guidelines suggest that sustained weight loss of just 3% to 5% achieved through lifestyle interventions can result in clinically significant reductions in plasma fasting glucose concentrations, hemoglobin A_{1c}, triglycerides, and the risk of developing type 2 diabetes.[61] The adverse outcomes from the use of pharmacological agents to treat obesity have led to the withdrawal from the market of some of them, such as fenfluramine developed in the 1970's and sibutramine used worldwide from the 1990's.[62] The current pharmacologic options for overweight and obesity approved by the United States Food and Drug Administration for long term use are the pancreatic lipase inhibitor orlistat, the serotonin 2-C receptor agonist lorcarserin, the combination of the sympathomimetic phentermine and the anticonvulsant

topiramate, the combination of naltrexone, an opioid receptor antagonist and bupropion, a catecholamine reuptake inhibitor, and the recently approved glucagon-like peptide-1 analogue, liraglitude 3.0 mg.

Except for orlistat, none of these drugs have long term post-marketing safety and efficacy data as they were all approved between September, 2012 and December, 2014.[63] Orlistat has adverse side effects resulting from anal leakage which makes its use unpleasant. While pharmacological approaches have their place in obesity treatment, it was only post-marketing surveillance that identified the risks involved with the previously approved and subsequently withdrawn drugs; hence, the newly approved drugs have yet to pass this test of long term safety and efficacy.[64] Moreover, the recommendation is that pharmacotherapy should be considered as an adjunct to comprehensive lifestyle management in individuals with a BMI $\geq$ 30 kg/m^2 or BMI $\geq$ 27 kg/m^2 having at least one co-morbidity who are motivated to lose weight.[65]

Bariatric surgery has been shown to be an effective weight loss treatment for the obese. The Roux-en-Y gastric bypass surgery is a procedure that separates the stomach into a small upper pouch, which is anastomosed to a jejunal limb leaving the rest of the stomach attached to the biliary limb into which the bile still drains. The surgery is restrictive yet metabolic and results in a speedy delivery of nutrients to the distal parts of the GI tract.[66] Meal-stimulated increases in peptide YY and glucagon-like peptide 1, gut hormones with anorectic effects have been observed after the Roux-en-Y gastric bypass.[67, 68] These signals act at vagal afferent fibers, which rather than responding to a single modality or peptide, integrate signals from multiple sources. However, bariatric surgery is only approved for individuals with a BMI $\geq$ 40 kg/m^2 or $\geq$ 35 kg/m^2 if they also have a co-morbid condition. Moreover, for the average individual the cost may be prohibitive. Roux-en-Y gastric bypass costs $25,000 to $30,000. The annual health care

cost for a patient with BMI of 35 kg/m^2 is $3,000 to $10,000. Even if health care costs following surgery are reduced by 50%, it could take up to 20 years to achieve cost neutrality. Given the high inpatient costs for surgical patients partly due to high complication rates, bariatric surgery is not cost saving.[69] Therefore, research relating to garnering these signals without the need for surgical rearrangement of the GI tract is far from trivial.

Any obesity treatment involves creating a negative energy balance. Finding the optimal diet composition for achieving a negative energy balance has proven elusive. What appears to be important is not the type of diet, but adherence to a diet regimen that promotes energy restriction.[70] Popular diets such as the Ornish (low fat),[71] Atkins (low carbohydrate without fat restriction),[72] Weight Watchers (restriction of portion sizes and calories),[73] and Zone (modulation of macronutrient composition and glycemic load)[74] do not work for the majority of people who are simply unable to sustain a high dietary adherence level.[75] The negative impact of weight loss on circulating levels of hormones involved in the regulation of body weight may in part explain the reason for non-adherence to calorie restriction over time.[68] Incorporation of whole foods or extracts from foods, to increase satiety and influence reward-induced eating behavior, offers a relatively safe means of overcoming the hurdle of non-adherence to the diet with minimal burden on the consumer. Thus, the use of dietary strategies involving β-glucan from oats or thylakoids from spinach to potentially increase satiety and reduce food cravings through activation of GI mechanisms is a proposition that merits consideration.

Objectives

The objectives of these studies were to:

1. Compare the effect of single servings (150 kcal) of instant and "old fashioned" oatmeal on

subjective ratings of satiety, with an isocaloric serving of the most widely consumed oat-based ready-to-eat cereal (RTEC).

2. Compare the effect of instant oatmeal on subjective ratings of satiety and energy intake, with an isocaloric serving (250 kcal) of the RTEC.
3. Determine the β-glucan content of the oat-based cereals.
4. Examine the physicochemical and rheological properties of the different oat-based cereals.
5. Determine the effect of thylakoid supplementation on subjective ratings of satiety, energy intake, the reward value of food stimuli, concentrations of blood lipids, and the glycemic response.

Research Questions

Study 1: Comparison of Instant Oatmeal, "Old Fashioned" Oatmeal, and a Ready-to-eat cereal

1. Do single servings of instant oatmeal and "old fashioned" oatmeal increase subjective satiety compared to an isocaloric serving of the RTEC?
2. Do the molecular weights and radii of gyration of the β-glucan in instant oatmeal, "old fashioned" oatmeal and the RTEC differ?
3. Do the viscosities generated by isocaloric servings of instant oatmeal (cooked in water), "old fashioned" oatmeal (cooked in water) and the RTEC (with the addition of cold milk) differ?

Study 2: Comparison of Instant Oatmeal and a Ready-to-eat cereal

1. Does a 250 kcal serving of instant oatmeal increase subjective satiety and reduce energy intake compared to an isocaloric serving of the RTEC?
2. Do the molecular weights and radii of gyration of the β-glucan in instant oatmeal and the RTEC differ?

3. Do the viscosities generated by isocaloric servings of instant oatmeal (cooked in water) and the RTEC (with the addition of cold milk) differ?

Study 3: Comparison of a Spinach Extract and a Placebo

1. Does consumption of an extract from spinach rich in thylakoids result in an increase in subjective satiety and a reduction in energy intake compared to a placebo?
2. Does consumption of an extract from spinach rich in thylakoids influence the reward value of food stimuli?
3. Does consumption of an extract from spinach rich in thylakoids affect postprandial blood concentrations of lipids and glucose when compared with a placebo?

Hypotheses

Study 1

1. Null: Single servings (150 kcal) of instant oatmeal and "old fashioned" oatmeal (cooked in water and served with milk) will have the same effect on subjective satiety as an isocaloric serving of the RTEC (with the addition of cold milk and served with water) over a four hour period following consumption, in healthy humans.

   Alternative: Single servings (150 kcal) of instant oatmeal and "old fashioned" oatmeal (cooked in water and served with milk) will have greater effects on subjective satiety than an isocaloric serving of the RTEC (with the addition of cold milk and served with water) over a four hour period following consumption, in healthy humans.
2. Null: The content, molecular weight, and radius of gyration of the β-glucan in instant oatmeal and "old fashioned" oatmeal will be the same as that of the β-glucan in the RTEC, evaluated using in vitro mechanisms.

Alternative: The content, molecular weight, and radius of gyration of the β-glucan in instant oatmeal and "old fashioned" oatmeal will be greater than that of the β-glucan in the RTEC, evaluated using in vitro mechanisms.

3. Null: Instant and "old fashioned" oatmeal (cooked in water) as well as the RTEC (with the addition of cold milk) will have the same viscosity in isocaloric servings, evaluated using an in vitro gastric simulation study.

   Alternative: Instant and "old fashioned" oatmeal (cooked in water) will have greater viscosity than an isocaloric serving of the RTEC (with the addition of cold milk), evaluated using an in vitro gastric simulation study.

Study 2

1. Null: Compared to consumption of a 250 kcal serving of the RTEC (with the addition of cold milk and served with water), consumption of an isocaloric serving of instant oatmeal (cooked in water and served with milk) will induce: 1) the same subjective satiety over the four hour post-prandial period; and 2) the same energy intake at an *ad libitum* meal served four hours after consuming the cereals, in healthy humans.

   Alternative: Compared to consumption of a 250 kcal serving of the RTEC (with the addition of cold milk and served with water), consumption of an isocaloric serving of instant oatmeal (cooked in water and served with milk) will: 1) induce greater subjective satiety evaluated over the four hour post-prandial period; and 2) reduce energy intake at *an ad libitum* meal served four hours after consuming the cereals, in healthy humans.

2. Null: The content, molecular weight, and radius of gyration of the β-glucan in instant oatmeal will be the same as that of the β-glucan in the RTEC, evaluated using in vitro mechanisms.

Alternative: The content, molecular weight, and radius of gyration of the β-glucan in instant oatmeal will be greater than that of the β-glucan in the RTEC, evaluated using in vitro mechanisms.

3. Null: Instant oatmeal (cooked in water) and the RTEC (with the addition of cold milk) will have the same viscosity in isocaloric servings, evaluated using an in vitro gastric simulation study.

   Alternative: Instant oatmeal (cooked in water) will have greater viscosity than an isocaloric serving of the RTEC (with the addition of cold milk), evaluated using an in vitro gastric simulation study.

Study 3

1. Null: Consumption of a spinach extract rich in thylakoids will induce the same subjective satiety compared to the placebo, over the four hour period following consumption, in overweight and obese individuals.

   Alternative: Consumption of a spinach extract rich in thylakoids will induce greater subjective satiety compared to the placebo, over the four hour period following consumption, in overweight and obese individuals.

2. Null: Consumption of a spinach extract rich in thylakoids will have the same reward value relating to food stimuli, and induce the same energy intake compared to the placebo, four hours after consumption, in overweight and obese individuals.

   Alternative: Consumption of a spinach extract rich in thylakoids will result in a decrease in the reward value related to food stimuli and energy intake compared to the placebo, four hours after consumption of the extract, in overweight and obese individuals.

3. Null: In overweight and obese individuals, the change from baseline (fasting) in postprandial blood concentrations of lipids and glucose will be the same with consumption of the spinach extract rich in thylakoids or the placebo.

   Alternative: In overweight and obese individuals, the change from baseline in postprandial blood concentrations of lipids will be lower, and the glycemic response will differ with consumption of the spinach extract rich in thylakoids, compared to the placebo.

Strengths

The strengths of these studies are:

1. The physicochemical properties of the fiber affect the viscosity it generates, which is recognized as promoting physiologic responses that induce satiety; yet, studies that investigate the satiety effects of viscous soluble fibers for the most part do not measure the physicochemical or rheological properties of the fiber. By addressing this issue, the results of Study 1 and Study 2 facilitate the drawing of inferences particularly those related to the mechanisms of action of β-glucan and the effects of processing on the fiber.
2. The studies were adequately powered to detect differences in the primary outcomes using a within-subject, repeated measures design, which is particularly important in the measurement of subjective ratings of satiety, involving introspection by study subjects.
3. Given the dubious accuracy of self-reported intakes, food intake was measured in a laboratory setting under supervision of study staff. The limitations of external validity imposed by laboratory conditions are offset by the limitations of intervening contextual conditions that may influence intake in a real world setting.
4. In vitro data and potential mechanisms were supported by direct (behavioral) evidence.

Limitations

The limitations of these studies are:

1. Adults in different sub-groups may or may not display disparate responses to the interventions. However, the relatively small sample sizes using a crossover design while providing sufficient power to detect differences in the primary outcomes in a diverse sample were insufficient to compare treatments among sub-groups.
2. In the studies investigating the oat-based cereals, oatmeal was compared with a popular oat based cereal; hence, the nutrient compositions of the two products were not matched. However, it is possible that differences in the protein and sugar content although insignificant in their individual effects may have exerted a cumulative effect on satiety.
3. In vitro measurements may not fully reflect the dynamic nature of the GI tract.
4. The evaluation of subjective ratings of satiety was done using visual analog scales which, despite the vagaries surrounding them, are a validated measure sensitive to dietary manipulations; however, the subjective measurements of liking and wanting in response to food reward stimuli may not have had the same statistical validity.
5. Post-prandial measurements of blood glucose and endocrine markers of satiety may have helped to elucidate the mechanisms of action of β-glucan; however, blood concentrations of glucose or hormones were not evaluated in the studies investigating the satiety effects of the oat-based cereals.
6. In the study investigating the effects of the spinach extract on eating behavior, blood concentrations of lipids were measured at a single time-point. Given the kinetics of lipid absorption a single measurement was insufficient to clearly elucidate the mechanism of

action of the thylakoids. Moreover, satiety related hormones and enterostatin concentrations in the blood were not measured.

Journal Articles

The first three articles comprise the literature review and include:

1. The Relevance of Appetite Research: A Review on Satiety and Reward-induced Eating Behavior. This article is under review at Critical Reviews in Food Science and Nutrition.
2. Gut Fat Sensing and Signaling with Special Emphasis on the Effect of Thylakoids on Eating Behavior. This article is published in International Journal of Obesity (Advance online publication: August 25, 2015).
3. Dietary Fiber and Satiety: A Review of the Effects of Oats on Satiety. This article has been accepted for publication by Nutrition Reviews.

The articles relating to the clinical trials include:

1. The Role of Meal Viscosity and Oat β-glucan Characteristics in Human Appetite Control: A Randomized Crossover trial. This article is published in Nutrition Journal (2014;13: 49).
2. Instant Oatmeal Increases Satiety and Reduces Energy Intake Compared to a Ready-to-eat Oat-based Breakfast Cereal: A Randomized Crossover Trial. This article is published in Journal of the American College of Nutrition (Advance online publication: August 14, 2015).
3. Acute Effects of a Spinach Extract Rich in Thylakoids on Satiety: A Randomized Controlled Crossover Trial. This article is published in Journal of the American College of Nutrition (2015:34: 470-477).

CHAPTER 2
THE RELEVANCE OF APPETITE RESEARCH: A REVIEW ON SATIETY AND REWARD-INDUCED EATING BEHAVIOR

Introduction

Worldwide, overweight and obesity defined as body mass index (BMI) ≥ 25 kg/m^2 and 30 kg/m^2 respectively, were estimated to cause 3.4 million deaths in 2010.[76] The global prevalence of overweight and obesity is estimated to have risen by 27.5% among adults and 47.1% among children from 1980 to 2013.[8] Despite evidence for a levelling off in developed countries,[77] the prevalence of overweight and obesity, estimated to be 68.5% in 2011 - 2012, remains high in the United States (US).[78] More than 50% of obese individuals live in ten countries. The US heads this list accounting for 13% of obesity. In 2013, approximately one third of men (31.6%) and women (33.9%) in the US were obese.[8]

Globally, the leading causes of death are cardiovascular disease (CVD), especially, coronary heart disease and stroke.[52] Overweight and obesity are important risk factors of CVD,[61, 79, 80] operating in part through mechanisms such as elevated levels of blood pressure, dyslipidemia, and abnormal glucose metabolism. However, interventions that address these abnormalities resolve approximately half of the excess risk for coronary heart disease that may be attributed to a high BMI.[50] Thus, maintenance of optimum body weight through strategies that can curb or reverse adiposity is of paramount importance in reducing CVD and type 2 diabetes.[50]

Economic growth and technological developments are important for prosperity, but the trade-off to business and trade by way of less regulated global markets has led to the creation of cheap, easily available food sources.[81] Thus, strong economic forces foster reluctance on the part of policy makers to regulate the marketing of foods that do not promote health or reduce the risk

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of disease.[82] However, stemming from major success in tobacco control which included taxation and smoke free policies,[83] there is a particularly resonant call for policy strategies to control and prevent obesity.[84]

While solutions based on governmental policy may have the potential to reverse environmental triggers of energy intake, implementation of the policies are fraught with difficulty. Taxes and subsidies to promote healthy food choices have been proposed and opposed. [85, 86] To maximize outcomes, a sensible food policy that includes taxes and subsidies has to strike a fine balance among issues such as accessibility to subsidized foods, equitability of the tax structure, and the capacity of the policy to satisfy all stakeholders (consumers, farmers, industry, and restauranteurs).[86, 87] Further, the strength of the evidence for policy strategies such as fiscal measures must be substantiated by intervention trials rather than modelling or observational studies.[88] Even so, policy decisions can only be directed at the environment and cannot compel an individual to make healthy choices. The final decision on what and how much to eat rests with the consumer. Unfortunately, in an environment that presents a plethora of inviting food choices the consumer is often prodded to disregard dietary recommendations.[51] For food policies to be effective, strategies that help individuals to control appetite must be considered.

Appetite and Satiety

One definition of appetite refers specifically to the qualitative aspects of eating that can be contrasted with the homeostatic control of eating. However, a more encompassing definition includes the entire field of food intake, selection, motivation, and preference.[89] By this definition, appetite reflects a complex interaction among the external environment, the behavioral profile, and subjective states as well as the storage and metabolism of energy.[90] The expression of

appetite manifests the interplay of events and processes that occur at three levels: i) psychological events such as perceptions of hunger, food cravings, or hedonic sensations, and corresponding behavioral actions; ii) responses in the peripheral physiologic system and metabolic events which stem from nutrient absorption, metabolism, and storage; and iii) central neural processes that translate the physiologic events.[1] Thus, neural activity triggers a behavioral response that is accompanied by a response in the peripheral physiologic system. The physiologic events and processes transduce information to the brain that results in neurochemical activity. The brain activity reflects the strength of the motivation to engage in or refrain from eating.[1]

The hypothalamus is the cerebral appetite center, integrating peripheral humoral signals that transmit information about food intake and energy expenditure with neuronal signals from the brainstem and higher cortical centers.[91] Within the arcuate nucleus of the hypothalamus there are two neuronal populations with opposing effects on food intake. The neurons which co-express neuropeptide Y and agouti-related peptide increase appetite, whereas the neurons that co-express the cocaine-and-amphetamine-regulated transcript (CART) and proopiomelanocortin decrease appetite and food intake.[92, 93] Following the ingestion of food, sensory information is transmitted from the gastrointestinal (GI) tract to the central nervous system either through vagal and somatosensory afferent fibers or via bloodstream signals which may be the gut hormones.[94] The presence of an incomplete blood brain barrier in the regions of the brain such as the area postrema, permits many circulating signals, including the gut hormones, direct access to the central nervous system.[95] Thus, complex neuronal pathways with reciprocal connections between the hypothalamus, brainstem, and higher cortical areas[91] are involved in the control of appetite

acting through endocrine and neuronal feedback signals from the periphery to synchronize appetite perception, food intake behavior, and energy homeostasis (Figure 2.1).

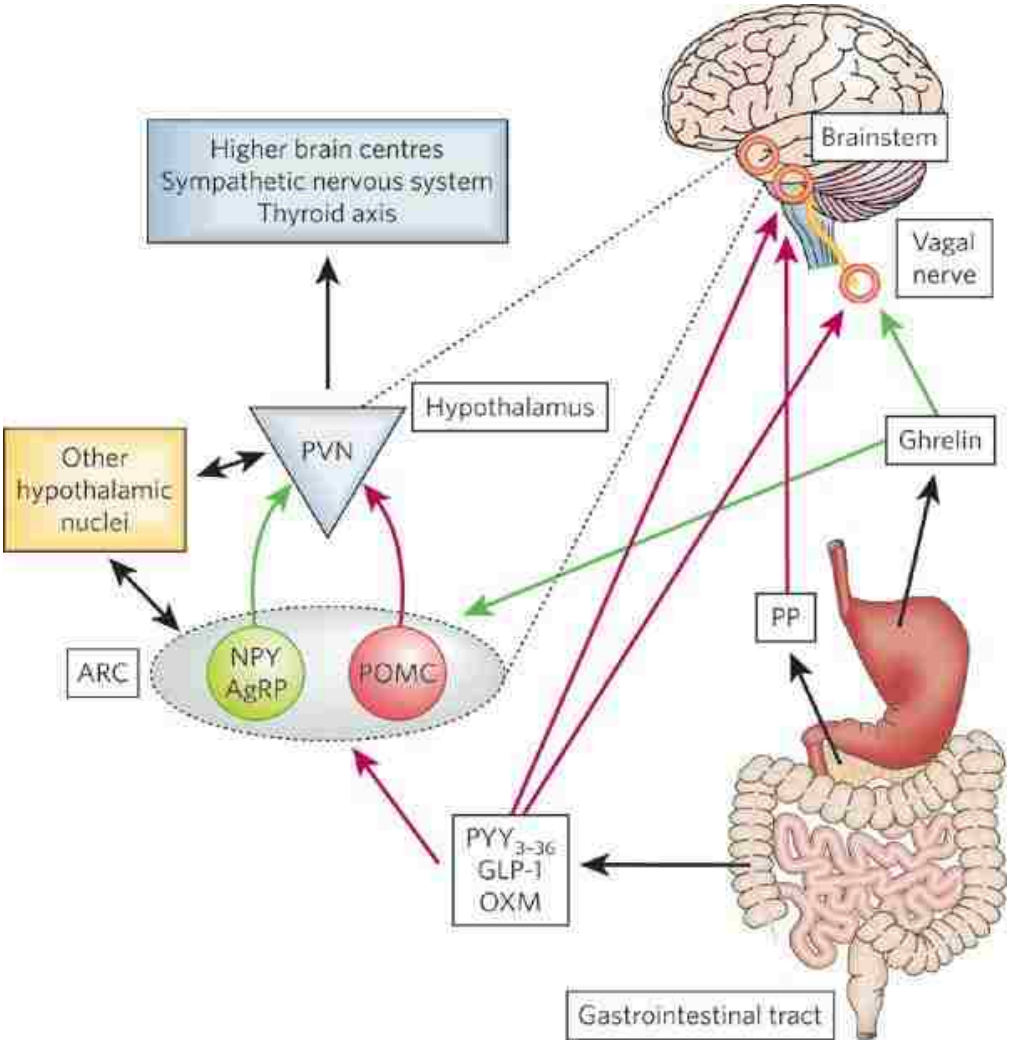

Figure 2.1 Peptide YY ($PYY_{3–36}$), Glucagon-like peptide-1 (GLP-1) and oxyntomodulin (OXM) are released from gut L cells after a meal. They can directly stimulate anorectic pathways in the hypothalamus and brainstem, and may also act through the vagus nerve. Pancreatic polypeptide (PP) is released from the pancreas after a meal and is thought to reduce appetite by directly signaling to neurons in the brainstem. Ghrelin is released from the stomach with fasting and might signal directly to the hypothalamus or through the vagus nerve to stimulate food intake. The arcuate nucleus (ARC) is important in integrating gut hormone energy homeostasis signals. Neuropeptide Y (NPY)/Agouti related protein (AgRP) neurons and proopiomelanocortin (POMC) neurons signal to the paraventricular nucleus (PVN) and other hypothalamic nuclei to increase or decrease appetite, respectively. NPY/AgRP and ghrelin are orexigenic signals and POMC, PYY, GLP-1, OXM, PP are anorectic signals. Reprinted with permission from Macmillan Publishers: Nature, Murphy KG, Bloom SR, 2006;444:854-859.

Energy homeostasis is coordinated by two sets of signals. Long-term or tonic signals emanate from tissue stores, predominantly adipose, and include chemical signals such as leptin, insulin, adiponectin, and certain cytokines. Short-term or episodic signals are generated periodically as food intake occurs and for the most part arise in the GI tract. The chemical components of episodic signals include, cholecystokinin (CKK), peptide YY (PYY), glucagon-like peptide-1 (GLP-1), ghrelin, and oxyntomodulin released from cells in and around the GI tract. Cerebral integration of tonic and episodic signals reflects the current state of energy stores and the flux of nutrients prompted by an eating episode.[96]

Human appetite involves a conscious sensation reflecting an urge to eat that is expressed as hunger. It is the drive or state of motivation to eat that impels an individual to seek out food. Perceptions of hunger are an important component of food choice, timing of meals, and quantity of food eaten.[90] However, humans eat for reasons other than to satisfy hunger, such as sensory hedonics, sensory stimulation, tension reduction, and boredom.[97] Thus, hunger must be considered in the context of environmental, social, and physiologic factors.

When food consumption reduces hunger it involves two processes, satiation and satiety. The complex processes that terminate an eating episode are regarded as satiation. Hunger declines as satiation occurs and is usually lowest at the end of a meal.[90] Satiation determines the size of an eating occasion and is important because it helps to determine meal size.[89] The events following consumption of food that suppress hunger are termed satiety.[90] Thus, satiety is a state in which eating is inhibited and it follows the end of a meal. Satiety occurs over a period of time and is mediated by the sensory, cognitive, post-ingestive (but pre-absorptive), and post-absorptive events and processes that comprise the satiety cascade, proposed as a conceptual

framework for examining how food influences the processes of satiation and satiety [98] (Figure 2.2).

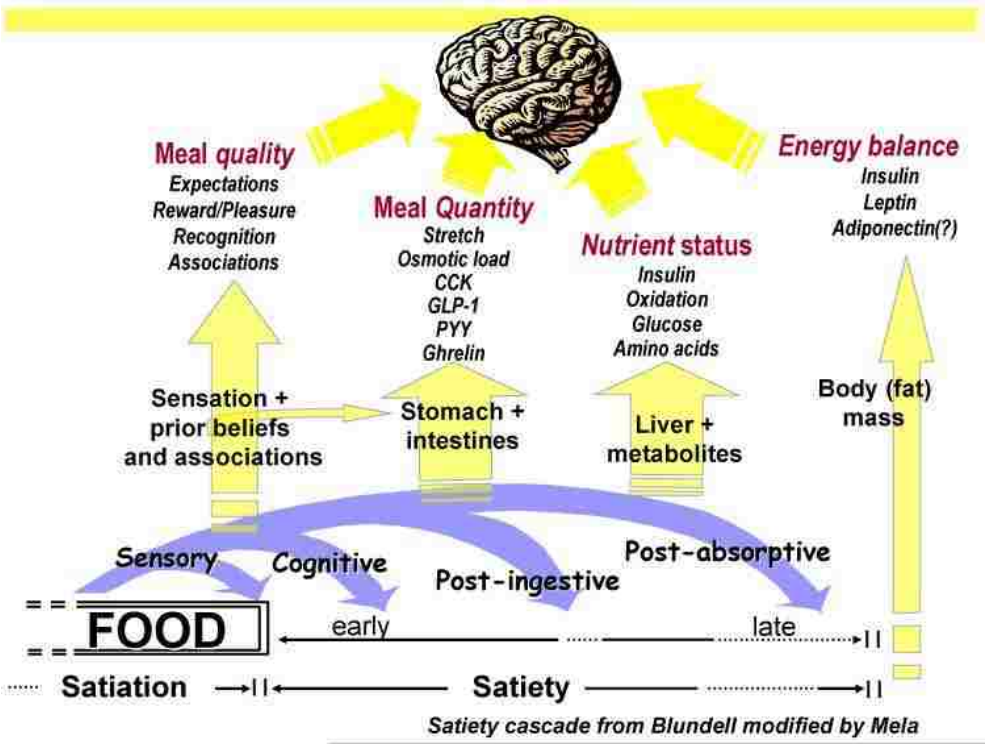

Figure 2.2 The satiety cascade is a conceptual framework that links the motivation to eat to cognitive and physiologic responses. The power of a food to influence eating behavior depends on its mediation of the right balance (one that results in satiation or satiety) between the sensory, cognitive, post-ingestive (but pre-absorptive), and post-absorptive events and processes that comprise the satiety cascade. Reproduced with permission from Wiley Publishers, Blundell J *et al*, Obesity Rev, 2010;11: 251- 270

Physiologic signals are generated by the orosensory aspects of food even before it touches the mouth. These events which constitute the cephalic phase of appetite are a prelude to the eating experience and function to anticipate and prepare the body for the ingestion of food [99]. The major control over appetite occurs during and after ingestion through information from ingested food.[98] Mechano-receptors signaling gastric distension resulting from the presence of food serve to indicate the quantity of food consumed whereas chemo-receptors detect the presence of nutrients and convey this post-ingestive information on the nutritional composition

of the food to the brain. Prandial and post-prandial signals generated by nutrients absorbed from the GI tract into peripheral circulation or metabolized in peripheral tissues and organs acting on the central nervous system, form the post-absorptive satiety signals.[22] The neural and hormonal signals that are transported from the GI tract to the brain mediate satiation and satiety; however, eating behavior is governed by metabolic as well as sensory, cognitive, reward, and emotional aspects.

Sensory specific satiety is a phenomenon that refers to a decline in the pleasure derived from a consuming a particular food in comparison to foods not consumed.[100] The decline in the reward value occurs due to repeated exposure to a particular sensory signal.[89] This decline in pleasure or boredom with a food on repeated exposure occurs when there has been little opportunity for digestion and absorption, and is related primarily to the sensory aspects of the food. Texture, flavor, and color influence the degree of sensory specific satiety.[101, 102] Foods high in protein have been shown to exhibit this effect on satiety.[103-105]

Sensory specific satiety is a key element in the search for variety when making food choices whereas satiety oversees the need to meet the energy needs of the body. The two concepts are closely tied to learning. Sensory signals are linked to metabolic consequences in the brain, from which arises sensory mediated satiety, or learned satiety. The association between the sensory quality of a food and its post-ingestive effects produces a conditioned response that may influence meal size. Thus, the cephalic phase response occurring when food is tasted provides an indication of its satiety value.[89, 106, 107] Cognitive factors such as an estimation of the satiating effect of foods, and the timing of the next meal contribute to making eating largely a learned behavior.[89]

Reward-induced Eating Behavior

Homeostatic control of eating is designed among other things to protect the lower limits of adiposity; but, in the modern world humans often eat in the absence of any metabolic feedback indicating a diminution of reserves.[108] This non-homeostatic or hedonic eating involves cognitive, reward, and emotional aspects. The neurologic pathways that govern this overriding process have yet to be clearly elucidated.[109] To compound the problem, the hedonic circuitry in the brain interacts with food to influence appetite. Evidence points to the existence of hedonic hotspots that magnify the hedonic impact of natural sensory rewards such as sweet tastes.[110]

The psychological components of reward include: i) learning which includes knowledge resulting from associative and cognitive processes; ii) affect (emotion) or liking which reflects the immediate experience or eagerness to experience pleasure from hedonic value of consuming a food; and iii) motivation to actually eat, or wanting.[111] Components of affective and motivational processes can exist objectively, without conscious awareness of them. Therefore, they can be implicit which assumes importance because individuals can react to a rewarding stimulus without conscious awareness of either the stimulus or their hedonic response to it.[111] Understanding the brain systems of food reward has important implications in appetite research.

The pleasure of a sweet taste arises from neural systems overlaying the sensation of sweetness with feelings of pleasure to produce liking. Thus, liking is not merely contained in the intrinsic hedonic sensation, rather it has an associated pleasurable sensation, essentially a hedonic reaction to the pleasure of a reward. Hence, in the absence of brain systems which activate the associated pleasure, a sweet taste may be sweet but not pleasurably sweet.[112] Neurochemical systems such as opioid, endocannabinoid and gamma-aminobutyric acid-benzodiazepine neurotransmitter systems enhance liking reactions when they act on sites

identified as hedonic hotspots. These hotspots are capable of generating increases in liking reactions compared with other areas even in the same structure of the brain.[21]

Wanting is a type of incentive motivation that prompts approach toward and consumption of a reward.[21] It refers to an underlying implicit and objective drive process, called incentive salience, that mediates an intent or desire to consume a food.[113] Incentive salience is attributed to rewards and their predictive cues, which gives them their motivational value.[112] Incentive salience does not require explicit cognitive expectations and is focused on direct reward related stimuli. An unfortunate outcome is that excessive incentive salience can lead to irrational wants for products that are not cognitively wanted.[114, 115] Foods and their cues can precipitate incentive salience driven wanting.[116-118]

Rewards and their predictive cues can powerfully stimulate wanting as may occur when a food is craved simply by imagining the sight, smell, and taste of the food.[116] Dopamine signaling plays an important role in translating motivation into action.[119] Based on research on drug abuse,[120] it appears that reward stimulants engender an imbalance between phasic (action potential dependent dopamine release) and tonic (escape of extracellular dopamine with repeated reward stimulus) neurotransmission. In an effort to correct the disequilibrium the subject increases administration of the drug. Analogous to drug administration, food cravings which are a form of wanting, may lead to seeking out food and initiating intake to correct the imbalance in dopamine signaling.[121] Susceptibility to food cravings appears to distinguish between individuals successful at adhering to dietary energy restriction and those for whom regulating energy intake poses a problem.[122, 123] Thus, targeting food cravings has significant implications for public health initiatives directed at curbing overconsumption.[124]

In most instances, the brain likes the rewards that it wants, but it may be that wanting occurs in the absence of liking. Thus, wanting and liking are dissociable both psychologically and neurobiologically.[21] Liking and wanting are thought to reflect the core processes that can operate implicitly, as well as explicitly in the form of subjectively expressed hedonic feelings arising from the ingestion of a specific food (conscious liking) or intent to consume the food (conscious wanting).[121] Neurochemical wanting mechanisms are more abundant and easily activated than liking mechanisms which may be the basis for being able to want a reward without equally liking the same reward.[112] Imaging studies to link neurochemical events to specific brain activity may not always be feasible. Thus, measuring and differentiating between food wanting and liking in humans may be difficult.[125] Research suggests that liking and wanting can be separately manipulated to produce patterns of eating behavior that are driven purely by affect or by motivation.[121]

The relationship between liking and wanting is illustrated in Figure 2.3. In this model, liking which is the immediate experience or anticipation of pleasure from the orosensory stimulation of the food is an essential component of desire or wanting. Desire is also influenced by external associations and cues such as the situation, which may increase or decrease the motivation to eat. The third element influencing the conscious desire to eat a particular food is the physiologic state such as hunger, thirst, and particular food cravings, which may also reinforce liking. Thus, the relative inputs of each of these drivers modulate the magnitude of desire which in turn determines what is eaten.[126]

Cues that have a reward associated with them, once learned, trigger motivational wanting to secure these rewards.[90] When that cue is consumption of a food and the reward is a predicted level of satiety it can have significant effects on the regulation of food intake and energy balance.

Unlike other dietary measures such as those to regulate blood cholesterol concentrations, the time frame to learn the association between consumption of the food and increase in satiety is short and the effects are easily monitored by the consumer which can greatly facilitate the learned association.[127]

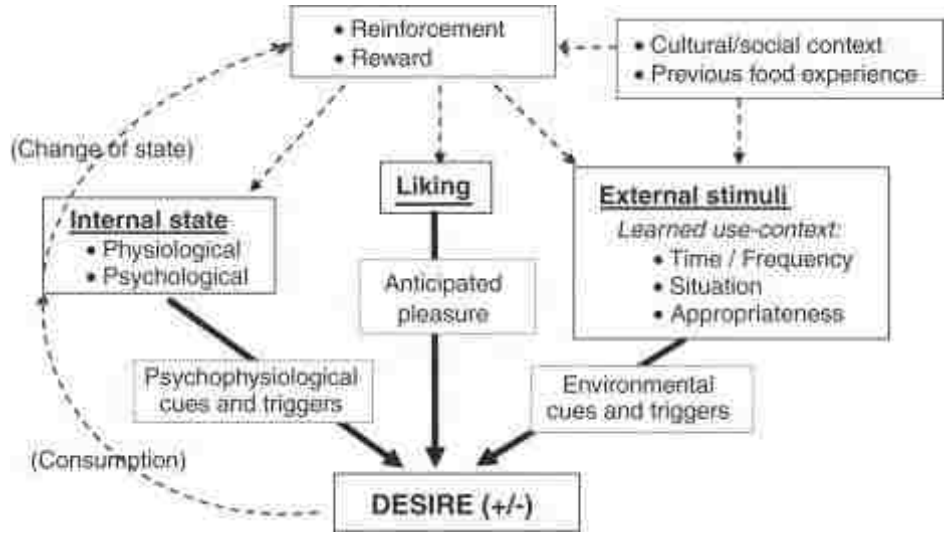

Figure 2.3 Scheme representing the effect of liking (pleasure), the internal state (psychophysiology) and external stimuli (learned cues) on wanting (desire). Solid lines reflect proximate drivers and dashed lines are underlying processes. Reproduced with permission, from Elsevier Publications, Mela DJ, Appetite, 2006;47:10-17.

