THE EFFECTS OF CHRONIC ALMOND CONSUMPTION IN ADULTS WITH DIFFERENT BODY FAT DISTRIBUTIONS ON CARBOHYDRATE AND LIPID METABOLISM

by

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To my Dad. I wish you were here.

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ABSTRACT

Acute feeding trials indicate that almond consumption can lower the glycemic response to a meal, evoke a second meal effect, and help lower glycemia throughout the day, especially when they are consumed at breakfast or as an afternoon snack. However, the literature is mixed with regard to the effect of almond consumption on HbA1c, hampering the acceptance of a beneficial role for almond consumption on glycemic control. Different body fat distributions carry different risks for insulin resistance, independent of body weight, and thus may respond differently to dietary interventions. Testing people with different body fat distributions may be a reason for the inconsistent evidence on almond consumption on HbA1c. This dissertation had two primary aims. The first primary aim was to determine the acute effect of almond consumption on the glycemic response to a meal tolerance test in adults with different body fat distributions. The second primary aim was to determine the chronic effect of almond consumption on fasting glucose, insulin, HbA1c, triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, body weight, body composition, appetite, and calculated HOMA-IR and HOMA-%β in adults with different body fat distributions. A secondary objective of this dissertation was to determine the effect of substituting almonds, a wholesome snack food, for a more traditional, less nutrient dense snack food on total diet quality.

A 6-month randomized, controlled trial in 134 adults was conducted. Participants were randomly assigned to the almond or control treatment group based on their body fat distribution. Participants in the almond treatment group consumed 42.5 grams of almonds with their breakfast and as their afternoon snack every day, and were instructed not to consume any other nuts or nut products. Participants in the control treatment group continued their habitual breakfast and afternoon snack routines, but were instructed not to consume any nuts or nut products. Body composition was measured and blood samples were collected for determination of fasting glucose, insulin, HOMA-IR, HOMA-%β, HbA1c, triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, and meal stimulated glucose and insulin at months 0 and 6. Appetite and dietary intake data to assess total diet quality were collected at months 0, 2, 4 and 6 as was a blood sample for compliance testing. Body weight was measured every 2 weeks. An intention-to-treat and complier linear mixed model analysis with Bonferroni correction for pairwise comparisons was performed.

The findings from this dissertation research demonstrate that compared to participants in the control treatment group who consumed their habitual breakfast and afternoon snacks without nuts or nut products, consumption of almonds every day for 6 months has no effect on body weight. However, participants with high android subcutaneous adipose tissue in the almond treatment group had a decrease in android fat mass percentage and an increase in android lean mass percentage, and tended to have an attenuation in gain of visceral adipose tissue mass compared to participants with high android subcutaneous adipose tissue in the control treatment group, but there were no effects in participants with high android visceral adipose tissue or high gluteal femoral adipose tissue. Participants who consumed almonds for 6 months had a higher seafood and plant protein score and fatty acid scores compared to the participants in the control treatment group, and total diet quality, measured by total HEI score, increased during the intervention compared to baseline in participants in the almond treatment group, but total diet quality was not higher in participants in the almond treatment group compared to participants in the control treatment group over time. There were no differences in appetite, fasting or meal stimulated glucose and insulin, HbA1c, fasting triglycerides, LDL cholesterol, HDL cholesterol, or HOMA-IR and HOMA-%β between participants in the almond and control treatment groups for any body fat distribution. Thus, testing people with different body fat distributions may not explain the mixed evidence of almond consumption on HbA1c.

CHAPTER 1. LITERATURE REVIEW

1.1 T2DM Prevalence

Despite a 35% decline in new cases since 2008 and stabilizing prevalence of type 2 diabetes (T2DM) in the United States, the prevalence of T2DM is still high (1). 34.2 million Americans, or 10.5% of the population had diabetes in 2018, with an estimated 7.3 million Americans having undiagnosed diabetes, and an additional 88 million Americans have prediabetes (2). While many Americans live with diabetes, the disease is also a major killer, ranking as the 7th leading cause of death in the United States in 2017. Therefore, an active pursuit of preventative and management approaches of T2DM and its risk factors is warranted.

1.2 Risk Factors for T2DM

1.2.1 Glycemia and Insulinemia as Risk Factors for T2DM and Chronic Disease

T2DM is characterized by having elevated glycemia and insulin resistance (3). A fasting blood glucose concentration greater than 126 mg/dL, or an oral glucose tolerance test blood glucose concentration over 200 mg/dL after two hours, or a blood glucose concentration of 200 mg/dL taken at any random time, is indicative of T2DM. However, these measurements reflect plasma glucose in the moment it is measured, and can be affected by when the person last exercised, when the person ate last, how long they have been fasting, what their last meal was composed of, medications they are taking, the time of day, and/or the status of their home glucose meter. A long-term measure of blood glucose concentration, glycated hemoglobin (HbA1c), is an indicator of average blood glucose over the past three months. HbA1c is used (either alone or in combination with fasting or postprandial blood glucose concentrations), to diagnose, treat, and monitor diabetes and glycemia. A HbA1c of less than 5.7% is normal, between 5.7% and 6.5% is indicative of prediabetes, and above 6.5% is indicative of diabetes.

The American Diabetes Association has recommended HbA1c as a possible substitute for fasting plasma glucose for diagnosing T2DM (4). HbA1c concentrations are strongly correlated with fasting plasma glucose concentrations (5), provide a reliable measure of long term glycemia, and correlate well with risk of complications from diabetes, such as risk of cardiovascular disease and stroke, retinopathy, and microvascular complications, whereas fasting

plasma glucose is a poor predictor (4, 6, 7). However, the cutoff values for normal, prediabetes, and diabetes HbA1c are controversial. A HbA1c of 6.5% is reported to be more reliable for diagnosing diabetes because it is associated with fewer false-negatives than fasting plasma glucose (8). Since HbA1c increases with increasing blood glucose long term, assays are not affected by short term modifications in meals, stress, or exercise, as fasting plasma glucose or 2-hour oral glucose tolerance tests are. This makes HbA1c measurements more indicative of lifestyle in relation to glycemia. The biological variability of HbA1c is lower than that for fasting plasma glucose, so two measurements (as is recommended) provide more reliable information than two measurements of fasting plasma glucose (6).

Although HbA1c is a long-term measure of glycemic control and not acutely altered by elevations in glycemia, sustaining a diet that leads to an increase in fasting, post-prandial, and post-absorption blood glucose concentrations over time will affect HbA1c. Studies indicate that postprandial glucose has a greater effect on HbA1c than fasting glucose in individuals with T2DM (9, 10), especially in those whose diabetes is fairly well controlled (11). However, fasting blood glucose concentrations may contribute more to HbA1c in uncontrolled diabetes (11). Thus, consuming a diet rich in foods that elicit a low glycemic response, especially a low post-prandial glycemic response, could lead to lower HbA1c.

1.2.2 Blood Lipids and Risk for T2DM

Elevated blood lipid concentrations are also characteristic of T2DM. Desirable blood lipid concentrations are less than 200 mg/dL of total cholesterol, less than 100 mg/dL of LDL cholesterol, less than 150 mg/dL of triglycerides, and greater than 60 mg/dL of HDL cholesterol. Elevated blood triglyceride concentrations and low concentrations of HDL cholesterol are risk factors for diabetes (12). In addition, people with T2DM typically have smaller and denser LDL particles than those without T2DM because of their high triglyceride concentrations (13). Small, dense LDL particles are associated with increased risk for coronary artery disease (13). Thus, people with T2DM or those at risk must not only manage their risk factors for diabetes but also their blood lipid concentrations to prevent cardiovascular disease (CVD). CVD is the most common cause of morbidity and mortality in those with T2DM (14). Therefore, maintaining healthy concentrations of blood lipids or decreasing blood lipids can help prevent or manage T2DM and CVD.

1.2.3 Obesity and Body Fat Distribution as Risk Factors for T2DM

Having overweight or obesity is a risk factor for T2DM. Losing 5-10% of body weight through lifestyle interventions reduces T2DM incidence and can help improve HbA1c, blood pressure, and blood lipid concentrations (15). Even 1 kg of weight loss can lead to a 16% reduced risk of T2DM (16). Alternatively, even modest weight gain (greater than 5 kg) during adulthood can increase risk of T2DM (17), and 1 kg of weight gain can increase the risk of T2DM by 7.3% (18). Thus, losing weight or maintaining a healthy weight is one way to prevent T2DM.

In addition to and independently of body weight, different body fat distributions carry different risks for T2DM. A large android visceral adipose tissue (VAT) depot is consistently positively associated with insulin resistance, metabolic syndrome, T2DM, and CVD (19-21). However, a causal role is debated (22, 23), and whether insulin resistance causes VAT accumulation (reverse causation) cannot be ruled out (24). Whether large amounts of android subcutaneous adipose tissue (SAT) is associated with T2DM is disputed (25-30). A large gluteal femoral fat depot is not considered to be problematic and is consistently associated with insulin sensitivity (31-33).

There are several mechanisms by which large amounts of VAT may lead to hepatic dysfunction eventually resulting in T2DM. They may act alone but it is likely they act in combination (25). VAT is directly connected to the liver through the portal vein, rendering the liver vulnerable to its secretions (34). VAT is positively correlated with percentage of hepatic free fatty acid (FFA) delivery from VAT (35), VLDL production (36), liver fat (37), and gluconeogenesis (38). VAT is also more responsive to the lipolytic activity of catecholamines due to increased beta adrenergic and decreased alpha-2 receptor function (39), and less responsive to the antilipolytic effect on insulin due to reduced IRS-1 protein expression (40) than SAT, which also contributes to high hepatic FFA delivery. This results in dyslipidemia (41, 42) and hepatic insulin resistance (43). Additionally, macrophages may be trafficked into VAT in obesity, which secrete inflammatory cytokines, such as TNF-alpha and IL-6 that are positively associated with insulin resistance (44). TNF-alpha and IL-6 act in a paracrine manner on surrounding adipocytes, impairing insulin action and promoting the release of FFA (45). Inflammation and high FFA concentrations can impair insulin signaling by stimulating NFkB and AP-1 Fos/Jun pathways, activating serine kinases, Ikkb, and JNK proteins that reduce the

signaling of IRS in the insulin signaling pathway (45). In summary, the location of VAT and its secretion of FFA and inflammatory cytokines, likely contribute to the pathogenesis of VAT, particularly at the liver.

Large amounts of SAT may be protective or problematic compared to VAT. Evidence indicates that each one standard deviation (SD) increase in VAT increases the odds of being insulin resistant by 80%, whereas each one SD increase in SAT decreases the odds of being insulin resistant by 48% (46). Other work in Chinese adults noted that the odds ratio of newly diagnosed diabetes per one SD increase in SAT and VAT were 1.29 and 1.61, respectively, in men, and 1.10 and 1.56, respectively, in women (20). However, with adjustment for BMI and VAT, the effect of SAT on newly diagnosed diabetes disappeared in men and was reversed in women. VAT was still positively associated with newly diagnosed diabetes in men and women with adjustment for BMI and SAT. Other studies have reported that SAT is a moderately more robust correlate of insulin resistance than VAT, even after controlling for VAT mass (47). A negative correlation between SAT and glucose disposal rate was also observed in men, and this trended towards significance when controlling for total body fat (48). Notably, these studies used different methods of measuring body fat distribution and insulin resistance/T2DM and statistical methodology. It has been argued that controlling for body weight, BMI, or total fat mass leads to differences in SAT findings, and that these should not be controlled for because they are a proxy for SAT and therefore attenuate any effect on insulin resistance (28). Consistent methodology may yield more conclusive answers.

SAT may be protective not just due to its location, but also due to its metabolic activity. Unlike VAT, which drains directly to the portal vein, SAT drains into the systemic circulation through the vena cava (34). Thus, lipid drainage from SAT does not directly contribute to increased gluconeogenesis and VLDL production in the liver like VAT. Studies in mice indicate that SAT transplantation into VAT compartments decreases body weight, total fat mass, and improves glucose metabolism, whereas the reverse is not observed with VAT (49). SAT is less susceptible to lipolysis and is more responsive to the antilipolytic effect of insulin than VAT (50), and secretes and expresses more favorable adipokines, such as leptin and adiponectin, than VAT. Leptin and adiponectin are negatively correlated with BMI, fasting insulin, and HOMA-IR, and positively associated with HDL (51-53). Adiponectin increases fatty acid oxidation in the skeletal muscle which can improve insulin resistance (50). Leptin reportedly prevents

overproduction of insulin and hyperinsulinemia, promotes fatty acid oxidation, and increases glucose uptake in skeletal muscle (54). It has also been suggested that SAT acts as an 'energy sink' (55, 56), particularly by increasing the number of new adipocytes (57), to buffer excess energy from being deposited ectopically. Support for this hypothesis comes from studies in lipodystrophy, where animals (58) and humans (59) have insulin resistance. When adipose tissue is surgically implanted in lypodistrophic mice, there is an increase in insulin sensitivity in the liver and muscles (60). Additionally, thiazolidinediones, a drug used to treat T2DM, increases body weight and SAT by promoting differentiation of new adipocytes in SAT, but not VAT, by activation of PPAR-gamma receptors (61, 62), and improves insulin sensitivity (63). The exact mechanism of the protective role of SAT is unclear, however it is likely that the location, responsiveness to insulin, adipokines, and buffering capacity by increasing adipocyte number all contribute.

The potential size of the truncal SAT depot may make it problematic. VAT is a relatively small fat depot, while SAT can expand much larger. Due to the size, each contributes different amounts of FFA to the liver or systemically. VAT contributed 6±1%, 13±2%, and 17±2% of total FFA release in men and women who are lean, women with obesity, and men with obesity, respectively, whereas upper body SAT contributes more than 50% of total FFA release in men and women who are lean or have obesity (35). While there is a positive correlation between VAT mass and hepatic FFA delivery (35), adults with small amounts of VAT have as little as 5-10% hepatic FFA delivery from VAT lipolysis, and over 30% of hepatic FFA delivery stems from VAT lipolysis in those with larger amounts of VAT (35). Yet, even in adults with high VAT, 50-60% of hepatic FFA delivery comes from the systemic circulation (35). Thus, large amounts of SAT may still substantially contribute to systemic FFA concentrations.

Gluteal femoral fat is considered protective and associated with insulin sensitivity (64, 65). Similar to the protective mechanisms of SAT, gluteal femoral adipose tissue acts as an energy sink to store excess lipids until they are needed during energy deficit (66). Storage in this depot helps to prevent hyperlipidemia or ectopic fat deposition. Gluteal femoral adipose tissue has an even lower FFA flux than SAT and VAT (66). Gluteal femoral fat depots are also lost more slowly than abdominal depots during energy deficit (67), consistent with gluteal femoral fat depots having lower FFA flux. High gluteal femoral fat depots are also associated with lower

inflammatory markers than other fat depots (68), and higher serum adiponectin (69) and leptin (65), which are associated with insulin sensitivity.

There are various genetic and lifestyle factors positively associated with high VAT. Different genders and race/ethnicities are associated with having different body fat distributions. Men typically have higher android adiposity. Females typically have high gluteal femoral adiposity until menopause, where android adiposity becomes more common. The mechanisms for this redistribution are not clear, however it suggests that estrogen likely plays a role in the high gluteal femoral body fat distribution as opposed to VAT. Estradiol increases the expression of the antilipolytic α2A receptor in gluteal femoral adipose tissue but not VAT, which may promote the accumulation of gluteal femoral adipose tissue (70, 71). Activation of Estrogen Receptor alpha (ERα) could also stimulate adrenergic β receptors in VAT that promote lipolysis and decrease VAT (70, 71). Thus, it is presumed that lack of estrogen during menopause would not have this effect and lead to VAT accumulation. When estrogen is administered during hormone replacement therapy in menopausal women, there is a decrease in abdominal fat (72). White men and women have greater VAT compared to black men and women, but white women have significantly lower SAT than black women (73). Asians also tend to have higher android adiposity than Caucasians, with South Asians/Indians having higher SAT, and East and Southeast Asians have higher VAT (19). While genetics plays a large role in these differences, genetic predisposition may also interact with environmental, intrauterine, diet, and physical activity patterns to determine body fat distribution (19). Increased age is also associated with a redistribution in body fat from subcutaneous to visceral adipose tissue, independent of BMI (74). SAT preadipocytes replicate and differentiate more rapidly than VAT preadipocytes, and telomere length and the capacity of preadipocytes to express adipogenic transcription factors decreases the more the cell divides (75). Thus, the redistribution of body fat from SAT to VAT with age may be due to the greater decline in capacity for adipogenesis in SAT compared to VAT preadipocytes (75, 76). Thus, different body fat distributions contribute different risk for metabolic disease among genders and race/ethnicities. In addition, diet is associated with VAT mass. Fried foods and red and processed meat are positively associated with large amounts of VAT, whereas consumption of fruit, fermented dairy, whole grains, and fiber are negatively associated VAT when BMI is controlled (77, 78).

1.2.4 Diet as a Treatment for Obesity

Diet and lifestyle modifications are recommended to prevent and manage T2DM. While individual treatment plans are developed with a doctor or registered dietitian, often times, eating plans that result in an energy deficit in combination with enhanced physical activity to achieve at least a 5% weight loss are recommended. Commonly, there is an emphasis on appropriate portion sizes, consuming non-starchy vegetables, minimizing added sugars and refined grains, and choosing whole foods over highly processed foods as much as possible to improve body weight and glycemic control (79). Additionally, replacing saturated fat and foods higher in carbohydrates with unsaturated fats, and reducing sodium may help manage diabetes complications, such as elevated blood cholesterol concentrations and blood pressure (79). Diet and lifestyle changes may also reduce the need for medications if they are already prescribed (79).

Medication may also be prescribed in combination with diet and lifestyle modifications to reduce body weight. Dietary restrictions can help with weight loss initially, but is difficult to sustain long-term, as 95% of weight lost is regained within one year in most patients (80). For those who fail to achieve or sustain clinically significant weight loss, anti-obesity medications can be used in combination with lifestyle modifications to reduce body weight and T2DM risk (81). Current anti-obesity medications approved for long-term weight loss include Orlistat, Phentermine, Bupropion, Liraglutide, and Gelesis100 (81). Just as diet and lifestyle modifications require lifelong adherence to reduce body weight and maintain weight loss, medications also require lifelong use to prevent weigh regain (81).

1.3 Healthfulness of Almonds and their Potential to Prevent and Treat T2DM

Nuts are a nutrient dense food that can be incorporated into many dietary patterns (82) and are recommended by the Dietary Guidelines for Americans within calorie limits (83, 84). The Food and Drug Administration has a qualified health claim that "scientific evidence suggests but does not prove that eating 1.5 ounces per day of most nuts as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease." 1.5 ounces of almonds contains about 246 kcals, with 21.2 g of total fat, 13.4 g of monounsaturated fatty acids (MUFA) (predominantly oleic acid) and 5.2 g of polyunsaturated fatty acids (PUFA) (predominantly linoleic acid), 1.6 g

of saturated fat and no cholesterol, 9 g of protein, 9.2 g of carbohydrates, 5.3 g of fiber and 1.8 g of sugar, and many vitamins, minerals, phytosterols, and polyphenols, including 10.9 mg alpha-tocopherol, 114.8 mg magnesium, 204.4 g phosphorus, and 55.25 mg of beta-sitosterol (85). These bioactive nutrients, in addition to nutrients they may replace if substituted into the diet, may not only reduce the risk of heart disease, but they may also aid in a diet to prevent and treat risk factors associated with T2DM (82).

1.4 Almonds and Risk for T2DM

1.4.1 Observational Data on Tree Nuts (Including Almonds) and Peanuts

Epidemiological studies often group nuts together, rarely measuring a single nut or seed on outcomes. However, with few exceptions, nuts are more similar to each other in nutrient content and health outcomes than they are different. Thus, it is not expected that epidemiological outcomes would markedly differ between a combination of nuts and almonds alone.

Epidemiological studies report an inverse association between nut consumption and risk of T2DM, especially in women. This has been observed in the Nurses' Health Study (NHS) (86), with walnuts and total nuts in the NHSII (87), and with peanuts in the Shanghai Women's Health Study (SWHS) (88). An inverse association between nut consumption and risk of T2DM has also been observed in men and women in the MESA (89), and TLGS studies (90), while the PREDIMED study (91) reported an inverse association between nut consumption and the prevalence of T2DM, and NHANES (92) observed an inverse association with nut consumption and HOMA-IR in men and women. However, other studies have reported no association between nut consumption and risk of T2DM (93, 94). These associations are often made between the lowest and highest servings of nut consumption. While classifications vary between studies, those in the highest serving of nut consumption are still consuming a small portion of nuts, often far less than is provided during clinical trials (e.g. in (91), average intake was 0.48 and 25.48 g/d in those who consumed less than 1 serving of nuts/week compared to those who consumed more than 3 servings of nuts a week), and there are far less participants that fall in the highest category of nut intake compared to the lowest in observational studies.

1.4.2 Almonds and Glycemia and Insulinemia

Almonds may reduce the risk of T2DM due to their beneficial effects on postprandial glycemia. Compared to a control meal without almonds, almond consumption decreases postprandial glucose concentrations in both healthy adults (95, 96) and those with elevated glucose concentrations (97, 98). The timing, form, and dose of almonds have different effects on postprandial glycemia. In adults at risk for T2DM who consumed 43 g of almonds with breakfast or lunch, or as a morning or afternoon snack, almonds decreased blood glucose iAUC 60 minutes post almond consumption more when they were consumed as morning or afternoon snacks compared to no almond consumption at those times (99). Additionally, when whole almonds were consumed with breakfast, glucose concentrations at lunch and daylong blood glucose concentrations were lower compared to the control group, indicative of a second meal effect (98). Thus, almonds have the potential to exert the most pronounced effects on postprandial glucose concentrations when consumed with breakfast or as a snack. In the same study, the form of almonds on glycemia was tested. Mean daylong blood glucose concentrations were lower after a test breakfast with whole almonds compared to the breakfast with almond butter, defatted almond flour, or no almonds. After a standardized lunch, almond butter produced a greater glucose peak than whole almonds, almond flour, and the control group without almonds, and afternoon blood glucose concentrations were lower when whole almonds were consumed with breakfast compared to almond butter and almond oil. These results suggest that whole almonds are better than other forms at reducing postprandial blood glucose concentrations. Lastly, the dose of almonds may have different effects on blood glucose concentrations. Almonds decreased 2 hour post prandial blood glucose concentrations in a dose response pattern when 30, 60, and 90 g of whole almonds were consumed with 50 g of white bread, with 90 g of almonds decreasing postprandial glucose significantly compared to when 50 g of white bread was consumed without almonds (95). Additionally, a half-ounce (about 14g) of almonds consumed as an “appetizer” 30 minutes before a glucose tolerance test decreased blood glucose concentration post-prandially in adults with impaired glucose tolerance (97). Thus, while almonds reportedly decrease glycemia post-prandially in a dose respond manner, even a small amount of almonds can have an effect on moderating glycemia in those with elevated glucose concentrations. A higher dose of almonds may be required for decreasing postprandial glycemia in adults with controlled glycemia (100).

While the effect of almonds on lowering postprandial glycemia is apparent, the effects of almonds on other measures of glycemia are not as clear. Studies indicate almonds have no effect (100-105) or decrease (99, 106, 107) fasting glycemia, no effect on fasting insulin (99, 100, 102, 103), and increase (98) or decrease (96, 103) postprandial insulin.

Despite evidence that almonds decrease postprandial glycemia, which has a strong influence on HbA1c, the evidence for the effect of long-term almond consumption on HbA1c is inconsistent. All studies to date on the effects of almond consumption on HbA1c have been conducted in adults with prediabetes or T2DM. In a small pilot study in adults with T2DM who consumed 28 g (1 oz) of almonds or 2 cheese sticks 5 days a week for 12 weeks, HbA1c significantly decreased in participants in the almond group compared to participants in the control group (100). However, another study in adults with T2DM who consumed the NCEP Step II diet with almonds replacing 20% of energy (average 60 g of almonds) or the NCEP Step II diet without almonds for three months, there was no effect of almond consumption on HbA1c after three months compared to participants in the control treatment (103). However, in a subgroup analysis of participants whose baseline HbA1c was less than 8%, there was a significant decrease in HbA1c in participants in the almond treatment versus participants in the control treatment. Lastly, adults with prediabetes who followed an ADA diet for 16 weeks with 20% of energy from almonds (about 2 oz per day) did not have lower HbA1c compared to participants in the control group who followed the ADA diet without almonds after 16 weeks (105). Thus, almond consumption may be beneficial for lowering HbA1c in those with elevated but controlled concentrations, although this finding is not consistent.

There are multiple mechanisms by which consumption of almonds may moderate glycemia. The nutrient composition of almonds may decrease their post-prandial glycemic response. Almonds are naturally low in carbohydrates, and therefore do not elicit a large glycemic response. The magnesium content in almonds may have benefits for glycemic control, because magnesium is a cofactor in insulin-mediated glucose uptake (108). The phytochemical content in almond skin may also contribute to decreased glycemia post-prandially by inhibiting alpha-glucosidase in the intestine, thus interfering with the bioavailability of glucose (109-111). The phytochemicals, fiber, and fat in almonds may beneficially influence the gut microbiome by increasing alpha diversity (112) and butyrate producers (113) that may be associated with improved glycemia (112). Additionally, the fiber in almonds may disrupt the absorption of

carbohydrates in the small intestine, aiding in decreased post-prandial glycemia (114). In addition to their nutrients that can lower glycemia, almonds may be especially beneficial in lowering postprandial glucose concentrations by replacing foods in the diets that contribute to increased glycemia, such as foods high in sugar and saturated fat but low in fiber (115).

The rate of gastric emptying is another mechanism in which almonds may lower glycemia. A slower gastric emptying rate decreases the rate at which sugars are absorbed and elicit a post-prandial response. Almonds contain fat and protein, which promote the release of gut hormones GLP-1, CCK, and PYY, that delay gastric emptying (116). The release of GLP-1 can also increase post-prandial insulin concentrations because of its incretin effect to lower post-prandial glycemia (116).

Almonds contain plant protein. Moderate to high amounts of animal protein reportedly improve glycemic control in diabetic individuals, however whether plant protein or the protein content of almonds would have the same effect is not known (117, 118). Dietary protein also enhances insulin action by increasing insulin receptor mRNA expression in the liver and adipose tissue (119), and increases GLUT4 translocation (120).

Almonds are notoriously known for their high fat content, but their fat influences glycemic control acutely by its effect on gastric emptying and GLP-1 release, and chronically, by increasing membrane fluidity and insulin receptor number (121). The MUFA content in almonds can also help with glycemic control, and diets high in MUFA may lower HbA1c (122). The fat content, in combination with their phenolic content, also likely plays a role. When whole almonds, almond oil with defatted almond flour, or high oleic acid sunflower oil (as a control) were consumed in muffins as a test meal, post-prandial glycemia was significantly lower when participants consumed almond oil plus defatted almond flour compared to when participants consumed whole almonds or the control oil (123). The difference in post-prandial glycemia between participants consuming the almond oil plus defatted almond flour and control oil suggest that the effect on glycemia is not due to the lipids alone. The almond flour provided phenolic components from the almond skin that likely aid the lipids in decreasing post-prandial glycemia.

1.4.3 Almonds and Lipidemia

People with diabetes are at an increased risk of cardiovascular disease compared to those without diabetes (124). Nut consumption has been associated with lower risk of coronary heart disease (125, 126). This may be due to their effect on blood lipid concentrations. Consumption of tree nuts significantly decreases total cholesterol (TC) (127, 128), and LDL cholesterol (127, 128), LDL:HDL cholesterol (128), and TC:HDL cholesterol (128) especially at doses greater than 60g per day (127) and in those with elevated cholesterol (128). However, tree nut consumption did not significantly improve triglycerides or HDL cholesterol (127, 128). Different nuts may have different effects on blood lipids as well, although more comparison data are needed. In a meta-analysis that compared the effect of consuming walnuts, pistachios, cashews, hazelnuts, almonds, and a control for at least three weeks on triglycerides, TC, LDL and HDL cholesterol in healthy adults or those at risk for CVD, participants consuming the pistachio and walnut enriched diets had the most beneficial effects on triglycerides and TC, whereas participants consuming the pistachio enriched diet and the almond enriched diet had the most beneficial effects on LDL cholesterol (129).

