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The effects of including almonds in an energy-restricted diet on weight, body composition, visceral adipose tissue, blood pressure and cognitive function

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THE EFFECTS OF INCLUDING ALMONDS IN AN ENERGY-RESTRICTED DIET ON WEIGHT, BODY COMPOSITION, VISCERAL ADIPOSE TISSUE, BLOOD PRESSURE AND COGNITIVE FUNCTION

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To my family, who's always had my back and supported me no matter what.

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ABSTRACT

Dhillon, Jaapna. Ph.D., Purdue University, August 2016. The Effects of Including Almonds in an Energy-restricted Diet on Weight, Body Composition, Visceral Adipose Tissue, Blood pressure and Cognitive Function. Major Professor: Richard Mattes.

Inclusion of almonds in an energy restricted diet has been reported to enhance or have no effect on weight loss. Their effects specifically on visceral fat stores during energy restriction have not been widely examined. Additionally, almond consumption has been associated with reduced blood pressure, but whether this is linked to or is independent of changes of body composition has not been examined. Moreover, almond consumption during energy restriction may be an effective strategy for reversing the negative effects of dieting on cognitive performance. The unique nutrient profile of almonds also has the potential to influence cognitive function post-prandially. The post-lunch dip in cognition is a well-established phenomenon of decreased alertness, memory and vigilance after lunch consumption and can be affected by lunch composition. Almonds which are higher in fat and lower in carbohydrate may be able to reduce this post lunch dip in cognition. Consequently, this dissertation had three primary aims. The first aim was to evaluate the effects of almond consumption as part of an energy-restricted diet on weight, visceral and subcutaneous adipose depots and blood pressure compared to a nut-free energy restricted diet. The second aim was to evaluate the effects

of almond consumption as part of an energy-restricted diet on cognitive function. The third aim was to evaluate the acute effects of almond consumption on the post-lunch dip in cognitive function. A secondary objective of this dissertation was to develop an analytical approach to identify metabolic profiles associated with almond consumption to ascertain compliance in long term clinical trials.

Overweight and obese adults (n=86, age: 31.4±12.9 yr, body mass index: 30.4±3.9 kg/m^2) were randomly assigned to a 12 week energy restricted (500 kcal/d deficit) almond-enriched diet (AED) (n=43) or a comparably energy restricted nut-free diet (NFD) (n=43). Participants in the AED group were asked to consume dry-roasted, lightly salted almonds providing 15% of energy in individualized energy-restricted diets. Participants in the NFD group were asked to avoid all nuts during the intervention period. The same participants were also randomized to receive either a high fat lunch (A-HFL) (>55% energy from fat, almonds contributing 70-75% lunch energy) or a high carbohydrate lunch (HCL) (>85% energy from carbohydrates) at the beginning and end of the intervention.

To isolate biomarkers and metabolite changes related to almond consumption, representative samples (pools) of erythrocytes from individuals consuming almonds for 12 weeks (n=31) or no almonds (n=30) as well as unmasticated almond seeds were screened by an untargeted and unbiased metabolomics approach, namely flow injection mass spectrometry (FIA-MS). Data on full scan mode, specific neutral loss and precursor ion scan modes for shotgun lipidomics, and *m/z* values compatible with nut flavonoids from literature were used. The informative *m/z* values detected in pooled samples were combined into single ion monitoring (SIM) and multiple reaction monitoring (MRM)

methods intended for individual sample screening. This shotgun metabolomics study identified specific ratios and combinations of membrane lipids that were discriminatory of almond consumption from the nut-free diet at the end of the 12 week intervention. Eight out of the 31 participants (25.8%) in the almond group and 3 out of the 30 (10%) participants in the control group were misclassified indicating a possibility of non-compliance which was supported by self-reported dietary intake data. All subsequent analyses of the primary study outcomes were based on an intention-to treat approach and a compliers analysis i.e. analysis of participants compliant to the energy-restriction as well as their respective intervention groups as determined by the metabolomics study and self-reported dietary intake data.

The findings from this dissertation research demonstrate that consumption of almonds during a weight loss regimen resulted in greater proportional reductions of trunk and total body fat as well as diastolic blood pressure compared to the nut-free weight loss regimen. Both intervention groups lost similar amounts of weight and visceral adipose tissue and had similar reductions in systolic blood pressure over time. Hence, almond consumption during energy restriction may help to reduce metabolic disease risk in overweight and obese individuals. In addition, both intervention groups demonstrated similar improvements in memory and attention indices of cognitive function over time. Moreover, consumption of a midday meal caused a post-lunch dip leading to a decline in cognitive function. The almond-enriched high-fat lunch reduced the post-lunch dip in memory but did not affect the dip in attention.

CHAPTER 1. INTRODUCTION

1.1 Study Rationale

With obesity recently being recognized as a disease[1] there is a concerted effort towards finding nutrition therapies that can treat obesity and its related health disorders. Data from epidemiological and clinical studies suggest that consumption of nuts such as almonds may be an effective strategy for lowering the incidence of obesity and cardiovascular disease[2,3]. Despite their high energy content, the inclusion of almonds in an energy-restricted diet does not hinder and may augment weight loss[4,5]. This may occur by several mechanisms, but probably most importantly, improved dietary compliance which is likely attributable to greater sensory variety resulting in higher palatability of the diet[6]; stronger locus of control[8] leading to a sense of empowerment; and possibly, through their slow and sustained energy release thus ameliorating physiological process that promote hunger and feeding. The satiating effects of almonds may prolong inter-meal intervals, promote smaller meal sizes and reduce the desire to eat when not hungry and, hence, contribute to purposeful weight loss[7]. Additionally, according to the U.S. Food and Drug Administration, consumption of 42.5g nuts, such as almonds, in conjunction with a diet low in saturated fat and cholesterol, could diminish cardiovascular disease risk[9]. Almonds contain a range of nutrients such as unsaturated

fats, vitamins, minerals, arginine, polyphenols, fiber etc. that could benefit cardiovascular health. The primary goal of weight loss is to maximize the reduction of fat mass while retaining fat-free mass. Dietary factors can be equally or even more effective than exercise in achieving this outcome. Increasing the proportion of protein in an energy-restricted diet enhances satiety, energy expenditure and greater relative fat mass loss[10]. In addition, different types of dietary fat may influence substrate oxidation. Monounsaturated fats are oxidized preferentially[11,12], and a diet higher in the unsaturated:saturated fat ratio may reduce subcutaneous adipose tissue (SAT)[13], but more importantly, visceral adipose tissue (VAT) during weight loss[14]. The promotion of VAT loss is important clinically as this may translate into the greatest reduction in the risk for metabolic diseases. Almonds are a good source of protein and monounsaturated fats and their effects on visceral fat loss, in conjunction with energy restriction, have not been widely examined.

Additionally, food-derived peptides reduce the risk of cardiovascular disease[15]. Arginine is among these peptides that help to reduce blood pressure. It is the physiological precursor of nitric oxide which is a vasodilator[16]. Certain nuts and seeds are good plant-based sources of arginine and studies with peanuts (2.8g arginine per 100g peanuts, as indicated in the USDA National Nutrient Database) reveals their ingestion for 12-weeks leads to a significant reduction in diastolic blood pressure[17]. Almonds also contain high levels of arginine (2.4g arginine per 100g almonds, as indicated in the USDA National Nutrient Database) and an augmentation of reduction in blood-pressure could be achieved with almond consumption during weight loss.

The process of dieting to lose weight is associated with cognitive function impairments[18]. Impairments of cognition may be a result of food restriction or anxiety related to maintaining a weight loss regimen. Negative effects of dieting on cognitive performance have been reversed with nut-supplemented diets higher in fat and lower in carbohydrate (LCHF)[19]. Hence, learning and memory could be enhanced with the inclusion of almonds during weight loss. In addition, a decrease of memory and vigilance is often reported after lunch, independent of weight loss[20]. This post-lunch dip in cognitive performance is a well-established phenomenon. It typically occurs in the early afternoon hours after lunch consumption and may partly be exacerbated by high carbohydrate meals[21]. There is very limited evidence on the effects of nut consumption on the post-lunch dip in cognitive function. However, the unique nutrient profile of almonds, which are lower in carbohydrate and higher in protein and unsaturated fats, may lessen the post-lunch dip.

Assessing compliance to dietary interventions in long-term clinical trials is problematic. Although self-reported measures of intake such as 24-hour food recalls and food frequency questionnaires are widely used, these are subjective measures of dietary intake[22] and only capture a snapshot of individuals' dietary patterns. Currently, there are no established analytical methods to ascertain compliance to long-term almond consumption. Hence, there is a need to develop analytical approaches that can identify metabolic profiles associated with almond consumption that are discriminatory of individuals consuming almonds and those that do not in long-term clinical trials. Nutritional metabolomics is one such tool that can capture the phenotype of dietary exposure in biological samples[23] such as erythrocytes and can be used for this purpose.

1.2 Study Objectives

The main objectives of this dissertation are to:

- Review the literature on effects of nut consumption on body weight, body composition, blood pressure and cognitive function, as well as literature pertaining to the post-lunch dip in cognitive function.
- Determine the effects of almond consumption during energy restriction on weight, body composition, visceral adipose tissue and blood pressure in overweight and obese adults.
- Determine the effects of almonds on cognitive function and the post lunch dip in energy-restricted overweight and obese adults.
- Develop a method for the assessment of compliance to long-term almond consumption using a shotgun metabolomics approach.

1.3 Organization of dissertation

This dissertation is organized into chapters that contain published manuscripts or manuscripts submitted to peer-reviewed journals. The details of the publications are provided at the beginning of the chapters.

Chapter 2 reviews the effects of nut consumption on appetite, dietary compensation, energy expenditure, fat oxidation and body weight. The focus in this chapter is on how nut consumption can aid weight management due to their strong satiating effects, the inefficient absorption of their energy, and possibly by elevating energy expenditure and fat oxidation.

Chapter 3 reviews literature on the effects of nut consumption on body composition, blood pressure, glycemic control, other cardiovascular disease risk factors and cognitive function including the post-lunch dip in cognitive function. This chapter establishes the foundation for the outcomes of the almond weight loss clinical trial that will be discussed in subsequent chapters.

Chapter 4 investigates changes in weight, body composition, specifically visceral fat and resting blood pressure with almond consumption during 12 weeks of energy restriction. Changes in secondary outcomes such as anthropometric measures of visceral adiposity such as waist circumference and sagittal abdominal diameter, 24-hour ambulatory blood pressure, 24-hour free living appetite ratings, and blood glycemic and lipid profiles were also assessed.

Chapter 5 investigates the acute effects of almond consumption on the post-lunch dip in cognitive function as well as changes in cognitive function with almond consumption during 12 weeks of energy-restriction. The focus in this chapter is on the memory and attention domains of cognitive function.

Chapter 6 describes the development of a shotgun metabolomics method to determine compliance to almond consumption in a weight-loss regimen. The focus was on 1) screening for prospective biomarkers that were discriminatory of almond consumption from the nut-free diet 2) conducting a performance analysis of specific combinations and ratios of prospective biomarkers, 3) determining non-compliance to the almond and nut-free diets via group misclassifications observed in the ratio performance analysis and 4) confirming possible non-compliance by cross-checking dietary intake data.

Chapter 7 summarizes the main dissertation findings and presents future directions for research.

1.4 Study aims and hypotheses

Specific aim 1: To evaluate the effects of almond consumption as part of an energy-restricted diet on weight, body composition, visceral adipose tissue and blood pressure compared to a nut-free diet matched on energy restriction (control).

Hypothesis 1: Inclusion of almonds in an energy-restricted diet will augment the rate of weight loss, lead to greater fat mass loss especially in the visceral depot and reduce blood pressure compared to the control.

Specific aim 2: To evaluate the effects of almond consumption as part of an energy-restricted diet on cognitive function compared to a nut-free diet matched on energy restriction (control).

Hypothesis 2: Inclusion of almonds in an energy-restricted diet will improve the attention and memory domains of cognitive function compared to the control.

Specific aim 3: To determine the post-prandial changes in cognitive function with almond consumption at lunch compared to a high-carbohydrate control lunch.

Hypothesis 3: Inclusion of almonds in a midday meal will ameliorate the post-lunch decline in memory and attention compared to a high carbohydrate control meal by lowering the percentage of dietary energy from carbohydrates and increasing the percentage of energy from fat.

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CHAPTER 2. A REVIEW OF THE EFFECTS OF NUTS ON APPETITE, FOOD INTAKE, METABOLISM, AND BODY WEIGHT

Tan S.Y., Dhillon, J. and Mattes R.D. (2014) A review of the effects of nuts on appetite, food intake, metabolism, and body weight. Am J Clin Nutr 100 (Supplement_1), 412S-422S.

2.1 Abstract

Tree nuts and peanuts are good sources of many nutrients and antioxidants, but they are also energy dense. The latter often limits intake because of concerns about their possible contribution to positive energy balance. However, evidence to date suggests that nuts are not associated with predicted weight gain. This is largely due to their high satiety value, leading to strong compensatory dietary responses, inefficiency in absorption of the energy they contain, a possible increment in resting energy expenditure, and an augmentation of fat oxidation. Preliminary evidence suggests that these properties are especially evident when they are consumed as snacks.

2.2 Introduction

The long-standing pillars of nutrition advice to optimize health are to practice balance, moderation, and variety. If followed, no food must be excluded from the diet and each food can make some useful contribution, the value of which is determined by the health status and needs of the individual. In an era in which positive energy balance has dominated health concerns in Western nations and is a growing problem globally, high-fat, energy-dense foods have often been identified as especially problematic. Nuts are an example, and one in which the science does not support this perspective. This review focuses on the role of nut consumption on appetite, energy intake, energy metabolism, and body weight. Recent studies that report the effects of nuts on various aspects of human energy balance are summarized in Table 2-1.

2.3 Nuts in the context of the whole diet

Nuts contribute to energy and nutrient intake directly and indirectly via multiple mechanisms. First, nuts themselves are rich sources of energy, various nutrients (eg, tocopherols, magnesium, potassium), and antioxidants (1, 2). Each form of nut has its own inherent sensory profile that is more or less appealing to individual consumers and so will influence their ingestive decisions. However, the sensory profile of the raw nut is commonly modified through processing. Roasting and frying darken the color, increase brittleness, and develop new flavor compounds (3–5). Changes in physical properties are of particular importance for the acceptability of nuts (6). A wide array of flavor compounds (eg, salt, sugar, cinnamon, and capsaicin) is also added directly to the surface of nuts to enhance their appeal. Broadly, such modifications increase sensory variety and,

by ameliorating monotony effects (7), may facilitate regular nut consumption and intake of the nutrients they contain. Sensory properties are among the strongest determinants of ingestive decisions (8, 9).

There are also indirect effects of nut consumption on total energy and nutrient intake. The sensory, nutrient, and/or physical properties of nuts alter gut hormone secretion (10, 11) and appetitive responses by consumers (12). In addition, nuts are frequently incorporated into the matrix of other foods (eg, confections, baked goods, ice cream), changing the flavor profile of both and creating a unique new unified sensory stimulus (13) that may guide intake of that item or influence the acceptability and selection of other items in the broader diet (14, 15). Whether this promotes greater energy intake remains to be determined.

2.4 Appetite and energy intake

With a few exceptions (16, 17), human feeding trials have shown that nut ingestion moderates appetite postprandially. Specifically, the inclusion of almonds and peanuts suppresses hunger (18, 19) and desire to eat (19) and increases fullness ratings after ingestion (17). Daily consumption of peanuts for 4 d also increased fasting satiety and fullness levels (20). These are important properties in weight management because a postprandial reduction in hunger may prolong meal latency; a decrease in desire to eat may prevent eating in the absence of hunger, and higher fasting satiety and fullness levels may translate into smaller meal sizes.

The satiating effects of nuts depend on 2 important factors. First, the form of nuts exerts differential effects on appetitive sensations. Smaller hunger suppression and greater hunger rebound (180 min postingestion) were observed when peanuts were consumed in the form of butter compared with whole nuts (18). However, whole almonds reportedly induce fullness levels comparable to those of almond butter (17). Second, the timing of nut consumption can affect appetite. The consumption of almonds together with a meal does little to augment the appetite-modulating effects of that meal, whereas consuming almonds alone as snacks blunts hunger and desire-to-eat ratings compared with individuals who received no nuts (19, 21). Other work noted that the ingestion of peanuts or peanut-containing snacks (300 kcal/d) tended to induce greater energy compensation when ingested as snacks relative to when they were consumed as part of a lunch meal (22). Hence, the form and timing of nut consumption may modulate appetitive sensations, with suggestive evidence that satiation/satiety effects may be greater for whole nuts consumed as snacks.

The underlying mechanisms for the appetitive effects of nut consumption are not well understood due to a paucity of studies on the issue. However, the available evidence indicates that the satiating effects of nuts are not likely to be mediated by delayed gastric emptying (23) or the release of selected appetite-regulating gut peptides including glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), or ghrelin (10, 17, 20). However, the effect of nut consumption on protein YY (PYY) secretion is mixed (10, 20). Furthermore, feeding 3 g of pine nut oil (in the form of fatty acids or triglycerides) reportedly decreased prospective food-intake ratings and increased the secretion of cholecystokinin (CCK) (11). Together, these studies suggest that the

satiating effects of nuts may be mediated by CCK and/or PYY secretion and that this effect may stem from the dietary protein or fat content of nuts. Their high unsaturated fat content has been proposed as the primary driver of satiety (24, 25). This hypothesis was based on evidence that unsaturated fat is oxidized more readily than saturated fats (26) and so would generate a more rapid and stronger satiety cue. However, several trials have directly tested this hypothesis and have not provided experimental support (27–30).

An expectation of appetite modulation by nuts is that it will translate into reduced energy intake from the balance of the diet by evoking a strong compensatory dietary response. Compensation data from trials that used almonds (31–33), hazelnuts (34, 35), macadamia nuts (36), peanuts (37, 38), pecans (39, 40), pistachios (41, 42), and walnuts (43) suggest that values range from 54% to 104% (18, 31, 33, 37). Thus, the majority of the energy provided by nuts is offset by spontaneous adjustments in the total diet. Dietary compensation may depend on the form of nuts consumed. Peanuts, in the form of peanut butter, produced higher dietary compensation than whole peanuts (104% compared with 151%) (18), despite evoking a weaker satiety effect (44). In summary, nut ingestion suppresses hunger and desire to eat and promotes fullness. These sensations may aid dietary compensation that offsets much of the energy contributed by nuts. However, strong compensation can also occur independently of reported appetitive effects. This may reflect imprecision in appetite measurement or a truly independent uncharacterized mechanism.

2.5 Mastication and the efficiency of nutrient absorption

Although the nutritive value of nuts is well documented, a growing body of evidence indicates that the published values may be substantively modified by the nut's physical properties (45). In particular, the structure and high fiber content of nuts modify the bioaccessibility and bioavailability of the nutrients they contain. To access the nutrients in nuts, their parenchymal cell walls must be disrupted. This may occur by enzymatic or microbial degradation or mechanical processing in the mouth (chewing) and stomach. The fiber nuts provide may bind with food constituents such as fatty acids, reducing the efficiency of their absorption (46). Fiber may also alter gastric emptying and gastrointestinal transit times and gut hormone secretion, with implications for appetite and energy intake (47). In addition, the cell walls may serve as a source of fermentable fiber in the colon, affecting energy balance and gut health (48, 49). Because these dynamic processes will largely determine the nutritional impact of nut consumption, they are attracting increasing research attention (50).

Nuts require considerable oral processing effort and this may, in part, account for the often-noted less-than-predicted effect of their consumption on body weight (31, 35, 39, 43). The mechanical act of chewing reportedly generates satiation signals through cognitive (51), neural (52), endocrine (12, 53), and physical (eg, gastric emptying) (54) mechanisms; augments cephalic phase responses linked to appetite (55–58); influences digestion efficiency (12, 59–61); modestly increases energy expenditure (62); and elicits dietary compensation (63).

A number of studies have evaluated the efficiency of energy absorption from ground and tree nuts through feeding trials. All showed substantive increases in fecal fat

loss with nut consumption, although the values ranged widely from ~5% to >20% (12, 61, 64–70). One early trial, in which peanut products constituted 95% of daily fat intake, reported the percentage of dietary fat excreted was 17.8% for whole peanuts, 7.0% for peanut butter, and 4.5% for peanut oil (68). These results documented a food form effect. However, there was also an effect of background diet because these fecal fat-loss values were observed when participants were consuming 20 g crude fiber daily, but decreased to 16.8%, 4.2%, and 1.8% for the 3 peanut forms when the background dietary fiber content was reduced to 5 g/d. Presumably, there was greater binding of energy-yielding nutrients, especially fatty acids, to the fiber in the high-fiber condition, leading to greater fecal excretion. The contribution of background diet to treatment effects may account, in part, for differences in absorption efficiency across studies. Subsequent work showed elevated fecal fat excretion with nut consumption across different types of nuts, including almonds (12, 65, 67, 70), pecans (66), pistachios (69), and peanuts (64). In some (67, 70), but not all (61, 69), studies, the increment in fecal fat followed a dose-response pattern. In the latest studies conducted in the same laboratory under comparable conditions, fecal fat losses associated with 42 g almond and pistachio loads/d were comparable at ~4.5 g/d (61, 69). However, they were higher with almonds (~9.1 g/d) compared with pistachios (~6.7 g/d) at higher nut intakes (84 g/d). This suggests relative consistency across nuts for effects at suggested levels of consumption and possibly a nonlinear dose-response relation for some types of nuts.

These trials raise a number of nutritional issues. First, the inefficiency of absorption is a double-edged sword. Whereas this may be beneficial with respect to moderating energy intake and the possible contribution of nuts to positive energy balance

and weight gain, it also likely reduces the absorption efficiency of lipid-soluble nutrients and other macronutrients. Fat accounts for ~55% of the increment in fecal energy loss when nuts are included in the diet (64), so other energy-yielding substances must also be affected. Vitamin E extraction is lower in whole compared with finely ground and defatted almonds with added almond oil (45). The availability of all nutrients is increased with longer gastrointestinal residence time due, in part, to swelling of cell walls and leakage of nutrients out of the parenchymal cells (45). Consequently, nut form can be used for selective purposes: that is, consumption of whole nuts when moderating energy intake is of primary concern; consumption of chopped, sliced, or finely ground nuts or butter or oils when maximization of nutrient intake is the priority. The delayed absorption of lipid from whole nuts may also moderate postprandial lipemia (71) and glycemia (17). Processing of nuts is an additional factor that influences nutrient bioaccessibility (4). Roasting of almonds leads to smaller fragments with greater bioaccessibility of cell contents when chewing. Mastication studies of peanuts confirm that the physical properties of nuts influence their disintegration characteristics (13). They also show that the matrix in which peanuts are embedded modifies chewing behavior but without modifying the final nut particle size. Simulated gastric processing of peanuts shows graded disintegration rates, from the fastest to the slowest in the following order: frying > roasting > boiling > raw (5).

Second, the data on energy bioaccessibility raise questions about the use of Atwater conversion factors for nutrient labeling. A goal of labeling based on standardized servings is to permit consumers to make informed choices about the energy content of different foods to meet their nutritional goals. The structure of nuts may be different

enough from other foods to render labels sufficiently inaccurate to warrant another basis for determination of their energy contribution to the diet (61, 69, 72).

Third, with the introduction of flavorings to nuts to enhance their appeal, questions have been raised as to whether flavors modify chewing and health outcomes. Because flavors are applied to the outer surface of nuts, they exert a strong sensory impact (73) and the actual amount added is limited. Consequently, they would not be expected to directly influence health risks associated with the flavor principle (eg, exacerbation of hypertension by sodium or hyperglycemia by sugar). However, they could theoretically alter oral processing and modify nutrient availability from the nuts themselves. The limited available data suggest that among the varieties tested (ie, raw, roasted unsalted, roasted salted, and honey roasted), no effects of flavor were reported on masticatory outcomes including particle size distribution (74, 75).

Issues of bioaccessibility raise questions about nutrient delivery between types of nuts. Almonds and peanuts have markedly different hardness. The initial break forces for almonds and peanuts are reported as follows: raw nut (7442 ± 332 compared with 3046 ± 380 g), honey roasted (5981 ± 172 compared with 1834 ± 232 g), roasted unsalted (5004 ± 209 compared with 1545 ± 337 g), and roasted salted (4940 ± 267 compared with 1195 ± 289 g) (74, 75). These differences in hardness lead to variation across forms and between nut types for indexes of oral processing effort such as number of chews, chewing rate, and time spent chewing, but the end result is a strikingly similar profile of particle sizes (Figure 2-1). No differences related to BMI have been reported, but processing effort is stronger in the fasted compared with sated states (74, 75). Thus, to the extent that particle size proxies for nutrient availability, the present literature suggests

more commonalities across nuts than differences. This coincides with a large body of literature showing similar effects of different nuts on cardiovascular disease risk, postprandial glycemia, and body weight (15, 76–78).

2.6 Energy expenditure

A limited number of trials have explored the effects of nut consumption on thermogenesis, either postprandially (also known as diet-induced thermogenesis) or on resting energy expenditure (REE). The fatty acid composition of nuts has been the target of much of this work. In an acute-feeding setting, the consumption of a meal containing walnuts (33% of energy from PUFAs) increased diet-induced thermogenesis significantly when compared with a dairy-containing meal (32% of energy from saturated fat) (16). An isoenergetic meal containing olive oil (31% of energy from MUFAs) yielded comparable results to the walnut meal. The test diets were not perfectly matched, so the authors' attribution of the elevation of energy expenditure and fat oxidation to the higher PUFA content of the walnut and the higher MUFA content in the olive oil meals cannot be verified. In addition, the inclusion of almonds [60 g (74) or 54.3 g (31)] and peanuts [52.5 g (37)], both rich sources of unsaturated fats, into meals has not led to elevated thermogenesis. The acute postprandial thermogenic effect of nuts is yet to be confirmed.

Mixed findings on thermogenesis have been reported from short- and longer-term (ranging from 4 d to 12 mo) trials of nut consumption. No effects have been documented with walnuts (20, 79). One study with almonds reported no effect, whereas another observed an increase that accounted for ~14% of the energy contributed by the almonds (31, 33). Several studies with peanuts have noted an increase in REE. In one trial, there

was an 11% increase after 8 wk of peanut consumption (52.5 g/d). When lean and overweight adults were supplemented with peanut oil (as 30% of REE) for 8 wk in another study, REE was elevated by 5%, but only in overweight individuals (80). Collectively, there is some evidence that nut consumption increases thermogenesis, but the data are not robust and there is no clear mechanism. One possibility is that the lipid from nuts is absorbed over a prolonged period of time, leading to a small but sustained source of substrate that fuels thermogenesis and could appear as an increase in REE.

2.7 Adipose tissue and fat metabolism

It has been proposed that nut consumption elevates fat oxidation and preferentially reduces body fat mass, especially in the viscera. These actions are attributed to their high unsaturated fat content. If true, their inclusion in the diet could help to prevent or mitigate the effects of metabolic syndrome. Animal studies have shown that higher PUFA intakes suppress adipocyte differentiation and downregulate adipocyte P2 and adipsin genes (81). However, PUFAs did not have an effect on adipose tissue size (82). In rodent retroperitoneal (but not subcutaneous) adipose tissues, fatty acid synthase (FAS), hormone-sensitive lipase (HSL), phosphoenolpyruvate carboxykinase (PEPCK), lipoprotein lipase (LPL), CCAAT/enhancer binding protein α (C/EBPα), and leptin mRNA concentrations decrease with higher PUFA intake, suggesting targeting of the visceral fat pool (82). There are also indications that fat metabolism is enhanced by higher PUFA intake (83). Mitochondrial protein gene expression is upregulated in the epididymal fat of mice fed PUFAs (84). The expression of genes that regulate oxidative metabolism [eg, peroxisome proliferator-activated receptor α (PPARα), peroxisome

proliferator-activated receptor γ coactivator 1α (Ppargc1a/Pgc1α), and nuclear respiratory factor 1 (Nrf1)] are also elevated (84).

In one acute-feeding study, a high-PUFA diet (33% of energy) enriched with walnuts increased fat oxidation in humans (albeit not significantly) (16). In another study, fat oxidation was significantly elevated (~50% higher compared with a control diet) when 30–35 g of walnuts were ingested by overweight and obese adults (85). The stronger effects noted in this trial may reflect the difference in participant BMI status. Blunted fat oxidation has been reported in adults with high body weight (86), but the inclusion of walnuts appears to reverse or normalize impaired fat oxidation. Notably, in the latter study, the dietary fat concentration and the composition of fat subtypes were matched, suggesting that the fat-oxidizing property of walnuts was not limited to their PUFA content alone. There are no human studies of fat oxidation with other nuts, which limits extrapolation of the walnut findings. Other nuts are richer sources of MUFAs compared with the high PUFA content of walnuts, and MUFAs are reported to induce comparable or higher fat oxidation rates (87, 88). This suggests that equal or greater effects on fat oxidation may be expected with other nuts.

Several clinical trials have examined whether elevated fat oxidation induced by walnuts translates into fat mass loss over time. In one trial (89), the inclusion of walnuts in a weight-maintenance diet of type 2 diabetic adults led to a small reduction in body fat, although body weight remained stable during the 6-mo study period. Body fat increased in individuals who adhered to the control diet (low-PUFA), and the difference between the groups approached significance ($P = 0.057$). The trend persisted when the

intervention period was extended to 1 year, where the inclusion of walnuts produced greater fat mass loss relative to the control group despite comparable weight in the groups (79). Early evidence suggests that the loss of fat mass derived predominantly from the subcutaneous and less from the visceral fat pools, although the size of both fat depots decreased over time in the walnut group (79). The effects of PUFAs on different body fat pools have been studied more intensively with the use of animal models (81, 90–95). This work consistently shows that higher PUFA intake reduces visceral fat. However, findings in humans, to date, do not replicate these results (79). Indeed, just the opposite has been reported, in which PUFAs appeared to preferentially reduce subcutaneous fat. No explanation for the inconsistent outcomes between human and rodent studies is apparent. Because subcutaneous and visceral fats are part of total body fat, evidence has shown that visceral fat loss is primarily determined by total fat mass loss (96). Human studies incorporating different nuts into the diet at realistic doses are needed to determine the effect of nut consumption on body composition. Findings may yield insights for management of body fatness and risk of metabolic syndrome.

2.8 Nut consumption and body weight

The literature on nut consumption and body weight has been the topic of several reviews (76, 97–104). Generally, epidemiologic studies indicate that incorporating nuts into diets on a regular basis does not compromise, and may aid, weight maintenance (14, 105–111). Because energy balance is the ultimate determinant of body weight, not surprisingly, controlled feeding studies using almonds, walnuts, pecans, and macadamia nuts all indicate that nut consumption does not cause changes in body weight when

energy intake is continually adjusted (14, 40, 112–115). More important, when total energy intake is less controlled, studies that involve the inclusion of nuts in habitual diets of free-living individuals have also shown that nut consumption does not lead to weight gain (36, 116–119). However, it must be noted that these were relatively short-term trials with limited power to detect small changes in body weight. Although there are reports of small, but significant increases in body weight with nut consumption (30, 120–122), the preponderance of evidence indicates that under controlled or free-living situations, nut consumption does not promote weight gain.