In the neural control of appetite, the interaction between the reward and homeostatic processes bears consideration. Neural systems, located primarily in the brainstem and hypothalamus represent the homeostatic regulators of food intake. Although not as well characterized as the homeostatic regulators, the neural pathways and functions located in the cortico limbic structures are responsible for the hedonic control of eating.[109] Nevertheless, hypothalamic signals regulating energy intake can influence the activity of the hedonic or reward systems in the cortico-limbic structures.[109] Similarly, signals generated in the cortico-limbic structures processing sensory, cognitive and reward information can modulate hypothalamic processes involved in homeostatic control.[128] Obesity trends suggest that overriding of

homeostatic control by cortico-limbic processes may be the cause of energy imbalance. Although, more evidence on the mechanisms underlying reward processes is needed, a strategy to target these processes offers tremendous opportunity for curbing overindulgence in food.

Measures of Appetite

Short-term regulation of appetite and satiety is often measured using the pre-load test meal paradigm. Foods, the effects of which are under investigation, are presented to subjects. These foods are matched on certain qualities such as energy density,[129] macronutrient composition,[130] and absence or presence of an appetite regulating substance.[131] These qualities may be the overtly or covertly manipulated depending on whether the outcome of the investigation is the determination of the physiologic or cognitive aspects.[132] How these characteristics are manipulated by the experimental conditions is generally dictated by the hypotheses under investigation. After a variable time delay, satiety is evaluated using food intake at a meal. However, self-reported food intakes through dietary records tend to be imprecise and prone to underreporting. Individuals required to maintain a food record tend to reduce their intake; but, they also tend to underreport by approximately 10% to 12%, with females underreporting to a significantly greater extent than males. Age and BMI do not appear to influence food records.[133, 134] The pre-load meal paradigm also permits measurement of the subjective expression of the satiety and the reward components of eating behavior.

Food Intake and Subjective Ratings of Satiety

The onset and termination of meals can be quite variable with myriad factors coming into play and either allowing, disallowing, or compromising the influence of an investigational product on food intake. Food intake could be habitual, occurring at certain times of the day. It could be opportunistic when a desirable food is encountered, or it could be reflexive resulting

from a reduction in energy availability at a critical site in the body, each of which may be modified by learning or experience.[135] Does that mean that measuring food intake as a determinant of appetite control is a futile exercise? Deviations from the ideal are a hazard of any intervention, more so in ingestive behavior. The decision to start or end a meal reflecting the homeostatic control of food intake is the result of adiposity and satiety signals integrated at several levels of the neuroaxis.[135] Compounds tested on the basis of their ability to manipulate these systems may not always be able to do so. The likelihood of failure to demonstrate an effect even if previously demonstrated is inescapable. Further, it is important to realize that a food component evaluated in isolation may not display the same functional properties when administered within a food product or when eaten with foods other than those along with which it was evaluated. These limitations only serve to highlight the complexity of appetite control. To dissect and understand the phenomenon, it is imperative that studies are designed with care and ingenuity within the constraints of pragmatism and financial considerations.

Some factors to consider in the measurement of food intake as a determinant of appetite and satiety are using a control to rule out trivial explanations, selecting an appropriate meal in relation to the time of testing, identifying the customs of eating, and replicating interventions. Imposing a food and alcohol fast, and controlling physical activity prior to testing to ensure compatibility of glycogen stores can help reproduce the results.[89] However, it has been demonstrated that standardizing through imposition of a uniform diet the day before the testing does not alter reproducibility of the experimental meals appreciably.[136] Although employing laboratory conditions make external validity in relation to eating in the usual circumstances circumspect, intervening contextual conditions such as social situations, dietary restraint, and

accessibility to food may influence intake in a real world setting; hence, measuring under controlled conditions serves as a good proxy indicator of appetite.[97]

Subjective measures of appetite are usually assessed before the preload meal and at subsequent time-points over the course of several hours.[89] Subjective ratings usually measure perceptions of hunger, fullness, desire to eat, and the prospect of future consumption, terms relating to the differing aspects of the motivation to eat.[137, 138] These measures include an element of introspection which may not always be amenable to capture, and people may not always eat when hungry or eat beyond the point of satiation.[97] In a study investigating the experience of hunger and satiety conducted among 603 individuals, no clear pattern of traits, sensations, or characteristics emerged as being indicative of hunger.[139] The nature and complexity of eating behavior cannot be overstated, and it may be expected that behavioral responses of individuals will differ not only with other individuals but also within subjects. Nevertheless, the use of within subject designs, where the same subjects participate in more than one experimental condition along with repeated measures help to mitigate these differences in perceptions.[89, 132]

Short term control of eating is influenced by episodic signals that arise largely from the GI tract and are generated periodically as food intake occurs. Long term control of eating, also referred to as tonic signaling, reflects the metabolic state of adipose tissue. Traits are predispositions exhibited by an individual that are relatively stable across days and months but not over extensive periods; whereas, states fluctuate across the course of a day and reflect an episodic or meal-related control of eating. An individual's traits exert a tonic influence over the inclination to eat.[96] Traits are measured using psychometric tests such as the Eating Inventory [140] which assesses dietary restraint or the tendency to deliberately refrain from food intake as a

measure of deliberate control over body weight, disinhibition which reflects the ability to lose control over eating behavior and ingest copious quantities of food, and emotional eating which represents a vulnerability to eat in response to emotional states or external cues. States reflect the drive to eat and are measured using visual analog scale (VAS) ratings of subjective satiety.[137]

Visual analog scales are usually administered using an electronic form. Participants rate each subjective state along a continuous line that is anchored by descriptors reflecting the state as it would be at either end of the spectrum. The advantage of these scales is that they are easy to use, quickly administered, and do not require the subject to formulate an expression of a state allowing for standardization and ease of interpretation. Principal component analysis has been used to show that the ratings of hunger, fullness, desire to eat, prospective intake, and preoccupation with thoughts of food essentially relate to a general motivation to eat and a sense of fullness.[141]

Visual analog scale ratings as a measure of perceptions are not without limitations. Unequivocal demonstration of the validity of a rating scale is not easy to demonstrate because of the inherent difficulty in finding an objective measure of a state with which one may compare the rating system. The power of the scale to predict changes in behavior and the sensitivity of the scale to dietary manipulations can be used as measures of its reliability and have been shown to provide results in the expected direction.[137, 142 143 144, 145] However, subjective perceptions of hunger may not always predict eating behavior. Hunger by virtue of being an innate representation of several factors may reflect changes in the factors other than those being manipulated. It could be the physiologic systems regulating appetite that are manipulated under the experimental conditions, a learned response, or other triggers. Variations in subjectively expressed ratings are greatly influenced by time reflecting a trained response arising from an

established eating pattern.[146, 147] However, most of the variation occurs from inter-subject variation.[141] Therefore, the need to use a within-subjects design cannot be overemphasized.

Under controlled conditions, there is insufficient evidence to suggest that subjective ratings measure something other than what they are purported to measure. Moreover, it is important to emphasize that subjective ratings are not defined by or dependent on their correlation with behavioral measures of food intake.[89] They may not be used to quantify food intake;[146] but, that does not in any way detract from their validity as a reflection of the intensity of a particular appetite sensation. Moreover, subjective sensations usually parallel with the timing and magnitude of physiologic responses such as gastric distension and post-prandial metabolic consequences.[89]

Visual analog scales administered to individual participants under controlled conditions vary in their reproducibility; however, values averaged over a group and comparisons among specific foods can generally be replicated.[137, 141, 146, 148] For instance, subjective hunger tracked hourly correlated with reported intake in the hour in a group ($r = 0.5$, $p < 0.02$), but the correlation was not significant when the analyses were conducted within individuals.[146] For specific foods such as oatmeal, bread, croissants, and yogurt, the mean difference in satiety ratings showed generally consistent findings in two studies.[148, 149] The prediction value of subjective appetite ratings when applied to free living individuals is constrained by external cues and entrained responses; nevertheless subjective ratings appear to be reproducible.[137, 150]

Despite the vagaries surrounding subjective ratings of appetite, the capacity to engage in introspection appears to be sensitive to experimental manipulations and serves to provide a means of discriminating between the satiety values of specific foods. Although valid in itself and not requiring substantiation, a subjective rating serves to provide a more panoramic view of

something as complex as eating behavior when coupled with other measures such as quantitative food intake assessments or physiologic data. Parallel methodologies assessing the mind and other aspects of the body are needed to advance theoretical models that attempt to explain food intake.

Liking and Wanting

The study of food reward in human appetite behavior for the most part approaches reward as a unitary process expressed as a rating of palatability or pleasantness of the food.[113] The assumption is that liking and wanting co-vary and that a change in liking will lead to a proportional change in wanting and vice versa.[121] In one study, three levels of palatable foods were consumed each day for five days for each level of palatable food. After a reduction in the desire to eat the least palatable food relative to the other levels on the first day of exposure, the decline slowly dissipated until all levels of the food had the same rating of desire to eat after a few days. However, the rated pleasantness did not display the same trend.[151] Thus, the sequence of liking leading to wanting and wanting leading to liking may not always hold true and underscores the need to account for a dissociation of liking and wanting in the brain, while evaluating the reward value of food.

Researchers use different methodologies to measure liking and wanting. One study asked participants to rate on a linear scale how pleasant it would be to experience a mouthful of a specific food (photographic food stimuli, varying along the dimensions of high or low fat and sweet or savory taste) to assess food liking. Wanting was assessed through a forced choice method whereby participants repeatedly had to choose between a pair of the same food items (each item from a food category was paired with a stimulus from another food category), the food they would "most want to eat right now." Mean liking scores were used to detect food

liking and mean frequency of choosing from each category was used to assess wanting. Measured before and after consumption of a meal, the researchers found that changes in liking and wanting did not always match.[121] In a subsequent study, the wanting measure was modified to include the reaction time of choices for each food category as a measure of implicit wanting, and explicit wanting was measured by presenting single foods and asking participants to rate "How much do you want some of this food now"? For the most part implicit wanting correlated with the frequency of choice measures used in the earlier study; however, it did not correlate with any of the other explicit ratings, and arguably the dissociation was not as clear as in the previous study. The authors contended that wanting measured through mean frequencies of wanting scores contained elements of both implicit and explicit wanting. The authors did concede that a possible learning occurred to hasten the reaction time when performing the task for a second time.[152]

Using another approach, the dissociation of liking and wanting was evaluated using a progressive ratio computer task whereby subjects were rewarded points that could be exchanged for quantities of a tasty snack food or time that could be spent playing an enjoyable computer game. In a comparison among obese and lean individuals it was found that the subjective ratings of the foods did not differ between the obese and lean participants; however, the obese were willing to work harder for the snack food relative to time allotted for playing the computer game, compared to the lean individuals.[151] In another study, the same investigators assigned individuals to a food deprived or fed state. Participants were four hours post prandial and both groups performed a taste test of the food; however, only participants in the fed group were also given a meal. Participants had to pull a joystick and feedback provided on the computer screen would indicate whether any points had been received which could be traded for a snack. In the food

deprived state, it was found that the participants worked longer for the snack food. Liking ratings for the food did not change between the groups; however, the same foods that comprised the snack were not the same foods whose liking was assessed.[153]

There is some debate as to whether the constructs of liking and wanting can be dissociated in humans. Most of the research relating to the division between food liking and wanting stemmed from animal studies. In rats, treatment with neurotoxin 6-hydroxydopamine left them depleted of dopamine. These rats became aphagic; but, they exhibited a liking for sucrose and a dislike for quinine.[154] An excess of dopamine triggered by amphetamine administration or a genetic mutation increases the motivation to consume sweet food but not the liking for it.[117, 155] In rodent models it is possible to investigate the neural substrates involved in the expression of food likes or dislikes, the disposition to eat, and the separation between the two by imposing specific brain lesions.[125] However, dissecting these food reward components in humans relies for the most part on subjective evaluations.

In a study investigating the dissociation of liking and wanting with sensory specific satiation, using a progressive ratio task, sensory specific satiation for chocolate milk relative to chips was first demonstrated. Half the subjects then had to work for chocolate milk by pressing a response key allocating them points representing a certain amount of chocolate milk. The other half of the participants were assigned the same progressive ratio task but had to work for chips. There was a positive correlation between the liking and wanting measures for chips and chocolate milk.[156] However, using this methodology of the relative reinforcing value of food, some studies have successfully demonstrated the dissociation between liking and wanting.[157-160]

Progress has been made in characterizing the liking and wanting phenotypes. In a study investigating the interaction between relative reinforcement value of food or wanting and the

dopamine genotype in obese and non-obese individuals, previous reports of differences in wanting among obese and non-obese individuals were confirmed. The presence of the *TaqIA* allele interacted with obesity to influence food reinforcement or wanting, and interacted with wanting to influence energy intake whereas liking did not.[158, 161]

The subjectivity of food and liking and wanting assessments notwithstanding, the concept of the parsing of food reward into the components of liking and wanting is not without merit. Neuroimaging using functional magnetic resonance imaging to permit measuring and mapping of brain activity that is specific to food stimuli in the reward circuitry is a possible way to measure motivational signals.[162, 163] The dissociation of liking and wanting emphasizes the importance of controlling the food environment and keeping exposure to triggers of food intake at a minimum in order to prevent excessive consumption when wanting occurs regardless of the perceivable qualities of liking. More importantly, partitioning food reward into its individual components lends credence to the need for developing foods that are perhaps low in energy or contain a component that enhances satiety. These foods are likely to be consumed even if they are not liked as much as they are wanted as opposed to consuming more energy dense foods or less satiating foods.

Hormonal and Biochemical Measures

Enteroendocrine cells are highly specialized cells scattered throughout the GI tract including the stomach, proximal small intestine, distal ileum, and colon [164] These cells secrete a host of peptide hormones which have numerous functions including blood glucose regulation, GI motility and growth, and adipocyte function.[95] The hormones are sensitive to the macronutrient components of the luminal contents. Different transporters such as the sodium-glucose linked transporter-1, taste receptors, and G-protein coupled receptors have been identified in

enteroendocrine cells as regulators that sense specific chemical signals arising from food components to trigger peptide release.[165-167] Short term hunger and satiety is in part mediated by changes in the circulating levels of these hormones.[95]

The role of gastric distension in stimulating satiation has been demonstrated in a series of studies in humans showing that gastric capacity measured by filling a balloon in the stomach correlates with food intake.[168-170] Gastric control of satiety may largely be volumetric mediated by afferents some of which may be mechanoreceptors, while others transduce chemical or other signals.[171] In addition, gut hormones such as GLP-1, and CCK by exerting a measure of control on the influx of nutrients from the stomach into the intestine, delay gastric emptying and increase stomach distension by acting on pyloric pressure and stomach motility.[172, 173]

Research characterizing the complex neuroendocrine interactions that underpin the gut-brain axis to regulate appetite has advanced tremendously.[174, 175] Although the precise physiology relating to appetite regulation is yet to be clearly elucidated, gut hormones undoubtedly play a key role in the regulation of energy homeostasis.[176]

Appetite: Is Evaluating it Right?

It is not uncommon to say "I am full" and yet proceed to consume more food or to express that one is "hungry" when that hunger is not associated with the intense uncomfortable sensations in the abdomen that characterize hunger pangs. Qualitative research suggests that consumers have varied views of what constitutes hunger and fullness, and these views are not detailed enough to capture the psychological and physical components.[177] In using an introspective approach to evaluating mental processes words are used to convey the desire for food which in reality may be merely getting food at a time when one is habituated to eating in the belief that the body needs a source of energy. Moreover, the amount a person may eat or

drink is the result of a string of instances of fleeting personal experiences being integrated, which are liable to change with each mouthful. Therefore, an appetite rating measures a biological or social influence that is operative at the moment that the wanting for food is expressed whether as a feeling of hunger, desire to eat, or lack of fullness. The dynamics that cause a particular state reflecting appetite are unique to each food and the stage in the meal, all of which are highly complex and perhaps under-investigated. Hence, it is illogical to think that a psychological phenomenon such as experiencing epigastric pangs from lack of food can be explained by the chemistry of a blood sample. These issues relating to appetite research have been the subject of much debate.[178-182]

While there may be some truth in the criticisms levelled against the concept of satiety and the evaluation of satiety, a whole field of research cannot be peremptorily dismissed as being based on a scientific premise that is either erroneous or completely lacking a sound foundation. This is especially so, when the data are substantiated using the scientific method, as is evident in the vast amount of literature disseminating the advances made in the field of appetite research.

Post-prandial hunger and fullness ratings do not always produce reductions in energy intake nor do they predict future weight loss which is clearly dependent on a wider dietary context. A single food or food component may have remarkable effects on satiety when eaten in isolation or when delivered in a particular food matrix.[38, 39] A satiety response depends on individual responsiveness to physiologic signals that are competing with environmental cues to eat. Further, it may not always be possible for an individual to incorporate the food into a customary dietary pattern. Thus, the satiety enhancing food may deliver the purported benefits within an explicit behavioral or usage context.[179] These considerations notwithstanding, in a review that included nearly 80 studies, ratings of satiety and energy intakes were correlated, as

were the effects of foods on satiety hormones and subjective satiety ratings.[97] Thus, satiety ratings are stand-alone methods of evaluating appetite and may not need to be substantiated by physiologic or food intake data. It is important to note that biomarkers of satiety cannot be quantitatively related to satiety ratings analyzed using a scale such as the VAS. However, patterns in these markers could explain the kinetics relating to the presence of satiety in the post-prandial period, or assess the mechanisms believed to be mediating the satiety effects.[183]

Assuming that an increase in satiety demonstrated in a single instance of acute dosing of a food will occur on repeated exposure, or that a demonstrated effect on satiety will translate into weight loss over the long-term is clearly untenable. For a satiety claim to be valid it must deliver the enhanced satiety in comparison to a control product on repeated exposure. Whether consumption of a single product will lead to sustained satiety and weight loss would need to be examined through a multi-step proof of concept. Nevertheless, quantitative research suggests that consumers are fairly discerning in that they understand that personal efforts are required if they are to reap any benefits from the satiety attributes of a product.[184] Learned satiety suggests that associations between the sensory properties of the food and the post-ingestive effects result in an acquired control of meal size.[185]

Even if the product does not have enduring benefits in relation to promoting satiety, the advantages accruing to a consumer in the short-term cannot be dismissed lightly. Compliance with energy restriction or specific diet plans, and perhaps making the weight loss experience more pleasant than it would otherwise be are likely outcomes that can contribute to success in meeting behavioral goals such as managing weight or inculcating eating habits that promote health. Assessments of the motivation to eat provide valuable insights that aid development of interventions targeting eating behavior that alters energy balance in the desired direction.

Conclusions

Visual analog scales are a valid and reproducible method of evaluating subjective ratings of satiety and can only enrich the results obtained through biochemical and food intake measures. Functional magnetic resonance imaging can examine the neural circuitry involved in food craving that makes food especially appetitive, and thereby drives liking and wanting. If wanting can occur in the absence of liking, parsing of reward into its components could provide the impetus for the development of foods and an environment that are more conducive to being lean. Dissecting the complexities of eating behavior in humans is no mean task, and attaining some understanding of its various dimensions can only occur if research is conducted to validate theoretical models.

CHAPTER 3
DIETARY FIBER AND SATIETY: A REVIEW ON THE EFFECTS OF OATS ON SATIETY

Introduction

The prevalence of overweight and obesity have been increasing globally over the past several years. Worldwide, the prevalence of overweight and obesity rose by 27.5% among adults and 41.5% among children, between 1980 and 2013 with a reversal of these trends far from been achieved by any country; although, some regions have achieved a stabilization of the average body mass index.[8] Obesity does not discriminate. It is evident in countries with high as well as low income levels and across all strata of society.[186] Progress in the reduction of obesity can only be described as abysmal.

The chronic nature of obesity and its related diseases makes a case for comprehensive management approaches to achieve and maintain weight loss.[187] There is some debate as to who should be responsible for taking action. Advocates of the "hard approach" envision a strong role for society involving government regulatory and fiscal interventions. A "soft approach" involves education and voluntary action undertaken by industry.[11] The multidimensional nature of obesity[186] makes the solutions to prevent obesity complex. Undoubtedly, the food environment interacts with personal vulnerabilities to foster a situation that promotes overconsumption. Little is achieved by laying the blame or the responsibility on either the individual or the environment. Individuals may be susceptible to the allures of the environment; but, they still have to make their own food choices. Hence, there will always be an element of personal responsibility.[188]

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There are important physiologic barriers to losing excess weight, once gained. Weight loss induces neuroendocrine changes that synchronize appetite perception, food intake behavior, and energy homeostasis. These changes make both weight loss and maintenance incredibly difficult.[189] The strong biological resistance to weight loss and predisposition to weight regain prompts a vicious cycle of failed attempts and personal misgivings.[188] Counter-regulatory adaptations that occur in response to energy deprivation include an increase in the drive to eat.[190] Controlling appetite in order to adhere to dietary recommendations is for most individuals a daunting task, especially, in an environment rife with enticing food choices.[51]

Human appetite is controlled by central and peripheral mechanisms that interact with the environment. The nutrient composition of foods is especially important. Foods varying in their nutrient content engage differently with the mediating processes to exert different physiologic effects. Some of these effects are signals that induce satiety, which is the inhibition of hunger after a meal is eaten.[90] Foods that increase satiety such as fiber-rich foods have been an area of active investigation.[37, 191-195] The results have been promising and offer an avenue by which the scientific community and industry can together work towards reversing the obesity trends and countering the impending adverse health effects.

The 2010 Dietary Guidelines for Americans[196] recommend that whole grains comprise at least half of the six to eleven daily servings (one ounce equivalent per serving) of grains, to reduce the risk of chronic diseases such as obesity, type 2 diabetes, and cardiovascular disease. Whole grains contain a host of nutrients, most notably, omega-3 fatty acids, dietary fiber, minerals (magnesium, iron, zinc, manganese, copper, selenium, phosphorus, calcium, sodium, and potassium), vitamins (vitamin E, thiamin, niacin, pantothenic acid, biotin, pyridoxine, and folate), and phytochemicals.[197] The precise nature of the physiologic effects of dietary fiber is

not well understood mostly because whole grains are abundant in many bioactive components.[198] Nevertheless, there is evidence to suggest that the dietary fiber component of whole grains may mediate the effects of whole grains on chronic diseases such as obesity, type 2 diabetes, and cardiovascular disease.[199-201]

Dietary fiber which for the most part consists of carbohydrate polymers that are undigested by human enzymes, has never been formally proposed as an essential component of the diet.[202] However, the scientific report of the 2015 Dietary Guidelines Advisory Committee[203] recognizes the potential role of dietary fiber in preventing coronary heart disease, colorectal and other cancers, type 2 diabetes, and obesity. Biomarkers for fiber intake are singularly lacking; hence, based on very low consumption across all sectors of the population in the United States (US), dietary fiber is designated as a nutrient of public health concern.

Advocates of whole foods argue that the relationship between diet and disease cannot be clearly identified from the effects of individual nutrients.[204] Thus, isolating dietary fiber from the overall field of nutrition from foods of plant origins is suggestive of assigning preeminence to one component.[202] Even the 2015 Dietary Guidelines Advisory Committee scientific report recommends consumption of high-fiber cereals, whole grains, fruits, and vegetables to meet the dietary fiber advice.[203] However, there is evidence to support a role for dietary fiber delivered in supplements or added to foods, in weight loss and cardiovascular disease.[200, 205] Thus, whole foods or fiber-enriched foods each have a place in the diet. It is important to recognize that while there may be a synergy among the bioactive components of a whole grain, there is no value in ignoring the potential contribution of foods that contain added fiber.

Oats are usually processed as a whole grain and are particularly high in a type of dietary fiber called β-glucan.[36] There is evidence to suggest that oat β-glucan reduces low density

lipoprotein cholesterol.[206-208] The United States Food and Drug Administration (FDA) allows a health claim for an association between consumption of rolled oats, oat bran, whole oat flour, oatrim and reduced risk of coronary heart disease.[209] There is growing evidence to suggest that oat products reduce the human glycemic response, compared to similar wheat foods or a glucose control.[210] This review will discuss the effects of dietary fiber on the regulation of energy balance and explore the effects of oats and oat β-glucan on appetite control.

Dietary Fiber

In human nutrition, the term dietary fiber was first described by Hipsley in the 1950's as the non-digestible components of the plant cell wall.[211] The properties of dietary fiber, such as its chemical composition, physiologic functions, and the food matrix in which it is delivered can be very diverse with different types of fibers sharing some, all, or none of these characteristics.[23] Varied definitions of dietary fiber have been proposed in an attempt to capture its multifaceted nature.

The American Association of Cereal Chemists International defines dietary fiber as "the edible part of plants and analogous carbohydrates that is resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances." The definition also acknowledges that dietary fiber promotes beneficial physiologic effects.[212] The Institute of Medicine (IOM) defines dietary fiber as consisting of non-digestible carbohydrates, and lignin that are intrinsic and intact in plants; and, it is distinguished from functional fiber which consists of isolated, non-digestible carbohydrate with beneficial physiologic effects in humans. The sum of dietary fiber and functional fiber is total fiber.[213] Thus, the IOM reserves the

term ‘dietary fiber’ only for materials that are intrinsic and intact or inherent within food as opposed to extracted, modified or synthesized fibers which are termed functional fibers.[23]

The term used in labeling and nutrient databases is ‘dietary fiber’ which includes fiber from foods and fiber added to the food which cannot be distinguished analytically when the food inherently contains the fiber that is added, thereby introducing an element of ambiguity. However, the proposed amendments to nutrition labeling regulations recognize these analytical limitations. A single definition for dietary fiber that is equivalent to the IOM’s definition for total fiber including carbohydrates of three or more monomeric units, rather than a separation of the definition into dietary fiber and functional fiber has been proposed. The isolated and synthetic non-digestible carbohydrates would qualify as dietary fiber only pursuant to the FDA’s approval of a citizen’s petition or health claim petition providing evidence of a physiologic effect beneficial to human health. Under the proposed provisions, β-glucan soluble fiber added to foods meets the definition of added fiber.[214]

Non starch polysaccharides (NSP) are complex polysaccharides other than starch which are comprised of several thousand monosaccharide units joined through glycosidic linkages.[215] The NSP definition essentially includes plant cell wall components and excludes synthetic resistant carbohydrate polymers or those extracted from foods by physical, enzymatic, or chemical means.[23] This definition excludes resistant starch which is starch or starch degradation products that are indigestible by enzymes in the small intestine.[216] Moreover, the NSP definition excludes the term dietary fiber altogether.[23]

The Codex Alimentarius definition includes dietary fibers that are intrinsic and intact, extracted from food, and synthesized or modified. The Codex definition captures the essence of the American Association of Cereal Chemists International and IOM definitions and further

stipulates that isolated or synthetic fibers must show a proven physiologic benefit to health. This definition attempts to harmonize the definition of dietary fiber among countries. However, the Codex Alimentarius places the decision on whether to include polymers of three to nine monomeric units in the definition of dietary fiber, on national authorities.[23, 217] Thus, the efforts to arrive at a definition that has international unanimity may miss the mark when countries do not accept short chain oligomers as dietary fiber.

Whether defined using the term dietary fiber or NSP, all of the definitions characterize dietary fiber as carbohydrate polymers or oligomers that escape digestion in the small intestine and are partially or fully fermented on reaching the large intestine.[23] The origin of the fiber as inherent in a food or added to the food does not change the way it is metabolized in the body; although, it has been argued that fiber in its original matrix (such as the NSP) have other nutrients attached which may influence its effects.[216] Nevertheless, most definitions include non-digested carbohydrate components when they are extracted from foods, synthesized, or modified if they exhibit a beneficial physiologic effect. The Codex definition including carbohydrate polymers of three to nine monomeric units appears to encompass the nuances of accepted definitions and will define dietary fiber in this review.

Physicochemical Properties

The physical and chemical properties of dietary fiber such as hydration, solubility, viscosity, and adsorption to organic molecules determine its physiologic effects. Polysaccharide networks are formed by an ordered packing of chain segments as in insoluble fibers. However, hydration and swelling are promoted by interconnecting sequences that are disordered as found in solution. The non-covalent bonds stabilizing these ordered junctions are individually weak and therefore the junctions are stable only above a certain critical minimum length. The length

requirement for ordered packing makes the network properties of specific polysaccharides highly dependent on the spacing of minor structural irregularities. The formation and disruption of the junctions can occur in response to relatively small changes in factors such as the temperature, pH, ionic environment, or Maillard reaction products formed during processing or cooking.[218] Processes such as grinding, drying, heating, or extrusion cooking that modify the physical properties of the fiber affect the hydration properties. The physicochemical properties of the matrix in which the fiber is delivered as well as the gut environment play a role in determining the hydration or swelling and water retention capacity of the fiber.[219]

The relative stability of the ordered and disordered forms of the polysaccharide networks determines the solubility of a polysaccharide. If the structure is such that the molecules fit together in a crystalline array as would occur in a linear structure such as cellulose, the polymer would be more energetically stable in a solid state than in solution. Polysaccharides with structural irregularities such as β-glucan would tend to be soluble. Some fibers that are insoluble in cold water will dissolve readily in hot water which promotes conversion to the disordered form.[219]

Viscosity of a fluid is described as resistance to flow.[219] Although the terms viscosity and gelling are often used interchangeably, their properties differ. A gel does not flow but it stretches elastically or breaks under a force.[220] When soluble polysaccharides are present in the digesta as disordered coils they confer viscosity by interpenetration of individual polymer chains to form an entangled network. The viscosity generated depends upon the number of coils present and their size. Viscosity will only occur at or above a critical polymer concentration.[221] Therefore, increasing the concentration or molecular weight will increase the viscosity. However, the structure and solubility also influence the viscosity.[215] Lowering the moisture

content and increasing the particle size have been shown to increase viscosity.[222] The food matrix in which the fiber is delivered[223] as well as the processing conditions to which the food has been subjected influence the viscosity generated by the fiber.[224]

Coil volume and hence viscosity may also be altered by other constituents in the digesta and the secretion or absorption of aqueous fluids along the gastrointestinal (GI) tract. For instance, the hydrodynamic volume of charged polysaccharides is reduced by salts which allow the coils to contract to a more compact form through reduced electrostatic repulsions. Further, the concentration in the lumen of the gut may be different from that ingested as the gut adapts to ingestion of a viscous solution, or as depolymerization of the polysaccharides occurs during transit in the GI tract.[219] Thus, measuring viscosity in vitro may not be fully indicative of true physiologic effects.[218] However, measuring viscosity generated by dietary fiber in vivo also has some limitations mostly due to the practical difficulties in obtaining access to the GI tract in humans and taking accurate and reproducible measurements of viscosity.[221]

Physiologic Effects

The primary physiologic effects of dietary fiber in the small intestine are: i) reducing the rate or extent of absorption of nutrients which is mediated by dietary fiber in part by physically trapping nutrients, and ii) increasing the viscosity of luminal contents to deter the transport of enzymes to their substrates, bile salts to fat for emulsification, and nutrients to the gut wall.[218] Intestinal nutrient contents are brought into contact with the intestinal mucosa by contractions creating turbulence that allows digesta from the center of the lumen to be transported to the vicinity of the epithelium. Diffusion across the thin unstirred layer of fluid close to the epithelium is then necessary for absorption to occur. This peristaltic mixing process is hampered with increased viscosity.[218]

At high polymer concentrations, dissolved polysaccharides present a physical obstacle to the diffusion of small molecules across the unstirred water layer.[218, 225] As the particle size of the grain is reduced, through cracking or milling the rate of digestion increases as the surface-to-volume ratio of the grain increases allowing greater access to enzymes. However, while this may occur with wheat and corn which contain larger proportions of insoluble fiber, increased digestion with reduced particle size occurs with oats in vitro but not in vivo because of the viscosity generated by its soluble fiber content which restricts digestive enzymes from coming into contact with their substrates.[226]

A vast and diverse microbial community inhabits the human GI tract with the greatest number in the distal gut. The constituency of the gut microbiota is determined by the host phylogeny and diet.[227] The genomes of these indigenous microbial communities, termed microbiome, encode myriad gene products providing a diverse range of biochemical and metabolic functions that humans have not had to evolve fully on their own,[227] including the processing of otherwise indigestible components of the diet such as plant polysaccharides.[228]

In response to changes in the diet, there are dramatic and rapid alterations in the cellular composition as well as the gene transcription network of microbiota.[229] Oat β-glucan supplementation for five weeks has been shown to promote the proliferation of bacterial species such as Bifidobacteria in healthy humans.[230] These bacteria are recognized as being associated with a beneficial impact on the host through their potential involvement in diabetes-related inflammation and the development of obesity.[231] In the colon, dietary fiber may be fermented by gut microbes to short chain fatty acids, namely butyrate, propionate, and acetate which activate the enteroendocrine cells of the gut to secrete a host of metabolically active peptides involved in food intake, lipid storage, and energy homeostasis.[232] However, as previously reviewed, fructan-

type oligosaccharides, lactulose, and galactooligosaccharides dominate prebiotic research in humans. In vitro studies provide the majority of the data to support the prebiotic potential of oat β-glucan, and more evidence is needed before a prebiotic effect may be attributed to oat β-glucan.[233]

Dietary fiber influences bowel function by increasing fecal volume and weight, to improve stool consistency and frequency which prevents constipation. This bulking effect is largely due to the non-fermentable fiber but fermentable fiber can also contribute by increasing bacterial mass, thereby increasing stool weight to promote laxation.[234] Soluble fibers are for the most part completely fermented by colonic bacteria and have a higher viscosity than insoluble fibers. However, not all soluble fibers are viscous and some insoluble fibers may be fermented.

