EFFECT OF CHIA SEED (SALVIA HISPANICA L.) SUPPLEMENTATION ON
POSTPRANDIAL GLUCOSE AND SATIETY

A Thesis
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Master of Science
In
Agriculture
Human Nutrition and Food Science

By
Anne C. Sung
2015
SIGNATURE PAGE

THESIS: EFFECT OF CHIA SEED (SALVIA HISPANICA L.) SUPPLEMENTATION ON POSTPRANDIAL GLUCOSE AND SATIETY

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Special thanks to Mr. Ralston form Salba Smart for your generous donation for this study. This research would not been possible without your kind support.
ABSTRACT

Background: A healthy diet and weight management are beneficial in reducing risk factors for type 2 diabetes. Increasing dietary fiber intake has been shown to lower post-prandial glucose (PPG) levels and increase satiety, but the majority of people do not meet the recommended fiber intake. Chia seed (Salvia Hispanica, L) contains an excellent source of dietary fiber and has been shown to be positively associated with lower PPG levels and decreased appetite.

Objective: The proposed study was to determine the effect of supplementing 20% of daily calories from chia seeds on satiety and PPG levels.

Design: This was a free-living, randomized cross-over design study with 5-week treatment periods separated by a 5-week washout period. Twenty-three healthy females, between the age of 19-50 years and with a normal BMI from Cal Poly Pomona were recruited. Fasting glucose and PPG levels were measured using finger prick testing at baseline and 60 minutes after consuming 1/3 of the allotted chia seed in 6 ounces of 100% fruit juice. Satiety was measured by satiety rankings and visual analog scale (VAS) appetite questionnaires. Postprandial glucose levels were analyzed using a 2(condition) x 3(time) repeated measure ANOVA. Satiety was analyzed using one-way ANOVA and independent t-test was used for VAS appetite questionnaires.

Result: The before, during and after meal satiety, VAS appetite questionnaires and PPG levels were not significantly different between the control and chia seed supplemented period.

Conclusion: Twenty percent chia seed supplementation was not associated with lowering PPG levels or increased satiety in this study.
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CHAPTER 1
INTRODUCTION

Background

*Chia seed, (Salvia hispanica L.)*, also known as "chia sage" or "Spanish sage", is an ancient grain that originated from Central America and was widely consumed by the Aztecs. It has been reintroduced as a new functional food due to its high amount of polyunsaturated fatty acids as well as the high dietary fiber content and presence of phenolic compounds (Reyes-Caudillo, E., Tecante A. & Valdivia-Lopez, M.A, 2007).

Current research on the physiological benefits of chia seeds are limited, but studies have indicated the benefits of increasing the intake of dietary fiber, unsaturated fatty acids and antioxidants from fruits, vegetables, nuts and seeds. These foods are associated with reducing disease risk factors such as cardiovascular diseases and diabetes (Jiang et al, 2002; Esposito et al, 2004 ; Jenkin et al, 2011; Tan et al; 2013)

Statement of the Problems

Type 2 diabetes mellitus is a chronic disease that is characterized by abnormally high blood glucose as a result of impaired secretion of insulin from the pancreas and insulin resistance. It is the seventh leading cause of death in the United States (US), a major risk factor for cardiovascular disease and stroke, as well as the leading cause of kidney failure, non-traumatic lower-limb amputations and blindness as a result of poor glycemic control (CDC, 2014). In the last 16 years, there has been a significant increase of type 2 diabetes incidence in the US (Figure 1).

In 2010, an estimated 29 million people (9.3%) in the US were affected with type 2 diabetes, while another approximately 8.1 million people are undiagnosed. The Center for
Disease Control (CDC) and Prevention estimates one in every seven people in the US will develop type 2 diabetes by 2025 (CDC, 2014). Diabetes is a growing epidemic (Figure 1) and also creates a significant toll on the cost of healthcare. The medical expenses associated with diabetes and its complications reached a cost of $174 billion in 2007, with $116 billion directly related to medical costs such as hospitalization, supplies and diabetes care (CDC, 2014).

![Type 2 Diabetes Map of Trend from 1994 to 2010.](image)

**Figure 1.** Type 2 Diabetes Map of Trend from 1994 to 2010.

Prevalence of type 2 diabetes has markedly increased over a 16-year period. The incidence of type 2 diabetes has increased in virtually every state in the country, but at a higher rate in the Southeast United States (CDC, 2012). Of the two types of diabetes, type 2 is far more prevalent, comprising some 90-95% of all diabetic cases (CDC, 2014). Age, family history, being overweight or obese, hyperglycemia, hypertension, and hyperlipidemia are all risk factors for type 2 diabetes development. According to CDC reports from 1994 to 2013, the increasing prevalence of obesity correlates with the increase of diabetes prevalence (CDC, 2013; Bo et al, 2011).
In addition to genetic predisposition, environmental factors such as stress, smoking, physical inactivity and diet can play important roles in the onset of disease. Prospective population based cohort studies showed that males without pre-existing hyperglycemia or obesity are more likely to develop hyperglycemia with significantly less fiber intake and physical inactivity. People who eat restaurant meals more than four times a week have the highest fasting blood sugars compared to other groups (Bianco et al., 2013). The lowest incidence of hyperglycemia was observed in the group with the highest dietary fiber intake (Bianco et al., 2013). Higher than normal body mass index (BMI) was one of the most significant risk factors of type 2 diabetes development in a seven-year follow up study (Poulsen, Cleal, Clausesn & Anderosn, 2014).

Lifestyle changes such as behavior modification and weight and diet management are effective in preventing or delaying the onset of type 2 diabetes and decreasing the mortality rate of diabetes mellitus (CDC, 2014; Sluik et al, 2014). Diet modification such as increasing dietary fiber intake has been shown to improve postprandial glucose (PPG) and insulin resistance (Weickert et al., 2002, 2005; Bodinham, Smith, Wright, Frost & Robertson, 2012). However, the majority of people do not achieve an adequate daily dietary fiber intake (Bazzano, He, Ogden, Loria & Whelton, 2003; King, Mainous & Lambourne, 2012; Delahanty et al., 2013). There has been a public health effort to encourage people to consume an adequate fiber intake. The current dietary recommendation for an adequate intake of dietary fiber is 25g for females and 38g for males between the ages of 19-50 (Institute of Medicine, 2005). According to the National Health and Nutrition Examination Survey (NHANES) statistics and the trends that documented the dietary fiber intake from participants from 1999-2008, the average fiber
intake was 15.6–15.9gm per day; and obese participants reported a lower fiber intake than normal weight individuals, which is far below the current recommended guidelines (King et al., 2012).

Statement of the Purpose

Diet and Risk Factor Prevention

A healthy diet is the foundation for disease prevention and the lowering of disease risk factors. Higher consumption of fruit, legumes, nuts and seeds, pasta, poultry and vegetable oil food groups were associated with lower mortality rates in healthy individuals and significant reduction in diabetic mortality in a nine-year prospective study (Sluik et al, 2014). Healthy diet patterns, such as the Mediterranean diet, encourages the higher consumption of fruit, vegetables, whole grains, nuts and olive oil, which in turn increases the overall intake of monounsaturated fat, polyunsaturated fat and dietary fiber. Studies have found that individuals with metabolic syndrome who followed the Mediterranean diet had better glycemic control, greater weight loss, improved endothelial function and reduced markers of inflammation (Esposito et al., 2004; Giugliano, Ceriello & Esposito, 2008). Nuts and seeds are frequently consumed in the Mediterranean diet and are both high in unsaturated fatty acids and dietary fibers. Increasing dietary unsaturated fatty acid intake through nuts has been shown to reduce HgA1C levels and improved glucose homeostasis in diabetic patients (Jiang et al., 2002; Jenkin et al., 2011; Tan et al., 2013). Although nuts are naturally higher in calories, studies have not linked increasing nut consumption with significant weight gain, such as the eight-year prospective study from The Nurses' Health Study II (Bes-Rastrollo et al., 2009), as well as randomized controlled trials (Tey, Brown, Gray, Ghisholm & Delahunty, 2011; Tan et
Increasing dietary fiber intake has also been shown to reduce disease risk factors and improve glucose tolerance tests, serum glucose, and insulin response (Wiström, Hilding, Gu, Ostenson & Bjorklund, 2013). Therefore, consuming foods such as chia seed may be beneficial for lowering disease risk factors. Chia seed can easily be incorporated into a Mediterranean diet plan as the seed is high in polyunsaturated fatty acid (6.71g/28.35g serving), yet its total fat content is still lower than most other oil seeds (USDA, 2014; Alfredo et al, 2009; Porras-Loaiza, Jimenez-Munguia, Sosa-Morales, Palou & Lopez-Malo, 2014). It is an excellent source of fiber (9.8g/28.35g serving), and it can easily be incorporated into diets to increase daily fiber intake. Five tablespoons of chia seeds (24.5g fiber) meets 98% of the daily recommended fiber intake for women.

The purpose of this study was to quantify the effect of chia seed consumption on PPG levels and satiety. The primary objective was to examine whether chia seed consumption can positively affect PPG levels and increase satiety levels. A second objective was to determine how supplementing chia seeds to the diet may influence food choices, and which food groups, if any, might be replaced when 20% of the daily caloric intake is derived from chia seeds. The broader impact of this proposal was to increase the public awareness of the health benefits of chia seeds, and to promote healthy eating habits that incorporate foods high in dietary fiber and unsaturated fatty acids as a step towards reducing chronic disease risk factors.

**Hypothesis**

**Null Hypothesis**

H01: Chia seed supplementation will not affect post-prandial glucose levels.

H02: Chia seed supplementation will not affect satiety.
Research Hypothesis

HA1: Chia seed supplementation will reduce post-prandial glucose levels.

HA2: Chia seed supplementation will increase satiety after meals.
CHAPTER 2

LITERATURE REVIEW

Background

Oil seeds, such as flax seed, chia seed, psyllium seeds, contain more plant protein and polyunsaturated fatty acid contents than whole grains. Although the kilocalorie content of seed oil is higher than most whole grains or fruits and vegetables per serving due to its lipid profile, the high content of fiber and protein have also aided in increased satiety which in turn may help people ingest fewer kilocalories (Ribeiro, Alfenas, Bressan & Brunoro Costa, 2013). Studies found oil seeds' high unsaturated fatty acids may also improve insulin sensitivity by modulating the phospholipids in the muscle cell membrane (Ribeiro et al., 2013) as well as stimulating glucagon 1-like peptide (GLP-1) secretion (Vuksan et al., 2010). Therefore, seed oil consumption might prevent insulin resistance and type 2 diabetes. Moreover, as Rieiro et al. (2013) suggested, high magnesium, high fiber content and low glycemic index properties of oil seeds also play important roles in reducing risk factors for type 2 diabetes.

Chia Seed Physical and Chemical Properties

Chia is an annual herbaceous plant of the Lamiaceae (mint) family, it contains high amounts of polyunsaturated fatty acids, omega 3 (n-3) and omega 6 (n-6) essential fatty acids, α-linolenic acid (ALA), soluble and insoluble fibers, vegetable protein, calcium, magnesium, iron, and polyphenols such as caffeic acid, chlorogenic acid and quercetine (Vuksan, Whitham& Sievenpiper, 2007; Porras-Loaiza et al., 2011). An analysis of chia seeds showed the seed contains approximately 30-33% fat, 15-25% protein, 26-41%
carbohydrate, 18-30% dietary fiber and 4-5% ash. Nearly 83% of oils are polyunsaturated fatty acids; of which ~64% are ALA (n-3) and 18% are linoleic acid (n-6) (Ayerza & Coates, 2011). There are various species of chia that are grown in different regions, which result in variation in seed colors, and physical and chemical properties. An analysis by Ayerza et al. (2013) found a significant difference in the protein and fatty acid composition of chia grown in five different ecosystems. Also, two different genotypes did not differ significantly in fatty acid composition, protein content, fiber content or antioxidant compounds (Ayerza, 2013).

Chia seeds contain superior levels of dietary fiber compared to traditional fiber sources such as barley, wheat and flaxseed seeds (Ayerza, 2013; USDA, 2014). Figure 2 below provides the visible difference in fiber content between chia seeds and other common grains and seeds. Chia seed has better fiber content than flaxseed, which is commonly used as a fiber supplement. The amount of dietary fiber in chia seed is nearly two times greater than that of oat bran, wheat and barley. (USDA, 2014)
Figure 2. Comparison of dietary fiber contents between chia seeds and other common higher fiber crops.

A dietary fiber content analysis done by Reyes-Caudillo (2007) showed the differences were not significant in seeds grown in different regions. Insoluble dietary fiber is the predominant fiber in chia, which accounts for 39-41% of the total dietary fiber, while 13.79-14.97% is cellulose and 3.05-3.60% are hemicellulose. The polysaccharide lignin accounted for 6.9-8.1% as reported by Capitani, Spotorno, Nolasco & Tomas (2012) and approximately 6% is soluble fiber as reported by Reyes-Caudillo (2007). Therefore, in comparing to traditional fiber sources such as barley, wheat, flaxseed and sesame seeds that contain less dietary fiber than chia, chia is considered a better source of dietary fiber (USDA, 2015). The fibrous fraction of chia seeds is high in insoluble fiber as well as soluble fiber with the latter being able to form viscous mucilage. Alfredo et al. (2009) analyzed the fiber rich fraction (FRF) of chia, which is obtained from the defatted dry flour, and showed the insoluble fiber proportion is similar to legume C. ensiformis. They also determined that the water holding capacity of chia was higher than soy bean, wheat
and maize hulls. The chia mucilage contributed to greater water holding capacity than many fruits such as passion fruit and oranges contain, even though these fruits have higher soluble fiber content than chia. This mucilage could not be quantified as soluble dietary fibers due to lack of precipitation during ethanol treatment for soluble dietary fiber determination and resulted in an under-reported total soluble dietary fiber content of chia (Alfredo et al., 2009, Capitani et al., 2012). Chia mucilage is formed when placed in contact with water. The chia gum is slimy because it contains β-D-xylopyranosyl, β-D-glucopyranosyl, and 4-O-methyl-α-D-glucopyranosyluronic acid unit in the ratio 2:1:1 (Segura-Campos et al., 2014). The gum contributed to the stability and emulsifying ability of commercial products; furthermore, recent research studies have begun to investigate its potential health benefits such as increasing satiety, decreasing bowel transit time and cholesterol lowering effects (Queenan et al., 2007; Kristensen & Jensen, 2011).

The majority of phenolic compounds in chia are flavonols, specifically quercetin, kaempferol (Reyes-Caudillo et al., 2007), myricetin (Capitani et al., 2012) and small amounts of chlorogenic and caffeic acid, which are crucial in preventing lipid peroxidation. Polyphenols have been shown to not only scavenge free radicals as a hydrogen donor, but also metal-chelating abilities to prevent iron and copper free radicals. (Reyes-Caudillo et al., 2007). Ayerza (2013) recently identified another phenolic compound, secoisolaricresorcinol diglucoside (SDG), also known as lignans, in both genotype seeds. This antioxidant also contributes to the overall stability of the lipids present in the seed.

**Current Research**

There are various species of chia that are grown in different regions, which result in
variation in seed colors, physical and chemical properties. ‘Salba’ is a registered variety of *Salvia hispanica* L. by the Salba corporation (Core Natural LLC, Buenos Aires, Argentina). This variety is characterized by a higher content of polyunsaturated fatty acids that are more stable, particularly the ALA, as compared to chia seed whose genetic background and purity are unknown (Vuksan et al., 2007). Several research studies were conducted using ‘Salba’ instead of general *Salvia hispanica* L. Table 1 summarized a list of chia seed related researches. Vuksan et al (2007) conducted a randomized controlled trial, supplementing ‘Salba’ chia seed based on 15g/1,000kcal intake in addition to conventional therapy in type 2 diabetic patients (n=20). This study used wheat bran bread as the placebo control and the ‘Salba’ chia seeds were baked into bread as the intervention. The energy level was estimated based on the Harris-Benedict equation multiplied by a 1.3 activity factor. Anthropometric measurements, symptom diary and 3-day diet records were collected every 2 weeks during the two 12-week treatment periods. Fasting blood samples were collected at weeks 0 and 12. The study found a significant reduction in systolic blood pressure and hcCRP (high sensitive C-reactive protein) after ‘Salba’ supplementation in type 2 diabetic patients. Fasting blood sugar, and fasting blood insulin was reduced by ~3% and Hg 1C (glycated hemoglobin) level was significantly lower compared to baseline; however, these results were not significant when compared to the control group. Vuksan et al. (2010) conducted another study using a double-blind, placebo-controlled, randomized, crossover study design, and sought to determine the effect of ‘Salba’ supplementation on postprandial glucose and appetite in 11 healthy subjects (n=11). The study intervention used 0, 7, 15 and 24 g of ‘Salba’ baked into breads. The control group consumed regular white breads. Capillary blood samples
were collected at fasting and 15, 30, 45, 60, 90 and 120 minutes post meal consumption. Satiety was measured using a 100mm visual analog scale (VAS) and calculated based on ratings for the following questions: How full do you feel? How strong is your desire to eat? How hungry do you feel? How much do you think you could eat right now? This study found that ‘Salba’ supplementation had a dose-dependent relationship with postprandial blood sugar and satiety. The result demonstrated a reduction of postprandial glycemia by 2% with every gram of ‘Salba’ baked into the white bread compared with the control group that was white bread without ‘Salba’. The appetite rating was decreased with an incremental increase of ‘Salba’ intake compared to the control white bread. Significant reduction in appetite was seen at 60, 90, and 120 minutes after meal ingestion at highest Salba dosage. Lowest and intermediate dosages produced a significant appetite reduction at 120 minutes (check this value, it might be 60 minutes), and 90, 120 minutes respectively. There were no significant difference between the control and treatment bread at 15, 30, 45 minutes after a meal, even though the incremental area under the curve (IAUC) showed a reduction in appetite rating. There was a concern that high amounts of n-3 polyunsaturated fatty acid intake may affect clotting factors, however the safety evaluation of chia supplementation did not find a significant impact on the liver enzymes, kidney function, or coagulation. Current studies have agreed that 37g of ‘Salba’s daily dosage is considered safe (Vuksan et al., 2007, 2010).

Ho et al. (2013) conducted a randomized controlled crossover study comparing the difference between whole and ground ‘Salba’ seeds and its effect on postprandial glucose levels in 13 healthy subjects (n=13). ‘Salba’ was baked into bread at 7, 15 and 24 g
increments for low, intermediate and high dosage test meals. Regular white bread without ‘Salba’ addition was used as a control. Capillary glucose was collected at fasting, 15, 30, 45, 60, 90 and 120 minutes after meal consumption. It was assumed that ground chia seeds might be more bioavailable than whole chia seed as seen in studies using flaxseed. Contrary to this assumption, the study found that both whole and ground ‘Salba’ seeds were effective in reducing postprandial blood sugar in a dose dependent manner. The form of seeds did not make a significant difference in the results. It was concluded the difference between ‘Salba’ and flaxseed was a result of the structural differences. ‘Salba’ fiber is located in the outer layers of the seed coat as compared to the inner spermoderm layer of the flaxseed, which makes ‘Salba’ fiber more bioavailable regardless of the seed form (Ho et al., 2013).

Another randomized, double-blind, placebo controlled study conducted by Neiman et al (2009) used a different brand of chia seeds (Dole Packaged Foods, LLC), both whole and ground, to determine the relationship between chia seed supplementation and the disease risk factors in 76 overweight post-menopausal women (n=76). Supplementation of 25g whole chia, ground chia and placebo (poppy seed) were provided twice daily at breakfast and dinner. Seeds were mixed in 0.25L fluid and consumed on an empty stomach after the liquid mixture sat for 10 minutes. Only the plasma ALA levels elevated significantly in the milled chia seed group compared to the placebo group. Other risk factors such as serum glucose, blood pressure, and inflammation markers were not significantly affected in either whole or milled chia seeds group when compared to the placebo group. This study did not measure appetite directly; instead, the hunger rating was measured as part of the symptom log that was collected on a bi-weekly basis. The
symptom logs surveyed symptoms related to digestive health, hunger levels in the morning, after and evening, and overall well-being such as stress, pain or energy levels. The study did not find a significant reduction in hunger rating based on the symptom log reported by subjects.

Another double blind, randomized study conducted by Nieman et al (2012) assessed the effectiveness of milled or whole chia seeds in altering disease risk factors in 56 overweight or obese postmenopausal healthy women (n=56) using a metabolomics approach. A total of 25g milled or whole chia seed was provided and subjects could add it freely into foods or liquid of their choice. Subjects were to consume the seeds raw without any cooking or heating involved. Poppy seed was used as the placebo control. A comprehensive chemistry panel and lipid panel were collected; plasma ALA, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA) as well as the plasma cytokine C-reactive protein (CRP) were analyzed. Symptom log was filled out at week 5 and 10, and the results did not show any significant impact on hunger. The findings were similar to the 2009 study conducted by Neiman et al as both whole and milled chia seed posed no significant effect on body composition, macro and micronutrient intakes, serum glucose, CRP, blood pressure, lipid panel and comprehensive metabolic panel (CMP). When comparing milled to whole seeds and placebo, plasma ALA and EPA were significantly higher in the milled seed group.

The current research yields conflicting results between ‘Salba’ and regular chia seeds; however, study design and outcome measurements were different between these studies. ‘Salba’ studies have focused specifically on postprandial glucose and satiety levels, whereas regular chia seed studies conducted by Neiman et al (2009, 2012) only assessed
hunger twice as part of the overall symptoms reported, and no postprandial glucose was evaluated. Although the different methodologies and subjects of the studies can contribute to the differences, studies have also shown different oil extraction methods such as using solvent or mechanical pressing will affect the lipid content as well as the fibrous fractions (Capitani et al., 2012). It was shown that the defatted fibrous fraction had a higher fiber content compared to the regular chia. As previously mentioned, there was a significant difference in the protein and fatty acid composition of chia grown in five different ecosystems (Ayerza, 2011). Therefore, the origin of chia seeds and processing methods can potentially contribute to the conflicting results of current research.