Several studies assessing the role of nut consumption in weight-maintenance programs have noted a decrease in body weight from baseline (32, 36, 40, 79, 113, 114). Whether this is due to a greater thermic effect of food or REE effect of the nuts compared with the foods they displaced in the diet has not been established. Nevertheless, current data indicate that the inclusion of nuts in a weight-maintenance program will not lead to weight gain and may aid weight loss.

The inclusion of nuts in energy-restriction regimens does not impede weight loss (115, 123–126). In several trials in which nuts did not augment weight loss (125, 126), there was a reduction in cardiovascular disease risk indexes in the nut-consuming groups, suggesting that such benefits derive from properties of the nuts rather than just weight change. There is a need for long-term randomized intervention studies with body weight as a primary outcome to establish the effect of nuts consumed daily in realistic quantities on maximal and sustainable weight loss.

2.9 Conclusions

It is now well established that body weight and fatness are functions of energy balance rather than the macronutrient content of the diet (125, 127, 128). Nuts are a high-fat, energy-dense food, but the evidence indicates that they pose little challenge to and may even aid weight management. This is attributable to the strong dietary compensation effects they elicit, inefficiency in the absorption of the energy they provide, and possibly an elevation of energy expenditure and fat oxidation. Although energy is the determinant of body weight and composition, the greater health effects of diets will be determined by their macronutrient content and other constituents. Nuts are rich sources of unsaturated fats, minerals, vitamins, antioxidants, and fiber, which can contribute to a healthful diet.

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Table 2-1: A summary of recent trials on human energy balance using a variety of nuts

Type of nut and first author (ref)	Year	Design	Length	*n*	Treatments	EI	Appetite	Fat absorption	Diet compensation	EE	Fat oxidation	Weight	Body fat
Almonds													
Kirkmeyer ([18])	2000	Randomized, crossover	180 min and 24 h	24	500 kcal almonds vs no load condition and preloads matched on weight and volume	—	↓	—	57%	—	—	—	—
Fraser ([31])	2002	Randomized, crossover	6 mo	81	320 kcal almonds/d vs no almonds	↑	—	—	54–78%	↔	↔	↔	—
Jenkins ([32])	2002	Randomized, crossover	4 wk	27	22.2%E from almonds or 11.1%E from almonds + 11.1%E from muffins vs 22.2%E from muffins	↑	—	—	—	—	—	↔	—
Wien ([115])	2003	RCT weight loss	24 wk	65	84 g almonds/d vs isocaloric complex carbs	—	—	—	—	—	—	↓	↓
Hollis ([33])	2007	Randomized, crossover	10 wk	20	344 kcal almonds/d vs no almonds	↓	—	—	74%	↔	—	↔	↔
Mori ([17])	2011	Randomized, crossover	490 min	14	43 g almonds vs no almonds, almond flour, and almond oil groups, all matched on 75 g available carbohydrate in breakfast meals	—	↑ Fullness	—	—	—	—	—	—
Novotny ([61])	2012	Randomized, crossover	18 d	18	42 and 84 g almonds/d vs no almonds	—	—	−4.5 g (42 g) −9.1 g (84 g)	—	—	—	—	—
Foster ([125])	2012	RCT weight loss	18 mo	123	56 g/d vs no almonds	—	—	—	—	—	—	↓	—
Tan ([19,21])	2012	RCT (suppl)	4 wk	137	43 g/d vs no almonds	↔	↓	—	—	—	—	↔	↔

Table 2-1 continued

Type of nut and first author (ref)	Year	Design	Length	n	Treatments	EI	Appetite	Fat absorption	Diet compensation	EE	Fat oxidation	Weight	Body fat
Hazelnuts													
Mercanligil ([34])	2007	Randomized, crossover	4 wk	15	40 g hazelnuts/d vs low-fat diet (no hazelnuts)	↑	—	—	—	—	—	↔	—
Yücesan ([35])	2010	Prospective (suppl)	4 wk	21	1 g hazelnuts/kg per day	↑	—	—	—	—	—	↔	—
Macadamia nuts													
Garg ([36])	2003	Prospective (suppl)	4 wk	17	40–90 g/d (15%E from macadamia nuts)	—	—	—	—	—	—	↓	—
Peanuts													
Kirkmeyer ([18])	2000	Randomized, crossover	180 min and 24 h	24	500 kcal peanuts and PB vs no load condition and preloads matched on weight and volume		↓	—	Peanuts: 104%; PB: 151%	—	—	—	—
Alper ([37])	2002	Crossover	30 wk	15	~500 kcal peanuts/d: FF vs ADD vs SUB	↔	↔	—	66% (FF)	↑	—	↔	↔ (SUB, FF) ↑ (ADD)
Griel ([14])	2004	Epidemiology	NS	14,262	Users vs nonusers		—	—	—	—	—	Negative assoc	—
Traoret ([64])	2008	Randomized, crossover	7–9 d	16	70 g peanuts/d vs no peanuts	↑	—	↓	—	—	—	—	—
Devitt ([22])	2011	Randomized, crossover	300 min	66	300 kcal peanuts vs isocaloric load	↔	↔	—	↑ As snacks	—	—	—	—
Pecans													
Morgan ([39])	2000	Prospective (suppl)	8 wk	19	68 g pecans/d	↑	—	—	—	—	—	↔	—

Table 2-1 continued

Type of nut and first author (ref)	Year	Design	Length	n	Treatments	EI	Appetite	Fat absorption	Diet compensation	EE	Fat oxidation	Weight	Body fat
Rajaram ([40])	2001	Randomized, crossover	4 wk	23	20%E from pecans vs no pecans, both treatments as part of Step 1 diet	↔	—	—	—	—	—	↔	—
Pistachios													
Kocyigit ([41])	2006	Prospective (suppl)	3 wk	44	20%E from pistachios	—	—	—	—	—	—	↔	—
Sheridan ([42])	2007	Randomized, crossover	4 wk	15	15%E from pistachios vs no pistachios	↔	—	—	—	—	—	↔	—
Li ([123])	2010	RCT weight loss	12 wk	59	53 g pistachios/d vs 56 g pretzels/d	—	—	—	—	—	—	↓	—
Baer ([69])	2012	Randomized, crossover	3 wk	18	42 and 84 g pistachios/d vs no pistachios	—	—	−4.5 g (42 g)−6.7 g (84 g)	—	—	—	—	—
Almario ([43])	2001	Crossover	6 wk	18	48 g walnuts/d vs no walnuts	↑	—	—	—	—	—	↔	—
Tapsell ([89])	2004	RCT weight maintenance	6 mo	58	30 g walnuts/d vs no walnuts	↔	—	—	—	—	—	↔	↓ (NS)
Sabaté ([120])	2005	Randomized, crossover	6 mo	90	28–56 g/d (12%E from walnuts) vs no walnuts	↑	—	—	—	—	—	↑	↑
Walnuts													
Casas-Augustench ([16])	2009	Randomized, crossover	5 h	29	33%E PUFAs from walnut-enriched meal vs olive oil (MUFAs)– and dairy product (saturated fat)–enriched isocaloric meals	—	↔	—	—	↑	↑ (NS)	—	—
Tapsell ([85])	2009	Randomized, crossover	8 h	16	30 g walnut-enriched meal vs olive oil–enriched isocaloric meal	—	—	—	—	↔	↑	—	—

Table 2-1 continued

Type of nut and first author (ref)	Year	Design	Length	n	Treatments	EI	Appetite	Fat absorption	Diet compensation	EE	Fat oxidation	Weight	Body fat
Tapsell ([79])	2009	RCT weight maintenance	12 mo	50	30 g walnuts/d vs lower-fat diet (no walnuts)	↓	—	—	—	↔	—	↓	↓ (NS)
Brennan ([20])	2010	Randomized, crossover	4 d	20	48 g walnuts/d vs isocaloric placebo	—	↓	—	—	↔	—	—	—
Pine nuts													
Pasman ([11])	2008	Randomized, crossover	240 min	18	3 g pine nut FFA or 3 g TGs vs 3 g placebo (olive oil), all in combination with breakfast	—	↓	—	—	—	—	—	—
Nut not specified													
Ellsworth ([108])	2001	Epidemiology	12-y follow-up	34,111 women	Eating frequency per week	—	—	—	—	—	—	Negative assoc (NS)	—
Albert ([106])	2002	Epidemiology	12-mo follow-up	21,454 men	Eating frequency per week	—	—	—	—	—	—	No assoc	—
Bes-Rastrollo ([107])	2007	Epidemiology	Median 28 mo	8865	Eating frequency per week	—	—	—	—	—	—	Negative assoc	—
Mozaffarian ([111])	2011	Epidemiology	NS	120,877	Serving size	—	—	—	—	—	—	Negative assoc	—

ADD, added to diet; assoc, association; EE, energy expenditure; EI, energy intake; FF, free feeding; FFA, free fatty acids; PB, peanut butter; RCT, randomized controlled trial; ref, reference; SUB, replaced equal amount of fat in diet; suppl, supplement; TG, triglyceride; ↑ ,significantly higher; ↓ , significantly lower, ↔, no change; %E, percentage of energy; —, did not measure

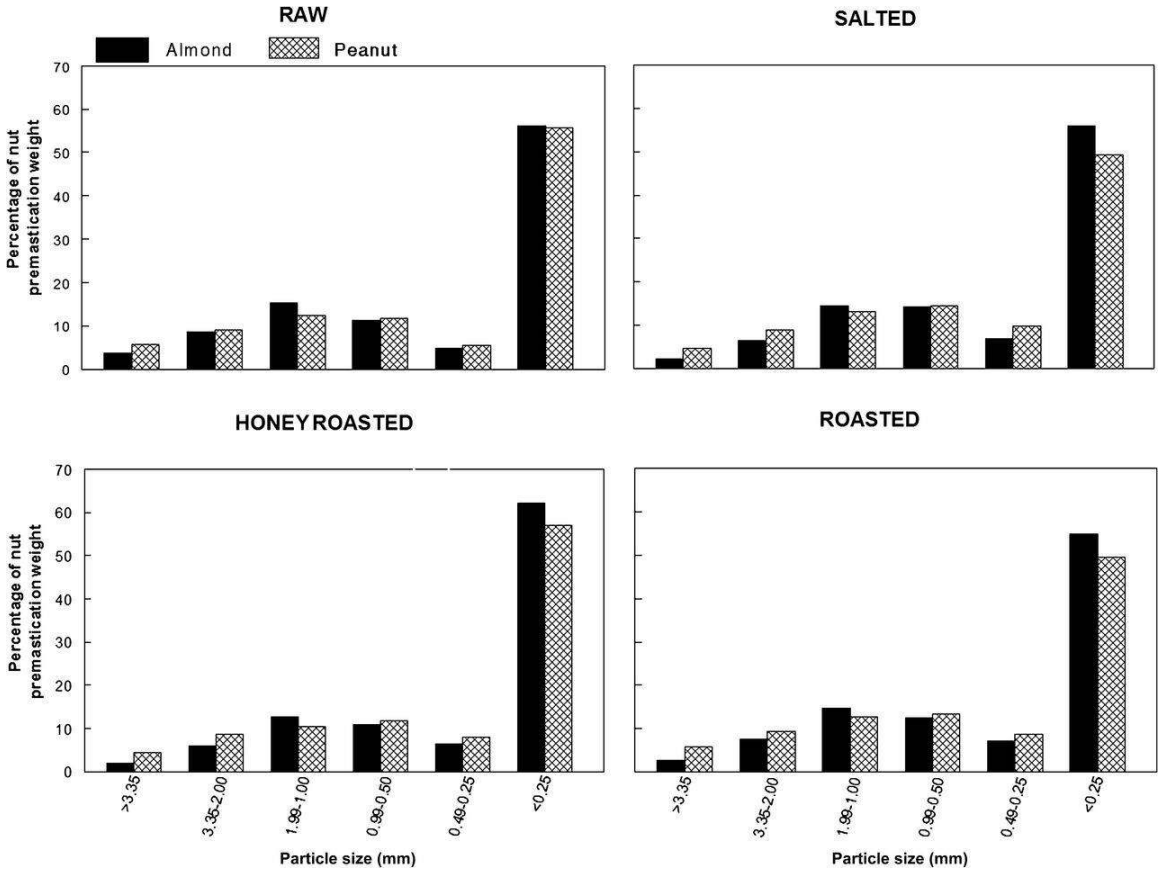

Figure 2-1: Particle sizes (in mm) of raw, salted, roasted, and honey roasted almonds and peanuts after mastication.

The x axis indicates the size of particles after mastication before swallowing.

CHAPTER 3. A LITERATURE REVIEW ON THE EFFECTS OF NUT CONSUMPTION ON BODY COMPOSITION, CARDIOVASCULAR HEALTH AND COGNITIVE FUNCTION

3.1 Introduction

Nuts are high in vegetable protein, fiber, monounsaturated fats (MUFAs), polyunsaturated fats (PUFAs), polyphenols, phytosterols and many vitamins and minerals[1]; nutrients that have beneficial effects on cardiovascular health and cognitive function. This chapter explores the literature on the effects of nut intake on other outcomes of the dissertation research such as body composition, specifically visceral fat, vascular health, lipid profile, glycemic control and cognitive function[1,2]. The potential role of nut consumption in ameliorating the post-lunch decline in cognitive function is also discussed.

3.2 Nut consumption and body composition

Epidemiological evidence indicates that nut consumption is associated with better adiposity measures[3]. Moreover, a recent meta-analysis of controlled interventional trials found that nut intake does not increase adiposity and, in fact, had an inverse effect on adiposity for interventions that imposed energy restriction compared to those focusing on weight maintenance[4]. This meta-analysis included trials that assessed adiposity using anthropometric measures such as BMI and waist circumference. Very few trials have

used direct measures to assess changes in adiposity after nut consumption and have reported conflicting results. While one weight-loss intervention noted that almond consumption for 24 weeks was associated with greater reductions in fat mass when assessed using bioelectrical impedance[5], another weight-loss trial of the same duration observed reductions in DEXA-assessed fat mass with almond consumption which were similar to that of the control[6]. Moreover, the Multi-Ethnic Study of Atherosclerosis (MESA) demonstrated that nut consumption was associated with decreased adiposity (as assessed by computed tomography (CT)) but only in the pericardial area and not in the subcutaneous and visceral depots[7].

Visceral obesity is correlated with various cardio-metabolic disorders and obesity-related complications. However, very few trials have examined the differential effects of nut consumption on subcutaneous and visceral fat loss. Two nut-based interventional trials have examined VAT directly and reported conflicting findings[8,9]. While one trial in diabetic individuals observed that walnut consumption led to significant reductions in fat in both visceral and subcutaneous depots[8], the other trial in individuals with metabolic syndrome found no reduction in visceral or subcutaneous adipose tissue (assessed by MRI) with pistachio consumption for 24 weeks[9]. Other studies have assessed overall abdominal obesity, but not VAT specifically. For example, in a 6- week crossover trial, almond consumption resulted in greater reductions in abdominal fat in individuals with elevated LDL-C compared to a control diet[10]. Hence, there are very few nut-based trials assessing VAT using direct measures such as DEXA, MRI or CT scans and no almond-based trials that have assessed VAT directly in the context of energy-restriction.

3.3 Nut consumption and vascular health

Dietary patterns that incorporate nuts such as the Mediterranean diet, DASH diet and the Nordic diet are associated with decreased blood pressure[11]. Nuts are high in L-arginine (2-3g arginine/100g), a precursor for nitric oxide which increases endothelial-dependent dilation. Nuts are also rich in K and Mg which could assist in reduction of blood pressure. However, findings from nut-based clinical trials are somewhat mixed. While one meta-analysis investigating cardiovascular outcomes after tree nut consumption suggests no effect of tree nuts on resting blood pressure in individuals without prevalent CVD[12], another meta-analysis published in the same year found that nut consumption led to reductions in systolic blood pressure but only in non-diabetic individuals[13]. The heterogeneity in the study inclusion criteria and analyses between the two meta-analyses could have led to the discrepant findings. The tree nut meta-analysis excluded peanuts from the analysis and standardized the effect size to 1 oz. of nuts per day[12]. In contrast, the other meta-analysis included all tree nuts, peanuts and soy nuts in the analysis and did not standardize the nut dose response[13]. Nevertheless, the preponderance of clinical evidence shows the greatest effects of nuts on blood pressure in individuals with some CVD risk factor. For example, peanut consumption for 12 weeks was associated with reduction in diastolic blood pressure in individuals at high CVD risk[14]. Moreover, in the PREDIMED trial, consumption of a nut-based Mediterranean diet for one year led to a decrease in 24-hour ambulatory systolic and diastolic BP but only in high CVD risk individuals[15]. Although there is some data to suggest that almond consumption in the context of energy restriction may augment reductions in blood pressure[5,16] it is not well characterized.

Nut consumption may also have beneficial effects on other indices of vascular health such as endothelial function and arterial compliance. A literature review indicates that nut consumption results in a 19.7% relative increase in vasodilation when computed as weighted average change across 9 studies[2]. Although there are benefits, particularly with walnut consumption, conclusions for other types of nuts cannot be quantified. There is limited evidence regarding the effects of nut consumption on arterial compliance. While 40g of pistachio consumption for 3 months led to reduced arterial stiffness[17], 15g of walnut consumption for 4 weeks did not[18] suggesting perhaps a dose-dependent and/or long-term response. Walnuts are the most extensively researched nuts and are high in ALA which is associated with improved endothelial function and neuro-protective effects in animal models[2]. Almonds and pistachios are high in MUFAs which also reduce arterial stiffness[19] and endothelial dysfunction[20]. In general, the cholesterol lowering effects of nuts can reverse endothelial dysfunction as hypercholesterolemia impairs endothelial function[21]. This may have important implications for cognitive function as discussed later.

3.4 Nut consumption, lipids and inflammation

Nut consumption promotes cholesterol-reduction[22] and reduces cardiovascular risk factors, inflammation and oxidative stress[23]. The preponderance of nut-based evidence shows the greatest improvements in blood cholesterol in individuals with high LDL cholesterol and those with low BMI. Improvements in triglycerides are greatest in individuals with hypertriglyceridemia[22]. The cholesterol lowering effects of nut consumption appear to be dose-dependent with consumption of 60g of nuts or more per

day having the greatest effects[12]. Nut consumption is also associated with reductions in inflammatory markers such as CRP, IL-6, ICAM-1, VCAM-1, E-selectin and interleukins [2]. These effects too appear to be dose dependent with doses greater than 30g per day having beneficial effects[2]. In addition, when nuts replace olive oil in Mediterranean diets, improvements in some inflammatory markers are demonstrated[24–26] suggesting that nuts have beneficial effects over and above that of the traditional olive oil Mediterranean diet.

3.5 Nut consumption and glycemic control

Several epidemiological studies have demonstrated a protective effect of nut consumption on diabetes risk[27,28]. Evidence from these studies has led many researchers to conduct trials investigating the effects of nut consumption on glycemic control. The regulation of glycemia is a critical factor in reducing the risk of diabetes. Nut consumption improves postprandial glycemic control in a dose-dependent manner[29–32]. In addition, nut consumption has second-meal effects on glycemic control[33,34]. The second meal effect is a phenomenon where a prior meal attenuates postprandial blood glucose responses to a subsequent meal[35,36]. The form of nuts may determine the accessibility of lipids leading to differential effects on glycemic control[33,34,37]. For example, lipids in more processed forms of almonds such as almond meal and almond butter are more accessible for absorption than lipids in whole almonds[38,39]. Hence, altering the physical form of nuts may have metabolic effects that warrant further study.

Although the findings from studies examining the chronic effects of nut consumption are inconclusive, evidence indicates that nut consumption for 12 weeks or

longer has beneficial effects on glycemic control through improvements in insulin sensitivity and/or beta-cell function[40,41] and HbA1c concentrations[24,42,43]. In contrast, short-term studies[44–47] suggest that the consumption of nuts for periods less than 12 weeks may not be effective in enhancing overall glycemic control or measures of insulin sensitivity. Although nut consumption when eaten alone or as a part of meals, has positive effects on glycemic control[1], the long term effects are typically more favorable in prediabetic[41], and diabetic individuals[42] or those with metabolic syndrome[40].

3.6 Nut consumption and cognitive function

Epidemiological evidence indicates beneficial effects of nut consumption on cognitive function, but mostly in middle aged and older adults. A five year prospective cohort study found a positive association between nut consumption and cognitive performance[48]. The difference in cognitive performance between the lowest and highest consumers of nuts was equivalent to a 5 to 8 year age difference[48]. In addition, they found no cognitive decline in the highest nut consumers over 5 years (amount of nuts unspecified). In the PREDIMED study, walnut (but not other nuts) consumption was associated with increased working memory in adults aged 55-80 years[49]. In another cross-sectional study, nut consumption was associated with increased executive function and semantic memory in older adults, although the association was non-significant[50]. A recent prospective cohort study utilizing data from the Nurses' Health Study found that higher long-term nut intake was associated with better cognitive outcomes[51]. In addition, women who consumed more than 5 servings of nuts per week had higher scores than non-consumers; the difference in scores was equivalent to the difference found in women

2 years apart in age. The only interventional trial conducted exclusively in young adults observed an 11% increase in inferential verbal reasoning after consumption of 60g walnuts/day for 8 weeks[52]. They observed no changes in non-verbal reasoning and memory scores. Another trial observed improvements in working memory and speed of processing with nut-supplemented diets in the context of energy-restriction[53]. The effects of almond consumption on the memory and attention domains of cognitive function in energy-restricted adults has not been explored before.

The beneficial effects of nuts on cognitive performance are likely due to the improvements in cardiovascular risk factors such as lipid profile, arterial compliance, glucoregulation, oxidative stress, blood pressure and inflammation, with subsequent improvements in endothelial function and cerebral vascular function[2]. Improved endothelial function is important for regulating cerebral blood flow to deliver nutrient-rich blood to the brain. Researchers have hypothesized that cognitive performance can be improved by improving cerebral blood flow[54,55].

3.7 Potential role of nut consumption on the post-lunch dip in cognitive function

Although there are circadian variations in cognitive performance during the day time, there is an acute decrease in cognitive performance around midday, particularly after lunch[56]. This post-lunch dip in cognitive performance is a well-established phenomenon of decreased cognitive function in the early afternoon hours typically after lunch consumption. It is thought to begin approximately one hour after the start of lunch consumption[57]. During the post-lunch dip, memory and vigilance are the most severely affected domains, and decreased mood, alertness and anxiety are also reported[58,59].

These symptoms may negatively affect performance and increase personal and societal risks[57,60,61]. A dip in cognitive function after lunch may be caused by an increase in cortisol levels[62], insulin surges[63] and increased serotonin levels[64]. Although there are no studies investigating the effects of nut consumption on the post-lunch dip, nuts can potentially affect cognitive function post-prandially by manipulating the macronutrient composition of the meal. Lunch composition is reported to play a role in the post-lunch dip with high-fat lunches leading to slower[65,66] but more accurate[66] responses than low-fat lunches. High-carbohydrate meals are shown to have a sedative effect promoting drowsiness in females and calmness in males[64]. Moreover, high carbohydrate and low protein meals could increase brain serotonin levels that could affect cognitive function[64]. The unique nutrient composition of nuts such as almonds which are higher in fat and protein and lower in carbohydrate may ameliorate this post lunch dip in cognitive function.

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CHAPTER 4. EFFECTS OF INCLUDING ALMONDS IN AN ENERGY-RESTRICTED DIET ON VISCERAL FAT AND BLOOD PRESSURE IN OVERWEIGHT AND OBESE ADULTS

Dhillon, J., Tan S.Y. and Mattes R.D. Effects of including almonds in an energy-restricted diet on body composition, visceral adipose tissue and blood pressure in overweight and obese adults. *Adv. Nutr* (submitted on June 28, 2016) [1-3]

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FOOTNOTES

[1]The present study was supported by the Almond Board of California (Modesto, CA, USA). The funders had no role in the study conception, design and implementation, data collection, data analysis or interpretation of results.

[2]Author Disclosures: RDM discloses grants and non-financial support from Almond Board of California during the conduct of the study. SYT and JD have no conflicts of interest.

[3]Abbreviations used: VAT, visceral adipose tissue; BP, blood pressure; AED, almond-enriched diet; NFD, nut-free diet; ITT, intention-to-treat

4.1 Abstract

Background: Inclusion of almonds in an energy restricted diet has been reported to enhance or have no effect on weight loss. Their effects specifically on visceral body fat stores (VAT) during energy restriction have not been widely examined. Additionally, almond consumption has been associated with reduced blood pressure (BP), but whether this is linked to or independent of changes of body composition has not been examined.

Objective: To evaluate the effects of consuming almonds as part of an energy-restricted diet on body composition, specifically visceral adipose tissue and blood pressure compared to a nut-free energy restricted diet.

Methods: Eighty-six healthy adults (BMI: 25-40 kg/m2) were randomly assigned to a 12 week energy restricted (500 kcal/d deficit) almond-enriched diet (AED) or a comparably energy restricted nut-free diet (NFD). Participants in the AED group consumed almonds providing 15% of energy in their individualized energy-restricted diet. Participants in the NFD group avoided nuts during the energy restriction period. This study is registered on ClinicalTrials.gov (registration number: NCT02360787)

Results: Body weight, trunk fat percent, total fat percent, VAT and systolic BP decreased after 12 weeks of energy restriction ($P<0.05$). Compliant participants in the AED group had a greater reduction in trunk and total fat percentages ($P<0.05$), diastolic BP ($P<0.05$) and a greater trend for VAT loss over time compared to those in the NFD group ($P<0.05$).

Conclusions: Inclusion of almonds in a weight loss regimen resulted in greater proportional reductions of trunk and total body fat as well as diastolic blood pressure compared to a nut-free weight loss regimen. Hence, moderate almond consumption during energy restriction may help to reduce metabolic disease risk in obesity.

4.2 Keywords

Almonds; Nuts; Energy restrictions; Weight loss; Body composition; Body fat; Visceral fat; Blood pressure; Obesity

4.3 Introduction

Despite their high energy content, the inclusion of almonds in an energy-restricted diet does not compromise and may enhance weight loss (1–3). This may occur by several mechanisms, but probably most importantly through improved dietary compliance. This is likely attributable to greater sensory variety resulting in higher palatability of the diet (4); stronger locus of control (5) leading to a sense of empowerment; and management of appetitive sensations through their slow and sustained energy release (6).The satiating effects of almonds may prolong inter-meal intervals, promote smaller meal sizes and reduce the desire to eat when not hungry and, hence, contribute to purposeful weight loss (7).

A goal of weight loss is to maximize the reduction of fat mass while retaining fat-free mass. Traditionally, exercise was viewed as the primary way to achieve this outcome, but evidence shows that dietary factors can be equally or more effective. Increasing the proportion of protein in an energy-restricted diet enhances satiety, energy expenditure and greater relative fat mass loss (8). In addition, different types of dietary fat may influence substrate oxidization. Monounsaturated fats are oxidized preferentially (9,10), and a diet higher in the unsaturated: saturated fat ratio may reduce subcutaneous adipose tissue (SAT) (11), but more importantly, visceral adipose tissue (VAT) during weight loss (12). Few studies have investigated the effects of dietary fatty acid

composition on abdominal fat changes (13). VAT undergoes rapid lipolytic activity leading to increased liver fatty acid production. As a consequence, muscle insulin sensitivity reduces and insulin secretion is stimulated. Elevated peripheral insulinemia will suppress lipolysis and promote lipogenesis, resulting in the accumulation of more abdominal and visceral fat (14). The preferential loss of VAT is believed to minimize metabolic syndrome and hence improve metabolic fitness. Almonds are good sources of protein and monounsaturated fats and their effects on visceral body fat loss, in conjunction with energy restriction, have not been widely examined.

Being overweight increases the risk of hypertension by 46% to 75% (15). There is evidence implicating visceral adiposity as the primary cause of obesity-related hypertension (16). Losing weight may reduce blood pressure and this may be augmented by incorporating almonds into the diet. Additionally, food-derived peptides such as arginine reduce the risk of cardiovascular disease (17). Arginine is the physiological precursor of nitric oxide; a vasodilator (18). Nitric oxide inactivation increases blood pressure (19). Certain nuts and seeds are good plant-based sources of arginine and studies with peanuts (2.8g arginine per 100g peanuts, as indicated in the USDA National Nutrient Database) reveals their ingestion for 12 weeks leads to significant reductions in diastolic blood pressure (20). Almonds also contain a high level of arginine (2.4g arginine per 100g almonds), but their effect on blood pressure is not well characterized.

The purpose of this study was to evaluate the effects of almond consumption as part of an energy-restricted diet on weight, body composition, visceral adipose tissue and blood pressure compared to a nut-free diet matched on the level of energy restriction. We hypothesized that inclusion of almonds in an energy-restricted diet will augment the rate

of weight loss, lead to greater fat loss especially in the visceral depot and reduce blood pressure compared to a control diet matched on energy-restriction.

4.4 Methods

4.4.1 Participants

Eighty-six (21 men and 65 women) healthy adults (18–60 y/o) with overweight and obesity (BMI: 25-40 kg/m^2) were recruited. Eligibility criteria included the following: no nut allergies, willingness to consume almonds, not taking medications known to influence metabolism and appetite, non-smoker >1 year, consistent diet and activity patterns and weight stable (≤3 kg change over the last 3 months). This study is registered on ClinicalTrials.gov (registration number: NCT02360787). All procedures involving human subjects were approved by the Purdue Institutional Review Board. Participants were recruited via public advertisements. After expressing interest, potential participants were asked to complete health and personality questionnaires. Participants were excluded from the trial if they had diabetes or pre-diabetes, uncontrolled hypertension, cardiovascular disease or dyslipidemia requiring drug therapy. Informed consent was obtained from participants who met eligibility criteria prior to commencement of study visits. All participants were compensated for their time and effort.

4.4.2 Study protocol

The study was a 12-week randomized, controlled, parallel-arm clinical trial. Participants were randomized into one of two energy-restricted study arms: Almond-

enriched diet (AED) or nut-free diet (NFD). Both groups received dietary counseling using the MyPlate food guidance system (21) to reduce energy intake to achieve 500 kcal/day deficit to support weight loss. Their estimated energy requirement was calculated using the Schofield equations (22) with a physical activity level factor of 1.3. Participants met with a dietitian on a weekly basis for the first 4 weeks to establish their dietary prescription, and every 2 weeks after that to monitor dietary adherence. Weekly energy and nutrient analyses using 24-hour food recalls were conducted to determine participants' compliance to dietary recommendations. All participants were requested to maintain their usual physical activity habits throughout the study duration. Compliance to physical activity was tracked every 4 weeks on 2 days: one weekday and one weekend day, using a previously validated triaxial accelerometer (RT3, Stayhealthy Inc., USA) (23).

Forty-three participants were randomized into the AED and NFD groups each. They were asked to consume dry-roasted, lightly salted almonds providing 15% energy in their individualized energy-restricted diet. Energy from almonds was accounted for during dietary modeling so that a 500 kcal/day deficit was achieved. Participants in the AED group were asked to avoid consumption of other nuts and seeds. Participants in the NFD group were asked to avoid all nuts, seeds and nut products during the intervention period.