Effects on Appetite Regulation

Appetite reflects a complex interaction among the external environment, the behavioral profile, and subjective states as well as the storage and metabolism of energy.[90] The entire field of food intake including its selection, motivation, and preference are encompassed in the broad definition of appetite.[89] When food intake reduces hunger and inhibits further intake, two processes are involved, namely satiation and satiety. Satiation develops during the course of eating and eventually causes meal termination; whereas, satiety is the state in which further eating is inhibited and is preceded by an eating episode. Thus, satiety is not an instantaneous process but it occurs over a period of time. Satiation and satiety are mediated by sensory, cognitive, post-ingestive, and post-absorptive processes.[90]

The mastication of foods high in dietary fiber requires time and effort prolonging the oral exposure and allowing time for signals mediating satiety sensations.[235] The duration of oral exposure has an important role in reducing energy intake and may be comparable with signals of

gastric filling which have also been shown to promote a feeling of fullness.[236, 237] Approximately 20 g of NSPs and other carbohydrates are fermented in the human colon each day to produce approximately 200 mmol of short-chain fatty acids. Only 7-20 mmol of these fatty acids are excreted in feces. Therefore, it is estimated that fermentable fibers provide approximately 1-2 kcal/g which lowers the energy density or amount of energy per unit weight of a food or beverage.[218] Thus, dietary fiber increases the volume of foods while lowering the metabolizable energy.[238] Increasing the energy density has a positive impact on the rate of gastric emptying in humans and diets high in fiber appear to have a consistent effect on slowing gastric emptying.[239]

Using magnetic resonance imaging it has been shown that appetite decreases with increased viscosity of locust bean gum solutions, which may be related to the increase in gastric volumes and decrease in gastric emptying. Although there was substantial dilution of viscosity possibly due to salivary and gastric secretions, thereby minimizing differences in gastric emptying between meals of varying doses of locust bean gum, the initial meal viscosity significantly influenced satiety.[240] The addition of nutrients to the locust bean gum solution resulted in an additive effect in delaying gastric emptying and increasing satiety sensations.[241] Magnetic resonance imaging also showed that guar gum added to a milk-based beverage increased viscosity compared to similar beverage without the fiber, to produce greater satiety.[242]

The increased viscosity of intestinal contents prolongs transit time and the absorption rate of nutrients. The prolonged presence of nutrients in the GI tract raises the possibility of interaction between nutrients and the intestinal mucosa to stimulate the release of peptides involved in appetite regulation[33] (Table 3.1).[243] Additionally, short chain fatty acids produced from colonic fermentation of non-digestible carbohydrates activate G-protein coupled receptors which are present in the colon.[244] It is postulated that short chain fatty acids may mediate satiety

through activation of these receptors to modulate the release of peptides involved in appetite regulation.[245, 246] In mice, acetate has been shown to cross the blood brain barrier and suppress appetite through hypothalamic mechanisms.[247]

Table 3.1. Major gut hormones: sites of synthesis and mechanism of action relating to appetite.

Hormone	Primary Sites of Synthesis	Major Effects on Appetite
CCK	I-cells of the duodenum, jejunum; widespread CNS expression	Slows gastric emptying and reduces food intake
Ghrelin	A-cells of gastric fundus; small and large intestine; hypothalamic nuclei	Promotes gastric motility and increases food intake
GLP-1	L-cells of distal small and large intestine; hypothalamus, dorsovagal complex, pituitary	Inhibits gastric emptying and reduces food intake
PYY_{3-36}	L-cells of distal small and large intestine; hypothalamus, medulla, pons	Reduces gut motility and reduces food intake

CCK = Cholecystokinin, GLP-1 = Glucagon-like peptide-1, PYY = Peptide YY
Adapted from Chaudhri et al, Philos Trans R Soc Lond B Biol Sci, 2006; 361:1187-209

The intake of whole-grains and dietary fiber in the diets of Americans falls dismally short of the recommendation. Based on the National Health and Nutrition Examination Survey (NHANES) data from 2001 to 2010, the average whole grain intake among adults in the United States is approximately 0.61 to 0.86 ounce equivalents/day which is not even close to the recommended three to six ounce equivalents.[248] The average dietary fiber intake is approximately 16.1 g/day, far short of the 25-38 g/day recommended by the IOM.[196, 248] Consumption of whole grains and cereal fiber-rich foods such as bran is a good way to increase the intake of dietary fiber.[249]

Oats

Whole oats have a hard outer hull. The hulls of cereal grains are designed to protect the seed from harsh environments and can pass through the digestive system with little or no digestion.[36] The hull must be removed to obtain maximum nutritional benefits. Hulled oats

known as oat groats have three fractions: the bran, the starchy endosperm, and the germ. The outer layers of the groats form the bran and typically include the pericarp, the testa or seed coat, the nucellus, the aleurone layer, and a large portion of the subaleurone layer of the starchy endosperm[250] (Figure 3.1).[250]

The aleurone cell wall contains some β-glucan, but the amount is small compared with the underlying starchy endosperm which is the primary storage site of starch, protein, lipid and β-glucan. As with most cereals, starch is the main component of the groats. The germ contains high levels of proteins and lipids but very little starch.[250] Oat is usually processed as a whole grain because its groats are softer than other grains such as wheat, and therefore cannot be easily separated into the germ, endosperm, and bran fractions. Milling is designed to remove foreign materials, isolate and stabilize the groats and convert them into a form suitable for cooking.[36] This involves cleaning, dehulling, and kilning (heat denaturing of lipase and lipoxygenase released during milling). After milling the oats may be cut, flaked, or ground to produce steel-cut oats, oat flakes, oat flour, and oat bran.[36]

Steel-cut or pinhead oats are made by passing the groats through steel cutters which cut each groat two to four times. Rolled oats are made by steaming the groats and then flattening them into oat flakes using rollers. Flake thickness can be controlled and in general quick-cooking oats are rolled thinner than whole-oat flakes.[36] Instant oats are prepared in a similar way to quick-cooking oats; however, they are steamed for a longer period and rolled more thinly.[251] Oat bran, the coarse fraction of oat flour consists of the outer aleurone and subaleurone layers of the groats and is higher in fiber than the fine fraction of oat flour.[36] The hull contains insoluble fiber which is commonly called oat fiber in some countries including the US as opposed to oat hull fiber in other countries, and may cause some confusion.[252] The fiber from the hulls if finely

ground has applications in animal feed, some human food ingredients, and as biomass for power plants.[36]

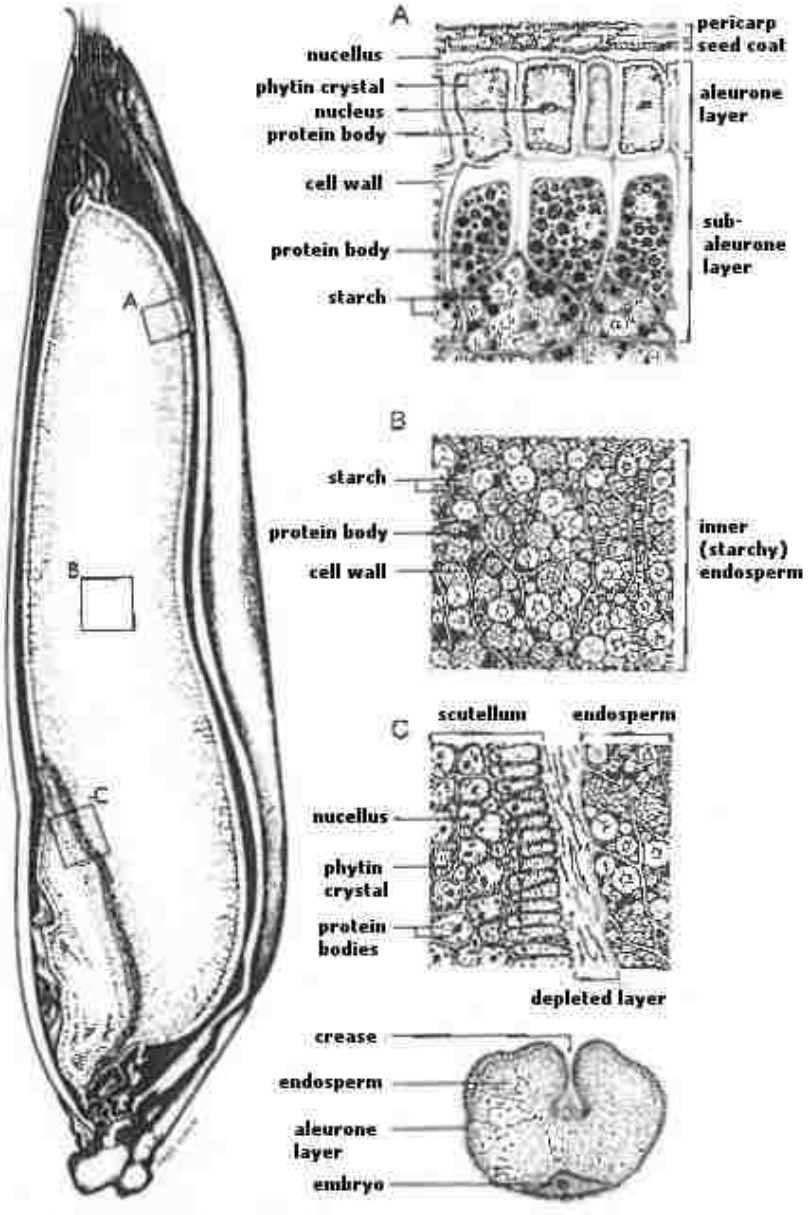

Figure 3.1 Diagram of the oat caryopsis (with the hull) that has been split longitudinally to display the major fractions of the groats: a) bran which includes the pericarp, seed coat, nucellus, aleurone layer, and a large portion of the subaleurone layer of the starchy endosperm ; b) starchy endosperm; c) germ-endosperm interface. At lower right is a cross section of the groat taken at the level of box c. Reproduced, by permission, from Miller SS, Fulcher RG. Microstructure and Chemistry of the oat kernel. In:Webster FH, and Wood PJ, ed. Oats: Chemistry and Technology, 2nd ed. AACC International: St. Paul, MN; 2011:77-94.

Oat β-glucan

Oat β-glucans are linear polysaccharides which can be viewed as a cellulose chain. Approximately 70% are 4-O-linked units interrupted by 3-O-linked β-D glucopyranosyl units. The (1→3) linkages occur singly leading to a structure of predominantly β (1→3)-linked cellotriosyl and cellotetraosyl units [220] (Figure 3.2). Structure affects the water solubility of β-glucan. Soluble β-glucans have a greater ratio of (1→4) linkages and cellotriosyl units than the insoluble form.[253]

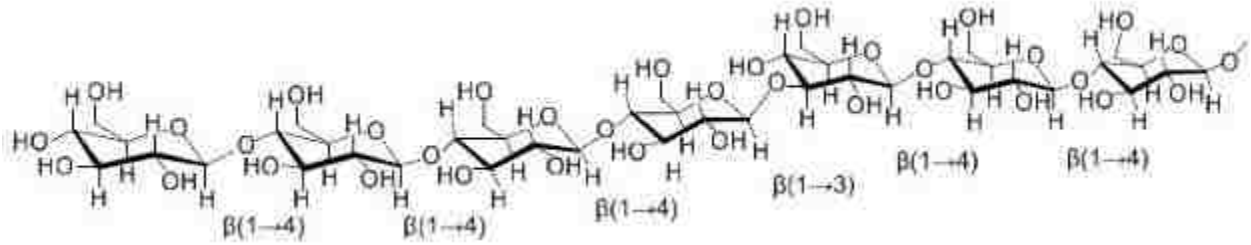

Figure 3.2 Oat β-glucan, a linear polysaccharide consisting of 4-O-linked units interrupted by 3-O-linked β-D glucopyranosyl units.

Oats typically contain 3-5% (dry weight basis) of β-glucan.[254] Oat β-glucan has a native chain length of approximately 20,000 glucosidyl units and it has molecular weights up to 3 million Daltons.[224, 255] A considerable range in the average or peak molecular weight of cereal β-glucans has been reported in the literature. The average molecular weight of oat β-glucan is above 1 million g/mol and probably in the range of 2 million g/mol.[220] However, the chain of glucopyranosyl units is easily disrupted by enzymatic or chemical hydrolysis, by mechanical shear or by heat treatments.[224] Thus, the molecular weights in commercial food products range from 0.4 to 2 million Daltons.[255]

The solubility of β-glucan is influenced by the structure of the polymer and by the properties of the solute. The amount of β-glucan dissolved depends on temperature, ionic strength, and pH of the solvent.[256] Impediments to the penetration of water and diffusion of

dissolved substances also affects the solubility.[224] Viscosity is a property of fluids; therefore, it is the amount of β-glucan solubilized in food and not just the total β-glucan content that is pertinent. Thus, the manner in which a fiber will modify solution properties depends upon the amount, the solubility or extractability under physiologic conditions, and the molecular weight and structure of the fiber. Changes in these properties of β-glucan in a food product can greatly influence the physiologic response.[220]

Food processing operations can influence the degree of polymerization, the molecular interactions within the structure, and the physicochemical properties of the fiber depending upon the processing methods employed.[257] For instance, hydrothermal treatments such as extrusion by preventing fragmentation of β-glucan arising from enzymatic hydrolysis can substantially improve the molecular weight and thereby viscosity; whereas, physical disruption of the cell wall material can increase the pool of soluble β-glucan.[258] However, the molecular size of the fiber may also be altered by conditions of high shear arising out of mechanical processing which reduces the viscosity.[259] Adverse structural changes such as depolymerization of the linear polysaccharides can also occur during commercial purification processes.[260] Nevertheless, there is a range in which the physiologic response is sensitive to viscosity and at higher levels little change in response will be observed despite large changes in viscosity.[30, 254]

Oats, β-glucan, and Satiety

In animal studies, β-glucan has been shown to increase satiety-related hormones as well as reduce energy intake and body weight.[261, 262] In one study using diet-induced-obese mice, the satiety effects of a six-week supplementation of the diet with different concentrations (0.7%, 3.5%, and 7%) of β-glucan from oat bran, were investigated. Energy intake and body weight reduced while plasma peptide YY (PYY) concentrations increased in a dose-dependent manner;

whereas, the expression of neuropeptide Y mRNA in the arcuate nucleus of the hypothalamus decreased with the highest concentration of β-glucan. The neurons which co-express neuropeptide Y and agouti-related peptide increase appetite.[94] The molecular weight of the β-glucan was approximately 1.7 million g/mol with solubility of approximately 13% dry weight basis. However, the viscosity was not measured.[261] In another study using high-fat fed mice, similar results were obtained using β-glucan doses of 0.5, 1, and 1.5 g/kg of body weight. At the end of three weeks of β-glucan supplementation, energy intake, and body weight reduced in a dose-dependent manner, whereas expression of neuropeptide Y mRNA in the hypothalamic arcuate nucleus decreased in the groups receiving the β-glucan supplementation compared to mice on the high-fat diet without β-glucan. However, no details on the physicochemical properties of the fiber were provided in this study.[262]

Human studies evaluating the effects of oat β-glucan on satiety have for the most part evaluated acute effects, and tested whole foods as well as β-glucan extracts added to food products. In a crossover study, breakfast meals containing a control (0 g), low (2.16 g), medium (3.82 g) and high (5.45 g) doses of oat β-glucan in extruded cereals, and a cereal with an ethanolic extract of β-glucan (added in equivalence to the highest dose among the extruded cereals), were compared for their effects on satiety.[37] Consistent with previous reports of the effects of extrusion on β-glucan,[258, 263] a decrease in molecular weight was offset by an increase in solubility to increase viscosity as the concentration of β-glucan in the cereals increased. There was no significant effect on suppression of ghrelin. However, regression analysis identified a significant relationship between the dose of β-glucan and the cholecystokinin (CCK) response ($R^2 \geq 0.97$, $p = 0.002$); but, there was no difference in the CCK response in the entire sample between the various doses and the control condition. Although among females the CCK response

was statistically significant between the control condition and the low, medium, and high fiber conditions ($p = 0.036$, 0.032, and 0.006 respectively), the sample size of seven females was small.

A reduction in energy intake was significant between the cereal containing the ethanolic extract of β-glucan and the control; although the repeated measures analysis of variance for the overall effect on energy intake at lunch was not significant. However, subjective satiety increased at all doses compared to the control; although, there was no dose response relationship. Despite the crossover design of this study, the differences between the overall effects and the effects among the conditions suggest that larger sample sizes may have been required to detect differences at all levels of the analysis. In an extension of this study, PYY was measured. Regression analysis showed a significant correlation between PYY concentrations and the fiber dose ($p = 0.003$, $R^2 = 0.994$). The total levels of plasma PYY increased linearly with increasing doses of β-glucan from 2.2 g to 5.45 g four hours following the meal.[193]

In studies evaluating the effects of oat-based breakfast cereals on satiety a 250 kcal serving of "old fashioned" oatmeal containing 2.6 g of β-glucan increased perceptions of satiety compared to an isocaloric oat-based ready-to-eat cereal (RTEC) containing 1.7 g of β-glucan. However, when a single serving (150 kcal) of "old fashioned" oatmeal was compared to an isocaloric serving of the RTEC, the effect on satiety was far less potent than that of the 250 kcal serving.[38, 39] Nevertheless, both serving sizes of instant oatmeal increased subjective satiety, while the 250 kcal serving of instant oatmeal also reduced energy intake. Unlike "old fashioned" oatmeal, instant oatmeal displayed a higher initial meal viscosity (after oral and initial gastric digestion) than the RTEC.[39, 45] It is likely that initial meal viscosity mediates the induction of signaling through orosensory stimuli to influence the overall satiety response. Thus, these

studies[39, 45] corroborate the results of other studies using magnetic resonance imaging in which initial meal viscosity influenced satiety possibly through the oral, gastric and intestinal signals working in concert.[240, 242] The sugar content of the RTEC in each of the studies [38, 39, 45] was higher than that of the oatmeal. Although, the kinetics of starch digestion and glucose release measured using in vitro mechanisms were not different among the breakfast cereals used in these studies, the possibility of differences in the nutrient composition influencing the results cannot be completely ruled out.

In another study using a crossover design, the satiety effect of isocaloric breakfast meals (352 kcal, including milk) consisting of oatmeal (4 g β-glucan and 4 g insoluble fiber), frosted cornflakes (under 1 g of fiber/110kcal, per Kelloggs™ Nutrition Facts panel), and water were compared. Perceptions of satiety increased, and energy intake was lower at an *ad libitum* lunch meal consisting of a liquid formulation, after consuming the oatmeal breakfast compared to the cornflakes breakfast meal and water. These effects were more pronounced among overweight individuals. Gastric emptying was slower after consuming oatmeal compared to the cornflakes meal or water which may have contributed to the increase in satiety. Although, the sugar content of the frosted cornflakes (35.5 g) in this study was higher than that of oatmeal (8.9 g), the effects on satiety were independent of the glycemic area under the curve which did not differ between the oatmeal and frosted cornflakes conditions. However, the physicochemical properties of the fiber were not measured.[41] Further, when the meals were eaten every day for four weeks, the group not eating breakfast lost more weight than the other groups, but there was no difference in body weight between the oatmeal and cornflakes groups despite increased satiety reported by participants in the oatmeal group.[40] This study conducted in 1998-1999, used the Likert-type rating scale to measure subjective satiety.[40] Unlike the visual analog scale (VAS) which is

continuous rather than interrupted by non-equivalent scale point ratings, the Likert-type scale has unknown magnitudes of satiety at equally spaced intervals along the scale.[264]

Other studies evaluated the effects of the viscosity generated by oat β-glucan on satiety by delivering the preload meal containing β-glucan in a beverage.[42-44] In a comparison among beverages (each approximately 167 kcal) containing 0 g, 5 g (2.5 g β-glucan) or 10 g (5 g β-glucan) fiber from oats, satiety over three hours following consumption of the beverages was measured. The fiber containing beverages increased satiety but it was not dose-dependent. Other conditions included in the study were a high viscosity beverage (167 kcal) containing 10 g fiber from oats (5 g, β-glucan), the fiber containing beverage treated with β-glucanase enzyme to reduce the viscosity, and a 0 g fiber beverage. The viscosity of the beverages was measured to ensure a difference in viscosity; however, the molecular weight or solubility of β-glucan was not measured. The enzymatically treated beverage and the high viscosity beverage increased perceptions of satiety and reduced hunger compared to the 0 g fiber beverage but there was no difference between the fiber-containing beverages on hunger ratings.[44] This study also evaluated the effects of energy levels of 167 kcal and 334 kcal at β-glucan doses of 0 g and 10 g and found that at both levels of energy the fiber containing beverages increased satiety with no significant differences between the energy levels. The fiber was added to the beverages just prior to serving to prevent viscosity levels that might make it unpalatable to ingest, which raises questions as to practical significance of delivering β-glucan in a liquid formulation.

Contrasting results were obtained when two isocaloric beverages (300 kcal) equal in volume, but differing in measured viscosity were compared.[42] Each beverage contained 5 g of soluble and 5 g of insoluble fiber from oat bran concentrate; however, the viscosity of one test product was reduced enzymatically using β-glucanase. The study had a crossover design with 20

subjects; but, no details of a power analysis were provided. The low viscosity beverage produced significantly greater post-prandial CCK, glucagon-like peptide-1, and PYY responses compared to the high viscosity beverage. There was no difference in energy intake at the *ad libitum* test meal; although, the low viscosity beverage did produce an increase in one of the subjective measures, which was the response to the question "How satiated are you?" However, the high viscosity beverage delayed gastric emptying. It is likely that consistent with previous research the physiologic response may not change in proportion to viscosity,[30] or that above a certain viscosity level, the response is insensitive.[254]

In another study subjects were served four different breakfast meals consisting of biscuits with a juice drink to investigate the influence of the food matrix on the effects of β-glucan. Four g β-glucan were either added or not added to the biscuits and juice drink (55% orange juice and 45% water). Each type of biscuit was combined with each type of juice drink. The viscosity increased as the β-glucan content of the meal increased regardless of the food form. While the addition of β-glucan increased perceptions of satiety compared to the control, the fortified juice drink and fortified biscuits combination produced the strongest effects on satiety. Moreover, the addition of oat bran was more effective in increasing satiety when added to the juice drink than when added to biscuits.[43] However, when a comparison was made among solid forms, arabinoxylan, oat β-glucan, as well as rye kernels in bread increased subjective satiety compared to refined wheat bread, although there was no effect on energy intake.[265] Thus, the food matrix may have a role to play in mediating the effects of viscosity on appetite as demonstrated by the lack of effect in studies using a semi-solid form.[266-268]

When delivered in a semi-solid pudding, isocaloric servings (300 kcal) containing 1.5 g of dietary fiber, 10.3 g of insoluble fiber from wheat bran, 10.2 g of fiber from oat bran (5 g β-

glucan) and a combination of wheat and oat bran providing 10.1 g fiber (2.5 g β-glucan), there were no significant differences in the post-prandial ghrelin, or PYY responses. Appetite ratings and energy intake at a subsequent meal were also not significantly different between the conditions. The meal with no added fiber when rated by subjects before eating was expected to be more filling. Cognitive factors such as an estimation of the satiating effect of foods, contribute to making eating largely a learned behavior,[89] and could have influenced the results which only underscores the complex nature of appetite and the responses to dietary manipulations. In this study, the physicochemical properties of the fiber were not evaluated.[266]

Similarly, when delivered in semi-solid form such as yogurt, no differences in satiety or gastric emptying were observed in a comparison between oat bran containing 4 g β-glucan and cornflakes.[267] Satiety was assessed at 15 and 90 minutes following the meal using a single numerical scale ranging from extreme hunger to extreme satiety punctuated with phrases describing various degrees of hunger and satiety. Using a similar design, the effects of breakfast cereals consisting of wheat bran flakes (7.5 g fiber), oat flakes (4g total fiber, 0.5 g β-glucan), and cornflakes (1.5 g fiber) were compared with similar results as the previous study; however, in this study the β-glucan content of oat flakes was almost negligible.[268] Subjective ratings usually measure perceptions of hunger, fullness, desire to eat, and the prospect of future consumption, terms relating to differing aspects of the motivation to eat.[89, 137, 138] These measures include an element of introspection which may not always be amenable to capture. Therefore, measuring several states repeatedly provides a better measure of satiety than the single scale used in this study. However, even when delivered in a breakfast cereal bar, fiber from oat bran had no effect on subjective satiety or energy intake compared to a control product.[269] In these studies,[267-269] the physicochemical properties of the fiber were not evaluated.

A three month intervention evaluated the effect of an energy-restricted meal plan supplemented with β-glucan from oat bran in RTEC's and snacks. The control diet had 0.2 g/day oat β-glucan, while the intervention groups had similar products with β-glucan at a moderate (5-6 g/day) and high (8-9 g/day) dose. The molecular weight and solubility of β-glucan altered as expected with food processing; however, the viscosity increased with β-glucan content. The average total dietary fiber consumption at the end of three months based on self-reported food intakes in the control, moderate, and high fiber groups was 21.6 g, 27.4 g, and 33 g respectively. There were no differences in body weight, or satiety hormones among the three groups.[270] However, self-reported food intakes tend to be imprecise and prone to underreporting.[133, 134] Further, compliance with the diet was likely compromised by imposing a diet including the same foods for a period of three months. A review of the human trials is presented in Table 3.2.

While oats are the focus of this review other sources of β-glucan, especially barley, are worth mentioning. Barley contains 3% to 7% of β-glucan and is also considered a good source of this fiber.[271] Oat and barley β-glucan are very similar in structure and properties; although, some differences exist. The molar ratio of (1→3) linked cellotriosyl units to (1→3) linked cellotetraosyl is lower in oats than in barley. Oat and barley β-glucans of the same molecular weight at the same concentration exhibit the same viscosity behavior but have different gelation characteristics largely due to the higher proportions of (1→3) linked cellotriosyl in barley which induces more rapid gelation, especially, at low molecular weights of the fiber.[220] Several studies investigating the effects of β-glucan on satiety used extracts of the fiber from barley. While a number of studies found a positive impact of β-glucan on satiety and energy intake,[191, 195, 272-275] some studies produced inconsistent results.[276-278] β-glucan is also found in cereals such as sorghum, rye, maize, triticale, wheat, and rice, as well as certain sea-weed and mushroom

Table 3.2. Summary of Human Studies Reviewed that Investigated the Effects of Oat β-glucan on Satiety

Source	Study Overview	Summary of Results	Conclusions
Beck *et al*, 2009[37,193]	*Subjects*:14 subjects, overweight/obese *Study Design*: Crossover *Intervention*: 5 breakfast cereals containing β-glucan in doses of 0, 2.2, 3.8, and 5.5 g, and a cereal with extracted β-glucan (5.7 g) *Length of Study:* 5 visits separated by 3 days *Food intake test:* Buffet lunch 4 h after breakfast *Subjective ratings:* VAS over 4 h *In vitro:* β-glucan content, MW, solubility, viscosity *Hormones*: Ghrelin, CCK, PYY	*Food Intake*: Only extracted β-glucan differed from control *Subjective Ratings*: Analyzed as a single response, difference between all doses and control, but no dose response relationship *Hormones*: Dose response relationship between β-glucan, and CCK. Correlation between β-glucan content and PYY release *Viscosity of extract:* Increased from 5.8 to 84.8 mPa.s as the β-glucan dose increased	*Conclusions*: β-glucan increases satiety possibly acting through CCK and PYY. Optimal dose of β-glucan, 4-6g
Rebello *et al*, 2013[38]	*Subjects*: 46 subjects *Study Design*: Randomized crossover *Intervention*: Old fashioned oatmeal (2.6 g β-glucan) and oat-based RTEC (1.7 g β-glucan) *Length of Study:* 2 visits with a one week interval between visits *Subjective Ratings:* VAS over 4 h *In vitro:* β-glucan content, MW, radius of gyration, viscosity	*Subjective Ratings*: Oatmeal increased fullness, and reduced hunger, desire to eat, and prospective intake compared RTEC *Viscosity:* Range over 2 h in cP 1 cP = 1 mPa.s Oatmeal: 1063.3 - 83.6 RTEC: 175.2 - 73.0	*Conclusions*: Oatmeal increases satiety which may be related to viscosity generated by β-glucan

(Table 3.2 continued)

Source	Study Overview	Summary of Results	Conclusions
Rebello *et al*, 2014[39]	*Subjects:* 43 subjects *Study Design:* Randomized crossover *Intervention:* Old fashioned oatmeal and instant oatmeal (1.6 g β-glucan) , oat-based RTEC (1 g β-glucan) *Length of Study:* 3 visits with a one week interval between visits *Subjective Ratings:* VAS over 4 h *In vitro:* β-glucan content, MW, radius of gyration , viscosity	*Subjective Ratings*: Instant oatmeal increased fullness, and reduced desire to eat, and prospective intake; whereas, old fashioned oatmeal reduced prospective intake compared to RTEC *Viscosity:* Initial and subsequent viscosity over 2 h in cP: Instant oatmeal: 7397.2, 87.9 Old fashioned oats: 1063.3, 85.1 RTEC: 175.2, 75.9	*Conclusions:* Oatmeal increases satiety compared to RTEC. Initial viscosity may be important for inducing satiety
Rebello *et al*, 2015[45]	*Subjects*:48 subjects *Study Design:* Randomized crossover *Intervention:* Instant oatmeal (2.7g β-glucan) and oat-based RTEC (1.7g β-glucan) *Length of Study*: 2 visits with a one week interval between visits *Subjective Ratings*: VAS over 4 h *Food Intake: Ad libitum* lunch meal 4 h after breakfast *In vitro:* β-glucan content, MW, radius of gyration, viscosity	*Food Intake*: Oatmeal reduced energy intake compared to RTEC *Subjective Ratings*: Oatmeal increased fullness, and reduced hunger, desire to eat, and prospective intake compared to RTEC *Viscosity:* Initial meal viscosity in cP Oatmeal: 7220.5 RTEC: 140.0	*Conclusions:* Oatmeal increases satiety and reduces energy intake compared to RTEC. Initial viscosity may be important for inducing satiety

(Table 3.2 continued)

Source	Study Overview	Summary of Results	Conclusions
Geliebter *et al*, 2015[41]	*Subjects*: 36 subjects, normal and overweight/obese *Study Design*: Randomized, controlled, crossover *Intervention*: Breakfast consisting of frosted corn flakes cereal (under 1 g fiber), quick cooking oatmeal (4 g soluble, 4 g insoluble fiber), and water *Length of Study:* 3 test visits with minimum 2 day interval between visits *Food intake test*: Lunch (liquid formula) 3 h after breakfast *Subjective Ratings*: VAS over 3 h *Gastric Emptying*: Acetaminophen in test meal	*Food intake*: Oatmeal reduced energy intake compared to frosted flakes or water. Overweight/obese subjects had lower energy intake than normal weight subjects *Subjective Ratings:* Oatmeal increased fullness and reduced hunger compared to frosted flakes, and water *Gastric Emptying:* Oatmeal slowed gastric emptying compared to frosted flakes and water.	*Conclusions:* Oatmeal increases satiety compared to cornflakes which may be related to delayed gastric emptying
Geliebter *et al*, 2014[40]	*Subjects:* 36 subjects, overweight/obese *Study Design:* Randomized, controlled, parallel *Intervention:* Breakfast consisting of frosted corn flakes cereal (under 1 g fiber), quick cooking oatmeal (4 g soluble, 4 g insoluble fiber), and water *Length of Study:* 4 visits over consecutive weeks *Subjective Ratings:* Likert-type scale over 3 h at each visit *Other measures:* Body weight, body composition, resting energy expenditure	*Subjective Ratings:* Oatmeal reduced hunger compared to frosted flakes or water and increased fullness compared to water. *Other measures:* No effect on body composition, or resting energy expenditure between the groups, but the control group lost more weight than the other groups	*Conclusions:* Oatmeal increases satiety; however, breakfast skippers lost more weight over 4 weeks

(Table 3.2 continued)

Source	Study Overview	Summary of Results	Conclusions
Lyly et al, 2010[44]	*Subjects:* 29 subjects *Study Design:* Crossover *Intervention:* Comparison of 3 beverages containing 0, 2.5, and 5 g oat β-glucan. Comparison of two 5 g oat β-glucan beverages (one reduced in viscosity), and a 0 g β-glucan beverage *Length of Study:* 1 visit per condition separated by one day *Subjective Ratings:*10-unit scale over 3 h *In vitro:* Viscosity	*Subjective Ratings:* Satiety increased at 2.5, and 5 g dose of β-glucan but no dose response relationship. Enzymatically treated and high viscosity beverages increased satiety compared to control. Enzymatically treated produced lower satiety but did not differ in hunger compared to high viscosity beverage *Viscosity*: 1.5, 31.6, and 661 mPa.s, for the 0 g, enzyme-treated, and 5 g β-glucan beverages respectively	*Conclusions:* β-glucan increases viscosity and enhances satiety but the effect may not be related to the amount of fiber.
Pentikainen *et al*, 2014[43]	*Subjects:* 30 females, normal weight *Study Design:* Randomized crossover *Intervention*: 4 conditions: Combinations of biscuits and juice providing 0, 4, 4, and 8 g β-glucan *Length of Study:* 4 visits separated by at least 2 days *Subjective Ratings:* VAS over 3.5 h *In vitro:* Viscosity	*Subjective Ratings:* Increased satiety with 8 g compared to 0, or 4 g β-glucan. β-glucan in juice had a greater impact on satiety than when added to biscuits *Viscosity:* 4 g β-glucan: 2920 - 8160 mPa. s 4 g β-glucan: 2100 - 6290 mPa. s 8 g β-glucan: 19890 - 22730 mPa. s	*Conclusions:* 4 or 8 g β-glucan increases satiety. Food matrix is an important factor influencing satiety

(Table 3.2 continued)

Source	Study Overview	Summary of Results	Conclusions
Juvonen *et al*, 2009[42]	*Subjects*: 20 subjects, normal weight *Study Design*: Randomized crossover *Intervention*: Two isocaloric beverages containing 5.1 g oat soluble fiber, one treated with β-glucanase *Length of Study*: 2 visits separated by >2 days *Food Intake: Ad libitum* meal 3 h after test meal *Subjective Ratings:* VAS over 3 h *Hormones:* Ghrelin, CCK, PYY, GLP-1 *In vitro:* MW and viscosity	*Food intake*: No differences in energy intake at *ad libitum* meal *Subjective Ratings:* No difference in hunger, fullness, desire to eat. Response to "How satiated are you?" greater with low viscosity beverage *Gastric Emptying:* Faster emptying after low viscosity beverage *Hormones:* Greater increase in CCK, PYY, and GLP-1 after low viscosity beverage. No effect on ghrelin *Viscosity:* >250 mPa.s, low viscosity >3000 mPa.s, high viscosity	*Conclusions*: Differences in viscosity may not clearly reflect differences in appetite sensations or energy intake.
Juvonen *et al*, 2011[266]	*Subjects*: 20 subjects, normal weight *Study Design*: Randomized crossover *Intervention*: Control, wheat bran, oat bran and combination puddings containing 0, 0, 5.1, and 2.5 g β-glucan respectively *Length of Study*: 4 visits separated by >2 days *Food Intake Ad libitum* meal 3 h after Test meal *Subjective Ratings:* VAS over 3 h *Hormones:* Ghrelin, PYY	*Food intake*: No differences in energy intake at *ad libitum* meal *Subjective Ratings:* No differences in appetite ratings *Hormones:* No differences in ghrelin or PYY	*Conclusions*: β-glucan in a semi-solid pudding has no effect on appetite sensations or gut hormone release. Food matrix may influence satiety response

(Table 3.2 continued)

Source	Study Overview	Summary of Results	Conclusions
Korczak et al, 2014[269]	*Subjects*: 42 females, normal weight *Study Design*: Randomized crossover *Intervention*: Breakfast bars:10 g oat bran, 10 g barley bran, 3 g dietary fiber *Length of Study*: 3 visits separated by at least one week *Food Intake: Ad libitum* pizza meal 4 h After breakfast *Subjective Ratings:* VAS over 4 h *Colonic Fermentation*: Breath samples	*Food intake*: No differences in energy intake at *ad libitum* meal *Subjective Ratings:* No differences in appetite ratings *Colonic fermentation:* No differences in hydrogen or methane production	*Conclusions*: Oat bran does not induce greater satiety than barley bran or a low fiber control
Hlebowicz et al 2007[268]	*Subjects*: 12 subjects, normal weight *Study Design:* Randomized crossover *Intervention:* Sour milk with oat flakes (0.5 g β-glucan), All-bran flakes, or cornflakes (0 g β-glucan) *Length of Study*: 3 visits, separated by > 1 week *Subjective Ratings*: 10 point single scale, over 2 hours *Gastric emptying*: Ultrasound, 15 and 90 minutes after test meal	*Subjective Ratings:* No differences in appetite ratings *Gastric emptying:* All-bran flakes and oat flakes were not different from corn flakes but All-bran flakes had lower gastric emptying rate compared to oat flakes	*Conclusions:* Presence of fiber in semi-solid meal has no effect on satiety despite reduction in gastric emptying rate

(Table 3.2 continued)

Source	Study Overview	Summary of Results	Conclusions
Hlebowicz et al, 2008[267]	*Subjects*: 12 subjects, normal weight *Study Design:* Randomized crossover *Intervention:* Yogurt with oat flakes (4 g β-glucan) or cornflakes (0 g β-glucan) *Length of Study*: 2 visits, separating period unavailable *Subjective Ratings*: 10 point single scale, 15 and 90 minutes after test meal *Gastric emptying*: Ultrasound, 15 and 90 minutes after test meal	*Subjective Ratings:* No differences in appetite ratings *Gastric emptying:* No differences in median values of gastric emptying	*Conclusions:* 4 g oat β-glucan in a semi-solid meal has no effect on satiety or gastric emptying
Beck *et al*, 2010[270]	*Subjects*: 56 subjects, overweight/obese *Study Design*: Randomized parallel *Intervention*: 5-6 g, 8-9 g β-glucan from oat bran, 0.2 g β-glucan (breakfast cereal + 2 snack items) *Length of Study*: 3 months *Subjective Ratings:* VAS over 2 h on 3 occasions *Anthropometry*: Body weight, waist circumference *Hormones:* Ghrelin, CCK, PYY_{3-36}, GLP-1 *In vitro:* MW, solubility, and viscosity	*Subjective Ratings:* No differences in appetite ratings among groups *Anthropometry:* No differences in body weight and waist circumference among groups *Hormones:* No differences in hormones among groups *Viscosity Range:* 14.6 - 57.0 mPa.s	*Conclusions:* Addition of oat β-glucan does not enhance the effect of energy restriction over 3 months

(Table 3.2 continued)

Source	Study Overview	Summary of Results	Conclusions
Hartvigsen *et al*, 2014[265]	*Subjects*: 15 subjects with the metabolic syndrome *Study Design:* Randomized crossover *Intervention:* Refined wheat bread, rye kernel bread, and wheat bread with added wheat arabinoxylan or oat β-glucan (2.9, 12.2, 11.2, and 13.4 g fiber respectively) *Length of Study*: 4 visits, separated by ~ 1 week *Subjective Ratings*: VAS over 4½ hours *Food Intake test: Ad libitum* pizza meal 4½ hours after test meal *Hormones:* Ghrelin, GLP-1	*Subjective Ratings:* Arabinoxylan, β-glucan, and rye breads reduced hunger, and prospective consumption, and increased satiety, whereas, only arabinoxylan and rye breads increased fullness compared to wheat bread. *Food Intake test:* No significant differences in energy intake among bread types *Hormones:* No differences in ghrelin, But GLP-1 was higher after rye compared to arabinoxylan or β-glucan breads 120 - 270 minutes post-prandial.	*Conclusions:* Breads with added fiber increase satiety with no effect on ghrelin concentrations, or energy intake

Visual analog scale (VAS); Cholecystokinin (CKK); Peptide YY (PYY); Ready-to-eat cereal (RTEC); Glucagon-like peptide- (GLP-1) Molecular weight (MW), millipascal-second (mPa.s); Centipoise (cP)

species; but, the β-glucan content is much lower than oats or barley.[279] Except for rye which has arabinoxylan as the dominant fiber,[280] the other sources have not been actively investigated for their effects on satiety.