The effects of consuming almonds on blood lipids are generally favorable. When consumed as part of an energy restricted diet, almonds decreased TG, TC, and TC:HDL in long (130) and short term trials (102, 106) in healthy adults compared to a nut free, energy restricted diet. However, there was no effect on LDL-cholesterol (102, 130), or almonds decreased LDL-cholesterol significantly less than a nut free, energy restricted diet (106). When almonds were added (131) or substituted (101, 132, 133)) in the diet, participants had decreased TC (101, 131, 134), and LDL cholesterol (101, 131, 132, 134), LDL:HDL (131), and TC:HDL (131), but there was not an effect of almond consumption on triglyceride concentrations (131, 135). While not consistent (103), the effect of almond consumption on blood lipids is primarily observed in those with elevated blood lipid concentrations (131-133) or risk of T2DM (134). A meta-analysis of five randomized controlled trials reported that almond consumption significantly decreased TC, but not LDL cholesterol, HDL cholesterol, triglycerides, or LDL:HDL (136), although there was a trend towards a significant reduction of LDL ($p=0.05$) and HDL ($p=0.08$) cholesterol with almond consumption. In a subgroup analysis of participants with hyperlipidemia, almonds significantly decreased TC, LDL, and HDL cholesterol, and in adults without T2DM, significantly decreased TC and LDL cholesterol. The authors concluded that their analysis “does

not support the ingestion of almonds solely for their lipid modifying effects", primarily due to the decrease in HDL in addition to TC and LDL cholesterol after almond consumption (136). Overall, while there are some inconsistencies, almond consumption generally has a beneficial effect on cholesterolemia.

Almonds may have a beneficial effect on blood lipid concentration due to their lipid profile. Almonds are low in saturated fat and high in unsaturated fat, especially MUFA. Normolipemic adults who replaced half of their habitual fat with either whole almonds or almond oil for six weeks had decreased plasma triglycerides, TC, and LDL cholesterol, and increased HDL compared to baseline, but there were no differences between those who consumed whole almonds compared to those who consumed almond oil, indicating that the fat component of almonds is responsible for the effects on blood lipids (137). Primarily, the high MUFA content of almonds is likely responsible for the beneficial effect on blood lipids (133). Compared to consumption of an isocaloric, weight maintenance diet high in carbohydrates, consumption of a high MUFA diet resulted in lower fasting plasma triglycerides, TC, and VLDL cholesterol, and increase HDL cholesterol, with no effect on LDL cholesterol in short term trials (138). Additionally, when a high or low fat diet with 10% of fat from almonds or olive or canola oil (high in MUFA) was consumed in adults with T2DM, there was no effect of fat source on TC, triglycerides, or LDL:HDL cholesterol, suggesting that almonds are similar to other high MUFA sources on blood lipids (101). However, there was a main effect of fat source on HDL cholesterol, such that consuming a diet rich in almonds resulted in lower HDL cholesterol compared to the control diet rich in olive or canola oil, and trends toward a significant main effect of fat source on LDL and TC:HDL cholesterol, with participants consuming the almond enriched diet having lower LDL cholesterol and higher TC:HDL cholesterol compared to participants consuming the control diet rich in olive or canola oil. In a systematic review and meta-analysis of randomized controlled trials and cohort studies investigating the effects of a high MUFA diet on cardiovascular disease and diabetes risk factors, consuming a MUFA rich diet increased HDL cholesterol and decreased TG, but there were inconsistent effects of consuming a MUFA rich diet on total and LDL cholesterol (122). However, no detrimental effects on blood lipids were observed following consumption of a MUFA rich diet. The MUFA may change the composition of VLDL and the expressed activities of enzymes and proteins involved in intravascular processing and catabolism of VLDL, which would decrease plasma

triglyceride concentrations (139). Consuming a high MUFA diet may also decrease hepatic apo C-III messenger RNA abundance, which is located on chylomicrons and VLDL, as a result of decreased production of VLDL (139, 140).

While MUFA are the predominant fat in almonds, there are also PUFA, mostly omega-6 and 9 fatty acids. The evidence for replacing sources of saturated fat with n-3 PUFAs for a reduced risk of CVD is stronger and more consistent than that for MUFA (83, 122). However, the evidence on the effect of n-6 PUFA's on blood lipids is also inconsistent (141). Thus, while the PUFA content in almonds likely aids in their beneficial effects on blood lipids, it is not a primary mechanism.

The phytosterols in almonds may play a role in their lipid lowering effect, by multiple mechanisms. Phytosterols decrease cholesterol absorption and increase cholesterol excretion (142), thus leading to decreased LDL cholesterol concentrations in the blood (143, 144). This may occur because phytosterols compete for incorporation into the mixed micelles in the gastrointestinal tract (145), thus displacing absorption of dietary cholesterol, leading to unabsorbed dietary cholesterol and biliary cholesterol that is not reabsorbed. The resulting decreased cellular cholesterol concentrations activate SREBP2, which upregulate the expression of the LDL receptor to decrease blood lipid concentrations (143, 146).

The fiber content of almonds may have direct and indirect effects on blood lipids. The small amount of soluble fiber in almonds could decrease LDL cholesterol by increasing the resistance to bulk diffusion in enterocytes, therefore increasing bile acid and cholesterol excretion (143, 147). The insoluble fiber in almonds may decrease fecal transit time in the intestine that decreases the absorption of nutrients (114). Indirectly, the insoluble fiber may contribute to decreased blood lipid concentrations through its effect on appetite, leading to decreased food intake. Insoluble fiber can increase the bulk and weight of the fecal bolus, causing gastric distension and increased satiation, which may decrease energy intake from other food sources that could contribute to increased cholesterol concentrations (143, 148).

Lastly, the protein and arginine content of almonds may also aid in lipid lowering effects. Consuming a diet low in saturated fat and cholesterol and rich in protein resulted in lower LDL cholesterol concentrations compared to consuming a diet rich in carbohydrates (149). Arginine, an amino acid high in plant protein, may also have beneficial effects on LDL cholesterol when substituted for animal protein sources high in lysine (143). Thus almonds, a rich source of plant

protein and arginine, may be especially beneficial for LDL cholesterol when they replace carbohydrates or animal sources of lysine in the diet.

1.4.4 Almonds and Body Weight

Despite their high fat content, studies indicate that higher nut consumption is inversely associated with body weight or associated with less weight gain over time (150). In the EPIC-PANACEA study, participants with the highest quartile of nut intake had less weight gain over the 5 year follow up and a 5% lower risk of becoming overweight or obese compared to non-nut consumers (151). In the NHSII, women who reported consuming nuts ≥2 times a week had lower mean weight gain than women who rarely ate nuts (152). Randomized controlled trials also indicate that consuming almonds does not lead to weight gain (103, 105, 107), and that consuming almonds in a hypocaloric diet can help with weight loss (102).

One reason almonds do not promote weight gain is because their energy is inefficiently absorbed. Atwater factors, which are used to calculate metabolizable energy (ME) and are displayed on the nutrition facts panel, overestimates the ME of whole natural almonds, whole dry roasted almonds, and chopped roasted almonds by 25%, 19%, and 17% (153). The ME of almond butter is not significantly different from the ME calculated by Atwater factors. Mastication ruptures parenchymal cells of almonds, making lipids bioaccessible. Raw almonds require a substantial amount of mastication to form a bolus that is able to be swallowed. The size of the particles following mastication influence their nutrient bioaccessibility and bioavailability downstream in the gastrointestinal (GI) tract (154, 155). When almonds are masticated into smaller particles, there are more fractured cells and therefore greater lipid release than when almonds are masticated into larger particles (155). Cell walls, which are the main source of dietary fiber in almonds (156), provide a barrier to nutrient release in the GI tract and therefore regulate bioaccessibility (123, 157). The lipids of almonds are stored within the cell walls, and become bioaccessible as a result of mastication, causing fissures in the cell walls. When cell walls are ruptured, the lipids within the cell diffuse out and become bioaccessible and bioavailable (157). Even when cells are not fully ruptured from mastication, some cells are fractured, providing digestive enzymes (i.e. lipases) access to the lipids within the cells. However, previous studies have indicated that many cells in whole, raw almonds are not ruptured or fractured during mastication (154). When raw almonds were masticated, only the cells at the

surface of the almond (directly exposed to the oral cavity) were ruptured. Most of the cells in the almond remained intact after mastication and through the GI tract; thus, the lipids encapsulated within the fiber of cell walls are not bioaccessible in the GI tract (154). Processing almonds (e.g. almond oil, butter, roasted, sliced almonds) affects cell rupture from mastication and ultimately their lipid bioaccessibility. Each form requires different amounts of mastication, and thus produce varying amounts of ruptured cells (155). Some of the processing techniques directly cause fractures in the cell walls of the almonds: lipids are more bioaccessible as oil and butter compared to when the cells are intact in roasted, sliced, or raw nuts. This is one reason the calculated ME of almond butter is closer to that predicted by Atwater Factors: the lipids are more freely bioavailable. When mastication does not fracture cells of almonds, they travel in-tact through the GI tract and are excreted in the feces. Scientists have also identified the same lipid droplets in an almond prior to being eaten as in the feces after it was consumed (158) to confirm that the lipids were indeed from the almond. Clinical studies observe an increase in fecal lipid concentration in participants on an almond rich diet compared to participants on a control diet without nuts. They quantified the lipid in the feces to be 21.1 ± 14.4 g in those consuming an almond rich diet, compared to 2.8 ± 1.5 g in those consuming a control diet without nuts (158). Fecal fat excretion contributes to the overestimation of ME by Atwater factors, and is one mechanism why nuts do not cause weight gain: even though nuts contain a lot of energy, especially due to their high fat content, a portion of the fat travels out of the body in the feces, and thus no energy from the lipids is absorbed.

Another reason almonds do not lead to weight gain is that they are highly satiating. Although not consistently (98, 99, 159), consumption of almonds results in decreased feelings of hunger (102), increased feelings of fullness (98), and decrease desire to eat and prospective consumption ratings (102). The effects of almonds on satiation and satiety can lead to spontaneous energy compensation from other energy sources throughout the day. When women were provided 344 kcals of almonds to consume each day for ten weeks but were not given advice on how to incorporate them into their diet, there was no change in body weight compared to when the women followed their customary diet (160). Body weight was not different primarily because the women compensated for the energy they consumed in the almonds by decreased energy intake from other food sources.

There are many properties of almonds that contribute to their effects on appetite. First, whole almonds require mechanical processing through mastication to break apart the almond into particles small enough to be swallowed. When whole almonds are consumed there are higher daylong fullness ratings compared to when processed almond forms, such as almond butter, flour, and oil are consumed (98). However, there is a higher expected satiation with whole nuts, thus a cognitive effect is also likely (161). Second, the mastication of almonds leads to the release of lipids and proteins from the cells, which lead to the release of gut hormones GLP-1, CCK, GIP, and PYY from the small intestine. While these hormones reportedly aid in satiation and satiety, it is also believed that they simply aid in the digestive process of these macronutrients due to their minimal effects on appetitive sensations at physiological levels. In any case, studies find that almond consumption does not increase GLP-1 or GIP compared to a control (98, 100), therefore the gut hormones likely don't play a large role in the appetitive effects of almonds. Lastly, the nutrient profile of almonds likely contributes to their effects on appetite. Almonds contain 3.5 g of dietary fiber per one ounce serving (162), which can increase GI transit time, and thus increase the duration at which satiety signals are sent to the central nervous system (163). Almonds are also rich in plant protein, the reportedly most satiating macronutrient (164), with 6 g per one ounce serving (162). Consuming snacks with a high protein content reduces hunger, increase fullness, and delays subsequent eating events compared to consuming lower protein snacks (165). Lastly, almonds contain 8.8 and 3.4 g of MUFA and PUFA per one ounce serving, respectively (162). Unsaturated fatty acids are more readily oxidized than SFA, and studies in mice suggest that fatty acid oxidation maintains satiety between meals and delays the onset of feelings of hunger in mice (166). Thus, the unsaturated fatty acid content of almonds could contribute to their effects on satiety, however studies testing nut loads of different fatty acid composition do not report different effects on appetite (167, 168). In sum, mechanical processing, the release of gut peptides, and their nutrient composition all contribute to the effects of almond consumption on appetite.

1.4.5 Almonds and Body Composition

There is mixed evidence of the role of almonds on body composition. However, their effect on body composition mimics changes in body weight. In studies where almond consumption did not change body weight, there were also no effects of almond consumption on waist

circumference (103, 169), waist to hip ratio (103), fat mass (135), fat free mass (135), abdominal fat (135), and percent body fat (103) compared to participants in the control group or compared to baseline values. However, most of these studies were of relatively short duration to observe changes in body weight and/or body composition (<12 weeks) (135, 169). In an 18-month study where adults consumed an almond enriched (56 g almonds/d) hypocaloric diet or a hypocaloric diet without almonds, participants in the nut-free group lost slightly but significantly more body weight than participants in the almond group at 6 months, but there were no differences in weight change after 18 months (130). There were also no differences in lean mass between participant groups at 6 or 18 months, and there was a trend towards significantly less fat mass in participants in the nut free group compared to participants in the almond group at 6 months, but not 18 months. In studies where almond consumption resulted in decreased body weight, there were also significant improvements in truncal fat, VAT, body fat percentage, fat mass, and waist circumference compared to baseline or the control group (102, 170). These studies were 12 weeks or longer. Interestingly, in a randomized crossover trial in adults with elevated LDL cholesterol, consuming 1.5 oz of almonds a day as a snack in a controlled diet for six weeks led to decreased abdominal fat and leg fat despite no differences in body weight compared to consuming a muffin as a snack after six weeks (132).

The MUFA content of almonds may be a mechanism for their improvements in body fat distribution. A randomized, crossover, controlled feeding study in adults with central obesity reported that participants consuming an isocaloric diet high in MUFA (canola oil and high oleic acid canola oil) decreased android fat mass in men, but not women, over 4 weeks of the intervention compared to adults consuming an isocaloric diet high in PUFA (a blend of flax and safflower oil) (171). In a randomized, crossover study in obese participants with insulin resistance, participants consuming a low fat-high carbohydrate diet for 28 days had reduced leg adipose tissue but elevated central adipose tissue compared to participants consuming an isocaloric high MUFA or high SFA diet for 28 days despite no changes in body weight (172). In another randomized, crossover trial, adults with T2DM consumed a low fat-high carbohydrate (23% energy from fat, 9% SFA, 9% MUFA, 4% PUFA, 49% energy from fiber rich carbohydrate) or a high MUFA modified fat diet (35% energy from fat, 10% SFA, 20% MUFA, 5% PUFA, 40% from carbohydrate) for three months each, with one month in between treatments (173). Participants in both treatments had a similar loss of body fat, but the upper

body to lower body fat ratio remained unchanged after consuming the high MUFA modified fat diet, whereas a disproportionate loss of lower body fat increased the upper body to lower bod fat ratio after consuming the low fat-high carbohydrate diet. This indicates that MUFA rich diets may decrease central adiposity compared to carbohydrate rich diets, suggesting that replacing carbohydrates with MUFA in the diet may be beneficial for decreasing central adiposity. The primary mechanism for this is greater fat oxidation rates and lipolysis due to activation of PPAR-delta and alpha receptors (172) and increased energy expenditure (174) in response to high MUFA diets (171). There is also evidence that MUFA may preferentially deposit in SAT, whereas saturated fatty acids preferentially deposit in VAT (171, 175, 176). However, as most studies report decreases in central adiposity, which consists of VAT and SAT, it is not clear if high MUFA diets decrease VAT specifically.

1.4.6 Almonds and Diet Quality

Nuts are an abundant source of nutrients, including MUFA, PUFA, protein, fiber, and vitamins and minerals (82). Substituting nuts into the typical American diet improves diet quality, as measured by the Health Eating Index (HEI), (177-180) which is a measure of diet quality used to assess how well a set of foods aligns with recommendations of the Dietary Guidelines for Americans (181). However, nuts themselves are used as a dimension in the HEI formula, falling under “seafood and plant proteins” and “total protein foods”. Therefore, consuming nuts, including almonds, even without any other alteration in the diet, improves diet quality based on HEI (180). In adults who substituted 1.5 oz of almonds per day into their diet for three weeks, HEI component scores for total protein foods, seafood and plant proteins, and fatty acids increased, whole fruit and intake of empty calories decreased, and total HEI-2010 score increased compared to adults in the control intervention who did not consume almonds (182).

An alternative method to using HEI sores to assess diet quality is to measure the intake of individual foods and nutrients. When adults substituted between meal snacks with tree nuts or almonds, solid fats, added sugars, dietary carbohydrate, and sodium all decreased in the diet, whereas oils, PUFA, alpha linoleic acid, MUFA, total fat, protein, potassium, magnesium, and dietary fiber all increased (177). This substitution significantly increased the overall diet quality, as measured by the HEI-2010 (177). In adults who substituted 1.5 oz. of almonds per day into

their diet for three weeks, energy intake, protein as a percent of energy, and fiber intake did not change in participants in the almond intervention, but carbohydrates as a percentage of energy intake and sodium consumption were lower and magnesium, vitamin E, monounsaturated fat and fat intakes were higher in participants in the almond intervention compared to participants in the control (182). NHANES 1999-2004 data indicate that tree nut consumers have greater intakes of total fruit, whole fruit, dark green/orange vegetables, whole grains, meat equivalents, nuts/seeds, and oils, and lower intake of total grains, meat/poultry/fish, solid fat and added sugars compared to non-consumers (183). This suggests that almonds may replace sources of carbohydrates, added sugars, solid fat, and sodium, or that those who consume almonds consume healthier diets in general than non-consumers.

1.4.7 Timing of Almond Consumption

Studies report that there may be an optimal time to consume almonds depending on the outcome of interest. For example, there was a trend toward a significant decrease in postprandial glycemia 60 minutes post snack in participants who consumed almonds alone as a morning or afternoon snack, compared to when participants did not consume any snack (99). However, there was no significant difference in glycemia 60 minutes post meal in participants when almonds were consumed with breakfast or lunch compared to the meal without almonds, suggesting that consuming almonds as snacks may confer special benefits for glycemic control. Another study reported that when almonds were consumed as a preload before meals for 16 weeks, there was a significant decrease in body fat mass and body fat percentage compared when a high carbohydrate control was consumed, but no significant difference from when almonds were consumed as a snack. There was also a significant decrease in visceral fat mass when almonds were consumed as a preload before meals for 16 weeks compared to when the control preload was consumed, and compared to when almonds were consumed as a snack (184). However, consuming almonds as a snack did not significantly decrease visceral fat mass or body fat mass compared to consuming the control, although there was a significant decrease in body fat percentage when almonds were consumed as a snack compared to when the control was consumed. Alternatively, when almonds were consumed as a snack, participants had a significant reduction in total cholesterol after 8 weeks compared to when participants consumed the control, but there was no significant difference compared to when participants consumed almonds as a

pre-load. LDL cholesterol concentrations were significantly lower in participants when almonds were consumed as a snack compared participants who consumed the control at 8 weeks, with no significant differences between LDL cholesterol when participants consumed almonds as a preload, but after 16 weeks there was a significant decrease in LDL cholesterol in participants when almonds were consumed as a snack compared to when participants consumed the control and when participants consumed almonds as a preload. Thus, one study reported that participants who consumed almonds as a morning or afternoon snack decreased postprandial glycemia compared to not eating a snack, while participants consuming almonds with a meal did not. The other reported that participants consuming almonds as a preload decreased VAT mass, body fat mass, and body fat mass percentage compared to participants in the control treatment; participants who consumed almonds as a snack decreased body fat percentage, and LDL cholesterol after 16 weeks compared to participants on the control treatment; and participants who consumed almonds as a preload or a snack decreased total and LDL cholesterol after 8 weeks compared to participants in the control treatment. These studies suggest that the optimal times to consume almonds for maximal benefits on glycemia and total and LDL cholesterol is as a snack, and as a preload for maximal benefits on VAT, however the underlying mechanisms are unknown. More studies need to be done to replicate and confirm these findings.

CHAPTER 2. STUDY AIMS AND HYPOTHESES

2.1 Study Rationale

The high incidence and prevalence of type 2 diabetes (T2DM) nationally and globally is well recognized (185, 186). The physical, emotional, and economic costs of T2DM require the active pursuit of preventive and management approaches to curb its adverse impacts. Although being overweight or having obesity is a risk factor for poor glycemic control that can lead to T2DM, not all fat depots pose the same risk. A large android visceral fat depot is strongly associated with insulin resistance and glucose dysregulation, independent of body weight (19), while a large android subcutaneous fat depot is less problematic than android visceral fat but still may carry some metabolic risk (25). A large gluteal femoral fat depot is not associated with metabolic risk (31).

Dietary interventions involving wholesome foods are the preferred route of management of T2DM because of the strong potential benefits, limited negative side effects, and such a diet may be sustainable. For example, almonds can be part of a wholesome diet to manage glycemia. Acute feeding trials indicate that almond consumption can lower the glycemic response to a meal (100), evoke a second meal effect (98), and help lower glycemia throughout the day, especially when they are consumed at breakfast or as an afternoon snack (98, 99). However, the literature is mixed with regard to the effect of almond consumption on HbA1c (100, 105), hampering the acceptance of a beneficial role for almond consumption on glycemic control. Why almond consumption improves postprandial glycemia, which contributes to HbA1c, but not HbA1c, is unknown. Testing people with different body fat distributions may be a reason for the inconsistent evidence on almond consumption on HbA1c. Different body fat distributions carry different risks for insulin resistance, independent of body weight, and thus may respond differently to dietary interventions. Thus, the purpose of this study is to determine whether body fat distribution plays a role in the physiological response to almond consumption and the mixed evidence of almond consumption on HbA1c.

2.2 Study Objectives

The main objectives of this dissertation are to:

- Determine the effects of long-term almond consumption in adults with different body fat distributions on body weight, body composition, and carbohydrate and lipid metabolism.

2.3 Study Aims and Hypotheses

The primary specific aims of this research are to:

1. determine the acute effect of almond consumption on the glycemic response to a meal tolerance test in adults with different body fat distributions
    a. Hypothesis: almond consumption will elicit a significant moderation of glycemia in individuals with high android visceral adiposity, an intermediate effect in individuals with a high android subcutaneous adiposity, and a limited effect in individuals with high gluteal-femoral adiposity compared to a control.
2. determine the chronic effect of almond consumption on fasting glucose, insulin, HbA1c, triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, body weight, body composition, appetite, and calculated HOMA-IR and HOMA-%β in adults with different body fat distributions.
    a. Hypothesis: Almond consumption will elicit a significant moderation of fasting glucose, insulin, HbA1c, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, calculated HOMA-IR and HOMA-%β in individuals with high android visceral adiposity, an intermediate effect in individuals with a high android subcutaneous adiposity, and a limited effect in individuals with high gluteal-femoral adiposity compared to a control. Substitution of almonds for other common snacks will decrease appetite and not promote weight gain.

The secondary specific aims of this research are to

1. determine the effect of substituting almonds, a wholesome snack food, for a more traditional, less nutrient dense snack food on total diet quality.
    a. Hypothesis: almond consumption will improve total diet quality compared to the control.

CHAPTER 3. METHODS AND EXPERIMENTAL DESIGN

3.1 Study Population

Participants were recruited by flyers, online advertisements, and social media advertisements from the greater Lafayette and Indianapolis, IN areas from August 2017 to October 2019 (Appendix A). Eligibility criteria included healthy men and women with a BMI of 27 kg/m^2 or greater, 18-60 years old, weight stable (±5 kg) for 6 months prior to the start of the study, non-smokers, not taking medication for diabetes, not allergic to tree nuts or peanuts, and regular breakfast and afternoon snack (weighted nutrient density score <8) consumers. Habitual nut consumption was not an exclusion criterion. Participants were randomly (random.org) assigned to treatment groups within body fat distributions. All procedures involving human subjects were approved by the Purdue University (PU) and Indiana University School of Medicine (IUSM) Institutional Review Boards. Informed consent was obtained from participants who met eligibility criteria (Appendix B). This study is registered in clinicaltrials.gov (NCT03236116).

3.2 General Protocol

This study was a 6-month randomized, controlled, parallel arm clinical trial. After the baseline appointment (month 0), participants were randomly assigned to the almond or control treatment based on their body fat distribution (BFD). Those in the almond treatment were provided about 21-gram packets of roasted, unsalted almonds to consume twice a day: once with their habitual breakfast, and once as their afternoon snack. The total amount of almonds consumed per day was 42.5 grams, based on the FDA's qualified health claim for nuts and coronary heart disease (187), which provided 270 kcals (Appendix C). Participants were instructed not to consume any other nuts or nut products throughout the study. Those in the control treatment continued their habitual breakfast and afternoon snack routines, and were instructed not to consume any nuts or nut products throughout the study. Approximately every two weeks, body weight was measured. At this time, subjects in the almond treatment were provided a two-week supply of almonds, and were reminded to consume them with their breakfast and as their afternoon snack, and not to consume any other nuts or nut products. Those

in the control treatment were reminded to continue their habitual breakfast and afternoon snacking routines, and not to consume any nuts or nut products. Blood samples for fasting and meal-stimulated glucose and insulin, and fasting HbA1c, HOMA-IR and HOMA-%B calculations, triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol, and body composition measurements were collected at months 0 and 6. Dietary recalls, red blood cells, and appetite measurements were collected at months 0, 2, 4, and 6.

3.3 Study Outcomes

The primary outcomes for this study were the acute effects of almond consumption on the glycemic response to a meal tolerance test, and the chronic effects of almond consumption on fasting glucose, insulin, HbA1c, triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, body weight, body composition, calculated HOMA-IR and HOMA%β in adults with BFD associated with different risks of insulin resistance and onset of diabetes. Other outcomes were the effect of substituting almonds for a more traditional, less nutrient dense snack food on total diet quality. We hypothesized that almond consumption will elicit a significant moderation of glycemia in response to a meal tolerance test, fasting glucose, insulin, HbA1c, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, and calculated HOMA-IR and HOMA-%β in individuals with high android visceral adipose tissue, an intermediate effect on outcomes in individuals with a high android subcutaneous adipose tissue, and a limited effect on outcomes in individuals with high GF compared to a control group with similar body fat distributions. We also hypothesized that substitution of almonds for other common snacks will not promote weight gain, and that almond consumption will improve total diet quality compared to the control treatment.

3.4 Assessment of Afternoon Snack

Study eligibility was based on self-reported customary snacking on items of low nutrient density. Snacks of low nutrient density were determined by an algorithm proposed by Arsenault et. al. (188), which assigns positive weighting factors for protein, fiber, calcium, unsaturated fat, vitamin C and negative weighting factors for saturated fat, added sugars, and sodium (Appendix D). The weighted nutrient density score (WNDS) cut-off was set at a value of 8.0, which is the

mean WNDS of normal weight, healthy adults in the NHANES 2005-2008 database. Those with an afternoon snack with a WNDS less than 8 were eligible for participation.

3.5 Assessment of Body Fat Distribution

BFD groups were determined by waist circumference to hip circumference ratio (WH) at the baseline appointment. WH was measured using a flexible tape measure. Waist circumference was measured around the smallest portion of the waist, and hip circumference was measured around the widest portion of the buttocks (189). Men and women who had a WH of <0.85 and <0.8, respectively, were grouped as having high gluteal femoral adipose tissue (high GF). Men and women who had a WH greater than 0.85 and 0.8, respectively, were grouped as having high android adipose tissue. Once 80 participants with high android adipose tissue completed the intervention, the visceral adipose tissue (VAT) ratio (VAT mass (g)/android fat (g)), determined by dual-energy x-ray absorptiometry (DEXA), was used to divide the group into subgroups having high android VAT (high VAT) and high android subcutaneous adipose tissue (high SAT). Those above the 50^{th} percentile of the VAT ratio were grouped as having high VAT, and those in the lower 50^{th} percentile of the VAT ratio were grouped as having high SAT for analysis. The rationale for this classification was based on a study by Kursawe et al 2010 (190).

3.6 Anthropometrics

Height was measured once at the baseline appointment using a portable stadiometer (PU: Seca, Chino, CA; Model 213 1821009; IUSM: Quick Medical Wall Mounted Stadiometer). Body weight and BMI were measured using a Body Composition Analyzer (PU: Tanita, Arlington Heights, IL; Model TBF-410; IUSM: Scale-Tronix) with participants wearing minimal, lightweight clothing. Body composition (total and regional) was assessed using DEXA in a Lunar DPX-IQ 240 densitometer (Version Encore GE 15, GE Healthcare, Madison, WI). CoreScan software was used to determine android visceral and subcutaneous tissue. The precision coefficient of variation (CV) is approximately 2% for the proportion of fat and approximately 1% for lean tissue mass.

3.7 Blood Assays

Participants arrived at the laboratory after an overnight fast of at least ten hours. An indwelling catheter was placed and fasting blood samples were collected 10 minutes after catheter placement. Next, the participant consumed an 8-ounce chocolate nutrition shake (Ensure Original, Milk Chocolate, Abbot Laboratories, Chicago, IL) (Appendix E) within ten minutes. 8 ml of blood was drawn at 10, 20, 30, 60, 120, and 180 minutes after completing consumption of the chocolate nutrition shake.