Limitations of the current research included small sample size, short study duration and sample selection. For example, the 2009 study conducted by Nieman et al included overweight/obese subjects and the BMI requirement was 25 kg/m² and higher without specifying an upper limit. Morbidly obese subjects can be included and contribute to variations in the results. Subjects between the ages of 20-70 years may include postmenopausal females, which can be another possible confounding factor when determining the effect of chia seeds on weight reduction.
### Table 1. Summaries of Chia Seed Related Research

<table>
<thead>
<tr>
<th>Author/Year /Country</th>
<th>Purpose</th>
<th>Design/Length</th>
<th>Sample</th>
<th>Methodology</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vuksan et al. 2007 Canada</td>
<td>Assess whether the addition of Salba to conventional treatments improves cardiovascular risk factors in type 2 diabetics</td>
<td>Randomized, Single blind crossover design</td>
<td>n=20 Well-controlled type 2 diabetes (11 males, 9 females) Age: 64 +/- 8 years BMI 28 +/- 4kg/m² A1C 6.8 +/- 9%</td>
<td>Treatment: Salba Control: wheat bran Dose: 15g/1000kcal Estimated using harris-benedit equation with 1.3 activity factor. Fasting glucose at week 0 and 12. Every 2 weeks anthropometric and clinical data taken</td>
<td>Significant reduction in SBP, hs-CRP, but not DBP, between Salba and control. A1C, fasting glucose, insulin, TG, LDL, HDL, total cholesterol were not significantly different. ~3% reduction in fasting glucose and insulin. A1C significantly reduced from baseline, but not compared with control.</td>
</tr>
<tr>
<td>Ho et al. 2013 Canada</td>
<td>Assess the effect of whole or ground form of Salba on postprandial glycemia</td>
<td>Randomized crossover study</td>
<td>n=13 Healthy subjects (6 males, 7 females) Age not specified BMI: 25.4 +/- 2.6 kg/m²</td>
<td>10-12 hour fast Finger prick collected at fasting, 15, 30, 45, 60, 90 and 120 minutes. Dosage: 7, 15, 24g whole or grounded Salba baked into white breads. Control: white bread</td>
<td>Significant in increasing doses and reducing postprandial glycemia in both ground and whole Salba bread. No difference in glucose response seen between ground or whole Salba.</td>
</tr>
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Table 1. Summaries of Chia Seed Related Research (Continued)

<table>
<thead>
<tr>
<th>Author/Year/Country</th>
<th>Purpose</th>
<th>Design/Length</th>
<th>Sample</th>
<th>Methodology</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vuksan et al. 2010 Canada</td>
<td>Analyze the effect of incremental dosage of Salba on glycemia and satiety</td>
<td>Randomized, double-blind study</td>
<td>n=11 Healthy subjects (6 males, 5 females) Age: 30+/- 3.6 years BMI: 22.2+/- 1.3kg/m²</td>
<td>10-12 hour fast Finger prick and subjective appetite ratings (100mm VAS) collected at fasting, 15, 30, 45, 60 and 90 minutes. Dosage: 0, 7, 15, 24g grounded Salba baked into white breads. Control: white bread</td>
<td>Found dose-dependent relationship of Salba and lowering postprandial glycemia. There was a 2% reduction in postprandial glycemia per every gram of Salba baked into white bread compared with control. Significant appetite reduction at high dose of Salba group. No significant appetite change seen at 15, 30, 45 minutes</td>
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Table 1. Summaries of Chia Seed Related Research (Continued)

<table>
<thead>
<tr>
<th>Author/Year /Country</th>
<th>Purpose</th>
<th>Design/Length</th>
<th>Sample</th>
<th>Methodology</th>
<th>Findings</th>
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<tbody>
<tr>
<td>Nieman et al. 2012 USA</td>
<td>Effect of milled or whole chia seed on disease risk factors in overweight postmenopausal women</td>
<td>Randomized, Double blind study</td>
<td>n= 56 Overweight or obese postmenopausal healthy women Age: 49-75 BMI: 25kg/m²</td>
<td>25g/day milled or whole chia seed v.s poppy seed (control) Body composition and blood samples taken prestudy and after 10 weeks. Diet record and symptom/hunger questionnaires taken at 5th and 10th week. Questionnaires using 12-point Likert scale</td>
<td>No significant difference between milled, whole chia or placebo group in body composition, macro and micronutrient intakes., Serum glucose, cholesterol, CRP, BP, LDL, HDL, TG, CMP, 9 plasma cytokines. Plasma ALA and EPA increased significantly in the milled chia seed group compared to whole chia and placebo. No significant change in bi-weekly symptom logs which included hunger levels, energy levels, sickness, digestive health, allergies, stress level, focus, overall wellbeing.</td>
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<tbody>
<tr>
<td>Nieman et al. 2009 USA</td>
<td>Effect of chia seed on weight loss and cardiovascular risk factors in overweight/obese adults</td>
<td>Randomized single-blind study</td>
<td>n= 76</td>
<td>25g chia seed powder or placebo powder twice a day in 0.25L water, ingested on an empty stomach before breakfast and dinner. Blood samples taken 9 hours fasting at 12th week. Diet records, symptom log questionnaires taken every 2 weeks</td>
<td>Significant increase in plasma ALA level</td>
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No significant change in BMI, body composition, serum CRP, plasma cytokines, blood lipoprotein and blood pressure. No significant change in bi-weekly symptom logs which included hunger levels, energy levels, sickness, digestive health, allergies, stress level, focus, overall wellbeing.
Importance of Glycemic Control

Postprandial hyperglycemia is one of the diabetes risk factors. It is determined by using an oral glucose tolerance test (OGTT), in which serum glucose is above 7.8mmol/L (140 mg/dL) after a 2-hour post 75g glucose load (WHO, 2006). Persons with impaired fasting blood glucose levels between 100-125mg/dl, impaired glucose tolerance test of 140-199mg/dl, or HgA1C level between 5.7%-6.4% often indicate insulin resistance and therefore have a higher chance of developing diabetes (CDC, 2014). According to WHO and the DECODE study, the prevalence of hyperglycemia is higher in women than in men and the incidence of type 2 diabetes development is 24% higher among persons with impaired glucose tolerance. They also have a relative risk for all-cause mortality that is 1.48 fold higher, and 1.66 higher for fatal cardiovascular events than those with a normal OGTT (DECODE study group 2001; WHO, 2006). An 8-10 year follow-up study found that about 30-40% of middle age people with pre-diabetes eventually develops type 2 diabetes (Alvarsson, Hilding & Ostenson, 2009).

Postprandial hyperglycemia is affected by many factors such as the timing, quantity, composition and carbohydrate content of the meal and it is also a risk factor for cardiovascular disease (CVD) (Giugliano, Ceriello & Esposito, 2008). The investigation of the Framingham Heart Study by Port et al (2005) found that elevated serum glucose has been shown to increase the risk of CVD mortality even in non-diabetic patients. In this study, 15.6% male and 17.4% female were glucose intolerant. Although the Framingham Heart Study subjects were mainly white middle class Americans, other studies have confirmed that acute hyperglycemia causes the overproduction of super-oxides such as peroxinitrite and nitrotyrosine, which lead to oxidative stress in
tissues resulting in endothelial dysfunction. (Ceriello, Esposito, Piconi, Ilhtat & Thorpe, 2008; Giugliano et al., 2008). Oxidative stress is a result of an imbalance between pro-oxidants and antioxidants, and the over-production of reactive oxygen species (ROS) that causes cellular and molecular damage. It is increased in many disease states such as diabetes and metabolic syndrome. Chronic oxidative stress damages pancreatic β-cells which lead to insulin resistance and ultimately type 2 diabetes (Sies et al, 2005; Ceriello et al, 2008; Tangvarasittichai, 2015). Hyperglycemia can generate oxidative stress via different pathways such as increased advanced glycation end products (AGE) formation, polyol pathway, and PKCβ1/2 kinase (Giugliano et al., 2008; Tangvarasittichai, 2015).

In the presence of excess glucose, such as hyperglycemia, excess glucose oxidation increases NADH and FADH$_2$ generation in the electron transport chain and increases superoxide production. The superoxide reacts with nitrogen forming ROS and the toxicity generated from the ROS damages the endothelial cells and leads to micro- and macro-vascular complications (Giugliano et al, 2008).

**Dietary Fiber and Glycemic Control**

Studies have shown that increasing dietary fiber intake is beneficial to glycemic control. One cohort study conducted by Wirström et al. (2013) found that the intake of whole grain reduces the risk of developing pre-diabetes, particularly in men, as well as reduces insulin resistance. This study also determined that there is a 20% reduction in OGGT results for every 60g of whole grain intake per day.

According to the Institute of Medicine (IOM), dietary fiber is defined as the non-digestible carbohydrate and lignin that are intrinsic and intact in plants, and functional fiber is isolated or extracted non-digestible carbohydrate that has beneficial
physiological effects on humans (IOM, 2005). Insoluble fiber does not dissolve in water and does not form a gel; whereas soluble fiber dissolves in water and is gel forming. Insoluble fiber has been shown to decrease stool transit time, increasing stool weight and frequency (Raninen et al., 2011) and reduce postprandial glucose, insulin and incretin responses (Weickert et al., 2002, 2005, Raninen et al., 2011). Weickert et al. conducted two studies examining the glucose and insulin attenuating effects of different cereal fibers in 2002 and 2005. In the first randomized, single-blind, cross-over study (2002), 17 normal glucose tolerance overweight or obese women consumed oat fiber enriched breads (total 31g insoluble fiber/day) vs control white breads three times a day for three days. This study primarily examined the effect of insoluble fiber, particularly cellulose and hemicellulose; therefore, dietary lipids, protein and soluble fiber were removed. After 72 hours, the study found an 8% improvement in insulin sensitivity in the fiber enriched bread group by measuring the whole body glucose disposal. While mean insulin concentrations were not significantly different between the test and control group, the insulin actions were significantly enhanced (12%) in the test group by measuring the post-hepatic insulin clearance rate. The rate was not reduced as fiber intake increased and the author suggested the improved insulin response was due to improved insulin action and not related to hepatic insulin clearance. Fasting insulin concentration was slightly lower, but not statistically significant, which might be limited by the smaller sample size of the study. In this study, no significant changes were detected in other blood parameters such as plasma glucose, lipids, serum insulin, C-peptide, ghrelin, adiponectin or magnesium between the test and control group. A second single blind randomized crossover study conducted by Weickert et al. (2005) compared control white bread to test
breads fortified with oat-fiber (OF), wheat-fiber (WF), and a subgroup using resistant starch (RS). All test meals removed the additional soluble fiber, protein and lipids from oats and wheat in order to see the primary effect of insoluble dietary fibers on glucose-regulating factors in 14 healthy, normal glucose tolerance female subjects. This study found that highly purified insoluble dietary fiber enhanced the insulin response and GIP release post fiber test bread ingestion. Capillary glucose levels were reduced in the fiber enriched group as compared to the control group. In addition, postprandial glucose level was significantly lower when ingesting control bread on the second day when the subjects had consumed fiber enriched bread the day before (24 hr). Colonic fermentation was only observed in the OF and RS-fiber groups compared to control and the author concluded the postprandial glucose lowering effect was not a result of colonic fermentation as both WF and OF group had a reduction in postprandial glucose response.

Previous studies only examined the effects of insoluble fibers, but viscous soluble fibers that increase gut viscosity are also effective in attenuating serum lipids, slowing gastric emptying as well as modulating both the insulin and glucose responses (Raninen et al., 2011). It was believed that glucose and serum insulin responses vary depending on the type of soluble fiber due to varying viscosity, water holding capacity and fermentability (Granfeldt, Nyberg & Bjorck, 2008; Karhunen et al., 2010). Viscous fiber, such as beta glucan and guar gum, have been shown to have a positive effect of lowering postprandial glucose and serum cholesterol (Queenan et al. 2007; Granfeldt et al., 2008; Vitaglione, Lumaga, Stanzione, Scalfi & Fogliano, 2009). A randomized control trial found muesli with 4g of beta glucans significantly lowered postprandial blood sugar and insulin when compared to non-muesli supplemented control in healthy adults. While 3 grams of
the same test meals showed lower postprandial glucose and insulin, the effect was not significant (Granfeldt et al., 2008). This study highlights the importance of fiber type and amount to achieve significant physiological benefits. The author cited a study by Biorklund, Van Rees, Mensink & Onning (2005), which found that the same amount of beta glucan from oat but not from barley decreased postprandial glucose and insulin, and it was suggested that the molecular weight and solubility of beta glucan might play a role in the different effects between beta glucan from oat and barley. In addition, food processing and enzymatic degradation of beta glucan can affect the viscosity (Biorklund et al., 2005). The molecular weight and solubility and lipid metabolism as beta glucan is non-digestible but fermentable by the intestinal microbes. The short chain fatty acid produced from fiber such as oat beta glucan or guar gum fermentation has been shown to lower serum cholesterol (Queenan et al. 2007; Granfeldt et al., 2008) and increased dietary fiber intake has been correlated with a lower risk of metabolic syndrome in adolescents in a cross-sectional analysis.

**Weight Management and Glycemic Control.** Besides dietary intervention, weight management is one of the behavior modifications that reduces diabetes risk factors. A study conducted by Tuomilehto et al, (2001) investigated the effect of lifestyle intervention and weight loss in impaired glucose tolerance test subjects. Intervention goals included weight loss of 5% or more, reduction of total fat intake to <30% and saturated fat to <10% of energy consumed, an increase of fiber intake to 15g/1000kcal and moderate physical activity for at least 30 minutes a day. The intervention lasted 2 years with another 3-year follow-up study. There were significantly more subjects achieving the weight loss goal in the intervention group. Reduction in serum insulin, oral
glucose challenge, triglyceride concentration and blood pressure were more significant in the intervention group as well. An overall 58% lower incidence of diabetes development was observed in the 3-year follow-up study. The study findings remain consistent with other diabetes prevention program studies such as Penn, the Finnish Diabetes Prevention Study, and the Diabetes Research Program (Diabetes Prevention Program Research Group, 2009; Penn et al., 2009; Penn et al., 2013).

Penn et al. (2013) conducted an analysis of the European diabetes prevention programs: The Finnish Diabetes Prevention Study (DPS), European Diabetes Prevention Study (EDIPS) and the SLIM study (Study on Lifestyle intervention and Impaired glucose tolerance Maastricht) to determine the effect of dietary intervention, ≥ 5% weight loss and physical activity on impaired glucose test subjects and the incidence of type 2 diabetes in an average of 3.1 years of follow-up. Interestingly, regardless of whether subjects met the weight loss goal, there were no significant differences in weight, BMI, waist, hip, plasma glucose and insulin or HgAIC levels between the intervention and control groups. However, when comparing the diabetes incidences, the intervention group had a 57% lower incidence rate than the control group. Subjects who achieved ≥ 5% weight loss the first year had a 64% lower type 2 diabetes incidence rate. Those who maintained the weight through the 2nd year had a 79% lower risk, and a 89% lower risk was shown if subjects maintained the weight loss through the 3rd year. These follow-up studies have shown the importance of weight loss and maintenance.

Decreased adiposity has been shown to improve insulin sensitivity in proportion to the weight loss (Ferranini & Camastra, 1998). The exact mechanism remains unclear; however, studies have shown a strong association with improved insulin sensitivity with
weight loss. Insulin resistance is the impairment in insulin sensitive tissue that results in abnormal insulin-induced glucose uptake resulting in hyperinsulinemia when pancreatic β-cell islets need to work harder to produce more insulin for glucose control (Ye, 2013; Ferranini & Camastra, 1998). In addition to reducing adiposity, increased muscle mass has also been shown to improve glucose tolerance test in glucose intolerant subjects as skeletal muscle is one of the glucose uptake sites especially postprandially (Kwankaew, Saetung, Chanprasertyothin, Leelawattana & Rattarasarn, 2014). In the study conducted by Kwankaew et al. (2014) subjects performed a 75 g OGTT after an overnight fast, and glucose and insulin blood samples were analyzed at 30, 60, 120 and 180 minutes postprandially and insulin sensitivity was interpreted using the insulin sensitivity index (ISI) from the OGTT model of Matsuda & DeFronzo (Kwankaew et al, 2014). Total body fat and lean muscle mass were analyzed using dual-energy x-ray absorptiometry (DEXA) standard software. The study results showed a significant correlation between lean mass and OGTT results and fasting glucose, log 1st-phase insulin secretion and lean mass were the significant parameters predicting the AUCg0-180 min. However, these clinical findings cannot prove the causal relationship between muscle mass and glucose tolerance using a cross-sectional study design, thus the author suggested a prospective follow-up study or intervention study to validate the concept.

Diet is important in weight management and maintenance. Increased satiety may help reduce subsequent calorie intake which prevents excess calorie intake that leads to weight gain. Meal energy density and composition affect people’s appetites and perceived satiety as well as hunger. Satiety is described as the inhibition of further intake of foods, decline of hunger or an increase in fullness after consumption of a meal; whereas
satiation refers to a process that terminates eating and is related to the meal size (Blundell et al., 2010). Subjective satiety can be measured using visual analogue scales (VAS) from 100-150mm length, with extremes of sensation anchored at the two ends. Measuring the line from left to the mark indicated by subjects will quantify the subjects’ sensation (Chaput et al., 2010). Objective measurements such as biochemical markers include gastrointestinal peptides that involve in hunger and satiety regulation, such as cholecystokinin (CCK), peptide YY (PYY), GLP-1, amylin and ghrelin (Blundell et al., 2010). These peptide hormones provide signals to the arcuate nucleus (ARC) of the hypothalamus, which is believed to be one of the key regions responsible for regulating energy intakes. PYY and GLP-1 hormones rise during food ingestion, and the increasing hormone levels signal the increased satiety and fullness signals in the ARC and subsequent inhibition of food intake occurs. The hormones also delay gastric emptying, which is another signal for satiation and decreased food intake (Bewick, 2012). Ghrelin, on the contrary, stimulates appetite and increases food consumption. Meal composition affects postprandial ghrelin concentration and energy dense foods suppress ghrelin release (Karhunen, Juvonen, Huotari, Purhonen & Herzig, 2008).

**Dietary Fiber and Satiety**

Dietary fiber is associated with increasing satiety through several possible mechanisms such as increasing bulk, lower energy density, increasing chewing time that results in prolonged meal duration (Lee et al., 2006), slower gastric emptying time, longer transient time, and decreased fat absorption rate (Queenan et al., 2007; Vuscan et al., 2009, Lyly et al., 2010; Kristensen et al., 2011). Increasing dietary fiber through foods such as whole grains has been shown to increase satiety and decrease subsequent energy intake.
(Lee et al., 2006; Ibrugger et al., 2012). Studies have been conducted using various types of dietary fiber sources such as oat beta glucan, psyllium, flaxseed or lupin seed. Lupin is a novel whole grain that is also rich in plant protein and dietary fiber. Lee et al. (2006) used lupin kernel flour, which was derived from the endosperm of lupin and incorporated into the test bread. Lupin bread has ~6g/1000KJ more dietary fiber than white bread. When comparing lupin bread to commercial white bread, the study found lupin bread was able to increase satiety for up to 4.5 hours and produce a 15% reduction in energy intake at the subsequent meal in healthy individuals. Plasma ghrelin concentration, postprandial glucose and insulin levels were significantly lower in the lupin enriched test bread than white bread. Lupin enriched test bread consumption was able to suppress ghrelin secretion by up to 3 hours, whereas white bread consumption suppressed ghrelin secretion by up to 2 hours.

Current research remains inconclusive whether dietary fiber can significantly impact satiety and hormones likely due to multifactorial effects such as physical extraction, types of fiber, molecular weight and degree of viscosity (Kristensen et al., 2011). It was assumed that whole grain foods, which are higher in dietary fiber, should be more satiating than refined grains. However, in another experiment conducted by Kristensen et al. (2010) the results were inconsistent with this hypothesis. In this study, they compared meals consisting of whole grain bread and pasta vs. refined grain and pasta. While whole wheat bread increased satiety when compared to refined bread, it did not reduce ad libitum energy intake 3 hours later or postprandial glucose. Interestingly, while there was not a significant difference between whole wheat and refined products in postprandial glucose, the difference between bread and pasta was significant.
Postprandial glucose was significantly lower in the pasta group than the bread group. The study suggested the difference could be due to the different types of dietary fiber present in the bread and pasta. Wheat fiber is non-viscous and only increased bulk, which could explain the insignificant difference in postprandial glycemia and the immediate but not prolonged effect on the satiety level. The types of fiber and the meal food matrix affect satiety level differently. Dietary fibers that are gel forming and highly viscous have been shown to affect satiety positively. The water holding capacity of the dietary fiber also determines the transient time and gastric emptying rate, and the ability of viscous fiber that form stable gel in the stomach resulting in increased satiety without consuming large energy dense foods, which is also known as kilocalorie displacement (Lyon & Kacinik, 2012).

Current research on chia seed and satiety is limited; however, the effect of flaxseed and satiety are better studied. Flaxseed is similar to chia seed as it is gel forming and an excellent source of dietary fiber (7.6g/28g serving, USDA, 2014). Near 1/3 of flaxseed dietary fiber are viscous (Ibrugger et al, 2012). Kristensen et al. (2013) conducted a double blind randomized crossover study using whole flaxseed in high and low flaxseed mucilage test meals to determine the effect on postprandial glucose, insulin, lipids, satiety hormones and subjective satiety sensation using a VAS scale. The dietary fiber content of the whole flaxseed (WF) and low mucilage flax seed meal (LM) were the same (12g fiber per meal) and the high mucilage (HM) test meal had 17g fiber per meal. Control meals had only 7g fiber per meal. The study found the addition of 2g/MJ flaxseed dietary fiber can lower triglyceride levels and increase the subjective appetite rating significantly. A significant difference was also seen in ghrelin levels between 2-4 hours post meals.
Postprandial glucose, free fatty acid and CCK levels were not significantly different and serum insulin response had significantly decreased at 30 minutes and 180 minutes compared to the control. Subjective appetite ratings were all higher in the test groups compared to the control, and the HM meal yielded significantly greater satiety, fullness and less hunger than other test meals (WF and LM). According to the author, extracting mucilage from whole flaxseed can result in a lower molecular weight and alter the polymerization of the dietary fiber. This study also compared the difference between WF and LM test meals, which both had 12g of fiber per serving, in order to determine whether the difference in molecular weight (whole vs ground) can affect the variables differently. Based on the results, they found a greater reduction in insulin and an increase in the appetite rating in the mucilage group. The author pointed out the difference might be due to the encapsulation of dietary fiber in whole flaxseed which reduces its hydration and water holding capacity.

There were many factors that could affect subjective satiety and contributed to the inconsistent results among studies. Satiety is not only impacted by the macronutrient content or amount of dietary fiber present; other factors such as energy density, volume, particle size and palatability can all have an effect on perceived satiety. This effect is referred to as "Sensory-specific satiety", which is the satiating effects based on the taste, look and feel of the ingested foods (Gerstein et al, 2004). The satiating effect related to the form of dietary fiber was studied in a cross-over randomized, single-blind study conducted by Ibrugger et al, (2012). This study compared the difference between flaxseed fiber in liquid form to a non-fiber control drink vs solid flaxseed fiber tablets. Satiety was measured based on ad libitum percent meal intake and the 100mm VAS questionnaire.
was used to measure appetite sensation. The results demonstrated that the flax drink produced significantly greater satiety and fullness (~30%, P<0.05) than the control drink and subsequent meal intakes were 8% smaller than the control group. When comparing both liquid and tablet form of flaxseed fiber, there were no differences observed in the subsequent meal intake or reported VAS. However, the control group had a higher palatability rating than both the flaxseed drink and tablet groups.

A separate randomized crossover study by Wanders et al. (2014) used a different type of dietary fiber, pectin, and examined the difference between bulking pectin, viscous pectin or gelled pectin to a no-pectin control. In addition, the pectin was also administered either as gelled, capsule or liquid form to determine whether a difference in the form of fiber affected satiety differently. The pectin test products differed in their preload viscosity where viscosity increases gradually from bulking to viscous and lastly to the gelled pectin. Preload viscosity also differs in the form of supplementation, and the capsule form was lowest and gradually increases with liquid and gelled as highest. The gelled pectin had the highest viscosity and greatest water holding capacity and it was rated the most unpalatable and most difficult to consume based on the appetite rating. However, gelled pectin significantly reduced hunger, increased fullness, produced less desire to eat and demonstrated a lower subsequent food intake compared to the control, bulking and viscous pectin interventions. When comparing the different forms of pectin, gelled pectin also had the greatest effect on satiety, appetite and subsequent intake, as well as slower gastric emptying rate based on breath samples when compared to the capsule and liquid form. It is interesting to note that the ad libitum energy intake after three hours did not differ significantly between the various viscosities of pectin types;
however, the capsule form of supplementation had a greater reduction of ad libitum energy intake than liquid form.