Some studies investigating the effects of oat β-glucan supplementation on satiety have not been able to show a positive impact on satiety.[266, 267, 269, 270] Despite the inconsistencies, a majority of the human studies have demonstrated that oat β-glucan increases perceptions of satiety.[37-45] However, this may not always translate into a reduction in energy intake or body weight.[269, 270] A number of studies did not provide details on the physicochemical properties of the fiber; nevertheless, it is clear that viscosity is an important factor in stimulating the effects on satiety. Varying ranges of viscosity have been able to deliver the desired result. In some studies the increase in satiety was congruent with the increase in viscosity;[38, 39, 43] whereas, in others a beverage with a low viscosity produced greater effects on satiety than the beverage with higher viscosity.[42] In the study which demonstrated changes in gut hormones in the desired directions with the low viscosity beverage compared to a high viscosity beverage, 85% of the β-glucan in this beverage had a molecular weight < 100,000 g/mol.[42] At sufficiently high concentrations (above 3.5 - 4%), solutions of low molecular weight β-glucan (35,000 to 110,000 g/mol) tend to abandon the random coil flow behavior over time and form gels.[250] Thus, the contrary results of the rheological effects of β-glucan in beverages, needs further investigation in future trials.

Some of the inconsistencies in the results may be explained by differences in terms used to describe viscosity. For instance, apparent viscosity is defined as the viscosity of a non-Newtonian fluid expressed as if it were a Newtonian fluid. Fluids such as tea, coffee, edible oils or milk display a true viscous flow and are termed Newtonian fluids. A number of fluid foods as well as biological fluids have non-Newtonian flow behavior. Unlike Newtonian fluids, these

fluids increase in viscosity when the shear rate increases and are not disposed to being measured at a single shear rate. The literature on dietary fiber appears to favor the use of the apparent viscosity. Moreover, the use of different instrumentation could provide different results.[30] Thus, in addition to reporting physicochemical data, there is clearly a need for standardization of procedures used to measure viscosity.

Satiety has been shown to increase at doses of β-glucan ranging from 2.2 g to 5.5 g; however, the effects of dose on satiety are inconsistent.[38, 39, 44, 267] Viscosity depends upon the solubility or extractability as well as the molecular weight of the fiber and is an important determinant of the physiologic response.[271] Thus, it is of importance to ensure that the food product provides not only a sufficient dose but also a good extractability. Further, the preload meal must be of sufficient caloric value to sustain satiety during the period of evaluation. Since viscosity is important for bioactivity, any processing, cooking, or storage treatments that affect solubility and molecular weight of β-glucan must be considered.[220]

The sensation of hunger is an important factor determining what and how much is eaten.[90] However, the control of appetite is not merely a question of satisfying biological needs. Individuals eat for various reasons including customary eating patterns, the social context, or even boredom. The interaction of social and physiologic factors lends complexity to human appetite control. Eating patterns are maintained by habits, attitudes, opinions about the value or suitability of the food, liking for the food, and a motivational drive to actually engage in eating.[90] Individuals experience a decline in the pleasure derived from a consuming a particular food in comparison to foods not consumed.[100] This phenomenon known as sensory specific satiety is what prompts consumers to search for variety when making food choices and may explain why it

is difficult to comply with a study protocol that requires consumption of two or three test products for a length of time.

The psychological and biological factors exercise a control over appetite that is anything but tenuous. Therefore, the development of foods that promote satiety requires a certain amount of ingenuity and highlights the need to expand research relating to components with evidence to support their role in promoting satiety, such as β-glucan. Ideally, further studies of process and cooking effects are required. Understanding the relationships between viscosity of isolates, viscosity of in vitro extracts from foods, and physiologic responses would help clarify the mechanisms by which β-glucan affects satiety, and the processing techniques that could facilitate development of satiety-enhancing products.

Conclusions

The mechanisms by which soluble dietary fiber exerts its physiologic effects on satiety are biologically plausible. Increased viscosity, delays gastric emptying and reduces the absorption of nutrients. The increased interaction with the cells that release satiety hormones stimulates the release of peptides involved in appetite regulation. Delivered in a whole food or an extract from the food, oat β-lucan appears to have a positive impact on perceptions of satiety. Whether the effects are enduring with repeated exposure remains to be established.

Eating behavior is largely learned, arising from metabolic and sensory factors as well as the rewarding value of foods.[89] The sensory factors drive food choice; however, preferences are influenced by various exposures, availability, cultures, and social norms surrounding the food. Repeated exposure leads to the development of habits. Although preferences are resistant to change, they are amenable to modification.[281] Consumption of whole foods or extracts from

foods shown to promote satiety offers a means of helping individuals to adhere to diet regimens by controlling hunger and the desire to eat.

CHAPTER 4
GUT FAT SIGNALING AND APPETITE CONTROL WITH SPECIAL EMPHASIS ON THE EFFECT OF THYLAKOIDS FROM SPINACH ON EATING BEHAVIOR

Introduction

Human appetite is controlled by a complex sequence of events among elements that form the psychobiological system. This system broadly encompasses psychological experiences, peripheral physiologic signals, and brain mechanisms.[90] Eating when energy stores are depleted and refraining from eating when replete reflects a homeostatic model of eating, or metabolically driven eating which is controlled by neural circuits primarily in the hypothalamus and brainstem. Eating in the absence of such metabolic feedback, termed non-homeostatic eating, involves cognition, reward, and emotion. Non-homeostatic eating is controlled by neural circuits principally located in the cortico limbic structures and has parallels to drug addiction. The metabolic and non-homeostatic controls of eating interact to determine eating behavior.[109]

The metabolic controls of eating are entrenched in a neural system that permits an interaction with the environment to produce an integrated adaptive response that coordinates metabolic need with the prevailing environment.[108] The procurement of energy and essential nutrients is defended by complex redundant pathways, which are different from the neural circuitry involved in fulfilling the metabolic needs of the body. In the modern world procuring food is not difficult or dangerous. However, this system was designed to cope with evolutionary pressures arising from times of feasting and famine and is therefore predisposed to neural circuits that facilitate ease of securing and storing energy.[282]

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Emotions evolved as a mechanism to initiate advantageous and preempt potentially harmful stimuli or behavior.[108] To elucidate, satiation represents meal termination and satiety prolongs the interval between meals. The pleasurable taste of certain foods and the perceptions of satiation or satiety they invoke are integrated by the brain to build a complex representation of the food and provide an evaluation of its reward value.[283, 284] These representations are associated with positive emotions that prompt a motivational drive to find the food and engage in eating.[108] The associated pleasing of the senses that indulging in the food brings on is stored by the brain and results in a keen motivation to renew these pleasurable sensations. The reward value of a food and the positive emotions it induces is learned and thereby sought at a future time. The metabolic need is translated into behavioral action by a cognitive and emotional brain that operates on factors such as past experiences, cost, and availability.[108] Thus, the neural circuits regulating energy homeostasis are intertwined with the cortico-limbic mechanisms involved in learning and memory, reward, and emotions.[108] However, the cortico-limbic systems can override the homeostatic systems controlling energy balance as they both act to synchronize the internal milieu with the external world[108] (Figure 4.1).

In the prevailing food environment where shortages for the most part are conspicuous by their absence, the 27.5% rise in the global prevalence of obesity as assessed over the past 33 years[8] suggests that the non-homeostatic drive is prevailing over metabolic feedback control.[109] Activating the mental representation of a behavior outside of awareness prepares a person to rapidly initiate the corresponding behavior.[285] Thus, reward evaluation can occur outside of awareness and the corresponding action taken without conscious control.[109] Interventions that target this subliminal priming have tremendous potential to reduce the asymmetry between metabolic and non-homeostatic regulation of food intake.

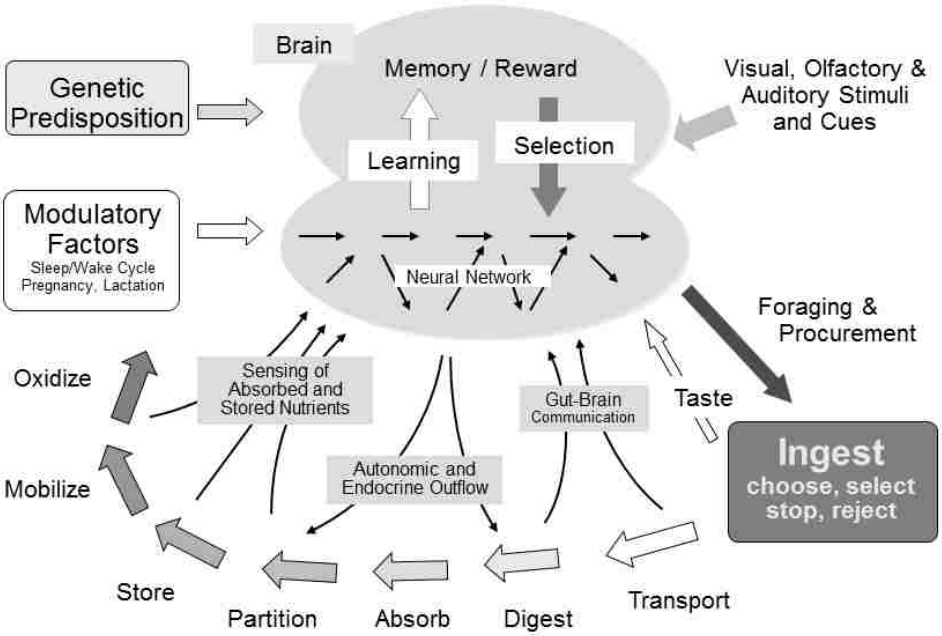

Figure 4.1 Flow diagram depicting the major factors and mechanisms determining the control of ingestive behavior and energy balance. Berthoud HR *et al*[282] Reproduced with permission from Annual Reviews of Psychology. http://www.annualreviews.org/

Fat is a concentrated source of energy that can contribute to increased energy intake. Foods high in fat especially those containing added sugars induce an undeniably pleasurable sensation that may increase their selection over other alternatives at the point of choice.[286] However, the presence of fats in the gastrointestinal (GI) tract reduces the drive for food.[49] Thus, fat contributes to increasing the energy density of the diet but fat can also influence appetite by inducing the release of hormones involved in regulating eating behavior.[287] However, the prolonged presence of fat in the GI tract is necessary for fat to exert its physiologic effects on appetite regulation. Delaying fat digestion is therefore crucial.[288]

Thylakoids are compartments inside the chloroplasts and are composed of membranes that form the internal photosynthetic membrane system of chloroplasts. By interacting with lipids and delaying fat digestion thylakoid membranes from spinach promote the release of satiety hormones and may influence the reward system.[53, 60, 106, 289] This review will explore gut lipid

sensing and GI signaling in the context of appetite regulation. Further, the effects of thylakoid membranes on eating behavior will be reviewed.

Gut Lipid Sensing and Appetite Regulation

The GI tract initiates a wide range of responses that result in digestion, absorption, and metabolism of a meal. As a highly specialized chemosensory organ, with the capacity to sense ingested nutrients, the primary function of the gut is to optimize these processes. The ingestion of a meal precipitates neural and hormonal signaling from the GI tract in response to gastric distension and the chemical presence of nutrients. Interaction between the enteroendocrine cells located throughout the GI tract and nutrients stimulates the release of peptides that act locally, centrally, or peripherally to influence appetite regulation.[290]

Oral fat exposure acting through a number of receptors and molecules including G-protein coupled receptors (GPCRs), potassium channels, and cluster of differentiation 36 (CD36) may play a role in mediating dietary fat preference and intake.[49, 291] However, it is oral exposure to fatty acids rather than triacylglycerides that elicit this response; and, lipolytic activity in the saliva is sufficient to produce the range of fatty acid concentration that produces the signals.[292, 293] The mechanisms involved in intestinal detection of lipids involve molecular sensing elements that largely coincide with those implicated in oral signaling. Luminal contents activate several GPCRs in the enteroendocrine cell membranes. These receptors are activated by fatty acids of more than 12 carbons or their derivatives such as oleoylethanolamine (OEA). Fatty acid induced activation of these receptors results in secretion of gut peptides such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY), that precipitate appetite suppressant responses.[294-296]

The biochemical basis for gut lipid sensing and the induction of signaling to regulate GI function and energy intake are not well understood but it may be inferred through the identification of locally released or expressed factors potentially important in the gut-brain feedback axis.[297] Expression of markers of neuronal activity such as c-Fos, in the caudal brainstem nucleus of the solitary tract, the central nervous system terminus of gut vagal afferents has been demonstrated with intestinal lipid infusions at doses that inhibit feeding, but the effect is blocked by the administration of the serotonin (5-HT) receptor antagonist ondansetron.[298-300] Duodenal 5-HT release acting via vagal 5-HT inotropic receptors to increase gut vagal afferent neurophysiological activity is therefore a possible mechanism by which food intake is regulated. However, evidence points to fat-induced CCK release mediating the activation of duodenal 5-HT receptors.[297]

In enteroendocrine cells, another possible candidate is CD36, a receptor-like membrane protein, which may act as a lipid sensor to promote peptide release.[297] Cluster of differentiation 36 is also expressed in the ventromedial hypothalamic neurons where it mediates oleic acid signaling. Binding of oleic acid to CD36 alters neuronal activity in the same way as fat perception by taste receptor cells modulates neuronal action.[301] Oleic acid is generated in the gut during digestive hydrolysis of complex dietary lipids. The interaction of CD36 with oleic acid is necessary for the production of OEA which suppresses energy intake. The precise mechanisms by which OEA exerts its inhibitory effect on feeding are unknown; however, the activation of the nuclear receptor, peroxisome-proliferator-activated receptor-α, an OEA agonist has been implicated.[302-305] Further, the activation of peroxisome-proliferator-activated receptor-α triggers the production of apolipoprotein IV whose presence is critical for induction of gut to brain

signaling conveying information about the presence of fatty acids in the small intestine. The feedback responses initiated include the inhibition of gastric motility.[306]

Dopamine is a key neurotransmitter that modulates reward. The role of dopamine signaling in reward in highly debated.[154, 307] There are two prevalent hypotheses that explain dopamine signaling. The first hypothesis states that overindulgence in pleasurable stimuli occurs when there is a positive correlation between the amount of dopamine signaling and the pleasure derived from the hedonic experience.[109] The other hypothesis is that diminished responses within brain reward dopaminergic circuits leads to overeating as a compensatory response.[308, 309] High-fat fed GI dysfunction plays an important role in dopamine deficiency. Reduced synthesis of the appetite suppressant OEA may be the link between GI dysfunction and high-fat induced dopamine deficiency. Administration of OEA to high-fat fed mice has been shown to correct the signaling deficiencies and reduce oral intake of a high fat emulsion.[305, 310]

To summarize, fatty acids are sensed by GPCRs on enteroendocrine cells which triggers the release of GI peptides. Other mediators include CD-36 which contributes to mobilization of OEA and apolipoprotein IV. These lipid mediators precipitate the activation of receptors on vagal afferents to induce signaling to the central nervous system. Additionally, fats in the gut may activate dopaminergic systems affecting reward, to promote an inhibition over eating.

Gastrointestinal Signaling and Appetite Control

The GI tract is the largest endocrine organ with multiple peptides being produced and released as secretory granules from a single cell type and single peptides having several effects. The brain senses and responds to external cues relating to the availability of foods and coordinates it with internal signals conveying information about presence and composition of nutrients in the gut, the circulating nutrients, and the energy stored as fat.[282] The GI signals

released as a result of ingestion may be classified as: 1) short-term signals which are synchronized with episodes of eating; 2) long-term signals which reflect the metabolic state of adipose tissue. Both the episodic and long-term or tonic signals interact to determine eating behavior[96] (Figure 4.2).

Short-term or Episodic Signals

Ghrelin is a peptide hormone released into circulation from the stomach. Ghrelin acts via the growth hormone secretagogue receptor-1α. Acylation of ghrelin is necessary for it to bind to the receptor and for ghrelin to cross the blood brain barrier.[311] Circulating levels of ghrelin are elevated during fasting and fall after eating suggesting that ghrelin exerts an orexigenic effect, to stimulate hunger. However, acylated ghrelin as well as the enzyme responsible of activating ghrelin are reduced by high-fat feeding and may inform the central nervous system about the availability rather than the absence of energy.[312, 313] Further, evidence suggests that ghrelin may influence the mesolimbic dopamine circuitry to enhance the reward value or motivational wanting for highly desirable foods.[314, 315]

Cholecystokinin is released postprandially from the I cells in the proximal GI tract primarily in response to fat and protein hydrolysates.[316] Cholecystokinin appears to act by binding to CCK1 receptors on the vagal nerve,[317] causing early meal termination and reducing food intake in animals and humans.[318-320] Evidence suggests that CCK may not wholly rely on this pathway. Selective vagotomy does not fully attenuate the response, and the presence of CCK1 receptors in the hypothalamus suggests that a direct communication without vagal mediation is likely.[321, 322] In vagal afferent neurons, the absence of CCK1 receptors has been linked to an elevated ghrelin mediated drive to eat.[323] Also, GLP-1 release is affected by CCK1

receptor antagonism.[324] Thus, CCK may at least in part regulate the capacity of vagal afferents to respond to other appetite related signals.[325]

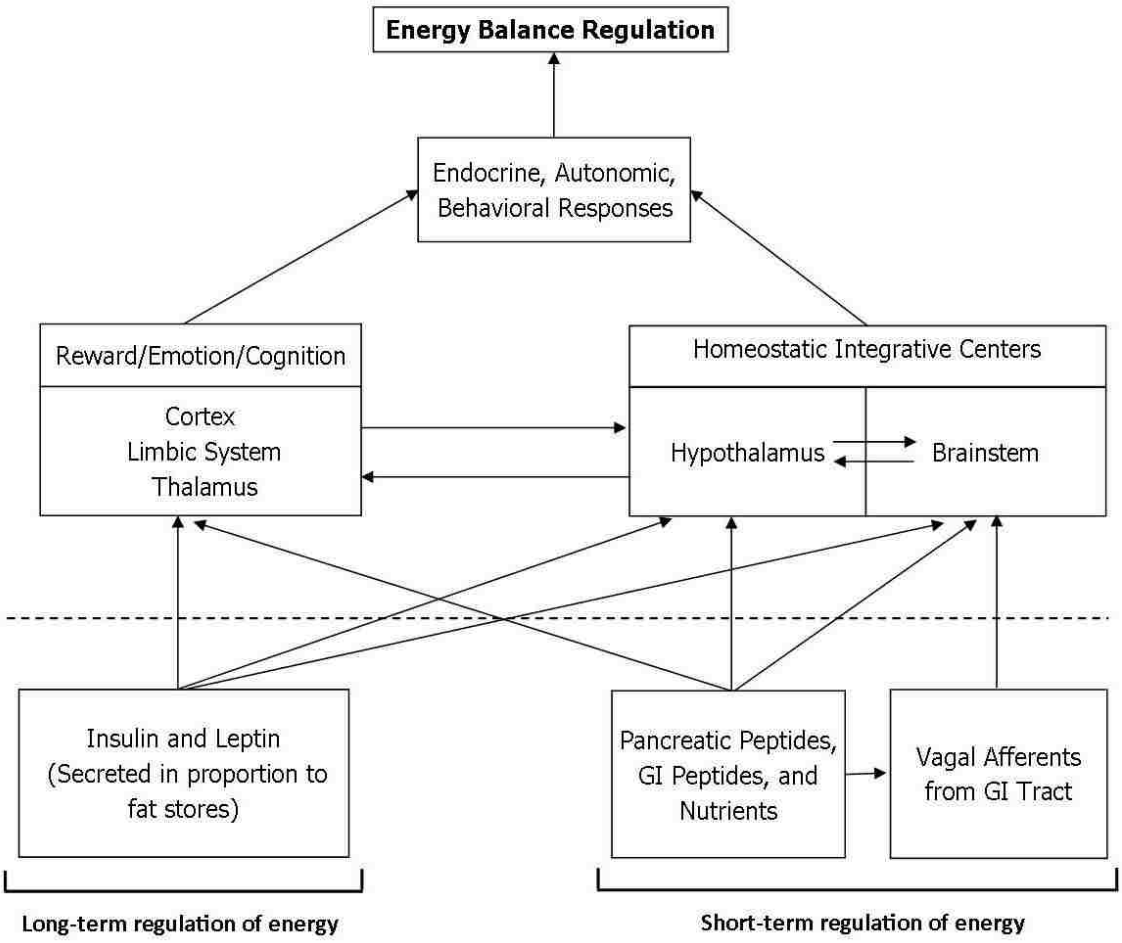

Figure 4.2 Schematic representation of the levels of homeostatic and non-homeostatic controls over the regulation of energy balance: Following ingestion of food, signals from the gastrointestinal (GI) tract including hormones such as ghrelin, cholecystokinin, glucagon-like peptide-1, peptide YY, and insulin as well as glucose, fatty acids, and enterostatin, act through vagal stimuli, directly, or both to inform the central nervous system of the nutritional and energy status of the body. Leptin and insulin released in proportion to adipose stores are involved in long term signaling reflecting the metabolic state of adipose tissue. Long term and short term signaling interact to determine eating behavior. Although the hypothalamus is a key player in the control of food intake, the integration of signals involves, important areas in the limbic system, cortex, midbrain, and brainstem to produce coordinated endocrine, autonomic, and behavioral responses that regulate appetite and energy balance. Adapted from Schneeberger *et al*[326] Copyright 2014, Biotechnica Ltd.

Co-secretion of PYY along with GLP-1 occurs in response to a meal from the L cells located throughout the gut, but present in highest concentrations in the distal regions. Peripheral administration of PYY_{3-36} reduces food intake in rodents and humans.[327-329] The secretion of PYY is stimulated by the various macronutrients; but fat appears to be the most potent stimulator of PYY release in humans.[330, 331] Using functional magnetic resonance imaging it has been shown that in humans PYY modulates neural activity in brain areas associated with homeostatic appetite control as well as the higher cortical areas involved in reward and hedonic control. Under conditions of low PYY, hypothalamic activation correlated with energy intake; however, under conditions of high PYY mimicking the prandial state, brain activity predicting energy intake switched from a homeostatic area (hypothalamus) to the orbito frontal cortex, a brain region implicated in reward processing.[332] Thus, PYY may diminish the rewarding value of food by modulation of the orbito frontal cortex.

Glucagon-like-peptide-1 is endogenously released by the L cells following nutrient entry, and by neurons of the nucleus of the solitary tract in the hindbrain.[333] GLP-1 receptors in the nucleus acumbens and in the ventral tegmental area appear to be responsible for an inhibitory effect of GLP-1 on the rewarding value of food in rats, as evaluated by a progressive ratio test.[334] With the identification of strong projections from GLP-1 neurons in the nucleus of the solitary tract to the nucleus acumbens core, it was shown that injections of GLP-1 into the nucleus acumbens reduced food intake.[335] Thus, this projection may link signals of satiety in the hindbrain with the forebrain signals of food reward. In the periphery, satiety-inducing effects of GLP-1 are likely mediated by vagal afferents originating in the intestine in combination with other mechanisms that may involve circumventricular organs (lacking the blood brain barrier).[336] Carbohydrate and fat appear to be more potent stimulators of GLP-1 than protein.[337]

Oxyntomodulin is processed from the same precursor as GLP-1 and is released postprandially. Although oxyntomodulin acts by binding to the GLP-1 receptors the binding affinity is far lower. When administered centrally or peripherally, oxyntomodulin has been shown to reduce weight gain in rats,[338, 339] and food intake in humans;[340] however, it has short circulating half-life which limits the use of exogenous oxyntomodulin as a means of appetite regulation.[341]

Pancreatic polypeptide is released from F-cells of the pancreatic islets in response to food intake. Pancreatic polypeptide has a high affinity for the Y4 receptor, which it is thought to reduce food intake by down-regulating the orexigenic neuropeptide orexin and increasing the anorexigenic brain-derived neurotrophic factor.[342] Intravenous administration of pancreatic polypeptide reduces food intake in humans.[343] However, pancreatic polypeptide too has a short half-life *in vivo*, which limits its potential as a treatment for obesity.[341]

In response to the ingestion of fat, procolipase is secreted from the exocrine pancreas. Following secretion it is converted to colipase by cleavage of the N-terminal pentapeptide enterostatin. Colipase facilitates the action of pancreatic lipase in the digestion of fat; whereas, enterostatin acts as a negative feedback signal regulating fat intake and inducing satiety.[344-347] Studies using immunohistochemistry have shown that procolipase and enterostatin are present in the gastric mucosa and certain brain areas such as the amygdala and hypothalamus.[348, 349] The effect of enterostatin on the regulation of energy intake involves both central and peripheral sites of action. The peripheral mechanisms involve vagal afferent signaling to hypothalamic pathways.[350]

It has been suggested that enterostatin may also influence the reward system by its actions on the opioidergic systems; but, clear evidence of the binding to opioid receptors has yet to be demonstrated.[351] Enterostatin's effects on appetite acting through the reward system may

also be influenced by changes in dopamine activity.[352] After an adaptive period of fat consumption, enterostatin has been shown to block dopamine reuptake transport to increase striatal dopamine release measured in rat striatal slices.[353] Acting via uncoupling proteins enterostatin may also raise thermogenesis to increase energy expenditure.[351] In humans, oral or intravenous enterostatin administration did not have an effect on subjective satiety ratings, hedonic scores, or energy intake;[354, 355] however, the effects of endogenously produced enterostatin on appetite have not been investigated.

Long-term or Tonic Signals

Insulin and leptin represent long lasting or chronic hormonal regulation of food intake. They are released into circulation proportional to body fat content and their plasma levels determine the rate at which they enter into the central nervous system.[92] However, insulin is secreted into the blood in response to changes in blood glucose concentrations. Incretin hormones such as GLP-1 can also stimulate its release. Thus, the relationship between insulin secretion and appetite regulation may not be completely straightforward.[97] Similarly, leptin secretion can become dissociated from body fat content. A decrease in leptin secretion during food deprivation is far greater than what may be expected by a decrease in body fat. The excessive response is designed to provoke the activation of compensatory mechanisms and prevent drastic depletion of body stores.[92] Thus, leptin and insulin are not useful as short-term biomarkers of satiety.[97] However, leptin signaling in the hippocampus, a brain structure involved in memory and learning may contribute to inhibiting the formation of associations between a rewarding event and the context in which the rewarding effects of the stimulus were experienced. In rats, it has been shown that leptin suppresses the expression and consolidation of learned appetitive behaviors.[356] Further, leptin may have a role in dopamine signaling but it is

not clear as to whether leptin decreases reward driven energy intake by inhibition of dopamine transmission or by making it more efficient.[109]

Thylakoid Membranes

Thylakoids found within the chloroplasts of plants, are flattened disc-like membranous vesicles in which the light-dependent reactions of photosynthesis occur.[357] Thylakoid membranes consist of a system of paired membranes encasing the lumen and separating it from the surrounding stroma of the chloroplast.[358] The grana are the cylindrical stacks of approximately 10 to 20 tightly appressed (where the membranes are held together by stacking forces) thylakoids that are interconnected by single unstacked stroma lamellae. Granum and stroma membranes differ in their protein composition; yet, the thylakoid membrane system is a continuous membrane and it encloses one inner luminal aqueous phase. The cylindrical granum of stacked membranes is surrounded by stroma lamellae that are interconnected through slits or junctions at the margins of the grana. This structure ensures contiguity of the membrane system across the entire grana-stroma network[357] (Figure 4.3).

The thylakoid membrane proteins are both intrinsic or membrane spanning, and extrinsic or attached to the surface of the membrane. The thylakoid membrane proteins and their bound pigments, chlorophyll and carotenoids contribute to 70% of the thylakoid mass.[359, 360] The proteins are found in large complexes spaced by lipids to maintain a tight molecular packing. The lipid-protein interface is comprised of solvation shell lipids around the integral membrane proteins that are weakly associated with the hydrophobic surface of the proteins, and lipids that are strongly bound to membrane proteins. The major lipids are the glycerolipids, such as galactolipids and phospholipids.[361] The photosynthetic thylakoid membrane system is the location of numerous biochemical reactions requiring regulation in response to light and

temperature conditions. Moreover, this system has to constantly battle and recoup from light and oxygen stress. It is of little wonder then that the thylakoid membrane system is a complex, yet, remarkable structure capable of accomplishing myriad functions.[358]

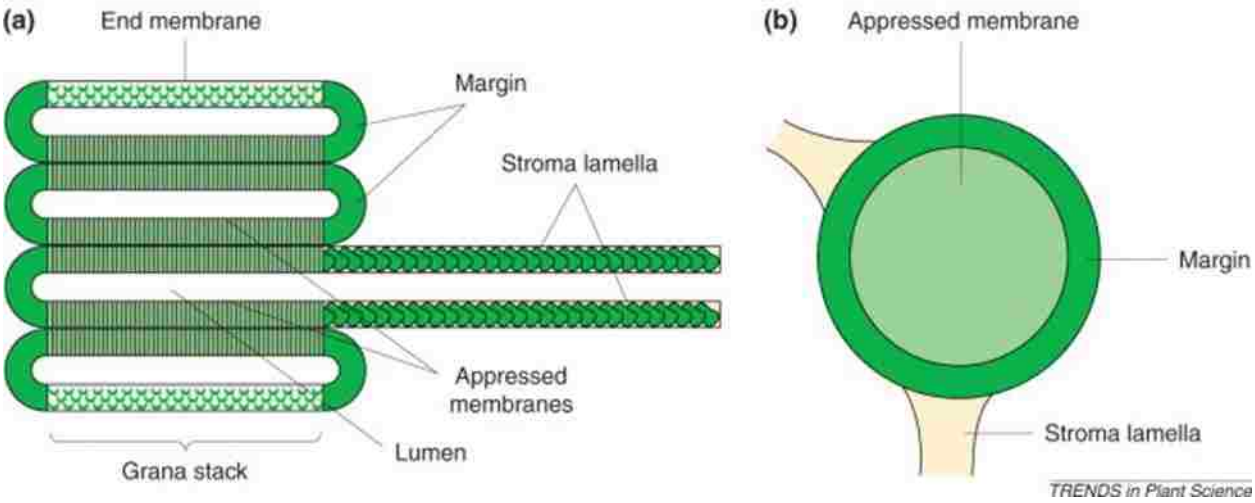

Figure 4.3 a) Cross section of grana. b) A grana disc viewed from above: Appressed domains, where the membranes are held together by stacking forces, are distinguishable from stroma-exposed domains (i.e. margins), end membrane, and stroma lamellae. The appressed domains and the end membranes of grana are essentially flat whereas the grana margins are curved. Several stroma lamellae are connected to one cylindrical granum of stacked membranes. Reproduced with permission from Elsevier, Albertsson P.[358]

Inhibition of Fat Digestion

Lipolysis requires that lipase and the lipid substrate are in close proximity at the oil-water interface. Therefore both lipase and the lipid droplet must partition from the aqueous and oil phases to the oil-water interface. However, the presence of various surface-active substances released from food, its digestion products, and GI secretions complicate the interfacial composition and influence the reaction.[55] For instance, the accumulation of bile salts containing highly surface-active bile acids at the interface has been shown to inhibit the adsorption of pancreatic lipase to its lipid substrate.[362] The inhibitory effect of bile salts can be subverted by the presence of colipase which forms a complex with lipase in the ratio of 1:1, and binds to the bile salt dominated interface to firmly anchor pancreatic lipase to the interface. Bile salts

promote disruption in the packing of the originally bound substances from food and GI secretions, and form their own clusters. The presence of bile salts as well as that of the lipase-colipase complex is required for hydrolysis to occur.[363] Thus, it is possible to regulate fat digestion by interfering with the oil-water emulsion interface or the interaction between the lipase-colipase complex and the lipid substrate.