Fasting blood for HbA1c analyses was collected in an EDTA Vacutainer (PU: 2 mL EDTA vacutainer; IUSM: 6 mL EDTA vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ). Whole blood was aliquoted and stored at -80^0 Celsius until further analysis. Blood samples for a fasting lipid panel (triglycerides, total cholesterol, HDL cholesterol, calculated LDL cholesterol) and fasting and meal stimulated glucose and insulin concentrations were collected in a Serum Vacutainer (PU: 5 mL Serum Vacutainer; IUSM: 6 mL Serum Vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ). The blood samples were allowed to sit for at least 30 minutes to allow the serum to clot. Blood samples were then centrifuged (PU: 4,000 RPM at 4^0 Celsius for 8 minutes; IUSM: 4,000 RPM at room temperature for 20 minutes). Serum was removed and divided into aliquots. Aliquots were stored at -80^0 Celsius until further analysis. Fasting and meal stimulated blood samples for GLP-1 and GIP were collected in a cooled EDTA vacutainer, and DPP-IV inhibitor was added within 30 seconds of collection. The blood samples were immediately centrifuged (PU: 4,000 RPM at 4^0 Celsius for 8 minutes; IUSM: 4,000 RPM at room temperature for 20 minutes). Serum was removed and divided into aliquots. Aliquots were stored at -80^0 Celsius. Due to budget limitations, GLP-1 and GIP samples were not analyzed. HbA1c, glucose, triglyceride, total cholesterol, and HDL cholesterol were determined on a Roche COBAS Integra 400 Plus analyzer. The repeatability and precision CV of the assays are 0.8 and 1.3% for HbA1c, 0.7 and 1.3% for glucose, 1.9 and 1.6% for triglycerides, and 1 and 1.13% for HDL. Insulin was determined on a Roche Cobas e411 analyzer. The repeatability and precision CV is 1.9 and 2.6%. LDL cholesterol was estimated using the Friedewald formula [LDL (mg/dL) = (Total Cholesterol – HDL Cholesterol – Triglycerides)/5]. HOMA-IR and HOMA-%β were calculated using the formula [glucose (mg/dL)*insulin (uU/mL) / 405] and [(360*insulin (uU/mL) / (glucose (mg/dL) -63)], respectively.

The fasting compliance sample was collected in a 6 ml EDTA Vacutainer (Becton, Dickinson and Co., Franklin Lakes, NJ). Erythrocytes were separated from the plasma by centrifugation at 4000 RPM for 10 minutes at 4^0 Celsius. The plasma was removed and the erythrocytes were hemolyzed in deionized distilled water, followed by another centrifugation. This was repeated two additional times. The erythrocyte aliquots were then stored at -80^0 Celsius until further analysis.

3.8 Dietary Recalls

Dietary intake was assessed using the web-based "Automated Self-Administered 24-hour Dietary Recall" (ASA24-2016) system (National Cancer Institute, Bethseda, MD). Participants were asked to record dietary intake for three non-consecutive days that included two weekdays and one weekend day for better representation of habitual intake. Participants were allowed to choose the days they recorded their dietary intake, and were instructed to record all food and beverages that they consumed for each 24-hour dietary recall. The Goldberg formula was used to determine if reported energy intake was physiologically plausible (191) (Appendix F).

3.9 Appetite Sensations

Appetite sensations were collected during all waking hours for 24 hours using an online Qualtrics questionnaire that poses questions on 100 mm visual analog scales (VAS) including: "How strong is your feeling of hunger?", "How strong is your feeling of fullness?", and "how strong is your desire to eat?", all from "not at all" anchored at 0 mm to "extremely" anchored at 100 mm, "How much food could you eat right now?" from "not at all" anchored at 0 mm to "an extremely large amount" anchored at 100 mm, "how strong is your preoccupation with food?", "how strong is your desire to eat something salty?", "how strong is your desire to eat something fatty?", and "how strong is your desire to eat something sweet?", all from "not at all" anchored at 0 mm to "extremely" anchored at 100 mm (Appendix G). Participants were instructed to complete appetite logs on their computer, smartphone, or electronic device using a Qualtrics link provided on the hour, every hour while awake for a 24-hour period, once every two months during the intervention. The mean 24-hour appetite ratings (in mm) were used for analyses. Logs with less than 6 entries were considered missing data and imputed with group means.

3.10 Diet Quality

Dietary intake data from ASA24 was used to calculate HEI-2015 scores to assess diet quality in a sample of participants (n=39 and 32 for the ITT and complier analysis, respectively) (Appendix H). All participants were from the Purdue location. HEI-2015 scores were calculated from the ASA24 totals spreadsheet using a SAS code provided by the National Cancer Institute (192). Briefly, the SAS program reads the ASA24 daily totals data, creates additional required variables from the ASA24 totals data for the HEI score, calculates total food group and nutrient intake over all possible days reported per participant, and then runs the HEI-2015 scoring macro, which calculates intake density amounts and HEI scores. There are 13 HEI-2015 components, which sum to a maximum score of 100 points. Nine of the score components are recommended to be consumed adequately, while four score components are recommended to be consumed in moderation (Appendix I). The thirteen score components (maximum points) are total fruits (5), whole fruits (5), total vegetables (5), greens and beans (5), whole grains (10), dairy (10), total protein foods (5), seafood and plant proteins (5), fatty acids (10), refined grains (10), sodium (10), added sugars (10), and saturated fats (10).

3.11 Compliance

Participants in the almond treatment were considered fully compliant if they reported consuming almonds with their breakfast and as their afternoon snack, and did not consume any other nuts or nut products. Participants in the control treatment were considered fully compliant if they consumed breakfast and an afternoon snack, and did not consume any nuts or nut products. Participants in the almond treatment were considered partially compliant if they consumed almonds at the wrong time (not at breakfast or as afternoon snack), or only reported eating almonds once, and did not consume any other nuts or nut products. Participants in the control treatment were considered partially compliant if they consumed breakfast but not an afternoon snack, and vice versa, and did not consume any nuts or nut products. Number of fully and partially compliant days was divided by number of recalls (12). If the ratio was greater than 0.57, participants were included in the compliance analysis. If the ratio was <0.57, participants were excluded from the compliance analysis. The ratio of 0.57 was chosen as a comparison of compliance 4 out of 7 days a week (4/7=0.57).

Participants were told that they would receive additional compensation if blood samples taken every two months during the study confirmed compliance based on plasma vitamin E testing, although this was a ruse and not actually tested (Appendix B). This provided additional incentive for participants to comply with the intervention.

3.12 Red Blood Cell Membrane Fatty Acid Analysis

A subset of red blood cell (RBC) samples (n=39) were analyzed to determine the fatty acid composition of the membrane. All participants were from the Purdue location. 1 mL of saline was added to 0.5 mL of the RBC aliquot. 10 uL of this solution was transferred into an autosampler vial (9mm S/T Vial Clear, part number 29000-U, Cap 9mm blue screw, part number 5182-0723). 0.5 mL of saponification solution (0.25M KOH in 90% ethanol) and 10 uL of internal standard (0.01 mg/mL heptadecanoic acid in ethanol) were added to the vial. The vials were then capped, vortexed, and heated at 80^{O} Celsius for 45 minutes. Vials were vortexed every ten minutes. After 45 minutes of heat, 0.3 mL 1M HCL and 0.5 mL hexane were added to the vial. Vials were vortexed vigorously for 20 seconds and inverted three times. This was repeated two more times. Vials were then centrifuged at 700 X G for 5 minutes. After centrifugation, the hexane top phase was transferred to another autosampler vial, dried at 65^{O} Celsius, and re-suspended in 750 uL of LC-MS sample diluent (90% ACN with 0.5 mM Ammonium Acetate). The vials were capped, and stored in the dark at room temperature until LC-MS analysis. Fatty acid composition was analyzed using LC-MS.

3.13 Statistical Analyses

Baseline data was assessed using linear mixed model in SPSS (version 24) to determine the effect of treatment, BFD, and treatment*BFD. Baseline data was also assessed between participants who completed the study compared to those who dropped from the study. The linear mixed model in SPSS (version 24) was used to determine the effect of Drop, treatment*Drop, BFD*Drop, and Treatment*BFD*Drop. Baseline categorical outcomes were assessed using chi-squared tests. The alpha level was set at 0.05 for all analyses. Data are reported as means and standard errors unless otherwise stated.

An intention-to-treat (ITT) analysis was conducted on all participants who provided baseline data (n=134), with the overall mean for each dependent variable imputed for missing values. Another analysis was performed on participants who complied with the intervention (n=101). Only participants with HbA1c data at month 0 were included in the analysis (n=119 in ITT analysis, n=101 in complier analysis - some participants who dropped early in the study did not have their HbA1c sample analyzed). Linear mixed model was used to determine the effect of time, treatment (Tx), body fat distribution (BFD), Tx*Time, and Tx*BFD*Time interaction effects with age as a covariate on body weight and anthropometric variables from the DEXA scan, blood biochemistries (fasting glucose, insulin, lipids, HOMA-IR, HOMA-%β, and meal stimulated glucose and insulin iAUC) between 0 and 6 months, and on HEI score components, energy intake, and 24 hour appetitive sensations between months 0, 2, 4, and 6 using proc mixed in SAS (version 9.4). Treatment, BFD, and time were treated as fixed effects, and participants were treated as random effects repeated over time using a repeated covariance matrix. iAUC was calculated using the Trapezoidal method, with any values below baseline omitted. Additional linear mixed models were used to determine the effects of treatment and Tx*BFD on change values of body weight and anthropometric variables in SPSS (version 24). One linear mixed model analysis was conducted using proc mixed in SAS (version 9.4) to determine the effect of treatment, time, and Tx*Time interaction effects on RBC fatty acid composition and nutrient intake from the subsample of participants included in the RBC analysis. BFD was not used as a factor in this analysis due to no participants being in the Control, High VAT group. The overall mean was recorded for missing values. In each analysis, when main or interaction effects were significant, pairwise comparisons with Bonferroni correction was carried out.

When data were not normally distributed, extreme outliers (>3 times the interquartile range) were removed or data was transformed. 1 extreme outlier in the Almond, High VAT group was removed from the HbA1c ITT analysis. Fasting and meal stimulated glucose data was log transformed, and fasting insulin, meal stimulated insulin, and HOMA-IR data was square root transformed in the ITT analysis. Meal stimulated glucose was log transformed, and fasting insulin and HOMA-IR data was square root transformed in the complier analysis.

The sample size calculation for this study was based on a power analyses that indicated a sample of 40 per BFD would be sufficient to detect treatment effects equal to 0.4 standard deviations of the mean with 80% power.

CHAPTER 4. RESULTS

4.1 Participants

134 participants were enrolled in the study. 16 withdrew during the intervention (Table 1), primarily due to lifestyle factors such as not being able to make appointments. Attrition rates were 15.7% for the almond treatment group, and 9.2% for the control treatment group. There were no significant differences in attrition rates between the almond and control treatment groups. The baseline characteristics of participants are presented in Table 2. Participants were primarily female and Caucasian. BMI, body weight, total body fat percentage, total fat mass and total lean mass did not differ between groups ($p>0.15$). Those with high VAT were older than those with high SAT ($p<0.001$) and high GF ($p<0.001$), and those with high SAT were older than those with high GF ($p=0.049$) but there were no differences in age between treatment groups ($p>0.6$).

Table 1. Participant flow.

Screened (n=3642)		
Enrolled (n=134)		
Almond Treatment (n=69) High Android (n = 47) High GF (n = 22)	**Allocated**	**Control Treatment (n=65)** High Android (n=45) High GF (n=20)
Almond Treatment **Discontinued intervention (n=10)** High Android (n=7) High GF (n=3) (7 lifestyle, 2 illness unrelated to study, 1 pregnant)	**Follow-Up**	**Control Treatment** **Discontinued intervention (n=6)** High Android (n=5) High GF (n=1) (6 lifestyle)
Almond Treatment **Intention-to-treat analysis (n=69)** High VAT (n=24) High SAT (n=23) High GF (n=22) **Complier Analysis (n=46)** High VAT (n=15) High SAT (n=14) High GF (n=17)	**Analysis**	**Control Treatment** **Intention-to-treat analysis (n=65)** High VAT (n=22) High SAT (n=23) High GF (n=20) **Complier Analysis (n=55)** High VAT (n=20) High SAT (n=17) High GF (n=18)

High GF = high gluteal femoral adiposity; High VAT = high android visceral adipose tissue; High SAT = high android subcutaneous adipose tissue. Lifestyle = dropped out due to time constraints or unwillingness to continue intervention.

Table 2. Baseline characteristics.

	High VAT		High SAT		High GF		P-Value		
Means ± SE	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Tx *BFD
N	22	24	23	23	20	22			
Dropped Out	2	3	3	4	1	3	0.348	0.686	0.872
Sex, n (%)							0.686	0.147	0.872
Men	6 (27.3)	9 (37.5)	3 (13)	4 (17.4)	6 (30)	5 (22.7)			
Women	16 (72.7)	15 (62.5)	20 (87)	19 (82.6)	14 (70)	17 (77.3)			
Race							0.057	0.527	0.193
%Caucasian	86.4	70.8	82.6	39.1	70	68.2			
Age, years	45 ± 2^{A}	44 ± 2^{A}	36 ± 2^{B}	35 ± 3^{B}	29 ± 2^{C}	32 ± 2^{C}	0.828	<0.001	0.647
BMI (kg/m^2)	32.6±1.1	34.6±1.1	33.0±1.1	34.2±1.1	33.3±1.2	33.1±1.1	0.284	0.909	0.599
Body Weight (kg)	93.3±3.6	99.2±3.4	91.7±3.5	94.3±3.5	92.4±3.8	90.4±3.6	0.456	0.389	0.541
Total Body Fat (%)	44.0±1.1	44.5±1.0	45.6±1.3	45.4±1.2	42.9±2.2	42.1±2.2	0.894	0.155	0.905
Total Fat Mass (g)	39004±1361	41907±1902	39955±2221	42015±2186	39099±3512	37584±2708	0.552	0.506	0.619
Total Lean Mass (g)	50242±2278	52617±2504	47211±1490	50616±1716	49683±1505	50364±2001	0.185	0.436	0.79

Values are means ± SE. ITT Linear mixed model of main effects of Tx and BFD, and the interaction of Tx*BFD with Bonferroni post hoc comparisons when main effects were significant in SPSS. Attrition rates and sex differences were determined by Chi Squared. Letters indicate significant difference between body fat distributions ($p<0.05$).
VAT=android visceral adipose tissue, SAT=android subcutaneous adipose tissue, GF=gluteal femoral adipose tissue, Tx=Treatment, BFD=body fat distribution.

Sex, race, age, BMI, body weight, total body fat percentage, and total fat mass were not different between those who dropped, or between treatment*drop, BFD*Drop, or Treatment*BFD*Drop ($p>0.1$) (Table 3). There was a significant main BFD*Drop effect in total lean mass ($p=0.004$), where those with high VAT who dropped had a higher baseline lean mass than those with high VAT who did not drop ($p=0.008$). There was no significant Drop, Treatment*Drop, or Treatment*BFD*Drop effect on total lean mass ($p>0.05$).

Table 3. Baseline characteristics between completers and dropped participants.

	High VAT		High SAT		High GF		Total		P-value					
Means ± SE	Control	Almond	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Drop	Tx *Drop	BFD *Drop	Tx *BFD *Drop
N	22	24	23	23	20	22	65	69						
Completed	20	21	20	19	19	19	59	59						
Dropped	2	3	3	4	1	3	6	10						
Sex, n (%)									0.686	0.147	0.866	0.909	0.118	0.562
Men														
Complete	5	7	3	4	6	5	14	16						
Dropped	1	2	0	0	0	0	1	2						
Women														
Complete	15	14	17	15	13	14	45	43						
Dropped	1	1	3	4	1	3	5	8						
Race									0.057	0.527	0.61	0.293	0.307	0.396
%Caucasian														
Complete	85	66.67	80	36.84	68.42	73.68	78	59						
Dropped	100	100	100	50	100	33.33	100	75						
Age, years									0.353	0.002	0.144	0.319	0.813	0.33
Complete	46±2	44±2	37±2	35±2	29±2	33±2	37±1	37±1						
Dropped	32±7	47±6	30±6	38±5	28±10	22±6	30±5	36±3						
BMI (kg/m^2)									0.636	0.802	0.906	0.808	0.841	0.572
Complete	32.2 ±1.2	34.9 ±1.2	33.1 ±1.2	34.4 ±1.2	33.5 ±1.2	32.9 ±1.2	32.9 ±0.7	34.0 ±0.7						
Dropped	36.5 ±3.8	32.7 ±3.1	32.6 ±3.1	33.5 ±2.7	30.2 ±5.4	34.3 ±3.1	33.1 ±2.4	33.5 ±1.7						
Body Weight (kg)									0.662	0.124	0.73	0.961	0.254	0.862
Complete	92.0 ±3.8	98.5 ±3.7	92.9 ±3.8	95.4 ±3.9	92.9 ±3.9	91.1 ±3.9	92.6 ±2.2	95.0 ±2.2						

Table 3 continued.

Dropped	105.5 ±12.0	103.8 ±9.8	84.1 ±9.8	89.2 ±8.5	83.8 ±17	86.2 ±9.8	91.1 ±7.7	93.0 ±5.4						
Total Body Fat (%)									0.765	0.249	0.599	0.845	0.133	0.979
Complete	44.4 ±1.6	45.1 ±1.6	45.3 ±1.6	45.1 ±1.7	42.6 ±1.7	41.3 ±1.7	44.1 ±1.0	43.8 ±1.0						
Dropped	40.0 ±5.2	40.0 ±4.2	47.8 ±4.2	46.9 ±3.6	49.0 ±7.3	46.8 ±4.2	45.6 ±3.3	44.5 ±2.3						
Total Fat Mass (g)									0.836	0.945	0.962	0.863	0.914	0.889
Complete	38869± 2544	42229± 2482	40170± 2544	42328± 2610	39028± 2610	37286± 2610	39356± 1481	40614± 1483						
Dropped	40357± 8043	39651± 6567	38520± 6567	40531± 5688	40437± 11375	39473± 6567	39771± 5134	39885± 3630						
Total Lean Mass (g)									0.441	0.004	0.861	0.901	0.004	0.968
Complete	49008± 2044[a]	51353± 1995[a]	47983± 2044	51615± 2097	50086± 2097	51287± 2097	49026± 1190	51418± 1191						
Dropped	62577± 6464[b]	61473± 5278[b]	42062± 5278	45871± 4571	42031± 9141	44514± 5278	48890± 4126	50619± 2917						

Values are means ± SE. ITT Linear mixed model of main effects of Tx, BFD, drop, and the interaction of Tx*drop, BFD*drop, and Tx*BFD*Drop with Bonferroni post hoc comparisons when main effects were significant in SPSS. Sex differences were determined by Chi Squared. Different letters indicate differences in total lean mass between participants with High VAT who completed the intervention compared to participants who dropped. VAT=android visceral adipose tissue, SAT=android subcutaneous adipose tissue, GF=gluteal femoral adipose tissue, Tx=Treatment, BFD=body fat distribution.

4.2 Body Fat Distribution Classifications

Body fat distribution (BFD) groupings at baseline resulted in participants differing in the amount of android (abdominal), android visceral, android subcutaneous, and gluteal femoral adipose tissue (table 4). The 50th percentile of the VAT ratio in the high android fat group was 0.33, which indicates that more than 33% of the fat in the android region is visceral adipose tissue in participants in the high VAT group, whereas less than 33% of the fat in the android region is visceral adipose tissue in participants in the high SAT group. Android mass was higher in participants with high VAT compared to participants with high GF ($p=0.001$). Participants in the high VAT and high SAT groups had significantly more android fat mass compared to participants in the high GF group ($p<0.0001$ and 0.018, respectively). Participants in the high VAT group had a significantly higher VAT ratio and a significantly lower SAT ratio than participants in the high SAT ($p<0.0001$ for both) and high GF ($p<0.0001$ for both) groups, indicative of having a greater percentage of android fat mass being visceral adipose tissue compared to subcutaneous adipose tissue compared to other BFD groups. Participants in the high GF group had a significantly lower WH ratio compared to participants in the high VAT ($p<0.0001$) and high SAT groups ($p<0.0001$), indicative of having more adipose tissue around the hips compared to the android region compared to other BFD groups. There were no differences between treatments within each BFD group ($p>0.05$).

Table 4. Baseline Body Fat Distribution Classifications.

	High VAT		High SAT		High GF		P-value		
Means ± SE	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Tx *BFD
Android Mass (kg)	7.5 ± 0.3^{A}	8.2 ± 0.5^{A}	7.0 ± 0.3^{AB}	7.7 ± 0.3^{AB}	6.5 ± 0.4^{B}	6.5 ± 0.3^{B}	0.106	0.001	0.529
Android Total Fat Mass (g)	3777 ± 155^{A}	4432 ± 318^{A}	3523 ± 248^{A}	4078 ± 240^{A}	3102 ± 324^{B}	3024 ± 253^{B}	0.081	<0.001	0.333
Android Total Lean Mass (g)	3692±190	3764±200	3369±111	3555±127	3346±129	3414±138	0.39	0.066	0.91
Android VAT mass (g)	1679 ± 149^{A}	2047 ± 142^{A}	855 ± 145^{B}	1068 ± 145^{B}	783 ± 155^{B}	633 ± 148^{B}	0.224	<0.001	0.379
Android SAT mass (g)	2097±168	2385±161	2669±164	3010±164	2319±176	2392±168	0.077	0.003	0.839
VAT Ratio	0.44 ± 0.02^{A}	0.45 ± 0.03^{A}	0.24 ± 0.02^{B}	0.26 ± 0.01^{B}	0.23 ± 0.02^{B}	0.21 ± 0.02^{B}	0.824	<0.001	0.609
SAT Ratio	0.56 ± 0.02^{A}	0.55 ± 0.03^{A}	0.76 ± 0.02^{B}	0.74 ± 0.01^{B}	0.77 ± 0.02^{B}	0.79 ± 0.02^{B}	0.821	<0.001	0.609
WH Ratio	0.91 ± 0.01^{A}	0.91 ± 0.01^{A}	0.88 ± 0.01^{A}	0.90 ± 0.01^{A}	0.77 ± 0.01^{B}	0.79 ± 0.01^{B}	0.2	<0.001	0.563

Values are means ± SE. ITT Linear mixed model of main effects of Tx and BFD, and the interaction of Tx*BFD with Bonferroni post hoc comparisons when main effects were significant in SPSS. Different letters indicate significant difference between body fat distributions ($p<0.05$). VAT=android visceral adipose tissue, SAT=android subcutaneous adipose tissue, GF=gluteal femoral adipose tissue, Tx=Treatment, BFD=body fat distribution, WH = waist to hip circumference ratio.

4.3 Compliance

The compliance rates were higher for participants in the control treatment (85%) compared to participants in the almond treatment (67%) (p=0.016). There were 23 non-compliers in the almond treatment group, 9 with high VAT, 9 with high SAT, and 5 with high GF. There were 10 non-compliers in the control treatment group, 2 with high VAT, 6 with high SAT, and 2 with high GF.

4.4 Blood Biochemistry Results

4.4.1 Fasting Glucose, Insulin, HOMA-IR, HOMA-%β, HbA1c

4.4.1.1 ITT Analysis

Participants with high VAT had higher fasting glucose compared to participants with high SAT (p=0.015) (figure 1A), however this did not vary between treatments or between treatments at any time point (p>0.2) (table 5). There was a significant main time effect on fasting glucose, where participants had a higher fasting glucose at month 6 compared to month 0 (p=0.048), however this did not differ between treatments (p>0.2). Participants with high VAT had higher fasting insulin (p=0.017) (figure 1B) and HOMA-IR (p=0.021) (figure 1C) compared to participants with high GF, and participants in the almond treatment had higher fasting insulin (p=0.023) and tended to have higher HOMA-IR (p=0.054) compared to participants in the control treatment, however these did not differ at any time point (p>0.1). Participants with high SAT tended to have higher HOMA-%β compared to participants with high GF (p=0.051), but there were no differences between treatments or at any time point (p>0.1) (figure 1D). There was no significant Treatment, BFD, Time, Treatment*BFD, Treatment*Time, or Treatment*BFD*Time effect for HbA1c (p>0.3) (figure 1E).

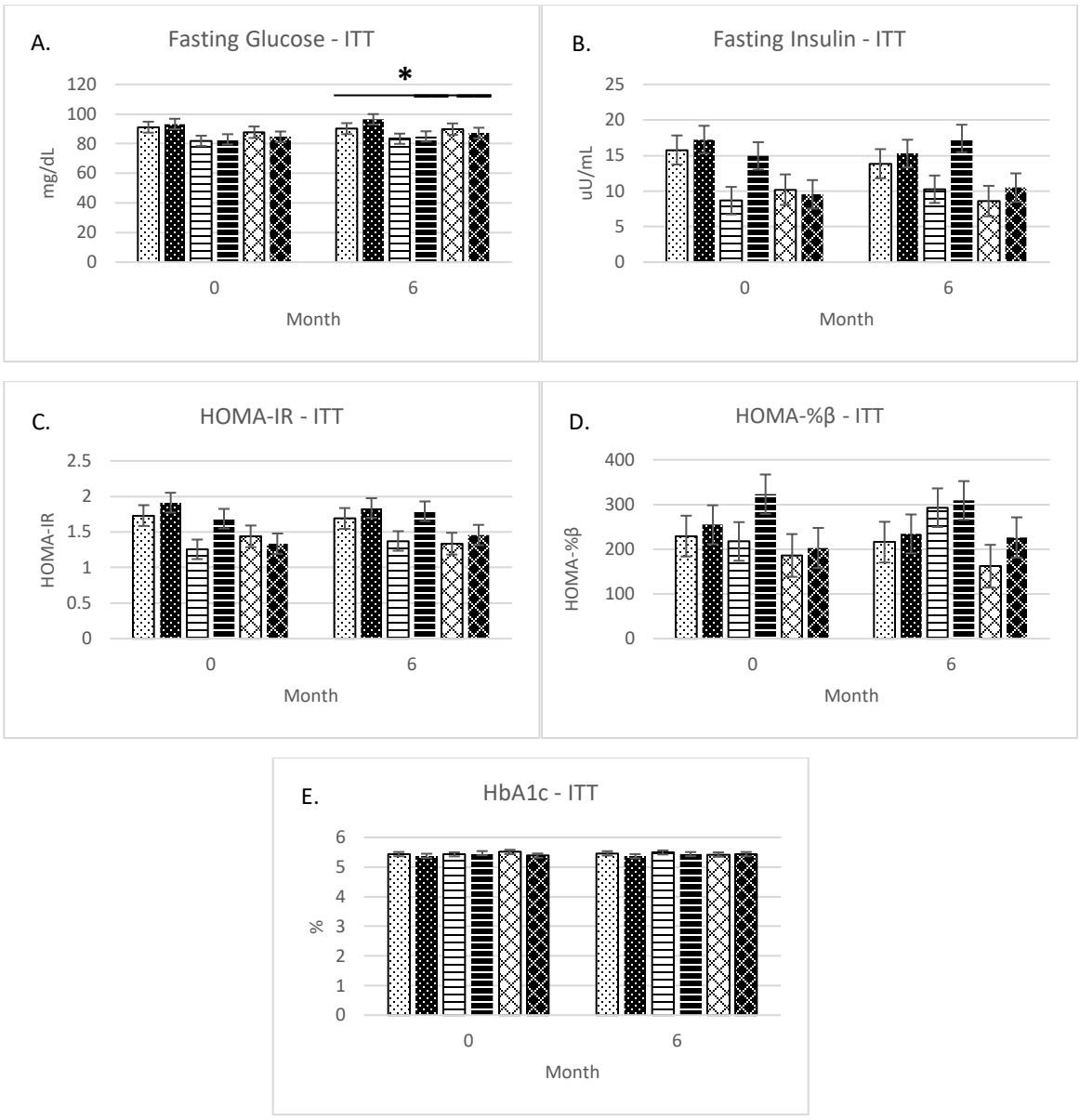


Figure 1. Blood biochemistry results at 0 and 6 months from the ITT analysis. A. fasting glucose, B. fasting insulin, C. HOMA-IR, D. HOMA-%B, E. HbA1c. *significantly higher compared to month 0 ($p<0.05$). Fasting insulin was significantly higher in participants in the almond treatment group compared to the control treatment group overall ($p=0.023$). Participants with high VAT had higher fasting glucose compared to participants with high SAT ($p=0.015$), and higher fasting insulin and HOMA-IR compared to participants with high GF ($p<0.03$). There were no other significant effects for fasting glucose, fasting insulin, HOMA-IR, HOMA-%B, or HbA1c ($p>0.05$). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

4.4.1.2 Complier Analysis

Participants with high VAT tended to have higher fasting insulin (p=0.069) (figure 2B) and HOMA-IR (p=0.078) (figure 2C) compared to participants with High GF (table 6). Participants in the almond treatment had higher HOMA-%β compared to participants in the control treatment (p=0.039), but this did not differ among BFD or at any time point (p>0.1) (figure 2D). There were no significant Treatment, BFD, Time, Treatment*BFD, Treatment*Time, or Treatment*BFD*Time effects for fasting glucose (figure 2A) or HbA1c (figure 2E) in the complier analysis (p>0.1).