In summary, studies have shown a positive association of dietary fiber, particularly those that are viscous, to improved glycemic control and increase satiety. Supplementing chia seeds to daily meals has been shown to reduce postprandial glucose levels and improve satiety in few clinical studies. To date, there has not been a study supplementing chia seeds at 20% calorie needs in a free-living environment. Although free-living studies may have research limitations due to the nature of fewer controls on individual calorie intake, exercise pattern, and food choices, this type of study generally has higher external validity, which refers to the degree research findings can be generalized to a different population, setting, or time other than the original research (Steckler & McLeroy, 2013). There has been an increasing emphasis on reporting external validity in public health related research. It is known that there are research gaps between highly controlled research settings and translating that intervention into effective and practical methods for the public to follow.
CHAPTER 3

METHODOLOGY

The study protocol 14-0105 (Appendix A) was reviewed and approved by the Cal Poly Pomona (CPP) Institutional Review Board (Appendix B), and addendums were submitted during the study to reflect any changes made during the study and recruitment periods.

Participants

Subject recruitment took place at CPP. A total of 55 healthy, normal weight females without pre-existing diseases were recruited in two separate recruitment periods in the Fall quarter 2014 and Winter quarter 2015. The inclusion criteria were as follows: healthy weight range (BMI between 18.5-25kg/m²), age between 18-45 years, free from pre-existing chronic diseases, without metal plates, pins or a pacemaker, not on a diet plan or taking omega-3 fatty acids or dietary fiber supplements. The exclusion criteria were as follows: BMI between 18.5-25kg/m² BMI<18.5kg/m² or >25kg/m², pregnancy, pre-existing gastrointestinal or chronic diseases, have metal plates or pins and/or a pacemaker inside the body, taking herbal or dietary supplements, and taking medication that can alter serum glucose and insulin values or affect appetite. Participants would be excluded during the study if they consumed less than 50% of the assigned chia seeds, became pregnant during the study period or started on a prescribed medication that could alter serum glucose levels.

Recruitment

Participants were recruited via flyers (Appendix E) sent through CPP e-mail invitations, posted on campus, distributed through school clubs and in-class. There were a
total of two recruitments in the Fall 2014 and Winter 2015 quarters. All participants
signed the informed consent form and completed the initial screening form prior to the
study to determine eligibility. A total of 24 participants was recruited initially; however,
due to unexpected dropped outs, only 18 participants stayed until the end of treatment
two. In order to maintain at least 30 participants to achieve the statistical significance for
the study, we recruited again in Winter 2015 quarter to accrue an additional 16
participants. One dropped out due to a minor allergic reaction to the chia seed. There
were 32 participants total from both recruitments that met the study criteria, but only 23
participants remained at the end of the study. Detailed subject recruitment and
characteristics are described in the Results section.

Study Design

The experiment was a randomized, cross-over study consisting of two five-week
treatment periods separated by a five-week washout period. Randomization was carried
out using randomizer.org web program. Baseline anthropometric measurements (height,
weight, BMI, waist and hip circumference) and food records at three randomly assigned
days were collected during the lead-in week. Finger pricks for postprandial glucose
measurements were performed every other week during Weeks 2, 3, and 4. A total of
nine food records and satiety questionnaires were collected during both treatment periods.
This was a free-living feeding study where participants have control over their daily food
intake and the freedom of incorporating chia seeds into any foods of their choices on a
daily (24h) basis.
The original protocol was an 18-week study, with six-week treatments and a six-week washout period. The postprandial glucose samples were obtained at 0, 30, 60, and 90-minute intervals. However, due to schedule conflicts, the 6th week of treatment one was during Christmas break and the CPP campus was closed. Therefore, participants were unable to meet with the primary investigators for data collection. In addition, there was insufficient funding prior to the beginning of the study. An addendum was submitted to the IRB to shorten the study and decrease the frequency of postprandial glucose checks to 0 and 60 minutes.

The last IRB addendum was submitted to add a second recruitment period and change the study design to remove the postprandial glucose checks from the study. The second recruitment was focused on testing hypothesis two to determine the effect of chia seed on satiety levels. There was more difficulty in recruiting participants due to the requirement for finger pricks as some potential participants expressed a fear of finger
pricking. Therefore, starting Spring 2015 quarter, finger pricks for postprandial glucose checks were removed from the study design, and the study was continued with the randomized diet records and satiety and VAS appetite questionnaires.

**Procedures**

Participants were randomly assigned to the treatment and control group in a 1:1 ratio. The treatment group consumed 20% of total daily calories from chia seeds. As this was a free-living study, participants had the freedom of incorporating chia seeds into any food of choice. During the lead-in week, we provided tips on when and how to incorporate the seeds. It was suggested to separate the seeds into 3 meal settings due to the amount of chia seeds assigned; however, it was not required. Participants' caloric needs were calculated using the Harris-Benedict equation with activity factors ranging from 1.2 to 1.7 depending on the activity levels reported by the participants. The participants were asked to fill out in detail the frequency, duration and types of physical activities they engaged in. Participants were asked to remain their normal diet and activity level during the study, and to avoid sudden changes in their activity levels, such as joining a school athletic team or marathon events during the study. The control group subjects consumed regular meals without chia seeds added, then crossed over to treatment after a five-week washout period.

Fasting and postprandial blood samples were collected in Weeks 2, 3, and 4 in Winter 2015 quarter for first recruited participants. They were required to fast overnight prior to their baseline blood glucose finger prick. Baseline blood glucose was obtained by finger pricks in the fasting state prior to seed consumption and analyzed using a glucometer, which was 98%-100% accurate based on the Food and Drugs
Administration's standard. Participants in the treatment group consumed one third of the daily-allotted chia seeds to simulate the amount of chia seeds eaten at one meal setting. Chia seeds were mixed in 6 oz. Capri Sun 100% juice and participants were required to consume the juice mixture in front of the primary investigator. The second finger prick was collected 60 minutes after the chia seeds were consumed. The control group drank the same amount and type of juice without chia seed added and had finger pricks at the same time interval as the treatment group.

Materials

Chia seeds, variety Salba, were donated by Salba Smart Natural Products, LLC. According to Salba Smart, this registered variety contains 65 kcal, 5g fat, 3g protein, 6g carbohydrates, 5g dietary fiber, 0.5g saturated fat, 4mg polyunsaturated fatty acid and 100mg potassium per 15g serving (Table 4). A carrier juice (Capri Sun 100% Juice TM) that contained 180ml fluid weight, 80calories and 20g of sugar without any additional fibers was used to mix with the chia seeds.

**Blood Glucose Analysis.** Capillary blood samples were drawn by finger pricking with single-use lancets and a portable glucose-monitoring device (FreeStyle Freedom Lite Glucose meter). This device was Food Drug Administration (FDA) approved and followed the International of Standard and Organization (ISO) guidelines. This device was 98% accurate to within 5% of true glucose value and 100% accurate for between 10% and 15% of the true glucose value under 100mg/dl glucose. One to two drops of blood samples were placed on test strips for glucose reading. We compared the pre- and post-test chia drink glucose levels between the treatment and control group for each participant as everyone had different amounts of chia seeds due to their different calorie
Satiety and Appetite Measurement. Participants filled out a food intake record with satiety ratings and VAS appetite questionnaire (Appendix H) at random points during each treatment period. A total of nine food records and appetite questionnaires were collected per treatment period. Satiety ratings were filled out before, during and after each meal on the scale of 1-10. We compared the before, during and after meal satiety ratings between chia-added and no chia-added diets. The appetite questionnaire was a 100mm VAS scale with a total of eight questions. Each question was rated from most negative on the left to most positive on the right. Participants were instructed to draw a line on where they felt their satiety level fell on the scale. The questions on the survey included: How hungry do you feel? How satisfied do you feel? How full do you feel? How much do you think you could eat right now? Would you like to eat something sweet? Would you like to eat something salty? Would you like to eat something fatty?

We analyzed the diet records using ESHA Food Processor nutrition analysis software to calculate total calories consumed and the major food groups such as fat, saturated fat, protein, carbohydrates, dietary fiber and sugar intake. Other nutrients analyzed included polyunsaturated fatty acids, cholesterol and sodium. We compared the data between chia added and no chia diet to evaluate what food groups, if any, were replaced when 20% of calories were consumed from chia seeds.

Compliance Monitoring. Participants were asked to return the unfinished chia seed bag and to document any usual diet pattern such as skipping meal due to illness or other activities in the unusual diet record form (Appendix I). Participants would also fill out the form if they accidentally ingested other chia seed containing products.
Statistical Method. Statistical analysis was performed using SPSS software and postprandial glucose levels, satiety ratings and VAS scale results were expressed as mean value +/- the standard deviation with a statistical significance level defined as p< 0.05. Postprandial glucose levels were analyzed using a 2(condition) x 3(time) repeated measure analysis of variance (ANOVA). Satiety data and nutrient analysis were analyzed using one way ANOVA and an independent T-Test was used for the VAS appetite questionnaire data. Age, weight and BMI were adjusted for using multivariable logistic regression.
CHAPTER 4
RESULTS

Recruitment

Thirty-three participants were screened and 24 were enrolled in the study during the first recruitment period. During the first treatment period, a total of 3 participants dropped out due to personal reasons and 4 were excluded due to poor response rates on the record keeping and frequently missed glucose check appointments despite phone call reminders, emails or texts. At the beginning of the second treatment period, one participant had pneumonia and her doctor ordered her to stop the study due to weakness. Two participants did not respond after the beginning of second treatment and did not come back to the study. One participant had difficulty coming to the glucose check appointments as a result of class schedule conflicts. This particular participant had a very long bus commute to school and she had all morning classes that did not allow her to fast or coming back for the 60 minute finger pricks. In addition, this participant did not turn in any food records, satiety or appetite questionnaires and was eventually excluded. By the end of the first treatment period, only 13 participants remained in the study.

In the second recruitment period, 22 participants were screened and 16 met the eligibility for enrollment. One dropped out due to a suspected minor allergy to chia seed and withdrew within the first week of consuming the chia seeds. At the start of treatment period two, two participants dropped out due to "busy school schedules". Another participant dropped out during the 3rd week because she felt there were "too many seeds to consume". One participant in the control group became ill and was hospitalized for two weeks. She did not complete any food records and was not well enough to continue with the study, and was excluded. At the end of treatment period 2, only 10 subjects remained
and completed the second phase of the study. Combining both recruitments, there were a total of 23 subjects who completed the entire study. Figure 4 below summarized the recruitment and analyses process of this study.

Figure 4. Recruitment and study flow chart.
Participant Characteristics

Table 2 provided the information of the participants' baseline characteristics and 3-day average nutrient intakes. Although 23 participants completed the study, baseline characteristics were based on the participants who had complete data to be entered in final the analyses.

Table 2

Baseline Participant Characteristics

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.67 (3.04)</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.84 (6.52)</td>
<td>146</td>
<td>170</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.11 (7.12)</td>
<td>44.4</td>
<td>69.69</td>
</tr>
<tr>
<td>BMI Avg (kg/m^2)</td>
<td>22.07 (2.07)</td>
<td>19.2</td>
<td>26.1</td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>1652.84 (369.69)</td>
<td>559.04</td>
<td>2838.37</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>226 (65.19)</td>
<td>57.6</td>
<td>422.05</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>66.35 (18.06)</td>
<td>30.82</td>
<td>126.13</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>22.55 (11.56)</td>
<td>6.25</td>
<td>82.57</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>72.24 (37.05)</td>
<td>15.59</td>
<td>177.04</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>56.55 (16.57)</td>
<td>12.71</td>
<td>148.98</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>18.03 (8.24)</td>
<td>1.7</td>
<td>50.42</td>
</tr>
<tr>
<td>PUFA (mg)</td>
<td>5.89 (4.32)</td>
<td>0</td>
<td>50.42</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>166.46 (109.42)</td>
<td>0</td>
<td>878.76</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>2679 (1086)</td>
<td>546.62</td>
<td>8176.53</td>
</tr>
</tbody>
</table>

Note: SD = Standard Deviation; BMI= Body Mass Index
Table 3 provided the estimated energy requirements for chia seed allotment and the amount of seeds used for postprandial glucose testing, which was 1/3 of daily allotted seeds.

**Table 3**

*Estimated Needs and Chia Seeds Allotment*

<table>
<thead>
<tr>
<th>Measures</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated Energy Needs (Kcal)</td>
<td>1885.09 (194.11)</td>
<td>1511.37</td>
<td>2282.48</td>
</tr>
<tr>
<td>Activity Factor</td>
<td>1.35 (0.11)</td>
<td>1.2</td>
<td>1.55</td>
</tr>
<tr>
<td>Chia seed assigned (g)</td>
<td>87.00 (8.96)</td>
<td>69.8</td>
<td>105.3</td>
</tr>
<tr>
<td>Chia seed calories (kcal)</td>
<td>377.02 (38.82)</td>
<td>302.77</td>
<td>456.5</td>
</tr>
<tr>
<td>Seeds allotted for postprandial glucose test (g)</td>
<td>29.29 (2.75)</td>
<td>24.8</td>
<td>35.1</td>
</tr>
</tbody>
</table>

Table 4 provided information of nutrient content of the chia seeds used for this study. The seeds were provided by Salba Smart Natural Products, LLC.

**Table 4**

*Salba Smart Chia Seeds Nutrient Profile*

<table>
<thead>
<tr>
<th>Serving per 15g</th>
<th>Calories (kcal)</th>
<th>Fat (g)</th>
<th>Prot (g)</th>
<th>CHO (g)</th>
<th>Fiber (g)</th>
<th>Sat. Fat (g)</th>
<th>PUFA (mg)</th>
<th>Potassium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>65</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>0.5</td>
<td>4</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: Prot= protein; CHO= carbohydrate; Sat. Fat= Saturated Fat; PUFA = polyunsaturated fatty acid
Data Collection

Postprandial Glucose

PPG measurements were collected only from the first recruitment participants. Although there were a total of 18 participants’ data collected from the first treatment period, only 12 had complete postprandial glucose data (n=12) available due to the dropouts and exclusions as previously discussed. The majority of students complied with the fasting requirement for finger pricks. One student was not fasting for one of the finger prick sessions, and on the following appointment, she did not remain fasting during the 60 minute time period prior to the 2nd finger prick. Therefore, that data was incomplete and excluded. The measurements were summarized in Table 7.

Randomized 3-day Food Records. Food record instruction and portion size explanations were discussed during the lead-in week. The random food record dates were e-mailed to the participants and followed up to ensure participants received the dates assigned. Only one participant did not turn in any food records despite frequent e-mails, phone calls and text reminders. Of all 22 food records, some records were not completed timely and participants had to be reminded via e-mails and phone calls. Questions regarding to details of the food records such as portion size clarification, specific brands, or type of products used were mostly e-mailed to the participants. Some participants provided food labels or took photos of the food consumed for reference. Two food records were incomplete due to missing information and the participants did not turn in all assigned food records at the end of the study hence they were excluded. One participant turned in the assigned food records altogether at the end of the entire study. However, her food records were complete, very detailed in food types and portion sizes,
thus we did not exclude this data. At the end of both study periods, 20 complete food records (n=20) were inputted into ESHA for the following nutrient analysis: total calories, protein, carbohydrates, dietary fiber, fat, saturated fats, polyunsaturated fatty acids, cholesterol and sodium.

**Satiety Rating.** Satiety rating instruction was discussed during the lead-in week, and examples were provided along with forms sent via e-mail. Participants were asked to rate their satiety for before, during and after each meal on their food records. Because not everyone had three meals a day, and some only ate snacks throughout the day, the satiety rating was calculated as an average of before, during and after each meal or snack. A few difficulties were encountered for instruction adherence. Some participants forgot the instructions provided during lead-in week, and filled out the satiety rating opposite from the direction given. For example, 1 is most hungry and 10 is most full; some used 10 as most hungry and 1 as most full. One participant forgot to fill out “during” meal satiety rating. Another participant used “0” as most hungry, which was not the number given in the instruction. These mistakes were corrected when the food records were received. However, some did not turn in their food records timely and those mistakes were not captured immediately; therefore, some satiety ratings results were based on recalls. The participant who turned in all food records at the end of the study did not complete any satiety rating as instructed, and it would be inaccurate to recall all 9 days at the end of the study; thus only 19 satiety ratings were analyzed (n=19).

**VAS Appetite Questionnaires.** Participants were asked to fill out a VAS appetite questionnaire at the end of dinner or the last meal of the day if it was not dinner. Two participants only turned in 8 appetite questionnaires; one had lost her food records, but
was able to find them during the next quarter and another one lost the appetite questionnaire. Both questionnaires were recalled by the participant based on the satiety level reported on that particular date. One participant did not turn in complete appetite questionnaires and she was unable to recall the missing days. Therefore, only 18 completed appetite questionnaires (n=18) were included for result analysis. The VAS scale was based on 100mm and measured using a ruler. The lower number represented less whereas a higher number represented more in the appetite sensation described on each question.

**Food Record Nutrient Analysis**

Participants’ randomized food records were analyzed to compare the difference between the control and chia supplementation period. The mean intakes and p-values were summarized in table 6. There was a significantly greater caloric intake (P=0.00) during chia seed supplementation than baseline and control. The results suggested that participants consumed on average nearly 500 more calories during the chia seed treatment period, indicating they added chia seeds in addition to their usual diet rather than displacing the calories from usual foods consumed. Subsequently, there was a significantly higher intake of protein (P= 0.01), carbohydrate (P=0.02), fiber (P=0.00), fat (P=0.00), and polyunsaturated fatty acids (P= 0.00) in the chia supplemented period than the control. Sugar, cholesterol and sodium intakes were not significant as seen in table 5.

When comparing chia supplementation to baseline, there was a significant difference seen in protein (P=0.032), fiber (P=0.00), fat (P=0.00) and polyunsaturated fatty acids (P= 0.00). Sodium (P=0.241), calorie (P=0.074) and carbohydrate ( P=0.737) intake were not significantly different.
There was a significantly higher calorie (P=0.005) and carbohydrate intake (P=0.02) during baseline when compared to control. Sodium intake was also significantly greater (P=0.028) during baseline. On average, participants consumed around 500 calories less during the control period when compared to the baseline. There were no significant differences seen in protein (P=0.38), fiber (P= 0.447), fat (P=0.189), saturated fat (P= 0.446), cholesterol (P= 0.998), sugar (P=0.779), and polyunsaturated fatty acid (P=0.888) intake between control and baseline.

Table 5

Nutrient Intake Result During Control and Chia Seed Supplementation Period (n=20)

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Mean (SD) CON</th>
<th>Mean (SD) CHIA</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>1342.92(248.5)</td>
<td>1864.30(266.35)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>177.57(46.41)</td>
<td>239.14 (52.99)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>59.72(13.96)</td>
<td>79.16(14.64)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>18.72(8.77)</td>
<td>48.92(9.25)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>65.40(29.43)</td>
<td>69.25(28.92)</td>
<td>0.924</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>47.12(15.26)</td>
<td>75.86(18.58)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>14.92(6.05)</td>
<td>20.17(20.17)</td>
<td>0.108</td>
</tr>
<tr>
<td>PUFA (mg)</td>
<td>5.36(2.59)</td>
<td>28.22(3.76)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>165.87(87.80)</td>
<td>157.88(59.45)</td>
<td>0.966</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>1972.80(603.01)</td>
<td>2244(767.75)</td>
<td>0.568</td>
</tr>
</tbody>
</table>

* Statistically significant
Hypothesis Analysis

Hypothesis 1

Table 7 summarized the fasting and PPG measurements during control and chia seed supplemented period. Hypothesis 1 posits that chia seed supplementation will reduce PPG levels. However, there was no main treatment effect for both fasting glucose (P=0.259) and PPG (P= 0.710). No significant differences were seen between treatment and time for fasting glucose (P= 0.805) and PPG (P=0.401). When comparing within subjects, no significant differences were seen in time and treatment in fasting glucose (P= 0.830) as well as in PPG (P=0.475). Therefore, research hypothesis 1 was not supported. Figure 5 shows the comparison between CON and CHIA glucose differences and Figure 6 illustrates the difference between fasting and postprandial glucose levels during CON and CHIA period.

Table 6

Fasting and Postprandial Glucose Summary (n=12)

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Mean (SD) CON</th>
<th>Mean (SD) CHIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting 1</td>
<td>91.17 (9.54)</td>
<td>92.5 (6.84)</td>
</tr>
<tr>
<td>Fasting 2</td>
<td>91 (9.18)</td>
<td>94.58 (8.47)</td>
</tr>
<tr>
<td>Fasting 3</td>
<td>87 (7.25)</td>
<td>88.67 (10.17)</td>
</tr>
<tr>
<td>Postprandial 1</td>
<td>95 (14.66)</td>
<td>98 (15.09)</td>
</tr>
<tr>
<td>Postprandial 2</td>
<td>97.5 (14.36)</td>
<td>96.33 (12.43)</td>
</tr>
<tr>
<td>Postprandial 3</td>
<td>94.75 (12.53)</td>
<td>89.42 (8.37)</td>
</tr>
</tbody>
</table>
Blood Glucose Comparison

Figure 5. Blood glucose difference during control and chia seed supplementation.

Figure 6. Blood glucose difference between fasting vs. postprandial glucose in control and chia seed supplementation.
Hypothesis 2

Hypothesis 2 posits that chia seed supplementation will increase the satiety level. Table 7 summarized the result for before, during and after meal satiety during baseline, control and chia supplemented period. The lower number indicated greatest in hunger; higher number indicated greatest in fullness. There were no significant differences between baseline, control and the chia supplemented period before, during and after meals. When comparing baseline to control, before, during and after meal p-value were 0.970, 0.969, 0.774 respectively. Comparing baseline to chia seed period, p value were 0.423, 0.395 and 0.655. CON and CHIA p-value comparisons were shown in table 7, which did not reach any statistical significance. Therefore, research hypothesis 2 was not supported.

Table 7

Satiety Rating Before, During and After Meal Summary (n=19)

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Mean (SD) Baseline</th>
<th>Mean (SD) CON</th>
<th>Mean (SD) CHIA</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>3.02 (1.08)</td>
<td>3.11 (1.16)</td>
<td>3.20 (1.10)</td>
<td>0.565</td>
</tr>
<tr>
<td>During</td>
<td>5.12 (1.44)</td>
<td>5.21 (0.93)</td>
<td>5.65 (1.35)</td>
<td>0.546</td>
</tr>
<tr>
<td>After</td>
<td>7.09 (1.42)</td>
<td>7.10 (1.42)</td>
<td>7.45 (1.16)</td>
<td>0.270</td>
</tr>
</tbody>
</table>

Note. P value indicated comparison between CON and CHIA
Figure 7. Comparison of satiety rating before, during and after meal between baseline, control and chia seed supplementation.