Thylakoid membranes from spinach have been shown to suppress lipolysis by pancreatic lipase-colipase in a dose dependent manner, using *in vitro* mechanisms. Using electron microscopy it was shown that thylakoid membranes cover the entire interface of an oil droplet in water. Moreover, at a sufficient concentration, thylakoid membranes bind to the lipase-colipase complex in the presence of bile salts. Therefore, it has been suggested that thylakoid membranes inhibit lipolysis by binding to the active site of the lipase-colipase complex and by adsorption to the oil water interface, thereby preventing interaction between the enzyme complex and its lipid substrate.[54] The inhibition of lipolysis following removal of the lipids from the membranes suggests that the inhibiting component may be the membrane-spanning region of the intrinsic proteins.[54] However, removal of the lipids reduced the binding 10-fold in strength suggesting that the membrane lipids may also have a role in the inhibition of lipolysis.[364]

Studies conducted *in vitro* show that galactolipids from thylakoids of spinach inhibit lipase activity, at the interface of the oil-water emulsion.[55, 363] The major galactolipids are monogalactosyldiacylglycerol and digalactosyldiacylglycerol which comprise 50% and 20% respectively of the total lipid content of the membranes. *In vitro* studies show that galactolipids derived from the thylakoids of spinach delay lipid digestion. Unlike monogalactosyldiacylglycerol, digalactosyldiacylglycerol has a large polar group which may sterically hinder the formation of the lipase-colipase complex or prevent the adsorption of the

lipase-colipase complex to the lipid droplet. Thus, digalactosyldiacylglycerol may alter the properties of the interface to inhibit pancreatic lipase activity.

The galactolipids are resistant to displacement by surfactants such as bile salts, which is crucial for their inhibitory action. Galactolipids are not easily digested by pancreatic lipase but are slowly hydrolyzed by pancreatic lipase-related protein 2.[55] Therefore, regardless of whether the inhibiting component is protein or lipid, fat digestion is delayed rather than completely inhibited because both components are ultimately digested in the GI tract, to relinquish their hold on fat digestion. Thus, unlike the lipase inhibitor orlistat, the unpleasant effects of fat malabsorption such as oily discharge and anal leakage are avoided.[365-367]

The term thylakoids was paired with satiety, appetite, obesity, fat, and food intake using the operator 'AND'. The paired terms were used to perform a literature search on PubMed and Google Scholar. Six human trials were identified for the period ending June 30, 2015. These trials are reviewed against a backdrop of a selection of animal studies, and a summary of the human trials is presented in Table 4.1.

Animal Studies

In rats fed a high-fat diet, supplementation with thylakoids from spinach for 13 days as well as 100 days reduced food intake and body weight, reduced circulating levels of triacylglycerol, and increased plasma concentrations of CCK compared to high fat fed controls. Further, there was an increase in lipase and colipase secretion in thylakoid-treated animals compared to controls following binding of the membranes to the lipase-colipase complex.[54, 368] Additionally, at the end of 100 days, there was a reduction in body fat, serum glucose, and free

Table 4.1. Summary of the effect of thylakoid supplementation in humans, on obesity and metabolic parameters

Study	Impact on Metabolic Parameters					Impact on Obesity			
	Glucose	Free Fatty Acids	GI Hormones	Insulin	Leptin	Subjective Satiety	Urge for Specific Foods	Energy Intake	Body Weight/Fat
Kohnke *et al*, 2009[53] 11 male and female subjects	↔	↓ In 50 g thylakoids condition	CCK↑ Ghrelin↓	↔	↓ In 25 g and 50 g thylakoid condition but not in delipidated thylakoid condition	NE	NE	NE	NE
Stenblom *et al*, 2013[57] 20 female subjects	↔	NE	CCK↑	↓	NE	Hunger↓ Fullness ↔.	NE	NE	NE
Montelius *et al*, 2014[60] 38 female subjects	↔	NE	GLP-1↑ Ghrelin ↔	↔	NE	Hunger ↔ Fullness↔	Sweet foods↓ Chocolate↓ High Carbohydrate ↔ Savory ↔	↔	Weight ↓

(Table 4.1 continued)

Study	Impact on Metabolic Parameters					Impact on Obesity			
	Glucose	Free Fatty Acids	GI Hormones	Insulin	Leptin	Subjective Satiety	Urge for Specific Foods	Energy Intake	Body Weight/Fat
Stenblom *et al* 2014[59] 26 female subjects	↔	NE	NE	↔	↓	Hunger ↔ Fullness↔	Sweet foods↓ Chocolate↓ High Carbohydrate ↔ Savory ↔	NE	Weight ↔ Fat ↔
Rebello *et al*, 2015[58] 60 male and female subjects	↑	↔	NE	NE	NE	Over two hours: Hunger, Longing for food, Prospective Intake↓, Fullness↑ Over four hours: No differences	No differences in liking and wanting measured at four hours	↔ Non-significant reduction in pizza intake among males	NE
Stenblom et al 2015[369] 32 female subjects	NE	NE	NE	NE	NE	Over nine hours: Hunger↓ Response to How satiated are you? ↑	After snack buffet: Wanting↓ Liking ↔	↔	NE

fatty acids; however, there was no change in PYY concentrations. There was a reduction in serum leptin which was consistent with the reduction in adipose tissue.[368]

In another study, rats were fed a high-fat, low-fat, or thylakoid-supplemented high-fat diet for 32 days. While there was no change in energy intake, the rats in the thylakoid-supplemented group lost significantly more weight and percent body fat than the rats in the other groups. Increased binding to pancreatic lipase-colipase by thylakoids was also demonstrated.[289] In rats fed a standard chow diet, supplementation with thylakoids from spinach for 10 days reduced food intake, with no effects on blood glucose, but a reduction in the insulin response compared to the controls.[366] Following a four week high fat diet, thylakoids fed to pigs increased plasma CCK concentrations over a period of six hours, whereas ghrelin concentrations were reduced in the two to four hour period compared to a control condition without thylakoid administration.[370] Similarly, in pigs fed a high-fat meal with or without thylakoids, lipase and colipase secretion and portal blood CCK concentrations increased; however insulin levels were lower with thylakoid supplementation.[371]

In animal studies, in periods ranging from 13 to 100 days, reductions in body weight and body fat have been demonstrated. The effects on food intake are less consistent. The increase in lipase and colipase secretion suggests that there was an increase in enterostatin, since it is produced in equivalent amounts to colipase. However, none of the studies measured enterostatin concentrations or thermogenesis to provide evidence of a role for enterostatin. The rise in CCK concentrations was consistent in the studies that evaluated CCK, but glucose and insulin concentrations were too inconsistent to arrive at any firm conclusions as to the effects of thylakoids on glucose metabolism from the animal studies. Although, ghrelin concentrations

declined in one study and PYY showed no change, the effects on body weight and body fat in conjunction with a consistent increase in CCK release warrants investigation in human trials.

Human Studies

In a crossover study including 11 subjects, the satiety effect of four pesto sandwich meals ranging in energy content from 2,277 to 3,006 kJ (544 to 718 kcal, 56% to 66% fat) and containing 0 g thylakoids, 25 g thylakoids, 50 g thylakoids, or 25 g delipidated thylakoids, were compared.[53] The thylakoid containing conditions increased serum CCK concentrations at four and six hours, while ghrelin concentrations were reduced at two hours compared with the control condition. The rise in CCK and decline in ghrelin occurred regardless of the thylakoid dose or type; however, leptin concentrations were lowered at six hours in the 25 g and 50 g thylakoid conditions but not in the 25 g delipidated thylakoids condition. There was no difference in the plasma glucose concentrations among the conditions; but, thylakoid supplementation reduced the insulin response. Free fatty acids were reduced only in the 50 g thylakoid condition during the four to six hour period.

Twenty women were included in a crossover study to test the effect of a high carbohydrate breakfast meal (71% energy from carbohydrates) supplemented with 0 g, 3.7 g, or 7.4 g of thylakoids from spinach on satiety and metabolic parameters.[57] There was no dose response relationship. Hunger motivation, which encompassed the scores for hunger, urge to eat, and thoughts of food presented as a single score decreased ($p = 0.05$), over the two to four hour period in the thylakoids group compared to the control group; however, the ratings for fullness were not significantly different.

There was a concurrent increase in the plasma CCK concentrations over the two to four hour period ($p = 0.05$). Although plasma concentrations of insulin increased, the area under the

curve for blood glucose concentrations was not significantly different between the groups. However, the blood glucose concentrations peaked at 90 and 120 minutes under the thylakoids condition; whereas blood glucose declined below baseline at 60 minutes and continued to decrease in the control group. The authors contended that the thylakoids were important for control of body weight by prevention of postprandial hypoglycemia; however, the lowest average blood glucose concentration in the control group over a four hour period was approximately 4.8 mmol l^{-1}. This concentration does not reflect hypoglycemia. Nevertheless, the results of the CCK evaluation confirmed previous evidence.

In a placebo-controlled study including 38 overweight and obese women, 5 g of thylakoids consumed for three months caused a 5 kg reduction in body weight in the thylakoid treated group which was significantly greater than the control group.[60] At study visits on days 1 and 90, participants consumed the thylakoids or a placebo prior to a standardized breakfast meal followed by *ad libitum* lunch and dinner meals six and eleven hours later, respectively. Blood was drawn before breakfast and over a six hour period following breakfast for assessments of glucose, insulin, ghrelin, and GLP-1.

There were no differences in energy intake at meal tests conducted on day 1 and day 90, or subjective measures of hunger, fullness, urge for high carbohydrate foods, and urge for savory foods, measured over the 11 hour period. However, there was a time by treatment interaction showing a decreased urge for sweet foods ($p = 0.05$) and chocolate ($p < 0.05$) over the three hour period following lunch in the thylakoid treated group on day 90. Additionally, the area under the curve for plasma concentrations of GLP-1 increased on day 90 ($p = 0.04$) in the thylakoids group; but, there were no differences in ghrelin, glucose, or insulin concentrations between the groups. The study demonstrates that the inconsistencies in the effects of thylakoids on glucose

and insulin displayed in single meal studies may stabilize over a period of 90 days or earlier. However, the effects on the glycemic response and body weight need confirmation in future studies.

In one study, 26 overweight women were placed on an energy restricted diet supplemented with 5.6 g thylakoids extracted from spinach leaves, for two months. Compared to the control group, supplementation with thylakoids reduced the urge for chocolate measured on the first and last days of the study (time and treatment interaction, $p < 0.05$). Serum leptin levels reduced ($p = 0.01$), but there were no differences in body weight, body fat, and subjective measures of satiety, urge for high carbohydrate foods, and urge for savory foods. Additionally, there were no differences between the groups in the blood glucose, insulin, and lipid concentrations.[59]

Regardless of the type of diet, adherence to a diet regimen that promotes energy restriction is difficult for most people.[70] Following menus that were prescribed to meet a 15% energy restriction may have imposed an additional burden on the subjects. Moreover, individual energy requirements were assessed using the Harris Benedict equation which may be lacking in predictive accuracy.[372, 373] Nevertheless, the inconsistent effect of thylakoids intake on body weight compared with the previous study iterates the need for long term studies evaluating the effect of thylakoids on body weight.

A placebo-controlled crossover trial was conducted to compare the effect of 5 g of thylakoids supplementation with a placebo on satiety and reward-induced eating behavior in 32 women.[369] The results showed that hunger decreased ($p < 0.05$) under the thylakoids condition over a period of nine hours, during which subjects were provided with standard breakfast and lunch meals as well as a snack buffet. The response to the question “How satiated are you?” was

also greater ($p < 0.01$) under the thylakoids condition. Treatment with thylakoids reduced wanting ($p < 0.05$) for snack foods considered highly desirable, including salty, sweet, and sweet and fat foods; however, the validity and reliability of the questionnaires used to measure wanting must be established in future studies. There was no effect on energy intake at the *ad libitum* snack buffet.

In a placebo-controlled crossover trial, 60 overweight and obese individuals ate a standardized breakfast and lunch meal.[58] Immediately before lunch, 5 g of thylakoids or a placebo were administered. Satiety was measured over a period of four hours following lunch, and reward-induced behavior was assessed at four hours. Blood was drawn before breakfast and two hours following lunch. Energy intake was assessed at an *ad libitum* pizza meal served four hours after lunch. Compared to the placebo, fullness increased ($p = 0.04$), and hunger ($p < 0.01$), longing for food ($p < 0.01$), prospective intake ($p = 0.01$), and desire for something savory ($p < 0.01$) decreased in the thylakoid group over two hours, but there was no difference in the desire for something sweet in these study participants. There were no effects on satiety measured over four hours. Energy intake at the pizza meal was not significantly different between the groups; however, males in the thylakoids group reduced energy intake of the high fat/savory pizza by 527 kJ (126 kcal). The difference was not statistically significant since the study was not sufficiently powered to detect gender differences.

There was a concurrent increase in plasma glucose concentrations; but, there were no significant differences in lipid concentrations between the groups. Further, subjective measures of liking and wanting measured at four hours were not significantly different. The method used for measuring liking and wanting has not produced results that were completely consistent in the past.[113] Although manipulating the reward system has great potential, it appears that

questionnaires are unable to capture the dynamic nature of the reward system, and other methods such as neuroimaging may provide more accurate results.

In human trials, the effects of thylakoids on perceptions of satiety measured using visual analog scales have been inconsistent. Three studies provided evidence of an increase in satiety[57, 58, 369] and two studies showed no effect on satiety.[59, 60] Two studies demonstrated a decrease in the urge for sweet foods and chocolate among women measured subjectively,[59, 60] and one study found that males reduced their intake at a pizza meal.[58] Males display a yearning for savory foods and females prefer high fat sweet foods such as chocolate.[374, 375] Thus, thylakoids may influence food cravings which are a form of wanting or eating behavior that prompts approach toward and consumption of a reward.[21]

The increase in CCK concentrations has been consistent in the human[53, 57] as well as the animal studies that measured the effect of thylakoids on CCK.[54, 368, 370] Cholecystokinin has been shown to reduce food intake in both human and animal species; but, the inhibitory effect is generally observed relatively shortly after food ingestion and is of a brief duration.[316] Thus, CCK has been shown to be effective in meal termination which reflects satiation rather than satiety.[376] In the studies that found an increase in CCK after thylakoids supplementation, the rise was two to three hours after ingestion which suggests a delay in fat digestion; but the effect on blood concentrations of free fatty acids has been inconsistent.[53, 58-60]

The increase in GLP-1at the end of 90 days of consuming the thylakoids was accompanied by a significant weight loss. Cholecystokinin may regulate the effects of other gut hormones such as ghrelin and GLP-1.[324, 325] Thus, it is likely that CCK released over a period of three months by influencing other hormones that mediate satiety, produced a sustained effect that promoted weight loss. Thus, thylakoids appear to have the potential to provide an effective

therapy for regulating appetite, particularly the reward system; however, the need for well-controlled trials to assess its effects on weight management cannot be overemphasized.

Conclusions

Humans have a strong innate drive to acquire food not only to survive the present but to sustain life during lean periods. Shaped by natural selection, human awareness and motivation produces behavioral characteristics that provide a survival advantage. Thus, humans are prone to eat beyond their energy needs and in the absence of a metabolic need to eat. Foods that appeal to the palate are in no shortage in the modern environment. These foods can activate brain reward circuitry beyond their evolved 'survival advantage' limits.[377] Foods high in fat invoke an undeniably pleasurable sensation and excessively stimulate the brain's reward pathways leading to overconsumption. Moreover, consumption could occur without conscious control through the activation of reward pathways outside of awareness.

The gut possesses an intricate system of lipid sensing and induction of signals to the brain that precipitate satiation, satiety, and modulation of reward induced eating behavior. The interplay between neuronal pathways acting through endocrine and neuronal feedback signals from the periphery often results in the potentiation of signals. For instance, the anorectic effects of central leptin and peripheral CCK potentiate each other.[164] Thus, the manipulation of fat metabolism to promote its presence in the GI tract may be a way to counteract forces that make resistance to its appeal especially difficult.

Thylakoid membranes act on ingested fat to stimulate satiety and perhaps blunt the rewarding value of fat. Although the results need to be confirmed in future studies, it is likely that thylakoids exert a sustained effect on appetite to reduce body weight. The mechanisms by which thylakoid membranes influence eating behavior are unclear. It could be the prolonged

presence of fats in the GI tract stimulating the release of peptides involved in appetite regulation, or the endogenous production of enterostatin. Well-controlled human trials that include neuroimaging assessments are needed to elucidate the precise mechanisms by which thylakoid membranes influence appetite, particularly, reward-induced eating behavior. Nevertheless, by its actions on fat, thylakoids do offer a means to strengthen the resolve to refrain from eating especially in an environment where there is superfluous access to foods processed to deliver qualities that some may find irresistible. Strategies that curtail the overriding of well-regulated adaptive behaviors cannot be overlooked.

CHAPTER 5
THE ROLE OF MEAL VISCOSITY AND OAT B-GLUCAN CHARACTERISTICS IN HUMAN APPETITE CONTROL: A RANDOMIZED CROSSOVER TRIAL

Introduction

The prevalence of obesity has increased in every region of the world, including several countries with low and middle incomes.[378] Evidence for a leveling off in the steep rises previously observed in high income countries, however, does not amount to a reversal of the obesity epidemic.[379] The US will have a projected 65 million more obese adults in 2030 compared to 2010.[380] Reversing this epidemic requires developing effective ways of curbing excessive energy intake. The role of dietary fiber in promoting satiation and satiety has been the focus of a vast amount of research, and there is evidence to indicate that consumption of fiber-rich foods has a modest long-term effect on weight loss.[26, 27]

Dietary fiber is classified as soluble and insoluble fiber based on its solubility in aqueous enzyme solutions similar to those in the gastrointestinal tract.[279] Some soluble fibers such as β-glucan form a viscous solution when mixed with liquids. Viscosity is an important rheological property of β-glucan, and is associated with beneficial physiologic responses that mediate appetite regulation such as delayed gastric emptying, increased stomach distension, and delayed intestinal transit.[33] The increased viscosity of intestinal contents prolongs transit time and the absorption rate of nutrients.[288] A thickening of the unstirred water layer in the intestine further impedes absorption.[381] Enhanced interaction between nutrients and the intestinal mucosa

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stimulates the release of appetite regulating peptides which either function distantly as hormones or activate nearby nerve fibers.[382] Some afferent neurons in the gastric mucosa are mechanoreceptors while others may transduce chemical or other signals of satiation.[171] Gastric and intestinal signals may also work in synergy to influence appetite.[171]

. When making choices of foods rich in fiber to enhance satiety it is also important to consider the manner in which the food is processed. β-glucan, is abundant in oat kernels and exhibits a high viscosity at relatively low concentrations.[383] However, the processing of oats, the concentration of soluble fiber, and the processing of products containing β-glucan affect the amount, solubility, molecular weight, structure, and functionality of β-glucan.[383, 384] Kilning, a hydrothermal treatment used in the processing of oats induces structural changes in the oats, as do the high shear forces of the flaking process. Old fashioned oats (SO) and instant oats (IO) have different kilning and flaking processes. Further, unlike old-fashioned oats, instant oats are cut which exposes the endosperm and affects the β-glucan, a major component of endosperm cell walls. In studies that investigated the effects of β-glucan on satiety, data on the physicochemical properties of the fiber are singularly lacking.[191, 193, 195, 267, 273] Thus, investigating the physicochemical properties of the fiber would help clarify the mechanisms by which β-glucan affects satiety, the amount of β-glucan that elicits an appetite response, and the processing techniques that could facilitate development of satiety-enhancing products to replace foods in the usual diet.

In a previous study [38] we demonstrated that a breakfast meal containing 250 kcal serving of SO increased satiety compared to an isocaloric meal containing 250 kcal of the most widely consumed oat-based ready-to-eat breakfast cereal (RTEC) in the United States ((based on IRI Liquid Data, 52 Weeks Ending March 11, 2012). The present study measured the satiety effect

of breakfast meals containing single servings (150 kcal) of oatmeal (IO and SO) and an isocaloric serving of the RTEC over four hours. Instant oats and SO differ in the manner in which they are processed; hence, in addition to portion size, the purpose of the present study was to determine if differences in processing influence the outcome. Since replacing foods in the usual diet with foods that increase satiety may be a means of reducing energy intake, a popular oat-based RTEC was used as a comparator even though it differed in nutrient composition and β-glucan content. The meal viscosity, starch digestion kinetics, and β-glucan characteristics of each breakfast meal were also determined. It was hypothesized that both the IO and SO breakfast meals would have a higher viscosity and would increase satiety more than the RTEC.

Methods

Subjects

Forty-eight healthy subjects 18 years of age or older were enrolled in a randomized, three treatment, crossover trial. All subjects participated in an initial screening that involved measurement of body weight; height; waist and hip circumferences; vital signs (blood pressure and pulse rate); chemistry-15 panel (glucose, creatinine, potassium, uric acid, albumin, calcium, magnesium, creatine phosphokinase, alanine aminotransferase, alkaline phosphatase, iron, cholesterol [total, high density lipoprotein, low density lipoprotein], and triglycerides); complete blood count with differential; and beta-human chorionic gonadotropin urine pregnancy test (in females of child-bearing potential). Health was further assessed through the administration of a medical screening questionnaire. Female subjects also completed a menstrual cycle questionnaire to ensure that test days would fall within the luteal phase of the menstrual cycle.[385] Inclusion criteria were: (i) healthy individuals taking no medication other than birth control or hormone replacement (ii) willing to use an effective method of birth control during the course of the study,

if female and capable of bearing children. Exclusion criteria were: (i) women who were pregnant or nursing, (ii) self-reported weight gain or loss of $\geq$ 4 kg in the last 3 months, (iii) fasting glucose >126 mg/dL, (iv) dietary restraint score $\geq$ 14, as assessed by the Dietary Restraint Scale of the Eating Inventory [140] and (v) allergy or intolerance to oats or milk.

This study was approved by the Institutional Review Board of the Pennington Biomedical Research Center, Baton Rouge, where the study was conducted. Participants provided written informed consent. The trial was registered on ClinicaTrials.gov with registration number NCT01666561. Recruitment for clinical trials conducted at The Pennington Biomedical Research Center is coordinated by the Recruitment Core which is responsible for the design and placement of advertisements, and screening of all incoming requests to determine study eligibility. Participants were recruited from Baton Rouge and the surrounding areas.

Study Design

The test breakfast meals consisting of Quaker Instant Oatmeal Flakes™ (IO), Quaker Old Fashioned Oatmeal™ (SO), and Honey Nut Cheerios™ (RTEC), were served to participants on three test days, separated by at least a week. There were six possibilities for assigning the order of the cereals for the three visits (abc, acb, bac, bca, cab, cba). Each subject was randomly assigned to one of these orders so that eight subjects were assigned to each order. The randomization was done by the study statistician and participants were enrolled by the study coordinator. The study dietitian who had no interaction with study subjects provided the test meals for the participants and had sole access to the random assignment until data analysis. The breakfast meals contained 217.5 total kcal, consisting of 150 kcal from the cereal, and 67.5 kcal from lactose-free, fat-free milk. The old fashioned oatmeal, (one standard serving, 40 g dry weight), was prepared by adding one cup room temperature water (240 g) and microwaving at

high power for three minutes. The instant oatmeal (40 g dry weight) was stirred, following the addition of one cup boiling water. Both were allowed to stand for one minute, and were served with 184.2 g of cold milk. The RTEC (38.2 g dry weight), was prepared by adding 184.2 g of cold milk, and was served with 240 g of water. The participants had the option of adding one g of Splenda™ and one-half teaspoon of cinnamon to the oatmeal. If the participant added the Splenda™ and cinnamon to the oatmeal, they were required to add the same amounts of both to the RTEC.

At each test breakfast visit, participants arrived at the center after fasting (except for water) for 10 hours overnight. They were also required to avoid alcohol and strenuous exercise for 24 hours prior to the test meal. To determine the presence of colds or allergies that might affect taste, participants were required to complete a questionnaire and were asked to return on another day if such a condition was present. Prior to serving the test meal hunger, fullness, desire to eat, and prospective intake were assessed using electronic visual analog scales (VAS).[89, 137] Participants rated each subjective state on a continuous line that was anchored using the descriptors 'Not at all' to 'Extremely', and displayed on a computer screen. Visual analog scales were scored by the computer on a 0 to 100 unit scale and the score was sent directly to the database. Hunger, fullness, desire to eat, and prospective intake, were assessed. The subjects were presented with their first breakfast test and given 20 minutes to eat it. Test meals were supervised to ensure that the entire breakfast was eaten. Visual analog scales were then administered at 30, 60, 120, 180, and 240 minutes following the start of the breakfast meal and subjects were asked an open ended question (How do you feel?) at each of these time-points to elicit any adverse events. Subjects were required to remain in the dining area during this period, and refrain from any food or drink.

Kinetics of In Vitro Glucose Release

Oatmeal was prepared as described in the study design, and allowed to rest for one minute. This oatmeal and dry RTEC were first analyzed for their sugar content by high performance liquid chromatography and total starch content by standard American Association of Cereal Chemists (AACC) procedures.[386] Thus, their total glucose content (G_{tot}) was determined. The cereals then underwent a three-stage *in vitro* digestion procedure described by Sopade and Gidley [387] to evaluate the kinetics of glucose release. The aim of this method which has been used extensively in the literature[388-390] is to explore differences in digestibility of starches caused by differences in their physicochemical and structural characteristics. During this procedure, α-amylase, pepsin, and pancreatin with amyloglucosidase were added in a timely order with adjustments to their corresponding working pH to mimic digestion in the oral cavity, stomach, and small intestine. Glucose release from about 500 mg of digested food in the simulated small intestine phase was monitored over the course of three hours using the Accu-Check Aviva glucometer (Roche Diagnostics, Indianapolis, IN) which was previously calibrated for glucose response and interactions with other sugars potentially present in the matrix. Data were compiled as digestograms ($G_{t=time} \times 100/G_{tot}$ versus time). Digestograms were fitted using a first order kinetic law, from where initial glucose release (glucose concentration at the beginning of the intestinal phase [$G_{t=0}$]), rate of glucose release (time constant of the first order law) time of half-of-total glucose release (time for $G_t \times 100/G_{tot} = 50\%$), and area under the curve (AUC) were calculated. Area under the curve values were normalized with a white bread control ($AUC_{white\ bread} = 100$).

Characterization of β-glucan

The RTEC, SO, and IO, were ground to flour. The β-glucan component was extracted from dry ground oat flakes and RTEC, according to the procedure described by Rimsten *et al.*[391] After extraction, dialysis purification and drying, the β-glucan was subjected to molecular weight, distribution, and radius of gyration analysis using high performance size exclusion chromatography with multi angle light scattering detection (sample preparation: dissolution at 0.1 % w/w in mobile phase; mobile phase: 50 mM $NaNO_3$ and 200 ppm NaN_3 in deionized water; flow rate: 0.8 $mL.min^{-1}$; columns: Agilent PL aquagel-OH MIXED-H 8μm 300×7.5 mm column and 50×7.5 mm guard column; Light Scattering detector: Wyatt Dawn Heleos II; Differential Refractive Index detector: Wyatt Optilab T-rEX, Wyatt Technology Europe GmbH, Dernbach, Germany). Molecular weight and radius of gyration calculations (parameters: second order Berry Model; dn/dc value: 0.146 $mL.g^{-1}$) were performed using Wyatt Astra V software (Wyatt Technology, Santa Barbara, California).

The β-glucan content was measured using standardized AACC procedures.[392] Briefly, β-glucan was hydrolyzed by lichenase into oligosaccharides, which were converted into glucose by β-glucosidase. The amount of glucose released was measured by UV absorbance with glucose oxidase/peroxidase. The β-glucan content was calculated based on the amount of released glucose.

Breakfast Viscosity

The viscosity of the two breakfast meals was measured in a process simulating the gastric phase of digestion *in vivo*. A 250 kcal serving of each breakfast meal (66.8 g dry weight of oatmeal and 63.6 g dry weight of RTEC) was prepared as described in the study design, using proportionate amounts of liquids. Although the *in vitro* experiment tested a larger serving than

the portion size used in the study, the relative effects are expected to be the similar, since the same ratio of cereal to liquid was used. Each sample underwent the first two phases (oral and gastric) of the three-stage *in vitro* digestion procedure described by Sopade and Gidley[387] and modified as follows: 61.2 mL of artificial saliva (250 $U.mL^{-1}$ α-amylase in carbonate buffer at pH 6) was used for the oral phase, and 334 mL of a 1 $mg.mL^{-1}$ pepsin in 0.02 M HCl was used for the gastric phase, for each sample. The viscosity of oatmeal and the RTEC was measured in triplicate at 0, 30, 60, 90, and 120 minutes in a 1000 mL beaker by using a viscometer (viscometer: Brookfield DV-I+ ; vane spindle #71 ; speed: 100 rpm [Brookfield, Middleboro, Massachusetts]).

Statistical Analysis

A mixed model analysis of variance for a crossover trial was performed to analyze the primary outcome. A strength of the crossover design is that significance of differences among treatments is evaluated in terms of pooled within subject comparisons. In parallel arm trials, pre-randomization covariates such as gender, age, and body mass index (BMI), can be included in analytical models to explain extraneous variability in outcome variables and increase precision in estimators of treatment effects. In cross-over trials pre-randomization covariates can influence only comparisons between groups containing different subjects in contrast to within subject comparisons where they have no influence on estimators of treatment effects or their precision.[393] Hence, covariates were not included in the models employed in this analysis.

Visual analog scale scores for hunger, fullness, desire to eat, and prospective food intake, were assessed at baseline and at 30, 60, 120, 180, and 240 minutes following the start of the breakfast meal. The AUC for each assessment was estimated using the linear trapezoidal rule and calculated as the area between the zero change line and the measured change curve which could

be either above or below the zero change line. The statistical model included fixed effects (treatment sequence effects [residual treatment carryover effects from test day 1 to test day 2 and test day 2 to test day 3], test day main effects, and treatment main effects) and random effects (subjects within treatment sequence groups). Scores at each assessment time were analyzed using a mixed model ANOVA for a doubly repeated-measures crossover trial, where the first repeated measures variable was the test day, and the second was the time since start of breakfast. Any baseline differences in VAS scores among treatments were normalized to zero, and the resulting changes from baseline were summarized as least squares means plotted for each cereal type across the assessment times. The p-values were adjusted for multiple comparisons using the Bonferroni method. Differential treatment effects with respect to AUC and per time point were compared using SAS (version 9.2, 2002-2008, PROC MIXED; SAS Institute, Cary, NC).

Significance of among-treatment differences in viscosity, molecular weight, and radius of gyration was assessed using ANOVA. Values were expressed as means ± standard error. The AUC was used to analyze the kinetics of starch digestion and glucose release and the values were expressed as means ± standard deviation. Statistical significance was set at $p < 0.05$.

During the planning phase of the study, sample size was estimated using G*Power, Version 3.1.2 (F. Faul, Universitat Kiel, Kiel, Germany) with the following assumptions: (i) power ≥ 0.75 was considered acceptable, (ii) the significance level under the null hypothesis was set at $\alpha = 0.05$, (iii) the primary outcome was VAS AUC with *a priori* standard deviation assumed to be 3047 mm×min based on previous research [394] and (iv) the null hypothesis was to be tested against a two-directional alternative. The study was sufficiently powered with 43 participants for detecting a minimum difference of 1258 mm×min between cereal types, which is

similar to observed differences in AUC (1213 mm×min) for desire to eat from a similar study that assessed appetite sensations.[394]

Results

Forty eight subjects were enrolled in the study, of which three were underweight (BMI < 18.5), 22 were normal weight (BMI from 18.5 to 24.9), nine were overweight (BMI from 25 to 29.9) and 14 were obese (BMI ≥ 30). Five participants were unable to complete the study. Two participants moved out of the area, one participant had difficulty in obtaining transportation to the Center, one participant had a conflicting schedule, and one participant had a change of mind. There were no adverse events. Descriptive characteristics of the subjects at baseline are summarized in Table 5.1. A nutrient analysis of the breakfast meals obtained from the nutrition facts label, and the β-glucan content which was measured are presented in Table 5.2.

Table 5.1 Subject characteristics at baseline including age, height, weight, body mass index, waist circumference, and gender

	n = 48	
	Mean	Standard Deviation
Age	29.8	9.9
Height (cm)	167.2	9.9
Weight (kg)	75.7	19.5
Body Mass Index (kg/m^2)	27.1	6.7
Waist Circumference (cm)	85.4	15.1
	n (%)	
Gender		
Female	28 (58.3)	
Male	20 (41.7)	

Hunger and Fullness

The four hour AUC for VAS ratings of hunger were not statistically different among the three breakfast cereals; however, IO consumption reduced hunger at 60 minutes significantly more than the RTEC (p= 0.01) (Figure 5.1, A). IO consumption increased fullness significantly

more than the RTEC over the four hour period following the meal (AUC IO: 9660.62 ± 885.5 mm × min ($p = 0.04$) and at 60 minutes ($p < 0.01$). IO consumption increased fullness more than SO at 60 minutes ($p = 0.04$), although fullness was not significantly different between these two meals over the four-hour period (Figure 5.1, B).