4.4.3 Study outcomes

The primary outcomes for this study were weight, body composition, visceral adipose tissue and resting blood pressure. Other outcomes were waist circumference,

sagittal abdominal diameter, serum lipids, insulin, glucose, 24-hour ambulatory blood pressure and 24-hour free-living appetite. All outcomes were assessed at baseline and 12 weeks after the intervention.

Anthropometric outcomes

Body weight was measured using a calibrated scale (Model ABC, Tanita Inc., USA) with participants wearing minimal light-weight clothing. Height was measured using a wall-mounted stadiometer. Body composition was assessed using dual-energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, UK). Sagittal abdominal diameter (SAD) was measured using a portable sliding caliper (Holtain-Kahn Abdominal Caliper, Holtain Limited, UK) placed at the level of the iliac-crest while participants were in a supine position. Waist circumference was measured using a measuring tape placed at the narrowest part of the torso.

VAT was predicted using two multivariate anthropometric models. The first model was based on proximal thigh circumference, waist circumference, age and/or BMI (24) i.e. women: VAT = 2.15 (waist circumference, cm) - 3.63 (proximal thigh circumference, cm) + 1.46 (age, y) + 6.22 (BMI, kg/m^2) - 92.713; men: VAT = 6 (waist circumference, cm) – 4.41 (proximal thigh circumference, cm) + 1.19 (age, y) – 213.65(24) (model 1). Proximal thigh height was measured using a measuring tape placed around the thigh just distal to the gluteal crease. The second model was based on sagittal diameter, age, waist circumference and trunk fat % i.e. VAT = -208.2 + 4.62 (sagittal diameter, cm) + 0.75 (age, y) + 1.73 (waist circumference, cm) + 0.78 (trunk fat, %) (25)

(model 2). Although this model has only been validated for women, we applied it for men as well and examined the correlations between the 2 models with regard to sex.

Resting blood pressure was assessed using an automated digital blood pressure monitor (Model 6013, American Diagnostic Corporation, USA). The participants rested for 5 min prior to blood pressure measurement. Three readings were taken and the average was used to determine resting blood pressure. Twenty-four hour ambulatory blood pressure in a free-living environment was assessed using an automated ambulatory blood pressure machine (ABPM50, Contec, China) that was programmed to measure blood pressure every hour from 8 am to 12 am then every 4 hours till 8 am the next day. This device was worn on the arm for 24-hours and the cuff periodically inflated and deflated to measure blood pressure.

Serum lipids, insulin and glucose

Fasting blood samples (8ml) obtained from participants were analyzed for serum lipids (total, LDL and HDL cholesterol and triglycerides), insulin and glucose using an automated sample analyzer COBAS (COBAS integra 400 plus, Roche Diagnostics Limited, USA).

24-hour free living appetite ratings

Hunger, fullness, desire to eat and prospective consumption ratings were measured on 100-mm visual analog scales on palm pilots with end anchors of “not at all” to “extremely”. These ratings were assessed hourly during waking hours over a 24 hour period. The mean of the respective appetite ratings were considered for analysis.

4.4.4 Almond acceptance and palatability

Participants randomized to the AED group rated the acceptability of almonds using a food action rating scale (26) and the palatability of almonds using a hedonic general labelled magnitude scale (gLMS) (27,28) one week after the start of the intervention and at the end of the intervention.

4.4.5 Compliance assessment

Participant's compliance to energy restriction (regardless of group) was assessed via self-reported intake (24-hour food recalls) and weight loss. Participant's compliance to their respective intervention groups (i.e. almond consumption or no nut consumption) was monitored via self-reported intake as well as by analyzing their erythrocyte membranes for lipids and flavonoids at baseline and 12 weeks after the intervention. The lipid extracts from participants' erythrocytes were prepared using a procedure by Rose and Oklander (29) and were analyzed by a shotgun lipidomics approach (J Dhillon, CR Ferreira, TJP Sobreira, unpublished data). The most informative lipids identified through the lipidomics scans and nut flavonoids reported in literature were combined into two MS methods i.e. single ion monitoring (SIM) and multiple reaction monitoring (MRM) using a triple quadrupole mass spectrometer. Data obtained from the targeted SIM and MRM methods were analyzed via univariate (volcano plots) and multivariate (PCA) statistics using Metaboanalyst (version 3.0, 2015) (30). The performance of the different m/z values and their combinations and ratios was evaluated by receiver operating characteristic (ROC) analyses.

4.4.6 Statistical analysis

We conducted two analyses, a primary intention-to-treat analysis followed by a secondary analysis of participants compliant to their respective intervention groups. Both analyses used a linear mixed model with time, intervention group and a time-by-intervention group interaction as factors for all absolute values of outcomes. An additional linear mixed model analysis on the change in outcomes as opposed to absolute values was also performed. In each analysis, when significant interactions were observed, pairwise comparisons with Bonferroni correction were carried out. Age range, sex and BMI range were also considered as between-subject factors for all the tests but there were no effects of the aforementioned factors on any of the outcomes.

The sample size calculations for this study were based on detection of a 7% difference in visceral fat between groups with 80% power and a 2-tailed alpha of 0.05. Complete data were required from forty participants per group.

Between-group differences were assessed at baseline by using independent-samples t tests. Categorical outcomes were assessed using chi-square tests. The alpha level was set at 0.05. SPSS (version 22, 2013, SPSS Inc.) was used for all the statistical analyses. Data are reported as means and standard errors (SEM) unless otherwise stated.

4.5 Results

4.5.1 Participants

Eight-six participants enrolled in the study, but 7 withdrew during the intervention (Figure 4-1). The attrition rates were 7% for the AED group and 9.3% for the NFD group at 12 weeks. There were no significant differences in attrition between the AED and NFD

intervention groups at 12 weeks (P=0.69). The baseline characteristics of all participants are described in Table 4-1. There were no significant differences in the baseline characteristics of participants between groups.

4.5.2 Compliance to energy restriction

The energy-restriction compliance rates were 65.1% for the AED group and 67.4% for the NFD group at 12 weeks. There were no significant differences in compliance to energy-restriction as assessed by the dietary intake data and weight loss between the AED and NFD intervention groups at 12 weeks (P=0.82).

4.5.3 Compliance to intervention groups

Data from 24-hour recalls indicated that total energy, carbohydrate, fat, protein and sodium intake decreased over time (P<0.05). The percentage of energy from fat and total MUFA, oleic acid, MUFA/SFA ratio, linoleic acid, total alpha-tocopherol, magnesium, copper and phytic acid intake (almonds are rich in these nutrients) was greater and percentage of energy from carbohydrate lower in the AED group compared to the NFD group at the end of the intervention (P<0.05) (Table 4-2).

The shotgun metabolomics analysis conducted on 61 participants whose erythrocytes were collected indicated that specific ratios and combinations of mainly membrane lipids such as phosphatidylcholine and sphingomyelin were discriminatory of almond consumption from the nut-free diet at the end of the 12 week intervention in the AED group (J Dhillon, CR Ferreira, TJP Sobreira, unpublished data). However, 8 participants in the AED group and 3 participants from the NFD group were misclassified

into the opposite group indicating possible non-compliance to their respective interventions which was supported by dietary intake data. These participants, as well as those not compliant to the energy restriction, were excluded from the secondary compliers analysis. There were no significant differences in overall compliance based on weight loss and metabolomics analysis to the AED and NFD intervention groups at 12 weeks (P=0.51). The baseline characteristics of the compliers are shown in Table 4-1**.**

4.5.4 Anthropometric outcomes

Body weight significantly decreased after the intervention in both analyses (P<0.05).There was no difference in weight loss between the AED and NFD groups in both the intention-to treat and compliers analyses (Table 4-3).

Trunk and total fat mass and percentage significantly decreased after the intervention (P<0.05) in both analyses while trunk and total fat-free mass significantly decreased only in the compliers analysis and trunk and total fat-free mass percentage significantly increased after the intervention in both analyses (P<0.05) (Figure 4-2). The compliers analysis indicated a significantly greater decrease in trunk fat mass and percentage and total fat percentage and a significantly greater increase in the trunk and total fat-free mass percentage in the AED group compared to the NFD group (P<0.05) (Figure 4-2).

SAD and waist circumference significantly decreased after the intervention in both analyses (P<0.05). No significant differences were observed between the AED and NFD groups after the intervention in both analyses (Table 4-3).

VAT predicted using both models significantly decreased after the intervention ($P<0.05$) in both analyses (Table 4-3). Although there was a trend for greater VAT loss in the AED group compared to the NFD group (Model 1: $P=0.097$) in the compliers analysis, this difference was not statistically significant (Table 4-3). Even though VAT model 2 was validated only for women, it strongly correlated with VAT model 1 for both men ($r=0.941$, $P<0.0001$) and women ($r=0.923$, $P<0.0001$) (Baseline correlations shown).

Resting systolic blood pressure significantly decreased after the intervention ($P<0.05$) while resting diastolic blood pressure remained unchanged for all participants. However, resting diastolic blood pressure significantly decreased in the AED group after the intervention ($P<0.05$) but not in the NFD group for participants compliant to the intervention (Table 4-3). Twenty-four hour ambulatory systolic and diastolic blood pressures remained unchanged at the end of the intervention in both analyses. In general, ambulatory blood pressure readings were significantly higher during waking hours i.e. from 8 am to 11 pm (systolic: 123.5 ± 0.6 mmHg, diastolic: 73.9 ± 0.46 mmHg) compared to sleeping hours i.e. 11 pm to 8 am (systolic: 113.5 ± 1.04 mmHg, diastolic: 64.08 ± 0.94 mmHg) ($P<0.05$).

4.5.5 Serum lipids, insulin and glucose

Fasting serum insulin, triglycerides, total cholesterol, HDL and LDL cholesterol remained unchanged after the intervention while fasting glucose increased significantly after the intervention regardless of intervention group ($P<0.05$) (Table 4-3). In the compliers analysis, fasting glucose remained unchanged after the intervention in both groups (Table 4-3).

4.5.6 24-hour free living appetite ratings

Twenty-four hour hunger, desire to eat and prospective consumption ratings significantly decreased at the end of the intervention ($P<0.05$) with no difference between the AED and NFD groups (Table 4-3). Fullness ratings remained unchanged at the end of the intervention (Table 4-3). In the compliers analysis, hunger, desire to eat and fullness ratings followed the same trend as in the intention-to-treat analysis but prospective consumption ratings remained unchanged at the end of the intervention (Table 4-3).

4.5.7 Almond palatability and acceptance

The almond palatability ratings significantly decreased after the intervention (-4.81 ± 2.2, (FACT scale units) $P<0.05$) while the almond acceptance ratings remained unchanged (-0.29 ± 0.28, (gLMS units) $P =0.308$) for participants in the AED group in the intention-to-treat analysis. The compliers analysis indicated no change in almond palatability ratings after the intervention (-3.8 ± 2.8, $P =0.194$) and the almond acceptance ratings remained unchanged as well (-0.16 ± 0.33, $P=0.632$).

4.5.8 Activity energy expenditure

Average activity energy expenditure was significantly higher on weekdays (0.7 ± 0.04 kcal/minute) compared to weekend days (0.59 ± 0.05 kcal/minute, $P<0.05$) but remained unchanged over the intervention in both AED and NFD groups.

4.6 Discussion

The present study had several important findings. Despite similar weight loss with the two diets, almond consumption was associated with significantly greater proportional improvements in overall body composition and greater fat loss in the trunk area in compliant participants. Estimates of trunk fat (DEXA) are typically strongly correlated with abdominal visceral fat (r=0.86-0.89) (31), hence a reduction in trunk fat could reduce metabolic disease risk. One possible explanation for the greater fat loss with almond consumption stems from their high unsaturated fat content. Unsaturated fats have high fat oxidation rates which can preferentially reduce visceral fat (7).

Although we did not see a statistically significant difference in VAT loss between the two diets, there was trend for greater reduction in VAT with almond consumption when assessed using models that took into account DEXA estimates of trunk fat and other measures of central obesity such as SAD and waist circumference. To our knowledge two nut-based interventional trials have examined VAT directly and found conflicting results (32,33). While one trial in overweight diabetic individuals observed that walnut consumption led to significant reductions of fat in both visceral and subcutaneous depots (33), the other trial in individuals with metabolic syndrome found no reduction in visceral or subcutaneous adipose tissue (assessed by MRI) with pistachio consumption for 24 weeks (32). Although the walnut trial was conducted in the context of weight maintenance, participants still lost weight. Hence, nut consumption in the context of weight loss might have greater effects on VAT loss. Nut-specific effects are also possible though for most clinical outcomes and similarities are generally greater than differences between nut types.

Our intervention demonstrated a decrease in resting systolic BP in both groups, but only the almond-enriched diet was associated with a reduction in resting diastolic BP (-3.6%) in compliant participants. Our findings are in contrast to the preponderance of clinical evidence that suggests no effect of tree nuts on resting BP in individuals without prevalent CVD (34). Similar reductions in diastolic BP have been observed with peanut consumption but only in individuals with elevated BP at baseline (20). Moreover, in the PREDIMED trial, consumption of a nut-based Mediterranean diet for one year led to a decrease in 24-hour ambulatory systolic and diastolic BP but only in high CVD risk individuals (35). It is possible our findings reflect the use of almonds under energy-restricted, weight loss conditions which could augment reductions of diastolic blood pressure (36).

In the present study, serum insulin and glucose remained unchanged with almond consumption for 12 weeks. Although nut consumption has positive effects on glycemic control (37), the long term effects are typically more favorable in prediabetic (38), and diabetic individuals (39) or those with metabolic syndrome (40). In addition, there were no changes in serum triglycerides, total cholesterol, HDL and LDL cholesterol with almond consumption. The participants in our study were healthy adults with overweight and obesity with no other CVD risk factors and the preponderance of nut-based evidence shows the greatest improvements in blood cholesterol in individuals with high LDL cholesterol and those with low BMI, and improvements in triglycerides in individuals with hypertriglyceridemia (41). Moreover, these improvements were observed with an average daily nut consumption of 67g whereas the participants in our study consumed only 38g of nuts on an average.

The satiating effects of almond consumption in acute feeding trials are well documented (7). These properties have important implications for weight management as they can translate into strong dietary compensatory responses. Whether these satiating effects can be sustained chronically has yet to be established. In this 12-week clinical weight loss trial, almond consumption reduced 24-hour hunger and desire to eat ratings to a similar degree as the nut-free diet. But it is important to note that both intervention groups underwent structured dietary counseling to make healthier and satiating food choices.

Another consideration in long-term feeding studies is that of monotony effects arising as a result of repeated daily consumption of specific foods (42). This is important as it might undermine compliance to a dietary recommendation to increase consumption of a given food. In our study, almond palatability ratings significantly decreased over time but remained within acceptable range i.e. from over strongly palatable at baseline to over moderately palatable at the end of the intervention. However, individuals compliant to the intervention demonstrated no decline in almond palatability ratings (rated consistently over strongly palatable) suggesting that these individuals may have been more resistant to the monotony effects and hence more compliant.

In conclusion, incorporating modest quantities of almonds in a 12 week weight-loss regimen led to significant reductions in weight and improvements in body composition and blood pressure in healthy adults with overweight and obesity. The clinical benefits of moderate almond consumption among individuals with or at a high risk for metabolic syndrome and/or CVD have been repeatedly confirmed and the present

findings indicate positive health effects among overweight, but otherwise healthy adults as well.

4.7 Acknowledgements

The authors' responsibilities were as follows—JD, SYT and RDM: designed and conducted the study, analyzed data and shared equal responsibility in writing the manuscript and of its final content. All authors read and approved the final manuscript.

4.8 References

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Table 4-1: Baseline characteristics of participants

Characteristic	Intention-to-treat (ITT) analysis		Compliers analysis	
	Almond-enriched diet (AED) (n=43)	Nut-free diet (NFD) (n=43)	Almond-enriched diet (AED) (n=23)	Nut-free diet (NFD) (n=27)
Sex [n (%)]				
Male	11 (25.6)	10 (23.3)	8 (34.8)	7 (25.9)
Female	32 (74.4)	33 (76.7)	15 (65.2)	20 (74.1)
Age (y)	31.05±12.9[1]	31.77±13.15	33.57±12.86	34.93±13.11
BMI (kg/m^2)	29.91±3.2	30.97±4.48	30.33±3.22	30.56±3.87
BMI Category [n (%)]				
Overweight (BMI: 25-29.9 kg/m^2)	23 (53.5)	21 (48.8)	10 (43.5)	13 (48.2)
Obese (BMI: 30-40 kg/m^2)	20 (46.5)	22 (51.2)	13 (56.5)	14 (51.8)
Body weight (kg)	82.79±12.9	84.71±14.12	83.46±12.94	82.22±14.59
Waist circumference (cm)	88.05±8.69	90.23±9.80	90.09±8.69	90.21±9.38
SAD (cm)	21.91±2.65	22.24±3.09	22.63±2.81	22.41±3.01
Resting systolic BP	123.11±11.39	121.12±8.62	124.67±9.25	121.19±8.15
Resting diastolic BP	75.22±8.79	73.85±7.15	74.52±6.45	76.32±9.63
Total fat mass (kg)	31.67±7.49	33.09±8.93	31.63±8.56	32.04±8.67
Trunk fat mass (kg)	16.14±4.53	16.96±5.42	16.63±4.81	16.71±5.16
Total fat-free mass (kg)	50.08±9.62	50.60±8.81	50.82±9.78	49.26±9.63
Trunk fat-free mass (kg)	22.47±4.41	22.84±3.72	22.82±4.54	22.34±3.87
VAT (model 1)[2]	92.42±43.95	106.62±51.01	104.59±45.14	114.83±42.69
VAT (model 2)	100.96±32.29	107.14±40.12	109.95±33.18	110.54±38.59
Almond palatability ratings (gLMS)	68.26±1.85	-	72.46±2.17	-
Almond acceptance ratings (FACT Scale)	6.86±0.17	-	7.102±0.19	-

Mean ± SD (all such values), [2] ITT analysis- AED: n=36, NFD: n=37; Complier's analysis- AED: n=20, NFD: n=23. - Refers to not assessed. There were no statistically significant differences in outcomes between the 2 intervention groups at baseline assessed using independent-samples t tests.

Table 4-2: Mean change in nutrient intakes over 10 weeks

Nutrients	Intervention groups Almond-enriched diet (AED) (n=43)	 Nut-free diet (NFD) (n=43)
Energy[1] (kcal)	-233.31±102.29	-292.28±105.09
Carbohydrate[1] (g)	-39.35±15.93	-23.05±16.37
Fat[1] (g)	-3.81±5.41	-19.02±5.56
Protein[1] (g)	-11.18±5.50	-9.02±5.64
Dietary Fiber (g)	1.40±1.55	0.59±1.59
% energy from carbohydrate[2]	-3.46±2.24	3.46±2.30
% energy from protein	-1.20±1.22	1.04±1.26
% energy from fat[2]	3.67±1.88	-5.02±1.93[1]
Total MUFA[2] (g)	2.87±2.05	-8.16±2.10[1]
% energy from MUFA[1,2] (g)	4.06±.82	-2.52±.84
Total PUFA (g)	.47±1.76	-4.29±1.81
Total SFA[1] (g)	-6.39±2.27	-5.40±2.33
MUFA/SFA ratio[1,2]	.77±.12	-0.03±.13
PUFA/SFA ratio[1]	.31±.13	0.11±.14
Oleic acid[2] (g)	3.26±1.95	-7.63±2.01[1]
Linoleic acid[1,2] (g)	.79±1.61	-4.01±1.65
Total alpha-tocopherol[1,2] (mg)	6.36±1.09	-1.72±1.12
Magnesium[2] (mg)	51.63±20.09[1]	-14.89±20.65
Copper[2] (mg)	0.13±.098	-0.24±.10[1]
Phytic Acid[2] (mg)	203.39±92.54[1]	-74.97±95.07
Sodium[1] (mg)	-400.29±269.87	-417.22±277.25

Values are mean ± SEM obtained from a linear mixed effects model with time as within-subject factor and intervention group as between subject factor. [1] $P<0.05$ over 12 week intervention, [2] $P<0.05$ AED vs. NFD

Table 4-3: Mean change in outcomes over the 12 week intervention

Characteristic	Intention-to-treat (ITT) analysis		Compliers analysis	
	Almond-enriched diet (AED) (n=43)	Nut-free diet (NFD) (n=43)	Almond-enriched diet (AED) (n=23)	Nut-free diet (NFD) (n=27)
Body weight[1] (kg)	-2.22±0.46	-1.09±.46	-3.55±0.47	-2.46±0.44
VAT (model 1)[1,2] (cm^2)	-4.31±1.48	-1.40±1.49	-8.19±1.81	-3.99±1.68
VAT (model 2)[1] (cm^2)	-9.04±1.31	-6.69±1.33	-12.82±1.60	-9.23±1.48
Waist circumference[1] (cm)	-4.36±1.78	-2.57±1.77	-3.24±0.44	-2.54±0.41
SAD[1] (cm)	-0.797±0.15	-0.59±0.15	-1.15±0.18	-0.88±0.16
Resting BP (mmHg)				
Systolic BP[1]	-3.11±1.35	-1.56±1.35	-3.23±1.85	-2.20±1.70
Diastolic BP	-1.07±0.92	0.07±0.92	-2.71±1.15[1,3]	0.815±1.06
Fasting blood profile (mg/dL)				
Insulin	-1.17±2.32	-2.79±2.33	-3.54±3.81	-3.22±3.52
Glucose[4]	3.20±1.80	2.57±1.81	2.61±2.63	4.41±2.43
Triglycerides	-18.10±11.43	-7.57±11.47	-17.65±18.70	-15.11±17.26
Total cholesterol	-2.43±3.54	2.57±3.56	-1.61±4.5	2.26±4.15
HDL	2.65±1.40	0.57±1.41	1.99±2.03	1.69±1.87
LDL	-0.79±2.96	1.60±2.98	.212±4.14	1.22±3.82
Appetite ratings (mm)				
Hunger[1]	-3.15±2.61	-6.07±3.81	-3.42±3.54	-6.71±4.12
Fullness	0.09±2.89	1.88±4.19	0.25±3.97	1.89±4.60
Desire to eat[1]	-5.23±2.69	-5.63±3.93	-6.76±3.59	-4.87±4.19
Prospective Consumption[4]	-3.45±2.47	-7.02±3.57	-3.29±3.41	-6.92±3.94

Values are mean ± SEM obtained from a linear mixed effects model with time as within-subject factor and intervention group as between subject factor. [1] $P<0.05$ over time (12 week intervention) for both analyses, [2] ITT analysis- AED: n=36, NFD: n=37; Complier's analysis- AED: n=20, NFD: n=23, [3] $P<0.05$ AED vs. NFD, [4] $P<0.05$ over time (12 week intervention) for ITT analysis only

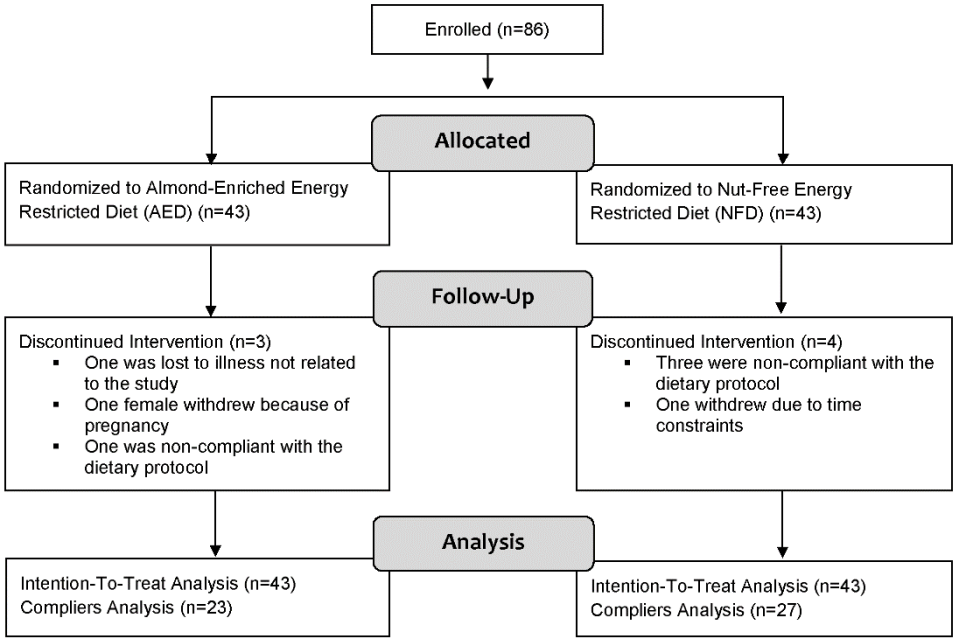

Figure 4-1: Participant flow throughout the almond weight loss study.

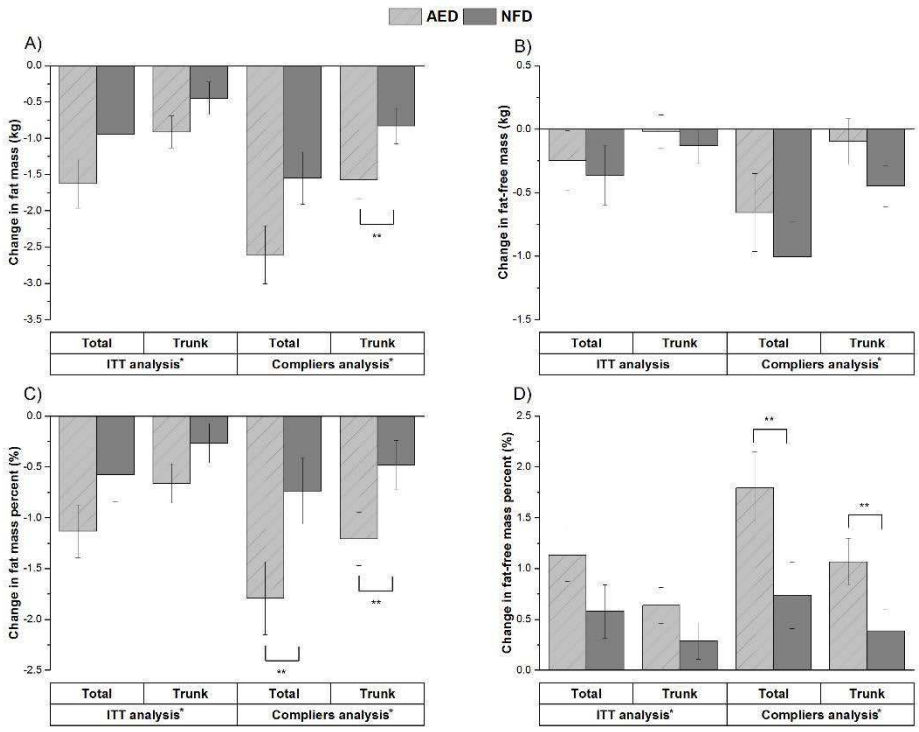

Figure 4-2: Mean change in total and trunk A) fat mass and B) fat-free mass C) fat mass percent and D) fat-free mass percent over the 12 week weight loss intervention in the AED and NFD groups.

Values are mean ± SEM obtained from a linear mixed effects model with time as within-subject factor and intervention group as between subject factor. * P<0.05 over time (12 week intervention). ** P<0.05 AED vs. NFD groups. ITT analysis-AED: n=43, NFD: n=43; Complier's analysis- AED: n=23, NFD: n=27. ITT: Intention-to-treat analysis. AED: Almond-enriched energy restricted diet, NFD: Nut-free energy restricted diet.

CHAPTER 5. EFFECTS OF ALMONDS ON COGNITIVE FUNCTION AND THE POST LUNCH DIP IN ENERGY-RESTRICTED OVERWEIGHT AND OBESE ADULTS

Dhillon, J., Tan S.Y. and Mattes R.D. Effects of almond consumption on the post-lunch dip and short-term cognitive function in energy-restricted overweight and obese adults. *Br. J. Nutr* (submitted on June 22, 2016)

The manuscript has been submitted to the British Journal of Nutrition and formatted according to the requirements of the journal.

5.1 Abstract

The post-lunch dip in cognition is a well-established phenomenon of decreased alertness, memory and vigilance after lunch consumption. Lunch composition reportedly influences the post-lunch dip. Moreover, dieting is associated with cognitive function impairments. The negative effects of dieting have been reversed with nut-supplemented diets. The aims of this study were to 1) evaluate the acute effect of an almond-enriched high fat lunch or high carbohydrate lunch on the post-lunch decline in cognitive function and 2) evaluate the effects of chronic almond consumption as part of an energy-restricted diet on the memory and attention domains of cognitive function. Eighty-six overweight and obese adults were randomized to consume either an almond-enriched diet or a nut-free control diet over a 12-week weight loss intervention. The same participants were also

randomized to receive either a high fat lunch (A-HFL) (>55% energy from fat, almonds contributing 70-75% energy) or a high carbohydrate lunch (HCL) (>85% energy from carbohydrates) at the beginning and end of the intervention. Memory and attention performance indices such as concentration and qualitative performance decreased after lunch consumption ($P<0.05$). The A-HFL group had smaller declines in memory scores compared to the HCL group ($P<0.05$). Both lunch groups had similar declines in attention. Hence, consumption of an almond-enriched lunch reduced the post-lunch dip in memory. However, the macronutrient composition of the lunch did not affect the post-lunch dip in attention. Moreover, memory and attention performance indices increased after the intervention ($P<0.05$). But, there were no additional improvements in these outcomes with almond supplementation.

5.2 Keywords

Cognitive function: Cognition: Post-lunch dip: Almonds: Nuts: Attention: Memory

5.3 Introduction

The post-lunch dip in cognitive performance is a well-established phenomenon of decreased cognitive function in the early afternoon hours typically after lunch consumption[1]. It is thought to begin approximately one hour after the start of lunch consumption. During the post-lunch dip, memory and vigilance are the most severely affected domains, and decreased mood, alertness and anxiety are also reported[2,3]. These symptoms may negatively affect performance and increase personal and societal risks[1,4,5]. Lunch composition is reported to play a role in the post-lunch dip with high-fat

lunches leading to slower[6,7] but more accurate[7] responses than low-fat lunches. High-carbohydrate meals have a sedative effect promoting drowsiness in females and calmness in males[8]. Other studies have reported longer reaction times after consumption of lunches higher in fat or carbohydrate than normal[9]. There is heterogeneity to these responses in terms of age. For example: adults ≥ 40 years show impaired cognitive performance after a high-carbohydrate lunch whereas adults < 40 years do not[8]. However, in healthy young adults, tasks that demand greater cognitive function are more sensitive to nutrient manipulations[10]. The unique nutrient profile of almonds, which is lower in carbohydrate and high in protein and unsaturated fats, may lessen this post-lunch dip in young and middle aged adults. If almonds are able to ameliorate the post-lunch dip, they can enhance productivity (e.g., accuracy of written tasks) and safety (staying awake while driving or working with machines).