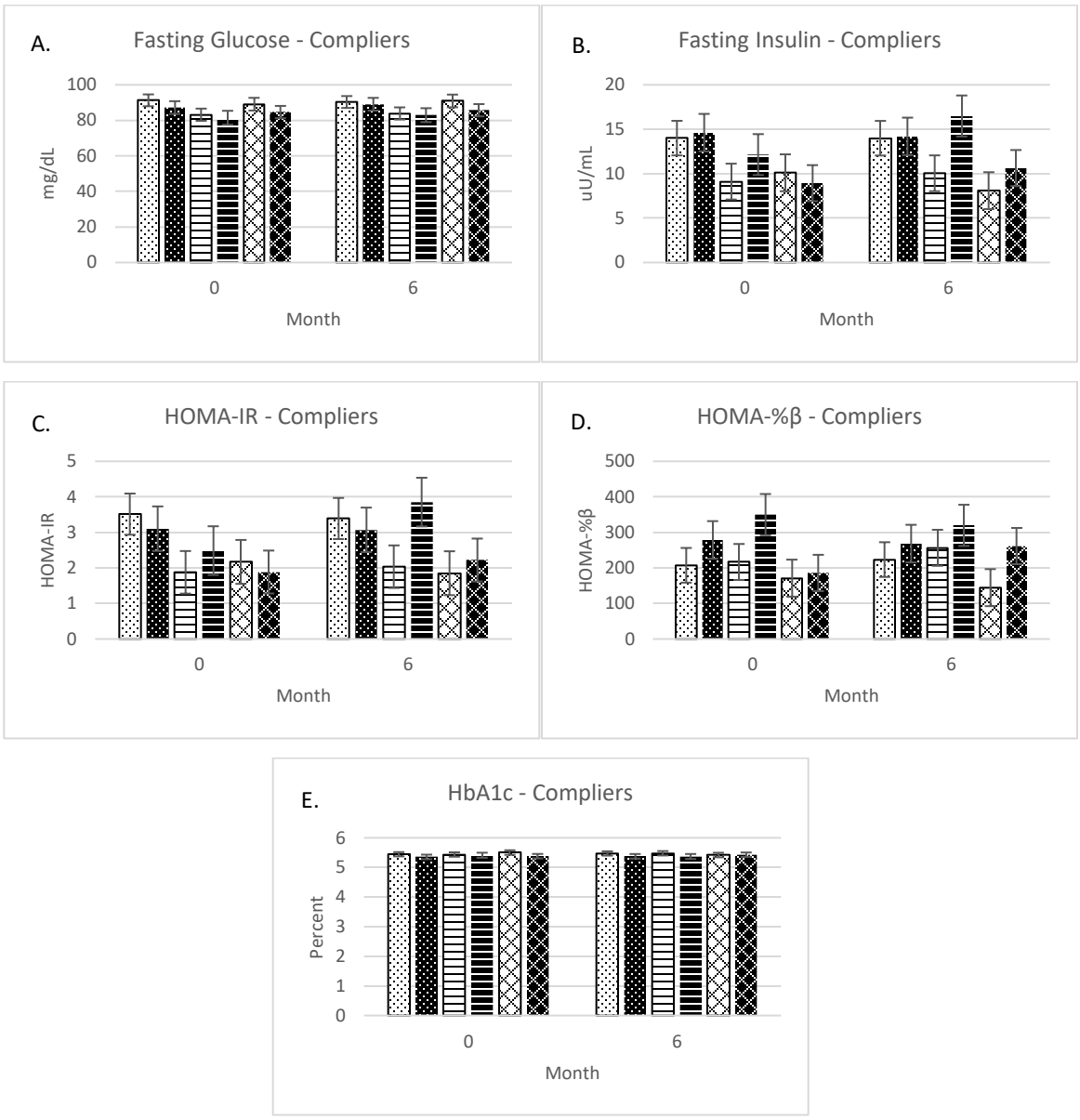


Figure 2. Blood biochemistry results at 0 and 6 months from the complier analysis. A. Fasting glucose, B. fasting insulin, C. HOMA-IR, D. HOMA-%B, E. HbA1c. Participants in the almond treatment group had significantly higher HOMA-%B scores overall compared to participants in the control treatment group ($p=0.039$). There were no other significant effects for fasting glucose, insulin, HOMA-IR, HOMA-%B, or HbA1c ($p>0.05$). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

4.4.2 Meal Stimulated Glucose and Insulin

4.4.2.1 ITT Analysis

Participants with High VAT had a higher iAUC meal stimulated glucose compared to participants with high SAT ($p=0.016$), but this did not differ between treatments or at any time point ($p>0.1$) (figure 3E) (table 5). Participants with high VAT and high SAT had higher iAUC meal stimulated insulin compared to participants with high GF ($p<0.001$) (figure 3F). Participants in the almond treatment had higher iAUC meal stimulated insulin compared to participants in the control treatment ($p=0.024$). Participants in the almond treatment tended to have higher iAUC meal stimulated insulin compared to participants in the control treatment at month 0 ($p=0.081$), however there were no differences at month 6 ($p>0.1$). There was a significant main time effect on meal stimulated glucose, where participants had lower meal stimulated glucose at month 6 compared to month 0 ($p=0.031$).

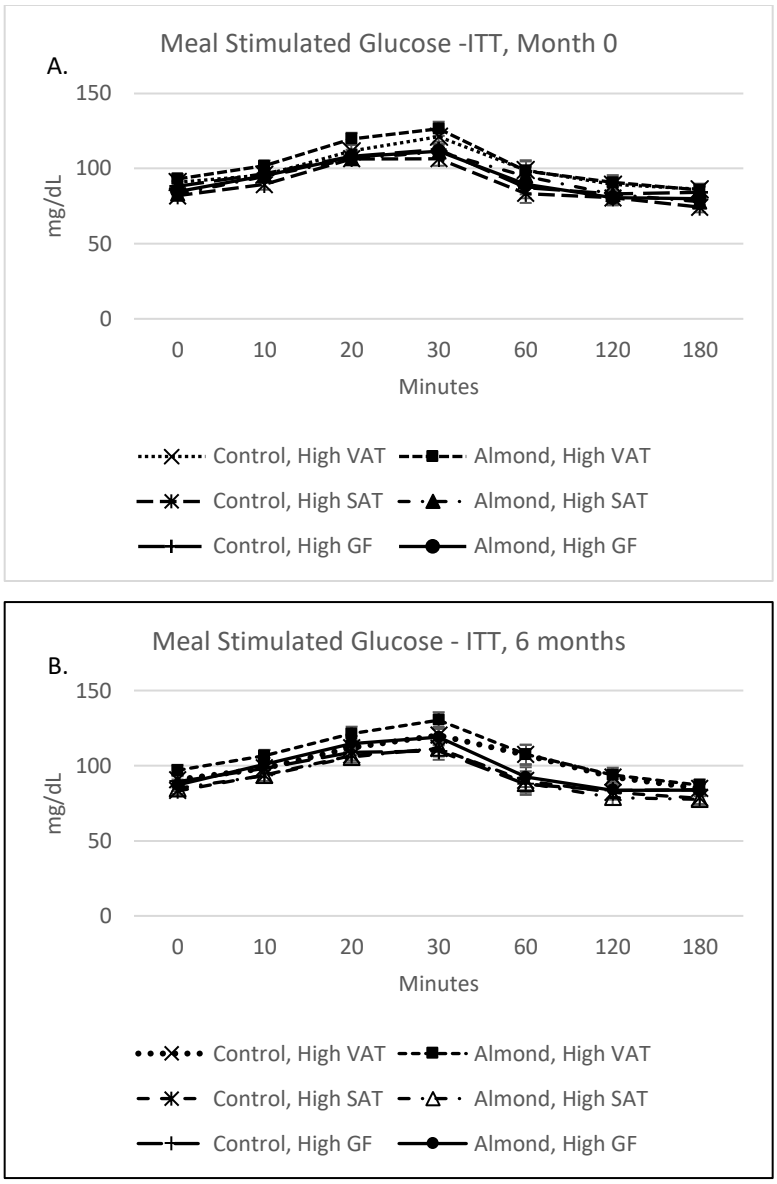
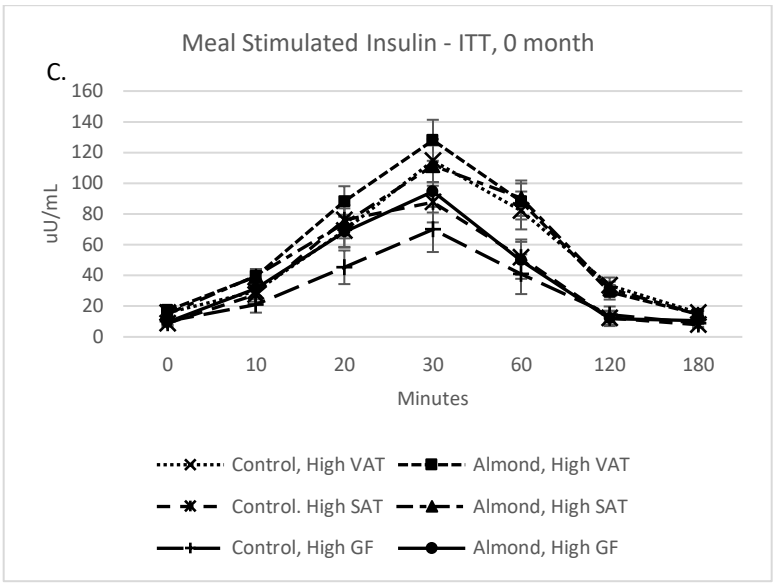
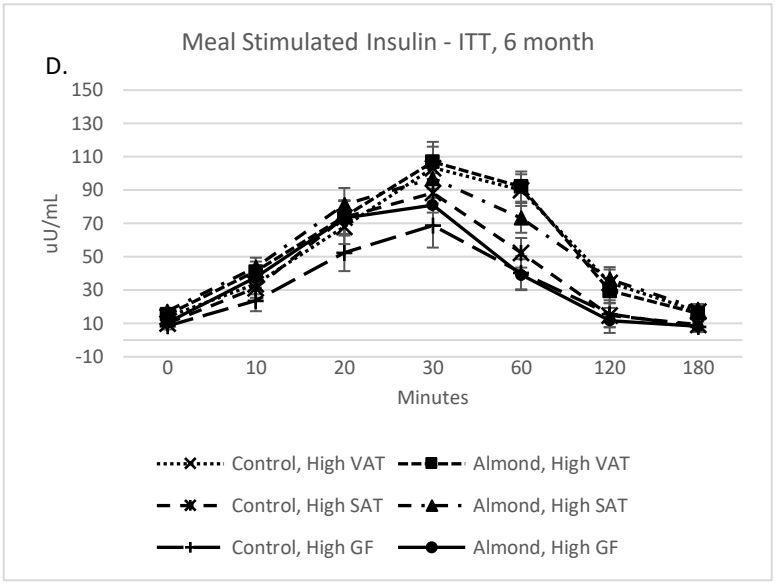
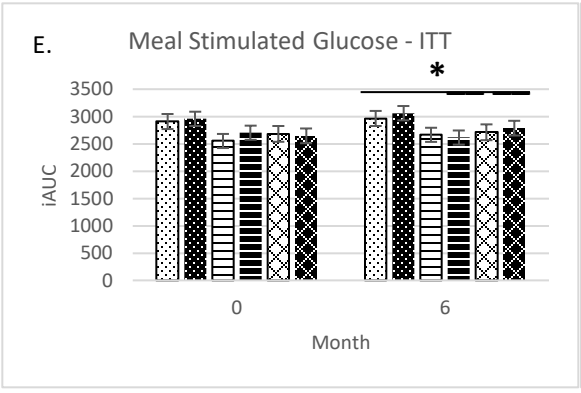


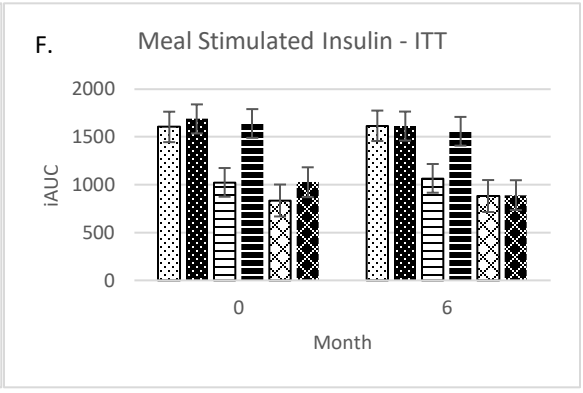
Figure 3. Meal stimulated glucose (A, B, E) and insulin (C, D, F) at 0 and 6 months from the ITT analysis. *significantly lower from month 0 ($p<0.05$). Participants with high VAT had significantly higher meal stimulated glucose compared to participants with high SAT ($p=0.016$), and participants with high VAT and SAT had higher meal stimulated insulin than participants with high GF ($p<0.001$). Participants in the almond treatment group had higher meal stimulated insulin compared to participants in the control treatment ($p=0.024$). There were no other significant differences ($p>0.05$). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

Figure 3 continued.









4.4.2.2 Complier Analysis

Participants with High VAT had higher iAUC meal stimulated insulin compared to participants with high GF ($p=0.002$) (figure 4F), and tended to have higher iAUC meal stimulated glucose compared to participants with high SAT ($p=0.052$) (figure 4E). There were no other significant Treatment, Time, Treatment*BFD, Treatment*Time, or Treatment*BFD*Time effects ($p\geq0.09$) (table 6).

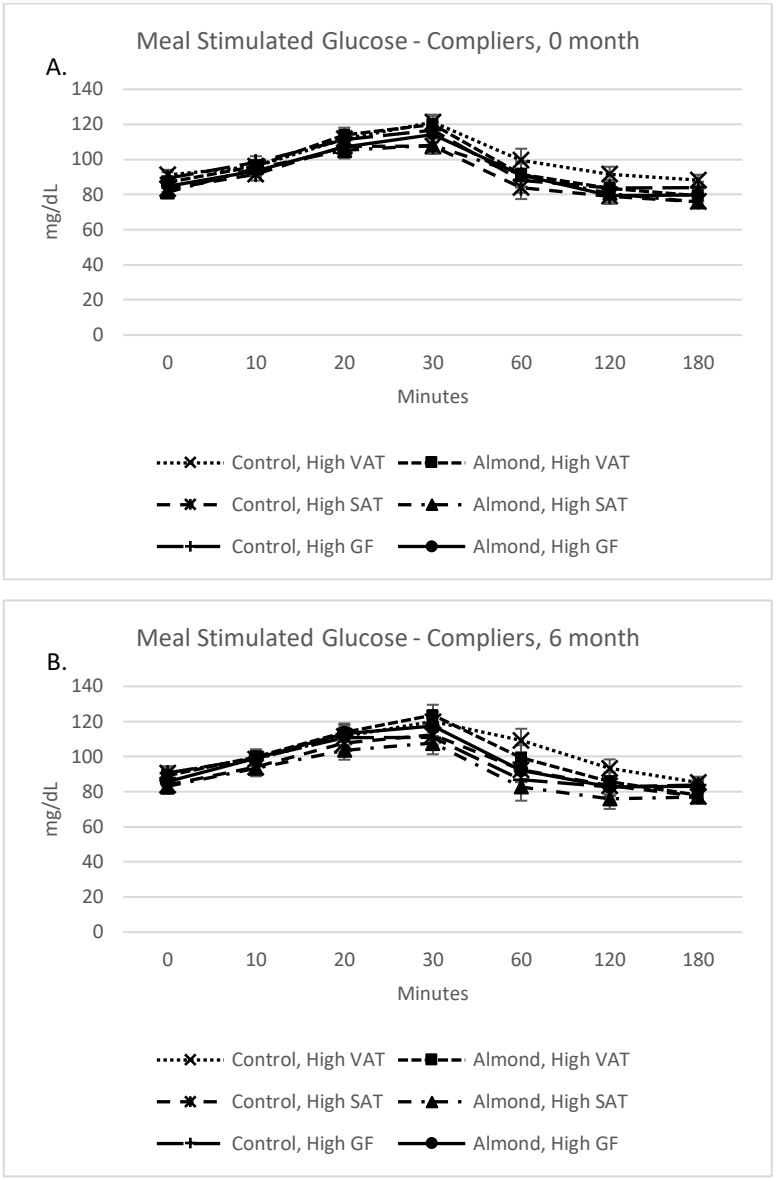
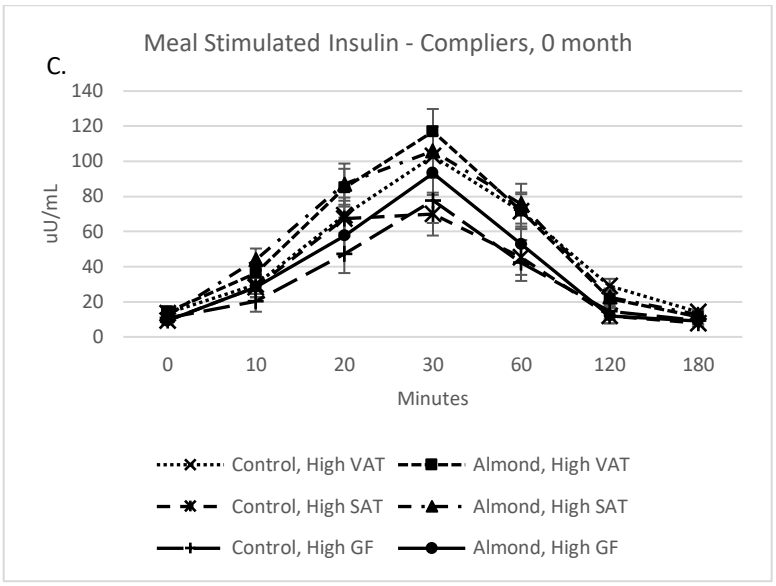
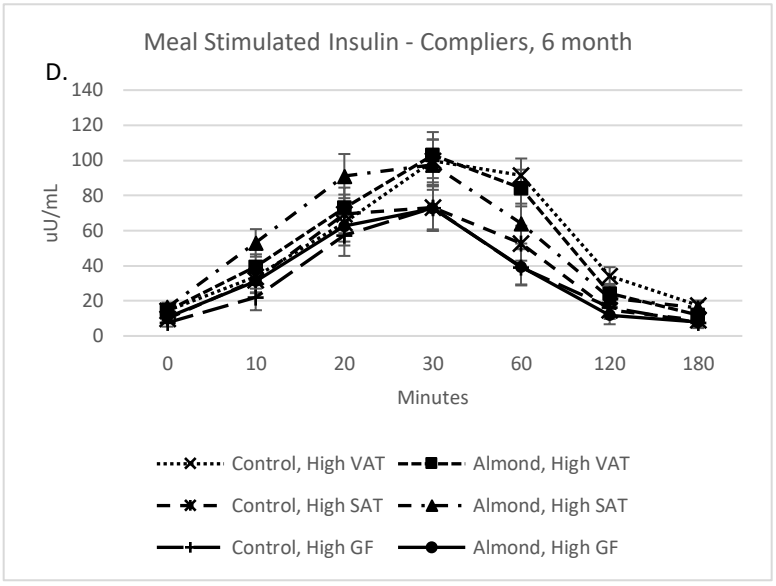
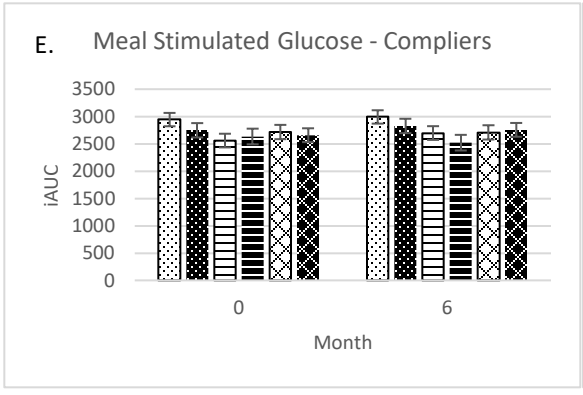


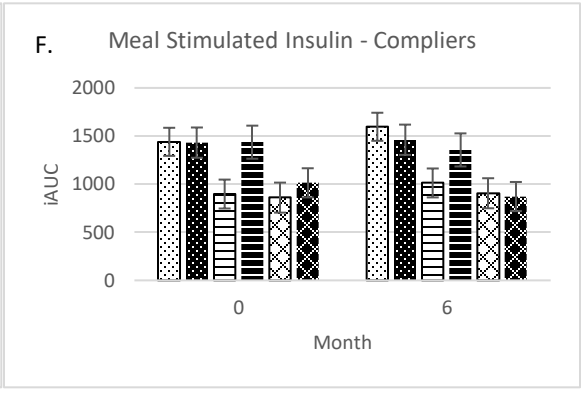
Figure 4. Meal stimulated glucose (A, B, E) and insulin (C, D, F) at 0 and 6 months from the complier analysis. Participants with high VAT had significantly higher meal stimulated insulin compared to participants with higher GF (p=0.002). There were no other significant results (p≥0.09). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

Figure 4 continued.









4.4.3 Lipids

4.4.3.1 ITT Analysis

Participants with high VAT had higher triglyceride concentrations compared to participants with high SAT and high GF (p=0.001), but this did not differ between treatments (p>0.2) (figure 5C). There was a significant Treatment*Time interaction effect on HDL (p=0.042), but pairwise comparisons did not indicate any significant differences (p>0.05) (figure 5B). There were no significant Treatment, BFD, Time, Treatment*BFD, Treatment*Time, or Treatment*BFD*Time effects on total cholesterol (p>0.1) (figure 5A) or LDL cholesterol (p>0.09) (figure 5D) (table 5).

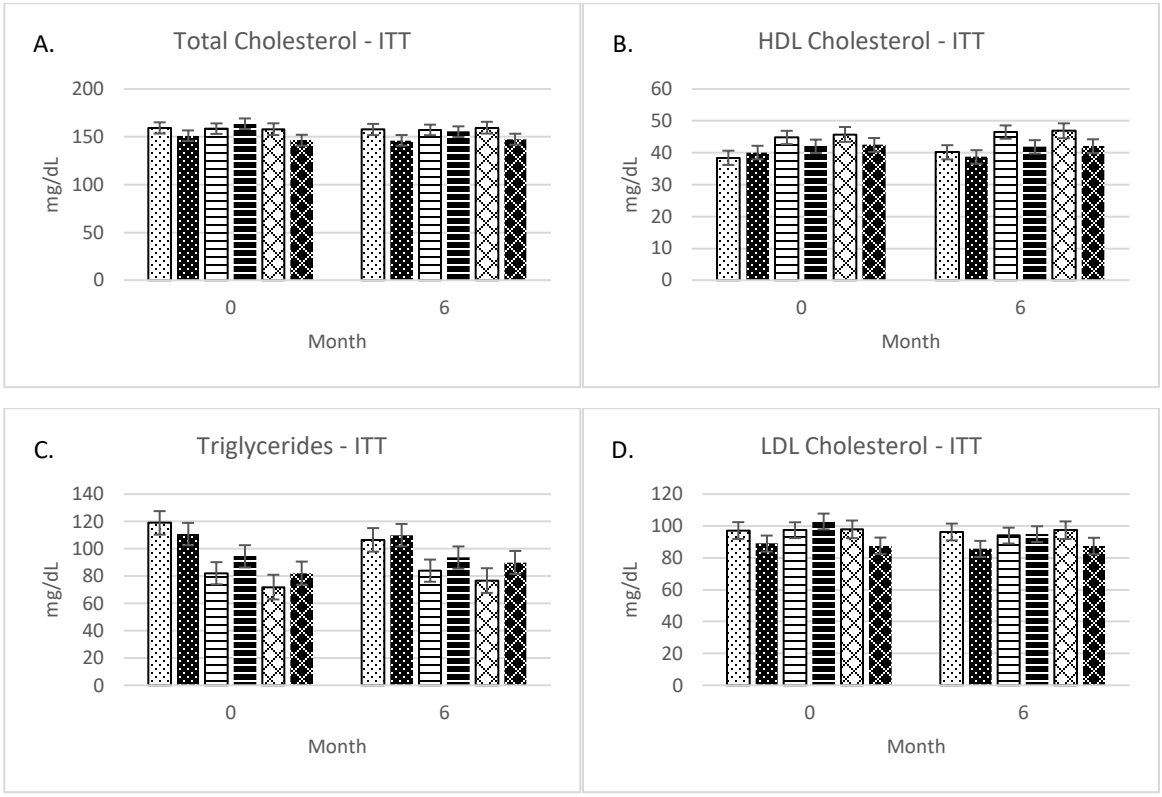


Figure 5. Blood lipid concentrations at 0 and 6 months from the ITT analysis. total cholesterol, B. HDL cholesterol, C. triglycerides, D. LDL cholesterol. Participants with high VAT had significantly higher triglyceride levels compared to participants with high SAT and high GF (p=0.001). There were no other significant differences in blood lipid concentrations (p>0.05). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

4.4.3.2 Complier Analysis

Triglyceride concentrations varied by BFD, where participants with high VAT had significantly higher triglyceride concentrations compared to participants with high SAT and high GF (p=0.007) (figure 6C). LDL concentrations varied over time, with participants having lower LDL concentrations at month 6 compared to month 0 (p=0.009), but this did not differ between treatments or BFD (p>0.1) (figure 6D). There were no significant Treatment, BFD, Time, Treatment*BFD, Treatment*Time, or Treatment*BFD*Time effects on total cholesterol (p>0.09) (figure 6A) or HDL cholesterol (p>0.07) (figure 6B) in the complier analysis (table 6).

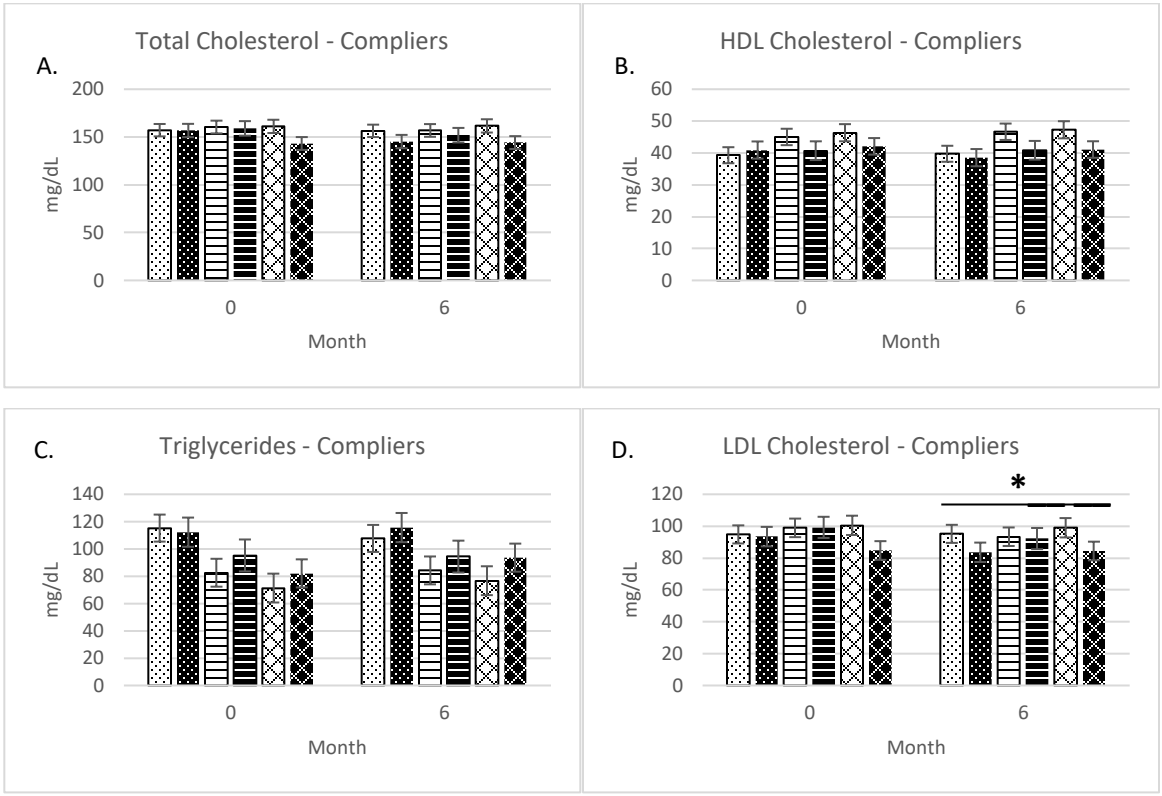


Figure 6. Blood lipid concentrations at 0 and 6 months from the complier analysis. A. Total cholesterol, B. HDL cholesterol, C. Triglycerides, D. LDL cholesterol. *significantly lower from month 0 (p<0.05). Participants with high VAT and significant higher triglyceride concentrations compared to participants with high SAT and GF (p=0.007). There were no other significant differences in blood lipid concentrations (p>0.05). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

Table 5. Blood biochemistries at 0 and 6 months from the ITT analysis.