**VAS Appetite Questionnaires**

There were no significant differences between control and the chia supplemented group for all eight questions on the VAS questionnaire (See table 8). The questionnaire was filled out after meals, which was consistent with the satiety rating result where no significance was detected before and after chia supplementation.
### Table 8

**VAS Appetite Questionnaire Result (n=18)**

<table>
<thead>
<tr>
<th>Questions</th>
<th>Mean (SD) CON</th>
<th>Mean (SD) CHIA</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>How hungry do you feel</td>
<td>22.85 (14.27)</td>
<td>18.07 (10.88)</td>
<td>0.411</td>
</tr>
<tr>
<td>How satisfied do you feel</td>
<td>73.88 (14.15)</td>
<td>84.94 (24.2)</td>
<td>0.661</td>
</tr>
<tr>
<td>How full do you feel</td>
<td>74.49 (11.63)</td>
<td>89.88 (11.5)</td>
<td>0.752</td>
</tr>
<tr>
<td>How much do you think you can eat</td>
<td>28.33 (12.42)</td>
<td>22.99 (13.20)</td>
<td>0.713</td>
</tr>
<tr>
<td>Would you like to eat something sweet</td>
<td>37.54 (19.79)</td>
<td>29.36 (17.32)</td>
<td>0.667</td>
</tr>
<tr>
<td>Would you like to eat something salty</td>
<td>19.91 (17.53)</td>
<td>16.98 (15.69)</td>
<td>0.485</td>
</tr>
<tr>
<td>Would you like to eat something savory</td>
<td>22.25 (16.54)</td>
<td>21.73 (17.39)</td>
<td>0.874</td>
</tr>
<tr>
<td>Would you like to eat something fatty</td>
<td>14.64 (15.32)</td>
<td>11.15 (12.37)</td>
<td>0.274</td>
</tr>
</tbody>
</table>

Note. P Values were calculated using Independent T-Test.
Figure 8. Comparison of VAS appetite questionnaire between control and chia seed supplementation.
CHAPTER 5
DISCUSSION

This study sought to determine the effect of chia seed supplementation on postprandial glucose and satiety in healthy females. Participants were recruited from CPP with a mean age of 21.67 years, and a baseline mean BMI of 22.07. This was a free-living randomized crossover study with 20% calories supplemented from ‘Salba’ brand chia seeds as intervention for 5 weeks and separated by a 5-week a washout and crossover to 5 weeks of control period. Participants had the freedom of eating the assigned chia seeds at any time and any method they desired, either in liquids such as water, juice or smoothie, or adding it as cooking ingredients or toppings for snacks. The majority of participants (91%) that remained in the study were in compliance with consuming the chia seeds assigned and returned unfinished bags as instructed. One participant returned less than 1/3 of a bag during the intervention period, and one participant missed three days due to illness. Some participants who did not finish all the assigned seeds in one day were instructed to allocate the seeds on other days that did not have assigned food record, satiety and VAS questionnaire. For PPG testing, 1/3 of allotted chia seeds were consumed on site and monitored by the primary investigator (PI); therefore, the intake compliance for this study was assumed to be 100%.

Based on the nutrient analysis results, participants supplemented chia seeds in addition to their usual diet pattern. During the chia seed intervention period, the overall intake of calories, protein, carbohydrates, fiber, fat, polyunsaturated fatty acids were significantly higher as Salba chia seed is high in plant protein, dietary fiber and polyunsaturated fatty acids (Table 4). One chia seed study done by Neiman et al, (2009)
assessed macro- and micro-nutrient intake and did not find any significant differences between chia supplemented and control period. However, this study used only 50g of chia seeds per day, which is 52% lower than our study (mean 87g+/- 8.96 SD). In this study, the hunger rating was measured as a part of the overall symptom questionnaire such as digestive health, stress, overall wellbeing on a biweekly basis, and questions were not specific to satiety. The mean fiber intake in the current study was 48.92g +/- 9.25 SD during the intervention period, which nearly doubled (196%) the daily recommended guideline of 25g for women 19-50 years old. Participants' usual fiber mean intake in the control period (18.72g +/- 8.77 SD) was slightly higher than the reported national average of 15.6-15.9 gm/day (King et al, 2012). Despite significantly greater fiber intake during the chia supplementation period, there was no significant difference in increased satiety or decreased appetite after meals between the chia supplemented periods when compared to control. This finding was contrary to the research findings of improved satiety when consuming high fiber foods, particularly those with high mucilage such as flaxseed (Ibrugger et al, 2012; Kristensen et al, 2013). However, this lack of significance after meal was in line with the ‘Salba’ study conducted by Vuksan et al (2010) where appetite was not significantly lowered at 15 and 30 minutes after chia seed consumption. In that study, a significant reduction of appetite was only seen at 60, 90 and 120 minutes post meal. For our study, the appetite questionnaire was filled out right after a meal, thus the prolonged satiating effect of chia seed might not reflect on the questionnaire. The lack of significance might be due to a smaller sample size (n=19) in this study and the study design difference. Studies that measure subjective satiety often use subsequent meal intake as another indicator in addition to the VAS questionnaire, such as seen in the Lupin
fiber fortified bread conducted by Lee et al (2006), or flaxseed fiber studies conducted by Ibrugger et al (2012) and Kristensen et al (2013) as well as the pectin study done by Wanders et al (2014). Moreover, these studies require participants to consume a pre-measured amount of test products on-site, then consume a subsequent meal at a specific time. Our study design did not restrict the time, method and amount of chia seeds taken at one setting; as a result, the actual satiety sensation immediately after ingesting chia seeds might not be reflected at the time of filling out the satiety rating or VAS appetite questionnaire and it is one of the limiting factors in satiety and appetite measurement.

Methodological differences, such as how chia seeds were taken, might also be a contributing factor to the perceived satiety. ‘Salba’ studies conducted by Vuksan et al. (2010) and Ho et al. (2013) both baked chia seeds in bread and used matching calorie white bread as a placebo control. Nieman et al. (2009) mixed chia seeds in liquid, and in another study (2012), participants were allowed to put chia seeds in any foods or drinks without cooking and heating. In this current study, the majority of participants (70 %) reported taking chia seeds in liquids due to preference or convenience, 2 participants added them to foods and 3 participants added them to both liquids and foods. Due to the viscous mucilage, chia seed turns liquid into gel and make the liquids more difficult to drink. Some participants drank the liquids right away before liquids could gel, but more than half of the participants reported pre-mixing seeds in liquids and consumed them slowly throughout the day, which indicated the degree of gelling varied depending on how soon the chia seed mixture was consumed when the satiety and appetite questionnaires were completed. The degree of gelling vs non-gelled liquids, as well as the
method of supplementation such as in capsule or liquid form, could affect satiety and
gastric emptying differently as shown in the study that used different preload viscosity
and forms of pectin (Wanders et al, 2014).

It is interesting to note that when interviewing participants after data collection some
participants reported "less satisfaction" when consuming chia seeds and wanted to
consume usual foods or snacks despite experiencing increasing fullness after consuming
chia seeds. As the nutrient analysis results indicated, the mean amount of chia seeds
assigned contained 377.02 kcal (+/-38.82) and the mean chia seeds intake was 522kcal
(+/- 266.35) more than the control period, which suggested participants consumed
slightly more calories (145kcal) while supplementing chia seeds than their usual diet
during the control period. This might be in part due to the "sensory-specific satiety" as
mentioned by Gerstein et al. (2004) where the taste, look and feel of ingested foods can
influence perceived satiety rather than amount of fiber or nutrient density present. This
phenomenon might influence how participants would choose to consume familiar and
usual foods to achieve their perceived satiety sensation. This pattern could be seen in
the random three day food records where participants might repeatedly consume
particular breakfast items such as oatmeal, dry cereals, protein bars or snacks, and they
would continue eating the same pattern even during the chia supplementation period.

Despite the lack of statistical significance, the results showed a positive trend (figure
6 and 7) between chia seed intakes and improved satiety based on the satiety ratings and
VAS appetite questionnaire result. The mean satiety rating were 0.1-0.44 point greater in
the chia supplemented group before, during and after the meal (Table 7). The mean VAS
results showed that participants reported less hunger, more satiety and fullness, less
desire to eat, and less desire to eat sweet, salty, savory or fatty foods (Table 8).

Interestingly, despite the extra caloric intakes, the body composition, weight, fat and lean muscle mass were not significantly different pre and post chia seed supplementation as seen in the separate outcomes measured by the other PI of this study.

The postprandial glucose testing used 1/3 of assigned daily chia seeds; therefore, each participant received different amounts of chia seeds mixed with 6oz of 100% juice. While the carrier juice contained same calories and sugar content, the amount of fiber, protein, and fats present in the juice mixture were varied depending on the chia seeds assigned to each participant. The mean chia seeds used for postprandial glucose analysis was 29.29g (+/-2.75), which contained approximately 9.8g of dietary fiber from the ‘Salba’ chia seed. This study failed to find a significant difference between the control and chia seed supplementation on postprandial glucose. Our findings are inconsistent with the Salba study conducted by Vuksan et al. (2010), where incremental doses of 0, 7, 14, 25g of Salba seed in bread showed an inverse relationship with postprandial glucose levels in normal weight subjects. The highest dose (25g) of Salba in this study contained 8.35g of dietary fiber, which is less than our study. Ho et al. (2013) also found incremental doses of 7, 14, 25g of ‘Salba’ in both ground and whole form could significantly lower postprandial glucose levels. Both studies had a small sample size (n=11 and n=13 respectively), normal BMI subjects, and 80% power and significant level of p<0.05 to detect a change of 25+/− 18mmol x min/L in the IAUC blood glucose difference. The main difference between this study and other ‘Salba’ studies were the methodologies. Both ‘Salba’ studies mentioned above had a fixed amount of Salba seeds and postprandial glucose levels were measured incrementally from 15, 30, 45, 60, 90 and
120 minutes post ingestion. This study only had two time points, which were 0 and 60 minutes post prandial measurements.

There were also a few confounding factors that could implicate the current findings. All participants were CPP college students, and the meeting time for finger pricks varied as two treatment periods fell in two separate school quarters, which could contribute to the variation of the fasting glucose taken at the 0-minute finger prick. Participants were unable to come at the same time for each glucose appointment during the control and chia seed period. This time variation in finger pricking can vary up to three hours, as some participants came early morning between 7:30-9am and some came late morning around 11-12pm, thus the total hours of fasting were not equally controlled between participants and between treatment periods. During the 60-minute waiting time, participants were instructed to avoid vigorous activities that could have affected the serum glucose level; however, the actual activity during this 60-minute downtime was not monitored by the PI. In addition, many participants had all morning classes, and had to go back to the class while waiting for the 60-minute finger prick.

**Limitations**

In addition to the confounding factors mentioned above regarding the postprandial glucose analysis, there were age, weight, female gender and college student limitations in this study. Since this study was conducted only in females between ages 19-50 years with a mean normal BMI (<25.9kg/m²), the study results could not be generalized to other populations such as males and weight or age above and below the BMI study requirement. During the study, participants were asked to remain on their usual diets and activity patterns throughout the treatment, control and washout periods; however, it was not
possible to monitor the actual compliance of the activity levels and intake patterns, especially during the washout period.

Satiety and appetite questionnaires were based on subjective ratings, and the subjective perceived satiety varied between individuals. In the current study, the participants’ usual diet pattern was designated as their control and there was no blinding involved. Participants were fully aware of when and how much chia seeds were added to their daily intake, which could possibly affect their subjective satiety ratings. Some satiety and appetite ratings were based on recalls due to incomplete food record keeping, which might also influence the study results.
Future Research

The preliminary findings of the current research on subjective appetite and satiety can be used for further studies on the benefits of chia seed supplementation in different populations, age groups and genders. Besides subjective assessment, measurement of appetite hormones such as ghrelin, gastric inhibitory peptide (GIP), Glucagon-like peptide -1 (GLP-1) or peptide tyrosine tyrosine (PYY) can be included in a future study to reduce the bias from self-reported data. The current study is in conjunction with another primary investigator examining the effect of chia seed on body composition, and if the body composition assessment is included in the future research, a parallel double-blind study design will be appropriate to best assess body composition and reduce possible confounding during the washout period as participants’ activities and food intake cannot be visually monitored. If future studies focus on appetite and satiety assessment without body composition measurements, a cross-over design with a shorter washout period may be utilized as appetite hormones have a relatively short half-life (Bewick, 2012). For example, in the ‘Salba’ studies conducted by Vuksan et al. (2010) each participant visit was at least 48 hours apart for the test meal consumption and outcome measurements. A double-blind study using pre-measured chia seeds incorporated into baked goods can reduce the bias of the participants knowing when and how they are consuming the chia seeds. A subsequent meal intake assessment can be used in addition to the VAS questionnaire to further validate appetite and reduce the bias from relying solely on the subjective data. However, it is important to keep in mind, even though a well-controlled study that uses a fixed amount of chia seeds at a fixed timing can best assess the immediate effect of chia seed supplementation on satiety and appetite, it is less
likely to show generalizable results. Everyone consumes chia seeds differently, and while some prefer adding the seeds into foods, the majority put them into liquids out of convenience as seen in this study. A free-living study has the benefit of higher external validity where participants have freedom of incorporating test products into their usual meal routine and will be more likely to adhere to the practice in the long run.

Future studies that examine the postprandial glucose should be conducted in a well-controlled environment as there are many external factors that affect blood glucose levels. In the current study, there were many confounding factors that implicate the results. For example, participants that are mainly college students may have schedule conflicts; therefore, it is best to avoid intervention periods that will last over different school quarters. Specific fasting hours should be specified and the downtime activity should be monitored as blood glucose can easily fluctuate with different activities. The current study used 1/3 of allotted chia seeds for postprandial glucose testing; however, the amount of chia seed should be controlled to better assess the postprandial effect. Effects of incremental dosage and different forms of chia seeds on postprandial glucose relationships have already been investigated in other ‘Salba’ chia seed research. Further research may consider comparing the effect of taking chia seeds liquid mixture immediately vs. gelled chia seed liquid mixture and its effect on postprandial glucose. A comparison between chia seed and other fiber rich grains such as flaxseed or psyllium in a double-blind placebo controlled study may also be considered to evaluate the difference between various fiber rich grains and the different viscosity effect on postprandial glucose.
CHAPTER 6

CONCLUSION

Hyperglycemia and obesity are major risk factors for type 2 diabetes development. Healthy diet and weight are vital to disease prevention and to reduce risk factors. The majority of people in the US do not meet daily recommended fiber intake in spite of increasing research on its health benefits. Chia seeds contain a high amount of dietary fiber, which can be easily incorporated into usual diet patterns and supplement daily fiber needs. The current study's findings show a positive trend of improved satiety and reduced hunger with chia seed supplementation despite the lack of statistical significance. Supplementing 20% of calories from chia seeds significantly increase dietary fiber, PUFA, protein, calories, carbohydrate and fat intakes as seen in current study. Chia seed supplementation can be an effective method to increase daily dietary fiber intake, but further research is warranted to determine the immediate effect of chia seed in appetite control and their ability to attenuate postprandial glucose levels.
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Weickert, M., Mohlig, M., Koebnick, C., Holst, J. J., Namsolleck, P., Ristow, M.,...


APPENDIX A

IRB Approval Letter

State of California
Memorandum
California State Polytechnic University, Pomona
Office of Research

Date: July 30, 2014
To: Hilary Wu
Agriculture, Human Nutrition and Food
From: Dr. Jeffrey S. Mio
Chair, IRB (Human Subjects Protection Committee)
cc: IRB file
Anne Sung and Bonnie Burns-Whitmore PhD PhD
Subject: Protocol number 14-0105

Your de novo protocol entitled “Effects of chia seeds consumption on levels of triglycerides, total cholesterol, HDL, LDL, blood glucose and safety level” has been reviewed by the Cal Poly Pomona Institutional Review Board (IRB) by the expedited process. It was found to be in compliance with applicable federal and state regulations and Cal Poly Pomona policies regarding the protection of human subjects used in research. Thus, the Cal Poly Pomona IRB grants you approval to conduct the research. On its behalf, I thank you for your adherence to established policies meant to ensure the safety and privacy of your study participants. You may wish to keep a copy of this memo with you while conducting your research project.

You may initiate the project as of July 30, 2014, and it must be completed by July 29, 2015. Federal regulations limit the IRB approval of studies for up to one year. If you find the need to renew your protocol, please remember to submit a request to the IRB at least six (6) weeks before the end date to ensure continuous human subjects protection and IRB approval. It would be appreciated that you advise the IRB upon the completion of your project involving the interaction with human subjects.

Approval is conditional upon your willingness to carry out your responsibilities as the principal investigator under University policy. Your research project must be conducted according to the methods described in the final approved protocol. Should there be any changes to your research plan as described, please advise the IRB, because you may be required to submit an amendment. Additionally, should you as the investigator or any of your subjects experience any “problems which involve an undetermined element of risk” (adverse events in regulatory terms), please immediately inform the IRB of the circumstances.

If you need further assistance, you are encouraged to contact the IRB administrator, Bruce W. Kennedy MS RUTAG CMAR CPIA at 909-895-4216.

The committee wishes you success in your research endeavors.

Jeffrey S. Mio PhD
Professor, Psychology
College of Letters, Arts, and Social Sciences

Federated Assurance 00001769
IRB principles: respect for persons, beneficence, and justice
Version 1 Sep 10
### APPENDIX B

**Institutional Review Board (IRB)**

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<table>
<thead>
<tr>
<th>Primary (Principal) Investigator (PI) (faculty, staff, student, etc.)</th>
<th>Other “Investigators” as applicable (your faculty advisor, co-PIs, facilitator, sponsor, collaborators, etc.)</th>
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<tr>
<td>Your name:</td>
<td>Hilary Wu</td>
</tr>
<tr>
<td>Your status: (indicate faculty, staff, student, unaffiliated, or something else)</td>
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</tr>
<tr>
<td>Your affiliation: (college/dept)</td>
<td>Food and Nutrition Department, College of Agriculture</td>
</tr>
<tr>
<td>Phone contact: (office or cell)</td>
<td>909-274-8536</td>
</tr>
<tr>
<td>Email contact: (Cal Poly address preferred)</td>
<td><a href="mailto:hcwu@csupomona.edu">hcwu@csupomona.edu</a></td>
</tr>
<tr>
<td>Title of your IRB protocol:</td>
<td>Effect of chia seed consumption on body composition, blood glucose and satiety levels.</td>
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<td>Any vulnerable subjects (risks)?</td>
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<td>Any permissions needed? (see 5.G)</td>
<td>__ CPP health center __ place of employment __ school principal or board __ place of business __ other:</td>
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**DECLARATION BY ALL INVESTIGATORS:** This proposal is guided by the ethical principles regarding research involving human subjects as set forth in the Belmont Report. I/We agree to abide by the policies and procedures of the IRB at CPP, including obtaining appropriate training in human subject research for myself and those involved in its conduct. I/We will not initiate any research associated with this proposal on or off campus until authorized by the IRB. I/We will report to the IRB about any adverse events or
unanticipated problems (unexpected, possible greater risk, etc.) that occur. I/We will inform the IRB of a need to modify the study design requiring an amendment. I/We understand that approval, when granted, is valid for up to one year and will submit a renewal for its continuation if needed.

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<th>(PI) primary investigator:</th>
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<td>all others, including advisors:</td>
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**IRB office use**

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This protocol has been reviewed and approved for conduct by the IRB, California State Polytechnic University, Pomona.

Jeffery S. Mio PhD, Chair, IRB

Date

Answer each question in the sections below adequately enough so that ethical standards and human protection can be determined by an outside reviewer. Be sure to address all questions asked within each section, as this will help to speed the review and approval process of your protocol.

1. **RESEARCH FOCUS OR CONCEPT**

Research for IRB purposes is defined as “a systematic investigation including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge.”

http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html

**A. Purpose of this study** – Why are you conducting this study? What are the goal(s), objective(s) and outcome(s)? What hypothesis or hypotheses are you testing or what are the research questions? Explain the rationale and impetus for your research project. Provide enough detail such that: a) the IRB member(s) reviewing your protocol will understand your research plan and b) it supports a judgment of the risks and benefits in order to approve the “use” of the research participants.

Diet high in omega-3 polyunsaturated fatty acid (omega-3 fatty acid) has been shown to reduce the risk factors of cardiovascular diseases, which contributes to 26.8 and 28.1% in women and men respectively as the leading cause of death in the United States exceeding cancer12. In addition, diabetes epidemic continues to grow each year affecting millions of adults and children20. Diabetes has been shown to increase risk for heart disease10, 20, 38. Researchers have found dietary fiber improves postprandial glucose, insulin and incretin responses, attenuates serum lipids profile, and slows gastric emptying14,19, 47, 49, 50, 52. Therefore, dietary modifications to increase omega 3 fatty acid and dietary fiber consumption are important to reduce disease risk factors. Foods such as seeds are high in omega-3 fatty acid. Chia seeds in particular, have higher omega-3 fatty acid and dietary fiber contents than most other seeds such as flax seeds6,7,8,43. Chia seeds contain 23.67g omega-3 fatty acid/100g seeds, of which 54.8% are α-linolenic acid (ALA), and 34.4 g dietary fibers/100g seeds6,7,8.
Majority of the chia seed studies are animal studies. The human studies are limited to obese and overweight individuals or population with type 2 diabetes, and the results have been inconclusive\textsuperscript{27,39,40,49}. There are no studies that have evaluated the effect of chia seeds on percentage of body fat, levels of low density lipoprotein (LDL), high density lipoprotein (HDL), total triglyceride (TG), plasma fasting glucose, postprandial blood glucose, erythrocyte fatty acid and satiety level in healthy non-menopausal women. In addition, all the human studies to date were feeding studies by adding 25-50 g of chia seeds into participants’ daily diet\textsuperscript{27,39,40,49}. There has not been a study substituting 20% of the participant’s daily caloric intake with chia seeds. Thus, further studies are warranted to help define the condition under which chia seeds consumption might be beneficial to human health.

Our proposed study aims to evaluate whether supplementing omega-3 fatty acid and fiber rich chia seeds will affect the levels of LDL HDL, TG, fasting glucose, postprandial blood glucose, satiety levels and body composition while increasing the levels of erythrocyte omega-3 fatty acid. The study objectives are 1) evaluate the effect of consuming chia seeds on body composition, and satiety level. 2) evaluate the effect of chia seeds consumption on postprandial blood glucose level and fasting glucose level.

Null hypothesis:

\( H_{01} \): Fasting blood glucose and postprandial blood glucose will not be affected by chia seeds consumption.

\( H_{02} \): Level of satiety will not be affected by chia seeds consumption.

\( H_{03} \): Body composition will not be affected by chia seeds consumption.

\( H_{04} \): Waist and hip circumference will not be affected by chia seeds consumption.

Research hypothesis:

\( H_{A1} \): Consumption of chia seeds will result in decreased fasting blood glucose and postprandial blood glucose.

\( H_{A2} \): Consumption of chia seeds will result in increased levels of satiety.

\( H_{A3} \): Consumption of chia seeds will result in decreased levels of percentage of fat mass.

\( H_{A4} \): Consumption of chia seeds will result in decreased in waist and hip circumference.