Nut consumption over the long-term is also positively associated with cognitive performance. In a five year prospective cohort study, the difference in cognitive performance between the lowest and highest consumers of nuts was equivalent to a 5 to 8 year lesser age-related difference in cognitive function[11]. In addition, individuals that ate the most nuts had no cognitive decline over the 5 year study period (amount and types of nuts unspecified)[11]. In the PREDIMED study, walnut consumption was associated with increased working memory in adults aged 55-80 years[12]. Data from the Nurses' Health Study revealed higher long-term nut intake was associated with better cognitive outcomes[13]. In addition, women who consumed more than 5 servings of nuts per week had higher global cognition scores than non-consumers; the difference in scores was equivalent to the difference found in women aged 2 years apart. There is very limited

data from interventional trials in humans to establish causation. In one trial, consumption of brazil nuts (one nut per day) for 6 months improved verbal fluency and constructional praxis in older adults with mild cognitive impairment[14]. The only interventional study to date conducted in young adults observed an 11% increase in inferential verbal reasoning after consumption of 60g walnuts/day for 8 weeks[15]. They observed no changes in non-verbal reasoning and memory scores.

The process of dieting to lose weight is associated with cognitive function impairments[16] that could be a result of food restriction, anxiety related to maintaining a weight loss regimen or preoccupying thoughts of hunger and desire to eat[17] and body esteem[18,19]. Negative effects of dieting on cognitive performance have been reversed with nut-supplemented diets higher in fat and lower in carbohydrate[20]. Hence, learning and memory may also be enhanced with the inclusion of almonds during weight loss.

The beneficial effects of nuts on cognitive performance in the long-term are likely due to the improvements in cardiovascular risk factors such as lipid profile, arterial compliance, glucoregulation, oxidative stress, blood pressure and inflammation, with subsequent improvements in endothelial function and cerebral vascular function[21]. Nut-rich Mediterranean diets reportedly increase brain-derived neurotrophin (BDNF) concentrations in people with depression[22]. High concentrations of BDNF are associated with retention of memory and reduced cognitive decline[23–25]. Nuts are high in neuroprotective compounds such as Vitamin E, folate, melatonin, alpha linoleic acid (ALA) (walnuts) and polyphenols[25]. Although most tree and ground nuts generally have similar nutrient profiles, some nut specific properties are purported to play a role in maintaining cognitive functions and preventing cognitive decline. For example, walnuts

have been widely studied in animal models[26,27] and in vitro[25], possibly because of their previously established anti-oxidative and anti-inflammatory effects. The effects of almonds on improving memory[28] in rats have been associated with their cholinergic, anti-cholinesterase and cholesterol lowering properties[25,28].

The purpose of this study was two-fold. The first aim was to determine the post-lunch changes in cognitive function with almond consumption at lunch compared to a high-carbohydrate control lunch. We hypothesized that inclusion of almonds in a midday meal will ameliorate the post-lunch decline in memory and attention compared to a high carbohydrate control meal by lowering the percentage of dietary energy from carbohydrates and increasing the percentage of energy from fat. The second aim was to evaluate the effects of almond consumption as part of an energy-restricted diet on cognitive function compared to a nut-free diet matched on energy-restriction (control). We hypothesized that inclusion of almonds in an energy-restricted diet will improve the attention and memory domains of cognitive function compared to the control.

5.4 Methods

5.4.1 Participants

Eighty-six healthy overweight or obese adults (21 men and 65 women) participating in an almond weight-loss clinical trial were recruited. Eligibility criteria was as follows: age of 18–60 years, BMI of 25-40 kg/m^2, no nut allergies, willingness to consume study foods and comply with the study protocol, no endocrine or metabolic disorders, non-smokers and consistent diet and activity patterns. This study was conducted according to the guidelines codified in the Declaration of Helsinki and is

registered on ClinicalTrials.gov (registration number: NCT02360787). All procedures involving human subjects were approved by the Purdue Institutional Review Board. Participants were recruited via public advertisements. Those meeting the eligibility criteria were contacted to schedule a screening visit. Participants provided written consent at the beginning of the screening session and were compensated for their time in the study.

5.4.2 Intervention

The study was a 12-week randomized, controlled, parallel-arm weight loss trial. In brief, participants were randomized into one of two energy-restricted study arms: Almond enriched diet (AED) or nut-free diet (NFD) groups. Both groups received dietary counseling to reduce energy intake to achieve 2092 kJ/day (500 kcal/day) deficits to support weight loss. Weekly energy and nutrient analyses using 24-hour food recalls were conducted to determine the participants' compliance to the dietary recommendations.

Forty-three participants were randomized into each of the AED and NFD groups. Participants in the AED group were asked to consume dry-roasted, lightly salted almonds providing 15% of the energy in their individualized energy-restricted diet. Energy from almonds was accounted for during dietary modeling so that a 2092 kJ/day (500 kcal/day) deficit was achieved. Participants in the NFD group were asked to avoid all nuts and nut products during the intervention period. Cognitive function outcomes were assessed at the beginning (baseline) and end of the 12-week intervention.

5.4.3 Test meals for post-lunch dip assessment

Participants were randomized into either an almond-enriched high fat lunch group (A-HFL), or a high-carbohydrate lunch group (HCL) at the beginning of the study. Lunch provided 23-25% of the participants' estimated daily energy intake. Their individualized energy intake at lunch was determined from the *What we eat in America*, NHANES 2011-2012 survey that reports the percentage of energy intake at lunch according to sex and age[29]. Participants in the HCL meal were fed a combination of spaghetti in tomato sauce (SpaghettiOs, Campbell, USA), white bread, jelly and apple juice providing greater than 85% energy from carbohydrate. Participants in the A-HFL meal were fed almonds with some Spaghetti Os and/or white bread, jelly and apple juice providing greater than 55% energy from fat. The almonds provided 70-75% energy in the A-HFL meal. An example of a sample lunch is provided in Table 5-1. Participants were instructed to eat the entire test meal. Cognitive function outcomes were assessed immediately after lunch and 30-35 minutes later. In addition, the post-lunch dip assessments were performed at baseline and at the end of the 12-week intervention.

5.4.4 Cognitive function test protocol

The cognitive function test protocol is shown in Figure 5-1. Participants arrived in the laboratory in a fasted state for measurement of other variables pertaining to the almond weight loss study such as body weight, body fat, blood pressure, serum insulin and glucose etc. (reported elsewhere). Instead of assessing cognitive outcomes before lunch in the fasted state, we assessed cognitive performance immediately after lunch in

lieu of the pre-lunch time point as there is some evidence that breakfast skipping is associated with negative cognitive consequences[30].

After measurement of the aforementioned variables, participants were provided with a midday meal according to their randomized lunch group (A-HFD or HCL). They were given 15 minutes to eat their meal after which they were taken into a quiet room for cognitive function testing. Testing included memory and attention domains of cognitive function. The tests for memory: immediate memory, delayed memory and verbal list recognition tests were adapted from the repeated battery for the assessment of neuropsychological status (RBANS) tests[31] while attention was assessed using the 'd2' test of attention[32] respectively.

In the *immediate memory test*, participants were read a list of 10 words immediately after lunch prior to the onset of the post-lunch dip. They were asked to remember and recall the words after 2 minutes. The number of correct responses was recorded. In the *delayed memory test* participants were asked to recall the same 10 words read to them and record them on a sheet of paper 35 minutes after lunch consumption. The number of correct responses was tallied.

In the *attention test*, participants were asked cross out the letter d accompanied with 2 dashes (above the letter d, below the letter d or one dash above and one below the letter d) on a recording blank with 14 lines of 47 letters comprised of either d or p with 1, 2, 3 or 4 dashes. They were given 4 minutes to do this task. The errors of omission (skipping target d's) and errors of commission (marking inappropriate targets) were recorded. This test was used to assess quantitative performance (total number of items processed, TN), qualitative performance (the total number of items processed minus total

errors, TNE) and concentration performance (the total number of correct items marked minus errors of commission, CP). The attention tests were conducted immediately- and 30 minutes after lunch.

Finally, the *verbal list recognition test* was used to assess the participant's ability to recall the 10 words read out to them immediately after lunch. The tester read out a list of 20 words and participants had to acknowledge verbally which words were present in the initial list. The correct responses were marked.

Distractor tests adapted from the "d2 test" of attention were used to fill in the 30 minute interval after lunch to ensure that participants did not fall asleep. Participants were asked to cross out different combinations of irrelevant letters with dashes over 28 minutes. These tests were not graded.

5.4.5 Statistical analysis

A linear mixed model analysis was performed on the correct responses obtained from the immediate and delayed memory tests, and the outcomes from the 'd2' test of attention (i.e., total number of items processed, concentration performance, quantitative performance and qualitative performance. The intervention period (before vs. after) and the lunch period (immediately after vs. 35 minutes after) were used as within-subject factors and the intervention and lunch groups as between-subject factors. The correct responses from the verbal list recognition test were analyzed using a linear mixed model with intervention period as the within-subject factor and the intervention and lunch groups as between-subject factors. An additional linear mixed model analysis on the change in outcomes as opposed to absolute values was also performed. Age range, sex

and BMI range were also considered as between-subject factors for all the tests. When significant interactions were observed, pairwise comparisons were carried out with Bonferroni correction.

Between-group differences were assessed at baseline by using independent-samples t tests. Pearson statistics were used to determine correlations. The alpha level was set at 0.05. SPSS (version 22, 2013, SPSS Inc.) was used for all the statistical analyses. OriginPro (version b9.3.226, 2016, OriginPro Corporation) was used to graph the data. Data are reported as means and standard errors (SEM). The sample size calculations for this study were based on visceral fat which was one of the primary outcomes for the almond weight loss study (reported elsewhere).

5.5 Results

5.5.1 Participants

The baseline characteristics of all participants are reported in Table 5-2. Of the 86 participants that were enrolled in the study, 7 withdrew during the intervention. Four participants withdrew due to non-compliance with the dietary protocol, 1 withdrew due to illness not related to the study, 1 female participant withdrew because of pregnancy and 1 withdrew due to lack of time to devote to the study. There were no significant differences in attrition between the AED and NFD intervention groups at 12 weeks.

5.5.2 Compliance to intervention

Total energy intake decreased in both AED (-976.17±427.98 kJ) and NFD groups (-1222.9±439.7 kJ) during the energy restriction period ($P<0.05$). The percentages of

energy from fat (36.62±1.42 vs. 29.36±1.46 (%, AED vs. NFD) and total MUFA (27.70±1.43 vs. 15.43±1.47 g), total alpha-tocopherol (15.15±0.8 vs. 6.43±0.82 mg), magnesium (311.98±16.85 vs. 245.36±17.31 mg), and phytic acid (850.86±64.13 vs. 614.42±65.89 mg) intake were greater and the percentage of energy from carbohydrate (46.34±1.65 vs. 51.93±1.70 %) was lower in the AED group compared to the NFD group at the end of the intervention ($P<0.05$). These nutrient intake patterns reflect the nature of the dietary intervention.

5.5.3 Cognitive function outcomes

Memory outcomes

Memory scores did not differ between the A-HFL and HCL meals immediately after lunch. However, memory scores decreased significantly 35 minutes after consumption of lunch in both groups ($P<0.05$). The A-HFL meal was associated with a significantly smaller decline in memory score 35 minutes after lunch consumption compared to the HCL meal ($P<0.05$) (Figure 5-2).

In addition, there were no significant differences between the mean number of correct words recorded immediately after lunch (immediate memory score) and mean number of correct words recorded 35 minutes after lunch (delayed memory score) between the AED and NFD groups at baseline. Both memory scores increased significantly after the 12-week weight loss intervention period ($P<0.05$), but the difference between the groups was not significant (Figure 5-3).

Verbal list recognition (VLR) outcomes

There was no significant difference in the VLR score between the A-HFL and HCL meals 35 minutes after lunch consumption. In addition, there was no significant differences between the mean number of words correctly recognized verbally (VLR score) between the AED and NFD groups at baseline. Although the VLR score increased after the 12-week weight loss intervention in both groups (AED: 0.47±0.25, NFD: 0.63±0.25, no. of words, $P<0.05$), the difference between the groups was not significant.

Attention outcomes

The performance indices i.e. concentration performance (CP), quantitative performance (TN) and qualitative performance (TNE) did not differ between the A-HFL and HCL meals immediately after lunch. However, while CP and TNE decreased significantly 35 minutes after consumption of lunch ($P<0.05$) (Figure 5-4), TN decreased significantly 35 minutes after consumption of lunch only after the weight loss intervention (Figure 5-4). There were no significant differences in the performance indices between the AED and NFD groups at baseline. Although the performance indices increased after the weight loss intervention ($P<0.05$), the difference between the groups was not significantly different (Figure 5-5).

Participants aged 18-39 years had higher scores for all performance indices than participants aged 50-60 years regardless of intervention group and post-lunch dip group ($P<0.05$, data not shown). Age was also moderately correlated with all performance indices regardless of group. The correlation coefficients between age and performance indices (CP, TN and TNE) ranged from -0.348 to -0.487 ($P<0.05$)

There was no effect of sex, weight category (BMI) or amount of weight loss on memory, VLR or attention outcomes.

5.6 Discussion

The present study confirmed the presence of a post-lunch dip as evidenced by a decline in the memory and attention performance domains of cognitive function 35 minutes after lunch consumption. An important finding of the study was the effect of lunch composition on cognitive function. As hypothesized, the almond enriched high fat lunch was followed by smaller declines in memory compared to the high carbohydrate lunch. This acute effect of almond consumption may be attributable to their high fat and fiber content and lower carbohydrate content. Almond consumption has previously been shown to have a moderating effect on postprandial blood glucose concentrations[33,34]. Fat and fiber can lower postprandial glycemia by delaying gastric emptying[35] and intestinal transit time[36] respectively. Lower than normal blood glucose concentrations impair cognitive function[37]. Hyperglycemia has also been associated with impaired performance but primarily among individuals with diabetes[38]. In one study, a low glycemic index breakfast improved memory performance over a high glycemic index breakfast indicating that the rate of glucose release into the circulation may be responsible for these effects[39]. Consumption of almonds with a meal can reduce the glycemic impact of carbohydrate, thereby maintaining optimum blood glucose concentrations for memory tasks.

Dietary factors other than lunch composition can influence the post lunch dip as well. Some researchers believe that the midday meal size may influence the decline in

cognitive function after lunch. Larger lunches are associated with an increased number of errors in selective attention[40] and decreased cognitive performance in general[41] compared to smaller lunches. In the present study, the amount of food given to participants was based on 23-25% of their estimated daily energy requirements. This is in line with customary intake levels in the population[29], so was ecologically valid though perhaps not a test of the extent of the effect. Others argue that the post-lunch dip is more attributable to endogenous rhythms[42] or is a consequence of conditioned lunch effects[43] rather than being causally link to lunch composition or size. For example, some studies have observed differences in cognitive function between late morning and early afternoon even in individuals that hadn't eaten lunch[2,44] but individuals who ate lunch had a greater post-lunch dip in sustained attention compared to the lunch skippers[2]. Although we did not control the time at which lunch was provided to participants, there are limited data indicating that the post-lunch dip in attention and reaction to a new stimulus is not affected by the time at which lunch is eaten[2,44]. Other causes of the post-lunch dip in cognitive function have been proposed including insulin surges[45] increased cortisol [46], or serotonin concentrations[8] among others.

In our study, almond consumption at lunch did not ameliorate the post-lunch dip in attention. However, the attention domain of cognitive function may not be as sensitive to macronutrient manipulation compared to the memory domain[47].

This twelve week randomized weight loss trial improved memory and attention performance in overweight and obese energy restricted adults. This is contrary to what has been observed in most[17,18,48] but not all[20] studies on dieting adults. The improvement in cognitive function could stem from the intervention itself with

participants having undergone dietary counseling to make healthier choices and lose weight. However, in spite of restricting the testing occasions to before and after the 12 week intervention, there is a possibility of practice effects[49]. There were no additional improvements in memory and attention with almond supplementation. Although a previous nut-based trial has demonstrated improvements in working memory and speed of processing in energy restricted adults consuming nut-supplemented diets[20], this study did not have a control group. Hence, a comparison of the magnitude of improvements in cognitive function between a nut supplemented and a nut-free diet could not be made.

Although the present study showed no effects of BMI on cognitive function, a recently published study observed a protective effect of obesity on cognitive function in people aged 45 years and older, particularly in women[50]. The lack of an effect noted here may be attributable to the absence of normal weight individuals for comparison. Interestingly, others have found a positive association between weight loss (over 12 years) and cognitive decline in older adults. While this is contrary to the improved effects on cognitive function with weight loss observed in the present study, we did not exclusively recruit older people and our intervention may not have been long enough to observe these changes. We also did not find any BMI, age or gender interaction effects on cognitive function. However, we did find a negative association between age and attention performance on the 'd2' test regardless of diet or lunch groups. Cognitive decline with ageing is a common occurrence[51].

In conclusion, almond consumption acutely ameliorated the post-lunch dip in memory and may be an effective means to maintain memory following the midday meal. However, almond consumption over 12 weeks did not further enhance the improvements

in cognitive function outcomes with weight loss. Nevertheless, literature indicates that almond consumption enhances satiety[52] and reduces hunger and desire to eat[33], properties that may minimize hunger-related thoughts that can acutely impair cognitive function in dieters[17].

5.7 Acknowledgements

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Table 5-1: Nutrient composition of a sample lunch provided to a 28 year old male participant on a 7431.2 kJ/day (1800 kcal/day) diet in both lunch groups

	Almond enriched high fat lunch (A-HFL)	High carbohydrate lunch (HCL)
Food items*	53g almonds (75% energy of lunch) ⅓ 12 oz. can spaghetti Os 1 cup water	⅔ 12 oz. can spaghetti Os 1 slice white bread (25g) 1 tbsp. jelly ½ cup apple juice 1 cup water
Energy (% daily intake)†	26	26
Carbohydrate (%)	31	86
Fat (%)	56	4
Protein (%)	13	10

* The study was not a crossover design, but this is exemplary of two meals for participants with similar energy intake levels. †Calculated from the 'what we eat in America survey'[29]

Table 5-2: Baseline characteristics of participants

	Intervention groups				Post-lunch dip experimental groups			
	Almond-enriched diet (AED) n=43		Nut-free diet (NFD) n=43		Almond-enriched high fat lunch (A-HFL) n=43		High carbohydrate lunch (HCL) n=43	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (y)	31.05	12.9	31.77	13.15	30.30	12.89	32.51	13.08
Body weight (kg)	82.79	12.9	84.71	14.12	84.38	14.50	83.12	12.51
BMI (kg/m^2)	29.91	3.20	30.97	4.48	30.93	4.42	29.94	3.29
	n	%	n	%	n	%	n	%
Sex								
Male	11	25.6	10	23.3	9	20.9	12	27.9
Female	32	74.4	33	76.7	34	79.1	31	72.1
Age range								
18-39 y	32	74.4	31	72.1	33	76.7	30	69.7
40-49 y	5	11.6	4	9.3	3	7.0	6	14.0
50-60 y	6	14.0	8	18.6	7	16.3	7	16.3
Weight Category								
Overweight (BMI: 25-29.9 kg/m^2)	23	53.5	21	48.8	21	48.8	23	53.5
Obese (BMI: 30-40 kg/m^2)	20	46.5	22	51.5	22	51.2	20	46.5

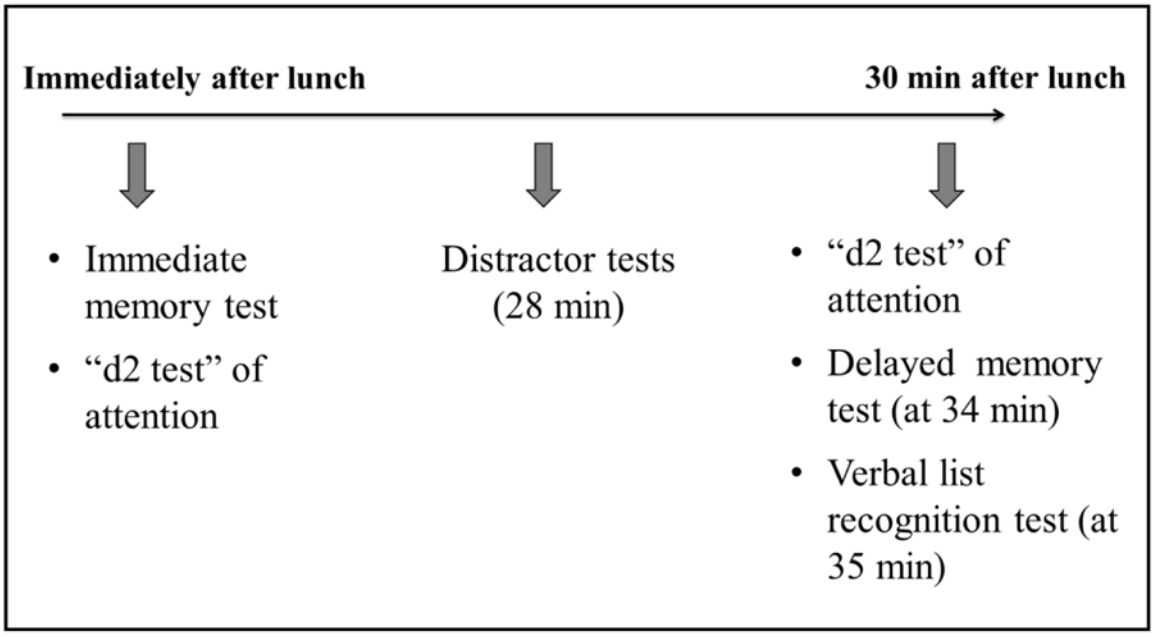

Figure 5-1: Cognitive function test protocol at the beginning and end of the 12 week almond weight loss intervention

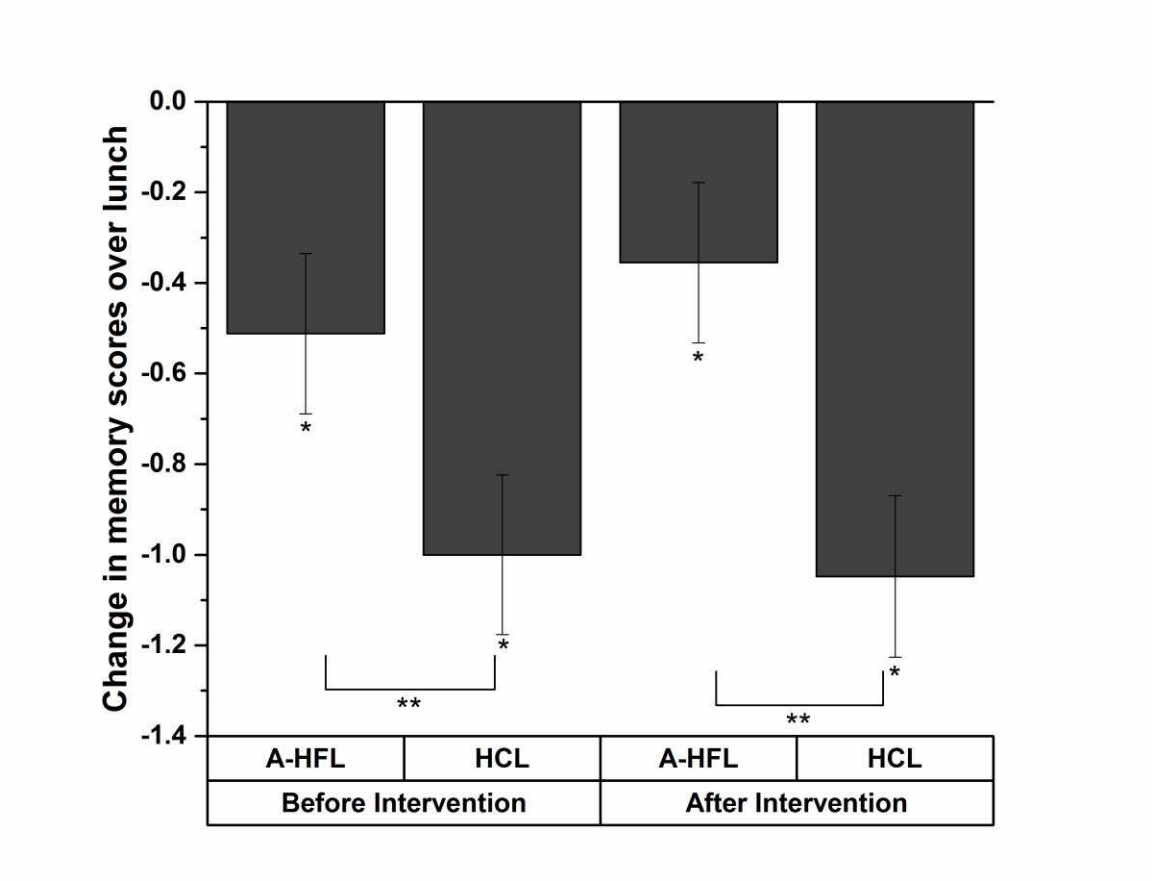

Figure 5-2: Mean change in memory scores immediately after lunch and 35 minutes after lunch consumption in the almond enriched high fat lunch (A-HFL) and high carbohydrate lunch (HCL) groups before and after the 12-week intervention.

Values are means and standard errors obtained from a linear mixed effects model with lunch period and intervention period as within-subject factors and lunch group and intervention group as between subject factors. * Significant differences for change in memory scores over lunch period ($P<0.05$). ** Significantly different between the A-HFL and HCL groups ($P <0.05$). A-HFL: n=43; HCL: n=43.Values are means and standard errors. * Significant differences for change in memory scores over intervention period ($P<0.05$). AED: n=43; NFD: n=43.

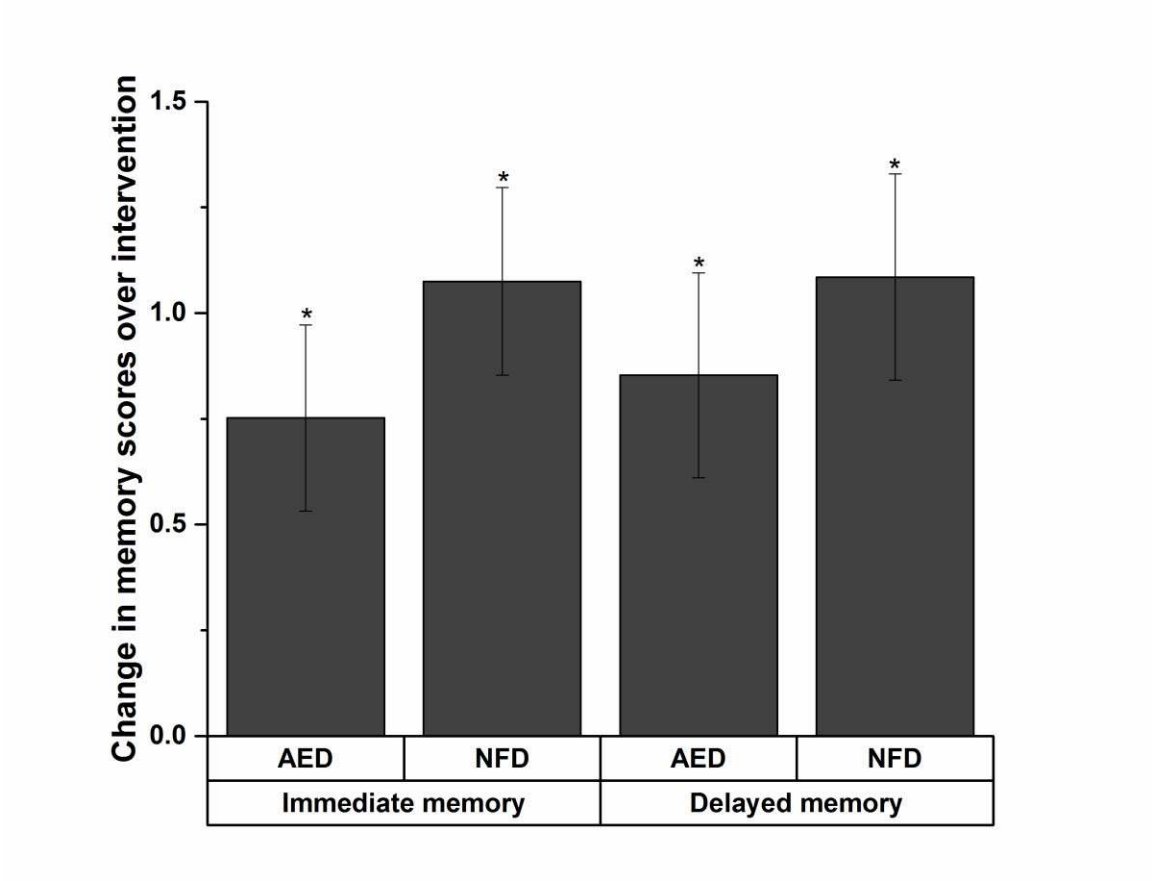

Figure 5-3: Mean change in immediate and delayed memory scores before and after the 12-week intervention in the almond enriched diet (AED) and the nut-free diet (NFD) groups.

Values are means and standard errors obtained from a linear mixed effects model with intervention period and lunch period as within-subject factors and intervention group and lunch group as between subject factors. * Significant differences for change in memory scores over intervention period ($P<0.05$). AED: n=43; NFD: n=43.

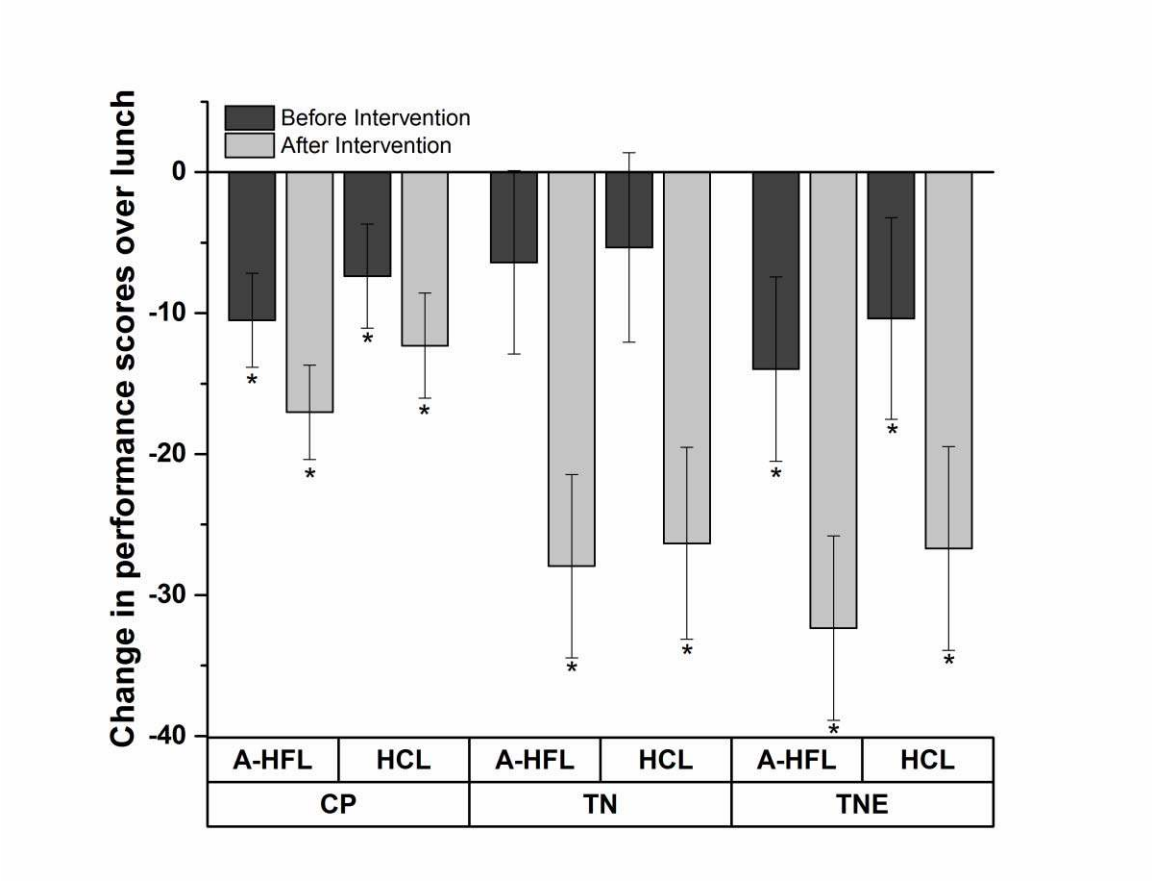

Figure 5-4: : Mean change in performance indices immediately after lunch and 35 minutes after lunch consumption in the almond enriched high fat lunch (A-HFL) and high carbohydrate lunch (HCL) groups before and after the 12-week intervention.