		High VAT		High SAT		High GF		Total		P-value					
		Control	Almond	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Time	Tx*BFD	Tx*Time	Tx*BFD *Time
	Month	n=22	n=24	n=23	n=23	n=20	n=22	n=65	n=69						
Fasting Glucose (mg/dL)	0	91.2 ±3.7^{A}	93.3 ±3.5^{A}	81.9 ±3.5^{B}	83.0 ±3.5^{B}	87.7 ±3.9^{AB}	84.6 ±3.6^{AB}	86.9 ±2.1	87.0 ±2.0	0.782	0.015	0.048	0.579	0.262	0.88
	6	90.2 ±3.7^{A}	96.5 ±3.5^{A}	83.4 ±3.5^{B}	85.0 ±3.5^{B}	89.8 ±3.9^{AB}	87.2 ±3.6^{AB}	87.8 ±2.1	89.5 ±2.0						
Fasting Insulin (uU/mL)	0	15.8 ±2.0 ^{A}	17.2 ±1.9 ^{A}	8.7 ±1.9 ^{AB}	14.9 ±1.9 ^{AB}	10.2 ±2.1 ^{B}	9.5 ±2.0 ^{B}	11.5 ±1.2	13.9 ±1.1	0.023	0.017	0.883	0.156	0.672	0.419
	6	13.9 ±2.0 ^{A}	15.3 ±1.9 ^{A}	10.3 ±1.9 ^{A,B}	17.4 ±1.9 ^{AB}	8.6 ±2.1 ^{B}	10.5 ±2.0 ^{B}	10.9 ±1.2	14.4 ±1.1						
HOMA-IR	0	3.8 ±0.7 ^{A}	4.5 ±0.7 ^{A}	1.8 ±0.7 ^{AB}	3.1 ±0.7 ^{AB}	2.2 ±0.8 ^{B}	2.0 ±0.7 ^{B}	2.6 ±0.0.4	3.2 ±0.4	0.054	0.021	0.684	0.254	0.562	0.501
	6	3.3 ±0.7 ^{A}	4.0 ±0.0.7 ^{A}	2.1 ±0.7 ^{AB}	4.0 ±0.7 ^{AB}	2.0 ±0.8 ^{B}	2.3 ±0.7 ^{B}	2.4 ±0.4	3.4 ±0.4						
HOMA-%B	0	229.3 ±45.6	254.7 ±43.4	217.4 ±43.2	323.0 ±44.0	186.0 ±47.8	202.8 ±44.8	210.9 ±25.7	260.2 ±25.1	0.108	0.051	0.932	0.756	0.661	0.586
	6	215.7 ±45.6	234.3 ±43.4	292.8 ±43.2	309.0 ±43.2	162.0 ±47.8	226.4 ±44.8	223.5 ±25.7	256.6 ±24.9						
HbA1c (%)	0	5.4±0.1	5.4±0.1	5.4±0.1	5.5±0.1	5.5±0.1	5.4±0.1	5.5±0.04	5.4±0.04	0.368	0.779	0.962	0.849	0.98	0.402
	6	5.5±0.1	5.4±0.1	5.5±0.1	5.4±0.1	5.4±0.1	5.4±0.1	5.5±0.04	5.4±0.04						
iAUC Glucose	0	2908.1± 138^{A}	2956.9±1 31.3^{A}	2551.7± 129.3^{B}	2703.0± 129.5^{B}	2682.0± 144.6^{AB}	2645.9± 134.9^{AB}	2714.0± 77	2768.6± 74.7	0.584	0.016	0.031	0.98	0.702	0.113
	6	2964.5± 138^{A}	3060.6±1 31.3^{A}	2664.8± 129.3^{B}	2614.9± 129.5^{B}	2711.4± 144.6^{AB}	2786.9± 134.9^{AB}	2780.2± 77	2820.8± 74.7						
iAUC insulin	0	1605.3 ±159.4 ^{A}	1689.3 ±151.8 ^{A}	1025.3 ±150.2 ^{A}	1641.3 ±150.4 ^{A}	835.8 ±167.0 ^{B}	1026.8 ±156.2 ^{B}	1689.3 ±151.8	1452.4 ±86.7$^{\#}$	0.024	<0.001	0.551	0.216	0.081	0.975
	6	1617.0 ±159.4 ^{A}	1614.3 ±151.8 ^{A}	1067.3 ±150.2 ^{A}	1559.8 ±150.4 ^{A}	882.4 ±167.0 ^{B}	891.5 ±156.2 ^{B}	1188.9 ±89.5	1355.2 ±86.7						
Total Cholesterol (mg/dL)	0	159.3 ±5.9	151.2 ±5.6	158.6 ±5.5	163.7 ±5.5	158 ±6.1	146.4 ±5.7	158.6 ±3.3	153.8 ±3.2	0.117	0.4619	0.218	0.369	0.329	0.628
	6	157.7 ±5.9	146.3 ±5.6	157.2 ±5.5	155.4 ±5.5	159.6 ±6.1	147.5 ±5.7	158.2 ±3.3	149.8 ±3.2						
HDL (mg/dL)	0	38.4 ±2.2	40.1 ±2.1	44.8 ±2.1	42.0 ±2.1	45.8 ±2.3	42.4 ±2.2	43.0 ±1.2	41.5 ±1.2	0.124	0.061	0.408	0.522	0.042	0.956
	6	40.1 ±2.2	38.7 ±2.1	46.5 ±2.1	41.9 ±2.1	46.9 ±2.3	42.1 ±2.2	44.5 ±1.2	40.9 ±1.2						
Triglycerides (mg/dL)	0	118.9 ±8.6 ^{A}	110.7 ±8.2 ^{A}	82.0 ±8.1 ^{B}	94.4 ±8.1 ^{B}	71.9 ±9.0 ^{B}	82.1 ±8.5 ^{B}	90.9 ±4.8	95.8 ±4.7	0.258	0.001	0.984	0.552	0.528	0.458
	6	106.5 ±8.6 ^{A}	109.9 ±8.2 ^{A}	83.9 ±8.1 ^{B}	93.5 ±8.1 ^{B}	76.7 ±9.0 ^{B}	89.9 ±8.5 ^{B}	89.0 ±4.8	97.8 ±4.7						

Table 5 continued.

LDL (mg/dL)	0	97.1 ±5.3	88.9 ±5.0	97.3 ±5.0	102.8 ±5.0	97.9 ±5.5	87.5 ±5.2	97.4 ±3.0	93.1 ±2.9	0.153	0.461	0.099	0.272	0.505	0.686
	6	96.2 ±5.3	85.6 ±5.0	93.9 ±5.0	94.8 ±5.0	97.3 ±5.5	87.4 ±5.2	95.8 ±3.0	89.3 ±2.9						

Values are means ± SE. ITT linear mixed model of main effects of Tx, BFD, and Time, and the interaction of Tx*BFD, Tx*Time, and Tx*BFD*Time with Bonferroni post hoc comparisons when effects were significant in SAS. Age was used as a covariate. Missing values were imputed with overall mean values for each measure and time point. All n=134, except HbA1c n=119. Only values below baseline were included in AUC calculation. Fasting glucose and iAUC glucose were log transformed. #Trend significant difference from control treatment at same time point (p=0.081). Different uppercase letters indicate a significant difference between BFD (p<0.05). VAT=android visceral adipose tissue, SAT=android subcutaneous adipose tissue, GF=gluteal femoral adipose tissue, Tx=treatment, BFD=Body Fat Distribution, HDL=high density lipoprotein, LDL=Low density lipoprotein.

Table 6. Blood biochemistries at 0 and 6 months from complier analysis.

		High VAT		High SAT		High GF		Total		P-value					
		Control	Almond	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Time	Tx *BFD	Tx *Time	Tx *BFD* Time
	Month	n=20	n=15	n=17	n=14	n=18	n=17	n=55	n=46						
Fasting Glucose (mg/dL)	0	91.3±3.3	87.2±3.6	83.2±3.4	81.5±3.9	89.1±3.6	84.7±3.5	87.9±1.9	84.4±2.1	0.279	0.144	0.254	0.872	0.593	0.897
	6	90.3±3.3	89.1±3.6	83.9±3.4	82.9±3.9	90.9±3.6	85.7±3.5	88.4±1.9	85.9±2.1						
Fasting Insulin (uU/mL)	0	14.0±1.9	14.6±2.1	9.1±2.0	12.1±2.3	10.1±2.1	8.9±2.0	11.1±1.1	11.9±1.2	0.152	0.069	0.518	0.513	0.345	0.581
	6	14.0±1.9	14.2±2.1	10.0±2.0	16.5±2.3	8.1±2.1	10.6±2.0	10.7±1.1	13.8±1.2						
HOMA-IR	0	3.5±0.6	3.1±0.6	1.9±0.6	2.5±0.7	2.2±0.6	1.9±0.6	2.5±0.3	2.5±0.4	0.347	0.078	0.447	0.47	0.309	0.74
	6	3.4±0.6	3.1±0.6	2.0±0.6	3.9±0.7	1.9±0.6	2.2±0.6	2.4±0.3	3.0±0.4						
HOMA-%B	0	207.4 ±48.8	278.8 ±52.9	216.7 ±50.7	350.1 ±57.9	171.2 ±52.1	185.9 ±51.0	198.5 ±28.3	271.6 ±31.0	0.039	0.128	0.721	0.897	0.877	0.577
	6	223.5 ±48.8	268.5 ±52.9	256.6 ±50.7	319.8 ±57.9	144.6 ±52.1	261.5 ±51.0	208.2 ±28.3	283.3 ±31.0						
HbA1c (%)	0	5.4±0.1	5.4±0.1	5.4±0.1	5.4±0.1	5.5±0.1	5.4±0.1	5.5±0.04	5.4±0.1	0.188	0.959	0.947	0.956	0.81	0.615
	6	5.5±0.1	5.4±0.1	5.5±0.1	5.4±0.1	5.4±0.1	5.4±0.1	5.5±0.04	5.4±0.1						
iAUC Glucose	0	2943.9 ±122.3	2747.7 ±131.5	2559.5 ±125.4	2634.3 ±143.4	2716.1 ±130.9	2658.1 ±126.45	2739.8 ±70.0	2680.0 ±76.8	0.552	0.052	0.237	0.819	0.418	0.12
	6	2992.1 ±122.3	2827.1 ±131.5	2697.6 ±125.4	2521.0 ±143.3	2709.0 ±130.9	2755.5 ±126.5	2799.6 ±70.0	2701.2 ±76.8						
iAUC Insulin	0	1439.8 ±146.0 ^{A}	1431.4 ±157.4 ^{A}	897.1 ±150.3^{AB}	1436.1 ±171.8^{AB}	859.8 ±156.0 ^{B}	1013.5 ±151.4 ^{B}	1065.5 ±83.8	1293.7 ±92.0	0.213	0.002	0.678	0.183	0.09	0.27
	6	1596.1 ±146.0 ^{A}	1461.3 ±157.4 ^{A}	1012.5 ±150.3 ^{AB}	1355.6 ±171.8 ^{AB}	905.4 ±156.0 ^{B}	871.1 ±151.4 ^{B}	1171.3 ±83.8	1229.3 ±92.0						
Total Cholesterol (mg/dL)	0	157.2 ±6.5	156.8 ±7.0	160.5 ±6.7	159.0 ±7.6	161.1 ±6.9	143.2 ±6.7	159.6 ±3.7	153.0 ±4.1	0.091	0.781	0.055	0.489	0.212	0.354
	6	156.5 ±6.5	145.2 ±7.0	156.9 ±6.7	151.9 ±7.6	161.6 ±6.9	144.2 ±6.7	158.3 ±3.7	147.1 ±4.1						
HDL (mg/dL)	0	39.3±2.5	40.9±2.7	45.0±2.6	40.7±2.9	46.4±2.7	42.1±2.6	43.6±1.4	41.2±1.6	0.105	0.217	0.942	0.467	0.07	0.77
	6	39.7±2.5	38.5±2.7	46.7±2.6	40.8±2.9	47.3±2.7	41.1±2.6	44.6±1.4	40.1±1.6						
Triglycerides (mg/dL)	0	115.2 ±9.9 ^{A}	112.3 ±10.7 ^{A}	82.6 ±10.2 ^{B}	95.2 ±11.7 ^{B}	71.3 ±10.6 ^{B}	82.1 ±10.3 ^{B}	89.7 ±5.7	96.5 ±6.3	0.224	0.007	0.558	0.808	0.531	0.74
	6	107.7 ±9.9 ^{A}	115.6 ±10.7 ^{A}	84.3 ±10.2 ^{B}	94.4 ±11.7 ^{B}	76.7 ±10.6 ^{B}	93.6 ±10.3 ^{B}	89.6 ±5.7	101.2 ±6.3						

Table 6 continued.

LDL (mg/dL)	0	94.8 ±5.7	93.4 ±6.1	98.9 ±5.8	99.2 ±6.6	100.5 ±6.1	84.7 ±5.9	98.1 ±3.2	92.5 ±3.6	0.112	0.728	0.009	0.433	0.243	0.284
	6	95.2 ±5.7	83.6 ±6.1	93.4 ±5.8	92.2 ±6.6	99.0 ±6.1	84.4 ±5.9	95.8 ±3.2	86.7 ±3.6						

Values are means ± SE. Complier linear mixed model of main effects of treatment, BFD, and time, and the interaction of Treatment*BFD, Treatment*time, and Treatment*BFD*Time with Bonferroni post hoc comparisons when main and interaction effects were significant in SAS. Age was used as a covariate. Missing values were imputed with overall mean values for each measure and time point. All n=101. Fasting and iAUC glucose were log transformed, and fasting insulin and HOMA-IR were square root transformed. Only values below baseline were included in iAUC calculation. *Significant difference from Control treatment group at same time point. Different uppercase letters indicate a significant difference between BFD ($p<0.05$). VAT=android visceral adipose tissue, SAT=android subcutaneous adipose tissue, GF=gluteal femoral adipose tissue, Tx=Treatment, BFD=Body Fat Distribution, HDL=high density lipoprotein, LDL=Low density lipoprotein.

4.5 Anthropometric Outcomes

4.5.1 Absolute Values

4.5.1.1 ITT Analysis

As expected, participants with high VAT had significantly higher android VAT mass (figure 7K) and VAT ratios (figure 7M), and significantly lower android SAT mass (figure 7L) and SAT ratios (figure 7N) compared to participants with High SAT and High GF ($p<0.001$) (table 7). Similar differences between BFD were found within treatments, where the participants in the almond, High VAT group had a higher android VAT mass and VAT ratio at months 0 and 6 compared to participants in the almond, High SAT and almond, High GF groups ($p=0.016$). Participants in the control, High VAT group also had significantly higher android VAT mass at months 0 and 6 compared to participants in the control, High SAT and control, High GF groups ($p=0.016$). Furthermore, participants in the almond, High VAT group had a significantly lower SAT ratio at months 0 and 6 compared to participants in the almond, High SAT and almond, High GF groups, and participants in the control, High VAT group had a significantly lower SAT ratio at months 0 and 6 compared to participants in the control, High SAT and control, High GF groups ($p=0.007$). There were no significant differences between treatments within BFD ($p>0.05$). Since participants were recruited by BFD based on VAT and SAT ratios, which reflect different proportions of android VAT and SAT mass, these results indicate successful grouping of participants by BFD.

Trends towards significant main treatment effects indicated that participants in the almond treatment group tended to have higher android mass ($p=0.083$) (figure 7F), android fat mass ($p=0.073$) (figure 7G), and android SAT mass ($p=0.079$) (figure 7L) compared to participants in the control treatment group, however this was not different over time, between treatments over time, or between treatments of similar BFD over time ($p\geq0.1$). Participants with high SAT had significantly higher android fat mass percentage ($p=0.015$) (figure 7H), and significantly lower android lean mass percentage ($p=0.016$) (figure 7J) compared to participants with high GF, but this did not differ between treatments ($p>0.1$). There were no significant treatment, BFD, time, treatment*BFD, treatment*time, or treatment*BFD*time effects on body weight ($p>0.1$) (figure 7A), total fat mass ($p>0.4$) (figure 7B), total fat mass percentage ($p>0.1$)

(figure 7C), total lean mass ($p>0.1$) (figure 7D), total lean mass percentage ($p>0.2$) (figure 7E), or android lean mass ($p>0.2$) (figure 7I) (table 7).

Table 7. Anthropometric data from the DEXA scan at 0 and 6 months from the ITT analysis.

		High VAT		High SAT		High GF		Total		P-Value					
		Control	Almond	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Time	Tx *BFD	Tx *Time	Tx *BFD *Time
	Month	n=22	n=24	n=23	n=23	n=20	n=22	n=65	n=69						
Body Weight (kg)	0	90.8±3.5	96.4±3.3	89.9±3.3	95.6±3.3	92.6±3.7	91.4±3.4	91.1±2	94.5±1.9	0.193	0.954	0.86	0.566	0.875	0.313
	6	89.1±3.5	94.8±3.3	91.2±3.3	96.2±3.3	92.5±3.7	92.4±3.4	90.9±2	94.5±1.9						
Total Fat Mass (g)	0	36893 ±2381	40039 ±2265	40205 ±2227	42463 ±2231	41286 ±2495	39013 ±2325	39462 ±1327	40505 ±1286	0.565	0.442	0.668	0.534	0.978	0.69
	6	36760 ±2381	39943 ±2265	40792 ±2227	42073 ±2231	40436 ±2495	39150 ±2325	39329 ±1327	40389 ±1286						
Total Fat Mass (%)	0	42±1	43±1	45.8±1.3	45.7±1.4	44.6±1.6	43.2±1.5	44.2±0.8	44±0.8	0.77	0.18	0.385	0.778	0.716	0.188
	6	43±1	44±1	45.7±1.4	44.6±1.4	43.9±1.6	42.9±1.5	44.1±0.8	43.7±0.8						
Total Lean Mass (g)	0	51256 ±1960	53515 ±1865	47091 ±1836	50401 ±1839	48632 ±2054	49677 ±1915	48993 ±1094	51198 ±1060	0.124	0.398	0.995	0.801	0.84	0.102
	6	49714 ±1960	52064 ±1865	47712 ±1836	51377 ±1839	49327 ±2054	50391 ±1915	48918 ±1094	51277 ±1060						
Total Lean Mass (%)	0	56±1	55±1	52.8±1.3	53±1.3	53.7±1.5	54.9±1.4	54.2±0.8	54.4±0.8	0.795	0.2	0.463	0.777	0.813	0.253
	6	56±1	55±1	52.9±1.3	53.7±1.3	54.4±1.5	55.2±1.4	54.3±0.8	54.6±0.8						
Android Mass (kg)	0	7.3±0.4	8±0.4	7±0.4	7.7±0.4	6.7±0.4	6.6±0.4	7±0.2	7.5±0.2	0.083	0.073	0.531	0.625	0.718	0.221
	6	7.2±0.4	7.9±0.4	7.2±0.4	7.7±0.4	6.7±0.4	7±0.4	7±0.2	7.5±0.2						
Android Fat Mass (g)	0	3556±268	4236±255	3549±251	4125±251	3331±280	3174±261	3479±149	3845±145	0.073	0.053	0.95	0.392	0.977	0.099
	6	3506±268	4170±255	3698±251	4005±251	3220±280	3356±261	3475±149	3844±145						
Android Fat Mass (%)	0	49±2^{AB}	52±1 ^{AB}	50.1±1.5 ^{A}	52.8±15 ^{A}	47.7±1.6 ^{B}	46.6±1.5 ^{B}	48.9±0.9	50.4±0.8	0.246	0.015	0.088	0.496	0.55	0.101
	6	48±2 ^{AB}	51±1 ^{AB}	50.7±1.5 ^{A}	51±1.5 ^{A}	46.5±1.6 ^{B}	46.7±1.5 ^{B}	48.5±0.9	49.7±0.8						
Android Lean Mass (g)	0	3704±157	3774±149	3368±147	3553±147	3334±164	3407±153	3468±88	3578±85	0.272	0.203	0.21	0.846	0.521	0.235
	6	3648±157	3708±149	3410±147	3670±147	3406±164	3538±153	3488±88	3638±85						
Android Lean Mass (%)	0	51±2 ^{AB}	48±1 ^{AB}	49.1±1.4 ^{A}	46.6±1.4 ^{A}	51.6±1.6 ^{B}	52.5±1.5 ^{B}	50.4±0.9	48.9±0.8	0.256	0.016	0.131	0.54	0.62	0.108
	6	51±2 ^{AB}	48±1 ^{AB}	48.5±1.4 ^{A}	48.3±1.4 ^{A}	52.8±1.6 ^{B}	52.5±1.5 ^{B}	50.7±0.9	49.5±0.8						
Android VAT Mass (g)	0	1569 ±118^{a}	1741 ±115^{a}	867 ±111 ^{b}	1090 ±111 ^{b}	895.6 ±123.6 ^{b}	706 ±116 ^{b}	1110 ±66	1179 ±65	0.433	<0.001	0.98	0.42	0.965	0.016
	6	1450 ±118^{a}	1577 ±115^{a}	1024 ±111^{ab}	1095 ±111^{ab}	865 ±124^{b}	864 ±116^{b}	1113 ±66	1179 ±65						

Table 7 continued.

Android SAT Mass (g)	0	1994 ±174 ^{A}	2294 ±165 ^{A}	2681 ±163 ^{B}	3032 ±163 ^{B}	2426 ±182 ^{AB}	2462 ±170 ^{AB}	2367 ±97	2596 ±94	0.079	0.001	0.935	0.746	0.892	0.111
	6	2063 ±174 ^{A}	2399 ±165 ^{A}	2672 ±163 ^{B}	2907 ±163 ^{B}	2346 ±182 ^{AB}	2486 ±170 ^{AB}	2360 ±97	2597 ±94						
VAT Ratio	0	0.42 ±0.02 ^{a}	0.43 ±0.02 ^{a}	0.24 ±0.02 ^{b}	0.26 ±0.02 ^{b}	0.24 ±0.02 ^{b}	0.22 ±0.02 ^{b}	0.30 ±0.01	0.30 ±0.01	0.952	<0.001	0.998	0.79	0.591	<0.001
	6	0.39 ±0.02 ^{a}	0.40 ±0.02 ^{a}	0.27 ±0.02 ^{b}	0.26 ±0.02 ^{b}	0.25 ±0.02 ^{b}	0.25 ±0.02 ^{b}	0.31 ±0.01	0.30 ±0.01						
SAT Ratio	0	0.58 ±0.02 ^{a}	0.57 ±0.02 ^{a}	0.76 ±0.02 ^{b}	0.74 ±0.02 ^{b}	0.76 ±0.02 ^{b}	0.78 ±0.02 ^{b}	0.70 ±0.01	0.70 ±0.01	0.962	<0.001	0.796	0.865	0.761	0.007
	6	0.61 ±0.02 ^{a}	0.61 ±0.02 ^{a}	0.73 ±0.02 ^{b}	0.74 ±0.02 ^{b}	0.76 ±0.02 ^{b}	0.75 ±0.02 ^{b}	0.70 ±0.01	0.70 ±0.01						

Values are means ± SE. ITT Linear Mixed Model of main effects of Tx, BFD, time, and the interaction of Tx*Time, Tx*BFD, and Tx*BFD*Time with Bonferroni post hoc comparisons when main or interaction effects were significant in SAS. Age was included as a covariate. Missing values were imputed with overall mean values. All n=134. Different uppercase letters indicate significant difference between BFD ($p<0.05$). Different lowercase letters indicate significant difference between BFD within a treatment and time ($p<0.05$).

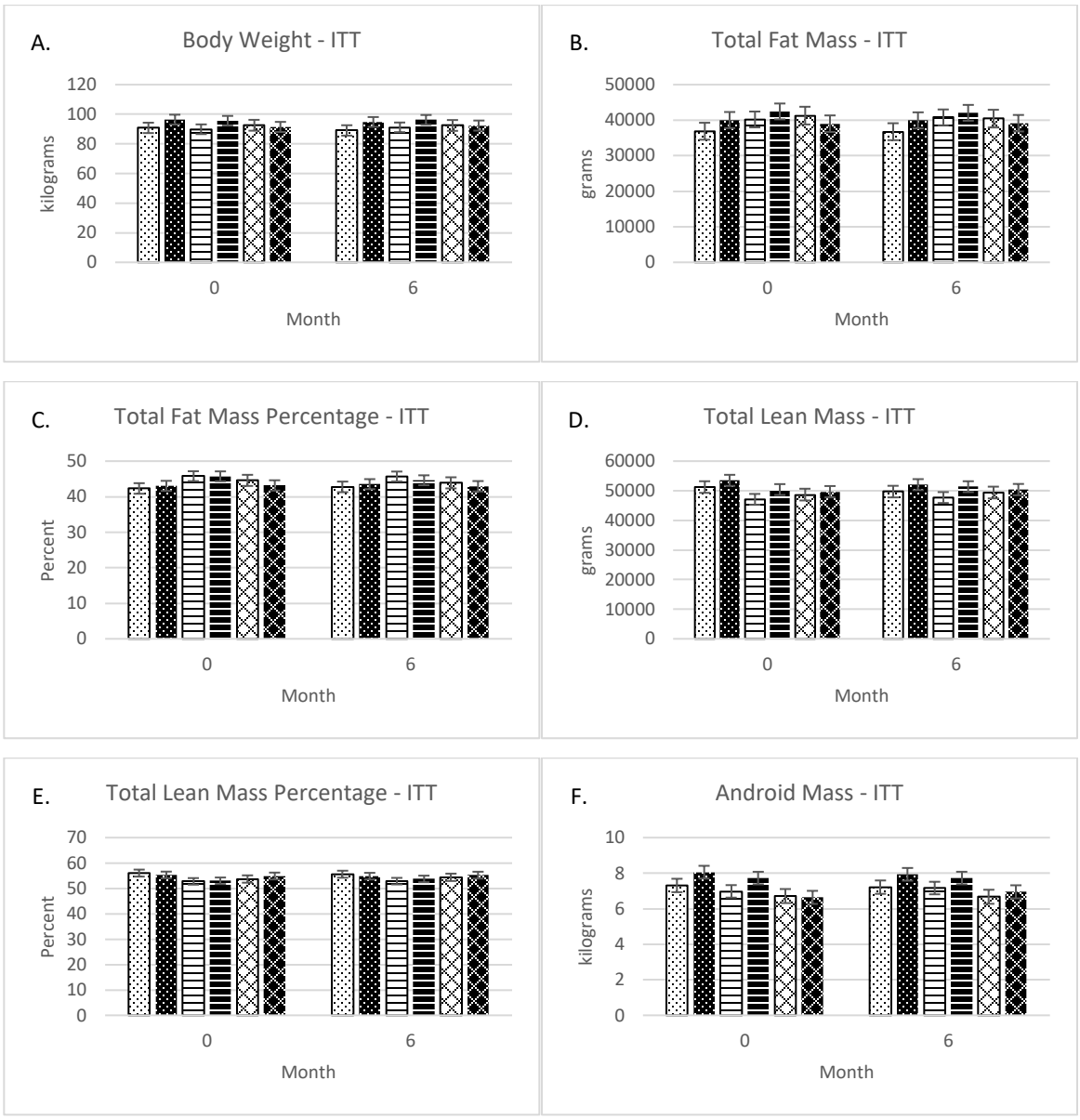
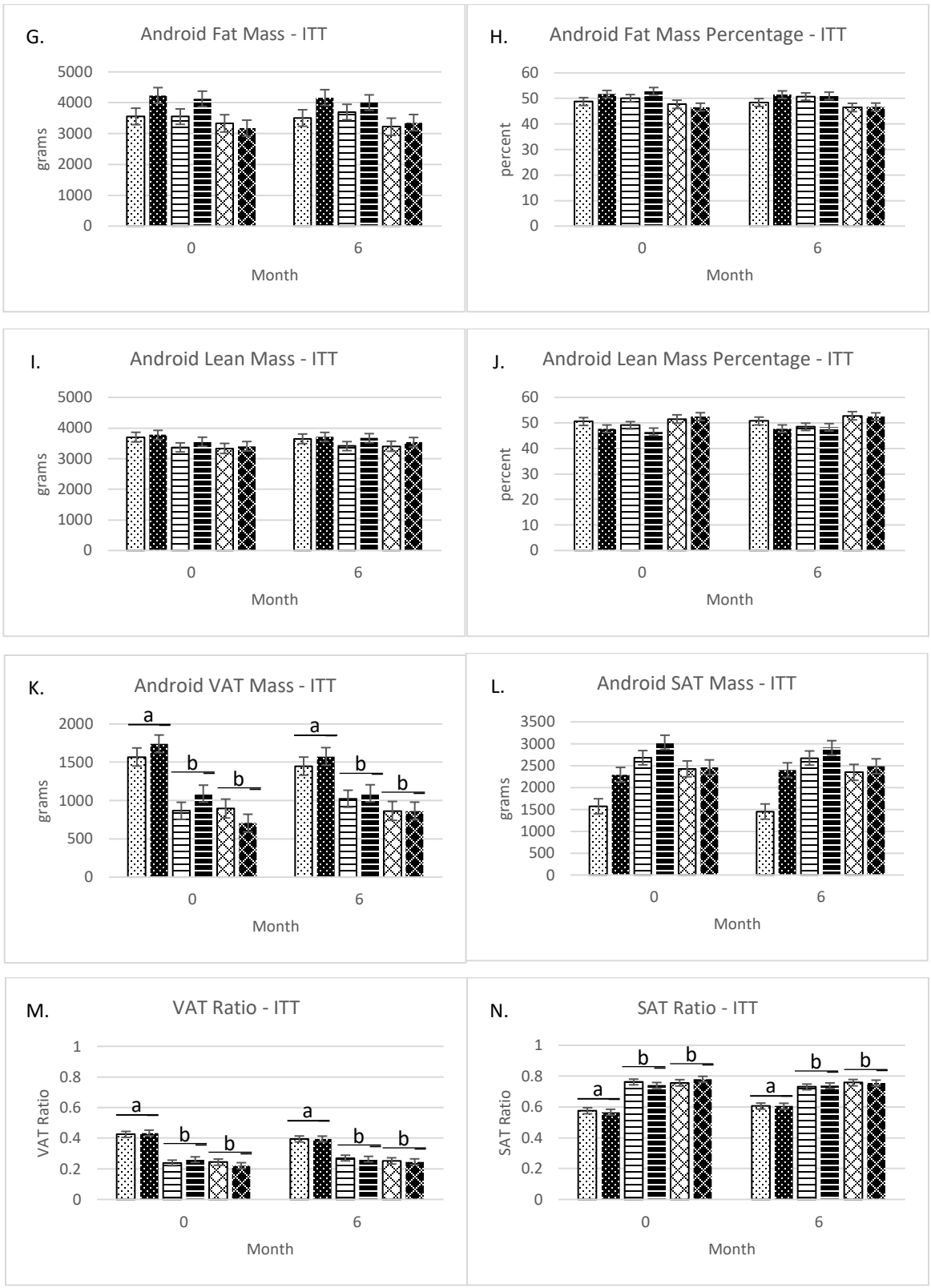


Figure 7. Anthropometric data from the DEXA scan at 0 and 6 months from the ITT analysis. A. Body Weight, B. Total Fat Mass, C. Total Fat Mass Percentage, D. Total Lean Mass, E. Total Lean Mass Percentage, F. Android Mass, G. Android Fat Mass, H. Android Fat Mass Percentage, I. Android Lean Mass, J. Android Lean Mass Percentage, K. Android VAT Mass, L. Android SAT mass, M. VAT Ratio, N. SAT ratio. Different lowercase letters indicate significant difference between BFD within a treatment and time ($p<0.05$). Participants with high VAT also had significantly higher VAT mass and ratios, and significantly lower SAT mass and ratios than participants with high SAT and GF ($p\leq0.001$). Participants with high SAT had significantly higher android fat mass percentages ($p=0.015$) and significantly lower android lean mass percentages ($p=0.016$) compared to participants with high GF. There were no other significant differences in anthropometric data ($p>0.05$). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

Figure 7 continued.