Table 5.2 Energy and nutrient content of breakfast meals

	SO*	IO†	RTEC‡	Lactose-Free, Fat-Free Milk
Energy (kcal)	150	150	150	67.5
Fat (g)	3.0	3.0	2.1	0
Protein (g)	5.0	5.0	2.7	6.0
Total Carbohydrates (g)	27.0	27.0	30.0	9.8
Total Fiber (g)	4.0	4.0	2.7	0
Soluble Fiber (g)	2.0	2.0	1.1	0
β-Glucan (g)	1.6	1.6	1.0	0
Sugar (g)	1.0	1.0	12.3	9.0
Sodium (mg)	0	0	218.3	93.8
Serving Size (g)	40	40	38.2	184.2

*Quaker Old Fashioned Oatmeal; (Pepsico Inc.Barrington IL)
† Quaker Instant Oatmeal Flakes; (Pepsico Inc.Barrington IL)
‡Honey Nut Cheerios; (General Mills Inc. Minneapolis MN)

Desire to Eat and Prospective Intake

IO consumption reduced desire to eat significantly more than the RTEC over the four hour period (AUC: IO: 9129.38 ± 884.33 mm × min versus RTEC: 7064.69 ± 886.3 mm × min, $p = 0.01$), at 60 minutes ($p < 0.01$), and at 120 minutes ($p = 0.01$) (Figure 5.1, C). Prospective intake, was significantly lower after both IO and SO consumption compared to the RTEC over the four-hour period (AUC IO: 7968.55 ± 769.24 mm × min, $p < 0.01$, versus RTEC; SO: 6954.12 ± 769.26 mm × min, $p = 0.04$ versus RTEC; RTEC: 5525.98 ± 771.02 mm × min). IO consumption reduced prospective intake at 30 minutes ($p = 0.01$), 60 minutes ($p < 0.01$) and 120 minutes ($p < 0.01$) more than consumption of the RTEC. SO consumption also decreased

prospective intake at 30 minutes (p = 0.02) and 120 minutes (p = 0.02) more than consumption of the RTEC. IO consumption lowered prospective intake at 60 minutes more than SO (p = 0.02), but prospective intake was not significantly different between the two over the four-hour period (Figure 5.1, D).

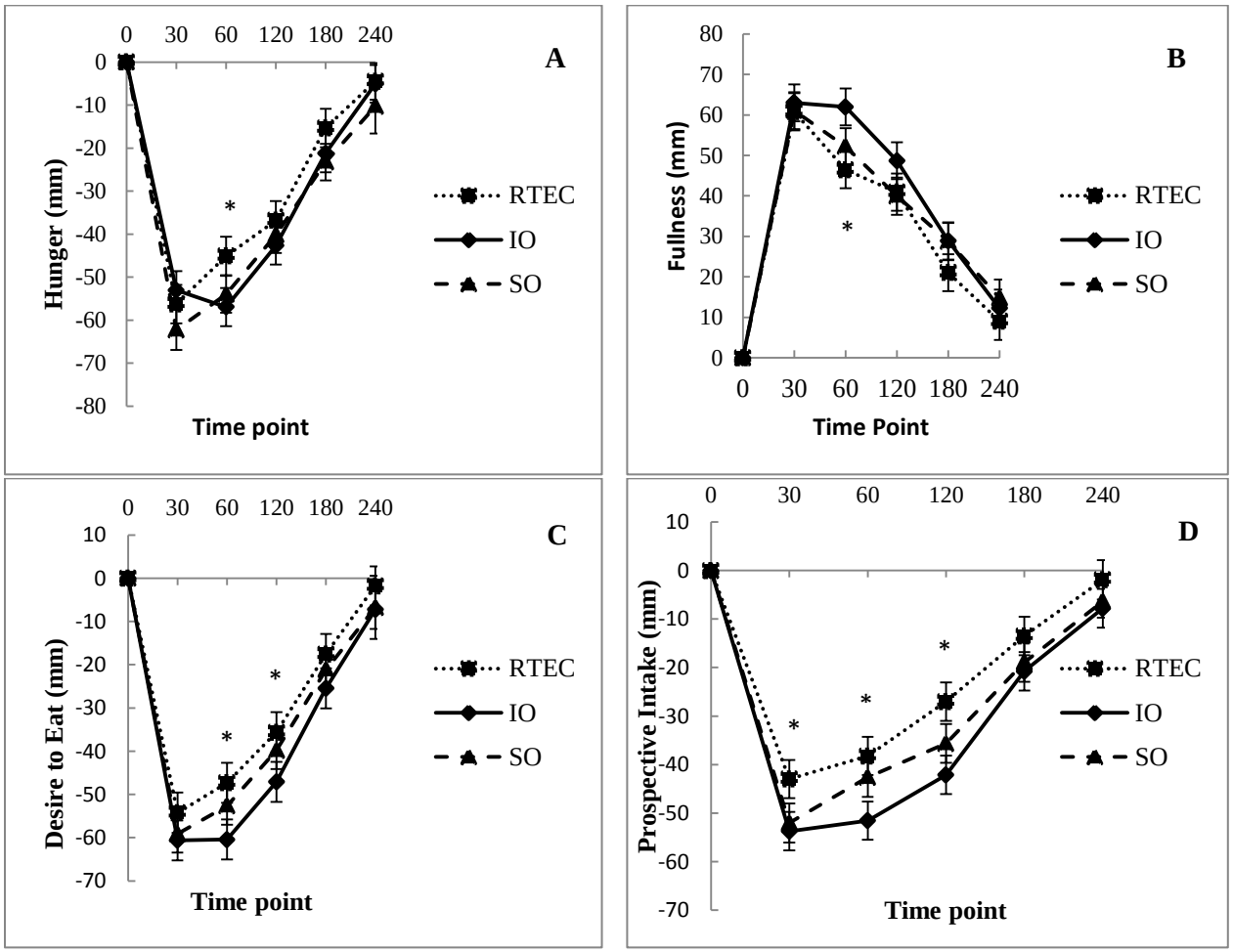

Figure 5.1 Visual analog scale ratings for hunger (n = 48) before and after consumption of instant oatmeal (IO), old fashioned oatmeal (SO) and a ready-to-eat breakfast cereal (RTEC): (A) Differences in hunger ratings among the three breakfast cereals as assessed by AUC were not statistically significant.*Least squares mean was different between IO and the RTEC at 60 minutes (p = 0.04). (B) Fullness ratings were different between IO and the RTEC by AUC. *Least squares mean was different between IO and the RTEC at 60 minutes (p < 0.01). (C) Desire to eat ratings were different between IO and the RTEC by AUC.*Least squares means were different between IO and the RTEC at 60 minutes (p < 0.01) and 120 minutes (p < 0.02). (D) Prospective intake ratings were different between the two types of oatmeal and the RTEC by AUC.*Least squares means were different between IO and the RTEC at 30 minutes (p < 0.02), 60 minutes (p < 0.01), and 120 minutes (p < 0.01)

Kinetics of Glucose Release

The kinetics of starch digestion and glucose release were not significantly different among the three breakfast cereals (IO: 99 ± 3 g × min, SO: 100 ± 3 g × min, RTEC: 98 ± 2 g × min, $p > 0.05$).

Physicochemical Characteristics of β-glucan

The molecular weight of β-glucan was higher in both varieties of oatmeal than in the RTEC (IO: $3.89x10^5 \pm 5.46x10^3$ Da, SO: $3.78x10^5 \pm 5.46x10^3$ Da, RTEC: $2.21x10^5 \pm 5.46x10^3$ Da ($p < 0.01$[IO versus RTEC], $p < 0.01$[SO versus RTEC]) (Figure 5.2, A). Additionally, the hydrated β-glucan molecules in oatmeal formed larger spheres than those in the RTEC as the radius of gyration was 50.23 ± 0.90 nm for IO, 48.2 ± 0.90 nm for SO, and 36.83 ± 0.90 nm for the RTEC ($p < 0.01$ [IO versus RTEC] and $p < 0.01$ [SO versus RTEC]) (Figure 5.2, B).

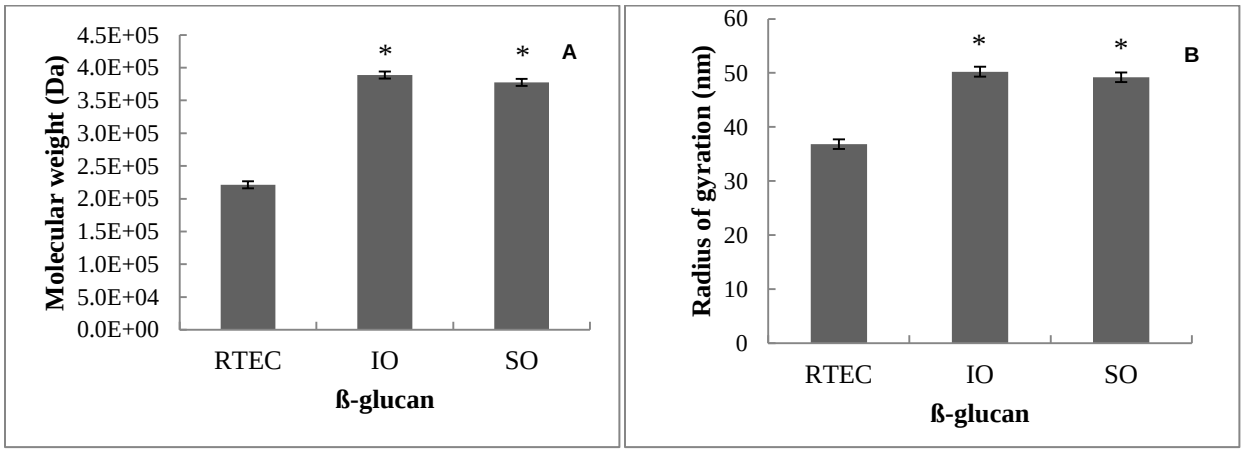

Figure 5.2 Least squares means of the molecular weight (Mw) in Daltons (Da) (A) and radius of gyration (Rg) in nanometers (nm) (B), of the β-glucan content of instant oatmeal (IO), old fashioned oatmeal (SO) and the ready-to-eat breakfast cereal (RTEC). Both varieties of oatmeal had higher molecular weight and radius of gyration that the RTEC ($p < 0.01$). Values are mean ± standard error

Meal Viscosities

IO (7397.17 ± 1564.51 centipoise) exhibited a higher initial viscosity (time = 0) than SO (1063.33 ± 1564.51 centipoise); and RTEC (175.17 ± 1564.51 centipoise), after oral and initial

gastric digestion (p = 0.03 [IO versus SO] and p = 0.02 [IO versus RTEC]) (Figure 5.3, A). IO (87.92 ± 3.12 centipoise) and SO (85.13 ± 3.12 centipoise) demonstrated a greater subsequent viscosity (time ≠ 0) than the RTEC (75.92 ± 3.12 centipoise) during the remainder of the *in vitro* gastric simulation process (p = 0.01 [IO versus RTEC] and p < 0.05 [SO versus RTEC]) (Figure 5.3, B).

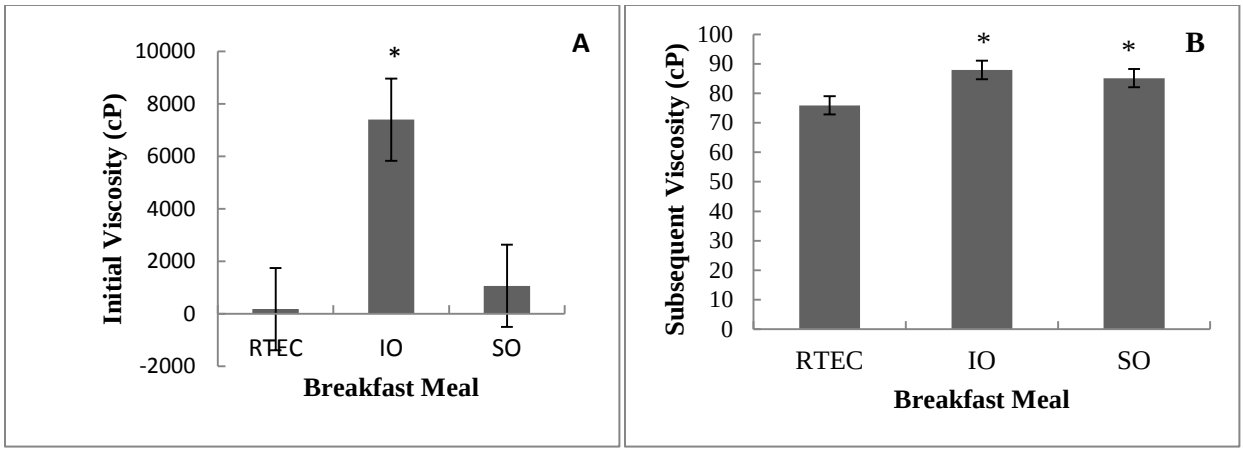

Figure 5.3 Mean viscosities of oatmeal and ready-to-eat-breakfast cereal (RTEC) meals observed at the *in vitro* simulation of digestion. Viscosity values are the means of three replicates and expressed in centipoise (cP) ± standard error. Instant oatmeal (IO) exhibited a higher viscosity than old fashioned oatmeal (SO) (p = 0.03) and the RTEC (p = 0.02) after oral and initial gastric digestion at time = 0 (A). IO (p = 0.01) as well as SO (p < 0.05) demonstrated significantly greater viscosity than the RTEC during the remainder of the in vitro gastric simulation process (B).

Discussion

Consumption of dietary fiber has been shown to increase satiation and satiety and have a modest effect on long term weight loss.[26, 27] In this study, the satiety effects, β-glucan characteristics, and meal viscosities of three different oat-based breakfast cereals were assessed. IO consumption increased fullness, suppressed desire to eat, and reduced prospective intake more than the RTEC did over a four-hour period, and consistently at the 60 minute time-point. SO consumption reduced prospective intake more than the RTEC did, but did not significantly improve any other satiety measures. The content, molecular weight, and radius of gyration of β-

glucan in both oatmeal varieties were higher compared to the RTEC, possibly contributing to greater viscosity of the oatmeal types.

Food processing may influence satiety by changing the viscosity and physicochemical properties of β-glucan. Viscosity (η) is a function of concentration (c) and molecular weight (Mw) of the polymers ($\eta \propto cM_w^{\alpha}$ where α is a parameter depending of the shape of the polymer).[395] Mechanical processing or excessive heat treatments can change the β-glucan structure reducing its molecular weight and viscosity. Extrusion, a process often used in the production of breakfast cereals can affect the physicochemical properties of the fiber based on the processing techniques employed, and the composition of the ingredients.[257] Both sugar and salt increase the apparent viscosity of β-glucan solutions.[396] Although the oatmeal had no sodium and no added sugar it had a higher molecular weight, radius of gyration, and viscosity than the RTEC, further indicating that the functional properties of β-glucan vary among products.

A serving size (150 kcals) of IO which was composed of thinly cut flakes increased all indicators of satiety, except hunger, compared to an isocaloric oat-based RTEC. However SO, which consisted of thicker flakes only decreased prospective intake compared to the RTEC. Using echo-planar magnetic resonance imaging, Hoad et al [242] showed that satiety increases as initial viscosity of the meal increases. Initial viscosity likely modulates a cephalic phase effect in which the orosensory factors play an important role in the overall satiety response. Previous research shows that intestinal infusion of a soup produces a weak effect on the control of appetite, which is progressively amplified with gastric and oral stimulation.[397] In the present study, IO had greater initial and subsequent viscosities compared to the RTEC, whereas SO only had greater subsequent viscosity compared to the RTEC. Thus, the greater initial viscosity of the IO may have increased oral stimulation and produced a greater satiety effect than SO, suggesting

that regulation of appetite works in concert with oral, gastric, intestinal, and post-absorptive mechanisms.

It is likely that the thinly cut IO flakes hydrated more easily with the addition of boiling water compared to the thicker SO flakes and may explain why the two oatmeal varieties displayed different viscosities when they first entered the stomach as estimated in the *in vitro* simulation. The addition of oat bran (4 or 8 g β-glucan) to biscuits and juice (enriched biscuits and enriched juice) increased satiety compared to the control meal without β-glucan, but, β-glucan added only to biscuits (enriched biscuits and juice) did not produce this effect.[43] In a comparison between two cereals, oatmeal (2.6 g β-glucan) prepared with hot water produced greater viscosity, larger hydration molecules, and increased satiety compared to a ready-to-eat oat based cereal (1.7 g β-glucan) served with cold milk.[38] Thus, sufficient hydration of the fiber is important for inducing the process of satiety.

Our previous study shows that larger portion sizes of IO and SO (250 kcals, 2.6 - 2.7 g β-glucan) increase satiety more than isocaloric servings of the RTEC (1.7 g β-glucan), particularly in the two- to four- hour period following consumption (CJ Rebello, et al; manuscript under review). Although we also found that IO and SO consumption increased some satiety measures in the current study, the effects were not as robust perhaps due to the smaller portion sizes. The low volume of food may have caused minimal stomach distension and quickly emptied from the stomach, and the energy content was perhaps insufficient for showing significant differences in satiety at all of the time-points past 60 minutes. Thus, portion size likely plays an important role in detecting satiety differences between two foods within a given time frame.

The effects of β-glucan on appetite and satiety have been assessed in several studies but the results have been inconsistent [37, 191-193, 195, 267, 268, 273] In a study investigating the effects of β-

glucan on satiety it was shown that consumption of 4 g oat β-glucan served with yogurt, had no effect on satiety despite a reduction in the post prandial blood glucose response.[267] Beck *et al* concluded that the optimal dose of β-glucan affecting satiety and other markers of appetite regulation were between 4 and 6 g and that the hormonal effects (peptide YY) were mediated through increased viscosity observed with increasing the concentration of β-glucan.[193] However, varying doses from 2.16 g to 5.68 g of oat β-glucan also increased satiety in a dose dependent manner.[37] Thus, the differences in the β-glucan content of a food, in addition to structural and functional differences of the fiber in different food products may influence satiety responses.

The sugar content of the oatmeal breakfast meals was lower than the RTEC breakfast meal. A sensory evaluation of the two breakfast meals was not conducted in this study to determine the palatability of the two test meals. Palatability is not a fixed property of a food. Rather, it is a momentary evaluation liable to change with the experience.[126] Moreover, it appears to affect satiation (meal termination) more than satiety (prolongation of the interval between meals).[398] While the sweetness of sugar is strongly hedonically positive and may stimulate eating rate, sugars in the gut could generate negative as well as positive feedback signals to influence satiation and satiety.[399, 400] Adults do not always equate good taste with sweetness, and their taste preferences are not always direct predictors of appetite regulation.[401]

Differences in viscosity arising from differences in physicochemical properties may influence the glycemic response.[402] However, the results obtained from the study of the kinetics of starch digestion and glucose release of the breakfast meals were not significantly different among the three cereals. *In vitro* studies of starch kinetics do not fully reflect the effects of viscosity, stomach motility, or nutrient interactions; but, they permit standardization of conditions. Although oatmeal has been shown to be a food with a high glycemic index [403] there

may have been differences in the glycemic indices of oatmeal and the RTEC. However, studies that investigated the effect of the glycemic response on satiety have shown inconsistent results.[404-407]

Oatmeal had higher protein content than the RTEC and protein-induced satiety has been demonstrated in several studies. In a study comparing a high protein meal (25% of energy) with a low protein meal (10% of energy) it was found that satiety significantly increased after the high protein meal.[408] In a comparison between breakfast skippers and those who ate a high protein breakfast (35 g protein, 40% of energy content) or a normal protein breakfast (13 g protein, 15% of energy content), both the protein breakfasts increased satiety compared to the breakfast skippers with the high protein breakfast meals eliciting a greater satiety response than did the normal protein breakfast.[409] These studies,[408, 409] compared meals or diets that differed by 15% to 25% in their energy content from protein. In the present study, the difference in protein content was 2.3 g or 4% of total energy, which is less than the proportion previously shown to increase satiety. Thus, the content and functionality of β-glucan likely influenced the satiety differences observed between the oatmeal types and the RTEC more than the differences in the protein content.

Hormones, neuropeptides, and the glycemic response following consumption of the breakfast meals were not measured in this study. Post-prandial measurements of glucose and endocrine markers of satiety may have helped to clarify the physiologic mechanisms influencing appetite responses, and provided additional support to the conclusions. In a previous study we showed that energy intake at lunch decreases after eating a larger portion size (250 kcal) of oatmeal at breakfast compared to an isocaloric serving of the RTEC; (Rebello CJ *et al*, manuscript under review) however, in this study food intake was not measured. Appetite scores

measured through VAS can be reproduced and are therefore feasible tools to measure appetite and satiety sensations.[89] Nevertheless, proof of concept would require that effects on energy intake and body weight be assessed in future studies. Further, adults in different subgroups may or may not demonstrate disparate treatment response. Thus, it is of interest to compare treatments in subgroups but there must be a sufficient number of participants within the subgroups to support making valid conclusions from such analyses. Because of the efficiency gained in crossover designs, relatively small sample sizes are usually justified. While this is an advantage for investigating the primary outcome in a diverse sample, the typically small sample employed in this study does not provide adequate power to enable drawing reliable conclusions from subgroup analyses.

Conclusions

The effects of instant oatmeal on satiety demonstrated in this study are similar to the effects that were observed in a previous study comparing the satiety effects of a larger portion size of IO with the oat-based RTEC, indicating that IO suppresses appetite, and increases satiety, over a range of portion sizes. SO consumption was less effective in appetite control than IO was when each was compared with the RTEC. Differences in β-glucan content, hydration, and physicochemical properties among the cereals are likely important factors influencing meal viscosity and therefore satiety. A high initial meal viscosity may be associated with increased satiety. Oatmeal provides a readily available source of viscous soluble fiber, and its consumption may be a means of reducing the motivation to eat at future meals. Replacing less-filling breakfast cereals with oatmeal can be an effective tool for promoting satiety.

CHAPTER 6
INSTANT OATMEAL INCREASES SATIETY AND REDUCES ENERGY INTAKE COMPARED TO A READY-TO-EAT OAT-BASED BREAKFAST CEREAL: A RANDOMIZED CROSSOVER TRIAL

Introduction

Overweight and obese individuals are at an increased risk for cardiovascular diseases, diabetes, musculoskeletal diseases, and certain cancers.[410] Worldwide, 3.4 million deaths per year are attributed to being overweight or obese.[410] Research spanning several years suggests that certain components of food can produce significant effects on the regulation of appetite in the short-term. A diet containing these foods could translate into reductions in body weight when coupled with other lifestyle changes.[411]

β-glucan, is a dietary fiber found in significant amounts in oats and barley.[257] Oat β-glucans are linear polysaccharides which can be viewed as a cellulose chain. Approximately 70% are 4-O-linked units interrupted by 3-O-linked β-D glucopyranosyl units. The (1→3) linkages occur singly whereas the 4-O-linked mostly occur in groups of two or three.[412] Approximately 75% to 78% of the β-glucan content of oats is found in the endosperm cell walls. β-glucan is generally water-soluble, and in solution is viscous even at low concentrations.[257]

Increased viscosity of gastrointestinal contents delays gastric emptying, prolongs the time during which nutrients are in contact with the small intestine, increases the intestinal area in contact with the nutrients, decreases postprandial glycemia, and promotes the release of gastrointestinal hormones that promote satiety such as peptide YY and glucagon-like peptide-1.

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[42, 193, 413-416] The manner in which a soluble fiber will modify viscosity depends on the amount, solubility under physiological conditions, and the molecular weight and structure of the fiber.[220]

The effects of β-glucan on appetite and satiety have been assessed in several studies. While some studies demonstrated a beneficial effect on satiety,[37, 191-195] others showed no effect. [267, 268] Thus, it appears that differences in the properties of β-glucan in each food may influence the physiological response. However, most studies do not provide data on the physicochemical properties of β-glucan used in the studies.

In the present study, the effect of an instant oatmeal breakfast on satiety and food intake was compared with that of an oat-based ready-to-eat breakfast cereal (RTEC). Replacing foods in the diet with foods that enhance satiety may be a means of reducing energy intake; hence, the comparator used was the most widely consumed (based on Information Resources Inc., Liquid data, 52 weeks ending March 11, 2012) oat-based RTEC in the United States, even though the products differed in their nutrient composition. The viscosity of the two breakfast meals, β-glucan content, molecular weight, and radius of gyration were determined. In a previous study using a 250 kcal serving of old fashioned oats [38] which is processed differently and has a thicker flake than instant oatmeal, it was shown that subjective measures of satiety increased after eating oatmeal compared to the RTEC. A single serving of instant oatmeal (150 kcal) also increased satiety compared to the RTEC.[39] In the present study, it was hypothesized that compared to the RTEC, consumption of a 250 kcal instant oatmeal breakfast meal would result in greater subjective satiety over four hours as well as greater reduction in food intake at the end of four hours.

Subjects and Methods

Subjects

Forty-eight subjects, 18 years of age or older were enrolled in a randomized, crossover trial. All subjects participated in an initial screening that involved measurement of body weight, height, waist circumference, vital signs (blood pressure, pulse rate), chemistry-15 panel (glucose, creatinine, potassium, uric acid, albumin, calcium, magnesium, creatine phosphokinase, alanine aminotransferase, alkaline phosphatase, iron, cholesterol [total, high density lipoprotein, low density lipoprotein], and triglycerides), complete blood count with differential, and β-HCG pregnancy test-urine (in females of child-bearing potential). For greater applicability of the hypothesized results, body mass index (BMI) was not a criterion for exclusion but participants were required to be otherwise healthy. Health was further assessed through the administration of a medical screening questionnaire. Female subjects also completed a menstrual cycle questionnaire so that test days would fall within the luteal phase of the menstrual cycle.[385] Exclusion criteria were: (i) Intake of regular medications other than birth control or hormone replacement therapy (ii) women who were pregnant or nursing, (iii) self-reported weight gain or loss of $\geq$ 4 kg in the last 3 months, (iv) fasting glucose > 126 mg/dL, (v) dietary restraint score $\geq$ 14, assessed by the Dietary Restraint Scale of the Eating Inventory,[140] and (vi) allergy or intolerance to oats or milk.

The study was approved by the Institutional Review Board of the Pennington Biomedical Research Center (PBRC), Baton Rouge where the study was conducted, and all procedures were in accordance with its ethical standards. Study records are available at PBRC. Participants provided written informed consent. The trial was registered on ClinicaTrials.gov with registration number NCT01666574.

Study Design

Each participant was tested on two days. On one occasion the breakfast meal consisted of Quaker Instant Oatmeal Flakes™ (PepsiCo Inc., Barrington, IL) and on the other occasion the breakfast meal consisted of the RTEC, Honey Nut Cheerios™ (General Mills Inc. Minneapolis, MN), served in random, balanced order. The randomization was done using a random number table, in blocks of two, by the study statistician and all study staff except the study dietitian who assigned participants to the groups, were blinded until the data were analyzed. The breakfasts contained 363 kcal, consisting of 250 kcal of cereal, and 113 kcal of lactose-free, fat-free milk. Oatmeal (66.8 g dry weight), was prepared by adding 1.5 cups boiling water (360 g), stirred, allowed to stand for a minute, and served with 307 g of cold milk to drink. The RTEC (63.6 g dry weight), was prepared by adding 307 g of cold milk, and served with 360 g of water to drink. The participants had the option of adding 1 g of Splenda™ and one-half teaspoon of cinnamon to the oatmeal. If the participant added the Splenda™ and cinnamon to the oatmeal, they were required to add both, in the same amounts to the RTEC.

At the first test breakfast visit, participants arrived at the Center after a 10 hour overnight fast (except water). Participants were also required to refrain from alcohol and strenuous exercise for 24 hours prior to the test meal. Before serving the test meal electronic visual analog scales (VAS)[89, 137] were administered. Visual analog scales were scored by the computer on a 0 to 100 unit scale and the score was sent directly to the database. Hunger, fullness, desire to eat, and prospective intake, were assessed. The subjects were presented with their first breakfast test and given 20 minutes to eat it. Test meals were supervised to ensure that the entire breakfast was eaten. Visual analog scales were then administered at 30, 60, 120, 180, and 240 minutes following the start of the breakfast meal. Four hours after the start of the breakfast meal,

subjects were presented with a lunch meal. Participants made a selection at the screening visit from among turkey, ham, roast beef, or vegetable patty sandwiches as well as a calorie-free or calorie-containing beverage. The same pre-selected sandwich and beverage was presented to the participant along with potato crisps and cookies on both test days, in quantities more than they could reasonably consume. Each sandwich, crisps, and cookie presentation ranged in energy content from 2600 - 2800 kcal.

The meals were pre-weighed and participants were presented with a meal that was in excess of what they could possibly eat. They were told to eat to satisfaction over 20 minutes, after which the remains of the meal were weighed. The food intake at lunch was determined by subtracting the weight of the uneaten food from its original weight. The energy and macronutrient intakes were calculated using the United States Department of Agriculture's Food and Nutrition Database for Dietary Studies 4.1,[417] and product information. Subjects were asked an open ended question (How do you feel?) at each of the time points that the VAS were completed, and after the lunch meal to elicit any adverse events. Subjects returned on another day separated by at least a week to repeat the breakfast and lunch tests.

***In vitro* Studies**

The conduct of the *in vitro* studies is previously described.[39] Oatmeal was prepared as described in the study design, and allowed to rest for one minute. This oatmeal and dry RTEC were first analyzed for their sugar content by high performance liquid chromatography and total starch content by standard American Association of Cereal Chemists (AACC) procedures.[386] To determine the β-glucan content and physicochemical characteristics, the RTEC and oatmeal were ground to flour. The β-glucan component was extracted from dry ground oat flakes and the RTEC, according to the procedure described by Rimsten *et al* [391] and the content was measured

using standardized AACC procedures.[392] After extraction, dialysis purification and drying, the β-glucan was subjected to molecular weight, and radius of gyration analysis. For the viscosity measurement, a serving of each breakfast meal was prepared as described in the study design, allowed to rest for one minute and the viscosity was measured in a process simulating the gastric phase of digestion *in vivo*.

Statistical Analysis

A mixed model ANOVA for a crossover trial was performed to analyze the following primary outcomes: total energy intake (kcal), weight of food consumed, and intake from fat, protein, and carbohydrate (g). The AUC for VAS ratings of hunger, fullness, desire to eat, and prospective food intake was determined. The model included factors with fixed effects (sequence effects, or residual treatment effects that carryover from test day 1 to test day 2 [hypothesized to be the same when either oatmeal or the RTEC is consumed on test day one]), test day main effects, and treatment main effects, in addition to the random effects of subjects within treatment sequence groups.

The secondary outcomes, changes in VAS ratings from time 0 to 30, 60, 120, 180, and 240 minutes following the start of the breakfast meal were analyzed using a mixed model ANOVA for a doubly repeated measures crossover trial where the first repeated measures variable was the test day, and the second variable was elapsed time since the start of breakfast. The changes from time 0 were summarized as least squares means plotted for each cereal type across the assessment times, and any baseline differences between VAS scores that existed despite randomization were normalized to zero.[89] Thus, differential treatment effects were compared with respect to AUC and per time point. Area under the curve was estimated using the linear trapezoidal rule and calculated in terms of change from baseline to account for the

differences at baseline. Results were summarized as least squares means. The differences in molecular weight, radius of gyration, and the AUC for glucose release were compared using t-tests. Median viscosities after oral and initial gastric digestion were determined, and then compared using t-tests. Results were summarized as mean ± standard deviation. The significance level under the null hypothesis was set at $\alpha = 0.05$ and the levels quoted are two sided. All analyses were performed using SAS 9.2 (SAS Institute, Cary, NC).

During the planning phase of the study, sample size was estimated using G*Power, Version 3.1.2 (F. Faul, Universitat Kiel, Germany). The study was sufficiently powered with 46 participants for detecting a minimum difference of 1,258 mm×min between cereal types, which is similar to observed differences in AUC (1,213 mm×min) for desire to eat from a similar study that assessed appetite sensations.[394]

Results

Forty eight subjects (29 females and 19 males) were enrolled in the study. One subject who determined she was pregnant was excluded before completion of the study. Six participants were obese (BMI ≥ 30), thirteen were overweight (BMI ≥ 25), twenty-six were of normal weight (BMI 18.5 – 24.9), and three were underweight (BMI < 18.5). Data relating to all enrolled participants were included in the mixed model analysis. There were no adverse events. Descriptive characteristics of the subjects at baseline are summarized in Table 6.1. A nutrient analysis of the breakfast meals including the β-glucan content of the cereals that was measured in the study is presented in Table 6.2.

Table 6.1. Characteristics at baseline of 48 participants who were enrolled in the study evaluating the effect of oat based products on satiety, and were included in the mixed model analysis

	Mean	SD[a]	Range
Age (years)	32.5	11.1	19 - 63
Height (cm)	168.9	9.1	153.3 - 190.2
Weight (kg)	71.2	16.7	46.8 - 112.8
BMI (kg/m^2)	24.9	5.0	16.6 - 38.7
Waist Circumference[b] (cm)	82.0	13.9	63.1- 124.2

[a]Standard deviation, [b]One missing value

Table 6.2. Energy and nutrient content of breakfast meals obtained from product information, except β-Glucan content which was measured

	Quaker Instant Oatmeal[a]	Honey Nut Cheerios[b]	Lactose-Free, Fat-Free Milk
Energy (kcal)	250	250	112.5
Fat (g)	5.01	3.41	0
Protein (g)	8.35	4.54	10
Total Carbohydrates (g)	45.09	49.97	16.25
Total Fiber (g)	6.68	4.54	0
Soluble Fiber (g)	3.34	1.84	0
β-Glucan (g)	2.68	1.73	0
Sugar (g)	1.67	20.43	15
Sodium (mg)	0	363.43	156.25
Serving Size (g)	66.8	63.6	307

[a]Quaker Oats; (Pepsico Inc.Barrington IL)
[b]Honey Nut Cheerios; (General Mills Inc. Minneapolis MN)

Hunger and Fullness

The reduction in hunger was significantly greater after consuming oatmeal compared to the RTEC based on the AUC analysis (oatmeal: 10,615 ± 727.15, mm × min, versus RTEC: 8,472.10 ± 733.92 mm × min, $p = 0.005$). Reductions in hunger were larger after consuming oatmeal compared to RTEC at 120 min ($p = 0.005$), 180 min ($p < 0.001$), and 240 min ($p = 0.012$) (Figure 6.1, A). Increase in fullness was significantly greater after consuming oatmeal compared to the RTEC based on the AUC analysis (oatmeal: 11,486 ± 708.67 mm × min, versus

RTEC: 9 442.54 ± 716 mm × min, p = 0.001). The increase was greater after consuming oatmeal compared to the RTEC at 120 min (p = 0.019), 180 min (p = 0.002), and 240 min (p = 0.049) (Figure 6.1, B).