Values are means and standard errors obtained from a linear mixed effects model with lunch period and intervention period as within-subject factors and lunch group and intervention group as between subject factors. * Significant differences for change in performance indices over the lunch period ($P<0.05$). CP: concentration performance; TN: quantitative performance; TNE: qualitative performance. A-HFL: n=43; HCL: n=43.

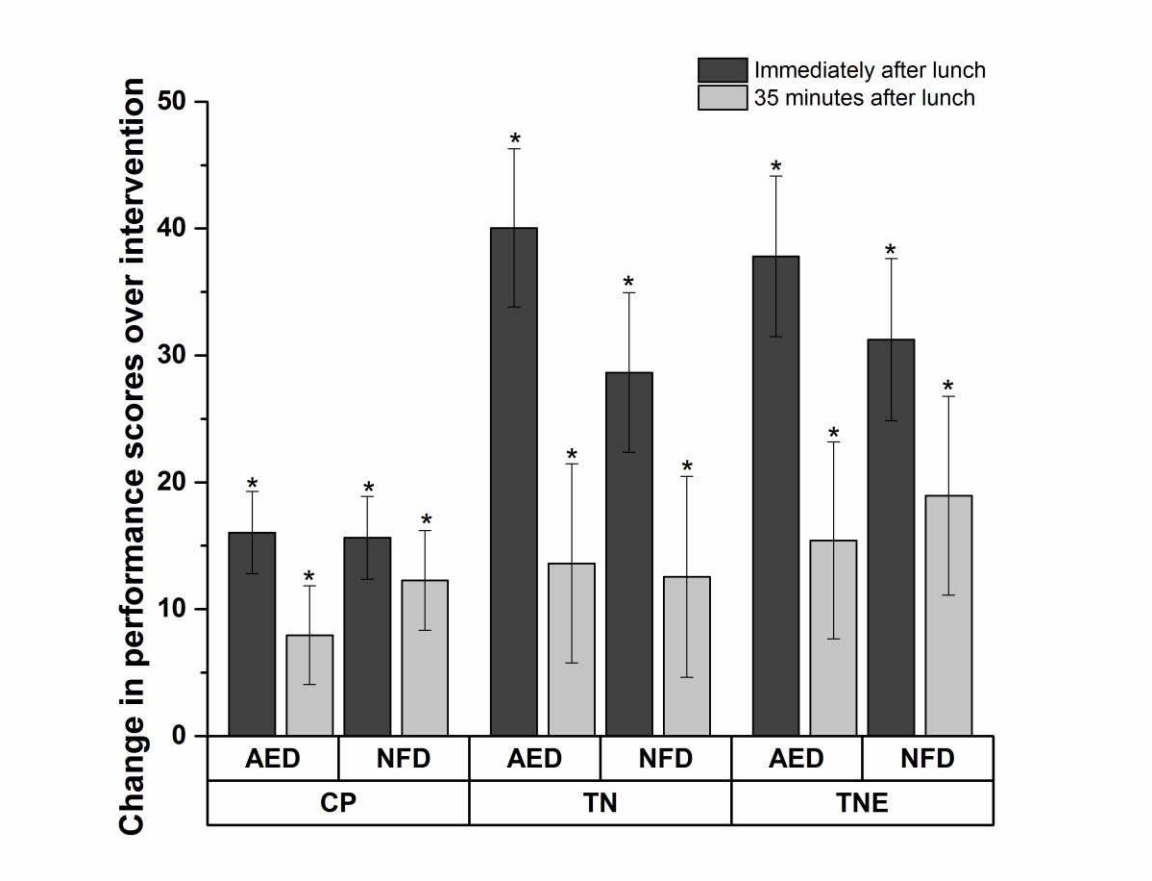

Figure 5-5: Mean change in performance indices before and after the 12-week intervention in the almond enriched diet (AED) and the nut-free diet (NFD) groups immediately after lunch and 35 minutes after lunch consumption.

Values are means and standard errors obtained from a linear mixed effects model with intervention period and lunch period as within-subject factors and intervention group and lunch group as between subject factors. * Significant differences for change in performance indices over the intervention period ($P<0.05$). CP: concentration performance; TN: quantitative performance; TNE: qualitative performance. AED: n=43; NFD: n=43.

CHAPTER 6. ASSESSING ALMOND CONSUMPTION COMPLIANCE BY FLOW INJECTION METABOLOMICS

Dhillon, J., Tan S.Y. and Mattes R.D. A shotgun fingerprinting approach to document compliance with an almond consumption intervention. *Metabolomics* (submitted on June 30, 2016)

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6.1 Abstract

Introduction: Almonds are extremely rich sources of lipids and flavonoids and their consumption is associated with several health benefits. However, there are no known analytical methods to document compliance to almond consumption in the long run.

Objective: To develop an analytical approach that identifies metabolic profiles associated with long-term almond consumption to ascertain compliance with prescribed consumption.

Methods: A shotgun fingerprinting strategy was designed to isolate metabolic changes in erythrocytes after 12-wk almond consumption. Representative samples (pools) of erythrocytes from individuals consuming almonds or no almonds were screened by flow injection mass spectrometry. Data based on scan modes for shotgun lipidomics, and *m/z* values compatible with almond flavonoid content were collected. Specific ion pairs and

m/z values detected in pooled samples were combined into single ion monitoring and multiple reaction monitoring methods intended for individual sample screening. Results: Out of the 243 values of *m/z* monitored by both methods, 5 ratios and combinations of specific ions with receiver operating characteristic (ROC) curves AUC>0.89 had sensitivity of 74.2% and specificity of 90%. Eight out of the 31 participants (25.8%) in the almond group and 3 out of the 30 (10%) participants in the control group were misclassified by all 5 ratios.
Conclusion: Ratios and combinations of specific ions mainly related to membrane lipids were discriminatory of almond consumption from the nut-free diet. The misclassifications observed as a result of ratio performance evaluation could be indications of possible non-compliance as supported by the dietary intake data.

6.2 Keywords

Almond Biomarkers; Human Erythrocytes; Metabolomics; Nuts; Shotgun Lipidomics

6.3 Introduction

Almond consumption is associated with several health benefits such as improvements in lipid profile, glycemic control and vascular health (Barbour et al. 2014; Kendall et al. 2010). In addition, despite their high energy content, almond consumption does not promote weight gain (Flores-Mateo et al. 2013) and recent evidence indicates that almonds may improve body composition (Hollis and Mattes 2007). Almonds are good sources of monounsaturated fatty acids (MUFAs), protein, fiber, arginine, vitamin E, polyphenols and magnesium. These nutrients have been implicated in the

aforementioned health benefits (Kendall et al. 2010) but characterization of their benefits requires additional clinical study.

Assessing compliance to dietary interventions in long-term clinical trials is frequently problematic. Although self-reported measures of intake such as 24-hour food recalls and food frequency questionnaires are widely used, these are subjective measures of dietary intake (Johnson 2002) and only capture an imperfect snapshot of an individual's dietary pattern. Compliance to long-term almond consumption is occasionally documented using plasma alpha-tocopherol (Hollis and Mattes 2007; Li et al. 2011). However, plasma alpha-tocopherol is more strongly associated with vitamin E supplement intake (Ascherio et al. 1992; Stryker et al. 1988) and might not reflect intake of vitamin E from dietary sources such as nuts (Kardinaal et al. 1995). Hence, there is a need to develop methods that can identify metabolic profiles associated with long-term almond consumption in order to ascertain compliance to an almond consumption prescription.

Nutritional metabolomics is a relatively new domain of nutrition that can capture the phenotype of dietary exposure in biological samples (Scalbert et al. 2014). Although urine and blood plasma samples are commonly used in the application of nutritional metabolomics, red blood cells (erythrocytes) may reflect longer-term dietary intake (Catalán et al. 2013) since the average erythrocyte lifespan is between 70 to 140 days (Franco 2012). Because almonds are a rich source of lipids, it is reasonable to screen for diverse lipids in the erythrocytes of individuals chronically consuming almonds.

Flow injection mass spectrometry (FIA-MS) is a simple, fast and informative mass spectrometry strategy for the targeted or untargeted screening of lipids and

metabolites that can be used for this purpose. It is based on the direct injection (without chromatographic separation) of crude lipid extracts on electrospray (ESI) ion sources. Targeted analysis by FIA-MS can be performed by screening for values of m/z or ion pairs related to specific metabolites or lipids. Untargeted analysis by FIA-MS can be performed by full mass scan or by shotgun lipidomics. Shotgun lipidomics takes advantage of neutral loss (NL) and precursor ion (PREC) scan mode experiments to profile diverse classes of lipids based on the fact that the lipid chemical structure is common in 'building blocks'. Therefore diverse lipid classes present characteristic fragment ions (detected by PREC experiments) or losses (detected by NL experiments)(Han and Gross 2005). However, because of ionization suppression, isotopic, isobaric and isomeric interferences likely occur during flow injection and may impair quantification accuracy, we did not quantify the lipids but rather used relative amounts of lipids and named this approach shotgun fingerprinting. This approach simplifies identification of relevant lipids by avoiding the necessity of internal standards, chromatographic separation and, by simplifying data and metabolite structural analysis, workflow.

The objective of the present study was to apply shotgun fingerprinting to assess compliance to almond consumption by screening for prospective biomarkers in erythrocytes that were discriminatory between individuals that consumed almonds and those that did not consume any nuts. In addition, because almonds also contain substantial amounts of flavonoids (Bolling et al. 2011), we screened the literature for flavonoids identified in almond skins (Bolling et al. 2009) and included them in the method for individual sample screening. Screening of the selected values of m/z, which

were related to lipids and metabolites in erythrocytes, indicated the presence of 19 molecule ratios highly discriminatory between individuals that consumed almonds and those that did not consume any nuts. Out of the 19 ratios, 5 were explored in more detail. The 10 m/z values present in the five selected ratios have been further analyzed by full MS/MS to obtain some structural identification. The results suggest that long term almond consumption impacts erythrocyte cell membrane composition. This information can be useful for assessing compliance to long-term almond consumption. Nonetheless, it is important to consider that the identified informative ion combinations related to lipids are only prospective biomarkers. Detailed structural identification (presence of isobars and isomers, location of unsaturation in the fatty acyl residues) and absolute quantification by LC-MS/MS and/or high mass resolution will come at later stages. Biomarker validation was not in the scope of this study.

6.4 Methods

6.4.1 Participants

Sixty-one healthy male and female overweight or obese individuals (age: 18–60 and BMI: 25-40 kg/m^2) participating in an almond weight loss study (ClinicalTrials.gov (registration number: NCT02360787)) were recruited.

6.4.2 Intervention

The study was a randomized, controlled, parallel-arm, 12-week, clinical trial. Participants were randomized into one of two 500 kcal energy-restricted study arms: Almond enriched diet or nut-free diet groups (Table 6-1). Weekly energy and nutrient

analyses using 24-hour food recalls were conducted to determine the participants' compliance to dietary recommendations. Participants randomized into the almond-enriched energy restricted diet group (n=31) were asked to consume dry-roasted, lightly salted almonds providing 15% energy in their individualized energy-restricted diet. Participants randomized into the nut-free energy restricted diet group (n=30) were asked to avoid all nuts, seeds and nut products during the intervention period.

6.4.3 Sample extraction and shotgun fingerprinting data acquisition

Fasting blood samples (5 ml) were collected from participants at baseline and the end of the 12 week intervention into vacutainers with EDTA. The erythrocytes were separated from the plasma by centrifugation and frozen at -80ºC. The erythrocytes were prepared by hemolyzing the cells two times in deionized distilled water followed by centrifugation at 4000 RPM for 10 minutes at 4ºC (Allegra 25R centrifuge; Beckman Coulter, Brea, CA). The erythrocyte aliquots were stored at −80 ºC until further analysis.

The lipid extracts were prepared using a procedure by Rose and Oklander (Rose and Oklander 1965). Erythrocytes (150uL) were mixed with 150uL of ultrapure water and the contents were allowed to stand for 15 min with occasional mixing (vortex). Isopropanol (1.0 mL) was added to the mixture with occasional mixing. After 30 minutes, 600uL of chloroform was added, mixed and allowed to stand for another 30 minutes. The tube was centrifuged at 10,000xg for 10min (Taylor Scientific Centrifuge). The lipid extract (bottom phase) was subsequently removed and transferred to another tube and the solvents were removed by evaporation in a Speed Vac concentrator. Dried Lipid extracts were resuspended in 270uL of acetonitrile (ACN)/methanol/ammonium acetate (300mM)

at 3:6.65:0.35 volume ratios. The solvent pumped by the microsampler between injections was ACN + 0.1% formic acid.

The lipid extract from almond seeds was prepared using a procedure by Bligh and Dyer (Bligh and Dyer 1959) for comparison purposes. Four almond seeds were individually homogenized in 1 mL of water using a Precellys®24 tissue homogeneizer (Bertin Technologies, Rockvielle, MD, USA). The homogenate (800 uL) was transferred to a 15mL Falcon tube and mixed with 1 mL of chloroform and 1.6 mL of MeOH. After mixing and 15 min incubation at room temperature, 1 mL of chloroform and 1 mL of water were added, causing the formation of a 2-phase solution. The lipid extract (bottom phase) was subsequently removed and dried under N2 stream. Aliquots of the four almond seeds were then combined for further experiments.

Lipid extracts (6 µL) from individual participants and the almond seeds were delivered using FIA-MS to a triple quadrupole mass spectrometer (Agilent QQQ 6460) equipped with a jet stream ESI ion source. The lipid extracts from participants were pooled into 3 groups for running the diverse scan modes and finding the lipids detectable in the samples. The first group comprised of lipid extracts of all participants at baseline (n=52) regardless of intervention group (Baseline), the second group comprised of lipid extracts of participants that consumed almonds at the end of the intervention (W12-almonds, N=30), and the third group comprised of lipid extracts of participants that did not consume any nuts or nut products at the end of the intervention (W12-control; N=31). These classifications were made based on the information obtained from the scans run on a selected few known compliant participants.

Full scan at the positive and negative ion modes and over 80 scan modes were run on the lipid extracts from the 3 pooled samples and almond seeds (data not shown); nonetheless, only 20 scans were informative, i.e. presented ions (Table 6-2). Initial data processing of the profiles obtained was carried out by MassHunter (B.06.00). The most informative ions identified through the scans on the pooled samples and the almond seeds were organized into two methods (one for the positive and another one for the negative ion mode) for multiple reaction monitoring (MRM) or single ion monitoring (SIM). With the inclusion of the m/z values related to anthocyanins, 243 values of m/z were monitored in the 114 individual samples of the three experimental groups. It is important to consider that some of the m/z values present in the shotgun lipidomics profiles and selected for screening may be isotopic patterns and some may have more than one molecule (isobaric and isomeric compounds). Therefore, structural identification of the m/z values was not pursued until the relevant ones were isolated by univariate and multivariate analysis. Also, we included in the MRM and SIM methods ions compatible with flavanoids present in almonds and nuts (Bolling et al. 2009; Gu et al. 2003). In total, 243 SIM or MRMs related to lipids and flavonoids were monitored in individual samples organized into two methods i.e. positive ion mode – 148 values of m/z, negative ion mode – 106 values of m/z.

6.4.4 Data analysis

For the informative shotgun fingerprinting scans (Table 6-2), the mass spectra had the background subtracted and values of m/z were exported for organization into the two screening methods. The files generated by the mass spectrometer were converted to

mzML format using MSConvert (http://proteowizard.sourceforge.net), and an in-house script was developed to sum the ion intensities from 0.4-1.0 min of data acquisition of each of the 243 m/z values monitored. The values of ion intensities of each m/z were normalized by total ion current (TIC) to obtain relative ion intensities. The dataset was then filtered out to remove any ions that did not appear in over 50% of the samples. Data were then auto scaled. The differences in the erythrocyte metabolome due to the diet factors (W12-Almond versus W12-Control) and time of intervention factors (Baseline versus W12) were analyzed via univariate (volcano plots) and multivariate (PCA) statistics using Metaboanalyst 3.0 (Xia et al. 2015). The performance of the identified metabolites in discriminating W12-Almond from W12-Control was evaluated by constructing receiver operating characteristic curves (ROCs) on the dataset, and estimating the area under the curve (AUC). The 50 most informative values of m/z i.e. those discriminating between W12-Almond versus W12-Control with AUC>0.75 were considered for computation of different combinations and ratios by hand and using the ROC feature of Metaboanalyst. The ratios with AUC>0.89 were considered as informative and submitted to PCA to visualize the difference between groups. The 50 informative values of m/z were also submitted for MS/MS analysis for lipid class and unsaturation level attribution (data shown only for the values of m/z present in the 5 selected ratios; Figure 6-5).

6.5 Results and discussion

The overall goal of this research was to assess compliance to an almond consumption protocol for weight loss using a novel concept of shotgun fingerprinting.

This analytical strategy is shown to be informative, rapid and a high-throughput strategy for erythrocyte metabolomic screening. The workflow utilized in the study (Figure 6-1) was based on fast sample preparation of erythrocytes, lipid profiling via FIA-MS including full scan, screening of flavanoids and multidimensional MS scans targeted at specific functional groups of metabolite classes.

Multivariate analyses of shotgun fingerprinting by principle component analyses (PCA) of all 243 ions showed no clear discrimination between the erythrocyte metabolome of the two intervention groups at baseline which was expected (Figure 6-2A). In addition, we did not see a discrimination between the metabolomic profiles of the two groups over time (Baseline vs. W12) (Figure 6-2A). This could be a result of an absence of a diet standardization phase prior to the start of the intervention. Hence, participants may have either been consuming nuts or abstaining from nut consumption prior to the intervention and this could have confounded identifying differences over time in each group. However, we did see a relatively distinct clustering of the almond and control groups at the end of the intervention (Figure 6-2B). This may indicate that either some participants were not complaint to their respective intervention groups or that the metabolites were not the best biomarkers of almond consumption.

Nevertheless, we evaluated the performance of the most informative ions indicated by ROC analysis (n=50, AUC>0.75) in discriminating between the almond and the control groups at the end of the intervention. In addition, we evaluated the performance of different combinations and ratios of all metabolites as relationships between multiple metabolites can capture information lost in univariate and multivariate methods and may provide more relevant information regarding a biological system than

individual metabolites alone (Steuer 2006). Out of the 243 ions monitored, 9 were observed in 19 ratios and/or combinations which presented ROC curves with AUC>0.89. A PCA of the 19 ratios demonstrated a distinct clustering with very little overlap of the almond and control groups at the end of the intervention (Figure 6-3). Out of the 19 ratios, 5 ratios that presented the most unique combinations of ions and those with the most informative fold changes were selected (Table 6-3).

Structural information obtained from the MS/MS experiments of the m/z values included in the 5 selected ratios is listed in Table 6-4. Most ions presented the fragment of m/z 184 which is diagnostic of phosphatidylcholine (PC) and sphingomyelin (SM) lipids. Also values of m/z coincident with flavonoids were included in the ratios. However, MS/MS was not diagnostic for these ions (Table 6-4).

All 5 ratios had a sensitivity of 74.2% and specificity of 86.7% (Figure 6-4) i.e. 8 out of the 31 participants in the almond group and 3 out of the 30 participants in the control group were misclassified indicating a possibility of non-compliance. To confirm whether these participants were non-compliers in their respective intervention groups, we evaluated their dietary intake data. Non-compliers in the almond group demonstrated a trend for either reduction in or inconsistent intake of MUFA, arginine, vitamin E and phytic acid (nutrients that are high in almonds) over the intervention period while non-compliers in the control group demonstrated a trend for either an increase in or inconsistent intake of the aforementioned nutrients over the intervention period (unpublished data). In contrast, participants that were categorized as compliers in the almond group had greater MUFA, arginine and vitamin E intake over the intervention (unpublished data). Although the extremely small number of possible non-compliers

precluded a statistical determination of the dietary intake data, it is reasonable to assume that based on shotgun fingerprinting and dietary intake data, they might not have been complaint to their respective intervention groups.

However, additional studies to are still needed to validate compliance to almond consumption in clinical trials using this approach. The concept of shotgun fingerprinting is similar to full mass scan profiling/fingerprinting used in a number of studies for food quality (Porcari et al. 2016; Saraiva et al. 2009; Sawaya et al. 2010) and proof of origin, adulteration (de Souza et al. 2007; Haddad et al. 2008), developmental biology (Ferreira et al. 2015), bacterial identification (Barreiro et al. 2012; Song et al. 2007) and disease diagnosis (Alfaro et al. 2016; Wiseman et al. 2005). We propose the use of multidimensional scans and a much simpler workflow compared to traditional metabolomics for biomarker discovery based on liquid (LC) or gas chromatography (GC), or capillary electrophoresis (CE). This analytical approach is mainly based on shotgun lipidomics features that use relative amounts of data and biomarker-discovery based statistic tools. The guiding principle of the method is the high-throughput utilization of the unique chemical and physical properties of small molecules present in the samples. Shotgun fingerprinting presents limitations for precise structural identification of the prospective biomarker combinations proposed due to the lack of chromatographic separation combined with frequent isomeric and isobaric nature of lipids. Therefore, more than one lipid isomer or even lipid class of compounds may be present at the same m/z and the molecular species. Even though we have acquired MS/MS data of the most relevant ions described in the study (Figure 6-5), they should be further evaluated as biomarkers in subsequent experiments including chromatographic

separation, high mass resolution MS or even more sophisticated tools such as ion mobility MS.

6.6 Conclusion

The exploration of the erythrocyte metabolome in 61 subjects (W12-Almond and W12-Control samples) by shotgun fingerprinting identified ratios and combinations of specific ions mainly related to membrane lipids which were discriminatory of almond consumption from the nut-free diet at the end of the 12 week intervention. While we cannot ascertain at this point whether the informative metabolites detected are biomarkers of almond consumption, the relationship between different metabolites indicates almond-induced changes in the metabolomics profile and the misclassifications observed as a result of the ratio performance evaluation could be indications of possible non-compliance as supported by the dietary intake data. Nevertheless, the repeatability and validity of this method will have to be tested in future studies with larger datasets and more certain control of dietary compliance.

6.7 Acknowledgements

The authors acknowledge Purdue's Bindley Bioscience Center for their analytical expertise. The authors' responsibilities were as follows—JD, CF, TS and RDM: conceived the study and shared equal responsibility in writing the manuscript and of its final content. All authors read and approved the final manuscript.

6.8 Conflict of interest declaration

The present study was supported by the Almond Board of California (Modesto, CA, USA). The funders had no role in the study conception, design and implementation, data collection, data analysis or interpretation of results. RDM receives research support from the Almond Board of California. CF, TS and JD have no conflicts of interest.

6.9 Compliance with ethical requirements

This study was conducted according to the guidelines stipulated in the Declaration of Helsinki. All procedures involving human participants were approved by the Purdue University Institutional Review Board. Informed consent was obtained from all individual participants included in the study.

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Table 6-1: Baseline characteristics of participants in the intervention

Characteristic	Almond-enriched diet (n=31)	Nut-free diet (n=30)
Sex [n (%)]		
Male	7 (22.6)	6 (20)
Female	24 (77.4)	24 (80)
Age (y)	32.06±13.61[a)]	33.40±13.21
BMI (kg/m^2)	29.97±3.34	30.93±4.52

a) Mean ± SD (all such values)

Table 6-2: Informative scan modes for screening diverse lipid classes in the shotgun lipidomics study

Scan mode	Ion detected	Targeted lipids/metabolites
full scan negative	[M-H]-	Free fatty acids, cholesterol sulphate
full scan positive	[M+H]+ and	Glycerolipids
full scan positive m/z	[M+H]+ and	Triacylglycerol (TAG)
NL 141	[M+H]+	Phosphatidylethanolamine (PE)
NL 183	[M+H]+	Phosphatidylcholine (PC), alkelnyl-acyl PC (ePC), sphingomyelin (SM) and LysoPC
PREC 184	[M+H]+	Phosphatidylcholine (PC), alkelnyl-acyl PC (ePC), sphingomyelin (SM) and LysoPC
PREC 255.2	[M-H]-	Glycerolipids containing Palmitic acid residue
PREC 264.3	[M+H]+	Ceramides (d18:1; sphingosines)/cerebrosides
PREC 266.4	[M+H]+	Ceramides (d18:0; sphinganines)
PREC 279.2	[M-H]-	Glycerolipids containing linolenic acid residue
PREC 281.2	[M-H]-	Glycerolipids containing oleic acid residue
PREC 282.2	[M+H]+	Ceramides (t18:0 4-hydroxysphinganines)
PREC 283.2	[M-H]-	Glycerolipids containing stearic acid residue
PREC 303.2	[M-H]-	Glycerolipids containing arachidonic acid
PREC 305.2	[M-H]-	Glycerolipids containing eicosatrienoic residue
PREC 327.3	[M-H]-	Glycerolipids containing docosahexaenoic (DHA) residue
PREC 329.3	[M-H]-	Glycerolipids containing Eicosapentaenoic acid (EPA) residue
PREC 331.3	[M-H]-	Glycerolipids containing docosatetraenoic acid residue
PREC 85	[M+H]+	Acylcarnitines
PREC 97	[M-H]-	Sulfatide (ST)

NL, neutral loss; PREC, precursor ion

Table 6-3: Fold change of the five selected *m/z* ratios and combinations between almond diet group and nut-free diet group at the end of the 12 week intervention i.e. (W12-almond/W12-control).

Selected m/z ratios[a)]	Fold change	log2(FC)	P-value
(447 X 930.5) / (703.5 X 988.5 X	2.5901	1.373	0.000000172
(447 X 930.5)/ (703.5 X 876.5)	4.7656	2.2527	0.00000546
(447 X 930.5 X 782.5)/ 703.5	8.5938	3.1033	0.0001237
(447 X 930.5 X 782.5)/ 813.5 -> 629.5	9.083	3.1832	0.00012746
(447 X 930.5)/ (703.5 X 814.5)	6.8211	2.77	0.0000514

a) Ion intensities have been measured by monitoring just the parent ion both in Q1 and Q3, except for the m/z 813.5, which has had the fragment of m/z 629.5 monitored. Ion modes of each ion or ion pair are displayed in Table 6-4.

Table 6-4: Metabolites associated with almond consumption in the shotgun fingerprinting of participants' erythrocytes.

m/z	Ion	Attribution (MS/MS)
447	$[M-H]^-$	Not attributed[a)]
703.5	$[M+H]^+$	Sphingomyelin SM (d18:1/16:0) or SM(d16:1/18:0)[b)]
930.5	$[M]+NH^+$	TAG(56:3)[c)]
782.5	$[M+H]^+$	Phosphatidylcholine PC(36:4)[b)]
814.5	$[M+H]^+$	PC(38:2)[b)]
813.5	$[M+H]^+$	SM (d18:2/24:0) or SM(d18:1/24:1)
813.5 -> 629.5	$[M+H]^+$	SM (d18:2/24:0) or SM(d18:1/24:1)
876.5	$[M]+NH^+$	Not attributed[d)]
988.5	$[M]+NH^+$	TAG (62:16)[c)]
1016.5	$[M+H]^+$ or $[M]+NH^+$	Not attributed[e)]

a) Value of *m/z* expected for diverse flavonols (such as Kaempferol 3-O-glucoside and Quercetin) but fragments expected were not found by MS/MS.

b) Attribution was based on presence of the fragment ion at *m/z* 184 corresponding to the glycerolipid polar head (phosphocholine), which is present in the product ion analysis of protonated choline-containing phospholipid molecular species such as phosphatidylcholine (PC) and sphingomyelin (SM). Metlin database (https://metlin.scripps.edu) was used to search attributions. See Figure 6-5 for MS/MS spectra.

c) TAG – triacylglycerol. In parenthesis are the number of carbon in the TAG fatty acyl residues and the number of unsaturations, separated by a colon. Tentative attribution due to lack of specific fragments for TAG lipids

d) MS/MS spectrum with no fragments up to collision energy of 35. Values of *m/z* not compatible with TAG.

e) Low intensity MS/MS spectrum, indicative of a dimer or adduct

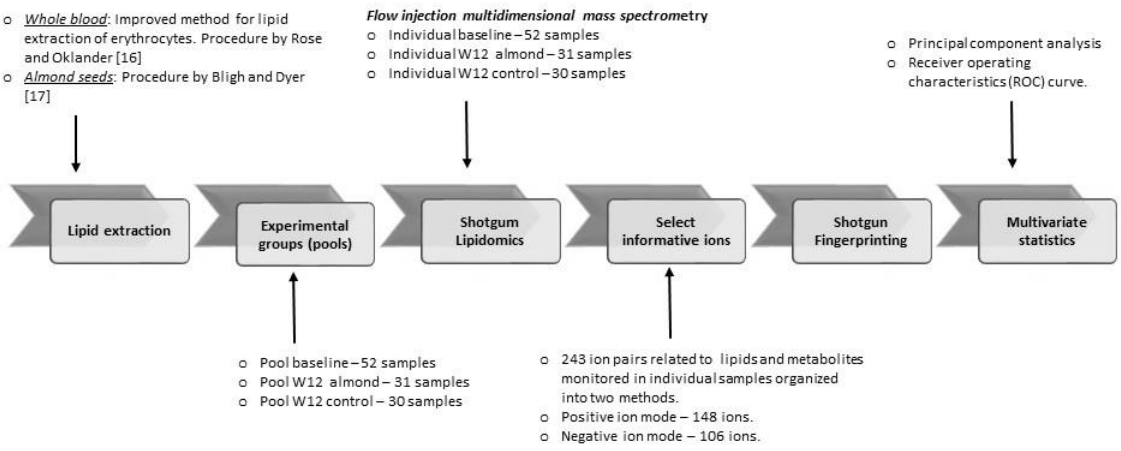


Figure 6-1: Shotgun fingerprinting study workflow for almond consumption compliance

Lipid extracts were injected without chromatographic separation into the ESI source (flow injection) and data on full mass scan and on diverse shotgun lipidomics scan modes were collected for each representative sample (pooled baseline, pooled W12-almond and pooled W12-control samples). The ions observed and m/z values of flavonoids reported in literature were organized into two methods including SIM or MRM. Since this workflow is mostly based on multidimensional MS experiments to perform chemical profiling, we refer to it as shotgun profiling

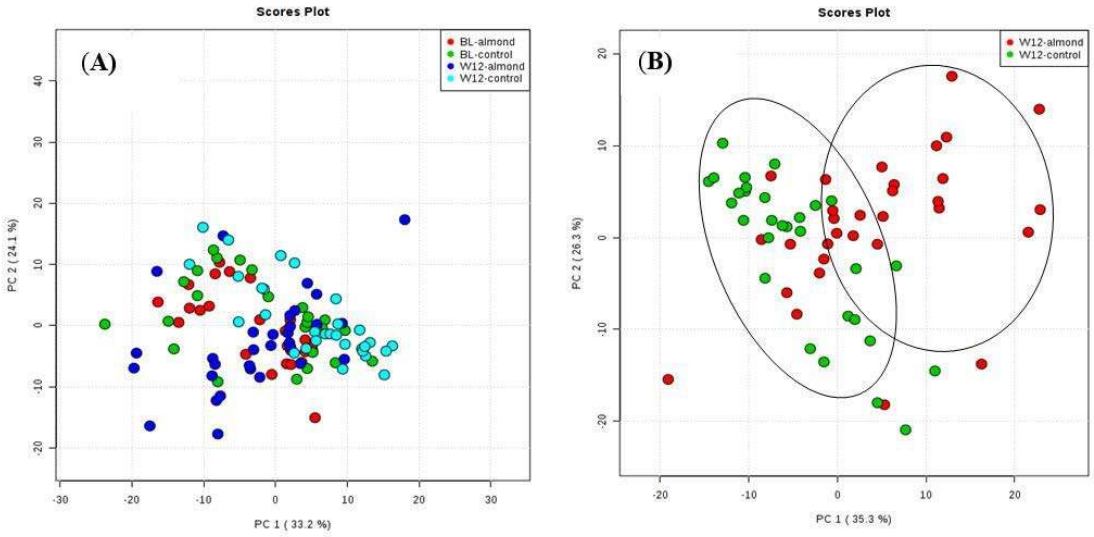


Figure 6-2: PCA score plots of all 243 ions

(A) PCA score plot of all 243 ions at baseline and W12. Colors indicate the experimental groups. Red dots are from the almond group before diet intervention (baseline); green dots are from the control group at baseline; dark blue dots are from the almond group after the 12 week intervention (W12-Almond); and light blue dots are from the control group after the 12 week intervention (W12-Control). (B) PCA score plot of all 243 values of m/z for the W12-Control and W12-Almond groups. Red dots represent W12-Almond group; and green dots the W12-Control samples. The circles are drawn for visualization (not related to statistical significance) of the W12-almond and W12-control clusters.