B. Relevance – State specifically the relationship of your proposed research to other, previous scientific and/or scholarly investigations in the field or to existing best practices. Provide full citations (APA or MLA reference styles are good). What literature is related to your research? On what are you basing your own work, pertaining to the use of human subjects? What are you doing that builds on existing research findings/best practices? What work has come before and what have you learned from it to inform your own methods and questions?

Literature reviews included current researches regarding chia seed supplementation and its relationship with blood sugar levels or satiety levels, body composition, plasma lipid levels and fatty acids levels. The available researches found mostly uses specific brand of chia seed- Salba in either whole or ground seeds with intake amount of 25g/ day. Study subjects were either healthy volunteers or diabetic patients. Animal studies show that by substituting chia seeds in feed, an increase level of alpha linolenic acid (ALA) is observed in those animals as well as an improved level of serum LDL and TG levels. Studies in which chia seeds have been consumed in human diet have been limited to obese and overweight individuals or population with type 2 diabetes and the result has been inconclusive.

Methods were reviewed from current chia seed related studies on postprandial glucose levels and satiety, as well as reference other whole grain studies that also investigated relationship of dietary fiber intakes and serum glucose levels. In order to understand the nutrient content and property of chia seeds, several researches on chia seed’s chemical and physical properties were reviewed to explore the differences between milled or whole seeds, differences in seed coat color, nutrition variation in different geographical regions. Due to limited research available on chia seeds, different seeds such as flaxseeds were investigated for its impacts on serum lipids, glucose levels
and satiety levels. To gain understanding related to whole grains and dietary fiber, literature reviews also included whole grain consumptions, different types of dietary fibers and its impact on human intestinal health as well as current recommendation related to fiber intake. Dietary fiber references were obtained from dietary reference intakes. Currently there has not been a study to date using chia seed to substitute 20% of energy needs. Using 2000kcal intake as reference, 400kcal will be supplemented with Chia seeds, which is equivalent to approximately 2.9 oz (81g) of chia seeds based on USDA Chia seed nutrient breakdown. This amount will contain a total of 28.4g dietary fiber, and 44.4g ALA, which meets the minimum requirement of female adequate intake for dietary fiber, and omega-3 fatty acids. (see attachment A for reference)

2. METHODS

It is important that the procedures to be applied – some might call these treatments - to the human subjects are thoroughly explained and outlined. Those who will review and approve your study must fully understand what will take place during its conduct. Once approved, it is necessary that the procedures be carried out in the way they are officially described in this protocol.

A. Summarize the overall design of your proposed study. Will you use an experimental, quasi-experimental, or correlational design? What are the independent variables, interventions, treatments, etc.?

We will employ a 2 period crossover design consisting of six weeks treatment period (chia seed added and a no chia seed added control diet), separate by a six weeks wash out period. Therefore, the participants will receive the treatment and serve as their own “perfect” control. Participants will follow their assigned diet for six weeks followed with a six weeks wash out period, followed by the crossover diet. There will be a lead-in week to obtain baseline measurement for each participant upon which the results will be normalized as described below.

<table>
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<th>Lead-in</th>
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A registered dietitian will calculate the daily caloric intake for each participant prior to lead-in period and 20% of the individual's daily caloric intake will be substituted with chia seeds supplied by Salba Smart Natural Products, LLC. The amount of the chia seeds will be pre-measured and pre-packaged specifically for each participant and will be given out to participants at the beginning of the week and empty bag from previous week will be collected. This process will take approximately 5 -10 minutes depending on if participants have any questions or concerns for the PI.

Independent variables are: treatment diet (substituting 20% daily caloric intake with chia seeds) and time

Postprandial blood glucose measuring starts on week 2 Monday. Baseline glucose level will be measured prior to seed consumption. One to two drops of capillary blood samples will be drawn by
finger prick with a single used lancet. Participants’ fingers will be disinfected with single used alcohol wipes prior to needle pricking. Capillary blood sample will be placed on test strip for analysis using a glucometer (Freestyle Freedom Lite). Participants may decide on which hand and fingers to be pricked; however, we will use the same fingers for the following sample collections on week 4 and week 6 to better control the variables. Participants will then consume the pre-measured samples, which is 1/3 of the daily seed samples mixed in 6oz fruit juice. Fruit juice will not contain additional fibers and variety of juice flavors with same calorie and sugar contents will be available to better meet participants’ preferences. Postprandial blood draw will be taken at 60 minutes after consuming the sample. Participants will be required to consume all samples in front of the PI and stay in the lab for the entire session. Light tasks such as reading or writing in between the finger pricking time interval may be permitted. This process will take approximately 60-70 minutes to complete and it will be taken during the 2nd, 4th, and 6th week of each study period.

For satiety ratings, participants will be asked to fill out 3 day food records in 3 randomly selected points during each study period. They will be asked to rate their hunger using a validated rating scale (VAS scale from 1-10) and questionnaire next to each snack or meal on the 3-day food records. (see attachment C for satiety questionnaire and Food records). The questionnaire and food records will be done in participants’ own time and should take approximately 15 minutes to complete.

For blood pressure, participants will be asked to sit down for 15 minutes after checking in with PI. After 15 minutes, PI will take the automatic blood pressure monitor and ask participants to relax one of their arms for blood pressure reading. The position of the blood pressure monitor will be facing away from participants to ensure participants will not obtain their reading from looking at the monitor. This process will take approximately 15-20 minutes and will only be taken during the 2nd and 6th week of each study period.

For body composition, PI will set the Tanita Body Impedance Analyzer on a flat, form surface. The screen of the scale will be covered using a piece of paper to prevent participants from looking at their result. PI will activate the scale and when the digital reading is 0.0, participants will be asked to step on the scale with both feet evenly distributing the weight. The scale will measured participant’s body fat percentage and weight. PI will record the reading. This process will take approximately 5-10 minutes depending on each participants and will only be taken twice at the 2nd and 6th week of each study period.

Waist circumference will be measured using a flexible measuring tape between the middle of the bottom rib (close to your belly button) and iliac crest (top of the hip bone) and recorded to the nearest 0.1 cm. Hip circumference will be measured using a flexible measuring tape around the largest part of the hips (i.e. buttocks) and recorded to the nearest 0.1 cm. It will take no longer than 5 minutes to do both measurements. This measurement will be taken twice during each treatment.

Height will be measured using a stadiometer and recorded to the nearest 0.1 cm. This will take no longer than 5 minutes and will only be done once at the beginning of the study.

**B. Provide a step-by step outline of the activities included in this study.** What events will occur and in what order? How will the information about the study be presented to the participants? Please note you are asked to describe the specific measures and data to be collected in Section 5 below.

**Recruitment:**
1. Send out recruitment e-mails to CPP students and post flyers on CPP campus.
2. Send out consent forms to prospective participants

**Prior to study:**
1. Screen participants to obtain weight, height, medical history, and food intakes for eligibility to be in the study. (see attachment for questionnaire)
2. Dietitians calculate each participant’s daily caloric need based on their age and activity level, and the amount of chia bread and micro-milled chia seeds required for each participant.

3. Randomized participants into 2 groups (chia seeds added treatment group and no chia seeds added control group)

4. E-mail enrolled participants to schedule a one on one orientation for the study.

**Orientation:**
1. Collect informed consent from each participant and address any questions or concerns they may have.
2. Explain overview of the study and the protocols for filling out food record sheet, satiety questionnaire, and accident sheet.
3. Schedule next meeting with each participant and ask participants to start baseline 3 day food intakes records.

**Lead-in period:**
1. Participants will record their 3-day food intake on the provided food record sheet during this week.
2. Send a reminder e-mail to participants to begin fasting on Sunday 12 hours prior to meeting on Monday, as well as meeting time and location on Monday.

**During Treatment 1 and 2 period:**
1. On first day of the study (Monday), all participants will check in with PI in nutrition lab located in building 7 between the allotted time periods.
2. After check in, participants will turn in their 3-day food records from the lead-in week to PI.
3. PI will obtain blood pressure from each participant using an automatic blood pressure monitor. Screen of the monitor will be faced away from the participants ensuring the results will not be seemed by participants.
4. Participants will be asked to stand on the Tanita Body Impedance Analyzer for their body composition reading by PI. PI will record the reading. The screen will be faced away from participants ensuring the results will not be seemed by participants.
5. Answer any questions participants may have.
6. Same activity will be repeated throughout treatment 1 and 2 every Monday for a total of 12 weeks.
7. Postprandial blood glucose will be collected on week 2, 4 and 6 during the two treatment periods.
8. To collect postprandial blood glucose, participants in treatment group will consume 1/3 of their daily assigned samples mixing in 6oz juice. Finger prick using glucometer (Freestyle Freedom Lite) will be used to collect and analyze postprandial blood glucose. Baseline reading will be obtained prior to sample consumption and 60 minutes after consuming the samples. Participants in control group will consume juice only without chia seeds added. Capillary blood samples will be collected at baseline and 60 minutes after drinking juice using same finger prick method described above.
9. Participants will be asked to fill out a three day food record and satiety questionnaire at 3 random points of each study period.

**Note:**
Participants will turn in a (3) three days food records per treatment period and washout period to assess participants’ compliance and diet composition.

3. **SUBJECTS AND THEIR RECRUITMENT**

The terms subjects and participants are interchangeable. A human subject is a “living individual about whom an investigator (whether professional or student) conducting research obtains data through intervention or interaction with the individual or identifiable private information.” (Dept. of Health and Human Services, 45CFR46)

**A. Briefly describe the characteristics of the subject group(s).** Who in a population, from which you will sample, are you trying to study? What are you looking for in your subjects? What
Inclusion criteria for the study are female students currently enrolled in California State Polytechnic University (CPP), is between the ages of 18 to 45 years, healthy without known chronic diseases, and is willing to avoid eating foods that contain high levels of omega-3 and omega-6 fatty acids such as walnuts, pistachios, flaxseeds, chia seeds, omega-3 fatty acid enriched eggs, fish oil or any omega-3 fatty acid supplement for the duration of the study. Participants should not be pregnant or plan to become pregnant during the study period. Exclusion criteria will include individuals with known allergies to chia seeds, a body mass index (BMI) less than 18.5 or greater than 25 kg/m² and any conditions or diseases that will require treatment of which might affect fat metabolism or normal dietary intake of fat. Pregnant women, women who are currently on a diet plan or consuming alcohol on a regular basis will not be included in the study. We will screen diet pattern and medication usage of potential participants. We will not include participants with high omega-3 and omega-6 containing food intakes, taking medicines or supplements that potentially interfere with essential fatty acid metabolism such as steroid or hormone medication (other than birth control pills) as well as other fiber containing supplements that can potentially affect serum glucose and satiety levels.

(see attachment B for screening form)

B. How many subjects (or participants) will be involved in the research project? How did you determine your sample size? It is acceptable to have a range, but it must be a close approximation. For projects with surveys (e.g., electronic, phone, written, door-to-door canvassing), indicate the number to be recruited, the anticipated response rate, and thus the estimated final number of actual participants.

We plan to recruit at least 30 female students from the Cal Poly, Pomona campus to participate in the study. With a 10% anticipated dropout rate, estimated final number of actual participants would be 24.

C. What are the benefits, if any, to the subjects from their participation in the study? Most studies have some kind of benefit, even if they are purely educational. Will the subjects personally gain something through the research by being a subject? This information must be included in the consent (and/or assent) form as well. If there is no direct benefit to the subject, this needs to be stated here.

Participants will receive free dietary counseling from a Registered Dietitian and free chia seed samples. They will be able to understand and apply current Dietary Guideline for Americans for healthier food choices and lifestyle. Participants will receive free blood tests and access their results at the end of the study.

D. Will the subjects be compensated? Will they be given something? If yes, in what way (token of appreciation, money, gift, cash card, course credit, food, lottery ticket, etc.)? This information must be included in the consent (and/or assent) form as well. If there is no compensation, then state that clearly.

For participants who finish ½ of the study, they will be given a five dollar farm store gift card. Upon completion of the study, participants will be given another five dollar gift card as a token of appreciation.

E. How will you gather your potential subjects to participate in the study? Where will you recruit them? (For example, will you recruit subjects using e-mails or flyers?) Include any recruitment materials you will be using with your application. As applicable, attach copies of flyers, e-mail or blog text, advertisements, etc., to be used for the recruitment of subjects. Review by the IRB is necessary for approval of your protocol. Include the statement as follows: The Cal Poly Pomona Institutional Review Board has reviewed and approved for conduct this research involving human subjects under protocol YY - ### (meaning
year and sequence number). Will translation of materials be necessary to other languages or to a different reading and comprehension level for recruiting purposes? Consider that children often need simplified language. Studies show that the average adult reads at a 5th to 8th grade level.

Participants will be recruited through e-mails and flyers. The email will be sent out through department databases, Recruitment flyers will be posted throughout campus especially in Department of Agriculture building. (Appendix B)

F. Describe your procedures for the recruitment of a representative sample of the population. Is your recruitment based upon race, ethnicity, gender, health status, or other characteristic? If this is not the case, discuss the reasons for not having such a balanced sample (such as, the research is focused on a certain subject group or it’s a case study).

Since our research focused on the effect of omega-3 rich chia seeds in health, non menopausal women, recruitment will be based on gender, age and health status.

4. VULNERABLE SUBJECTS

When a subject has limitations, is coerced or manipulated, there is a loss of capability to volunteer, and the subject may be vulnerable. According to regulations, vulnerable subjects include prisoners, pregnant women, minors and fetuses. The IRB considers other kinds of vulnerability, for example, the possibility that bosses can coerce at the workplace and teachers can manipulate in the classroom. Research conducted with regulated vulnerable subjects requires demonstration of your training and experience with that specific population (include in section 8).

A. Minors – Will children, minors, or wards be recruited for this research? Children in most circumstances are those less than 18 years of age. Research with children involving no greater than minimal risk requires the permission of one parent and the assent of the child (45 CFR 46.404). Please note: Research involving minors is typically subject to full IRB review.

No, all the participants in the study will be at least 18 years old.

B. Other kinds – Explain research involving other vulnerable subjects such as prisoners, pregnant women, or culturally or medically vulnerable groups?

Consider the circumstances, too. For example, a pregnant woman answering a survey about being a teacher may not be vulnerable, but she could be if it’s a study about baby furniture.

No, our participants will come from a convenience sample.

5. DATA COLLECTION AND PROCEDURES

Collection methodologies include, but are not limited to: surveys, interviews, focus groups, oral histories, participant observation, observations of public behavior, research in public schools, and the analysis of existing data. Data include: survey sheets and questionnaires, biological samples, audio and video tapes, transcripts of verbal communication, photographs, paper and electronic records, previously collected (existing) information, etc. Personal and private data deemed by the IRB to be a risk to subjects if revealed include: gender, income, number of children, age, religion, ethnicity, e-mail addresses, and more. Even when labeled as demographic data, it is still personal and private and could potentially identify an individual. This is not to say such data should not be collected, but mechanisms must be described in this protocol to protect the interests of the subjects should they be (somehow) identified.

The HIPAA Privacy Rule regulations [45 CFR 164.514(b)] list specific elements that are considered to be personal identifiers. These include: name and initials; street address, city, county, precinct, zip code, or equivalent geocodes; elements of dates (except year) directly related to an individual (date of birth, admission date, discharge date, date of death); elements of date including year for persons 90 or older; telephone and/or fax number; e-mail address; social security number; medical record or health plan identification number; account number; certificate and license number; vehicle identifier and serial number including license plate number; device identifier and serial number; web address (URL), internet IP address; biometric identifier including finger and voice print, full face photographic image and comparable image; other unique identifying number, characteristic, or code.

A. List the data that you will collect from the subjects. In what format will you collect the data?

Data such as name, age, medical history, medication uses, food intake will be collect from subjects
in form of questionnaires. Finger prick for capillary blood sample will be collected for postprandial
blood sugar levels. Body compositions will be taken via Tanita Body Impedance Analyzer, a (3)
three days food records per diet treatment and control from each participant. Participants will
also be asked to fill out a validated satiety questionnaire using the VAS scale per diet treatment
and control. Waist and hip circumference will be measured using a flexible measuring tape.
Height and weight will be obtained by measurement and by questionnaire. All information will be
put on an excel sheet by ID numbers only.

B. Describe each of your study’s measures, data collection tools/apparatus, and data
collection procedures. Describe in detail all procedures to be done with human subjects.
What types of test(s) will you perform on or with the subjects? How will you carry them out?

Postprandial blood glucose measuring starts on week 2 Monday. Baseline glucose level will be
measured prior to seed consumption. One to two drops of capillary blood samples will be drawn by
finger prick with a single used lancet. Participants’ fingers will be disinfected with single used
alcohol wipes prior to needle pricking. Capillary blood sample will be placed on test trip for analysis
using a glucometer (Freestyle Freedom Lite). Participants may decide on which hand and fingers
to be pricked; however, we will use the same fingers for the following sample collections on week 4
and week 6 to better control the variables. Participants will then consume the pre-measured
samples, which is 1/3 of the daily seed samples mixed in 6oz fruit juice. Fruit juice will not
contain additional fibers and variety of juice flavors with same calorie and sugar contents will be
available to better meet participants’ preferences. Postprandial blood draw will be taken at 60
minutes after consuming the sample. Participants will be required to consume all samples in
front of the PI and stay in the lab for the entire session.

For satiety ratings, participants will be asked to fill out 3 day food records in 3 randomly selected
points during each study period. They will be asked to rate their hunger using a validated rating
scale (VAS scale from 1-10) and questionnaire next to each snack or meal on the 3-day food
records. (see attachment C for satiety questionnaire and Food records)

For blood pressure, participants will be asked to sit down for 15 minutes after checking in with PI.
After 15 minutes, PI will take the automatic blood pressure monitor and ask participants to relax
one of their arms for blood pressure reading. The position of the blood pressure monitor will be
facing away from participants to ensure participants will not obtain their reading from looking at the
monitor.

For body composition, PI will set the Tanita Body Impedance Analyzer on a flat, form surface.
The screen of the scale will be covered using a piece of paper to prevent participants from looking
at their result. PI will activate the scale and when the digital reading is 0.0, participants will be
asked to step on the scale with both feet evenly distributing the weight. The scale will measured
participant’s body fat percentage and weight. PI will record the reading.

Waist circumference will be measured using a flexible measuring tape between the middle of the
bottom rib (close to your belly button) and iliac crest (top of the hip bone) and recorded to the
nearest 0.1 cm. Hip circumference will be measured using a flexible measuring tape around the
largest part of the hips (i.e. buttocks) and recorded to the nearest 0.1 cm. It will take no longer than
5 minutes to do both measurements. This measurement will be taken twice during each treatment.

C. If applicable, have you submitted a copy of the survey or questionnaire to the IRB?
A copy (actual hard copy or PDF) of the survey should be provided for electronic
surveys. If using a published survey with a copyright, do you need and have you provided
permission to use it? If any changes are made to the survey after approval, the IRB must be
notified.

✓ yes  ____ in development (only finalized surveys can be approved)
D. If using a survey, will it be conducted online?  Provide the URL for electronic survey.  This would include SurveyMonkey or other other professional internet-based data collection surveys.  Your survey will be tested during IRB review; so discard those data before ‘going live.’

E. Will you use any third (3rd) party online websites to collect data?  If so, which websites will be utilized?  This would include any social media websites where you post your survey link for potential subjects to access.  For example, if you post a SurveyMonkey link to Facebook, your ability to control what information is exchanged over the internet could vary and be limited.  What steps will you take to insure the privacy and security of data you collect online?  How might you prevent someone under the age of 18 participating in a study designed for adults?

F. What is the timeline for your research?  When do you plan to conduct your study?  Provide approximate beginning and ending dates.  If there are multiple time periods, indicate the dates for each period.

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G. Where will your research be conducted?  What kind of authorization or permissions do you need?  Will the research take place in another country?  The IRB must receive authorization/permission verification prior to the approval of your protocol.  Will you be conducting any experiments in a lab or classroom or collecting data in the field?  The IRB needs evidence that you are permitted to conduct the research in other venues for the protection of you, your subjects, and institutions.  For example, a signed letter or email authorizing a study at your work or in a business, or from a school principal or school board, or to use the CPP student health center will be required for protocol approval.  Provide information about the human subjects procedures that apply for your international studies (see the CPP IRB Policies and Procedures document).

H. For studies involving medical records, explain compliance with the HIPAA privacy rule (Health Insurance Portability and Accountability Act) and disclosure of protected health information (PHI).  See http://www.csupomona.edu/~research/irb/Hints_help_examples.shtml for the “Experimental subject's bill of rights – Medical research” consent form if any invasive procedures are to be performed.

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6. DATA SECURITY PROCEDURES

Per California law, CC 1798.24, the researcher must provide a plan sufficient to protect personal information from improper use and disclosures, including sufficient administrative, physical, and technical safeguards to protect personal information from reasonable anticipated threats to the security or confidentiality of the information.

A. Who will have access to and use the data? Describe who else will be involved in this research. What will their responsibilities be within the study? Will you have research assistants? All others involved and engaged in the research - including research assistants and associates - must complete CITI training before they may work with subjects. For example, a person who hands out ICFs to participants is considered to be engaged in research, while a statistician who analyzes the data is not engaged. Their CITI completion record must be included below in section 8: Researcher/PI Training.

Both PI, and advisor will have access to and use the coded data. PI will be responsible for all the data collection and input the raw data into an excel sheet with participants’ assigned ID through randomization. Although, we will have research assistants helping to input the food intake record, they will not be given the data with participants’ name on it instead, they will be given the excel sheet with participants’ randomization ID numbers preventing them from identifying the participants in any way.

B. How will the raw data be kept protected and secure? How will it be coded or identified? Will social security numbers or other personally identifiable information be used? What will become of the data at the end of the study (returned, destroyed, archived)? Keep in mind that some demographic data are considered to be identifiers. If you are collecting data online, know that an IP address is considered to be an identifier. If data like audio and video tapes are kept for any reason (such as archiving for publication), the subject must be told of the purpose (e.g., conference presentations) and for how long, as part of the informed consent process. Also, if you are going to be using audio and video tapes there must be a section on the ICF for the subject to initial that they agree to being recorded. The subject has the option, after the study is over, to contact the researcher to withdraw permission for continued use. This information – summarized – must be included in the consent form.

Participants will be assigned a code through computer randomization and all of the data collection sheet will only show the participant’s code instead of their name to ensure confidentiality. The collected data such as blood samples and information will be securely placed in a locked file cabinet, to which only the PI and advisor will have access. The sheet that records the code and participants’ name will also be placed in another locked file cabinet, separated from raw data. The raw data will be kept separated from other materials from the study in a locked file cabinet in one of PI’s home. Participants’ name will be coded in a series of randomization numbers. The consent form will be put in another locked file cabinet in room 119, building 2 to further prevent the possibility of identifying the participants linking the consent form with raw data.

We will not collect social security numbers, but we will collect demographic data such as age, height, and weight. The procedure as described above will be explained on the consent form so that all participants are aware. After the study ended, the data will be archived for five years after publication and will be destroyed.

C. How will the data, results, and conclusions be utilized? Do you plan to use it in a presentation, publication, or something else? Will the data be shared with any other researchers or funding agencies? Will these data appear in a published thesis or journal publication? This information – summarized – must be included in the consent form.