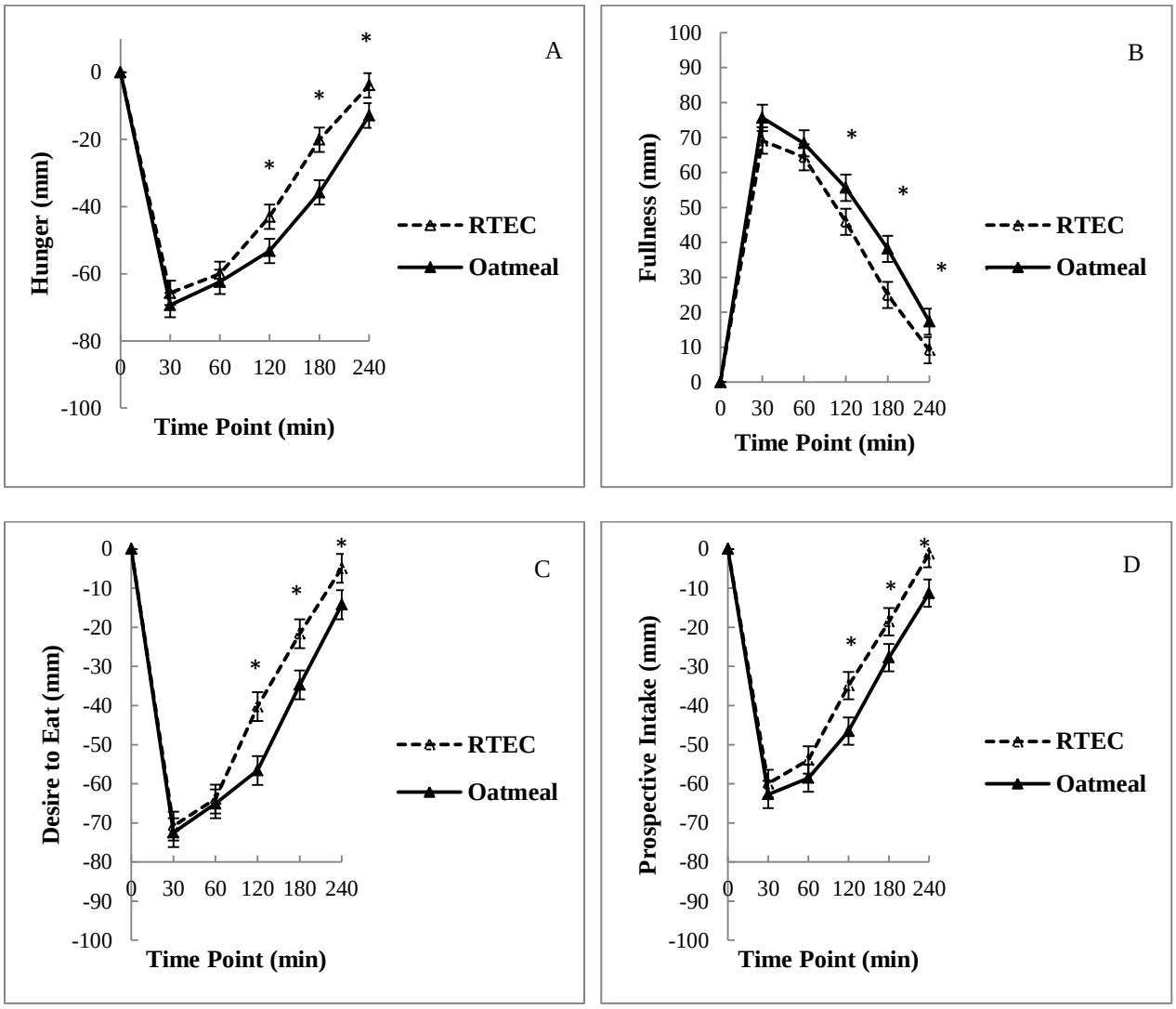

Figure 6.1 Visual analog scale ratings (n = 48) before and after consumption of oatmeal and a ready-to-eat breakfast cereal (RTEC). Oatmeal increased satiety across all measures: (A) Hunger ratings: *Differences in least squares means (LSM) were significantly different at 120 minutes (p = 0.005), 180 minutes (p < 0.001) and 240 minutes (p = 0.012) (B) Fullness ratings: *Differences in LSM were significant at 120 minutes (p = 0.019), 180 minutes (p = 0.002) and 240 minutes (p = 0.049). (C) Desire to eat ratings: *Differences in LSM were significant at 120 minutes (p < 0.001), 180 minutes (p < 0.001) and 240 minutes (p = 0.007). (D) Prospective food intake ratings: *Differences in LSM were significant at 120 minutes (p < 0.001), 180 minutes (p = 0.004) and 240 minutes (p = 0.002). Values are mean ± standard error.

Desire to Eat and Prospective Intake

Reduction in the desire to eat was significantly greater after consuming oatmeal compared to the RTEC, based on the AUC analysis (oatmeal: 11,010 ± 730.57 mm × min, versus RTEC: 8,854.71 ± 735.40 mm × min, $p = 0.001$). The reduction was greater after consuming oatmeal compared to the RTEC at 120 min ($p < 0.001$), 180 min ($p < 0.001$), and 240 min ($p = 0.007$) (Figure 6.1, C). A similar reduction in prospective food intake was determined after consuming oatmeal compared to the RTEC, based on the AUC analysis (oatmeal: 9,310.79 ± 717.41 mm × min, versus RTEC: 7,372.97 ± 723.08, $p = 0.006$). The reduction was greater after consuming oatmeal compared to the RTEC at 120 min ($p < 0.001$), 180 min ($p = 0.004$), and 240 min ($p = 0.002$) (Figure 6.1, D).

Energy, Food, and Macronutrient Intake

The food intake results were analyzed with and without intakes from the beverages. Results from both the analyses were similar; hence, the results of the intakes without the beverages are presented. Energy (kcal) intake at the *ad libitum* lunch meal was significantly lower (difference = 85 kcal, $p = 0.012$) following consumption of the oatmeal compared to the RTEC breakfast meal. Fat ($p = 0.02$) and protein ($p < 0.001$) intake were significantly lower after eating oatmeal, whereas carbohydrate intake and total weight of food consumed were not significantly different between the two conditions (Figures 6.2 and 6.3).

***In Vitro* Analyses**

The *in vitro* kinetics of starch digestion based on glucose release showed no differences between the two breakfast products (oatmeal: 99 ± 3 g × min, RTEC: 98 ± 2 g × min, $p > 0.05$). The molecular weight of the β-glucan in oatmeal was higher than that of the RTEC (oatmeal: $3.89 \times 10^5 \pm 8.96 \times 10^3$ Dalton, RTEC: $2.21 \times 10^5 \pm 4.16 \times 10^3$ Dalton, $p < 0.001$). Additionally,

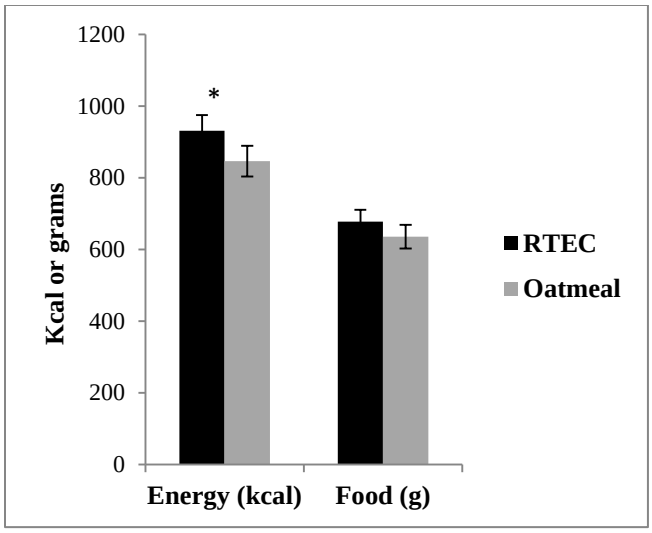

Figure 6.2 Energy and food intake at lunch meal following consumption of ready-to-eat breakfast cereal (RTEC) and oatmeal at breakfast (n = 48). Values are mean ± standard error. Asterisk indicates a significant difference (p = 0.012).

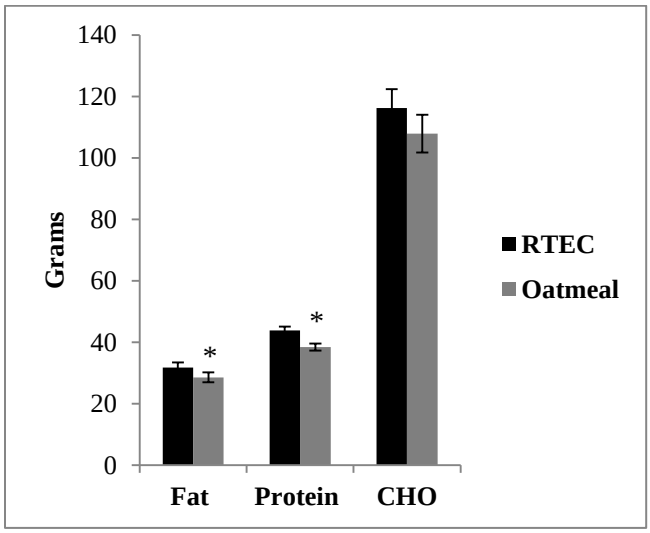

Figure 6.3 Macronutrient intake at lunch meal following consumption of ready-to-eat-breakfast cereal (RTEC) and oatmeal at breakfast (n = 48). Values are mean ± standard error. Asterisks indicate a significant difference (p < 0.05).

the β-glucan molecules in oatmeal, once hydrated, formed larger spheres than the β-glucan molecules in the RTEC. The radius of gyration of the β-glucan molecules in oatmeal measured as 50.23 ± 0.76 nm was greater (p < 0.001) than that of the RTEC measured as 36.83 ± 0.42 nm (Figure 6.4 A, B). Oatmeal exhibited a higher viscosity than the RTEC after oral and initial

gastric digestion (oatmeal median viscosity: 7,220.5, IQR: 2816.5 – 12,154.5 centipoise; RTEC median viscosity 140.0, IQR: 131.5 - 254.0 centipoise; (p = 0.025) (Figure 6.5).

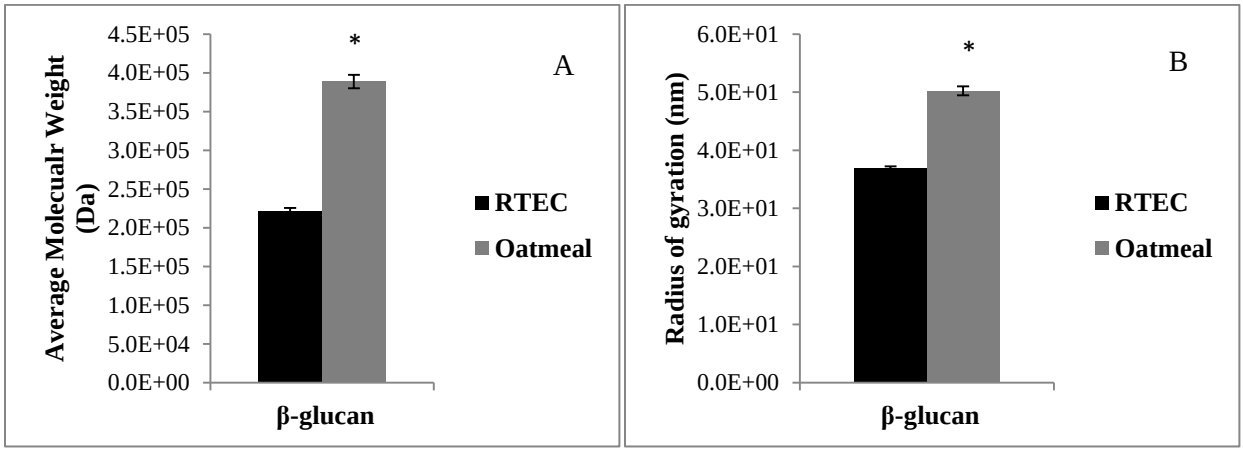

Figure 6.4 (A) Average molecular weight (Mw) in Daltons (Da) and (B) radius of gyration in nanometers (nm), of the ß-glucan content of oatmeal and the ready-to-eat breakfast cereal (RTEC). *Both outcomes were significantly different at $p < 0.001$. Values are mean ± standard deviation

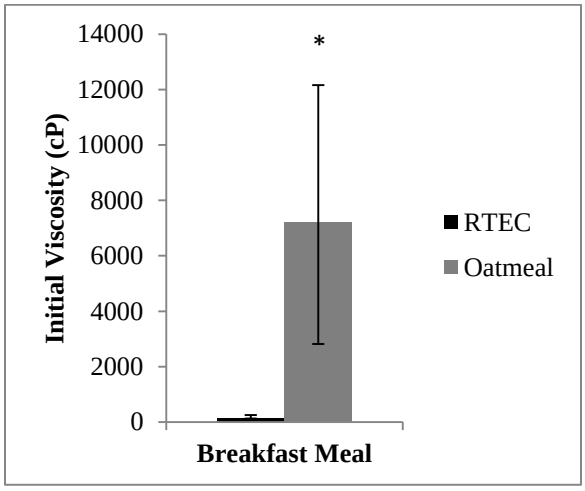

Figure 6.5 Median viscosities of oatmeal and ready-to-eat-breakfast cereal (RTEC) meals after oral and initial gastric digestion observed at the *in vitro* study. Viscosity values are the median of three replicates and expressed in centipoise (cP) ± interquartile range. Asterisk indicates a significant difference (p = 0.03).

Discussion

In this study the viscosity of two oat-based cereals was measured and its effects on satiety were evaluated. Consistent with the hypothesis, a breakfast meal consisting of oatmeal increased fullness, and suppressed hunger, desire to eat, and prospective intake resulting in a reduction in energy intake at lunch, compared to an oat-based RTEC. The content, molecular weight, and radius of gyration of the β-glucan in oatmeal were higher than that of the β-glucan in the RTEC which most likely contributed to the higher viscosity generated by oatmeal.

In order to form a viscous solution, β-glucan must be sufficiently solubilized. Thus, the difference in the hydration levels of β-glucan in these two oat-based cereals may have played an important role. Oat soluble fiber imbibes water, swells, and gradually dissolves at ambient or higher temperatures.[224] The instant oatmeal used in this study consisted of thin-cut flakes that hydrated easily after adding boiling water. In contrast, β-glucan in the RTEC prepared with cold milk did not hydrate as easily. This may have contributed to its lower viscosity and may explain why the difference in the viscosity of the two cereals was greatest when they first entered the stomach as estimated in the *in vitro* simulation. Although during transit through the gastrointestinal tract, β-glucan in the RTEC might be solubilized and finally hydrated completely, the initial high viscosity of oatmeal seemed to be important for its effects on satiety. The importance of initial viscosity was also demonstrated in a previous study comparing instant oatmeal, old fashioned oatmeal, and the RTEC. Instant oatmeal displayed greater initial viscosity than old fashioned oatmeal, and produced greater satiety than old fashioned oatmeal when both were compared with the RTEC.[39]

Consistent with research indicating that people tend to consume a constant weight of food,[418] in the present study, the weight of food consumed at lunch did not differ between the

two conditions. Although the carbohydrate intake did not differ, the fat and protein intake at the lunch meal were lower after consumption of the oatmeal breakfast compared to the RTEC which resulted in a reduction in energy intake.

A sensory evaluation of the two breakfast meals was not conducted in this study to determine the palatability of the two test meals. Palatability is a momentary evaluation liable to change with the experience,[126] and it appears to affect satiation (meal termination) more than satiety (prolongation of the interval between meals).[398] In one study preloads of beef consommé (10 kcals) varying in their rated palatability reduced appetite compared to a no soup control, but palatability had no effect on appetite ratings. Energy intake at a subsequent meal was not different among the conditions; but, the energy content of the soup may have been too low to affect subsequent food intake indicating that sensory stimulation alone is insufficient to reduce energy intake.[419] In another study although palatability of a preload test meal affected appetite ratings, there was no effect on subsequent food intake.[420]

The nutrient composition of the breakfast cereals was not matched; but, the purpose of the study was to determine whether oatmeal would be a good replacement for a ready-to-eat cereal, to keep consumers full for a prolonged period. Among the macronutrients, protein-induced satiety in the short term is well documented; however, the effects on food intake are less consistent.[421, 422] The difference in the protein contents of the meals or diets evaluated in studies that demonstrated protein-induced satiety ranged from 15% to 25% of the energy content.[408, 409, 423-425] In the present study, the difference in the protein contents of the breakfast meals (4%) was less than the proportion that has been shown to facilitate increased satiety.

The RTEC had higher sugar content than oatmeal. Instant oatmeal is composed of thinly cut flakes which facilitates quick hydration during cooking and increases the rate of digestion

and absorption. It has been shown to have a high glycemic index [403] and the *in vitro* studies examining the kinetics of starch digestion and glucose release showed that there was no difference between the two cereals. However, *in vitro* studies do not reflect effects such as gastric motility and nutrient interactions. Hence, it is likely that the glycemic indices of the two products differed. Nevertheless, studies that investigated the effect of the glycemic response on satiety have shown inconsistent results.[149, 405, 406, 407] Thus, based on the results of the *in vitro* studies, the fiber content of oatmeal especially, the β-glucan component, appears to be the most likely factor influencing appetite, satiety, and subsequent food intake.

Dietary guidelines emphasize consumption of whole grain foods and foods high in fiber and both the products tested in this study meet that description.[196] However, foods that enhance satiety offer quick gratification. Cues that have a reward associated with them, once learned, trigger motivational wanting to secure these rewards.[21] When that cue is consumption of a food and the reward is a predicted level of satiety it can have significant effects on the regulation of food intake and energy balance. Unlike other health promoting foods, the time frame to learn the association between consumption of the food and increase in satiety is short and the effects are easily monitored by the consumer which can greatly facilitate the learned association.[127] Even so, the crux of the issue is whether wanting can be sustained with repeated exposure. Nevertheless, foods that increase satiety provide consumers with appetite control strategies, especially, those susceptible to the wiles of an obesogenic environment. Foods that increase satiety also help to cope with mood states provoked by restricted energy intakes, facilitate compliance with healthy eating goals, and promote adherence to diet restrictions.

The main limitation of this study was that the macronutrient composition of the cereals was not matched; hence, it is possible that differences in the protein and sugar content although

insignificant in their individual effects may have exerted a cumulative effect on satiety. Further, it is possible that prior perceptions about the satiating properties of the cereals may have influenced the results. *In vivo* measurements of β-glucan viscosity might have provided a better understanding of the satiating effects of oat-based cereals; but, these measurements are difficult to obtain. While *in vitro* models may fall short of the dynamic environment of the gastrointestinal tract, the conditions can be standardized and provide a means for understanding physiological events.

It would have been interesting to compare treatments in subgroups but there must be a sufficient number of participants within the subgroups to support making valid conclusions from such analyses. The crossover design justified investigating the primary outcome in a diverse sample; however, it did not provide adequate power to enable drawing reliable conclusions from subgroup analyses. The strength of this study is that the physiochemical properties of β-glucan were analyzed and appeared to corroborate the satiety response.

Conclusions

The effects of instant oatmeal on satiety demonstrated in this study are similar to the effects that were observed in previous studies comparing the satiety effects of a thicker oat flake (old-fashioned oatmeal) with the oat-based RTEC,[38] and a smaller serving of instant oatmeal to an isocaloric serving of the RTEC.[39] In addition, energy intake was reduced following consumption of instant oatmeal compared to the RTEC. The results of this study add to the evidence provided through a multi-step proof of concept that oatmeal suppresses appetite, increases satiety, and reduces subsequent energy intake. The hydration and physicochemical properties of β-glucan are important factors influencing viscosity, a rheological property of soluble fibers that is thought to mediate satiety. Oatmeal could replace foods in the diet and keep

consumers full for a prolonged period. Thus, as a satiety-enhancing product oatmeal appears to be food that merits investigation.

CHAPTER 7
ACUTE EFFECTS OF A SPINACH EXTRACT RICH IN THYLAKOIDS ON SATIETY: A RANDOMIZED CONTROLLED CROSSOVER TRIAL

Introduction

Appetite reflects a complex interaction among the external environment, the behavioral profile, and subjective states as well as the storage and utilization of energy.[90] Thus, the initiation and termination of ingestive behavior has both metabolic and non-metabolic components. An eating episode can be sparked by metabolic need, hedonic drive, or an interaction between the two. A neural network sensitive to energy status signals has been identified as the homeostatic control system for the regulation of food intake and energy balance.[109] The system is powerfully designed to protect the lower limits of adiposity by modulating the processing of cognitive and reward functions.[109] However, in the modern world, humans, often eat in the absence of any metabolic feedback requiring replenishment of diminished reserves. This non-homeostatic or hedonic eating involves cognitive, reward, and emotional aspects. Cues that have a reward associated with them, once learned, trigger motivational wanting to secure these rewards.[21]

The photosynthetic membrane of chloroplasts consists of a system of paired membranes, the thylakoids. The thylakoid membrane system forms a physically continuous three-dimensional network that encloses an aqueous space, which is the thylakoid lumen.[426] Approximately 70% of the thylakoid mass consists of the membrane proteins and their bound pigments such as chlorophyll, carotenes, and xanthophylls. The remaining 30% largely consists of the membrane lipids such as galactolipids, phospholipids, and sulfolipids.[344]

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Thylakoid membranes are found in green plants such as spinach, A patented [427] extract of spinach containing significant amounts of thylakoids has been shown to have an inhibitory effect on lipase activity.[54] This inhibition is largely mediated by the protein fraction;[54] but, the membrane galactolipids may also have a role.[55] Delayed fat digestion increases the production of the satiety hormones cholecystokinin[53,57] and glucagon-like peptide-1 (GLP-1)[60] as has been demonstrated in human trials. Additionally, in humans[53] and pigs[370] ingestion of the extract has been shown to suppress the hunger hormone ghrelin. Among the gut hormones involved in appetite regulation GLP-1 in particular has been associated with the regulation of reward induced eating behavior.[334] Thylakoid-induced increase in the precursor for enterostatin a peptide involved in appetite suppression and thermogenesis has also been demonstrated.[54]

In studies with rats and mice,[289, 368] significant reductions in body weight and percent body fat occurred when the diet was supplemented with the spinach extract containing thylakoids. In overweight women, a breakfast meal supplemented with 3.7 g or 7.4 g of the spinach extract suppressed subjective hunger compared to a control in a cross-over study with no statistical difference between the two doses.[57] Overweight women, consuming 5 g of the spinach extract for three months demonstrated 43% greater loss of body weight compared to a placebo The women also exhibited a decreased the urge for sweet and chocolate by 95% and 87%, respectively. The reduced urge for sweets was significant after a single dose and was sustained throughout the study demonstrating that no tolerance developed during the 3 months of daily usage.[60] Further, unlike pharmaceutical lipase inhibiting drugs, the thylakoids temporarily delay but do not prevent fat digestion. Thus, the excretion of undigested fat which is an unpleasant side effect of lipase inhibitor drugs is avoided.

In this study subjective satiety ratings and food intake following a single administration of thylakoids from spinach leaves or a placebo, were measured. The hedonic and reward responses to food related stimuli were also evaluated. Plasma glucose and lipid concentrations were measured. It was hypothesized that thylakoid supplementation would produce an increase in satiety that would be accompanied by the appropriate changes in glucose and lipid measures.

Subjects and Methods

Participants

Sixty overweight or obese males and females between the ages of 18 and 65 years were recruited from Baton Rouge and the surrounding areas to evaluate the effect of thylakoid supplementation on appetite, satiety, and food intake. Inclusion criteria specified that all subjects should have a body mass index between 25 and 35 kg/m^2 and a waist circumference over 35 inches. Subjects were excluded if: (i) they had existing medical conditions or were taking medications that could influence their appetite, food absorption, body weight, or mood, and (ii) were currently or during the previous two months on a low calorie diet.

All subjects participated in an initial screening that involved measurement of body weight, height, waist circumference, and vital signs (blood pressure and pulse rate). At screening, health was assessed through the administration of a medical screening questionnaire and subjects underwent a medical examination to confirm their medical suitability for participation in the study. Sixty subjects passing the screening were randomized to two groups of 30 each. This study was approved by the Institutional Review Board of the Pennington Biomedical Research Center, Baton Rouge, where the study was conducted. Participants provided written informed consent. The trial was registered on ClinicaTrials.gov with registration number NCT01919814.

Design

The study followed a double blind placebo-controlled randomized cross-over design. Each participant was tested on two days. On one occasion the test product consisted of a concentrated extract of thylakoids from spinach (Appethyl™, Green Leaf Medical, Stockholm, Sweden) and on the other occasion the test product consisted of the placebo served in random, balanced order. At the first test visit, subjects arrived at the center after a 12 hour overnight fast, and having avoided strenuous exercise for 24 hours prior to arrival. Body fat was measured using bioelectrical impedance analysis (Tanita Corporation of America Inc. Arlington Heights, Illinois) and subjects were allowed to rest for a few minutes prior to having a blood sample drawn for assessment of serum triglycerides (TG), total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) free fatty acids (FFA), high-sensitivity C-reactive protein (hsCRP), and glucose. After eating a standardized 300 kcal breakfast meal they were advised to remain in the dining area of the metabolic kitchen and refrain from any food or snack consumption until lunch. Before eating lunch subjects were required to rate their satiety using electronic visual analog scales (VAS).[89, 137] They then consumed the spinach extract or placebo prior to being served a standardized 750 kcal lunch meal.

Breakfast and lunch were supervised to ensure that the entire meal was eaten. Visual analog scales assessing satiety were administered at 30, 60, 120, and 240 minutes after the start of the lunch meal. Two hours after lunch a second blood draw was conducted to obtain postprandial measures of TG, total cholesterol, HDL-C, LDL-C, FFA, hsCRP, and glucose. Liking and wanting[113] were assessed four hours after lunch followed by an *ad libitum* dinner meal consisting of pizza. The pizza meals were pre-weighed and participants were presented

with a meal that was in excess of what they could possibly eat. They were told to eat to satisfaction over 20 minutes, after which the remains of the meal were weighed. Subjects were required to remain at the Center between lunch and dinner. The food intake at dinner was determined by subtracting the weight of the uneaten food from its original weight. The energy and macronutrient intakes were calculated using product information.

Study Products and Meals

Five grams of the spinach extract were well mixed with two fluid ounces of diet Blueberry Pomegranate Juice (Ocean Spray) and one teaspoon of corn oil. For the placebo 2.5 g of cornstarch, 2.5 g of All-purpose flour, 5 g of glycerin, one teaspoon of corn oil, and food coloring were well mixed with two fluid ounces of diet Blueberry Pomegranate Juice. The test products were prepared and served immediately. A description of the standardized breakfast and lunch meals is provided in Table 7.1. Pepperoni pizza (Tombstone™ Original, Glendale, CA) was served at the *ad libitum* diner meal.

Subjective Satiety, Liking, and Wanting Assessment

Hunger, fullness, desire to eat, prospective intake, satisfaction, thirst, and appetite for sweet, salty, and savory foods were assessed using VAS. Liking refers to the affective reaction reflecting the hedonic response to a food and is the result of a central process that integrates the sensory properties as well as the individual's physiological state and associative history. Using the method developed by Finlayson *et al*,[113] liking was measured through VAS ratings associated with food image stimuli varying across the dimensions of fat (high or low) and taste (sweet or savory). Thus, using 16 pictures, the foods could be arranged into combined categories where four were high fat sweet, four were high fat savory, four were low fat sweet, and four were low

fat savory foods. These foods could also be arranged into generic groups with eight foods in each of the categories high fat, low fat, sweet, and savory. The images were relatively similar in size and in color. The order in which images were presented to subjects was randomized by time point using a random number generator. Each image was paired with the following questions administered using VAS: (i) How pleasant would it be to experience a mouthful of this food now? (ii) How often do you eat this food? (iii) How pleasant do you find this food? (iv) How much do you want some of this food now?

Table 7.1 Energy and Macronutrient Content of Standard Lunch and Breakfast Meals

Food	Energy (kcal)	Protein (g)	Carbohydrate (g)	Fat (g)
Breakfast				
Whole Wheat Bread	133.0	4.9	22.6	1.6
Butter, Salted	22.0	0.0	0.0	2.4
Cheese, Cheddar	52.0	3.2	0.2	4.3
Extra Lean Ham	43.0	5.7	0.7	1.4
Egg, Scrambled	50.0	3.3	0.7	3.7
Water	0.0	0.0	0.0	0.0
Total	300.0	17.1	24.2	13.4
Lunch				
Oven Roasted Turkey	75.0	15.0	1.5	0.8
Cheese, Swiss	120.0	8.5	1.7	8.8
Whole Wheat Bread	165.0	6.0	28.0	2.0
Lettuce	4.0	0.3	0.8	0.1
Tomatoes	5.0	0.3	1.2	0.1
Mayonnaise	115.0	0.2	0.6	12.5
Mustard, Yellow	7.0	0.4	0.5	0.4
Fritolay™ Sunchips	209.0	3.4	28.6	9.0
Fruit Salad	50.0	0.0	15.0	0.0
Water	0.0	0.0	0.0	0.0
Total	750.0	34.1	77.9	33.7

Wanting refers to an underlying implicit and objective drive process that mediates an intent or desire to consume a food.[113] Called incentive salience, it reflects a motivating desire that the brain attributes to reward-predicting cues.[428] Wanting was measured by presenting an image of a food stimulus paired with another image of a different food stimulus and asking subjects to select the food they "most want to eat now." The images used in the wanting procedure were the same images used for the liking procedure and each image in the set was uniquely paired with every possible image outside of its food category. For instance, a high fat sweet food was paired with every low fat sweet, high fat savory, and low fat savory food. Thus, using 16 images, 96 pairs of food images were presented and participants had to choose one of the two foods presented imagining they could eat as much or as little of that food as they wanted.

Statistical Analyses

To analyze the differences in the VAS ratings of satiety between the spinach extract and placebo conditions, a linear mixed model was used to estimate how the ratings for each of the questions changed over time. The covariates in the model were time and cross-over order effect, and an unstructured covariance matrix was used to model the relation between time points for each subject. The responses defined as change from baseline (the measure just prior to lunch) included measures at 30, 60, 120, and 240 minutes following the lunch meal. T-tests based on the least squares means from the model were used to determine differences between the two conditions.

The hedonic impact of each food category was assessed by calculating the mean liking scores and the differences in scores between the test and the control conditions, for each of the questions asked. The differences in wanting were assessed by calculating the mean frequency scores for food selection, in each food category. Food intake was evaluated as the difference in

mean scores between the between the spinach extract and placebo conditions using a linear model that adjusted for a crossover effect. A sub-analysis based on gender was also performed. Metabolic parameters were assessed as change from baseline using a linear model adjusted for a crossover effect. T-tests based least squares means were used to determine differences between the two conditions. All values are expressed as least squares means ± standard error. Statistical analyses were performed using SAS (version 9.4, SAS Institute, Cary, NC).

Results

Sixty participants, 30 males and 30 females were enrolled in the study. One female participant had a severe headache which the participant related to the temperature in the testing room. It was treated as a serious adverse event and the available data relating to this participant was not included in the analysis. Additionally, two other participants were dropped from the study due to one being diagnosed with diabetes and the other having a schedule conflict. Descriptive characteristics of participants included in the analyses are presented in Table 7.2.

Table 7.2**.** Characteristics at baseline of 59 participants who were included in the mixed model analysis

	Mean	SD[a]	Range
Age (years)	35.3	12.4	18 - 64
Height (cm)	171.4	9.6	153.2 - 194.5
Weight (kg)	88.0	10.9	67.3 - 114.0
BMI (kg/m^2)	29.9	2.6	25.2 - 34.5
Percent Body Fat	35.1	8.3	20.4 - 47.8
Waist Circumference (cm)	97.8	6.4	88.9 - 114.3

[a]Standard Deviation

Visual Analog Scales

Analysis of individual satiety ratings revealed that compared to the placebo, consumption of the spinach extract significantly increased fullness ($p = 0.04$), and reduced hunger ($p < 0.01$), longing for food ($p < 0.01$), and prospective intake ($p = 0.01$) over the two hour period following lunch (Figure 7.1, A, B, C, D), while satisfaction was not significantly different (Fig 7.1, E). In addition, consumption of the spinach extract reduced the desire for something salty ($p < 0.01$), desire for something savory ($p < 0.01$), and thirst ($p < 0.01$) over the two hour period following lunch, compared to the placebo (Figure 7.2, A, B, C); however, the desire for something sweet was not significantly different (Figure 7.2, D). At four hours thirst was still suppressed after consuming the spinach extract ($p = 0.02$), while there were no significant differences between the two conditions for the other ratings.

Intake at Dinner Meal

There were no significant differences in food intake at the dinner meal served four hours after lunch, between the two conditions. The total weight of food consumed and energy intake were also not significantly different. Males exhibited a trend towards decreased consumption, reducing their energy intake by 49.3 g (125.7 kcal, $p = 0.08$); whereas, females increased their intake by 3.2 g (8.1 kcal, $p = 0.85$).

Liking and Wanting

There were no significant differences in the mean scores for all the questions asked in each food category, in the test to assess differences in liking between the spinach extract and placebo conditions. No significant differences were found in the mean scores for frequency of choosing foods in each food category, in the test to assess the differences in wanting between the two conditions.