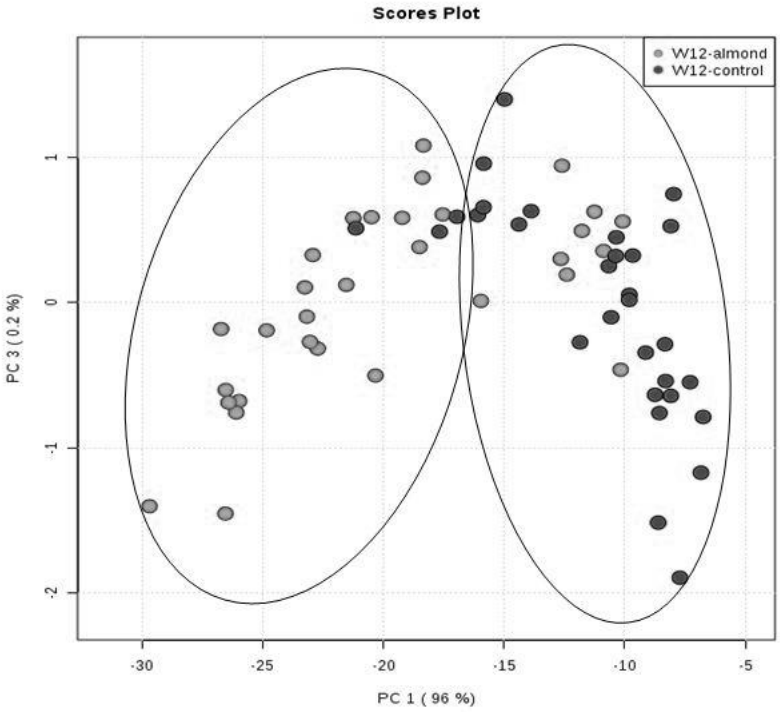


Figure 6-3: PCA score plot of the 19 *m/z* value ratios presenting AUC>0.89

Colors indicate the experimental groups. Grey dots are from the almond group after the 12 week intervention (W12-Almond); and black dots are from the control group after the 12 week intervention (W12-Control).

Ratio no.	Selected m/z ratios	AUC [95% CI]	Sensitivity	Specificity
1	(447 X 930.5)/(703.5 X 988.5 X 1016.5)	0.903 [0.816-0.959]	74.2% (23/31)	90% (27/30)
2	(447 X 930.5)/(703.5 X 814.5)	0.902 [0.808-0.961]	74.2% (23/31)	90% (27/30)
3	(447X 930.5X 782.5 /813.5 -> 629.5	0.90 [0.811-0.959]	74.2% (23/31)	90% (27/30)
4	(447X 930.5) /(703.5 X 876.5)	0.899 [0.806-0.955]	74.2% (23/31)	90% (27/30)
5	(447 X 930.5 X 782.5)/703.5	0.889 [0.792-0.955]	74.2% (23/31)	90% (27/30)

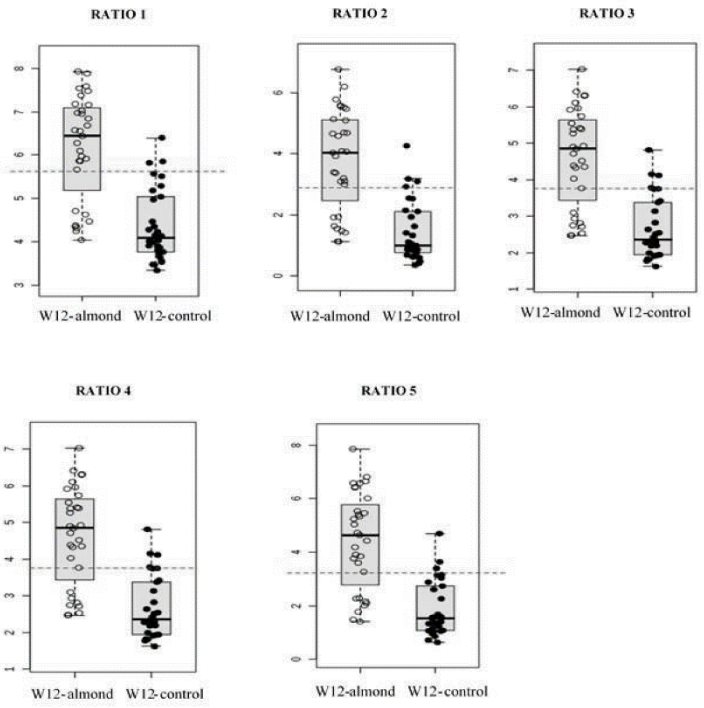
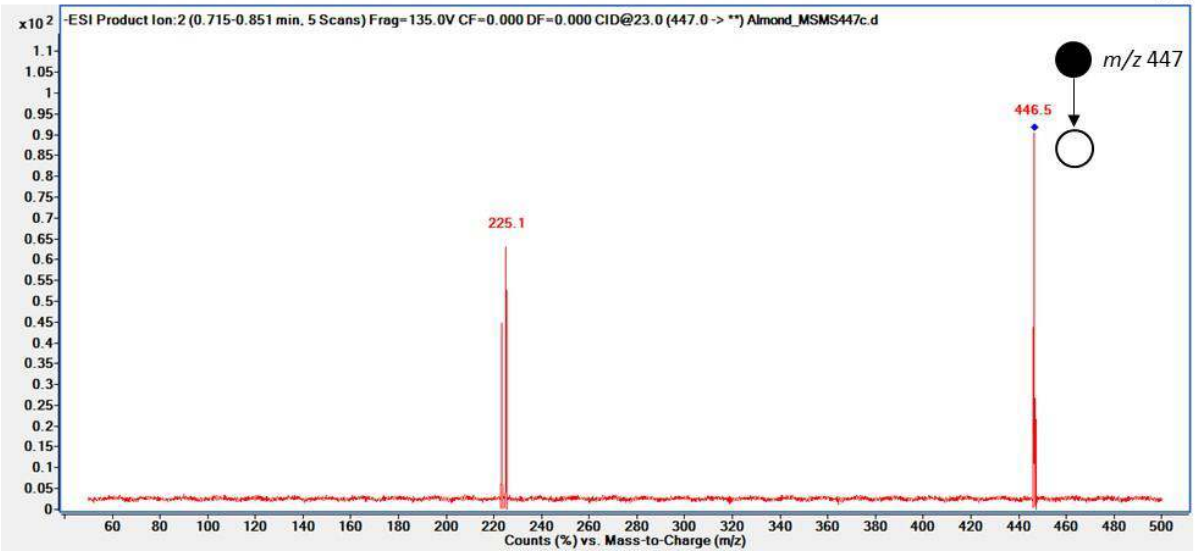
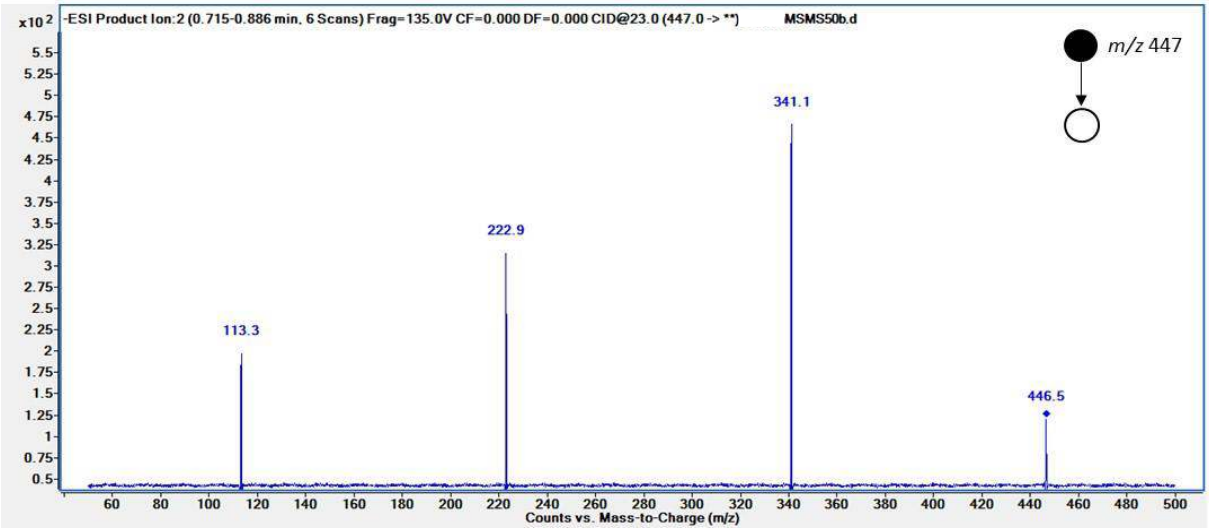


Figure 6-4: Performance analysis of selected *m/z* ratios using ROC univariate curves Graphs for ratios 1 to 5 show the averages, ±SD and error for the values. Individual samples of W12-Almond and W12-control are depicted as open and solid symbols, respectively. Lines are the ROC threshold used to classify samples in one of the two groups.

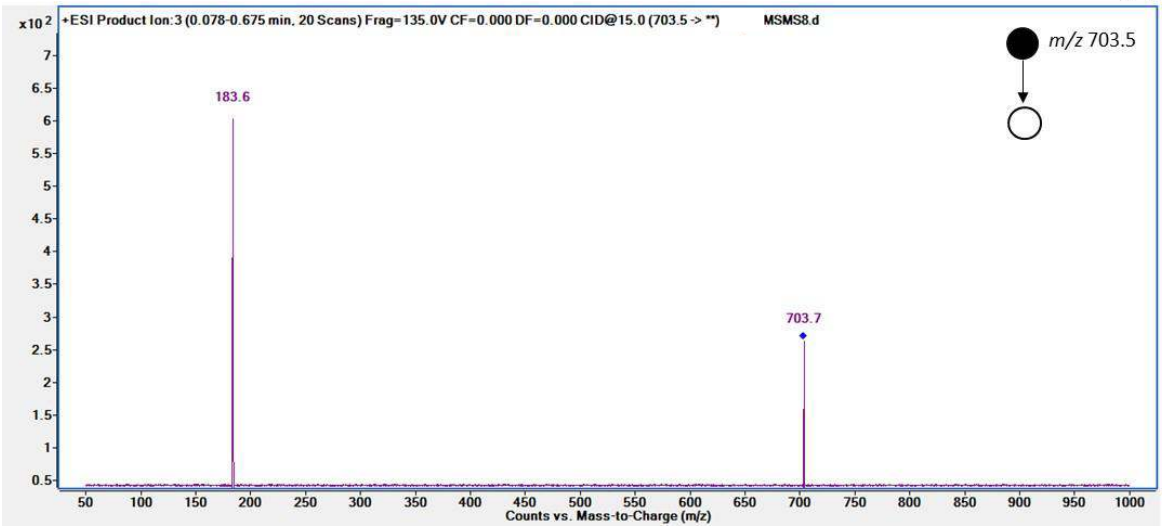
(A)


-ESI Product Ion:2 (0.715-0.851 min, 5 Scans) Frag=135.0V CF=0.000 DF=0.000 CID@23.0 (447.0 -> **) Almond_MSMS447c.d
x10 2
m/z 447
446.5
225.1
Counts (%) vs. Mass-to-Charge (m/z)

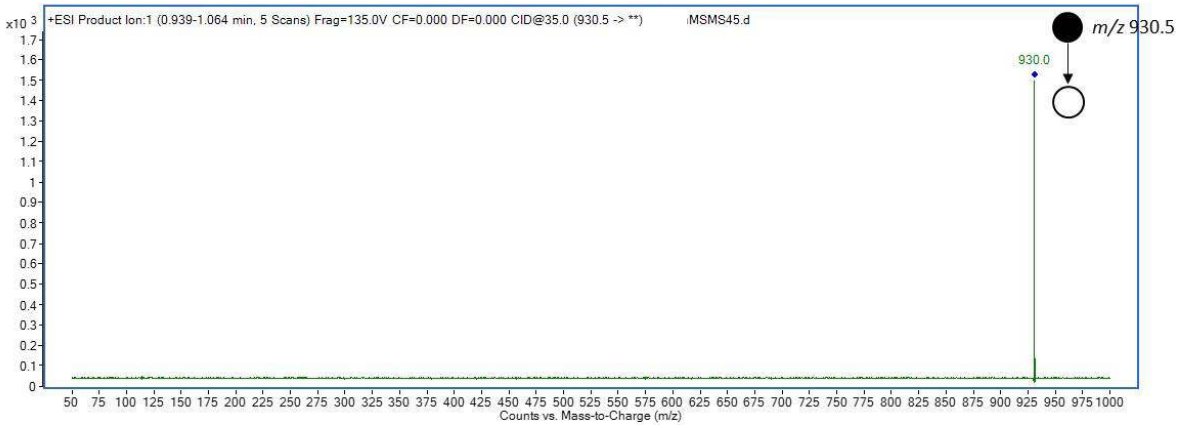
(B)


-ESI Product Ion:2 (0.715-0.886 min, 6 Scans) Frag=135.0V CF=0.000 DF=0.000 CID@23.0 (447.0 -> **) MSMS50b.d
x10 2
m/z 447
341.1
222.9
113.3
446.5
Counts vs. Mass-to-Charge (m/z)

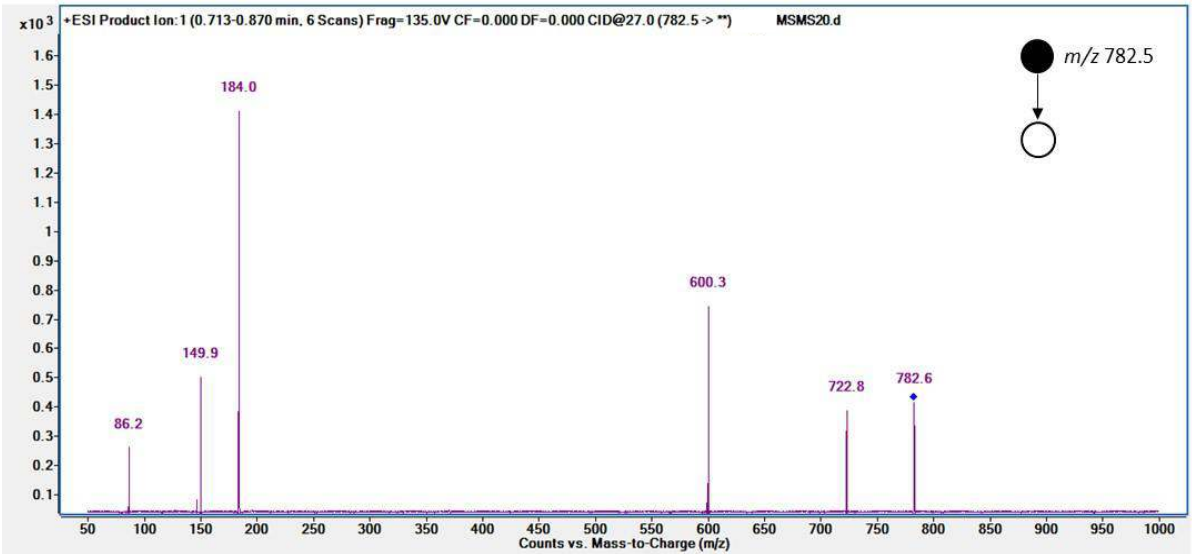
(C)


x10 2
+ESI Product Ion:3 (0.078-0.675 min, 20 Scans) Frag=135.0V CF=0.000 DF=0.000 CID@15.0 (703.5 -> **)
MSMS8.d
m/z 703.5
183.6
703.7
Counts vs. Mass-to-Charge (m/z)

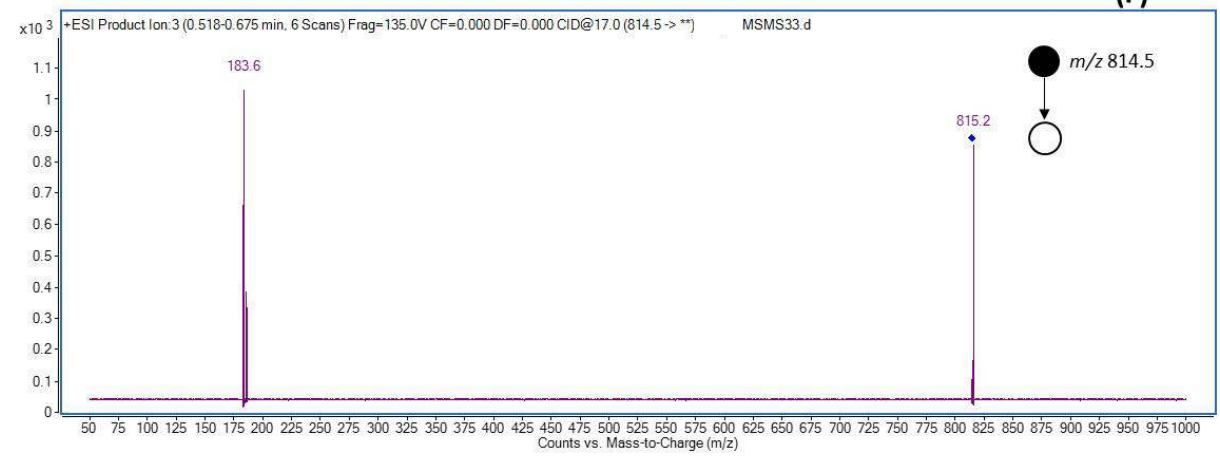
(D)


x10 3
+ESI Product Ion:1 (0.939-1.064 min, 5 Scans) Frag=135.0V CF=0.000 DF=0.000 CID@35.0 (930.5 -> **)
MSMS45.d
m/z 930.5
930.0
Counts vs. Mass-to-Charge (m/z)

(E)


x10 3
+ESI Product Ion:1 (0.713-0.870 min, 6 Scans) Frag=135.0V CF=0.000 DF=0.000 CID@27.0 (782.5 -> **)
MSMS20.d
m/z 782.5
184.0
600.3
149.9
722.8
782.6
86.2
Counts vs. Mass-to-Charge (m/z)

(F)


x10 3
+ESI Product Ion:3 (0.518-0.675 min, 6 Scans) Frag=135.0V CF=0.000 DF=0.000 CID@17.0 (814.5 -> **)
MSMS33.d
m/z 814.5
183.6
815.2
Counts vs. Mass-to-Charge (m/z)

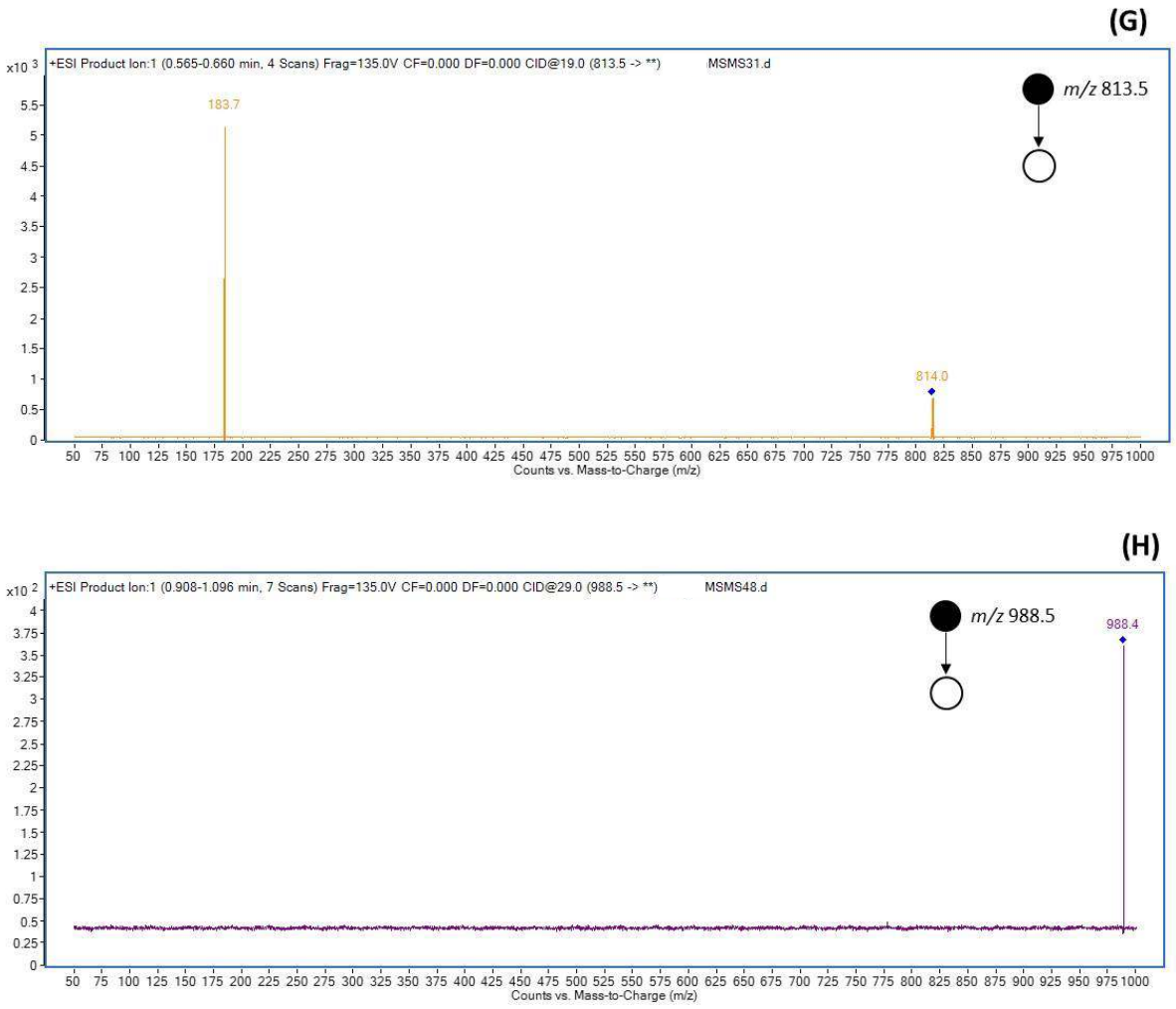


Figure 6-5: MS/MS spectrum of informative m/z values in ratios

(A) MS/MS spectrum of *m/z* 447 from almond seed extract; **(B)** MS/MS spectrum of *m/z* 447 from erythrocytes' lipid extract; **(C)** MS/MS spectrum of *m/z* 703.5 from erythrocytes' extract; **(D)** MS/MS spectrum of *m/z* 930.5 from erythrocytes' extract. Even under high collision energy no fragment characteristic of polar head fragment or neutral were observed. Therefore a tentative attribution as a TAG is proposed. **(E)** MS/MS spectrum of *m/z* 782.5 from erythrocytes' lipid extract; **(F)** MS/MS spectrum of m/z 814.5 from erythrocytes' lipid extract; **(G)** MS/MS spectrum of m/z 814.5 from erythrocytes' lipid extract; **(H)**

MS/MS spectrum of m/z 988.5 from erythrocytes' lipid extract. No fragment characteristic of polar head fragment or neutral were observed. Therefore a tentative attribution as a TAG is proposed. ***Note***: W12-almond and W12-control were pooled for the acquisition of MS/MS data from erythrocytes' lipid extract.

CHAPTER 7. SUMMARY AND FUTURE RESEARCH DIRECTIONS

7.1 Summary

Nut consumption can aid weight management due to their strong satiating effects, the inefficient absorption of their energy, and possibly by elevating energy expenditure and fat oxidation (Chapter 2). In addition, nuts have beneficial effects on body composition, cardiovascular health, and cognitive function (Chapter 3). The unique nutrient profile of nuts can also modulate the post-lunch dip in cognitive function (Chapter 3).

This dissertation focused on almond consumption with three overall primary aims. The first was to evaluate the effects of almond consumption as part of an energy-restricted diet on weight, body composition specifically the visceral depots and blood pressure compared to a nut-free energy restricted diet (Chapter 4). The second was to evaluate the effects of almond consumption as part of an energy-restricted diet on the memory and attention domains of cognitive function (Chapter 5). The third was to evaluate the acute effects of almond consumption on the post-lunch dip in the memory and attention domains of cognitive function (Chapter 5). This research also contributed to the methods literature by developing an analytical approach to identify metabolic profiles associated with almonds to ascertain compliance to almond consumption in long term clinical trials.

7.2 Major findings

This dissertation provides new evidence for the beneficial effects of almond consumption on body composition and blood pressure in the context of energy restriction as well as for the acute effects of almond consumption in ameliorating the post-lunch decline in cognitive function. The major findings of all the studies are presented below.

7.2.1 The effects of almond consumption on weight-related clinical outcomes

- Almond consumption during a 12 week weight loss regimen compared to a nut-free weight loss regimen resulted in:
    - Greater proportional reductions in trunk and total body fat mass in participants compliant to the intervention
    - Greater proportional increases in trunk and total body fat-free mass in participants compliant to the intervention
    - Greater reductions in diastolic blood pressure in participants compliant to the intervention
    - Similar reductions in weight, visceral adipose tissue, anthropometric measures of abdominal adiposity such as SAD and waist circumference, and systolic blood pressure for all participants
    - Similar reductions in twenty-four hour hunger and desire to eat ratings for all participants
- Both the almond-enriched and nut-free weight loss diets demonstrated no changes in the following outcomes over the 12 week weight loss intervention
    - Twenty-four hour fullness ratings.

  - Twenty-four hour ambulatory systolic and diastolic blood pressure
  - Fasting serum lipid profile i.e. HDL, LDL, total cholesterol, and triglycerides
  - Fasting serum insulin and glucose. Although there was a significant but not clinically relevant increase in fasting glucose levels for all participants, fasting glucose concentrations remained unchanged for participants compliant to the intervention.
- Almond palatability and acceptance ratings remained unchanged over the weight loss intervention in participants compliant to the intervention.
- Compliance rates to energy restriction were similar in the almond-enriched and nut-free weight loss diets
- Age, sex and BMI had no effects on the aforementioned outcomes.

7.2.2 The effects of almond consumption on cognitive function and the post-lunch dip in cognitive function

- Both the almond enriched diet and nut-free weight loss diets resulted in similar improvements in the memory and attention indices of cognitive function for all participants after 12 weeks.
- In the post-lunch dip analysis, consumption of an almond enriched high fat lunch compared to the high carbohydrate lunch resulted in:
  - Amelioration of the post-lunch decline in the memory domain of cognitive function
  - Similar post-lunch decline in the attention domain of cognitive function

- Age of participants was negatively correlated with attention performance indices of cognitive function
- Gender, BMI and amount of weight lost had no effect on the cognitive function outcomes over the 12 week intervention as well as in the post-lunch dip analysis.

7.2.3 The assessment of almond consumption compliance using an untargeted metabolomics approach

- Specific ratios and combinations of mainly membrane lipids such as phosphatidylcholine and sphingomyelin were discriminatory of the almond-enriched diet from the nut-free diet at the end of the 12 week intervention.
- The relationship between these metabolites indicates almond-induced changes in the metabolomics profile.
- The misclassifications, 25.8% in the almond group and 10% in the nut-free group observed as a result of the ratio performance evaluation could be indications of possible non-compliance as supported by dietary intake data.

7.3 Future research directions

- Would almond consumption elicit greater visceral fat loss in individuals with greater obesity?
    - Very few studies have directly assessed the effects of nut consumption on visceral fat and have found inconsistent results[1–3]. However, none of these studies exclusively recruited obese individuals. Although visceral adiposity varies greatly at a given BMI value [4], it is strongly correlated

with total body fat and hence is expected to be higher in obese individuals than overweight individuals. Although this dissertation research showed a trend for greater visceral fat loss with almond consumption (Chapter 3), it was not powered to detect a difference between overweight and obese individuals. Therefore, additional research is needed to establish if almond consumption in the context of energy restriction can result in significant visceral fat loss in obese individuals.

- Would the effects of almonds on blood pressure be more pronounced in individuals at high CVD risk?
    - The participants in this dissertation research were overweight or obese with no other CVD risk factors. The majority of research demonstrates no effects of nut consumption on blood pressure in individuals without elevated CVD risk[5]. Despite recruiting only low CVD risk individuals we observed a greater reduction in diastolic blood pressure with almond consumption compared to the nut-free diet (Chapter 3). However, the health impact of almond consumption may be greater among individuals at high CVD risk and needs to be investigated further.

- Can almond consumption in long-term clinical trials result in monotony effects?
    - Past research on almond influences on ingestive behavior has largely focused on the presumed hormonal regulation (ghrelin, CCK, PYY, etc.) of appetite and energy intake. However, the predictive power of these

hormones, measured in the circulation, for appetite and intake is weak[6]. This may be due to a dominating effect of the reward system over the appetitive system and poor correlation between circulating and brain hormone concentrations[7]. Emerging technologies, such as neuroimaging, allow for a more direct assessment of brain activity in appetitive and reward centers. Data from these studies indicate that increased activity elicited by visual food stimuli in the mesolimbic dopamine system and related brain structures is associated with increased appetitive drive and motivated behavior[8,9]. Documentation of almond effects on brain reward centers would add considerable strength to the evidence that they are palatable yet satiating and resistant to monotony effects, a combination ideal for weight management.