4.5.1.2 Complier Analysis

Participants with high VAT had higher VAT mass ($p<0.001$) (figure 8) and VAT ratios ($p<0.001$) (figure 8M), and lower SAT ratios ($p<0.001$) (figure 8N) compared to participants with high SAT and High GF. Participants with High VAT also had lower android SAT mass compared to those with high SAT ($p=0.013$) (figure 8L). These results are similar to those observed at baseline, and reflect successful grouping by BFD. They did not differ between treatments, or between treatments over time ($p>0.09$) (table 8).

There was a significant main time effect, where participants had higher android lean mass at month 6 compared to month 0 ($p=0.044$) (figure 8I). Participants in the almond treatment tended to have higher android lean mass at month 6 compared to month 0 ($p=0.07$), but there was no difference between BFD ($p>0.4$) (figure 8I). Trends for a main treatment effect indicated that participants in the almond treatment group tended to have higher android fat mass ($p=0.085$) (figure 8G), and had significantly higher android SAT mass ($p=0.04$) (figure 8L) compared to participants in the control treatment, but this did not differ at either time point, between treatments over time, or between treatments of participants with similar BFD over time ($p>0.1$). There were no significant treatment, BFD, time, treatment*BFD, treatment*time, or treatment*BFD*time effects on body weight ($p>0.2$) (figure 8A), total fat mass ($p>0.5$) (figure 8B), total fat mass percentage ($p>0.2$) (figure 8C), total lean mass ($p>0.1$) (figure 8D), total lean mass percentage ($p>0.3$) (figure 8E), android fat mass percentage ($p>0.1$) (figure 8H), android lean mass percentage ($p>0.3$) (figure 8J), or android mass ($p>0.1$) (figure 8F) (table 8).

Table 8. Anthropometric data from the DEXA scan at 0 and 6 months from the complier analysis.

	Month	High VAT		High SAT		High GF		Total		P-Value					
		Control	Almond	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Time	Tx* BFD	Tx* Time	Tx* BFD* Time
		n=20	n=15	n=17	n=14	n=18	n=17	n=55	n=46						
Body Weight (kg)	0	89±4	94.2±4.0	90.2±3.8	94.8±4.3	93.0±4.0	92.6±3.8	90.8±2.1	93.9±2.3	0.288	0.968	0.512	0.721	0.524	0.999
	6	89±4	94.2±4.0	89.7±3.8	94.9±4.3	92.5±4.0	92.4±3.8	90.3±2.1	93.9±2.3						
Total Fat Mass (g)	0	36383 ±2615	39022 ±2803	39404 ±2670	42321 ±3052	41509 ±2800	39996 ±2694	39098 ±1489	40446 ±1634	0.531	0.506	0.446	0.712	0.928	0.896
	6	36285 ±2615	39091 ±2803	39500 ±2670	41752 ±3052	40661 ±2800	39825 ±2694	38815 ±1489	40223 ±1634						
Total Fat Mass (%)	0	42.38±1.61	42.88±1.73	44.57±1.65	46.15±1.88	44.43±1.73	43.96±1.66	43.80±0.92	44.33±1.01	0.82	0.374	0.257	0.927	0.267	0.355
	6	42.48±1.61	43.03±1.73	44.89±1.65	44.96±1.88	44.01±1.73	43.63±1.66	43.79±0.92	43.87±1.01						
Total Lean Mass (g)	0	50254 ±2021	52319 ±2165	48087 ±2062	49765 ±2357	48849 ±2164	49772 ±2080	49063 ±1150	50619 ±1262	0.293	0.579	0.856	0.919	0.154	0.346
	6	49802 ±2021	52232 ±2165	47463 ±2062	50464 ±2357	49104 ±2164	49796 ±2080	48790 ±1150	50831 ±1262						
Total Lean Mass (%)	0	55.9±1.5	55.4±1.6	53.8±1.5	52.8±1.8	53.8±1.6	54.3±1.6	54.52±0.86	54.13±0.94	0.849	0.385	0.485	0.943	0.454	0.587
	6	55.8±1.5	55.2±1.6	53.4±1.5	53.4±1.8	54.3±1.6	54.6±1.6	54.5±0.9	54.41±0.94						
Android Mass (kg)	0	7.16±0.38	7.75±0.41	6.94±0.39	7.61±0.45	6.79±0.41	6.81±0.40	7.0±0.2	7.4±0.2	0.13	0.262	0.208	0.756	0.26	0.87
	6	7.21±0.38	7.85±0.41	7.02±0.39	7.74±0.45	6.69±0.41	6.97±0.40	7.0±0.2	7.5±0.2						
Android Lean Mass (g)	0	3644±158	3644±169	3417±161	3495±184	3385±169	3443±163	3482±90	3527±99	0.525	0.429	0.044	0.887	0.07	0.559
	6	3664±158	3675±169	3390±161	3645±184	3404±169	3507±163	3486±90	3609±99[#]						
Android Lean Mass (%)	0	50.8±1.6	47.9±1.8	50.2±1.7	46.4±1.9	52.0±1.8	51.6±1.7	51.0±0.9	48.6±1.0	0.111	0.1	0.501	0.758	0.451	0.227
	6	50.9±1.6	47.6±1.8	49.1±1.7	47.8±1.9	52.9±1.8	51.7±1.7	51.0±0.9	49.0±1.0						
Android Fat Mass (g)	0	3473±279	4060±299	3461±285	4061±326	3360±298	3307±287	3431±159	3809±174	0.085	0.233	0.626	0.573	0.571	0.574
	6	3483±279	4120±299	3569±285	4045±326	3231±298	3408±287	3428±159	3857±174						
Android Fat Mass (%)	0	48.6±1.7	51.6±1.8	48.9±1.7	53.0±1.9	47.3±1.8	47.6±1.7	48.3±0.9	50.7±1.0	0.106	0.1	0.354	0.717	0.492	0.254
	6	48.2±1.7	51.7±1.8	50.0±1.7	51.6±1.9	46.4±1.8	47.4±1.7	48.2±0.9	50.2±1.0						
Android VAT Mass (g)	0	1523 ±129 [A]	1710 ±138 [A]	867 ±132 [B]	1061 ±151 [B]	935 ±138 [B]	735 ±133 [B]	1108 ±74	1169 ±81	0.486	<0.001	0.539	0.385	0.636	0.157
	6	1473 ±129 [A]	1733 ±138 [A]	1008 ±132 [B]	1056 ±151 [B]	856 ±138 [B]	811 ±133 [B]	1112 ±74	1200 ±81						

Table 8 continued.

Android SAT Mass (g)	0	1950 ±190 ^{A}	2350 ±204 ^{A}	2594 ±194 ^{B}	3000 ±222 ^{B}	2425 ±204 ^{AB}	2572 ±196 ^{AB}	2323 ±108	2641 ±119	0.04	0.013	0.872	0.809	0.667	0.753
	6	2010 ±190 ^{A}	2387 ±204 ^{A}	2560 ±194 ^{B}	2989 ±222 ^{B}	2375 ±204 ^{AB}	2596 ±196 ^{AB}	2315 ±108	265 ±119						
VAT Ratio	0	0.42 ±0.02 ^{A}	0.42 ±0.02 ^{A}	0.24 ±0.02 ^{B}	0.26 ±0.02 ^{B}	0.25 ±0.02 ^{B}	0.22 ±0.02 ^{B}	0.30 ±0.01	0.30 ±0.01	0.574	<0.001	0.897	0.746	0.56	0.097
	6	0.40 ±0.02 ^{A}	0.41 ±0.02 ^{A}	0.28 ±0.02 ^{B}	0.26 ±0.02 ^{B}	0.25 ±0.02 ^{B}	0.23 ±0.02 ^{B}	0.31 ±0.01	0.30 ±0.01						
SAT Ratio	0	0.58 ±0.02 ^{A}	0.58 ±0.02 ^{A}	0.76 ±0.02 ^{B}	0.74 ±0.02 ^{B}	0.75 ±0.02 ^{B}	0.78 ±0.02 ^{B}	0.70 ±0.01	0.70 ±0.01	0.651	<0.001	0.879	0.83	0.776	0.12
	6	0.60 ±0.02 ^{A}	0.59 ±0.02 ^{A}	0.72 ±0.02 ^{B}	0.74 ±0.02 ^{B}	0.76 ±0.02 ^{B}	0.77 ±0.02 ^{B}	0.69 ±0.01	0.70 ±0.01						

Values are means ± SE. Complier Linear Mixed Model of main effects of Tx, BFD, time, and the interaction of Tx*Time, Tx*BFD, and Tx*BFD*Time with Bonferroni post hoc comparisons when main effects were significant in SAS. Age was included as a covariate. Missing values were imputed with overall mean values. All n=101. #Trend different from month 0 (p=0.067). Different uppercase letters indicate significant difference between BFD (p<0.05). Tx=treatment, BFD=body fat distribution.

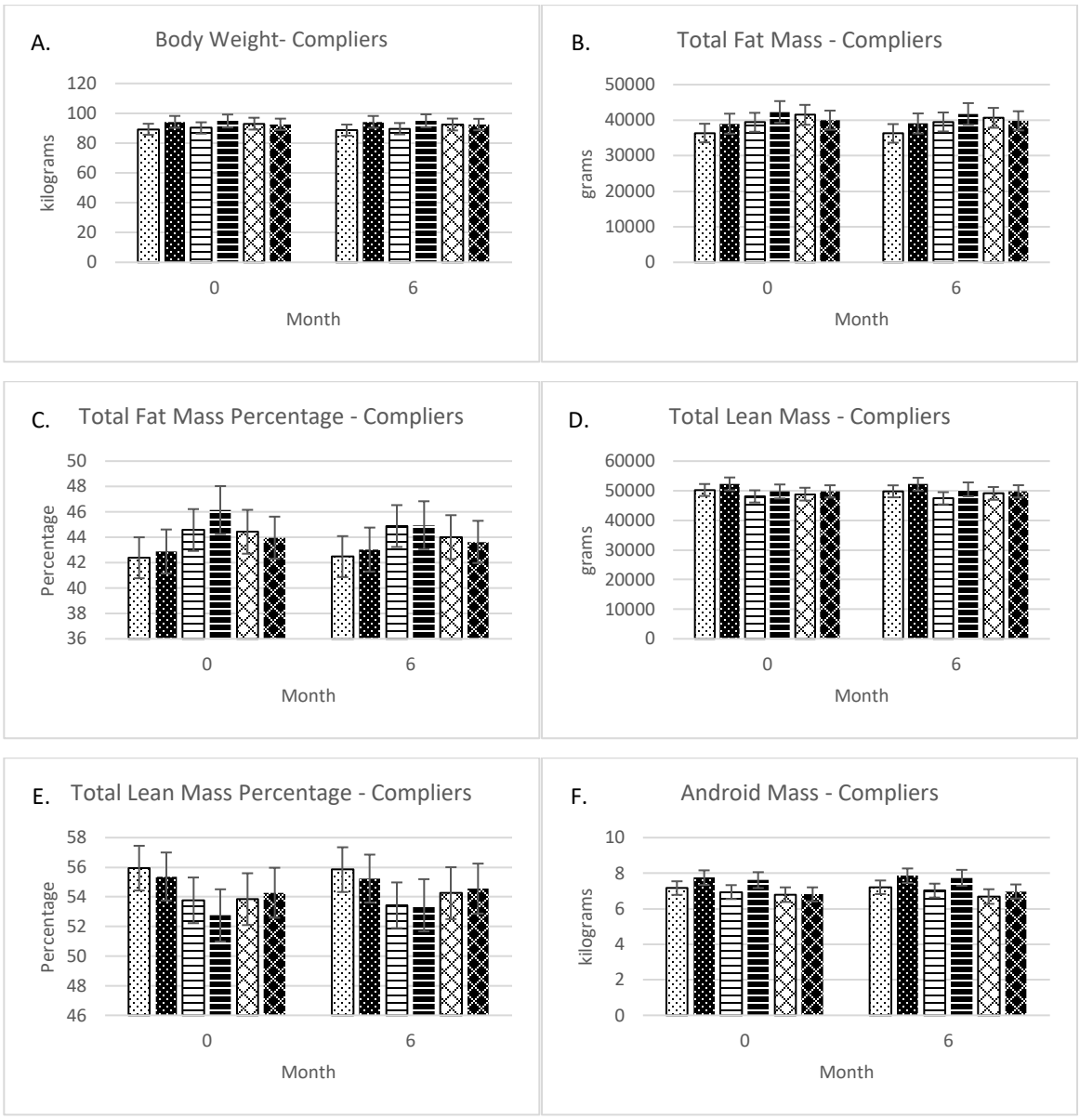
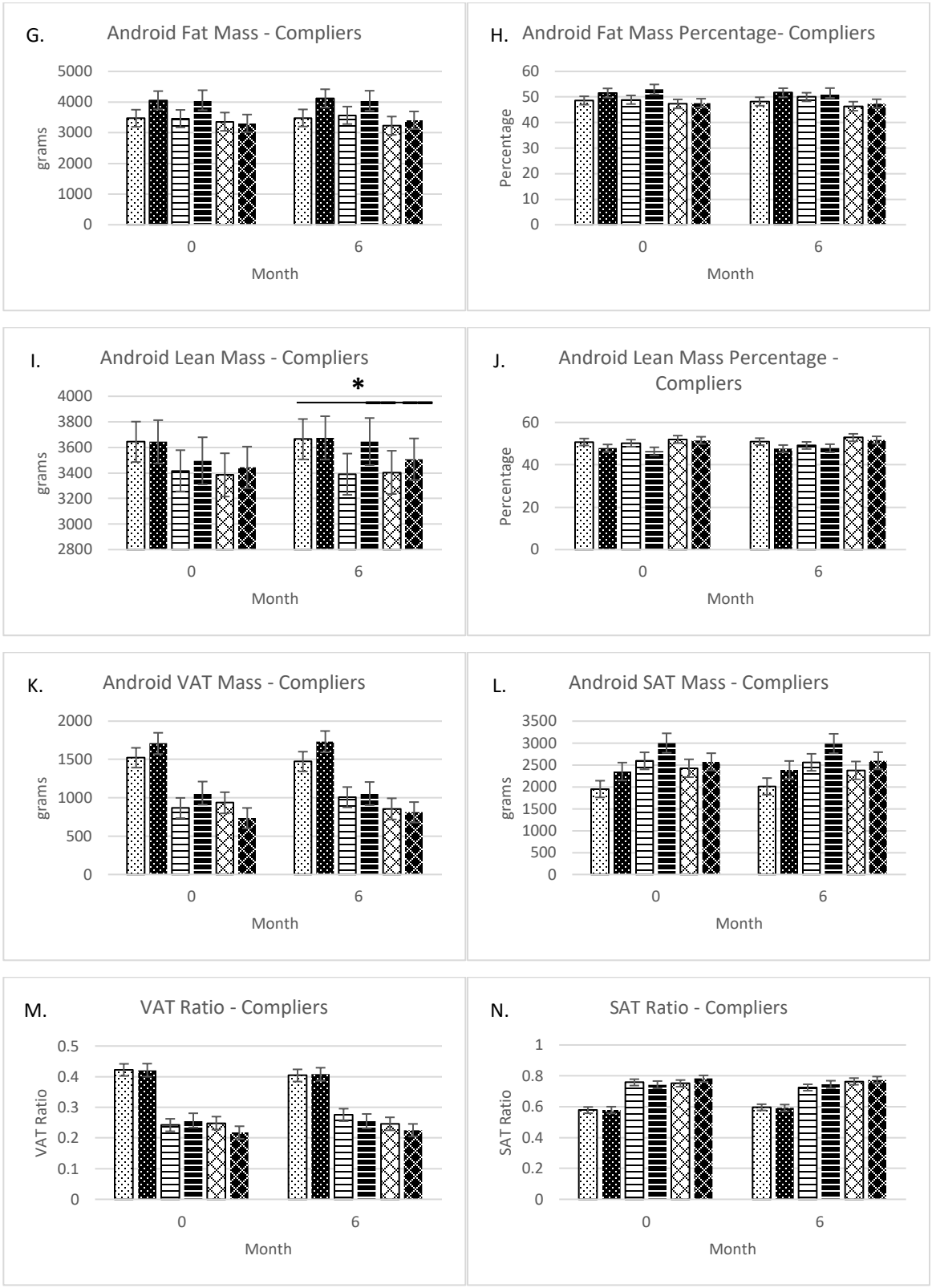


Figure 8. Anthropometric data from the DEXA scan at 0 and 6 months from the complier analysis. A. body weight, B. total fat mass, C. total fat mass percentage, D. total lean mass, E. total lean mass percentage, F. android mass, G. android fat mass, H. android fat mass percentage, I. android lean mass, J. android lean mass percentage, K. android VAT mass, L. android SAT mass, M. VAT ratio, N. SAT ratio. Participants with high VAT had significantly higher VAT mass and ratio ($p<0.001$), and significantly lower SAT ratios ($p<0.0.01$) compared to participants with high SAT and GF. Participants with high VAT also had significantly lower SAT mass compared to participants with high SAT ($p=0.013$). Participants in the almond treatment group had significantly higher SAT mass compared to participants in the control treatment group ($p=0.04$). There were no other significant differences in anthropometric data ($p>0.05$). *significantly higher at month 6 compared to month 0 ($p=0.044$). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

Figure 8 continued.



4.5.2 Change Values

4.5.2.1 ITT Analysis

Participants in the Almond, High SAT group had significantly decreased android fat mass percentage (p=0.038) (figure 9H) and significantly increased android lean mass percentage (p=0.042) (figure 9J) compared to participants in the Control, High SAT group (table 9). Furthermore, participants in the Almond, High SAT group tended to decrease android VAT mass (p=0.079) (figure 9K) compared to participants in the Control, High SAT group. There were no differences between participants in the almond and control treatment groups with high VAT or high GF (p>0.05). Within the control treatment, participants in the high GF group tended to decrease android fat mass compared to participants in the control, High SAT group (p=0.079) (figure 9G). There was a trend toward a significant treatment*BFD interaction for total lean mass (p=0.082) (figure 9D), however there were no differences after pairwise comparisons (p>0.1). There were no significant treatment, BFD, or treatment*BFD effects on change in body weight (p>0.3) (figure 9A), total fat mass (p>0.3) (figure 9B), total fat mass percentage (p>0.1) (figure 9C), total lean mass percentage (p>0.2) (figure 9E), android mass (p>0.1) (figure 9F), android lean mass (p>0.1) (figure 9I), android SAT mass (p>0.2) (figure 9L), VAT ratio (p>0.1) (figure 9M), or SAT ratio (p>0.1) (figure 9N) (table 9).

Table 9. Change in anthropometric data from the ITT analysis.

	High VAT		High SAT		High GF		Total		P-value		
	Control	Almond	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Tx *BFD
	n=22	n=24	n=23	n=23	n=20	n=22	n=65	n=69			
Δ Body Weight (kg)	0±0.8	0.3±0.7	-0.1±0.7	-0.2±0.7	-1.1±0.8	-1.0±0.8	-0.4±0.4	-0.3±0.4	0.868	0.346	0.960
Δ Total Fat Mass (g)	229±643	150±611	346±601	-380±602	-1179±673	-394±627	-201±358	-208±347	0.990	0.332	0.473
Δ Total Fat Mass (%)	0.2±0.4	0.1±0.4	0.4±0.4	-0.6±0.4	-0.5±0.5	0.2±0.4	0.01±0.2	-0.1±0.2	0.678	0.795	0.126
Δ Total Lean Mass (g)	-206±305	140±290	-476±285	164±286	58±320	-573±298	-208±170	-90±165	0.617	0.798	0.082
Δ Total Lean Mass (%)	-0.2±0.4	-0.1±0.4	-0.4±0.4	0.3±0.4	0.5±0.4	0±0.4	0±0.2	0.1±0.2	0.763	0.601	0.280
Δ Android Mass (kg)	0.1±0.1	0.2±0.1	0.1±0.1	0±0.1	-0.2±0.1	0.02±0.1	0±0.1	0.1±0.1	0.485	0.137	0.377
Δ Android Fat Mass (g)	54±88	69±84	126±82	-57±82	-182±92#	19±86	0±49	10±48	0.875	0.274	0.079
Δ Android Fat Mass (%)	-0.2±0.6	-0.1±0.6	1.1±0.6	-1.0±0.6*	-1.1±0.7	-0.03±0.6	-0.1±0.4	-0.4±0.3	0.571	0.629	0.038
Δ Android Lean Mass (g)	42±41	84±39	-17±39	82±39	2±43	1.0±40	9±23	55±22	0.154	0.401	0.453
Δ Android Lean Mass (%)	0±0.6	0±0.6	-1±0.6	0.9±0.6*	1.1±0.7	0.1±0.6	0±0.4	0.3±0.3	0.571	0.559	0.042
Δ Android VAT Mass (g)	-22±56	25±54	127±53	-13±53#	-73±59#	16±55	11±31	9±30	0.974	0.275	0.079
Δ Android SAT Mass (g)	76±63	44±60	-1±59	-44±59	-109±66	3±61	-11±35	1±34	0.802	0.230	0.365
Δ VAT ratio	-0.02±0.01	-0.01±0.01	0.03±0.01	0±0.01	0.01±0.01	0±0.01	0.01±0.01	0±0.01	0.340	0.107	0.232
Δ SAT ratio	0.02±0.01	0.01±0.01	-0.03±0.01	0±0.01	0±0.01	0±0.01	-0.03±0.01	0±0.01	0.548	0.136	0.253

Change values from ITT analyses with mean imputations for missing values were conducted using Linear Mixed Model in SPSS. If main or interaction effects were significant, pairwise comparisons with Bonferroni correction was performed. All n=134. Age was included as a covariate. Fixed Effects = Tx, BFD, Tx*BFD. Random Effects = Participant ID. Delta values = 6-month value - 0-month value. *Significant difference from Control, High SAT ($p<0.05$). #Trend difference from Control, High SAT ($p<0.08$).

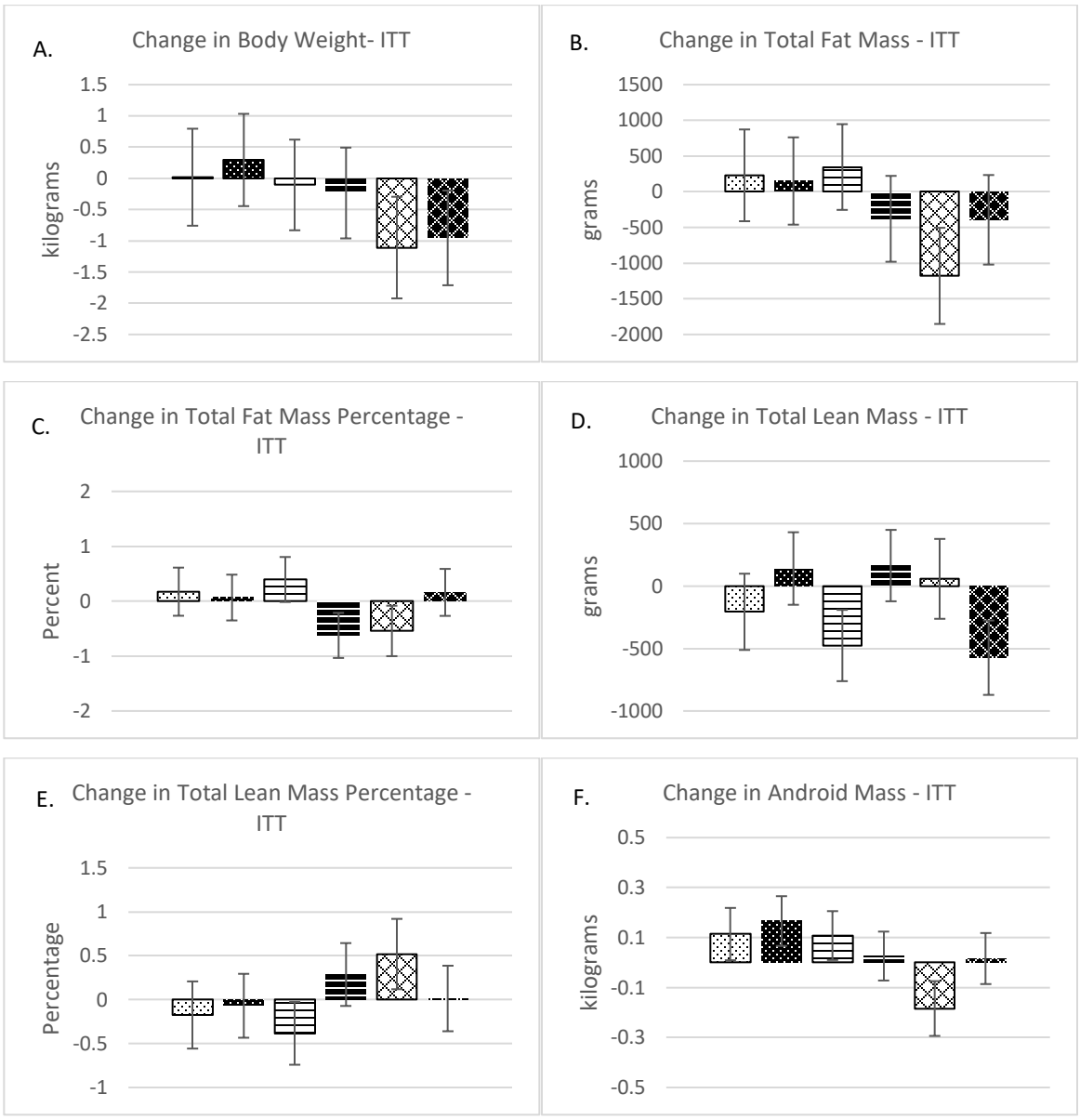
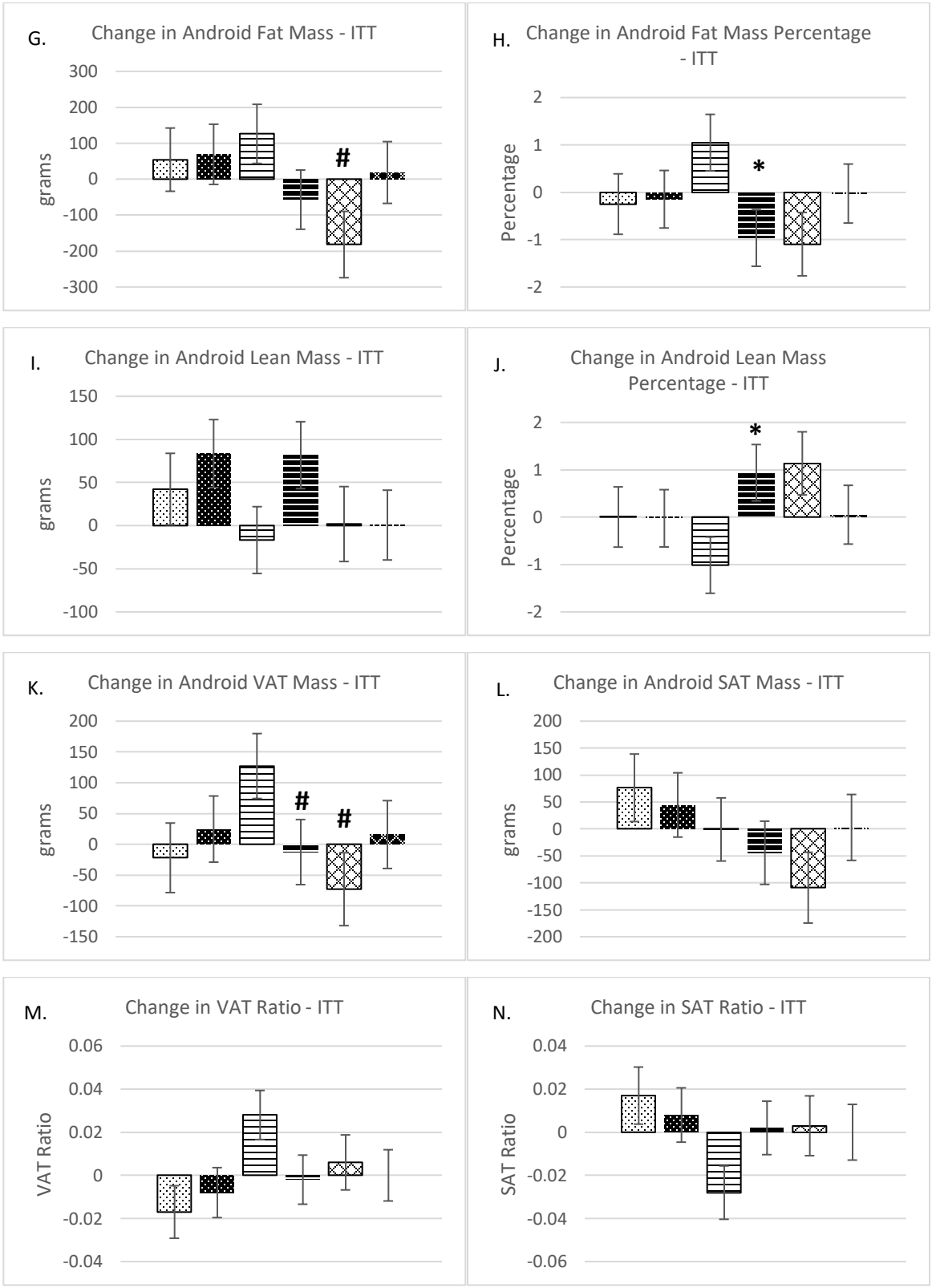


Figure 9. Change in anthropometric data from the ITT analysis. A. body weight, B, total fat mass, C. total fat mass percentage, D. total lean mass, E. total lean mass percentage, F. android mass, G. android fat mass, H. android fat mass percentage, I. android lean mass, J. android lean mass percentage, K. android VAT mass, L. android SAT mass, M. VAT ratio, N. SAT ratio. *Significant difference from control, high SAT group ($p<0.05$). #Trend significant difference from control, High SAT group ($p<0.08$). There were no other significant differences in change in anthropometric data ($p>0.05$). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

Figure 9 continued.