The dissemination plan for the results includes but is not limited to:

- Publication in newsletter articles and research bulletins
- Publication on the Cal Poly Pomona web site, Agriscapes, e-mail announcements,
7. POTENTIAL RISKS AND THEIR ASSESSMENT

Definition of risk: A potential harm, discomfort, or inconvenience associated with your research that a reasonable volunteer would be likely to consider significant in deciding whether or not to participate. Risks include legal, social, emotional, or psychological issues, physical or biological hazards, revealing an identity, damage to reputation, exposure of behavior or medical character, illness, injury, side effects of applied or consumed products, revealing or a loss of private information, etc. Risk comes at various orders of magnitude, ranging from mere inconvenience to perceptible bodily pain.

A. What are the risks? Describe any potential harm, discomfort, or inconvenience, however minimal, as you would explain them to the subjects. It can be said that everything has a risk. Think carefully about what may potentially happen during your research. This information – summarized – must be included in the consent form.

Some of the risks identified are as followed:
- Physical hazards such as 2 blood draws and a total of 12 fingers prick for postprandial blood glucose per treatment period. There is a possibility of anemia from the blood draw, which can cause dizzy or light-headed or weak after the blood draw.
- In occasion, blood draws may cause hematomas and ecchymosis after, which means that another vein may need to be used. It leaves a very large bruise, which should begin to dissipate after a couple of days.
- Possible risk of infection at the needle site, which is rare.
- Biological hazards such as consuming 6 weeks of chia seeds bread and/or micro-milled chia seeds per treatment period. Since chia seed is high in dietary fiber, participants may experience mild gastrointestinal discomfort such as bloatedness or flatulence when consuming the amount chia seeds that’s replacing 20% of their daily calories with increased dietary fiber intakes.
- Participants who are afraid of needles (trypanophobia) or seeing blood may cause psychological distress such as nervousness or anxiety when doing the blood draws and finger pricks.
- Emotional/Psychological impact: Knowledge of their body composition, weight, blood pressure, postprandial blood glucose reading, and abnormal blood test results might result in psychological discomfort when the results are not as anticipated by the participants.
- Participants will provide their demographic information such as age, height and weight so that there may be risk for possibility for identification.
- Inconvenience for participants because they will have to check in with PI once every week during the treatment periods.
-Possible allergic reaction to chia seeds that participants did not know prior to the study. Symptoms of an allergic reaction or intolerance include scratchy throat, difficulty breathing and/or swallowing, skin rash, blurred vision, persistent diarrhea, vomiting, and excessive gastrointestinal discomfort.

B. Describe your procedures for protecting against or minimizing the potential risks. Is a debriefing statement needed? Contact information for Counseling and Psychological Services (CAPS at CPP) might be necessary. Do you have procedures and contacts with medical emergency services for treadmill exercises or phlebotomy? Could someone else not affiliated with the study obtain the personal and private data that you collect? Should an adverse event like these occur – something you don’t anticipate or didn’t plan on – the IRB web site has a reporting form for this purpose.

- In order to protect participants’ information and identities, anyone who is not affiliated with the study will not have access obtaining the personal and private data collected by this study.
- A dietitians will go over tips on how to avoid any possible side effect such as bloating,
constipation, and stomach discomfort with all participants and will be available to answer any possible questions throughout the study period.

- Prior to the study, study methods will explain to each participant so that all are aware of the blood draws. Contact information for Counseling and Psychological Services (CAPS) will be provided to each participant during orientation. During the study, if participants have any emotional or psychological stress regarding any part of the study, contact information for the CAPS will be provided.
- We will avoid recruit subjects with known trypanophobia diagnosis; however, if participants express fear about needle or finger prick, we will suggest the participants to look away during the blood draw, or lie down in supine position during blood draw to prevent possible fainting or anxiety attack. The participants will continue to use same position for every blood sample collection to minimize variables.
- Only licensed phlebotomist (clinical laboratory scientist) from the Health Center will perform the blood draw; however, finger prick poses minor risks and does not require professional training and will be performed by the primary investigator. Single used lancets and single used alcohol disinfectant will be utilized to minimize risk of infection. Participants will be instructed to wash hands thoroughly with soapy water if fingers are soiled after the finger pricks during study period.
- If more severe or intolerable symptoms are experienced, participants will be sent to the Health Center, which is conveniently located next to building 7, or will be transferred to the emergency room at the nearest hospital if any medical emergency should occur during the study or lab work period.
- Participants who developed allergic reaction to chia seed consumption will be excluded from the study.

C. Explain why these risks should be determined as reasonable in relation to the anticipated benefits, if any, while conducting research with the subjects. Include in your response the importance of the expected gain in generalizable knowledge, when evaluated against the risks.

In the short term, this study will help those students who have enrolled to incorporate healthier fats in their diets, which could help them maintain their weight and have a healthier lipid profile and glucose control. Participants will receive free dietary counseling from a registered dietitian and free chia seed samples. They will be able to understand and apply current Dietary Guideline for Americans for healthier food choices and lifestyle. Participants will receive free blood tests and access their results at the end of the study. Immediate benefits include pilot data to submit for further funding. It is hope that the result of this pilot study will stimulate further interest in the biological results of human-related chia consumption, leading to additional funding from the National Institute of Health and the USDA for larger studies and different populations. Long-term benefit could include reducing the risk of obesity related chronic disease such as diabetes, hypertension, cardiovascular disease and certain cancers through the promotion of healthy eating habits and weight maintenance.

D. Is your study anonymous or confidential? The response should be consistent with that in section 6 about your procedures to assure the protection of subjects’ information, sensitive data, and privacy. In other words: If anonymous, how will you protect that status for the participants? If confidential, how will you protect the information and data from further release? See the CPP IRB web page for a discussion of what is confidential and what is anonymous. This is a highly significant point to understand and consequently to explain in the protocol (for the Board’s review) and in the informed consent form (for the subjects). Keep in mind that studies are either anonymous or confidential, almost never both. There are processes to de-identify data obtained in confidence, thereby making it anonymous.

Our study is confidential since participants will pick up their samples from PI once a week so that there is possibility to re-identified some participants. To ensure the risk of re-identified participants, we will assign participants with a code through randomization. The sheet containing the code and participants’ name will be placed in a locked file cabinet, which only the PI and advisor have access. All of the data collection sheet during the study will only contain
participants’ assigned code. The consent form will also be placed in another locked file cabinet separated from the data to minimize the risk of re-identified participants. Any raw data collected during study such as blood sample, and measurement will be placed in a third locked file cabinet separate from the consent form. The protocol on how to secured participants’ identifies and information will be thoroughly explained in the consent form so that all participants are aware.

(see attachment D for informed consent)

8. RESEARCHER (PI) TRAINING

Both formal training and practical experience in research with human subjects are critical for the protection of the participants and minimization of risk that might be associated with the conduct of your study. Federal regulations require that investigators possess training. The CPP IRB adopted in 2006 the on-line CITI program as required training in human subjects research (CITI training is valid for five years). All investigators submitting applications to the IRB must complete appropriate modules of CITI as a condition of approval of a protocol. Other formal training will be considered by the IRB on an individual basis. See https://www.citiprogram.org and the training section of the CPP IRB website.

A. Describe the training possessed by you as the primary investigator. Include when it was obtained. Provide your CITI completion report number. Will you obtain any additional training related to this proposal? If you are a student, your advisor (faculty mentor) must possess training as well, which you are to describe in the next section (B).

Hilary Wu’s CITI program training for Introduction to Human Subjects 101 was completed on 11/05/2013 with reference ID number: 11697482. Anne Sung’s CITI training for Introduction to Human Subjects 101 was completed on 10/14/2013, reference ID number: 38087977. We will complete any additional training that is required for this proposal.

(see attachment E for CITI program training report)

B. Describe the training possessed by others, including your co-investigators, collaborators, students, staff, faculty members, a student’s mentor or advisor, etc., from Cal Poly or elsewhere, working on this study. Include when it was obtained. Provide CITI completion report number(s). Provide a copy of this report if training was obtained from a different institution.

Dr. Bonny Burns-Whitmore completed her CITI training 9/13
Student staff: Jennifer Zagorski’s CITI program training for Introduction to Human Subjects 101 was completed on 11/02/2012 with reference ID number: 3188808, and will be valid until 11/02/2017.

9. AFFILIATIONS

These questions ask about how you are related to the institution and subjects where the research project is to be conducted. As examples: you are a teacher using your students in a classroom setting as your subjects, or you work for the company where a marketing survey is to be conducted, or you have a financial interest in a product being tested, or you are working with a colleague in another country. Each of these examples presents an element of risk. IRB reviewers will evaluate whether these risks are reasonable and whether they are sufficiently controlled, minimized, or eliminated by your procedures.

A. Are you collaborating with another group such as a school, community association, government agency, etc.? Is IRB approval necessary, or being obtained elsewhere (domestically or internationally)? Is the study being sponsored or supported through a grant, contract, or other financial arrangement? Does the funding agency require IRB approval? Describe as appropriate. The IRB is required to collect such data for OHRP (Office for Human Research Protections) on studies funded by the DHHS (NIH, FDA, etc.). The Cal Poly Pomona IRB can work with the ethical research boards at other institutions to negotiate approvals.

We do not collaborated with other group, but we are hoping to obtain sponsorship from Salba Smart Natural Products, LLC to provide chia seeds. We are still in progress securing the
sponsorship. If sponsorship is confirmed, Salba Smart Natural Products, LLC will accept Cal Poly Pomona’s IRB approval so that there is no additional IRB approval needed at this time. If Salba Smart Natural Products, LLC agrees to sponsor samples, we will provide monthly progress report of the study and a full report at the end of the study; however, raw data will not be given to Chia Brand Co. If we do not obtain sponsorship from them, we still plan to purchase samples, but they will not be granted privilege to access any results or information of this study.

B. Personal gain – As an investigator involved with the project, do you or any of your family members (e.g. spouse, child) have a financial or other “self” interest in this study? If yes, describe. For example: an MBA student may conduct a consumer survey about establishing a business (restaurant) she herself wants to open. In this case, there could be a need for disclosure of that fact in the informed consent form.

No, we do not have any financial or other “self” interest in this study. The only interest we may have is to investigate the effect of chia seeds on the biological parameters such as lipid panel. We will received a degree thesis, and perhaps a publishable paper for this study.

C. Are you a student? Is this project part of a classroom experience or a graduate program? Has your advisor/mentor reviewed your IRB application? Describe as appropriate. Student protocols cannot be reviewed by the IRB until there is evidence of 1) the advisor’s contribution to the protocol, 2) his/her training with human subjects, and 3) approval including his/her signature on the “Investigator Information and Signature Page” of this protocol application. See the Cal Poly Pomona guideline on “Undue Influence and Coercion in Protocol Consent” within the IRB website for acceptable model statements to use for degree completion.

Yes, both PI are graduate students in the Food and Nutrition department and completion of this study is a component of the Master Degree program at Cal Poly Pomona. Prior to submitting this application to IRB, our advisor has reviewed this application and necessary adjustment are made according to their suggestions.

D. Do you have any kind of pre-existing relationships with the subjects (participants) or institutions involved in conducting this study? Is there a possibility that collection of data from either the participant or institution may be seen as a favor and/or coercive when they are being asked to volunteer information? As appropriate, please describe. State any type of relationship apart from the study itself. If you work for an off-campus organization or entity and need to keep its identity confidential, note that here. See the Cal Poly Pomona guideline on “Undue Influence and Coercion in Protocol Consent” within the IRB website.

We do not have any pre-existing relationship with the participants other than some of them may be our classmates. However, the collection of data from those participants who were or are taking the same classes as the PI do not involved any favor for the PI nor forced into. If so, Dr. Burns-Whitmore will collect their data.

E. If you are not affiliated with Cal Poly Pomona, who is your co-PI or facilitator on campus? A co-PI applies when there is a collaborative research project being proposed in this protocol. A facilitator applies when there is a need for logistical support to conduct your study at Cal Poly Pomona. Not all studies need a facilitator, but the IRB may, upon review of your protocol, make it a condition for approval. See section 14.14 of the IRB policies and procedures. Obtaining a facilitator is the responsibility of the PI(s). Describe as appropriate. Specify the name, email address, and phone number of either your co-PI(s) or facilitator.

Not applicable

F. If you are collaborating with another group such as a school, community association, government agency, etc., do you have official approval to conduct research at this site? The IRB will need written confirmation of this approval.

Not applicable

G. Though there may not be one, could there be the perception of a conflict of interest for either you, as the investigator, or for the subjects in this study? If so,
how will you manage that? See http://www.csupomona.edu/~policies/Administrative/conflict_of_interest_and_financial_disclosure.html.

There will not be a conflict of interest. Both PI and advisor are not affiliated or employed by any Chia seed companies. Chia seeds will be obtained from Chia-brand, and will not be purchased from any possible prospective participants’ related business.

10. INFORMED CONSENT FORM FOR ADULTS AND ASSENT FOR MINORS
The informed consent form (ICF) is the means by which you as the PI convey not only the research, but also the principles of human subjects protections to your subjects: respect, beneficence, and justice. There are examples on the IRB website. Complete the accompanying consent form(s) below. Include it as part of the URL in electronic surveys. Include it when submitting this protocol application for review. Include the assigned IRB protocol number in your ICFs. Be sure that your ICF meets all of the requirements in the ICF Checklist listed below.

A. How will you obtain and document informed consent (for adults) or assent (for children)? Which study personnel will be involved in obtaining consent and/or assent? For certain types of research methods, like anonymous on-line surveys, it is possible to obtain a waiver of documentation of consent (implied or passive) from the subjects. Contact the IRB for a determination and the requirements. A justification must be provided to obtain the waiver during the IRB member review.

ICF will be given out to prospective participants during screening by the PIs. After PI has confirmed eligibilities of the participants, they will receive e-mail from the PI inviting them to participate in the study. Participants will meet with PI at orientation and turn in the consent form with signatures. Participants will receive a copy of the ICF and will be asked to keep it in a safe place for references. Participants will not be allowed to participate in the study if the consent form is not turned in to the PI prior to lead in period.

B. Will there be recruitment of subjects who cannot themselves provide informed consent? If so, how will informed consent be documented for this population? For example, the ability of minors to assent could be dependent upon their age and/or their circumstance. Persons in vulnerable situations could be impaired in their ability to understand the study and be able to consent.

Prospective participants will be informed regarding the overview of the study as well as the necessary data collection method, and the fine print in the ICF by PI or the advisor to ensure they fully understand the study and all the method collection procedures.

C. Describe how you will maintain the consent forms received from the subjects?
Where (the location) will they be kept? For how long/until when? Will they be kept separate from subject data and specimens? For anonymous studies, it is crucial to keep identifiers separated from the actual data.

All the consent forms will be placed in a locked file cabinet separate from all the study data and samples for 5 year after the study. After 5 years of the study, all the consent forms including the data will be destroyed.

D. How many consent and assent forms are you submitting? Have you reviewed the checklist below? The IRB requests the header on the following page be included in the ICF(s) of all Cal Poly Pomona approved protocols. It is also provided in Spanish. Complete the ICF based upon the elements in the checklist below. A properly written ICF will include the following elements. You, as the Principal Investigator, are responsible for addressing each when writing your consent and/or assent form. Both federal and California regulations require the inclusion of these elements to adequately inform subjects when participating in research. Incomplete forms will be returned to you for revision. See the IRB website for examples. You may submit the ICF as part of this protocol application or you may send it as an attachment, but it must have the informational header below.

We will only submit one consent form.
Informed Consent Form Checklist

- Title of the protocol (same as on the front page of this application)
- Protocol number as assigned by the IRB (it will be provided after the protocol is submitted to the IRB administrator); it must appear distinctly (e.g., bolded, its own line)
- A telephone number and/or e-mail address of all primary investigator(s) of this proposal, including faculty members and graduate students, who would be the point(s) of contact for the subjects
- Affiliations (professional and institution) of the contacts and investigators; use full names, thus don’t write Cal Poly Pomona - use California State Polytechnic University, Pomona
- Clarification of the contacts in research projects which involve multiple sites (there can be multiple offices of research for example)
- A statement that the study you are conducting involves research
- An explanation of the purpose(s) of the research; why it's being conducted by you
- A description of what the subject must do as part of the research, what data will be collected, what will happen to the data after the "active" phase of interaction with the subject is completed. (It has been found useful to include blocks in the ICF for subjects to initial when audio or video taping, so as to further document that these methods will be conducted.)
- The expected duration of the subject's participation on the study (e.g., 50 mins in one day, four visits between May 1 and June 30)
- The information about the procedures must be presented in layman's terms (at the 5th grade reading level); it must fully explain to the subjects what they are expected to do
- The entire consent and/or assent form may need to be translated into the subject's language of fluency
- Identification of any procedures or methods which are experimental
- A description of any reasonable and foreseeable risks or discomforts to the subject
- Changes of pronoun as appropriate to the subjects (e.g., you will be asked …; your child will do …)
- A description of any benefits to the subject or others which may reasonably be expected (or not) from the research
- A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject
- For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments would be available if injury occurs and, if so, what that would consist of and where further information may be obtained
- California law, under Health & Safety Code Section 24172, requires that any person asked to take part as a subject in research involving a medical experiment, or any person asked to consent to such participation on behalf of another, is entitled to receive the Experimental Research Subject's Bill of Rights written in the language in which the person is fluent.
- An explanation of whom to contact for answers to pertinent questions about the research and research subject's rights, and - as appropriate - to contact in the case of a research-related injury to the subject
- A statement that participation is voluntary, that declining to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits, to which the subject is otherwise entitled
- Printed name and signature lines for the subject and the date signed
- Printed name and signature lines for the principal investigators (e.g., faculty member or the graduate student conducting the research) and perhaps research associates; and the date signed
- A statement that the subject is entitled to receive a copy of the completed informed consent form.

California State Polytechnic University, Pomona
Informed Consent Form for Research Involving Human Subjects

You are being invited to participate in a research study, which the Cal Poly Pomona Institutional Review Board (IRB) has reviewed and approved for conduct by the investigators named here. This form is designed to provide you - as a human subject - with information about this study. The Investigator or his/her representative will describe this study to you and answer any of your questions. You are entitled to an Experimental Research Subject's Bill of Rights and a copy of this form. If you have any questions about your rights as a subject, complaints about the informed consent process of this research study, or experience an adverse event (something goes wrong), please contact the Compliance Office within Cal Poly Pomona's Office of Research at (909) 869-4215. More information is available at the IRB website, www.csupomona.edu/research/irb.
Forma de Consentimiento Informada para Investigación que Implica Sujetos Humanos

Usted está invitado a participar en un estudio de investigación que el Comité Examinador Institucional (CEI) de Cal Poly Pomona ha revisado y aprobado para ser conducido por los investigadores nombrados aquí. Esta forma está diseñada para proporcionarle información acerca de este estudio en su calidad de sujeto humano. El investigador o su representante le describirán este estudio y le contestarán cualquier pregunta que tenga. Usted tiene derecho a la Declaración de Derechos del Sujeto que participe en una Investigación Experimental y a recibir una copia de este documento. Si tiene alguna pregunta o quejas acerca del proceso descrito en dicho documento, por favor llame a la Oficina de la Conformidad que forma parte de la Oficina de Investigación de la Universidad de Cal Poly Pomona al (909) 869-4215. Más información esa disponible en sitio web en el CEI en el www.csupomona.edu/research/irb.
APPENDIX C1

IRB Addendum 1

October 7, 2014
IRB # 14-0105
IRB Addendum #1

This addendum was submitted to request revision of original IRB protocol # 14-0105.

**Addition to the IRB protocol are as followed:**

**Section 1A Hypothesis**

H04 : Waist and hip circumference will not be affected by chia seeds consumption. 
H04 : Consumption of chia seeds will result in decreased in waist and hip circumference.

**Section 2A Summary of overall study design**

Chia seeds are supplied by Salba Smart Natural Products, LLC. The amount of the chia seeds will be pre-measured and pre-packaged specifically for each participant and will be given out to participants at the beginning of the week and empty bag from previous week will be collected.

Participants will consume the pre-measured samples, which is 1/3 of the daily seed samples mixed in 6oz fruit juice. Fruit juice will not contain additional fibers and variety of juice flavors with same calorie and sugar contents will be available to better meet participants' preferences. Postprandial blood draw will be taken at 60 minutes after consuming the sample. Participants will be required to consume all samples in front of the PI and stay in the lab for the entire session. Light tasks such as reading or writing in between the finger pricking time interval may be permitted. This process will take approximately 60-70 minutes to complete.

**Section 2B During treatment 1 and 2 period**

To collect postprandial blood glucose, participants in treatment group will consume 1/3 of their daily assigned samples mixing in 6oz juice. Finger prick using glucometer (Freestyle Freedom Lite) will be used to collect and analyze postprandial blood glucose. Baseline reading will be obtained prior to sample consumption and 60 minutes after consuming the samples. Participants in control group will consume juice only without chia seeds added. Capillary blood samples will be collected at baseline and 60 minutes after drinking juice using same finger prick method described above.

**Section 5A Data collection**

Waist and hip circumference will be measured using a flexible measuring tape.
Section 5B Data collection measures, tool and procedure

Baseline glucose level will be measured prior to seed consumption. Participants will then consume the pre-measured samples, which is 1/3 of the daily seed samples mixed in 6oz fruit juice. Fruit juice will not contain additional fibers and variety of juice flavors with same calorie and sugar contents will be available to better meet participants' preferences. Postprandial blood draw will be taken at 60 minutes after consuming the sample. Participants will be required to consume all samples in front of the PI and stay in the lab for the entire session.

Waist circumference will be measured using a flexible measuring tape between the middle of the bottom rib (close to your belly button) and iliac crest (top of the hip bone) and recorded to the nearest 0.1 cm. Hip circumference will be measured using a flexible measuring tape around the largest part of the hips (i.e. buttocks) and recorded to the nearest 0.1 cm. It will take no longer than 5 minutes to do both measurements. This measurement will be taken twice during each treatment.

Section 9A Affiliations

We do not collaborated with other group, but we are hoping to obtain sponsorship from Salba Smart Natural Products, LLC to provide chia seeds. We are still in progress securing the sponsorship. If sponsorship is confirmed, Salba Smart Natural Products, LLC will accept Cal Poly Pomona's IRB approval so that there is no additional IRB approval needed at this time.

Deletion to the IRB protocol are as followed:

1. in 1A, paragraph 3, deleted sentence for blood lipid
2. in 1A, deleted the original hypothesis for blood lipid and erythrocyte fatty acid incorporation
3. in 2A, deleted the procedure to collect blood lipid
4. in 2 B, deleted procedure to collect blood lipid
5. in 5 A, deleted "we will collect two blood samples, health center will collect about 2 tubes of blood."
6. in 5 B, deleted description of blood lipid collection
This addendum was submitted to request revision of original IRB protocol # 14-0105. 

**Addition to the IRB protocol are as followed:**

**Section 2A. Summary of overall study design**

Body composition will also be measured by dual-energy x-ray absorptiometry (DXA) (Hologic Discovery-QDR Series Densitometer, Bedford, MA) which is a safe and reliable assessment that has been previously implemented in various subclinical and clinical subject populations. DXA scans are typically 7-10 minutes in duration and non-invasive with marginal radiation exposure. The effective dose for a whole body scan using the Hologic Discovery system is <5 uSv, which is less than half a day of natural background exposure and less than half the dose of a standard x-ray scan. Total body mass (TBM) will be quantified through the scan. A 3-compartment model of body composition will be applied through which FM (kg) and non-bone LBM (i.e. fat free mass – bone mineral content) (kg) will be analyzed for the whole body. Data for body fat percentage (%BF) will also be acquired from DXA measurements. The DXA machine will be calibrated before each scan using a manufacturer-provided phantom. All DXA measurements and analyses will be conducted by a single certified technologist with a California State x-ray permit.