- What approaches may be implemented to identify and validate the biomarkers of almond consumption?
    - The metabolomics study presented in Chapter 6 raises important questions concerning the unattributed metabolites that were discriminatory between the almond enriched diet and the nut-free diet at the end of the intervention. Those metabolites could be by products of microbial degradation of flavonols as described by Urpi-Sarda et al[10]. However, that study assessed the metabolomic profiles of phenolic metabolites in plasma and urine samples after consumption of almond skin polyphenols in only two individuals. While that study was different from ours in

several respects, including study design, biological samples (we used erythrocytes), and metabolites screened, it provides some insight into the identity of the unattributed metabolites. Nevertheless, there is a need for detailed structural identification and absolute quantification by LC-MS/MS and/or high mass resolution for biomarker validation which can be explored in future studies.

- Does almond consumption at lunch ameliorate the post-lunch decline in cognitive function by moderating glycemia?
    - This dissertation research showed that almond consumption at lunch reduced the post-lunch decline in cognitive function (Chapter 5) but the underlying mechanisms are unknown. Lower and higher than normal concentrations of plasma glucose are known to impair cognition[11]. Almonds moderate postprandial blood glucose concentrations[12,13]. There is also evidence to suggest that low glycemic index meals improve cognitive performance compared to higher glycemic index meals[14]. Hence, consumption of almonds with a meal can reduce the glycemic impact of carbohydrate, thereby maintaining optimum blood glucose concentrations for effective cognitive performance. However, monitoring plasma glucose concentrations during the post-lunch phase when cognitive function is waning might prove to be tricky as multiple measurements within a short period can interfere with the decline in cognitive function by promoting alertness. Hence, participants may need to be conditioned to

the study process prior to testing to avoid the confounding effects of study methods on cognition.

- How would almond form influence vascular health and cognitive function?
    - Individuals with vascular disease (VD) may have impaired endothelial dependent dilation of blood vessels[15,16]. Moreover, increasing evidence suggests that vascular disease is associated with cognitive dysfunction[17]. These dysfunctions may be mediated by structural and functional changes in the cerebral blood vessels. Epidemiological and clinical evidence suggests that almond consumption may be an effective strategy for lowering the incidence of VD and preventing cognitive decline[18]. Almonds contain a range of nutrients, the bioaccessibility of which are dependent on the form of almonds. For example, nutrients in more processed forms of almonds such as almond meal and almond butter are more accessible for absorption than nutrients in whole almonds[19,20]. Hence, the increased bioavailability of nutrients from more processed almond forms may reduce the risk of vascular disease and cognitive impairments further. The role of almond form on vascular indices such as endothelial function, vascular compliance and cognitive function has not been investigated.

- How would the textural attributes of nuts affect acceptability and cognition?
    - Although nuts have similar nutritional profiles, their textures are vastly different, for example almonds are harder and more brittle than walnuts. The textural properties of foods such as crispiness, crunchiness etc. can influence the acceptability and enjoyment of food[21]. The effects of different textural profiles of nuts on their acceptability has not been evaluated. Moreover, the relationship between mastication sounds and cognition has not been explored. Foods such as nuts that are harder to chew and those that elicit greater mastication sounds may increase alertness and potentially affect cognitive function acutely in comparison to foods that do not require considerable oral processing efforts.

- What are the effects of lunch skipping and timing of lunch consumption on the post-lunch dip in cognitive function?
    - The post lunch dip analysis described in Chapter 5 did not account for the timing of lunch consumption and the effects of lunch skipping on cognitive function. Although a few studies have shown that the post-lunch dip in cognitive function is not affected by the time at which lunch is eaten[22,23] there is very limited evidence to derive firm conclusions. In addition, there is inconsistent evidence regarding the effects of lunch consumption versus no lunch on the post-lunch decline in cognitive function[24]. Therefore, there is a need to determine whether the post-lunch dip induced by lunch intake in the early afternoon is different from that

induced by lunch in the late afternoon and whether lunch skipping elicits a post-lunch dip which is either more or less pronounced than that experienced by lunch eaters.

7.4 References

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APPENDICES

Appendix A Institutional Review Board Documents

Revised 10/10 Ref. # _____

APPLICATION TO USE HUMAN RESEARCH SUBJECTS
Purdue University
Institutional Review Board

1. Project Title: The effects of including almonds in an energy-restricted diet on weight, abdominal fat loss, blood pressure, and cognitive function

2. Full Review ☒ Expedited Review ☐

3. Anticipated Funding Source: Almond Board of California

4. Principal Investigator *[See Policy on Eligibility to serve as a Principal Investigator for Research Involving Human Subjects]*:

Name and Title	Department, Building, Phone, FAX, E-mail address
Richard D Mattes (Distinguished Professor)	Nutrition Science, Stone Hall, Ph:765-494-0662 Fax:765-494-0674, mattes@purdue.edu

5. Co-investigators and key personnel *[See Education Policy for Conducting Human Subjects Research]*:

Name and Title	Department, Building, Phone, FAX, E-mail address
Sze Yen Tan (Post-doctoral Research Associate)	Nutrition Science, Stone Hall, Ph:765-496-3067 Fax:765-494-0674, tan66@purdue.edu

6. Consultants *[See Education Policy for Conducting Human Subjects Research]*:

Name and Title	Department, Building, Phone, FAX, E-mail address
Judy George (Laboratory Manager)	Nutrition Science, Stone Hall, Ph:765-494-6192 Fax:765-494-0674, georgej@purdue.edu
Robin Rhine (Laboratory Technician)	Nutrition Science, Stone Hall, Ph:765-494-6192 Fax:765-494-0674, rrhine@purdue.edu

7. The principal investigator agrees to carry out the proposed project as stated in the application and to promptly report to the Institutional Review Board any proposed changes and/or unanticipated problems involving risks to subjects or others participating in the approved project in accordance with the HRPP Guideline 207 Researcher Responsibilities, Purdue Research Foundation-Purdue University Statement of Principles and the Confidentiality Statement. The principal investigator has received a copy of the Federal-Wide Assurance (FWA) and has access to copies of 45 CFR 46 and the Belmont Report. The principal investigator agrees to inform the Institutional Review Board and complete all necessary reports should the principal investigator terminate University association.

Principal Investigator Signature _____ Date _____

8. The Department Head (or authorized agent) has read and approved the application. S/he affirms that the use of human subjects in this project is relevant to answer the research question being asked and has scientific or scholarly merit. Additionally s/he agrees to maintain research records in accordance with the IRB's research records retention requirement should the principal investigator terminate association with the University.

Department Head *(printed)* _____ Department Name _____

Department Head Signature _____ Date _____

APPLICATION TO USE HUMAN RESEARCH SUBJECTS

9. This project will be conducted at the following location(s): (please indicate city & state)
   - ☒ Purdue West Lafayette Campus
   - ☐ Purdue Regional Campus (Specify): _____
   - ☐ Other (Specify): _____

10. If this project will involve potentially vulnerable subject populations, please check all that apply.
   - ☐ Minors under age 18
   - ☐ Pregnant Women
   - ☐ Fetus/fetal tissue
   - ☐ Prisoners Or Incarcerated Individuals
   - ☐ University Students (PSYC Dept. subject pool ___)
   - ☐ Elderly Persons
   - ☐ Economically/Educationally Disadvantaged Persons
   - ☐ Mentally/Emotionally/Developmentally Disabled Persons
   - ☐ Minority Groups and/or Non-English Speakers
   - ☐ Intervention(s) that include medical or psychological treatment

11. Indicate the anticipated maximum number of subjects to be enrolled in this protocol as justified by the hypothesis and study procedures: 100

12. This project involves the use of an **Investigational New Drug** (IND) or an **Approved Drug For An Unapproved Use**.
   ☐ YES ☒ NO
   Drug name, IND number and company: _____

13. This project involves the use of an **Investigational Medical Device** or an **Approved Medical Device For An Unapproved Use**.
   ☐ YES ☒ NO
   Device name, IDE number and company: _____

14. The project involves the use of **Radiation or Radioisotopes**:
   ☒ YES ☐ NO

15. Does this project call for: (check-mark all that apply to this study)
   - ☐ Use of Voice, Video, Digital, or Image Recordings?
   - ☒ Subject Compensation? Please indicate the maximum payment amount to subjects. $200
     Purdue's Human Subjects Payment Policy Participant Payment Disclosure Form
   - ☐ VO2 Max Exercise?
   - ☒ More Than Minimal Risk?
   - ☐ Waiver of Informed Consent?
   - ☐ Extra Costs To Subjects?
   - ☒ The Use of Blood? Total Amount of Blood 32 ml; Over Time Period (days) 84 days
   - ☐ The Use of rDNA or Biohazardous materials?
   - ☐ The Use of Human Tissue or Cell Lines?
   - ☐ The Use of Other Fluids that Could Mask the Presence of Blood (Including **Urine** and Feces)?
   - ☐ The Use of Protected Health Information (Obtained from Healthcare Practitioners or Institutions)?
   - ☐ The Use of academic records?

16. Does investigator or key personnel have a potential financial or other conflict of interest in this study?
☐ YES ☒ NO

APPLICATION NARRATIVE

A. PROPOSED RESEARCH RATIONALE

Background

Despite their high energy content, the inclusion of almonds in an energy-restricted diet can promote weight loss (1). This may occur by several mechanisms, but probably most importantly, improved dietary compliance. This is likely attributable to greater sensory variety resulting in higher palatability of the diet (2); stronger locus of control (3) leading to a sense of empowerment; better appetite regulation (4) minimizing negative affect; and possibly, through their slow and sustained energy release, moderation of physiological process that promote feeding.

The primary goal of weight loss is to maximize the reduction of fat mass while retaining fat-free mass. Traditionally, exercise was viewed as the only way to achieve this outcome, but evidence shows that dietary factors can be equally or more effective. Increasing the proportion of protein in an energy-restricted diet enhances satiety, energy expenditure and greater relative fat mass loss (5). In addition, different types of dietary fat may determine which substrates are oxidized to yield energy for the body. Monounsaturated fats are oxidized preferentially (6, 7), and a diet higher in the unsaturated:saturated fat ratio may reduce subcutaneous adipose tissue (SAT) (8), but more importantly, visceral adipose tissue (VAT) during weight loss (9). The promotion of VAT loss is important clinically as this may translate into the greatest reduction in the risk for metabolic diseases. Almonds are a good source of protein and monounsaturated fats and their effects on differential rates of visceral and subcutaneous body fat loss, in conjunction with energy restriction, have not been examined.

Emerging evidence indicates food-derived peptides reduce the risk of cardiovascular disease (10). Arginine is among these peptides that help to reduce blood pressure. It is the physiological precursor of nitric oxide; a vasodilator (11). Nitric oxide inactivation increases blood pressure (12). Certain nuts and seeds are good plant-based sources of arginine and preliminary results from one of our ongoing studies with peanuts (2.8g arginine per 100g peanuts, as indicated in the USDA National Nutrient Database) reveals their ingestion for 12-weeks leads to a significant reduction in diastolic blood pressure. Almonds also contain a high level of arginine (2.4g arginine per 100g almonds, as indicated in the USDA National Nutrient Database), and we hypothesize that a blood-pressure-reducing effect can be replicated using almonds.

Dietary intake also modulates cognition as measured by vigilance, short-term memory and reaction time tasks (13). Impairments of cognition may be a result of food restriction or anxiety related to maintaining a weight loss regime. Negative effects of dieting on cognitive performance have been reversed with a diet higher in fat and lower in carbohydrate (LCHF) (14). The LCHF diet included 40g mixed nuts as one of the primary fat sources and we hypothesize that vigilance and memory can be enhanced with the inclusion of 42.5g/day (1.5oz) of almonds eaten as snacks during weight loss. In addition, a decrease of memory and vigilance is often reported after lunch, independent of weight loss (15). This post-lunch dip in cognitive performance may partly be due to high carbohydrate meals (16). The unique nutrient profile of almonds, which are lower in carbohydrate and higher in protein and unsaturated fats, may lessen this post-lunch dip.

B. SPECIFIC PROCEDURES TO BE FOLLOWED

Study design

This will be a single-blinded randomized, controlled, parallel-arm study.

Primary objective

To investigate the efficacy of almonds in promoting preferential abdominal fat loss, improving blood pressure, and reducing a decline in post-lunch cognitive function during weight loss.

Experiment protocol

Participants will be randomly assigned to one of the two study arms:

a) Energy restriction (-500 kcal/day) (N=40), where participants will receive dietary counseling to reduce energy intake to achieve 500 kcal/day deficits (based on the estimated energy requirement calculated using the Schofield equations) to support weight loss. Participants will also be asked to avoid all nuts during the intervention period.
b) Energy restriction (-500 kcal/day) with almonds supplying 15% of estimated energy requirement (N=40). Participants will receive an energy prescription similar to Group (a), with 15% of estimated energy requirement from dry-roasted, lightly salted almonds. Energy from almonds will be accounted for during dietary modeling so that a 500 kcal/day deficit is achieved.

Participants will be seen by a dietitian on a weekly basis for the first 4 weeks to establish their dietary prescription, and every 2 weeks after that to monitor dietary adherence. At baseline, participant body weight, body composition (BOD POD), abdominal fat distribution (DEXA), habitual dietary intake, cognitive performance (tests outlined in Table below), blood biochemistry (glucose, insulin, lipids), ambulatory blood pressure, 24-hour free-living appetite ratings and physical activity levels (RT3 accelerometers) will be measured. These measurements will be repeated every 4 weeks during the intervention, except for abdominal fat distribution with DEXA which will be measured only pre- and post-intervention. A summary of the study activities is provided in the figure below.

Screening procedures

Potential participants will respond to public advertisements (see attached) by e-mail. They will be asked to complete a general online screening questionnaire for the Laboratory for Sensory and Ingestive Studies (www.cfs.purdue.edu/lsis, IRB approval #504002017). Potential participants meeting pre-set criteria will be enrolled and scheduled for the baseline visit at the laboratory.

Baseline visit

Written consent will be obtained from eligible participants during their baseline visit to the laboratory. Measurements at baseline include:

a. Anthropometric measurements
    - Height
    - Weight(Tanita)
    - Body composition using air displacement plethysmography (BOD POD® Model 2000A, Life Measurement Inc.)
    - Waist circumference and sagittal measurements
b. Blood biochemistry (fasting plasma vitamin E and fasting serum glucose, insulin, lipids)
c. Clinical (blood pressure) and physical activity (RT3 accelerometer) assessments
d. Dietary assessment (24-hour diet recall) and dietary counseling
e. Cognitive function assessments that include

- Visual Spatial Learning
- Visual Verbal Learning
- Corsi Block Tapping Test
- Tower of Hanoi Test
- Grooved Pegboard
- Psychomotor tests
- Source Monitoring Test
- Paragraph Recall

f. Appetite ratings (visual analog scales)

Participants will also be asked to complete questionnaires related to health, smoking behaviour, use of medication, alcohol consumption, physical activity, eating behaviour, and anxiety. Acceptability of the almonds will also be evaluated during the screening. DEXA scans will be used to measure total body fat composition. Together, all measurements will take approximately 3 ½ hours.

Week 1, 2 and 3

Participants will attend weekly visits to the dietitians, where dietary prescription will be re-emphasized and compliance to treatment will be checked. Participants will be given opportunities to discuss issues related to their dietary prescription. Body weight (Tanita) will be checked as a measure of dietary compliance. Almonds will be provided to participants during these visits. Each visit will take approximately 45-60 minutes.

Week 4, 8 and 12

All measurements at the baseline visit, except for height and health questionnaires, will be taken. Total body fat (DEXA) will be measured again only at Week 12. At weeks 4 and 8, participants will see a dietitian for dietary intake assessment and to check dietary compliance. Each visit at Week 4 and 8 will take approximately 2 hours, whereas visit at Week 12 will take 3 ½ hours due to the DEXA measurement that will be performed off campus.

Week 6 and 10

These visits are identical to visits at weeks 1, 2 and 3, except that dietary assessments (24-hour recall) will be taken to assess compliance to the dietary prescription. These visits will take approximately 45-60 minutes.

	Base line	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 6**	**Week 8**	**Week 10**	**Week 12**
Consent form	X								
Health questionnaires	X								
Almond liking & well-being questionnaires	X				X		X		X
Height	X								
Weight	X	X	X	X	X	X	X	X	X
Waist measurements	X				X		X		X
Fat % (BOD POD)	X				X		X		X
Total body fat (DEXA)	X								X
Glucose, insulin, lipids	X				X		X		X
Vitamin E	X				X		X		X
Dietary assessment	X				X	X	X	X	X
Dietary counseling	X	X	X	X	X	X	X	X	X
Appetite	X				X		X		X
Blood pressure	X				X		X		X
Physical activity monitor	X				X		X		X
Cognitive tests	X				X		X		X
Almonds	X	X	X	X	X	X	X	X	X

C. SUBJECTS TO BE INCLUDED

Describe:

Adult participants (age 18-60 years) will be recruited by advertisements in local newspapers and on notice boards at Purdue University. Additional eligible criteria include:

- Non-smoking
- Males and females of equal numbers
- Are overweight or obese (BMI 27-35 kg/m^2)
- Fasting blood glucose between 6.1-6.9 mmol/L via capillary finger-stick blood samples
- No nut allergies
- Willing to comply to study protocol and to eat test meals

D. RECRUITMENT OF SUBJECTS AND OBTAINING INFORMED CONSENT

Participants will be recruited through public advertisements on the Laboratory for Sensory and Ingestive Studies website: www.cfs.purdue.edu/lsis (IRB approval #504002017), newspaper ads, Purdue Today, Boiler TV, PurduE-board, and posted flyers (see attached). After expressing interest, subjects will be asked to complete the initial health and personality questionnaires described above. Those meeting the preset criteria, described above, will be contacted via their indicated preferred method (i.e., phone or e-mail) to schedule a screening visit. Participants will be asked to read and sign an informed consent form at the beginning of the screening session.

E. PROCEDURES FOR PAYMENT OF SUBJECTS

Participants will receive a payment of $180 as compensation for any inconvenience caused by participating in this study. For participants who do not meet pre-set criteria, a payment of $5 will be made following completion of the screening session. A payment of $20/12-day testing period will be made to participants should they withdraw or be withdrawn from the study for sessions completed. Participants will be told that they will receive an additional $20 if plasma vitamin E tests confirm that they are compliant with eating the diets. Thus, they will receive a total of $200 for completion of the study.

F. CONFIDENTIALITY

The record of participant progress in the study will be kept in a confidential file in a locked filing cabinet. The confidentiality of any computer record will also be carefully guarded by never including the participant's name on any data file. The information will be stored electronically in a password-protected file indefinitely. For participants who do not meet pre-set criteria, screening data will be immediately destroyed. The key linking ID numbers and data will be destroyed upon completion of the study. A copy of the written consent form will be retained for three years after termination of the study, at which time it will be destroyed. No information by which participants can be identified will be released or published. However, participants will be informed that to process their payments, it will be necessary to provide their name, social security number, and address to the university business office. In addition, participants will be notified that their research records may be reviewed by the National Cattlemen's Beef Association, and by departments at Purdue University responsible for regulatory and research oversight.

G. POTENTIAL RISKS TO SUBJECTS

DEXA entails exposure to a low dose of radiation. The maximum additional radiation exposure participants will receive in any given 12 month period from this study is 12.0 mRem. For comparison, exposure from a single chest x-ray is 6 mRem, an x-ray of your pelvis and hip would give an exposure of 65mRem. A single dental x-ray involves 1 mRem and a CAT scan would be 110 mRem. According to the American Nuclear Society, the average annual dose per person from all sources is approximately 360

mRem in the United States. The additional exposure to radiation from this study is well below the annual allowable exposure limit of 100 mRem (in addition to the annual radiation exposure) that is recommended by the United States Nuclear Regulatory Commission.

H. BENEFITS TO BE GAINED BY THE INDIVIDUAL AND/OR SOCIETY

There are no foreseeable direct benefits to participants. The knowledge gained from this study may provide new insights for the management of obesity – the nation's most pressing public health problem.

I. INVESTIGATOR'S EVALUATION OF THE RISK-BENEFIT RATIO

Participants will be faced with only a small level of risk greater than normally encountered on a daily basis. The findings may yield insights for obesity. Thus, the potential benefits outweigh the possible risks.

J. WRITTEN INFORMED CONSENT FORM *(to be attached to the Application Narrative)*

See attached consent form

K. WAIVER OF INFORMED CONSENT OR SIGNED CONSENT

If requesting either a waiver of consent or a waiver of signed consent, please address the following:

Not applicable

L. INTERNATIONAL RESEARCH

Not applicable

M. SUPPORTING DOCUMENTS *(to be attached to the Application Narrative)*

See attached:

- Study Advertisements
  - Recruitment Flyer
  - Newspaper/Newsletter Advertisement
- Brief Questionnaire
- Informed Consent Form
- gLMS for Almond Palatability
- Power of Food Scale
- Food Craving Questionnaire—State
- Perceived Stress Scale
- Zung Self-Rating Scale (Depression)
- Eating Attitudes Test (Disordered Attitudes Toward Foods)
- Eysenck Personality Questionnaire-Revised (Extraversion)
- Food Attitudes Survey (Finickiness)
- Brief Sensation Seeking Scale 4
- Food Action Rating Scale (Food Preferences)
- Physical Activity Questionnaire
- Appetite Logs

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Almonds & Weight Management

Participants are needed by the Purdue Nutrition Science Dept. to study the role of almonds in weight management

Primary Investigator: Prof. Richard Mattes

If you are interested and meet the criteria below, contact Yen at almeal@purdue.edu

Compensation: $200 upon completion of the study.

- Aged 18 – 60 years
- Overweight
- No nut allergies

The Almond Study almeal@purdue.edu
The Almond Study almeal@purdue.edu
The Almond Study almeal@purdue.edu
The Almond Study almeal@purdue.edu
The Almond Study almeal@purdue.edu
The Almond Study almeal@purdue.edu
The Almond Study almeal@purdue.edu
The Almond Study almeal@purdue.edu
The Almond Study almeal@purdue.edu
The Almond Study almeal@purdue.edu

Newspaper/Newsletter Advertisement

Men and women ages 18-60 years are needed by the Purdue Nutrition Science Department for a study of almonds and weight loss (Principal investigator: Richard Mattes, Professor of Nutrition Science). Participants must be in general good health, overweight, have no nut allergy, and are not taking medications that affect appetite or metabolism. The study involves 9 visits over a 12-week period. Compensation: $200 upon completion of the study. For more information, contact Yen at almeal@purdue.edu.

ALMOND AND WEIGHT MANAGEMENT STUDY
Brief Questionnaire

Please complete the brief questionnaire (below).
Type a response to each question, save the document, and e-mail it to almeal@purdue.edu

1. What is your full name? _____
2. What name do you like to be called? _____
3. What is your e-mail address? _____
4. What is your age? _____ years
5. What is your height? _____ inches
6. What is your weight? _____ lbs
   - How much has your weight changed within the last 3 months? _____ lbs
7. Do you exercise (yes/no)? _____
   - If yes, what do you do for exercise? _____
     _____
8. Has your exercise pattern changed within the last 3 months (yes/no)? _____
   - If yes, how has your exercise pattern changed? _____
     _____
9. Has your diet/eating changed within the last 3 months (yes/no)? _____
   - If yes, how has your diet/eating changed? _____
     _____
10. Do you enjoy eating almonds (yes/no)? _____
11. Are you willing to eat 1.5 oz, or about 1/3 cup, almonds daily for 12 weeks (yes/no)?
    _____
12. How often do you eat almonds? _____
13. How often do you eat tree nuts (e.g., almonds, cashews, macadamia nuts, pecans, pistachios, walnuts)? _____

14.Do you have food allergies (yes/no)? _____
- If yes, what food(s) are you allergic to? _____
  _____
- Are you allergic to almonds (yes/no)? _____
- Are you allergic to tree nuts (yes/no)? _____

15.Do you take medications (yes/no)? _____
- If yes, what medications do you take? Please list each medication's name, dose, and length of time taken. _____
  _____

16.Do you take dietary supplements (yes/no)? _____
- If yes, please indicate the type, brand, dose, and how long you have been taking them: _____
- If yes, are they self-prescribed or by your physician? _____

17.Do you have diabetes (yes/no)? _____

18.Do you have hypertension (yes/no)? _____

19.Do you currently, or have you ever, had gastrointestinal disease (e.g., diverticulitis, inflammatory bowel disease, impaired gag reflex, gastroesophageal reflux disease) (yes/no)? _____
- If yes, please list the name(s) of the gastrointestinal disease and when it/they were experienced. _____
  _____

20.Do you currently, or have you ever, smoked (yes/no)? _____
- If you smoked in the past, when did you quit? _____

**** REMEMBER ****

You must also complete the Laboratory for Sensory and Ingestive Studies questionnaire, which can be found by clicking the "Participate in a Study" link at: www.cfs.purdue.edu/lsis. After both questionnaires are evaluated, you will be contacted by e-mail and informed if you are or are not eligible for screening. If you are eligible, an appointment will be scheduled.

Research Project Number _____

b. Your height and weight will be measured using a scale, and your body fat percent will be measured using an air displacement plethysmography (BOD POD®). This measurement requires that you sit in an enclosed chamber in minimal clothing (e.g. a swimsuit) with a swim cap covering you head. You must provide the tight-fitting swimsuit or bike shorts for the body composition measurement. During the test, you will wear a nose clip and breathe fresh air through a plastic tube and you may notice a slight change in air pressure. The BOD POD measurement will take 20 to 30 minutes.
c. Your total body fat will be measured using dual-energy x-ray absorptiometry (DEXA) at an imaging center by a qualified technician. You will have repeated scans consisting of your whole body. During the scanning process, you will be lying on a padded table. You will need to remain still during the scan time. A padded block and a foot brace may be used to assist in positioning. You will be asked to do no more than 4 repeats of each scan.
d. Your waist circumference and sagittal abdomen diameter will be measured using a measuring tape and a caliper. This measurement requires you to lie in a supine position, and the caliper will measure the height of your abdominal region, which gives an indication of your abdominal fat.
e. A blood sample (8 ml or about 2 teaspoons) will be taken to determine glucose, insulin, lipids, and vitamin E concentrations.
f. Your blood pressure will be measured.
g. Your dietary intake will be assessed using a 24-hour dietary recall method.
h. You will see a dietitian for a dietary prescription that you will follow for 12 weeks.
i. You will complete standardized psychological tests that examine your cognitive function. These include:
    i. Visual spatial learning
    ii. Visual verbal learning
    iii. Corsi block tapping test
    iv. Tower of Hanoi
    v. Grooved pegboard
    vi. Psychomotor tests
    vii. Source monitoring test
    viii. Paragraph recall
j. At the end of this visit, you will be given:
    i. An ambulatory blood pressure machine that measures your hourly blood pressure in a free-living environment for 24 hours. This device is worn on your arm for a day and periodically the cuff inflates and deflates to measure your blood pressure.
    ii. An accelerometer that measures your free-living physical activity levels for two days (1 weekday and 1 weekend day). This is a device about the size of a pager (68 X 48 X 18 mm) that is worn on your waist level to monitor your daily physical activity levels and to predict your daily energy expenditure. You will wear this device for 48 hours, except when you go to bed and when you are taking a shower or swimming (as the device is not water resistant).
    iii. A palm pilot where you will be asked to record your hourly appetite ratings for 24 hours during waking hours.

Visit 2, 3 and 4 (Week 1, 2 and 3)

a. You will attend weekly visits to the dietitian, where your dietary prescription will be re-emphasized and your dietary compliance checked.
b. You will discuss issues related the dietary prescription.
c. Your weight will be measured using a conventional medical scale.

Research Project Number _____

Visit 5 and 7 (Week 4 and 8)

a. All measurements at Baseline Visit will be repeated, except for personality questionnaires and the whole body fat assessment using DEXA.

Visit 6 and 8 (Week 6 and 10)

a. All measurements at Visit 2, 3 and 4 will be repeated.
b. Your dietary intake will be assessed using a 24-hour dietary recall method. You will be required to write down your dietary intake on the previous day, and this information will be checked and clarified by a dietitian to ensure that all details are accurate and properly recorded.

Visit 9 (Week 12)

a. This visit is identical to Baseline Visit and you will repeat all measurements performed at baseline. There will be no dietary counseling.

Duration of Participation

In total, your participation in this study requires 13 weeks and includes 9 visits. You will be required to attend:

a. All testing visits which will be conducted at Purdue University
b. The length of each visit is:
    i. **Baseline visit**: 2 – 2 ½ hours at Purdue + 1 hour at imaging facility
    ii. **Visit 2, 3, and 4**: 45 – 60 minutes each
    iii. **Visit 5**: 2 – 2 ½ hours
    iv. **Visit 6**: 45 – 60 minutes
    v. **Visit 7**: 2 – 2 ½ hours
    vi. **Visit 8**: 45 – 60 minutes
    vii. **Visit 9**: 1 ½ – 2 hours at Purdue + 1 hour at imaging facility

Risks

The blood collections may result in pain, bruising, and/or infection at the site of collection. You may experience lightheadedness during blood collections and may faint. Appropriate techniques will be used to minimize these risks. There is a remote chance of breach of confidentiality, but records will be protected as described below.

The dual-energy x-ray absorptiometry measurement will expose you to a small amount of radiation of 12 mRem. For comparison, exposure from a single chest x-rays is 6 mRem, a pelvis and hip scan is 65 mRem, and a CAT scan is 100 mRem. In United States, the average annual exposure to radiation from all sources is about 360 mRem. The radiation from this study is well below the 100 mRem recommended by the United States Nuclear Regulatory Commission. If you have occupational or other routine exposure to radiation, you must consider the cumulative effects before enrolling in this study.

Repeated exposure to almonds can lead to a sensitivity or allergic response to nuts. If this occurs, you will be withdrawn from the study immediately. If you develop a rash or have difficulty breathing, please stop taking almonds and contact your physician immediately. Additionally, please contact the investigator of the study so that the occurrence can be further investigated.

Research Project Number _____

Body fat measurement using the BOD POD® requires you to sit in a confined chamber during the measurement. You may feel claustrophobic during the measurement, but we have not experienced such event. Each measurement takes approximately 20 – 30 minutes. The BOD POD machine has a window and the door will be opened regularly during the measurement. There is a button in the instrument that you can press and open the chamber if you feel uncomfortable.

Benefits
There are no expected benefits to you from your participation. However, the knowledge gained from this work may provide new insights for the management body weight.

Compensation
You will receive a payment of $180 as compensation for any inconvenience caused by participating in this study. If you do not meet the pre-set eligibility criteria, a payment of $5 will be made following completion of the screening session. A prorated payment of $20/session will be made should you withdraw or be withdrawn from the study (for example, for failure to report to study sessions). An additional $20 will be paid if urine analysis confirms compliance to the study protocol.

Injury or Illness
Purdue University will not provide medical treatment or financial compensation if you are injured or become ill as a result of participating in this research project. This does not waive any of your legal rights nor release any claim you might have based on negligence.