4.5.2.2 Complier Analysis

Participants in the Almond, High SAT group increased total lean mass (p=0.048) (figure 10D), and tended to gain android lean mass percentage (p=0.095) (figure 10J) compared to participants in the control, high SAT group (table 10). There were no differences between participants in the almond or control treatment groups with high VAT or high GF (p>0.05). Participants in the almond treatment group also tended to gain more android lean mass compared to participants in the control treatment (p=0.089) (figure 10I), but this did not differ based on BFD (p>0.4). There were no significant treatment, BFD, or treatment*BFD effects on change in body weight (p>0.3) (figure 10A), total fat mass (p>0.4) (figure 10B), total fat mass percentage (p>0.1) (figure 10C), total lean mass percentage (p>0.3) (figure 10E), android mass (p>0.2) (figure 10F), android fat mass (p>0.3) (figure 10G), android fat mass percentage (p>0.1) (figure 10H), android VAT mass (p>0.1) (figure 10K), android SAT mass (p>0.5) (figure 10L), VAT ratio (p>0.1) (figure 10M), or SAT ratio (p>0.1) (figure 10N) in participants in the complier analysis (table 10).

Table 10. Change in anthropometric data from the complier analysis.

	High VAT		High SAT		High GF		Total		P-Value		
	Control	Almond	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Tx*BFD
	n=20	n=15	n=17	n=14	n=18	n=17	n=55	n=46			
Δ Body Weight (kg)	0.1±0.9	0.4±1.0	-0.6±0.9	0.1±1.1	-1.3±1.0	-1.2±1.0	-0.6±0.5	-0.2±0.6	0.631	0.368	0.967
Δ Total Fat Mass (g)	223±774	265±829	72±789	-569±902	-1231±829	-593±796	-312±440	-299±483	0.984	0.425	0.738
Δ Total Fat Mass (%)	0.1±0.5	0.1±0.5	0.3±0.5	-1.2±0.6	-0.4±0.5	-0.2±0.5	0±0.3	-0.4±0.3	0.293	0.578	0.197
Δ Total Lean Mass (g)	-142±340	101±365	-646±347	699±397*	-114±365	-562±350	-301±194	79±213	0.190	0.579	0.048
Δ Total Lean Mass (%)	-0.1±0.4	-0.2±0.5	-0.3±0.5	0.7±0.5	0.4±0.5	0.2±0.5	-0.01±0.3	0.2±0.3	0.502	0.659	0.382
Δ Android Mass (kg)	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	-0.2±0.1	0.02±0.1	0±0.1	0.1±0.1	0.349	0.229	0.761
Δ Android Fat Mass (g)	49±104	84±112	105±106	-17±122	-176±112	13±107	-7±59	27±65	0.702	0.404	0.375
Δ Android Fat Mass Percentage	-0.4±0.8	0.1±0.8	1.1±0.8	-1.4±0.9	-0.9±0.8	-0.4±0.8	-0.1±0.4	-0.6±0.5	0.450	0.806	0.102
Δ Android Lean Mass (g)	49±46	48±49	-29±47	150±53	-15±49	7±47	2±26	69±29[&]	0.089	0.404	0.138
Δ Android Lean Mass Percentage	0.1±0.7	-0.3±0.8	-1.1±0.8	1.5±0.9[#]	0.9±0.8	0.4±0.8	0±0.4	0.5±0.5	0.396	0.693	0.095
Δ Android VAT Mass (g)	-10±65	48±70	139±66	-5±76	-128±70	15±67	0.4±37	19±41	0.737	0.221	0.110
Δ Android SAT Mass (g)	58±67	36±72	-33±68	-11±78	-48±72	-2±69	-8±28	8±42	0.787	0.556	0.879
Δ VAT ratio	-0.02±0.01	-0.01±0.02	0.03±0.01	0±0.02	-0.01±0.02	0±0.01	0±0.01	-0.01±0.01	0.433	0.148	0.310
Δ SAT ratio	0.02±0.02	0.01±0.02	-0.03±0.02	0±0.02	0.02±0.02	0±0.01	0±0.01	0.01±0.01	0.646	0.165	0.278

Means±SE. Change values from complier analyses with mean imputations of absolute anthropometric data were conducted using Linear Mixed Model in SPSS. If main or interaction effects were significant, pairwise comparisons with Bonferroni correction was performed. Participants who complied with the intervention were included. All n=101. Age was included as a covariate. Fixed Effects = Treatment, BFD, Treatment*BFD. Random Effects = Participant ID. Delta values = 6-month value - 0-month value. *Significant difference from control, High SAT ($p<0.05$). #Trend difference from Control, High SAT ($p<0.096$). &Trend difference from Control treatment ($p=0.089$).

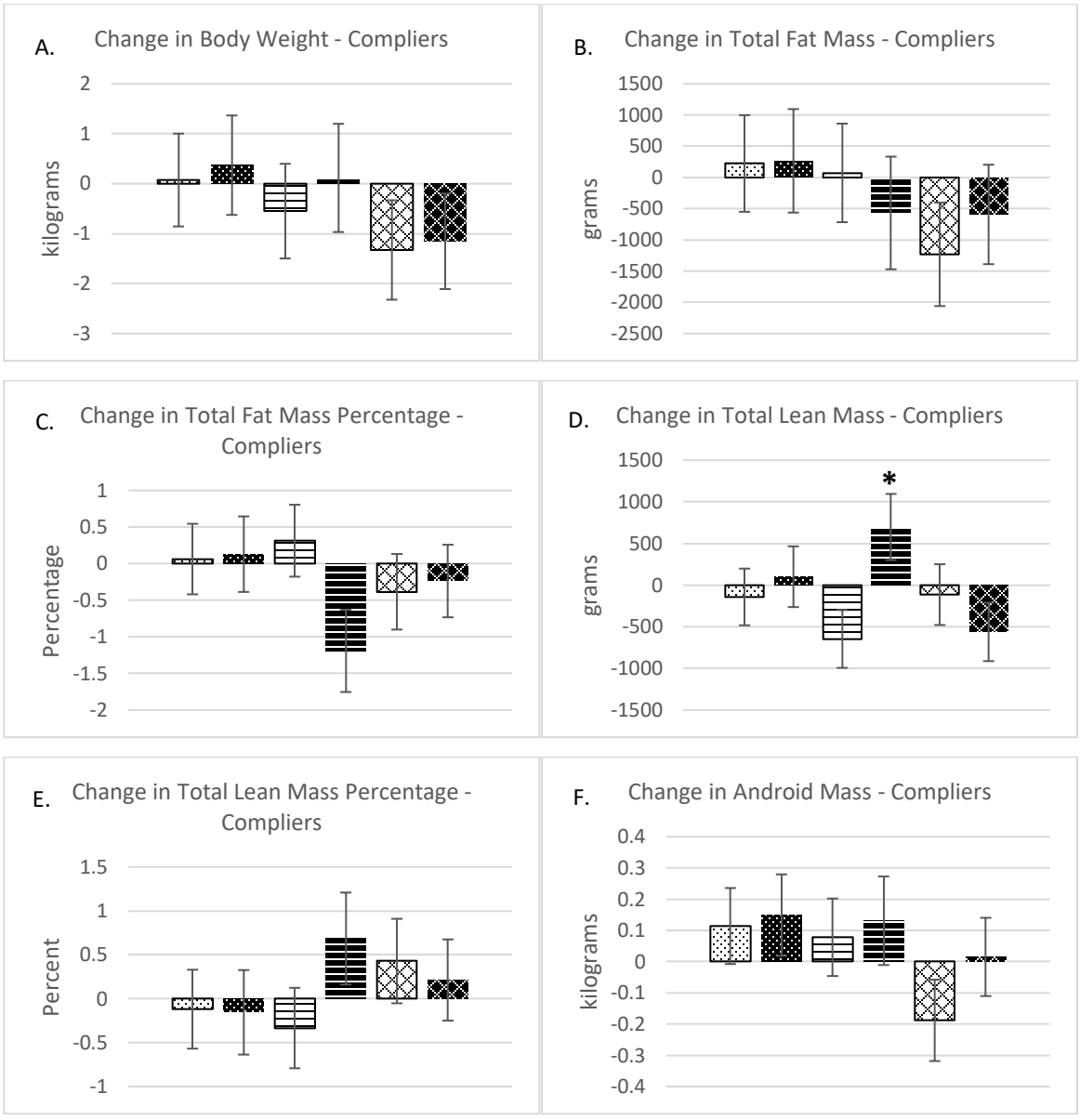
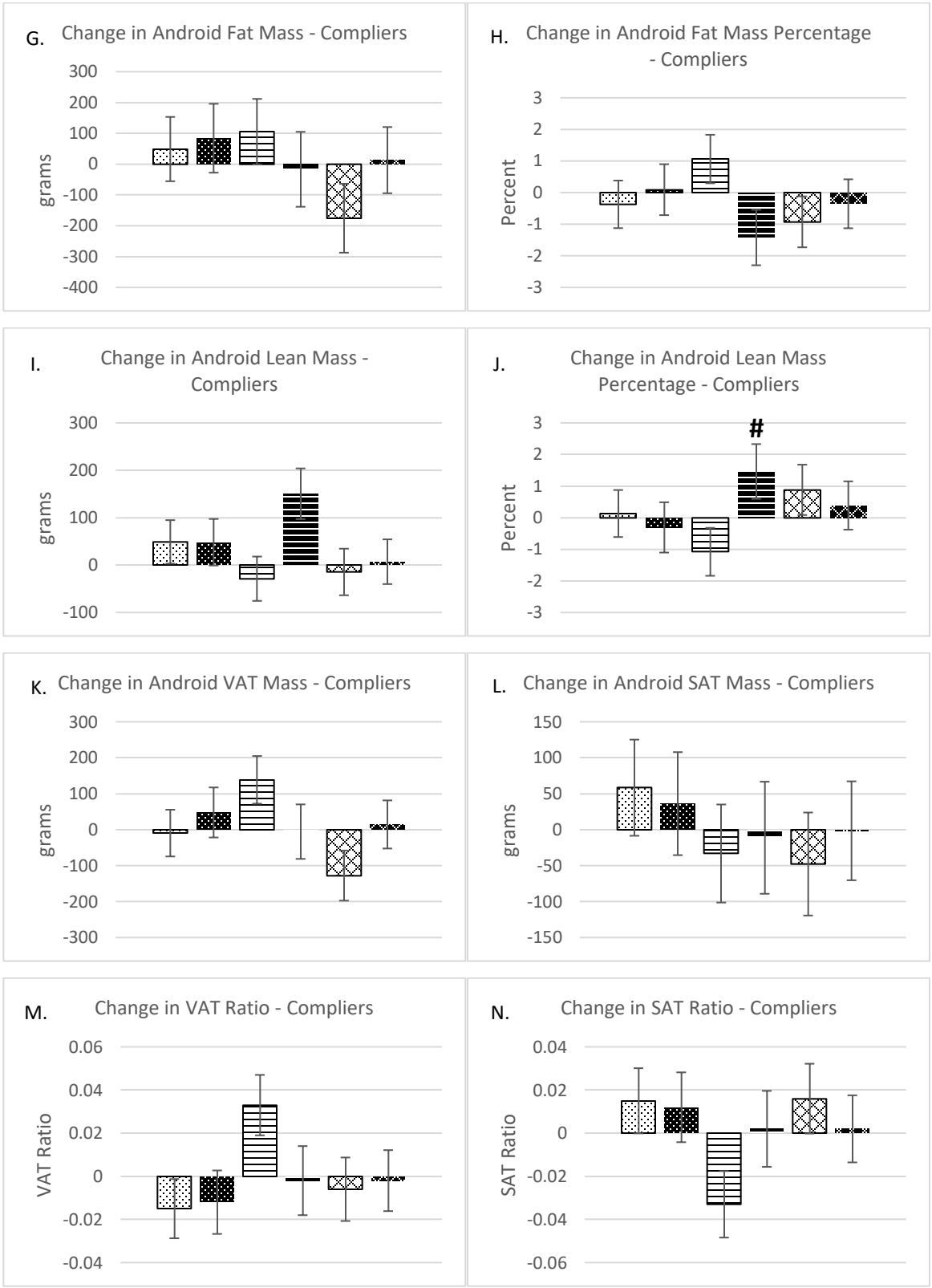


Figure 10. Change in anthropometric data in the Complier analysis. A. body weight, B. total fat mass, C. total fat mass percentage, D. total lean mass, E. total lean mass percentage, F. android mass, G. android fat mass, H. android fat mass percentage, I. android lean mass, J. android lean mass percentage, K. android VAT mass, L. android SAT mass, M. VAT ratio, N. SAT ratio. *Significant difference from control, High SAT ($p<0.05$). #Trend difference from Control, High SAT ($p<0.096$). There were no other significant differences in change in anthropometric data ($p>0.05$). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

Figure 10 continued.



4.6 Energy Intake

4.6.1 ITT Analysis

Participants in the almond treatment group consumed 195±87 kcals more overall compared to the control treatment group (p=0.027), however energy intake did not differ by BFD, time, Treatment*BFD, Treatment*Time, or Treatment*BFD*Time (p>0.6) (figure 11) (table 11). Notably, only 11% of the reported energy intakes fell within the Goldberg cutoffs.

Table 11. Energy intake (kcals) at months 0, 2, 4, and 6 from the ITT analysis.

	High VAT		High SAT		High GF		Total		P-value					
	Control	Almond	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Time	Tx* BFD	Tx* Time	Tx* BFD* Time
Month	n=22	n=24	n=23	n=23	n=20	n=22	n=65	n=69						
0	1725±143	2058±132	1803±134	1970±134	2027±148	2014±139	1852±80	2014±77	0.03	0.74	0.89	0.68	0.92	0.89
2	1786±143	2059±132	1752±134	1998±134	1770±148	1967±139	1769±80	2008±77						
4	1793±143	2052±132	1810±134	1941±134	1843±148	2048±139	1815±80	2014±77						
6	1859±143	2110±132	1668±134	1988±134	1951±148	1922±139	1826±80	2006±77						

Values are means ± SE. ITT Linear Mixed Model of main effects of Tx, BFD, time, and the interaction of Tx*Time, Tx*BFD, and Tx*BFD*Time with Bonferroni post hoc comparisons when main or interaction effects were significant in SAS. Age was included as a covariate. Missing values were imputed with overall mean values. All n=134. Tx=treatment, BFD=body fat distribution.

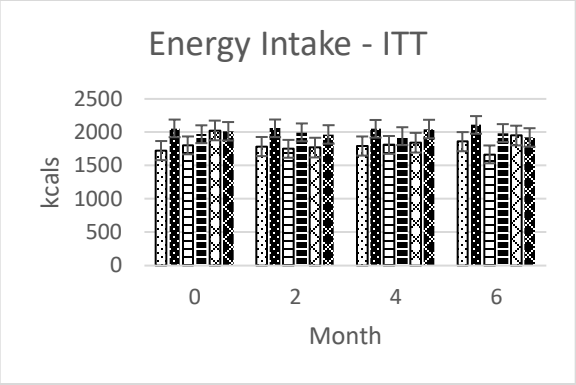


Figure 11. Energy intake at months 0, 2, 4, and 6 from the ITT analysis. Participants in the almond treatment group consumed significant more energy compared to participants in the control treatment group (p=0.03), but there were no other significant differences in energy intake (p>0.6). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

4.6.2 Complier Analysis

There were no significant treatment, BFD, time, treatment*BFD, treatment*Time, or treatment*BFD*time effects on energy intake in the complier analysis (p>0.15) (figure 12) (table 12). Only 12% of the reported energy intakes fell within the Goldberg cutoffs.

Table 12. Energy intake (kcals) at months 0, 2, 4, and 6 from the Complier analysis.

	High VAT		High SAT		High GF		Total		P-value					
Month	Control	Almond	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Time	Tx* BFD	Tx* Time	Tx* BFD* Time
	n=20	n=15	n=17	n=14	n=18	n=17	n=55	n=46						
0	1754±147	1969±149	1719±158	1952±175	2031±166	2127±152	1835±88	2016±89	0.16	0.43	0.44	0.50	0.60	0.70
2	1880±147	1850±149	1613±158	1878±175	1798±166	1997±152	1764±88	1908±89						
4	1886±147	1944±149	1618±158	1992±175	1842±166	2028±152	1782±88	1988±89						
6	1959±147	1861±149	1527±158	1937±175	1979±166	1827±152	1822±88	1875±89						

Values are means ± SE. Complier Linear Mixed Model of main effects of Tx, BFD, time, and the interaction of Tx*Time, Tx*BFD, and Tx*BFD*Time with Bonferroni post hoc comparisons when main or interaction effects were significant in SAS. Age was included as a covariate. Missing values were imputed with overall mean values. All n=101. Tx=treatment, BFD=body fat distribution.

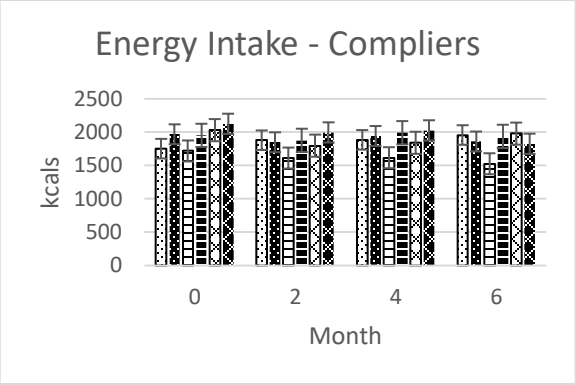


Figure 12. Energy intake at months 0, 2, 4, and 6 from the complier analysis. There were no significant differences in energy intake (p>0.1). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

4.7 Diet Quality

4.7.1 ITT Analysis

Since nuts, including almonds, are included into calculated a component of the total HEI score, there were expected differences in the 'seafood and plant protein' score. Participants in the almond treatment group had higher seafood and plant protein scores overall (p=0.001), and at months 2, 4, and 6 compared to participants in the control treatment group (p<0.001) (table 13) (figure 13H). Within treatments, participants in the almond treatment had significantly higher seafood and plant protein scores at months 2, 4, and 6 compared to month 0, whereas participants in the control treatment group had lower seafood and plant protein scores at months 2, 4, and 6 compared to month 0 (p<0.001) (figure 13H). There were also differences in the fatty acid score, where participants in the almond treatment group had higher fatty acid scores overall (p=0.019), and at months 2, 4, and 6 compared to participants in the control treatment group (p<0.001) (figure 13I). Within treatments, participants in the almond treatment had significantly higher fatty acid scores at months 2, 4, and 6 compared to month 0, whereas participants in the control treatment group had lower fatty acid scores at months 2, 4, and 6 compared to month 0 (p<0.001) (figure 13I). There were higher total vegetable scores at month 0 compared to month 4 (p=0.031), however this did not differ between treatments (p>0.6) (figure 13A). There were no other differences in HEI score components between participants in the almond and control treatment groups (p>0.08) (table 13). Participants in the almond treatment group had higher total

HEI scores, indicative of a higher diet quality, at months 2, 4, and 6 compared to baseline (p=0.001), however there were no differences in total HEI score between participants in the almond and control treatment groups at any time (figure 13N).

Table 13. HEI score components and total score at months 0, 2, 4, and 6 from the ITT analysis.

Month	Control	Almond	P-value		
	N=12	N=27	Tx	Time	Tx*Time
Total Vegetable			0.656	0.041	0.882
0 [1]	3.2±0.5	3.3±0.3			
2 [1,2]	2.6±0.5	3.0±0.3			
4 [2]	2.5±0.5	2.7±0.3			
6 [1,2]	2.7±0.5	3.1±0.3			
Green Bean			0.951	0.304	0.379
0	2.0±0.6	1.8±0.4			
2	1.9±0.6	1.8±0.4			
4	1.5±0.6	1.2±0.4			
6	1.5±0.6	2.3±0.4			
Total Fruit			0.798	0.427	0.197
0	1.2±0.6	1.8±0.4			
2	1.6±0.6	2.0±0.4			
4	1.3±0.6	1.5±0.4			
6	1.9±0.6	1.4±0.4			
Whole Fruit			0.926	0.165	0.361
0	1.6±0.7	1.8±0.4			
2	2.0±0.7	2.3±0.4			
4	1.6±0.7	1.7±0.4			
6	2.4±0.7	1.8±0.4			
Whole Grain			0.167	0.401	0.381
0	3.6±0.9	1.9±0.5			
2	2.8±0.9	1.5±0.5			
4	3.7±0.9	1.5±0.5			
6	2.5±0.9	1.8±0.5			

Values are means ± SE. n=39. ITT linear mixed model of main effects of treatment, time, and the interaction of treatment*time with Bonferroni post hoc comparisons when main or interaction effects were significant in SAS. Missing values were imputed with overall mean values. *Significant differences from control treatment ($p<0.05$). Different lowercase letters indicate a difference within treatment over time ($p<0.05$). Different numbers indicate a significant difference over time ($p<0.05$).

Table 13 continued.

Total Dairy			0.575	0.975	0.089
0	5.3±1.0	6.1±0.6			
2	6.1±1.0	4.9±0.6			
4	6.1±1.0	5.0±0.6			
6	6.3±1.0	5.0±0.6			
Total Protein			0.234	0.658	0.199
0	4.4±0.3	4.4±0.2			
2	4.2±0.3	4.7±0.2			
4	4.4±0.3	4.7±0.2			
6	4.2±0.3	4.8±0.2			
Seafood and Plant Protein			0.001	0.987	<0.001
0	3.3±0.4[a]	2.5±0.3[a]			
2	1.7±0.4[b]	4.3±0.3[b]*			
4	1.6±0.4[b]	4.3±0.3[b]*			
6	1.6±0.4[b]	4.2±0.3[b]*			
Fatty Acids			0.019	0.08	<0.001
0	5.1±0.9	4.2±0.5[a]			
2	3.3±0.9	7.5±0.5[b]*			
4	3.9±0.9	7.7±0.5[b]*			
6	4.0±0.9	7.7±0.5[b]*			
Sodium			0.694	0.908	0.267
0	4.9±0.9	3.3±0.6			
2	4.3±0.9	4.3±0.6			
4	4.3±0.9	4.5±0.6			
6	4.6±0.9	4.3±0.6			
Refined Grains			0.429	0.247	0.128
0	5.8±1.0	5.1±0.6			
2	5.9±1.0	6.9±0.6			
4	4.8±1.0	6.8±0.6			
6	4.8±1.0	6.1±0.6			
Saturated Fat			0.56	0.436	0.102
0	4.5±1.0	3.8±0.6			
2	3.8±1.0	5.3±0.6			
4	4.0±1.0	5.0±0.6			
6	4.5±1.0	5.4±0.6			

Table 13 continued.