**Section 5B Data collection measures, tool and procedure**

Body composition will also be measured by dual-energy x-ray absorptiometry (DXA) (Hologic Discovery-QDR Series Densitometer, Bedford, MA) which is a safe and reliable assessment that has been previously implemented in special subject populations. DXA scans are typically 7-10 minutes in duration and non-invasive with marginal radiation exposure. The effective dose for a whole body scan using the Hologic Discovery system is <5 uSv, which is less than half a day of natural background exposure and less than half the dose of a standard x-ray scan. Total body mass (TBM) will be quantified through the scan. A 3-compartment model of body composition will be applied through which FM (kg) and non-bone LBM (i.e. fat free mass – bone mineral content) (kg) will be analyzed for the whole body. Data for body fat percentage (%BF) will also be acquired from DXA measurements. The DXA machine will be calibrated before each scan using a manufacturer-provided phantom. All DXA measurements and analyses will be conducted by a single certified technologist with a California State x-ray permit.

**Section 7A Potential risk**

Radiation from DXA might have a carcinogenic effect, though the dose used for a whole body scan is <5 uSv, which is less than half a day of natural background exposure and less than half the dose of a standard x-ray scan.

Informed Consent form was revised to reflect the above change in study procedure and risk.
APPENDIX C3
IRB Addendum 3

December 1, 2014
IRB # 14-0105
IRB Addendum #3

This addendum was submitted to request revision of original IRB protocol # 14-0105.

1. Change study to 5 weeks instead of 6 weeks of each treatment period and wash out.

2. New recruitment in Winter quarter 2015 to remove glucose measurement. Changes made are as followed:

1A: delete the Null and Research Hypothesis for glucose
2A: delete postprandial glucose procedure
2B: under Under Treatment 1 and 2, delete bullet 7 and 8 for postprandial glucose
5B: delete postprandial glucose procedure
7A: delete postprandial glucose risk (bullet 2 and 3)

Informed Consent was revised to reflect the change:

Under Purpose of Study: delete postprandial glucose
Under Study Procedure: delete bullet 7 for postprandial glucose
under Risk: delete bullet 1-3 for postprandial glucose.
Effect of chia seed consumption on body composition, blood glucose and satiety level.
IRB Protocol # 14-0105(CPP)

RESEARCHERS:

Hilary Wu, (Principal Investigator), RD, hewu@csupomona.edu (909) 274-8536
Anne Sung (Principal Investigator), RD acsung@csupomona.edu (626) 246-2521
Master Students, Food Science and Nutrition Department, California State Polytechnic University, Pomona
Dr. Bonny Burns-Whitmore (Co-Investigator), RD, Professor, Department of Human Nutrition and Food Science, California Polytechnic University, Pomona; bburnswhitmo@csupomona.edu; (909) 869-3793

RESEARCHERS’ STATEMENT:

We are asking you to participate in a research study. The purpose of this consent form is to give you the information you will need to help you decide if you would like to participate. The study will begin on the week of October 20, 2014, and will continue for a total of 19 weeks. Please read the form carefully. If there is anything that is unclear, please do not hesitate to obtain clarification from the researchers. When we have answered all your questions, please decide if you would like to be included in the study. This process is called “informed consent”. You will be provided with a copy of this form for your records. The data from this study will be compiled from both campuses and analyzed. Your name will not appear on any of the data sheets.

PURPOSE OF THE STUDY

The overall purpose of this study is to examine the effects of milled chia seed consumption on body composition, fasting and postprandial blood glucose levels and satiety using a cross-over study with two 6-week treatments (chia seed added-20% of kcals and a no-chia control diet), separated by a 6-week washout period. We will recruit 30 female students from Cal Poly Pomona. We hope to show the health benefit of chia seed consumption as part of an overall healthy lifestyle. Chia (Salvia Hispanica, Family Lamiaceae) is native to California and has been safely used by Native American and the Aztec as a food source for its high levels of dietary fiber and omega-3 fatty acid. Chia seeds can be consumed raw or put into smoothies, breakfast cereals, or yogurt.

Hilary Wu will be looking at the effects of chia seed supplementation on blood pressure, body composition and waist and hip circumference.
Anne Sung will be looking at the effects of chia seed supplementation on postprandial blood sugar, fasting glucose levels, and satiety.
STUDY PROCEDURES

In the Fall quarter, you will be assigned to one of two groups, either consuming chia seeds for six weeks or not consuming chia seeds for six weeks. In the Winter quarter, you will crossover to the other group; for example, those who do not consume chia seeds during the Fall quarter, will consume chia seeds for six weeks. For those who consumed chia seeds during the Fall quarter, will consume a background diet/normal diet without chia seeds.

Treatment #1 will either be the chia seeds or chia seeds-free treatment. Treatment #2 will be the opposite of Treatment #1.

1. After signing the consent form, you will fill out a brief health-screening and qualification questionnaire.
   a. Examples of questions will be:
      - Are you currently suffering from or have you ever been diagnosed with a chronic disease (heart attack, cancer, diabetes, renal failure, asthma)?
      - Do you eat chia seeds on a regular basis? How much chia seeds do you add to your food?
   b. For important safety reasons, you will be asked to answer each and every question on the health-screening questionnaire prior to qualification for the study; especially the seeds-allergy and medication log.

2. You will be educated/instructed about the background diet and exercise plan that you must maintain for the duration of the study, by a registered dietitian. The background diet/habitual diet is your normal diet, but free from any food that has high level of omega-3 fatty acid like walnuts, pistachios, chia seeds, flax seeds, omega-3 enriched eggs, fish oil, or any omega-3 fatty acid, or fiber supplements. This instruction will require you to meet with the study personnel a total of fourteen study visits.

3. This study is composed of two distinct experimental treatments (six weeks each) with a six-week washout (rest period) diet between the treatment diets:
   a. The chia seed-free diet, in which you must maintain just the background diet.
   b. The chia seed-added diet, in which you must consume 20% of your calories of chia seeds per day in addition to the background diet.
   c. We will ask that you meet with the study personnel every week during the chia seed treatment to pick up your weekly allotment of seeds.
   d. There will be a six-week washout (rest period) after your first six weeks of treatment period. This includes the holiday break. We want you to continue to eat your regular diet during this time. The study will resume in the Winter quarter.

NOTE: The order in which you complete these two treatments will be randomly determined at the beginning of the study. The two experimental treatments will be separated by a six-week period in which only the background diet is consumed.
4. Before you begin the first experimental phase, you will complete a one-week “lead-in” period in which the background diet will be consumed and we will ask you to complete a 3-day food record.

5. Your body composition will be measured at the beginning and end of each distinct treatment using a Tanita brand bioimpedance analyzer. The body composition measurements will require you to remove your shoes and socks, and stand on a platform while the machine analyzes body composition. The analysis will take approximately 5-10 minutes. This measurement will be taken twice during each treatment.

6. Your blood pressure will be measured at these same intervals using an Omicron blood pressure cuff. You will be asked to sit down for 15 minutes before we can measure your blood pressure. The blood pressure measurement will require you to allow us to measure your blood pressure with a wrist cuff that will expand and then deflate, revealing your blood pressure and heart rate. You will be asked to sit down and have your blood pressure measured using a blood pressure cuff around your left wrist. This process will take about 15-20 minutes. This measurement will be taken twice during each treatment.

5. Your postprandial blood sugar will be obtained by finger pricking using a glucometer. Finger prick will be done at baseline prior to consuming chia seeds and at 60 minute after consumption. Each person will have individual sterile lancets and test strip for the finger pricks, and only 1-2 drops of blood are needed for each glucose reading. This process will take approximately 60-70 minutes to complete and it will be taken during the 2nd, 4th and 6th week of each study period. You may perform light tasks such as reading or writing in between finger pricks.

6. At three random points during each of the diet period you will be asked to fill out a 3-day food record. You do not need to be on-site to complete this task. Jennifer Zagorski will be collecting this data and input it in the computer.

10. You will be asked to rate your hunger using a validated rating scale (1-10) and questionnaire next to each snack or meal on your three required 3-day food records. You can turn in 3 day food record and questionnaire at the next nutrition lab visit.

11. Your waist circumference will be measured using a flexible measuring tape between the middle of the bottom rib (close to your belly button) and iliac crest (top of the hip bone) and recorded to the nearest 0.1 cm. Hip circumference will be measured using a flexible measuring tape around the largest part of the hips (i.e. buttocks) and recorded to the nearest 0.1 cm. It will take no longer than 5 minutes to do both measurements. This measurement will be taken twice during each treatment.

12. Your height will be measured using a stadiometer and recorded to the nearest 0.1 cm. This will take no longer than 5 minutes and will only be done once at the beginning of the study.

**INCLUSION CRITERIA**

You are qualified to participate in this study if you:

- are a female.
- are between the ages of 18 and 45.
- are not averse to consuming chia seeds on a regular basis (at least six weeks).
- have not taken any medication for any chronic disease (heart, diabetes, cancer) for twelve weeks.
- have not taken any steroid or hormone medication in the last eight weeks.
- are free of any other chronic diseases.
- do not consume excess amounts of nuts and seeds.
- do not consume alcohol on a regular basis.
- are not currently on a diet plan.
- are not pregnant or plan to become pregnant.
EXCLUSION CRITERIA

You are NOT qualified to participate in this study if you:
- are a child, teenager, woman, or male younger than 18 or older than 45.
- eat large quantities of chia seeds on a regular basis.
- are taking any steroid or hormone medication (other than birth control pills).
- are taking laxatives or fiber containing supplements on a regular basis.
- are pregnant or become pregnant.
- are diagnosed with any chronic diseases.
- are currently on a diet plan.
- have a known allergy to seeds.
- consume excess amounts of seeds or nuts.
- consume alcohol on a regular basis.
- have a pacemaker or metal pins or plates in the body.

This study will be conducted from the Cal Poly Pomona campus. You will be required to come to campus each week (place to be determined as per your schedule) to pick up your allotment of chia seeds, and will be required to visit the Student Health Center for a blood draw a total of 4 times. You will be asked to fast for 12 hours before the blood draw.

RISKS, STRESS, OR DISCOMFORT

- You will be asked to have two blood draws and a total of twelve fingers pricks for the study period. There is a possibility of anemia from the blood draw, which can cause dizziness or light-headedness or weakness after the blood draw.
- On occasion, blood draws may cause hematomas and ecchymosis, which means that another vein may need to be used. A collapsed vein leaves a large bruise, which should begin to dissipate after a couple of days. If the bruise does not go away or seems to spread, or you experience pain or numbness of arm, please contact the Health Center or your physician, and Dr. Burns-Whitmore.
- There can be a potential risk of infection at the needle, which is rare. If you experience persistent pain, swelling and redness at the needle site, please contact the Health Center or your physician, and Dr. Burns-Whitmore.
- You may experience mild gastrointestinal discomfort such as bloatedness or flatulence, when consuming the amount of dietary fiber from chia seeds that account for 20% of your daily calories for a period of six weeks.
- You may not know whether you have allergic reaction to consuming chia seeds prior to the study. Some possible symptoms of an allergic reaction or intolerance include: scratchy throat, difficulty breathing and/or swallowing, skin rash, blurred vision, persistent diarrhea, vomiting, and excessive gastrointestinal discomfort.
- There may be a chance that you will feel nervousness or anxiety when seeing needles or blood during blood draw and finger pricks. You may not be eligible for the study if you have a diagnosis of trypanophobia, which fear of needles.
- You may feel anxious or stressful to see your body composition, height, weight, blood pressure, postprandial blood glucose results and abnormal blood test result. Feel free to contact Principal Investigators (PIs), Hilary Wu or Anne Sung, and / or Counseling and Psychological Services (CAPS) to discuss the emotional stress.
- There may be a potential risk for personal identification, as you will provide demographic information such as age, height and weight. We will ensure maintaining confidentiality during the by assigning you an identification number.
You may feel inconvenience during the course of the study, as you will have to check in with PIs once every week during the treatment periods. Feel free to discuss potential schedule conflicts or concerns with PIs.

If you experience any of the above mentioned symptoms, intolerances, stress or discomfort in connection with this study, please do not hesitate to get in touch with Principal Investigators Hilary Wu at (909) 274-8536, or Anne Sung at (626) 246-2521, Dr. Burns-Whitmore at (909) 869-3793, or your physician to discuss options.

If more severe or intolerable symptoms are experienced, please contact the Student Health Center at (805) 231-0864-cell, Dr. Burns-Whitmore at (909) 869-3793, the emergency room at the nearest hospital, 911 or your physician, and immediately discontinue eating the chia seeds.

Symptoms of an allergic reaction or intolerance include: scratchy throat, difficulty breathing and/or swallowing, skin rash, blurred vision, persistent diarrhea, vomiting, and excessive gastrointestinal discomfort.

Dr. Burns-Whitmore can be contacted at (909) 869-3793 or bburnswhitmo@csupomona.edu
Hilary Wu can be contacted at (909) 274-8536 or hcwu@csupomona.edu
Anne Sung can be contacted at (626) 246-2521 or acsung@csupomona.edu

If your blood values are found to be not within the normal range, we will ask you to have the values re-checked at the Student Health Center.

It is possible that you might experience a weight gain, however, a number of published scientific studies show that adding 20% of your required calories in the form of seeds will not cause significant weight gain.

**BENEFITS OF THIS STUDY**

You will receive free dietary counseling from a Registered Dietitian and free chia seeds for six weeks. You will be able to understand and apply current Dietary Guideline for Americans for healthier food choices and lifestyle. You will receive compensation in the form of a gift card worth $5.00 when you finish half of the study. At the end of the study, there will be another compensation in the form of a gift card worth $5.00. You will also have access to the results of your blood test, body composition as well as other measurements.

**PARTICIPATION IN THE STUDY**

Your participation in this study is voluntary, and declining to participate will involve no penalty or loss of benefits to which you are otherwise entitled, and you may discontinue participation at any time without penalty. You will receive compensation pro-rated in the form of a gift card for your participation. For example if you only complete one treatment, you will receive a $5.00 gift card instead of $10. You will also receive a certificate of thanks from the study personnel at the end of the study or if you cannot complete the study.

**OTHER INFORMATION**

Any information provided by you will be confidential. In the event that the investigators learn that any participant intends to harm herself or others, this intent must be reported to the authorities. University staff sometimes reviews studies to ensure that they are being done safely, legally, and ethically. The reviewers will protect your privacy, and study records will not be used to harm any person or put a person at legal risk.

**COMPENSATION FOR INJURY**

There will be no compensation for lost wages, lost time, allergic reactions, adverse reactions, or pain. Payment for care resulting from adverse reactions is the sole responsibility of the participant and they
should consider having medical insurance to pay for such care. If your test results are abnormal, you may need follow-up medical evaluation and treatment. Payment for care resulting from abnormal test results is the sole responsibility of the participant and you should consider having medical insurance to pay for such care.

**PRINTED NAME OF STAFF/PERSON OBTAINING CONSENT**  
**DATE**  
**SIGNATURE**

**SUBJECTS’ STATEMENT**

This study has been explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have any questions later about the study, I can ask one of the listed researchers listed. If I have questions about my rights as a research subject or a research-related injury, I can call the Compliance Office within Cal Poly Pomona’s Office of Research at (909) 869-4215. I will receive a copy of this consent form.

**PRINTED NAME OF SUBJECT**  
**SIGNATURE**  
**DATE**

**EXPERIMENTAL RESEARCH SUBJECTS’ BILL OF RIGHTS**

California law, under Health & Safety Code Section 24172, requires that any person asked to take part as a subject in research involving a medical experiment, or any person asked to consent to such participation on behalf of another, is entitled to receive the following list of rights written in a language in which the person is fluent. This list includes the right to:

1. Be informed of the nature and purpose of the experiment.
2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized.
3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment.
4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable.
5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to the subject, and their relative risks and benefits.
6. Be informed of the avenues of medical treatment, if any, available to the subject after the experiment if complications should arise.
7. Be given an opportunity to ask any questions concerning the experiment or the procedures involved.
8. Be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation in the medical experiment without prejudice.
9. Be given a copy of the signed and dated written consent form.
10. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion, or undue influence on the subject’s decision.
APPENDIX E1

First Recruitment Flyer

CHIA SEED RESEARCH STUDY
DEPT. OF FOOD SCIENCE AND NUTRITION

Approved by Cal Poly Pomona Institution Review Board under protocol # 14-0105

Research Title:
"Effect of chia seed consumption on body composition, levels of triglycerides, total cholesterol, HDL, LDL, blood glucose and satiety levels"

We are looking for FEMALE volunteers who:
- ARE HEALTHY AND NOT PREGNANT
- BETWEEN 18-45 YEARS OLD
- HAVE A NORMAL BODY WEIGHT *
- WILLING TO AVOID EATING HIGH LEVELS OF OMEGA-3 FATTY ACID FOOD AND SUPPLEMENT SUCH AS FISH OIL, NUTS AND SEEDS

* Additional screening required to confirm eligibility to participate

Participation in this study involves:
- Consuming specific amount of chia seed bread and chia seed every day for 6 weeks.
- Measurement of body composition, plasma lipid, glucose levels and satiety.
- Before and after study blood works done at the health center.
- After meal blood sugar testing
  - This test will require consuming chia seeds on site and finger pricking prior to eating and at 30, 60, and 90 minute intervals after eating. The whole session will last about 90-100 minutes.
  - 4 Finger prick blood samples (1-2 drops each) in week 2, 4 and 6 of each study period.
- Filling out a 3-day food record and rate your satiety levels at three random points during each study period.
- Approximately 14 visits across 19 week study.

For more info or to sign up, please contact:

Hilary Wu
hcwu@csupomona.edu
Anne Sung
acsung@csupomona.edu
Dr. Burns-Whitmore
bburriswhitmo@csupomona.edu, (909)869-3793
APPENDIX E2

Second Recruitment Flyer

CHIA SEED RESEARCH STUDY
DEPT. OF HUMAN NUTRITION AND FOOD SCIENCE

Approved by Cal Poly Pomona Institution Review Board under protocol # 14-0105

Research Title:
"Effect of chia seed consumption on body composition and satiety levels"

We are looking for FEMALE volunteers who:
• ARE HEALTHY AND NOT PREGNANT
• BETWEEN 18-45 YEARS OLD
• HAVE A NORMAL BODY WEIGHT *
• WILLING TO AVOID EATING HIGH LEVELS OF OMEGA-3 FATTY ACID FOOD
  AND SUPPLEMENT SUCH AS FISH OIL, NUTS AND SEEDS
* Additional screening required to confirm eligibility to participate

Participation in this study involves:
• Consuming specific amount of chia seeds every day for 6 weeks.
• Measurement of body composition, waist and hip circumference,
  blood pressure and satiety.
• Filling out a 3-day food record and rate your satiety levels at three
  random points during each study period.
• Approximately 11 visits across 16 week study.

For more info or to sign up, please contact:

Hilary Wu
hchwu@csupomona.edu

Anne Sung
acsung@csupomona.edu

Dr. Burns-Whitmore
bburnswhitmo@csupomona.edu, (909)869-3793
APPENDIX F

Screening Form

CHIA SEEDS STUDY INITIAL SCREENING QUESTIONNAIRE
California State Polytechnic University, Pomona IRB # 14-0105

Date: ________________

Thank you for taking the time to fill out this questionnaire. Please be sure to read each question carefully, and then check the appropriate box. All information you provide on this form will be kept confidential. After filling out the form, please save a copy on your computer, then attach it to an e-mail and return it to: Hilary Wu at hcuw@csupomona.edu, Anne Sung at anneesung@gmail.com or Dr. Burns-Whitmore at bburnswhitmo@csupomona.edu. One of us will email/call you back and let you know if you qualify. Thank you again!

Name: ________________________________________________________________________

________________________________________________________________________

Date: ________________

________________________________________________________________________

Address: ________________________________________________________________________

________________________________________________________________________

Phone: ________________________________________________________________________

________________________________________________________________________

E-Mail: ________________________________________________

Preferred method of contact: ☐ Telephone ☐ E-Mail

1. What is your age? ____ years.

2. What is your weight? ____ pounds or ____ kilograms.

3. Have you experienced any weight changes in the last year?
☐ No
☐ Yes: ____ pounds gained or ____ pounds lost over a period of ____ months.

4. Do you currently have an exercise plan? If you answer “No”, please skip to question 6.
☐ No
☐ Yes, I exercise ____ day(s)/ week and _______hour(s)/ day using the following activities:

5. How long have you been following this exercise plan?
____ Days ____ Months ____ Years

6. What is your height? ____ inches or ____ centimeters.

7. Do you have any current medical problems (ex. diabetes, high blood pressure)?
☐ No
☐ Yes, I have the following: ________________________________________________________.

8. Do you have any gastrointestinal problems (ex. IBS, ulcerative colitis)?
☐ No
☐ Yes, I have the following: ________________________________________________________.
9. Are you currently taking any medications (prescription or over the counter)?
☐ No
☐ Yes, I am taking:

__________________________________________________________________

10. How often do you take the following medications?

<table>
<thead>
<tr>
<th>Types of Medication</th>
<th>Never</th>
<th>Daily</th>
<th>Weekly</th>
<th>Monthly</th>
<th>Yearly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<td>☐</td>
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<tr>
<td>Tylenol (Acetaminophen)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td>Advil (Ibuprofen)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Naprosin/Naproxin/Aleve</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Steroids/Steroidal drugs</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td>Hormone supplements</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td>Fiber supplements</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Statins</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

11. Are you currently taking any supplements (ex. vitamins, plant sterols, fish oil, fiber)?
☐ No
☐ Yes, I am taking:

__________________________________________________________________

12. Do you have high cholesterol?
☐ No
☐ Yes, my total blood cholesterol is ____ mg/dL.
☐ I’m not sure, but I think so.
☐ I don’t know.

13. Do you have high blood sugar?
☐ No
☐ Yes, my fasting blood sugar is ____ mg/dL.
☐ I’m not sure, but I think so.
☐ I don’t know.

14. Are you a(n):
☐ Omnivore
☐ Vegetarian
☐ Vegan/Strict Vegetarian

15. How long have you followed that diet?
   _____ Days _____ Months _____ Years

16. Do you have any food allergies (ex. peanuts, seeds, shellfish, dairy)?
☐ No
☐ Yes, I have the following allergies: ________________________________

17. Are you allergic to nuts or seeds?
☐ No
☐ Yes

18. How often do you eat Chia seeds?
☐ Never
☐ Daily
☐ Weekly
☐ Monthly
☐ Yearly
19. Do you like Chia seeds?
☐ Yes
☐ No
☐ I am indifferent.
☐ I don’t know what it is.