Confidentiality
If you are deemed ineligible for study after the screening session all of your data will be destroyed. The record of your progress in the study will be kept in a confidential file in a locked filing cabinet. The confidentiality of any computer record will also be carefully guarded by never including your name on any data file. The information will be stored electronically in a password-protected file indefinitely. The key linking ID numbers and data will be destroyed upon completion of the study. A copy of the consent form will be retained for three years after termination of the study at which time it will be destroyed. No information by which you can be identified will be released or published. However, to process your payments, it will be necessary to provide your name, social security number, and address to the university business office. In addition, your research records may be reviewed by the Almond Board of California, and by departments at Purdue University responsible for regulatory and research oversight.

Voluntary Nature of Participation
You do not have to participate in this research project. If you agree to participate you can withdraw your participation at any time without penalty.

Contact Information
If you have any questions about this research project, contact the principal investigator, Prof. Richard Mattes, at (765) 494-0662. If you have concerns about the treatment of research participants, you can contact the Institutional Review Board at Purdue University, Ernest C. Young Hall, Room 1032, 155 S. Grant St., West Lafayette, IN 47907-2114. The phone number for the Board is (765) 494-5942. The email address is irb@purdue.edu.

Research Project Number _____

Documentation of Informed Consent

I have had the opportunity to read this consent form and have the research study explained. I have had the opportunity to ask questions about the research project and my questions have been answered. I am prepared to participate in the research project described above. I will receive a copy of this consent form after I sign it.

Participant's Signature	Date
Participant's Name	
Researcher's Signature	Date

Test Meal Palatability

Please place a mark along each of the scales for the following questions.

Study Code _____

On the scale to the right, please rate your liking for the almonds you just tasted. You should rate this liking relative to other all kinds of liking that you have experienced.

SCORING

Measurement in millimeters (mm):

0 = no sensation

100 = strongest liking of any kind

POWER OF FOOD SCALE (WITH SCORING) **Study code:_____**

Response options are on a 5-point Likert scale ranging from (1) don't agree at all to (5) strongly agree. Possible range of scores 15 (lowest hedonic food score) to 75 (highest hedonic food score).

I find myself thinking about food even when I'm not physically hungry.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

I get more pleasure from eating than I do from almost anything else.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

If I see or smell a food I like, I get a powerful urge to have some.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

When I'm around a fattening food I love, it's hard to stop myself from at least tasting it.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

It's scary to think of the power that food has over me.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

When I know a delicious food is available, I can't help myself from thinking about having some.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

I love the taste of certain foods so much that I can't avoid eating them even if they're bad for me.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

Just before I taste a favorite food, I feel intense anticipation.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

When I eat delicious food I focus a lot on how good it tastes.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

Sometimes, when I'm doing everyday activities, I get an urge to eat "out of the blue" (for no apparent reason).

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

I think I enjoy eating a lot more than most other people.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

Hearing someone describe a great meal makes me really want to have something to eat.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

It seems like I have food on my mind a lot.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

It's very important to me that the foods I eat are as delicious as possible.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

Before I eat a favorite food my mouth tends to flood with saliva.

(1) I don't agree	(2) I agree a little	(3) I agree somewhat	(4) I agree quite a bit	(5) I strongly agree
1	2	3	4	5

Cappelleri JC, Bushmakin AG, Gerber RA, Leidy NK, Sexton CC, Karlsson J & Lowe MR (2009) Evaluating the Power of Food Scale in obese subjects and a general sample of individuals: development and measurement properties. *Int J Obes* **33**, 913-922.

FOOD CRAVING QUESTIONNAIRE—STATE (WITH SCORING) **Study code:_____**

Possible range of scores 15 (lowest food craving score) to 75 (highest food craving score).

1. **I have an intense desire to eat one or more specific foods.**
   ( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree
   1 2 3 4 5

2. **I'm craving one or more specific foods.**
   ( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree
   1 2 3 4 5

3. **I have an urge for one or more specific foods.**
   ( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree
   1 2 3 4 5

4. **Eating one or more specific foods would make things seem just perfect.**
   ( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree
   1 2 3 4 5

5. **If I were to eat what I am craving, I am sure my mood would improve.**
   ( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree
   1 2 3 4 5

6. **Eating one or more specific foods would feel wonderful.**
   ( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree
   1 2 3 4 5

7. **If I ate something I wouldn't feel so sluggish and lethargic.**
   ( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree
   1 2 3 4 5

8. **Satisfying my craving would make me feel less grouchy and irritable.**
   ( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree
   1 2 3 4 5

9. **I would feel more alert if I could satisfy my craving.**
   ( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree
   1 2 3 4 5

10. **If I had one or more specific foods, I could not stop eating it.**
    ( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree

1 2 3 4 5

11. My desire to eat [one or more specific foods] seems overpowering.

( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree

1 2 3 4 5

12. I know I'm going to keep on thinking about one or more specific foods until I actually have it.

( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree

1 2 3 4 5

13. I am hungry.

( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree

1 2 3 4 5

14. If I ate right now, my stomach wouldn't feel as empty.

( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree

1 2 3 4 5

15. I feel weak because of not eating.

( ) Strongly Disagree ( ) Disagree ( ) Neutral ( ) Agree ( ) Strongly Agree

1 2 3 4 5

Cepeda-Benito A, Gleaves DH, Williams TL & Erath SA (2000) The development and validation of the State and Trait Food-Cravings Questionnaires. *Behav Ther* **31**, 151-173.

PERCEIVED STRESS SCALE- 10 ITEM (WITH SCORING) **Study code:_____**

PSS-10 scores are obtained by reversing the scores on the four positive items, e.g., 0=4, 1=3, 2=2, etc. and then summing across all 10 items. Items 4, 5, 7, and 8 are the positively stated items. Possible range of scores: 0 (lowest stress score) to 40 (highest stress score).

1. In the last month, how often have you been upset because of something that happened unexpectedly?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often
0 1 2 3 4

2. In the last month, how often have you felt that you were unable to control the important things in your life?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often
0 1 2 3 4

3. In the last month, how often have you felt nervous and "stressed"?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often
0 1 2 3 4

4. In the last month, how often have you felt confident about your ability to handle your personal problems?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often
4 3 2 1 0

5. In the last month, how often have you felt that things were going your way?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often
4 3 2 1 0

6. In the last month, how often have you found that you could not cope with all the things that you had to do?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often
0 1 2 3 4

7. In the last month, how often have you been able to control irritations in your life?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often
4 3 2 1 0

8. In the last month, how often have you felt that you were on top of things?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often
4 3 2 1 0

9. In the last month, how often have you been angered because of things that were outside of your control?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often
0 1 2 3 4

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often
0 1 2 3 4

Cohen S, Kamarck T & Mermelstein R (1983) A global measure of perceived stress. *J Health Soc Behav* **24**, 385-396.

ZUNG SELF-RATING SCALE FOR DEPRESSION (WITH SCORING) **Study code:_____**

Possible range of scores 20 (lowest depression score) to 80 (highest depression score):
≤ 49 normal range; 50-59 mildly depressed; 60-69 moderately depressed; ≥ 70 severely depressed.

1. I feel down-hearted and blue.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
1	2	3	4

2. Morning is when I feel the best.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
4	3	2	1

3. I have crying spells or feel like it.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
1	2	3	4

4. I have trouble sleeping at night.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
1	2	3	4

5. I eat as much as I used to.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
4	3	2	1

6. I still enjoy sex.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
4	3	2	1

7. I notice that I am losing weight.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
1	2	3	4

8. I have trouble with constipation.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
1	2	3	4

9. My heart beats faster than usual.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
1	2	3	4

10. I get tired for no reason.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
1	2	3	4

11. My mind is as clear as it used to be.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
4	3	2	1

12. I find it easy to do the things I used to.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
4	3	2	1

13. I am restless and can't keep still.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
1	2	3	4

14. I feel hopeful about the future.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
4	3	2	1

15. I am more irritable than usual.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
1	2	3	4

16. I find it easy to make decisions.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
4	3	2	1

17. I feel that I am useful and needed.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
4	3	2	1

18. My life is pretty full.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
4	3	2	1

19. I feel that others would be better off if I were dead.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
1	2	3	4

20. I still enjoy the things I used to do.

( ) A little of the time	( ) Some of the time	( ) Good part of the time	( ) Most of the time
4	3	2	1

Zung WWK (1965) A self-rating depression scale. *Arch Gen Psychiatry* **12**, 63-70.

EATING ATTITUDES TEST (EAT-26) (WITH SCORING) **Study code:_____**

Possible range of scores 0 (lowest disordered eating score) to 78 (highest disordered eating score). Scores > 20 indicate disordered eating.

1. Am terrified about being overweight.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

2. Avoid eating when I am hungry.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

3. Find myself preoccupied with food.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

4. Have gone on eating binges where I feel that I may not be able to stop.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

5. Cut my food into small pieces.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

6. Aware of the calorie content of foods that I eat.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

7. Particularly avoid foods with a high carbohydrate content (i.e. bread, rice, potatoes, etc.).

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

8. Feel that others would prefer if I ate more.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

9. Vomit after I have eaten.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

10. Feel extremely guilty after eating.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

11. Am preoccupied with a desire to be thinner.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

12. Think about burning up calories when I exercise.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

13. Other people think that I am too thin.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never

3 2 1 0 0 0

14. Am preoccupied with the thought of having fat on my body.

( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

15. Take longer than others to eat my meals.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

16. Avoid foods with sugar in them.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

17. Eat diet foods.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

18. Feel that food controls my life.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

19. Display self-control around food.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

20. Feel that others pressure me to eat.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

21. Give too much time and thought to food.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

22. Feel uncomfortable after eating sweets.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

23. Engage in dieting behavior.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

24. Like my stomach to be empty.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

25. Enjoy trying new rich foods.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
0 0 0 1 2 3

26. Have the impulse to vomit after meals.
( ) Always ( ) Usually ( ) Often ( ) Sometimes ( ) Rarely ( ) Never
3 2 1 0 0 0

Garner DM & Garfinkel PE (1979) The Eating Attitudes Test: an index of the symptoms of anorexia nervosa. *Psychol Med* **9**, 273-279.

Study code:_____

EXTRAVERSION QUESTIONNAIRE –
EYSENCK PERSONALITY QUESTIONNAIRE-REVISED (EPQ-R) (WITH SCORING)

Are you a talkative person?	Extravert
Are you rather lively?	Extravert
Do you enjoy meeting new people?	Extravert
Can you usually let yourself go and enjoy yourself at a lively party?	Extravert
Do you usually take the initiative in making new friends?	Extravert
Can you easily get some life into a rather dull party?	Extravert
Do you tend to keep in the background on social occasions?	Introvert
Do you like mixing with people?	Extravert
Do you like plenty of bustle and excitement around you?	Extravert
Are you mostly quiet when you are with other people?	Introvert
Do other people think of you as being very lively?	Extravert
Can you get a party going?	Extravert

Eysenck SBG, Eysenck HJ & Barrett P (1985) A revised version of the psychoticism scale. *Pers Indiv Differ* **6**, 21-29.

Eysenck HJ, Eysenck SBG (1992) *Manual of the Eysenck Personality Questionnaire-Revised.* San Diego CA: Educational and Industrial Testing Service.

FINICKINESS QUESTIONNAIRE (WITH SCORING) **Study code:_____**

Possible score range 4 (least finicky) to 20 (most finicky).

I have been called a picky eater

(1) strongly agree	(2) agree	(3) neutral	(4) disagree	(5) strongly disagree
5	4	3	2	1

I consider myself a picky eater

(1) strongly agree	(2) agree	(3) neutral	(4) disagree	(5) strongly disagree
5	4	3	2	1

I find many foods distasteful

(1) strongly agree	(2) agree	(3) neutral	(4) disagree	(5) strongly disagree
5	4	3	2	1

I think that many foods are disgusting

(1) strongly agree	(2) agree	(3) neutral	(4) disagree	(5) strongly disagree
5	4	3	2	1

Raudenbush B, Van Der Klaauw NJ & Frank RA (1995) The contribution of psychological and sensory factors to food preference patterns as measured by the Food Attitudes Survey (FAS). *Appetite* **25**, 1-15.

BRIEF SENSATION SEEKING SCALE (BSSS) 4 –
SENSATION SEEKING QUESTIONNAIRE (WITH SCORING)

I would like to explore strange places.

(1) strongly agree	(2) agree	(3) neutral	(4) disagree	(5) strongly disagree
5	4	3	2	1

I like to do frightening things.

(1) strongly agree	(2) agree	(3) neutral	(4) disagree	(5) strongly disagree
5	4	3	2	1

I like new and exciting experiences, even if I have to break the rules.

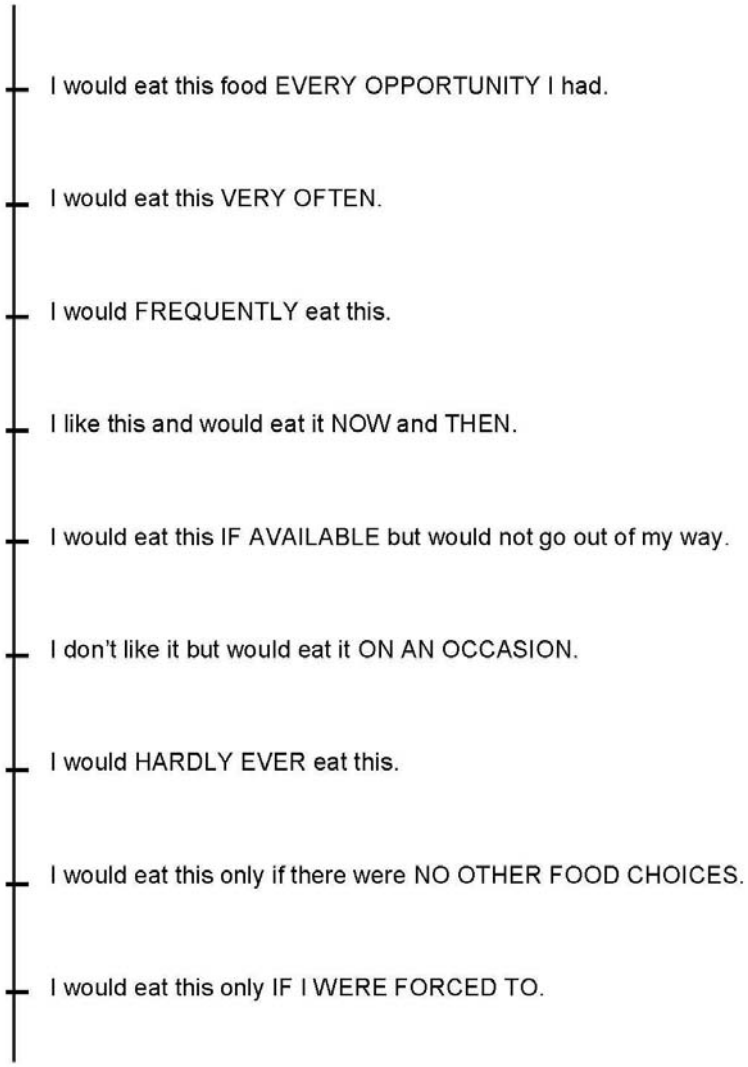
(1) strongly agree	(2) agree	(3) neutral	(4) disagree	(5) strongly disagree
5	4	3	2	1

I prefer friends who are exciting and unpredictable.

(1) strongly agree	(2) agree	(3) neutral	(4) disagree	(5) strongly disagree
5	4	3	2	1

Stephenson MT, Hoyle RH, Palmgreen P & Slater MD (2003) Brief measures of sensation seeking for screening and large-scale surveys. *Drug Alcohol Depen* **72**, 279-286.

FOOD ACTION RATING SCALE (FACT SCALE) **Study code:_____**



I have never tried this.

Schutz HG (1965) A food action rating scale for measuring food acceptance. *J Food Sci* **30**, 365-374.

PHYSICAL ACTIVITY QUESTIONNAIRE **Study code:**_____

Describe your physical activity at work (even work at home, sick leave at home and studying, for instance in a university)

1. Very light, e.g., sitting at the computer most of the day or sitting at a desk
2. Light, e.g., light industrial work, sales or office work that comprises light activities
3. Moderate, e.g., cleaning, staffing at kitchen or delivering mail on foot or by bicycle
4. Heavy, e.g., heavy industrial work, construction work or farming

Describe your physical activity at leisure time. If the activities vary between summer and winter, try to give a mean estimate

1. Very light: almost no activity at all
2. Light, e.g., walking, non-strenuous cycling or gardening approximately once a week
3. Moderate: regular activity at least once a week, e.g., walking, bicycling, or gardening or walking to work 10–30 min/day
4. Active: regular activities more than once a week, e.g., intense walking or bicycling or sports
5. Very active: strenuous activities several times a week

PHYSICAL ACTIVITY QUESTIONNAIRE SCORING

Table 2 The scheme for estimating physical activity levels

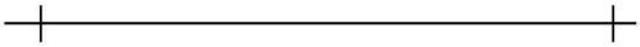
Physical activity in leisure time	*Physical activity at work*			
	Very light	*Light*	*Moderate*	*Heavy*
Very light	1.4	1.5	1.6	1.7
Light	1.5	1.6	1.7	1.8
Moderate	1.6	1.7	1.8	1.9
Active	1.7	1.8	1.9	2.1
Very active	1.9	2.0	2.2	2.3

Johansson G & Westerterp KR (2008) Assessment of the physical activity level with two questions: validation with doubly labeled water. *Int J Obes* **32**, 1031-1033.

APPETITE LOG **Study code:_____**

Please place one mark on each scale that best reflects your answer to each of the following questions at this time.

1. How strong is your feeling of hunger? 1. ____



Not at all Extremely

2. How strong is your feeling of fullness? 2. ____

Not at all Extremely

3. How strong is your desire to eat? 3. ____

Not at all Extremely

4. How strong is your "urge to eat"? 4. ____

Not at all Extremely

5. How strong is your preoccupation with thoughts of food? 5. ____

Not at all Extremely

6. How strong is your feeling of thirst? 6. ____

Not at all Extremely

7. How strong is your desire to eat something salty? 7. ____

Not at all Extremely

8. How strong is your desire to eat something fatty? 8. ____

Not at all Extremely

9. How strong is your desire to eat something sweet? 9. ____

Not at all Extremely

10. The shakiness of your hand is... 10. ____

Not at all Extremely

11. How strong is your grip? 11. ____

Not at all Extremely

12. How itchy is your scalp? 12. ____

Not at all Extremely

Appendix B Forms for Cognitive Function Testing

Participant instructions for cognitive testing

1. The researcher walks the participant in the room after lunch and asks the participant to put all distracting items like cell phones, ipods etc. in a box in another room.
2. The researcher then conducts the immediate memory test.
3. The researcher hands a recording blank to the participant and says. "With the help of this test I would like to see how well you can concentrate."
4. The researcher continues, "After the word 'Example' on your recording blank you see three small letters marked with dashes. These are the letter 'd' as in 'dog' and each is marked with two dashes. *The first 'd' has two dashes on the top, the second has two on the bottom, and the third 'd' has one dash on the top and one on the bottom, still making two dashes all together.* **I would like you to cross out every letter 'd' that has two dashes by making a single line through the letter.** Try doing this first with the three examples, and then try the practice line. You are not supposed to cross out the other letters. Thus, a'd' which has more than two or fewer than two dashes should not be crossed out, and the letter 'p' as in 'pig' should never be crossed out, no matter how many dashes it has. Do you have any questions right now?"
5. Researcher encourages the participant to try the practice line.
6. Once the participant is done with the practice line, the researcher may answer queries and assess whether the participant has in fact understood the instructions.
7. Researcher turns over recording blank and says, "On this side of the recording blank you will see 14 lines with the same letters you have worked on in the practice line. **For each one of the 14 lines you should start on the left side, work to the right** and cross out each 'd' with two dashes. You will have **four** minutes to do the test. Cross out as many 'd' with two dashes as you can. ***Work as quickly as you can without making any mistakes.*** The first line is on top. On the upper left hand corner you will see an arrow pointing to where you should start working on the first line."
8. The Researcher continues, "I will be handing to you eight such forms. You will have four minutes per form. The instructions will be given on top of each form. I will quietly come behind you and switch the forms when four minutes for each form are up. You will be wearing headphones to prevent distractions. From now on it'll take you about 35 minutes to complete all the tests. I will be standing in a corner. There will be no interaction between us and you have to sit in silence until I address you after 35 minutes. Do you have any questions for me? This is the last chance you get to ask questions."
9. Researcher then asks the participant to wear the headphones and begin the test.
10. After 30 minutes, the researcher hands the participant the form for the delayed memory test. Once the participant is done recalling the words, the researcher breaks the silence and conducts the verbal list recognition test.
11. Post the verbal list recognition test, the participant is done with the cognitive testing.

Immediate memory test

1. Researcher will read out 10 words to the participant.
2. Participant will recall words.
3. Researcher will place checks against words recalled by participant.
   a. Market
   b. Package
   c. Elbow
   d. Apple
   e. Story
   f. Carpet
   g. Bubble
   h. Highway
   i. Saddle
   j. Powder

Delayed memory test

Please recall and write down the words read out to you after lunch in the space provided.

Verbal list recognition test

1. Researcher will test participant's ability to recall words which were read out earlier.
2. Researcher will read out a list of words and participant will have to verbally acknowledge which words were present in the initial list (from the immediate memory test).

Word list	Circle yes or No
a. Apple	Yes / No
b. Honey	Yes / No
c. Market	Yes / No
d. Story	Yes / No
e. Fabric	Yes / No
f. Sailor	Yes / No
g. Velvet	Yes / No
h. Carpet	Yes / No
i. Valley	Yes / No
j. Elbow	Yes / No
k. Bubble	Yes / No
l. Prairie	Yes / No
m. Highway	Yes / No
n. Oyster	Yes / No
o. Student	Yes / No
p. Saddle	Yes / No
q. Powder	Yes / No
r. Angel	Yes / No
s. Package	Yes / No
t. meadow	Yes / No

VITA

VITA

ACADEMIC QUALIFICATIONS

Ph.D., Nutrition Science — August 2016
Purdue University, West Lafayette, IN

Applied Statistics Graduate Certification Program — August 2016
Purdue University, West Lafayette, IN

Applied Management Principles Program — June 2015
Purdue University, West Lafayette, IN

M.S., Nutrition Science — May 2012
Syracuse University, Syracuse, NY

B.Tech., Bioinformatics — June 2007
Jaypee University of Information Technology, Waknaghat, India

PROFESSIONAL EXPERIENCE

Bilsland Graduate Fellow, Purdue University	August 2015 – August 2016
Teaching Assistant, Purdue University	January 2015 – May 2015
Research Assistant, Purdue University	August 2013 – December 2014
Lynn Graduate Fellow, Purdue University	August 2012 – August 2013
Graduate Assistant, Syracuse University	August 2010 – May 2012
Project Engineer, Wipro Technologies, India	June 2008 – April 2010
Intern, Wipro Technologies, India	March 2008 – May 2008
Trainee, Indian Agricultural Research Institute, India	June 2005 – July 2005

PUBLICATIONS

Tan, S.Y., **Dhillon, J**., and Mattes, R.D. (2014). A review of the effects of nuts on appetite, food intake, metabolism, and body weight. *American Journal of Clinical Nutrition 100*, 412S–422S.

Dhillon, J., Running, C. A., Tucker, R. M., and Mattes, R.D. (2015) The effects of food form on appetite and energy balance. *Food Quality and Preference*.

Dhillon, J., Craig, B.A. et al. (2016) The effects of increased protein intake on fullness: a meta-analysis and its limitations. *Journal of Academy of Nutrition and Dietetics*.

Mattes, R.D., **Dhillon, J**. et al. (2016) The macronutrients appetite and energy intake. *Annual Review of Nutrition.* (In Press).

PRESENTATIONS

Dhillon, J. and Mattes, R.D. Effects of including almonds in an energy-restricted diet on weight, body composition, visceral adipose tissue and blood pressure in obese adults. *Experimental Biology.* 2016. San Diego, CA.

Dhillon, J. Effects of dieting on body composition. 2015. IBRC Journal Club. Purdue University, IN.

Dhillon, J. The role of nut consumption on abdominal obesity, cognitive function and brain reward systems. *Ingestive Behavior Seminar.* 2014. Purdue University, IN.

Dhillon, J. Dietary habits and practices of South Asian female students in the United States. *Food, Health, and Agriculture in South Asia: Contemporary Issues and Future Directions Conference*. 2012. Syracuse University, NY.

Auciello, A., **Dhillon, J**., Fischetti, N., and Sathyamurthy, M. Performance nutrition for military effectiveness. *Nutrition Education Presentation at Fort Drum*. 2011. Fort Drum, NY.

POSTERS

Dhillon, J. and Mattes, R.D. Effects of including almonds in an energy-restricted diet on weight, body composition, visceral adipose tissue and blood pressure in obese adults. ASN's Emerging Leaders in Nutrition Science Poster Competition. *Experimental Biology.* 2016. San Diego, CA.

Dhillon, J. and Mattes, R.D. Effects of including almonds in an energy-restricted diet on weight, body composition and visceral adipose tissue in obese adults. *The Obesity Society Conference.* 2015. Los Angeles, CA.

Dhillon, J. and Mattes, R.D. The cephalic phase insulin response to nutritive and non-nutritive sweetener exposure. *Ingestive Behavior Research Center Conference.* 2015. Purdue University, IN.

Dhillon, J. and Mattes, R.D. Effects of almonds on post-lunch cognitive function in overweight and obese adults. *The Office of Interdisciplinary Graduate Programs Conference.* 2015. Purdue University, IN.

Dhillon, J. and Mattes, R.D. Effects of almonds on post-lunch cognitive function in overweight and obese adults. *Health and Disease: Science, Culture and Policy.* 2015. Purdue University, IN.

Sayer, R.D., **Dhillon, J.** Tamer, G.G., Campbell, W.W. and Mattes, R.D. Effect of almond consumption on the neural response in the left insula. *Experimental Biology.* 2015. Boston, MA.

Dhillon, J. and Mattes, R.D. Effects of almonds on post-lunch cognitive function in overweight and obese adults. *The Obesity Society Conference.* 2014. Boston, MA.

Dhillon, J. and Mattes, R.D. Effects of macronutrient composition of midday meals on post-lunch cognitive function. *Ingestive Behavior Research Center Conference.* 2013. Purdue University, IN.

LEADERSHIP EXPERIENCE

President, Ingestive Behavior Graduate Student Association	2015 – 2016
Vice President, Nutrition Science Graduate Student Association	2015 – 2016
Salsa Instructor, Purdue Salsa Club	2015 – 2016
Event Planner, Purdue Salsa Club	2014 – 2015
Secretary, Nutrition Science Graduate Student Organization	2013 – 2014
Scrum Master -Software maintenance project, Wipro Technologies	2009 – 2010
Cultural Club Head, JUIT Youth Club	2005 – 2006

WORKSHOPS, VOULNTEER & MENTORING EXPERIENCE

Science Day Event April 2016
Sensory system activities for elementary school children and their families
Wea Ridge Elementary school, Lafayette, IN

GERI Purdue Outreach Event June 2015
Gifted education resource institute outreach event for 3rd and 4th graders on the sensory systems
LSIS, Purdue University, West Lafayette, IN

Undergraduate Research Project Mentor February 2015 – May 2015
Project: Chili pepper's effects on reactions to ostracism
LSIS, Purdue University, West Lafayette, IN

4-H Nutrition Workshop June 2014
Learning sessions and activities on the sensory systems
LSIS, Purdue University, West Lafayette, IN

Undergraduate Research Project Mentor February 2014 – May 2014
Project: Factors influencing food choice and their potential to moderate daily food acceptance
LSIS, Purdue University, West Lafayette, IN

Clinical Nutrition Experience August 2010 – April 2011
Crouse Hospital, Syracuse, NY

Early Sprouts August 2010 - December 2010
Gardening and nutrition experiences for the young child
Early education and child care center, Syracuse, NY

Orange WRAP member August 2010 - December 2010
Outreach and nutrition group education by wellness responsibility advocating peers
Nutrition education experience
Syracuse University, Syracuse, NY

ACADEMIC PROJECTS

- **PhD Dissertation:** The effects of including almonds in an energy-restricted diet on weight, abdominal fat loss, blood pressure, and cognitive function, October 2012 – Present
- The cephalic phase insulin response to nutritive and non-nutritive sweetener in solid and liquid form, December 2014 – Present
- **Master's Thesis**: Dietary habits and practices of South Asian female students in the United States, January 2011 – May 2012
- Nutrition education curriculum for elementary school children at Meachem Elementary School, Syracuse, NY, January 2011 – April 2011
- Database on bio-mineralization processes (BioMin)**,** July 2006 – June 2007
- Production of apple cider using upstream processing and downstream processing, July 2006 – November 2006
- Development of a hidden markov model tool in C++ in order to predict CpG Islands, July 2005 – November 2005
- Rational drug design project for atherosclerosis, July 2005 – November 2005
- A biological database management project on diabetes, July 2005 – November 2005
- A project on micropropagation techniques in Chrysanthemum (IARI, PUSA Institute, Delhi), June 2005 – July 2005
- A viral database of subtypes of Hepatitis-B virus and SARS-Corona virus, January 2005 – May 2005

SELECTED HONORS & AWARDS

- ASN's Emerging Leaders in Nutrition Science Poster Competition, Obesity RIS 2nd place winner, 2016
- Ingestive Behavior Research Center Travel Award, 2015
- Purdue Graduate Student Organization Travel Award, 2015
- Bilsland Dissertation Fellowship, Purdue University, 2015
- Honorable mention at the Health and Disease: Science, Culture and Policy graduate student poster competition, 2015
- The Obesity Society 2014 Early Career Investigator Award, 2014
- Health and Human Sciences Compton Graduate Student Travel Award, 2014
- Lynn Fellowship, Purdue University, 2012
- Outstanding Graduate Student Award in Nutrition Science and Dietetics, Syracuse University, 2012
- Graduate School Master's Prize, Syracuse University, 2012
- Nutrition Science Department Marshal, Syracuse University, 2012
- Certificate of Achievement, Lifestyle Oriented Nutrition Counseling, Syracuse University, 2011
- Excellence Award for Nutrition Education Presentation at Fort Drum, 2011
- Chancellor's Award for Orange Wrap – Outreach and Nutrition Group Education by Wellness Advocating Peers, Syracuse University, 2010
- Scotty Award for Best Debut – Team Rainbow, Wipro Technologies, 2008-2009
- Project Readiness Program Topper, Wipro Technologies, 2007-2008
- 3rd Rank, B.Tech Bioinformatics, Jaypee University of Information Technology, 2007
- 2nd Place in the Bio-Programming Contest, 2005

OTHER CERTIFICATIONS & SKILLS

Certificate Course in Dietetics and Nutrition, VLCC Institute, Kolkata, India. November 2009
Certificate Course in Sports and Fitness Nutrition, VLCC Institute, Kolkata, India. November 2009
Technical Skills

- Programming Languages: **C, C++, Java, Perl**
- Scripting languages**: PHP, HTML**
- Database Management System: **MySQL, Oracle 8i and 9i**
- Statistical Analysis Software: **SPSS, SAS, R**
- Computational Biology