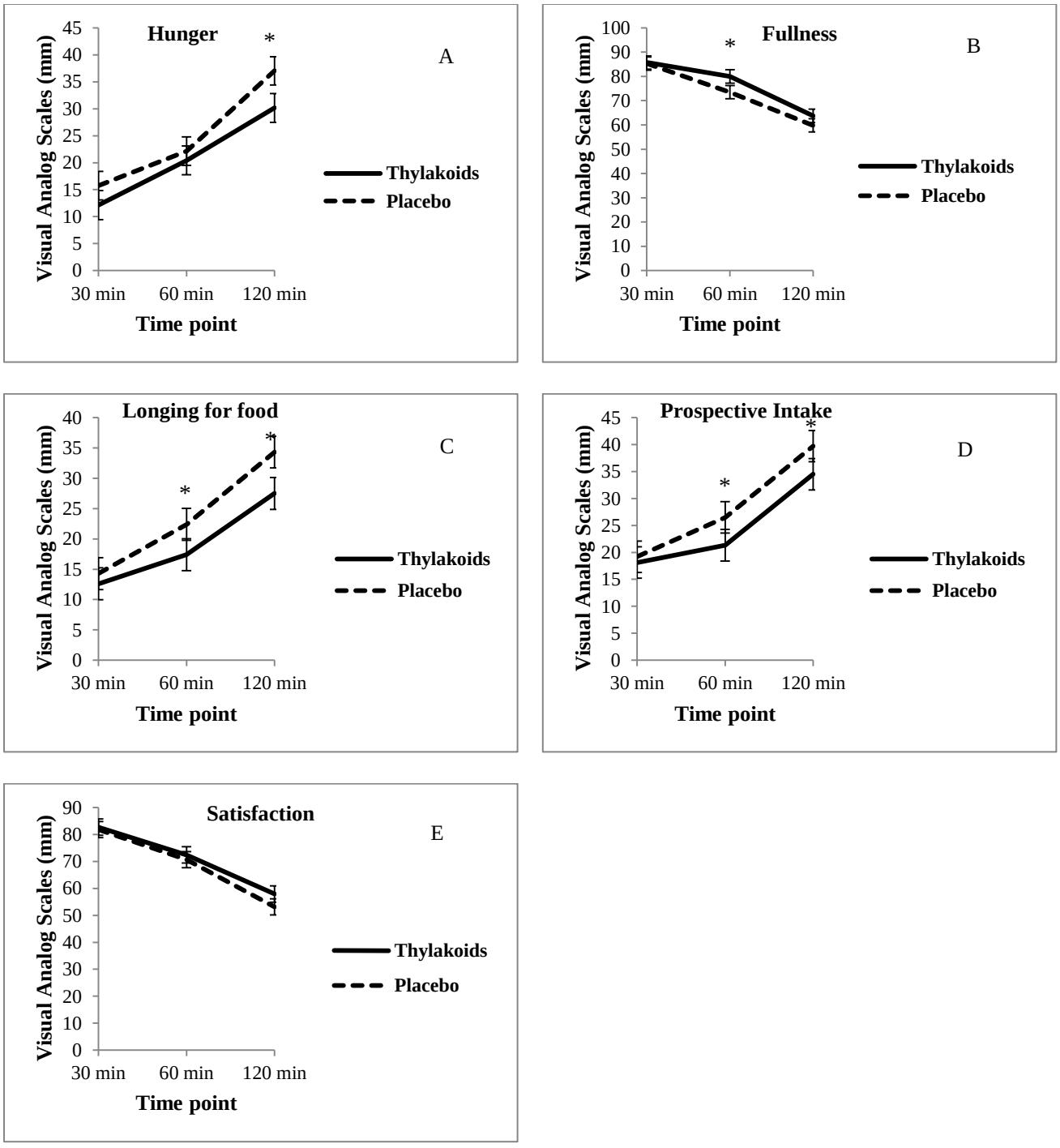

Figure 7.1 Visual analog scale ratings for satiety (n = 59) over two hours after consuming the spinach extract or a placebo. (A) Hunger: Overall differences, 4.0720 mm ± 1.52 ($p < 0.01$), *120 min ($p < 0.01$) (B) Fullness: Overall differences, 3.62 mm ($p = 0.04$), *60 min ($p = 0.03$) (C) Longing for food: Overall differences, 4.50 mm ± 1.32 ($p < 0.01$), *60 min ($p < 0.03$) and *120 min ($p < 0.01$). (D) Prospective intake: Overall differences, 3.83 mm ± 1.35 ($p < 0.01$), *60 min ($p = 0.03$) and *120 min ($p = 0.03$). (E) Satisfaction: Overall differences were not significant. Values are mean ± standard error

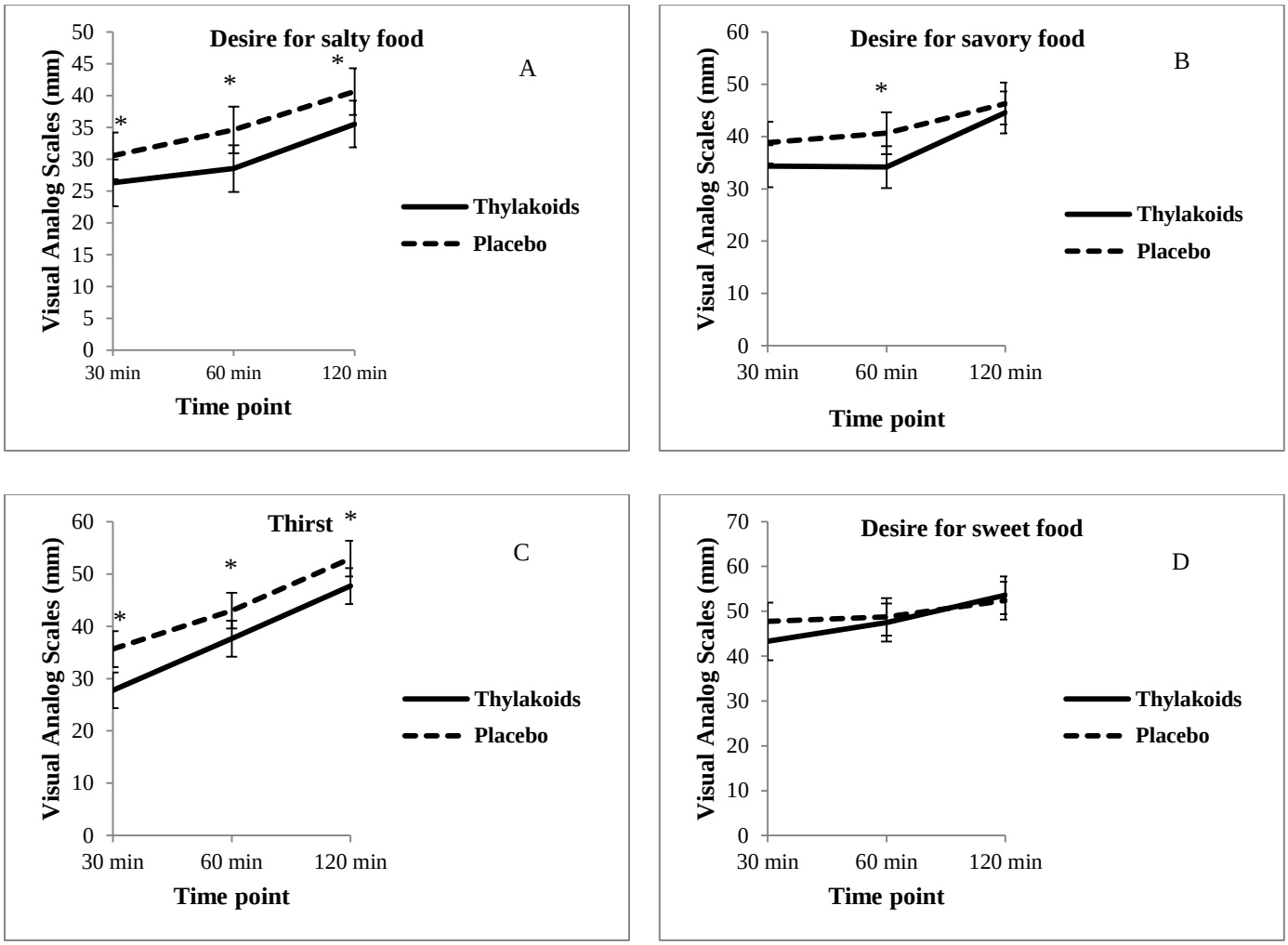

Figure 7.2 Visual analog scale ratings for satiety (n = 59) over two hours after consuming the spinach extract or a placebo. Values are mean ± standard error (A) Desire for salty food: Overall differences, 5.14 mm ± 1.09 ($p < 0.01$), *30 min ($p = 0.02$), *60 min ($p < 0.01$), and *120 min ($p < 0.01$). (B) Desire for savory food: overall differences, 4.22 mm ± 1.4 ($p < 0.01$) *60 min ($p < 0.01$). (C) Desire for sweet food: Overall differences were not significant. (D) Thirst: Overall differences, 6.16 mm ± 1.46 ($p < 0.01$) * 30 min ($p < 0.01$), *60 min ($p = 0.03$), and *120 min ($p = 0.04$).

Metabolic Parameters

There were no differences in the serum concentrations of TG, total cholesterol, HDL-C, LDL-C, FFA, and hsCRP. However, the difference in fasting and post-lunch serum glucose concentrations was greater ($p < 0.01$) in the spinach extract condition compared to the placebo (Figure 7.3).

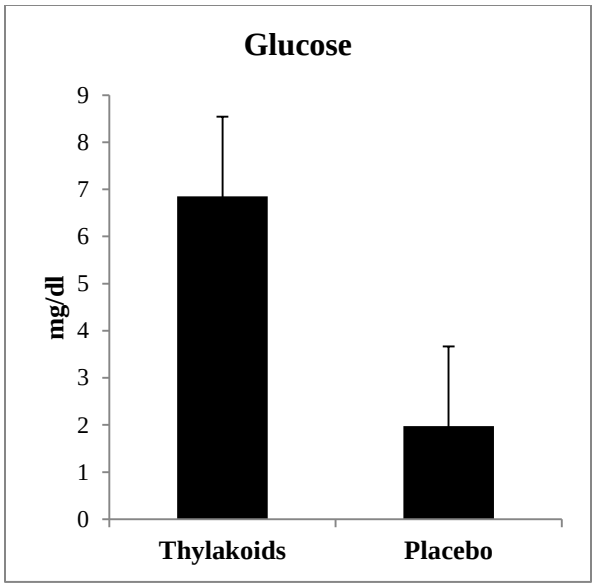

Figure 7.3 Difference in fasting and two hour post-lunch plasma glucose concentrations between the spinach extract and placebo conditions. *Significantly different at $p < 0.01$. Values are mean ± standard error.

Discussion

Compared to a placebo, a single supplementation with 5 g of thylakoids increased satiety measured subjectively over two hours. This was accompanied by a greater increase in the post-prandial plasma glucose response. Satisfaction, which introduces a hedonic component into the measurement of satiety,[264] was included to determine if satiety measures were judged from a comparable baseline during the repeated testing. As expected, it was not significantly different between the conditions. However, over a four hour period the differences in satiety measures were no longer significant. Differences in energy intake at the *ad libitum* meal and in the liking and wanting components of food reward measured at four hours after consuming thylakoids were also not significant between the two conditions.

The spinach extract (Appethyl™) contained concentrated thylakoids extracted from the choloroplasts of spinach leaves. By interacting with lipids and retarding fat digestion thylakoid membranes promote the release of satiety hormones such as cholecystokinin and reduce the

hunger stimulating hormone ghrelin.[53] *In vitro*, thylakoid membrane proteins bind to lipid droplets at the oil-water interface as well as to the lipase-colipase complex.[54] Thus, inhibition of lipolysis could occur through blocking the access of the lipase-colipase complex to the fat droplet or blocking of the active site of the lipase-colipase complex and preventing the enzyme complex from coming in contact with the fat droplet.[54] It has also been suggested that the thylakoid membrane lipid digalactosyldiacylglycerol, with its large polar head groups may shield colipase and sterically hinder the formation of the lipase-colipase complex necessary for lipolysis.[55]

Thylakoid membranes effectively suppressed lipolytic activity in a dose dependent manner during *in vitro* hydrolysis of an emulsion of TG dispersed in bile salts.[54] Suppression of lipase-colipase activity stimulates a compensatory increase in endogenous secretion and production of lipase and colipase. This compensatory release has been suggested as a mechanism for increasing the appetite suppressing peptide enterostatin in response to thylakoid supplemented high fat meals.[54]

In the present study, there was no difference in the serum lipid concentrations between the thylakoid and placebo conditions at two hours. Following a fat containing meal, TG reach their maximal concentrations three to four hours after consuming a single meal and return to baseline within six to eight hours.[429-432] Following sequential meals the plasma appearance of TG peaks at 60 minutes and thereafter falls up to 240 minutes.[431] Free or non-esterified fatty acid concentrations fall in response to a single meal and rise thereafter peaking between four and five hours post-prandially.[429-432] Following two sequential meals FFA increase within 30 to 60 minutes and thereafter fall.[431] Based on the kinetics of fat absorption it is difficult to determine if

there was a delay in lipid absorption without repeated testing before and after the two hour period.

The prolonged presence of nutrients in the gastrointestinal tract as well as cholecystokinin release prompts the release of GLP-1 from the distal regions of the intestine.[60] Supplementation with 5 g of the spinach extract significantly increased the difference between the pre- and post-prandial GLP-1 levels after a single instance as well as following 12 weeks of supplementation, compared to a placebo treated group.[60] GLP-1 acts on the reward system;[334] hence, it may provide an explanation for the decrease among males, in energy intake at the dinner meal served four hours after consuming the spinach extract. Males exhibit a yearning for savory foods,[374, 375, 433] whereas females prefer high fat sweet foods such as chocolate[374, 433, 434]. Although not significant, which may perhaps be because the study was not powered to detect differences in food intake by gender, males reduced their intake of savory food (pizza) by 126 kcal after consuming the spinach extract.

It has been demonstrated that thylakoids consumption reduces the urge for chocolate among women.[60] However, in the present study the option of sweet foods was not presented when food intake was evaluated at the *ad libitum* dinner meal. This resulted in an overall non-significant change in energy intake between the spinach extract and control conditions. The absence of a sweet food at the dinner meal may also explain why desire to eat something savory decreased with supplementation of the spinach extract; but, desire to eat something sweet was not different between the conditions. Nevertheless, it is estimated that weight gain in 90% of the adult population in the US is due to a positive energy balance of 100 kcal/day or less.[435] Thus, a reduction in energy intake of 126 kcal may have clinical relevance.

The increase in plasma glucose concentrations at the two hour time point may be explained by a decrease in insulin secretion. In humans, a single thylakoid-enriched high fat meal increased the satiety hormones cholecystokinin, and leptin while reducing levels of insulin and the hunger-stimulating hormone ghrelin.[53] A reduction in insulin secretion by supplementation with thylakoids has also been demonstrated in a porcine study.[370] In a previous study,[57] a high carbohydrate meal (71% of energy) supplemented with the spinach extract resulted in a tendency towards higher plasma glucose from 90 minutes following the meal, compared to a control meal. The greatest difference occurred at 120 minutes; although, over four hours the differences were not significant. In that study, the trend towards increased blood glucose was accompanied by suppressed hunger from 180 minutes.

In the present study, significantly higher plasma glucose concentrations coincided with greater satiety. Increasing extracellular glucose concentration inhibits the orexigenic agouti-related peptide/neuropeptide Y-expressing neurons and stimulates the anorexigenic proopiomelanocortin and cocaine-and amphetamine-related transcript expressing neurons.[436] Thus, the greater increase in plasma glucose concentration two hours post-lunch may in part explain the increase in satiety following supplementation with the spinach extract compared to the placebo. Longer term (90 days, 5g/day) consumption of thylakoids decreased body weight, but there were no differences in blood glucose or insulin concentrations between control and treated groups.[60] Thus, the decrease in insulin secretion and rise in two hour post prandial glucose concentrations do not seem to adversely affect glycemic or insulinemic control over time. Moreover, thylakoids administered with a low-carbohydrate breakfast had no effect on serum glucose concentration at two hours.[53] Therefore, the effect of thylakoids on glucose homeostasis may depend on the carbohydrate content of the meal. The glycemic and insulinemic

effect may also dissipate before 90 days; however, more studies are needed to establish these relationships.

The main limitation of this study is that metabolic parameters were assessed at a single time point. Frequent and more prolonged testing would have provided a better determination of the physiological mechanisms influencing satiety. Moreover, insulin and gut hormones were not measured. Further, males and females differ in their food cravings and the *ad libitum* meal did not cater to both gender preferences. A larger sample of males and females than the study provided would have helped to clarify potential mechanisms relating to the effects of thylakoids on the reward system.

Conclusions

Consistent with previous research a single meal supplemented with 5 g of a concentrated extract of thylakoids from spinach increases satiety over the two hour period following consumption. Thylakoids supplementation may influence food cravings by acting on the reward system, thereby offering a novel way to address a positive energy balance in a manner that is minimally burdensome on the consumer. Gender based studies of the effect of thylakoids consumption on appetite regulation, employing appropriate sample sizes, are needed.

CHAPTER 8
SUMMARY

The clinical trials evaluating the effects of oat-based cereals and the spinach extract rich in thylakoids on satiety showed that instant oatmeal and the spinach extract are foods that promote satiety. When consumed as a single serving (150 kcal) or as a 250 kcal serving, instant oatmeal produced greater satiety than an isocaloric serving of a popular oat-based ready-to-eat cereal (RTEC). A single serving of "old fashioned" oatmeal did not exhibit as potent an effect on subjective ratings of satiety as instant oatmeal, when compared to the RTEC. The physiochemical and rheological properties of β-glucan in processed cereal products, particularly the initial viscosity generated by the fiber, play an important role in appetite regulation. The clinical trial to determine the effect of thylakoids on eating behavior showed that 5 g of thylakoids increased the two hour subjective satiety response. Although not statistically significant because the study was not powered to detect gender differences, the reduction in energy intake by 126 kcal among males may have clinical relevance and provides circumstantial evidence of a possible effect of thylakoids on gender-specific food cravings.

Consumption of viscous soluble fibers can alter the viscosity of the digesta in the gastrointestinal (GI) tract and impede or inhibit the absorption of nutrients.[29, 30] Increased gastric volumes and reduction in gastric emptying rates have also been demonstrated.[240] The prolonged presence of nutrients in the GI tract stimulates the release of peptides which in turn affects gastric emptying and signaling to the central nervous system to promote satiety.[33]

In the present studies evaluating the effect of oat-based cereals, increased viscosity was demonstrated concurrently with an increase in satiety, suggesting that viscosity is a key factor stimulating satiety. The results are supported by other studies demonstrating that an increase in the viscosity generated by β-glucan is accompanied by an increase in satiety.[38, 43] However,

inconsistent results obtained from comparing a beverage with high viscosity compared to a beverage with low viscosity[42] suggests that there may be a level of viscosity beyond which the physiologic response is not sensitive to changes in viscosity. Further, consistent with previous research,[240, 241] the initial meal viscosity influenced satiety perhaps acting through a cephalic component driven by the direct response of the oral cavity and the gut to the tactile qualities of the meals.[437] Nevertheless, standardized procedures and instrumentation used to measure the physicochemical and rheological properties of β-glucan in trials to evaluate its satiety effect would greatly help to clarify the mechanisms by which β-glucan affects satiety and the processing techniques that would enhance the quality of the products delivering the fiber.

Receptors present on the surface of taste cells of the oral cavity sense fats and transduce chemical signals generated during feeding into electrical currents that are carried to the brain. These gustatory messages enter the nucleus of the solitary tract in the caudal brainstem, where they merge with information coming from the gut via the afferent vagus nerve. Neurotransmission in the form of reciprocal signals between the hypothalamus, the brain stem, and the higher cortical areas control reward-induced eating and energy homeostasis. The orosensory qualities of fat enhance its hedonic value and activate reward centers in the brain. Microdialysis experiments in sham feeding rats have shown that the mere oral exposure to fat triggers the release of dopamine in the nucleus accumbens, a key controller of reward.[438] Chemosensory and neural mechanisms are geared towards optimizing the sensing, procuring, and storage of fat, an evolutionary adaptation linked to archaic humans who were accustomed to uncertainty in food availability. The adaptive value of fat which once conferred a selective advantage likely contributes to over-consumption in contemporary societies where food is in

abundance.[439, 440] However, fats in the GI tract exert an anorexigenic effect arising from the release of hormones involved in satiety and other lipid mediators such as oleoylethanolamide.[440]

The slowing of GI transit by thylakoids likely contributes to the overall effects of the lipid mediators on satiety. However, enterostatin may also have a role to play and further investigation into the mechanisms of thylakoid action is warranted. The cumulative evidence from studies evaluating the effect of thylakoids from spinach suggests that thylakoids increase satiety. The evidence in support of the effect of thylakoids on reward-induced eating behavior shows promise. Two studies[59, 60] showed a reduction in the desire for sweet and chocolate among women, and the trial performed as part of this dissertation showed a decreased intake of a savory food among men.

Fat delivered into the GI tract has been shown to increase satiety;[441, 442] however, this effect is likely to be eclipsed when individuals consume large quantities of energy dense foods that invoke pleasurable sensations. Therefore, a strategy that not only increases satiety but also attenuates the rewarding stimuli of these foods thereby preventing passive over-consumption has tremendous potential in the regulation of appetite. Nevertheless, the crux of the issue in any obesity intervention is whether the treatment results in a reduction in body weight. Although thylakoids from spinach have been shown to reduce body weight,[60] the results need to be reproduced in future studies.

Measurement of subjective satiety using terms such as ‘hungry,’ ‘full,’ ‘desire to eat,’ that can neither be considered tangible nor construed as being objectively observed phenomena, might be circumspect. Therefore, the objective measure would be a quantitative evaluation of food intake. However, allowing subjects to indulge *ad libitum* within the constraints of the experimental design may reflect factors other than the physiologic systems being manipulated

under the hypothesis, such as the entrained response to the timing of a meal or desirability of the foods proffered at the meal. Gathering information about subjective motivation to eat as an aggregate description of several sensations that individual's recognize as predictors of their normal behavior, can yield important information.[141] Thus, events which cannot be directly observed, defined, or captured by objective measurements may be inferred by the subjective measures that conceptualize the physiologic signals and brain representations of previous experiences which influence behavior. Subjective measures provide insights which not only complement other measures, but are in themselves a valid measure of the effect of an intervention on different aspects of the motivation to eat, especially when measured using a repeated measures, within-subjects experimental design.

Psychometric ratings of appetite sensations such as the visual analog scale (VAS), though lacking an objective measure against which they may be compared, are easy to interpret, convenient, and have a universal scale that by being continuous do not predetermine the magnitude of a sensation. Moreover, VAS ratings are valid and reproducible which gives them statistical sanction. However, a large component of the motivation to eat is a learned response and demonstrating a concurrence between subjective measures of food intake and laboratory manipulations of physiologic systems relating to energy balance may not always be possible. This is especially so when the interventions involve relatively small manipulations. These caveats notwithstanding, the VAS ratings have a proven track record in demonstrating sensitivity to experimental manipulations.[141] However, subjective measurements of liking and wanting in response to food reward stimuli may not have the same practical or statistical validity.

Food reward is composed of both liking and wanting, each having psychological and neural correlates that are distinguishable from each other even if they occur in the same instance.

Thus, food liking and wanting can be segregated under certain circumstances and can contribute separately and differently to reward-induced eating.[112] However, if wanting and liking usually occur together, measuring them separately and subjectively is not the easiest of tasks. Unlike animal studies, where food liking and wanting can be studied separately by inflicting specific brain lesions, understanding the dichotomy of these two components of food reward in humans is particularly challenging. While the parsing of food reward has implications in that foods that promote health can be wanted without being liked, validated measures of food liking and wanting in humans that conclusively determine how liking and wanting contribute to eating behavior do not appear to be available at this time. Tests that measure aspects of reward may well be a surrogate for evaluation of reward-induced eating behavior, especially if correlated with results of neuroimaging studies.

Weight gain is influenced by a range of factors including inherited biological traits, social and environmental dynamics, as well as life experiences that impact behavior. Although the balance between energy intake and expenditure is controlled by a powerful unconscious biological system, the laws of thermodynamics predict that body weight can be controlled by consciously balancing food intake and energy expenditure. Therefore, an element of personal responsibility cannot be completely dismissed from the decisions on what and how much to eat. The environment clearly interacts with personal vulnerabilities making it difficult for people to make choices that promote health, and the food industry has been portrayed as the 'villain of the piece.' The debate that seeks to place the blame either on the environment or the individual is more constructively reframed by acknowledging that both positions have merit. Nevertheless, addressing the issue is a Herculean task. The demand of civil society for changes to the food environment will need multiple global accountability mechanisms, binding agreements to codify

the accountabilities, and substantial political will to make it happen[11] especially, given the competing interests of economic growth and prosperity.

Recent evidence links energy balance to the circadian clock which adjusts the energy needs of the body to the sleep/wake cycle. The molecular links between the circadian clock and energy balance have yet to be clearly elucidated. Nevertheless, the timing of food intake appears to have a significant role in the development of obesity. The most effective measure to align central and peripheral clocks is to adopt a lifestyle in which eating is restricted to around the same time and during the daylight hours.[15] Other evidence suggests that the chronic use of prescription medication such as psychotropic drugs that increase appetite are important drivers of obesity.[18] These issues only serve to highlight the importance of research related to the regulation of appetite. Whether it is dealing with the wiles of an obesogenic food environment, negotiating the evening hours without eating, or controlling appetite increases resulting from antidepressant medications, foods that empower individuals and help them overcome hunger, the desire to eat, or the rewarding value of food stimuli are far from inconsequential.

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APPENDIX I
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Journal Articles

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From: Candida Rebello [mailto:Candida.Rebello@pbrc.edu]
Sent: 24 July 2015 17:24
To: JOURNALS PERMISSIONS
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Emma,

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If an article is accepted for publication by Nutrition Reviews and I wish to include the article in my dissertation, will I need permission from OUP?
Thanks,
Candida

-----Original Message-----
From: Frank Greenway
Sent: Tuesday, August 18, 2015 12:47 PM
To: Candida Rebello
Subject: FW: Decision on Revised Manuscript ID NUTR-REV-099-ES-05-2015.R1

Candida,
Congratulations on the acceptance of your review.
Frank

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To: Frank Greenway
Cc: nutritionreviews@ilsi.org
Subject: Decision on Revised Manuscript ID NUTR-REV-099-ES-05-2015.R1
18-Aug-2015
Dear Prof. Greenway:
I am very pleased to report that your revised manuscript entitled "Dietary Fiber and Satiety: A Review on the Effects of Oats on Satiety" has been evaluated and is now accepted for publication in Nutrition Reviews.

If you have not already done so, please have all authors complete and submit the attached declaration of interest form. The corresponding author is kindly requested to obtain the signatures of all authors and to return the completed forms to the editorial office in a single batch via either e-mail (nutritionreviews@ilsi.org) or fax (+1-202-659-3859) .

Prior to publication, your files will be checked to ensure that they comply with the journal's style and production requirements. If anything further is needed, you will be contacted again.

Please note that due to the number of manuscripts currently awaiting publication, it may be up to six months before you receive final proofs for review. Your patience as your manuscript works its way through the queue will be sincerely appreciated.

Thank you again for the time and attention you devoted to this submission. We look forward to publishing this work in Nutrition Reviews and hope to receive other submissions from you in the future.

Sincerely,
Dr. Naomi Fukagawa
Editor in Chief, Nutrition Reviews
nfukagawa-nutr-rev@ilsi.org

Chapter 4

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Claire,

Thank you for your prompt response.

I have a couple more questions and I would appreciate it if you could answer them.

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Thanks,
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NUTRITION JOURNAL

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The role of meal viscosity and oat β-glucan characteristics in human appetite control: a randomized crossover trial

Candida J Rebello[1], **Yi-Fang Chu**[2], **William D Johnson**[1], **Corby K Martin**[1], **Hongmei Han**[1],**Nicolas Bordenave**[2], **Yuhui Shi**[2], **Marianne O'Shea**[2] and **Frank L Greenway**[1*]

*Corresponding author: Frank L Greenway Frank.Greenway@pbrc.edu

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[1]Pennington Biomedical Research Center, Louisiana State University System, 6400 Perkins Road, Baton Rouge, LA 70808, USA

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Nutrition Journal 2014, **13**:49 doi:10.1186/1475-2891-13-49

The electronic version of this article is the complete one and can be found online at:http://www.nutritionj.com/content/13/1/49

Received: 12 February 2014
Accepted: 22 May 2014
Published: 28 May 2014

Chapter 6

Journal of the American College of Nutrition

Instant Oatmeal Increases Satiety and Reduces Energy Intake Compared to a Ready-to-Eat Oat-Based Breakfast Cereal: A Randomized Crossover Trial

DOI:

10.1080/07315724.2015.1032442

Candida J. Rebello MS, RD[ab], William D. Johnson PhD[b], Corby K. Martin PhD[b], Hongmei Han MS[bc], Yi-Fang Chu PhD[d], Nicolas Bordenave PhD[d], B. Jan Willem van Klinken MD, PhD[d], Marianne O'Shea PhD[d] & Frank L. Greenway MD[*b]

Publishing models and article dates explained

Received: 23 Oct 2014

Accepted: 17 Mar 2015

Published online: 14 Aug 2015

Chapter 7

Journal of the American College of Nutrition

Acute Effects of a Spinach Extract Rich in Thylakoids on Satiety: A Randomized Controlled Crossover Trial

DOI:

10.1080/07315724.2014.1003999

Candida J. Rebello MS, RD[ab], Jessica Chu BS[c], Robbie BeylPhD[a], Dan Edwall PhD[d], Charlotte Erlanson-Albertsson PhD[e] & Frank L. Greenway MD[a*]

Publishing models and article dates explained

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Chapter 4

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EXPEDITED APPROVAL
PENNINGTON BIOMEDICAL RESEARCH CENTER
(Federalwide Assurance # 00006218)

FROM: PBRC Institutional Review Board

TO: Frank Greenway, M.D.

RE: PBRC IRB # **12029 QUAKER A & B**
The Effect of Oat Based Breakfast Cereals on Satiety (period 1 - QUAKER A); The Effect of Oat Based Breakfast Cereals on Satiety and Food Intake (period 2 - QUAKER B)

DATE: July 16, 2012

This is to document review of the above research proposal. In the judgment of this Board, the procedures delineated in said application conform to the pertinent DHHS and FDA rules and regulations regarding use of human subjects. Records regarding action of the Board, referable to said project, are on file in the IRB Office. This study is expedited through the expedited review procedure authorized in 46.110. Specifically, categories 2a & 7 apply to this study [(Federal Register: November 9, 1998 (Volume 63, Number 216)].

Documents approved: Protocol (version 7/9/12); Informed Consent period 1 (QUAKER A - 7/9/12); Informed Consent period 2 (QUAKER B - 7/9/12); HIPAA; Study Specific Information (A & B); Web Screener (A & B); Website Posting (A & B); Classified Ad (A & B); Email (A & B)

The investigator agrees to report to the Committee any emergent problems, serious adverse reactions, or procedural changes that may affect the status of the investigation, and that no such change will be made without Board Approval, except where necessary to eliminate apparent immediate hazards. The Investigator also agrees to periodic review of this project by the Board at intervals appropriate to the degree of risk to assure that the new project is being conducted in compliance with the Board's understanding and recommendation.

PLEASE NOTE:

1. Any advertisement to recruit subjects for this study must be approved by the IRB prior to posting, publication and/or distribution.
2. Other institutional approvals may be required before the study can be initiated.
3. Written notification (at the time this study is completed/canceled) must be sent to the IRB Office.

Date of Approval: July 16, 2012 **Approval Expiration Date: July 15, 2013**
Re-review frequency: annual, unless otherwise stated **Continuing Review report due: 5/31/13**
Number of subjects approved: 48 for period 1 (QUAKER A) and 48 for period 2 (QUAKER B)

Frank Greenway
Principal Investigator DATE: 7/18/12

Paula Geiselman, Ph.D., Chairman DATE: 7-16-12

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PENNINGTON BIOMEDICAL RESEARCH CENTER
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Submission Type: **Continuing Review**
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Principal Investigator: **Frank Greenway, M.D.**
IRB # PBRC **12029**
Title: **The Effect of Oat Based Breakfast Cereals on Satiety (period 1 - QUAKER A); The Effect of Oat Based Breakfast Cereals on Satiety and Food Intake (period 2 - QUAKER B)**
Sponsor: **Pepsico**

Expedited Approval Category: 2(a) – Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture from healthy, non-pregnant adults who weigh at least 110 pounds. For these subjects, the amounts drawn may not exceed 550 ml in an 8 week period and collection may not

7 – Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologiesoccur more frequently than 2 times per week

Approval Includes: **Study and Investigator(s) for an additional continuing review period. This approval expires on the date noted above.**

Investigators and study staff must comply with the Human Research Protection Program policies and procedures that apply to IRB members and staff, which can be found at www.pbrc.edu/HRPP

Paula Geiselman, Ph.D., Chairman

6400 Perkins Road, Baton Rouge, Louisiana 70808-4124 • Phone: (225) 763-2693 • Irb@pbrc.edu

PENNINGTON BIOMEDICAL RESEARCH CENTER
Louisiana State University System

IRB Certificate of Approval

FWA # 00006218

Date of Approval: **July 3, 2013**
Study Expiration Date: **April 16, 2014**
Submission Type: **Initial**
Review Frequency: annual
Number of Subjects Approved: **60**
Review Type: Expedited Approval of Board Requested Revisions
Approval Status: **Approved**
Continuing Report Due 60 days prior to expiration date

Principal Investigator: **Frank Greenway, M.D.**
IRB # PBRC **13022**
Title: **A Short Term Appetite Suppression Trial Using Appethyl: The Pizza Study**
Sponsor: **Greenleaf Medical**

Approval Includes: **Protocol (4/5/13); Informed Consent (5/21/13)**

Investigators and study staff must comply with the Human Research Protection Program policies and procedures that apply to IRB members and staff, which can be found at www.pbrc.edu/HRPP

Paula Geiselman, Ph.D., Chairman

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PENNINGTON BIOMEDICAL RESEARCH CENTER
Louisiana State University System

IRB Certificate of Approval

FWA # 00006218

Date of Approval: **3/21/14**
Study Expiration Date: **March 20, 2015**
Submission Type: **Continuing Review**
Review Frequency: **12 months**
Number of Subjects Approved: **60**
Review Type: Expedited
Approval Status: **Approved**

Principal Investigator: **Frank Greenway, M.D.**
IRB # PBRC **13022 PIZZA STUDY**
Title: **A Short Term Appetite Suppression Trial Using Appethyl: The Pizza Study**
Sponsor: **Greenleaf Medical**

Expedited Approval Category: 8c. Continuing review of research previously approved by the convened IRB where the remaining research activities are limited to data analysis.

Approval Includes: **Study and Investigator(s) for an additional continuing review period. This approval expires on the date noted above.**

Investigators and study staff must comply with the Human Research Protection Program policies and procedures that apply to IRB members and staff, which can be found at **www.pbrc.edu/HRPP**

Signed Friday, March 21, 2014 2:52:36 PM ET by Geiselman, Paula Ph.D.

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VITA

Candida Rebello received a Bachelor of Law degree (LLB) from the University of Mumbai, in March 1986. She began the Didactic Program in Dietetics at Louisiana State University (LSU), Baton Rouge in June 2005 and completed the program in May 2009. She became a registered dietitian in August 2010, following completion of a dietetic internship program and achieving success in the Registered Dietitian examination administered by the Commission on Dietetic Registration, the credentialing agency of the Academy of Nutrition and Dietetics, Chicago, Illinois. Candida began the Master of Science program with a concentration in human nutrition in June 2011, at LSU's School of Human Ecology. She began her research at the Pennington Biomedical Research Center (PBRC), LSU System, following enrollment in the Master's program. After graduating from the Master's program in December 2012, she continued to pursue her research and subsequently enrolled in the Ph.D. program in human nutrition at LSU's School of Nutrition and Food Sciences, in August 2013. She has been employed as a Research Dietitian at PBRC since December 2012 and has accepted a National Institutes of Health sponsored post-doctoral training award at PBRC, to begin on completion of the Ph.D. program.