20. How often do you eat any kinds of nuts or seeds?
☐ Never
☐ Daily
☐ Weekly
☐ Monthly
☐ Yearly

21. Are you willing to come to campus once a week to pick up your chia seeds?
☐ Yes
☐ No

22. Would you be willing not to eat any other nuts or seeds during this study?
☐ Yes
☐ No

23. Do you have any pins or metal objects in your body (other than piercing that can be removed)?
☐ Yes
☐ No

24. Where did you find out about this study?
☐ E-Mail
☐ Flyer
☐ Friend or coworker
☐ Professor
APPENDIX G

Chia Seed Intake Suggestion

WELCOME TO THE CHIA SEED STUDY

Hello,
Thank you for participating in the chia seed study. We are very excited to share this experience with you. **While on this study, it is important that you do not increase your exercise or activity and you should eat as normally as possible.** We also have some tips on helping you to incorporate chia seeds into your diet. You will come in one day a week to pick up your allotment of chia seed for the week and please return the empty bag to us.

Here are some tips:
- Mixed with water, juice or any beverage of your choices
- Mixed with smoothie
- Mixed with yogurt
- Mixed with milk to make pudding
- Sprinkle on top of your favorite hot or cold cereal
- Sprinkle on your salad
- Mixed with casserole
- Added to your soup as thickener
- Mixed with beef to make meatball
- You can always added them in your favorite baked good

Thank you 😊
APPENDIX H

Food Record Form

Diet Record Instructions

Directions: Record what you eat and drink on the sheets provided. As you record each food, make careful note of the amount and how it was prepared (fried, baked etc.). Estimate the amount to the nearest weight or fluid ounce, quarter cup, tablespoon, or other common measure. It is suggested to bring a measuring cup with you to meals.

In guessing at the sizes of meat portions, it helps to know that a piece of meat the size of the palm of your hand weighs about 3 or 4 ounces. It also helps to know that a slice of cheese (such as sliced American cheese) or a 1 1/2-inch cube of cheese weighs about 1 ounce. If you are unable to estimate serving sizes, measure out servings the size of a cup, tablespoon, and teaspoon onto a plate or into a bowl to see how they look. You will have to break down mixed dishes to their ingredients (for example: burrito = 10 inch whole wheat tortilla, ¼ cup black beans drained, ¼ cup Spanish rice, 1oz. Monterey jack cheese, 2 tbs. chopped tomatoes, 1 tbs. chopped onion, 1/8 medium avocado sliced).

The closer your approximations, the closer your actual intake will be reflected. Some common errors include using weight ounces instead of fluid ounces. Record the liquids as fluid ounces and the solids as weight oz. It is also very helpful to read the labels of the foods you consume. If you eat name brand foods, please also include the name brand of the food or the restaurant chain name if applicable. Be sure to list the actual amounts of foods eaten (ie. don’t include milk left in your bowl after eating cereal. Only count what you’ve consumed).

Please record any nutrient supplements you take.


Serving Sizes

Everyday Objects

1 cup of cereal = a fist

1/2 cup of cooked rice, pasta, or potato = 1/2 baseball
1 baked potato = a fist

1 medium fruit = a baseball

1/2 cup of fresh fruit = 1/2 baseball

1 1/2 ounces of low-fat or fat-free cheese = 4 stacked dice

1/2 cup of ice cream = 1/2 baseball

2 tablespoons of peanut butter = a ping-pong ball
Hunger/Satiety Scale directions: list 3 numbers by each meal/snack for your rating (1-10) before, during and after each meal/snack (ie. 1, 5, 10 for Breakfast).

<table>
<thead>
<tr>
<th>Hunger/Satiet y Rating**</th>
<th>Meal (B, L, D) or snack (S)</th>
<th>Food/Beverage (Brand)</th>
<th>Cooking Method</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

* Please fill out the separate Visual Analog Scale after your snack/meal with chia seeds or the designated snack/meal if you are not consuming chia seeds. (starting next week)

<table>
<thead>
<tr>
<th>Supplements Taken Today</th>
<th>Brand</th>
<th>Amount Taken</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>
APPENDIX I

Visual Analog Scale Appetite Questionnaire

Appetite Questionnaire

ID # ________ Date: ________ 0 ___ 1 ___ 2 ___ 3 ___ 4 ___ 5 ___

Instructions: Please quantify your feel for the aspects mentioned below.
Consider the line as the extremes of your feeling.
Draw a vertical line at the level that best represents your feeling at that moment.

How hungry do you feel?
I am not hungry at all | I have never been more hungry

How satisfied do you feel?
I am completely empty | I cannot eat another bite

How full do you feel?
Not at all full | Totally full
APPENDIX J

Unusual Diet Record Form

There will be some days that you will accidentally eat a food that is restricted during the treatments. Please use the form to document those accidents.

Also there will be days when you are not eating normally or you have had to take certain medications. Please list those in the next table.

Example:

Diet: Chia seed Treatment

<table>
<thead>
<tr>
<th>How much?</th>
<th>What kind of food?</th>
<th>Date</th>
<th>Time</th>
<th>Where</th>
<th>Activity</th>
<th>How did it happen?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 brownie and about ½ teaspoon of chia seeds</td>
<td>Brownie made with chia seeds</td>
<td>10-31-2014</td>
<td>3:25 p.m.</td>
<td>Office</td>
<td>Working on report</td>
<td>Co-worker gave to me, then I found out afterwards that it’s made with chia seeds</td>
</tr>
</tbody>
</table>

Example:

Diet: No Chia seed Treatment

<table>
<thead>
<tr>
<th>Medication</th>
<th>Illness</th>
<th>Date</th>
<th>Time</th>
<th>Describe diet or appetite changes?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin (Antibiotic)</td>
<td>Respiratory infection</td>
<td>12-20-2012</td>
<td>8 a.m., noon, 6pm</td>
<td>Stomach upset. Ate soup all day</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Headache</td>
<td>12-21-2012</td>
<td>10am</td>
<td>Slept all day-didn’t eat</td>
</tr>
<tr>
<td>Amoxicillin (Antibiotic)</td>
<td>Respiratory infection</td>
<td>12-20-2012 to 12-30-2012 (10 days)</td>
<td>8 a.m., noon, 6pm</td>
<td>No diet changes after 12-21-2012</td>
</tr>
</tbody>
</table>
### Diet: Chia seed Treatment #1

<table>
<thead>
<tr>
<th>Food</th>
<th>No Chia seed</th>
<th>Chia seed</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>How much?</th>
<th>What Kind?</th>
<th>Date</th>
<th>Time</th>
<th>Where</th>
<th>Activity</th>
<th>How did it happen?</th>
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### Diet: Chia seed Treatment

<table>
<thead>
<tr>
<th>Medication</th>
<th>Illness</th>
<th>Date</th>
<th>Time</th>
<th>Describe diet or appetite changes?</th>
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</tbody>
</table>

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APPENDIX K

Publishable Article

Effect of Chia Seed (Salvia Hispanica L.) Supplementation on Postprandial Glucose and Satiety
Anne Sung , Bonny Burns-Whitmore, Eddie Jo, Michelle Wien
Journal of the Academy of Nutrition and Dietetics

Abstract

**Background:** A healthy diet and weight management are beneficial in reducing risk factors for type 2 diabetes. Increasing dietary fiber intake has been shown to lower post-prandial glucose (PPG) levels and increase satiety, but the majority of people do not meet the recommended fiber intake. Chia seed (Salvia Hispanica, L) contains an excellent source of dietary fiber and has been shown to be positively associated with lower PPG levels and decreased appetite.

**Objective:** The proposed study was to determine the effect of supplementing 20% of daily calories from chia seeds on satiety and PPG levels.

**Design:** This was a free-living, randomized cross-over design study with 5-week treatment periods separated by a 5-week washout period. Twenty-three healthy females, between the age of 19-50 years and with a normal BMI from Cal Poly Pomona were recruited. Fasting glucose and PPG levels were measured using finger prick testing at baseline and 60 minutes after consuming 1/3 of the allotted chia seed in 6 ounces of 100% fruit juice. Satiety level was measured by self-reported food records, satiety rankings and visual analog scale (VAS) appetite questionnaires. Satiety was measured by satiety rankings and visual analog scale (VAS) appetite questionnaires. Postprandial glucose levels were analyzed using a 2(condition) x 3(time) repeated measure analysis of variance (ANOVA). Satiety was analyzed using one-way ANOVA and independent t- test was used for VAS appetite questionnaires.

**Result:** The before, during and after meal satiety, VAS appetite questionnaires and PPG levels were not significantly different between the control and chia seed supplemented period.

**Conclusion:** Twenty percent chia seed supplementation was not associated with lowering PPG levels or increased satiety in this study.

**Background**

Diabetes is a chronic disease that is characterized by abnormally high blood glucose as a result of impaired pancreas and insulin resistance. Not only it is the seventh leading cause of death in the United States, it is also a major risk factor for cardiovascular disease and stroke, the leading cause of kidney failure, non-traumatic lower-limb amputations and cause of blindness . According to Centers for Disease and Control, type 2 diabetes incidences nearly doubled in the past 16 years in the United States. This epidemic continues to grow and it also creates a significant toll on the healthcare cost. Obesity and hyperglycemia are one of the risk factors for the development of type 2 diabetes.
Lifestyle changes such as behavior modification, weight and diet management are effective in reducing diabetes risk factors\(^2-4\). Diet modification such as increasing dietary fiber intakes have been shown to improve postprandial blood sugar and insulin resistance\(^5-8\). Increased fiber intakes particularly viscous fibers are also associated with increased satiety and reduced appetite\(^9-12\). Yet, in the past decade, the average fiber intakes ranged from 15.5-15.9 gm per day, which is far below the recommended adequate intake of 25 gm for female and 38 gm for male between the age of 19-50\(^13\). Chia seeds, (Salvia hispanica L.), is an annual herbaceous plant of the Lamiaceae (mint) family that originated in the Central Americas\(^14\). It contains high amounts of polyunsaturated fatty acids and dietary fibers\(^15,16\). The fiber content of chia seeds is superior to the common high fiber crops such as flaxseed, and nearly two times greater than that of oat bran, wheat and barley\(^17\). Current chia seed researches have shown a positive outcome in reducing appetite and an incremental increase of chia seed dosage is associated with decreased postprandial glucose\(^18,19\). The objective of this study was to determine whether substituting 20% of daily calories from chia seed can reduce postprandial blood sugar and increase satiety.

**Method**

The experiment was a randomized, crossover study consisting of five-week treatments separated by a five-week washout period. A total of 32 healthy, normal weight females without pre-existing diseases were recruited in two separate recruitment periods. The inclusion criteria were: healthy weight range (BMI between 18.5-25 kg/m\(^2\)), age between 18-45, free from pre-existing chronic diseases, without metal plates, pins or a pacemaker, not on a diet plan or taking omega 3 fatty acids or dietary fiber supplements. Exclusion criteria: BMI between 18.5-24.5 kg/m\(^2\), BMI < 18.5 kg/m\(^2\) or >24.5 kg/m\(^2\), pregnancy, pre-existing gastrointestinal or chronic diseases, have metal plates or pins and/or a pacemaker inside the body, taking herbal or dietary supplements and medication that can alter serum glucose and insulin values or affecting appetite. Participants would be excluded during the study if they consumed less than 50% of assigned chia seeds, became pregnant during the study period or started on prescribed medication that could alter serum glucose levels.

Participants were randomly assigned to the treatment and control group in 1:1 ratio. The treatment group consumed 20% of total daily calories from chia seeds. As this was a free-living study, participants had the freedom of incorporating chia seeds into any food of choice. During the lead-in week, we provided tips on when and how to incorporate the seeds. Participants’ caloric needs were calculated using the Harris-Benedict equation with activity factors ranging from 1.2 to 1.7 depending on the activity levels reported by the participants. The participants were asked to fill out in detail the frequency, duration and types of physical activities they engaged in. Participants were asked to remain as normal a diet and activity level during the study, and avoiding sudden changes of their activity levels, such as, joining a school athletic team or marathon events, during the study. The control group participants consumed regular meals without chia seeds added, then crossed over to treatment after a five-week washout period. Of the two recruitments, only 23 participants remained at the end of the study.

**Materials**

Chia seeds, variety Salba, were donated by Salba Smart Natural Products, LLC.
Salba was a registered variety of chia seed. A carrier juice (Capri sun 100% Juice TM) that contained 180ml fluid weight, 80calories and 20g of sugar without any additional fibers was used to mix with chia seeds.

**Blood Glucose Analysis**

Postprandial blood samples were collected in week two, three and four in winter quarter, 2015 for first recruited participants. They were required to fast over-night prior to finger pricks. Baseline blood glucose was obtained by finger pricks in the fasting state prior to seed consumption and analyzed using a glucose meter (Freestyle Freedom Lite Glucose meter), which was 98%-100% accurate based on the Food and Drugs Administration's standard. Participants in the treatment group consumed one third of the daily-allotted chia seeds to simulate the amount of chia seeds eaten at one meal setting. Chia seeds were mixed in 6 oz. Capri sun 100% juice and participants were required to consume the juice mixture in front of the primary investigator. The second finger pricks were collected at the 60th minute. The control group drank the juice without chia seed added followed by finger pricks at the same time interval as the treatment group.

**Satiety and Appetite Measurement**

Participants filled out food intake record with satiety ratings and a separate Visual Analog Scale (VAS) appetite questionnaire after meals. A total of nine food records and appetite questionnaires was collected per treatment period. Satiety rating was filled out before, during and after each meal on the scale of 1-10. The appetite questionnaire was a 100mm VAS scale with a total of eight questions. Each question was rated from most negative on the left to most positive on the right. The questions on the survey included: How hungry do you feel? How satisfied do you feel? How full do you feel? How much do you think you could eat right now? Would you like to eat something sweet? Would you like to eat something salty? Would you like to eat something fatty?

**Compliance Monitoring**

Participants were asked to return the unfinished chia seed bag and to document any usual diet pattern such as skipping meal due to illness or other activities in the unusual diet record form. Participants would also fill out the form if accidentally ingested other chia seed containing products.

**Statistical Method**

Statistical analysis was performed using SPSS software and postprandial glucose levels, satiety ratings and VAS scale results were expressed as mean value (the standard deviation with statistical significance level defined as p< 0.05. Postprandial glucose levels were analyzed using a 2 (condition) x 3 (time) repeated measures analysis of variance (ANOVA) was used. Satiety was analyzed using one way ANOVA and independent T-Test was used for VAS appetite questionnaire. Age, weight and BMI are adjusted using multivariable logistic regression.

**Result**

Table 1 summarized the baseline characteristics of participants. The mean estimated energy needs were 1885.09 (+/- 194.11)kcal and mean chia seed supplemented were
377.02 (+/-38.82)g.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.67 (3.04)</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.84 (6.52)</td>
<td>146</td>
<td>170</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.11 (7.12)</td>
<td>44.4</td>
<td>69.69</td>
</tr>
<tr>
<td>BMI Avgas (kg/m²)</td>
<td>22.07 (2.07)</td>
<td>19.2</td>
<td>26.1</td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>1652.84 (369.69)</td>
<td>559.04</td>
<td>2838.37</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>226 (65.19)</td>
<td>57.6</td>
<td>422.05</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>66.35 (18.06)</td>
<td>30.82</td>
<td>126.13</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>22.55 (11.56)</td>
<td>6.25</td>
<td>82.57</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>72.24 (37.05)</td>
<td>15.59</td>
<td>177.04</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>56.55 (16.57)</td>
<td>12.71</td>
<td>148.98</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>18.03 (8.24)</td>
<td>1.7</td>
<td>50.42</td>
</tr>
<tr>
<td>PUFA (mg)</td>
<td>5.89 (4.32)</td>
<td>0</td>
<td>50.42</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>166.46 (109.42)</td>
<td>0</td>
<td>878.76</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>2679 (1086)</td>
<td>546.62</td>
<td>8176.53</td>
</tr>
</tbody>
</table>

**Postprandial Glucose**

Due to dropouts and incomplete data, only 12 participants from first recruitment were analyzed for postprandial glucose (n=12). There was no main treatment effect for both fasting glucose (P=0.259) and PPG (P=0.710). No significant differences were seen between treatment and time for fasting glucose (P=0.805) and PPG (P=0.401). When comparing within subjects, no significant differences were seen in time and treatment in fasting glucose (P=0.830) as well as in PPG (P=0.475).

**Satiety and Appetite Measurement**

A total of 19 satiety rating was submitted for analysis (n=19). One way ANOVA was conducted and there were no significant differences between baseline, control and the chia supplemented group before, during and after meals.
VAS appetite questionnaires
A total of 18 VAS appetite questionnaires was analyzed using independent T-Test (n=18). There were no significant differences between control and the chia supplemented group for all eight questions on the VAS questionnaire. How hungry do you feel? (P=0.411); How satisfied do you feel? (P=0.661); How full do you feel? (P=0.752); How much do you think you can eat? (P=0.713) would you like to eat something sweet? (P=0.667); Would you like to eat something salty? (P=0.485); would you like to eat something savory? (P=0.874); would you like to eat something fatty? (P=0.274). The questionnaire was filled out after meals, which was consistent with the satiety rating result where no significance was detected pre and post chia supplementation.

Nutrient Analysis
A total of 20 food records was analyzed using one way ANOVA. There were significantly greater caloric intake (P=0.00) in the chia supplement group than baseline and control. According to the result, participants consumed on average nearly 500 more calories during the chia seed treatment period, which indicated they added chia seeds in addition to their usual diet rather than displacing the calories from usual foods consumed. Subsequently, there was a significantly higher intake of protein (P=0.01), carbohydrates (P=0.02), fiber (P=0.00), fat (P=0.00), and polyunsaturated fatty acids (PUFA) (P=0.00) in the chia supplemented group than the control.

When comparing chia supplementation to baseline, there was a significant difference seen in protein (P=0.032), fiber (P=0.00), fat (P=0.00) and polyunsaturated fatty acids (P=0.00). Both sodium (P=0.241) and carbohydrate intake was not significantly different (P=0.737).

There was a significantly higher calorie (P=0.005) and carbohydrate intake (P=0.02) during baseline when compared to control. Sodium intake was also significantly greater (P=0.028) during baseline. On average, participants consumed around 300 calories more during the lead-in week. There were no significant differences seen in protein (P=0.38), fiber (P=0.447), fat and PUFA intake between control and baseline.

Discussion
The majority of participants (91%) was in compliance with allotted chia seeds based on self reported intake and returning of unfinished seeds. For PPG testing, 1/3 of allotted chia seeds were consumed on site and monitored by the primary investigator (PI); therefore, the intake compliance for this study was assumed to be 100%.

Satiety
Based on the nutrient analysis results, participants supplemented chia seeds in addition to their usual diet pattern. During the chia seed intervention period, the overall intake of calories, protein, carbohydrates, fiber, fat, PUFA was significantly higher as Salba chia seed is high in plant protein, dietary fiber and polyunsaturated fatty acids. The mean fiber intake in the current study was 48.92g +/- 9.25 SD during the intervention period, which nearly doubled (196%) the daily recommended guideline of 25g for women 19-50 years old. Participants' usual fiber mean intake in the control period.
(18.72g +/- 8.77 SD) was slightly higher than the reported national average of 15.6-15.9 gm/day (King et al, 2012). Despite significantly greater fiber intake during the chia supplementation period, there was no significant difference in increased satiety or decreased appetite after meals between the chia supplemented periods when compared to control. The finding was in line with the ‘Salba’ study conducted by Vuksan et al (2010) where appetite was not significantly lowered at 15 and 30 minutes after chia seed consumption. In that study, a significant reduction of appetite was only seen at 60, 90 and 120 minutes post meal. For our study, the appetite questionnaire was filled out right after a meal, thus the prolonged satiating effect of chia seed might not reflect on the questionnaire. The lack of significance might be due to a smaller sample size (n=19) in this study and the study design difference. Our study design did not restrict the time, method and amount of chia seeds taken at one setting; as a result, the actual satiety sensation immediately after ingesting chia seeds might not be reflected at the time of filling out the satiety rating or VAS appetite questionnaire and it is one of the limiting factors in satiety and appetite measurement.

Methodological differences, such as how chia seeds were taken, might also be a contributing factor to the perceived satiety. ‘Salba’ studies conducted by Vuksan et al. (2010) and Ho et al. (2013) both baked chia seeds in bread and used matching calorie white bread as a placebo control. Nieman et al. (2009) mixed chia seeds in liquid, and in another study (2012), participants were allowed to put chia seeds in any foods or drinks without cooking and heating. In this current study, the majority of participants (70 %) reported taking chia seeds in liquids due to preference or convenience, 2 participants added them to foods and 3 participants added them to both liquids and foods. Due to the viscous mucilag, chia seed turns liquid into gel and make the liquids more difficult to drink. Some participants drank the liquids right away before liquids could gel, but more than half of the participants reported pre-mixing seeds in liquids and consumed them slowly throughout the day, which indicated the degree of gelling varied depending on how soon the chia seed mixture was consumed when the satiety and appetite questionnaires were completed. The degree of gelling vs non-gelled liquids, as well as the method of supplementation such as in capsule or liquid form, could affect satiety and gastric emptying differently as shown in the study that used different preload viscosity and forms of pectin (Wanders et al, 2014).

Despite the lack of statistical significance, the results showed a positive trend between chia seed intakes and improved satiety based on the satiety ratings and VAS appetite questionnaire results. The mean VAS results showed that participants reported less hunger, more satiety and fullness, less desire to eat, and less desire to eat sweet, salty, savory or fatty foods.

Postprandial Glucose

The postprandial glucose testing used 1/3 of assigned daily chia seeds; therefore, each participant received different amounts of chia seeds mixed with 6oz of 100% juice. While the carrier juice contained same calories and sugar content, the amount of fiber, protein, and fats present in the juice mixture were varied depending on the chia seeds assigned to each participant. The mean chia seeds used for postprandial glucose analysis was 29.29g (+/-2.75), which contained approximately 9.8g of dietary fiber from the ‘Salba’ chia seed. This study failed to find a significant difference between the control
and chia seed supplementation on postprandial glucose. Our findings were inconsistent with the Salba study conducted by Vuksan et al. (2010) and Ho et al. (2013), where incremental doses of 0, 7, 14, 25g of Salba seed in bread showed an inverse relationship with postprandial glucose levels in normal weight subjects. The main difference between the current study and other ‘Salba’ studies were the methodologies. Both ‘Salba’ studies mentioned above had a fixed amount of Salba seeds and postprandial glucose levels were measured incrementally from 15, 30, 40, 60, 90 and 120 minutes post ingestion.

There were a few limitations study limitations. The study treatment periods were in two different school quarters; thus participants fasting glucoses were not measured at the same time during each visit. During the 60-minute waiting time, participants were instructed to avoid vigorous activities that could have affected the serum glucose level; however, the actual activity was not visually monitored. Both satiety rating and VAS analog scale were based on self reported data. In the current study, the participants’ usual diet pattern was designated as their control and there was no blinding involved. Participants were fully aware of when and how much chia seeds were added to their daily intake, which could possibly affect their subjective satiety ratings. Some satiety and appetite ratings were based on recalls due to incomplete food record keeping, which might also influence the study results.

**Conclusion**

Chia seed supplementations significantly increase calories, protein, carbohydrates, fiber, fat, PUFA intakes. It can be an effective method to increase dietary fiber and PUFA intakes. In this study, 20% of daily calories from chia seed were not associated with increase appetite or reduce postprandial glucose levels. However, positive trends were seen with improving satiety rating and reducing appetite.

**References**