Added Sugars			0.226	0.32	0.203
0	7.5±0.7	7.9±0.4			
2	6.9±0.7	8.4±0.4			
4	7.9±0.7	8.4±0.4			
6	6.9±0.7	8.3±0.4			
Total Score			0.31	0.662	<0.001
0	52.3±4.1	47.7±2.4[a]			
2	47.4±4.1	57.0±2.4[b]			
4	47.7±4.1	54.8±2.4[b]			
6	48.0±4.1	56.0±2.4[b]			

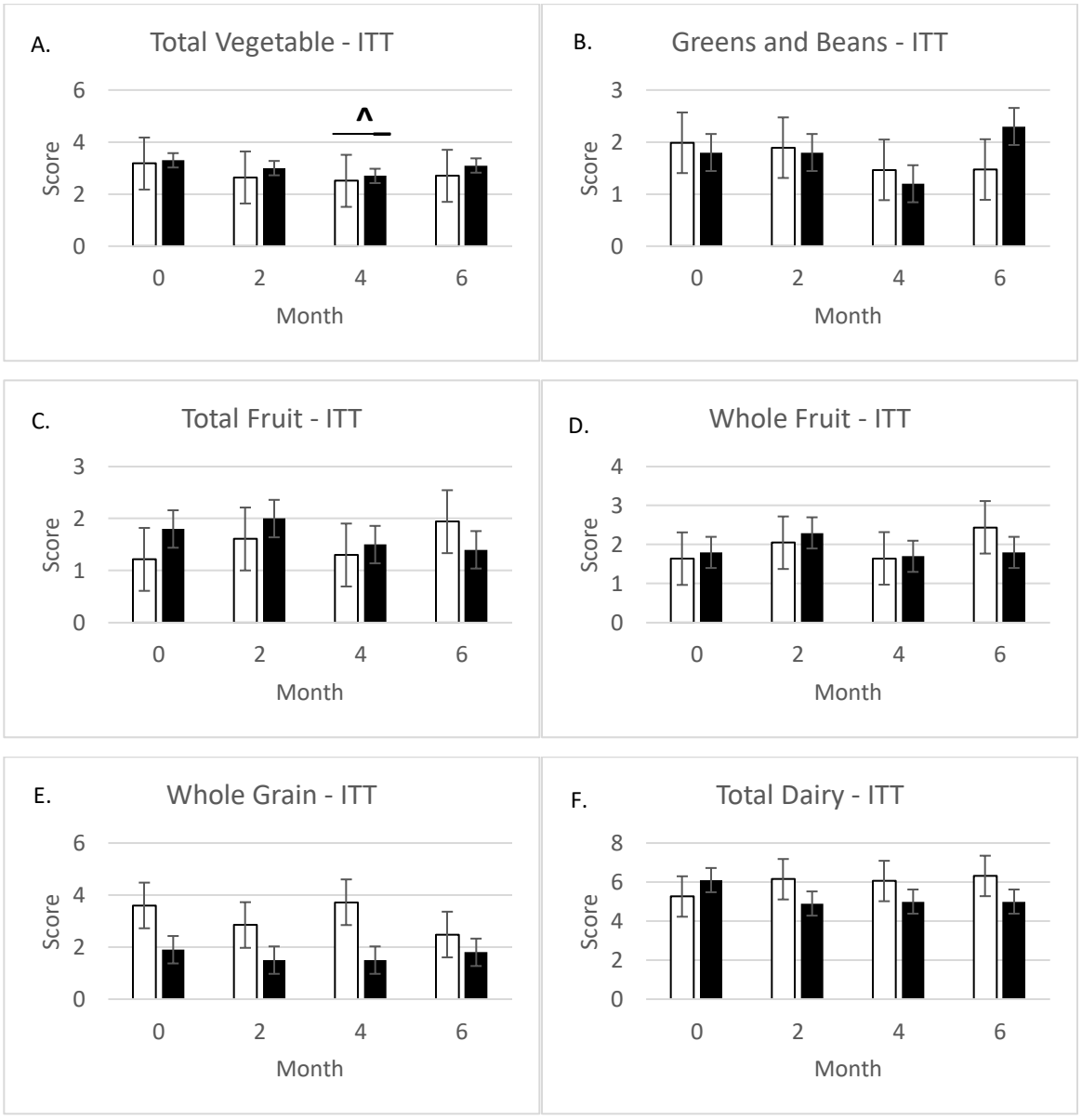
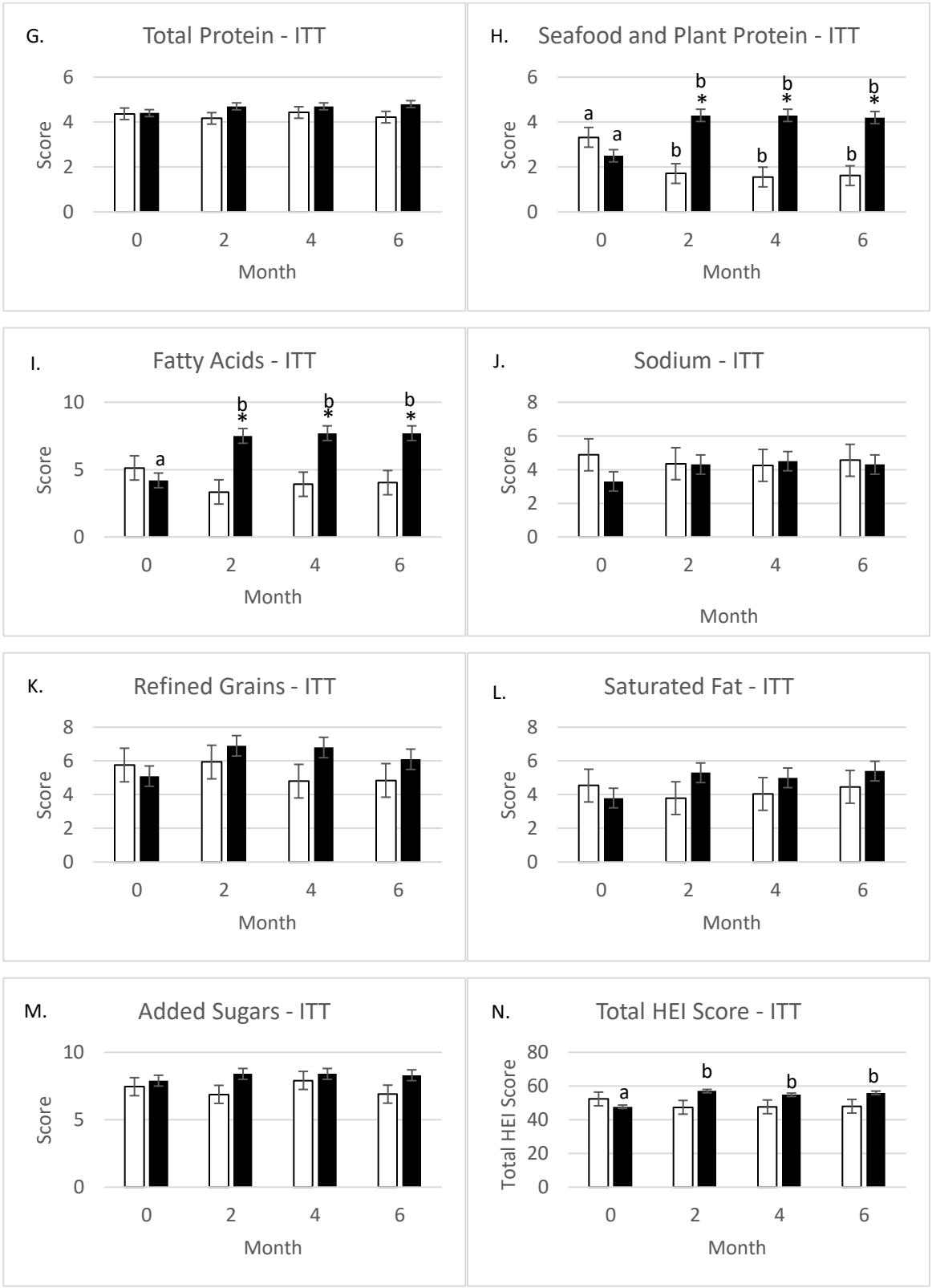


Figure 13. HEI score components and total score at months 0, 2, 4 and 6 from the ITT analysis. A. total vegetable, B. greens and beans, C. total fruit, D. whole fruit, E. whole grains, F. total dairy, G. total protein, H. seafood and plant proteins, I. fatty acids, J. sodium, K. refined grains, L. saturated fat, M. added sugars, N. total HEI score. *significant difference from control treatment at same timepoint ($p<0.05$). ^Significantly lower from month 0 ($p<0.05$). Different lowercase letters indicate significant difference within a treatment over time ($p<0.05$). There were no other significant differences in HEI score components ($p>0.05$). White bars=control treatment, Black bars=almond treatment.

Figure 13 continued.



4.7.2 Complier Analysis

Since nuts, including almonds, are included into calculated a component of the total HEI score, there were expected differences in the 'seafood and plant protein' score. Participants in the almond treatment group had higher seafood and plant protein scores overall ($p<0.001$), and at months 2, 4, and 6 compared to participants in the control treatment group ($p<0.001$) (table 14) (figure 14H). Within treatments, participants in the almond treatment had significantly higher seafood and plant protein scores at months 2, 4, and 6 compared to month 0, whereas participants in the control treatment group had lower seafood and plant protein scores at months 2, 4, and 6 compared to month 0 ($p<0.001$) (figure 14H). There were also differences in the fatty acid score, where participants in the almond treatment group had higher fatty acid scores overall ($p=0.024$), and at months 2, 4, and 6 compared to participants in the control treatment group ($p<0.001$) (figure 14I). Within treatments, participants in the almond treatment had significantly higher fatty acid scores at months 2, 4, and 6 compared to month 0 ($p<0.001$), but there were no differences in fatty acid score in participants in the control treatment over time ($p>0.05$) (figure 14I). There were higher total vegetable scores at month 0 compared to month 4 ($p=0.03$), however this did not differ between treatments ($p>0.1$) (figure 14A). Added sugar scores tended to be higher in participants in the almond treatment compared to participants in the control treatment ($p=0.07$), but there were no differences between treatments over time ($p>0.05$) (figure 14M). There were no other differences in HEI score components between participants in the almond and control treatment groups ($p>0.1$) (table 14). Participants in the almond treatment group had higher total HEI scores, indicative of a higher diet quality, at months 2, 4, and 6 compared to baseline ($p=0.001$), however there were no differences in total HEI score between participants in the almond and control treatment groups at any time ($p>0.3$) (figure 14N).

Table 14. HEI score components and total score at months 0, 2, 4, and 6 from the complier analysis.

Month	Control	Almond	P-value		
	N=11	N=21	Tx	Time	Tx*Time
Total Vegetable			0.195	0.03	0.857
0	2.9±0.5	3.7±0.4			
2	2.3±0.5	3.3±0.4			
4	2.2±0.5	3.0±0.4			
6	2.4±0.5	3.5±0.4			
Greens and Beans			0.892	0.362	0.368
0	1.9±0.7	1.6±0.5			
2	1.9±0.7	1.9±0.5			
4	1.5±0.7	1.1±0.5			
6	1.5±0.7	2.3±0.5			
Total Fruit			0.771	0.572	0.387
0	1.2±0.7	1.8±0.5			
2	1.5±0.7	2.0±0.5			
4	1.2±0.7	1.7±0.5			
6	1.9±0.7	1.6±0.5			
Whole Fruit			0.963	0.138	0.55
0	1.6±0.7	1.6±0.5			
2	2.1±0.7	2.3±0.5			
4	1.6±0.7	1.8±0.5			
6	2.5±0.7	1.9±0.5			
Whole Grain			0.338	0.48	0.382
0	3.5±1.1	1.9±0.8			
2	2.8±1.1	1.5±0.8			
4	3.7±1.1	1.2±0.8			
6	2.3±1.1	1.7±0.8			
Total Dairy			0.357	0.921	0.081
0	5.6±1.2	5.5±0.9			
2	6.7±1.2	4.3±0.9			
4	6.7±1.2	5.0±0.9			
6	6.9±1.2	4.5±0.9			

Values are means ± SE. n=32. Complier linear mixed model of main effects of treatment, time, and the interaction of treatment*time with Bonferroni post hoc comparisons when main or interaction effects were significant in SAS. Missing values were imputed with overall mean values. *Significant differences from control treatment ($p<0.05$). Different lowercase letters indicate a difference within treatment over time ($p<0.05$). Different numbers indicate a significant difference over time ($p<0.05$).

Table 14 continued.

Total Protein			0.467	0.402	0.115
0	4.4±0.3	4.3±0.2			
2	4.2±0.3	4.7±0.2			
4	4.5±0.3	4.6±0.2			
6	4.3±0.3	4.8±0.2			
Seafood and Plant Protein			<0.001	0.909	<0.001
0	3.5±0.4	2.5±0.3			
2	1.9±0.4	4.5±0.3			
4	1.7±0.4	4.5±0.3			
6	1.8±0.4	4.6±0.3			
Fatty Acids			0.024	0.194	<0.001
0	5.0±1.0	4.9±0.7			
2	3.1±1.0	8.4±0.7			
4	3.7±1.0	8.1±0.7			
6	3.8±1.0	8.2±0.7			
Sodium			0.617	0.859	0.375
0	5.0±1.1	3.3±0.8			
2	4.7±1.1	4.2±0.8			
4	4.6±1.1	4.8±0.8			
6	4.9±1.1	3.9±0.8			
Refined Grains			0.643	0.229	0.358
0	6.5±1.0	4.6±0.7			
2	6.8±1.0	6.5±0.7			
4	5.6±1.0	5.9±0.7			
6	5.7±1.0	5.3±0.7			
Saturated Fat			0.248	0.736	0.238
0	4.0±1.1	4.7±0.8			
2	3.2±1.1	6.0±0.8			
4	3.5±1.1	5.6±0.8			
6	3.9±1.1	5.9±0.8			
Added Sugars			0.07	0.508	0.052
0	7.3±0.6	8.2±0.5			
2	6.5±0.6	8.8±0.5			
4	7.6±0.6	8.7±0.5			
6	6.5±0.6	8.9±0.5			

Table 14 continued.

Total Score			0.398	0.624	0.004
0	52.3±4.7	48.4±3.4			
2	47.7±4.7	58.3±3.4			
4	48.1±4.7	55.8±3.4			
6	48.4±4.7	56.9±3.4			

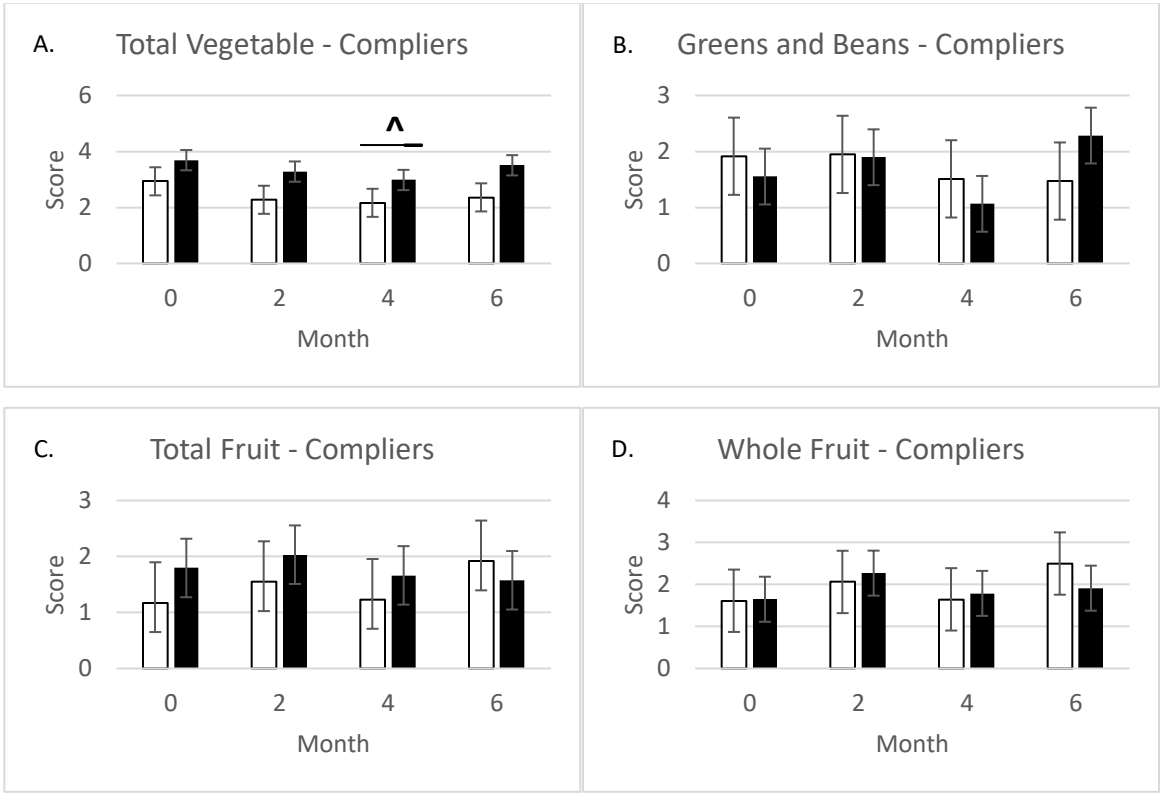


Figure 14. HEI score components and total score at months 0, 2, 4, and 6 months from the complier analysis. A. total vegetable, B. greens and beans, C. total fruit, D. whole fruit, E. whole grains, F. total dairy, G. total protein, H. seafood and plant proteins, I. fatty acids, J. sodium, K. refined grains, L. saturated fat, M. added sugars, N. total HEI score. *significant difference from control treatment at same timepoint ($p<0.05$). ^Significantly lower from month 0 ($p<0.05$). Different lowercase letters indicate significant difference within a treatment over time ($p<0.05$). There were no other significant differences in HEI score components ($p>0.05$). White bars=control treatment, Black bars=almond treatment.

Figure 14 continued.

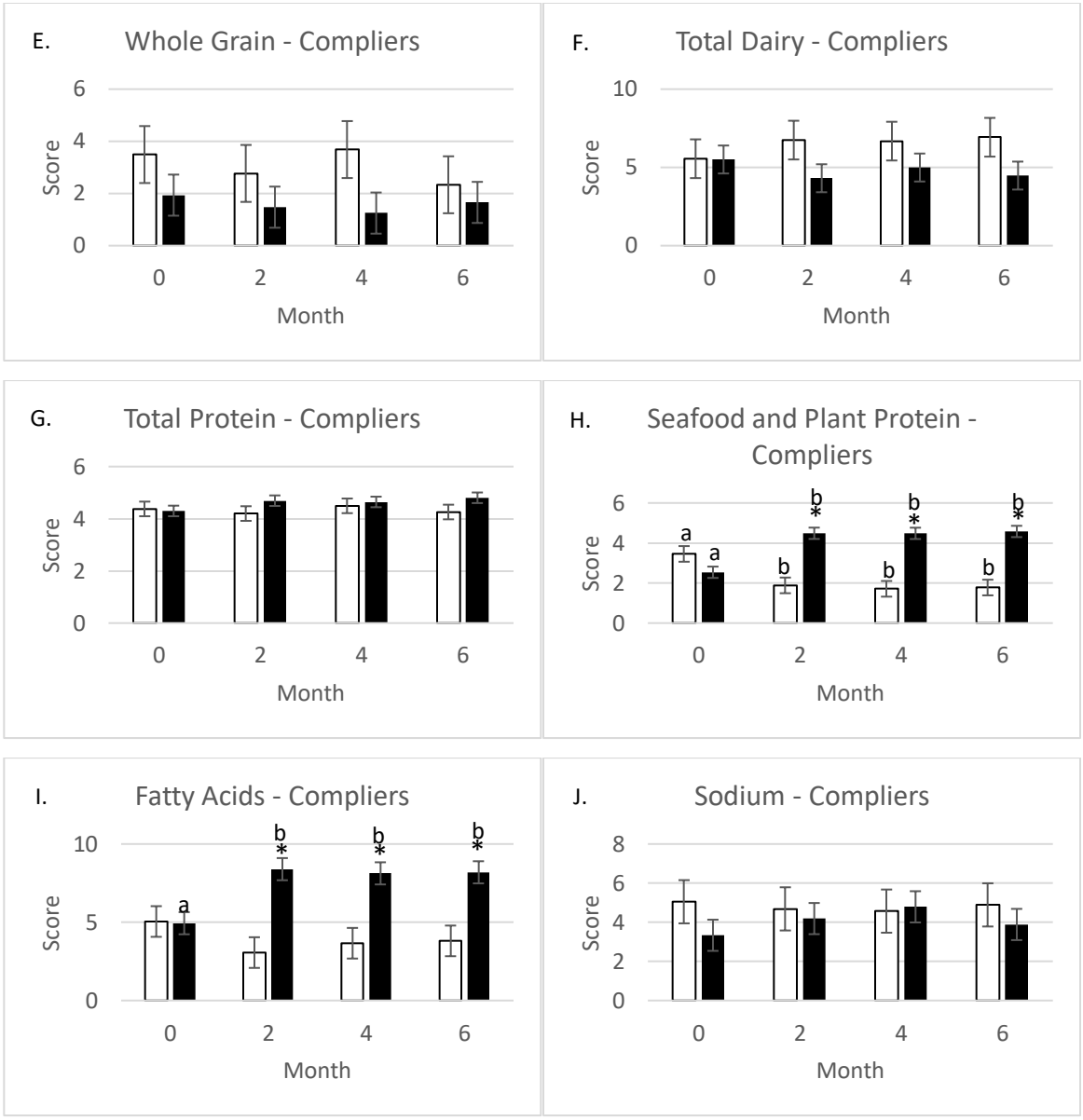
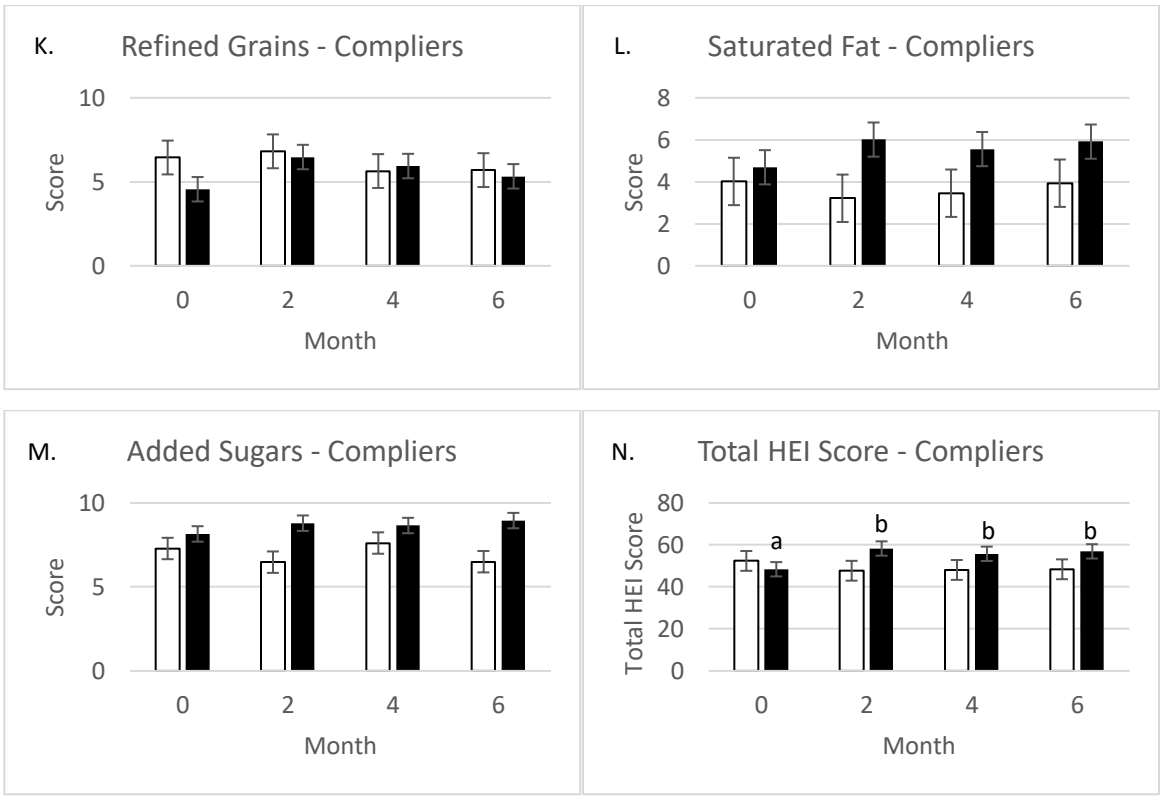


Figure 14 continued.



4.8 Appetite

4.8.1. ITT Analysis

There were significant main effects of time, where participants rated their feelings of hunger higher at month 6 compared to month 4 (p=0.29) (figure 15A), desire to eat salty foods higher at month 6 compared to months 0 and 4 (p=0.015) (figure 15F), and desire to eat fatty foods higher at month 6 compared to months 0, 2, and 4 (p=0.006) (figure 15G) (table 15). Additionally, participant's preoccupation with food ratings tended to be higher at month 0 compared to month 2 (p=0.077) (figure 15E), but these did not differ between treatments or BFD (p>0.1). There was a significant treatment*BFD*time effect on participants desire to eat sweet foods ratings (p=0.039) (figure 15H), however pairwise comparisons did not indicate any significant differences (p>0.05). There were no significant treatment, BFD, time, treatment*BFD, treatment*time, or treatment*BFD*time effects for participants ratings of

fullness ($p>0.1$) (figure 15B), desire to eat ($p>0.09$) (figure 15C), or prospective consumption ($p>0.08$) (figure 15D) (table 15).

Table 15. Average 24-hour appetite ratings at months 0, 2, 4, and 6 from the ITT analysis.

	High VAT		High SAT		High GF		Total		P-value					
	Control	Almond	Control	Almond	Control	Almond	Control	Almond	Tx	BFD	Time	Tx* BFD	Tx* Time	Tx* BFD* Time
Mean±SE	n=22	n=24	n=23	n=23	n=20	n=22	n=65	n=69						
Hunger									0.352	0.456	0.029	0.647	0.401	0.226
0 [1,2]	27.4±2.6	28.9±2.4	24.0±2.3	28.1±2.4	28.3±2.6	25.9±2.4	26.5±1.4	27.6±1.4						
2 [1,2]	28.9±2.6	27.5±2.4	24.3±2.3	27.1±2.4	26.6±2.6	26.8±2.4	26.6±1.4	27.1±1.4						
4 [1]	28.3±2.6	28.0±2.4	24.1±2.3	22.8±2.4	22.2±2.6	26.9±2.4	24.9±1.4	25.9±1.4						
6 [2]	28.9±2.6	28.5±2.4	23.7±2.3	31.7±2.4	26.7±2.6	29.4±2.4	26.5±1.4	29.9±1.4						
Fullness									0.268	0.419	0.139	0.798	0.713	0.411
0	47.6±3.2	46.5±3.0	49.7±2.9	45.4±3.0	46.2±3.3	45.5±3.1	47.8±1.8	45.8±1.7						
2	45.7±3.2	39.0±3.0	48.9±2.9	45.9±3.0	46.3±3.3	44.7±3.1	47.0±1.8	43.2±1.7						
4	45.7±3.2	42.3±3.0	45.7±2.9	47.6±3.0	47.0±3.3	42.8±3.1	46.1±1.8	44.2±1.7						
6	44.5±3.2	40.0±3.0	48.5±2.9	45.2±3.0	41.2±3.3	45.3±3.1	44.7±1.8	43.5±1.7						
Desire to Eat									0.361	0.565	0.667	0.421	0.723	0.097
0	30.9±2.7	30.8±2.5	26.5±2.4	32.8±2.5	30.5±2.7	30.8±2.6	29.3±1.5	31.5±1.4						
2	32.4±2.7	29.8±2.5	25.2±2.4	31.8±2.5	29.8±2.7	28.9±2.6	29.1±1.5	30.1±1.4						
4	32.5±2.7	31.3±2.5	28.4±2.4	25.9±2.5	26.0±2.7	31.2±2.6	28.9±1.5	29.5±1.4						
6	30.0±2.7	29.9±2.5	25.9±2.4	32.8±2.5	30.2±2.7	30.6±2.6	28.7±1.5	31.1±1.4						
Prospective Consumption									0.315	0.235	0.552	0.669	0.334	0.085
0	31.2±2.7	32.9±2.5	27.1±2.4	31.7±2.4	34.3±2.7	31.2±2.5	30.9±1.5	31.9±1.4						
2	32.4±2.7	31.0±2.5	25.8±2.4	32.2±2.4	32.4±2.7	31.3±2.5	30.2±1.5	31.5±1.4						
4	32.9±2.7	33.0±2.5	28.7±2.4	26.3±2.4	27.7±2.7	32.0±2.5	29.8±1.5	30.4±1.4						
6	30.4±2.7	33.0±2.5	26.0±2.4	32.7±2.4	30.8±2.7	32.7±2.5	29.1±1.5	32.8±1.4						

Values are means ± SE. n=134. ITT Linear Mixed Model of main effects of Tx, BFD, time, and the interaction of Tx*Time, Tx*BFD, and Tx*BFD*Time with Bonferroni post hoc comparisons when main effects were significant in SAS. Age was included as a covariate. Missing values were imputed with overall mean values. Different numbers indicate significant different pairwise time comparisons ($p<0.05$). Tx=treatment, BFD=body fat distribution.

Table 15 continued.

Preoccupation with Food									0.256	0.287	0.077	0.544	0.758	0.621
0	26.4±2.8	28.1±2.6	26.4±2.8	24.3±2.6	23.5±2.9	23.9±2.7	23.5±1.6	25.4±1.5						
2	23.1±2.8	23.5±2.6	23.1±2.8	25.8±2.6	21.8±2.9	20.3±2.7	20.7±1.6	23.2±1.5						
4	26.2±2.8	26.1±2.6	26.2±2.8	21.5±2.6	20.1±2.9	22.2±2.7	22.3±1.6	23.2±1.5						
6	24.6±2.8	25.2±2.6	19.4±2.5	25.6±2.6	21.5±2.9	23.3±2.7	21.8±1.6	24.7±1.5						
Desire to Eat Salty Foods									0.542	0.34	0.015	0.214	0.898	0.432
0 [1]	21.1±2.8	18.1±2.6	13.9±2.5	18.8±2.6	15.9±2.8	15.6±2.7	17.0±1.5	17.5±1.5						
2 [1,2]	21.1±2.8	16.1±2.6	13.7±2.5	19.9±2.6	14.8±2.8	16.0±2.7	16.5±1.5	17.3±1.5						
4 [1]	21.2±2.8	19.6±2.6	15.0±2.5	16.0±2.6	13.5±2.8	18.6±2.7	16.6±1.5	18.1±1.5						
6 [2]	22.5±2.8	19.6±2.6	14.9±2.5	21.6±2.6	18.3±2.8	19.5±2.7	18.6±1.5	20.2±1.5						
Desire to Eat Fatty Foods									0.398	0.677	0.006	0.322	0.465	0.595
0 [1,2]	20.2±3.0	18.5±2.8	14.4±2.7	20.5±2.8	18.8±3.1	16.2±2.9	17.8±1.6	18.4±1.6						
2 [1]	19.7±3.0	16.9±2.8	14.6±2.7	21.3±2.8	14.7±3.1	16.5±2.9	16.3±1.6	18.2±1.6						
4 [1,2]	21.0±3.0	19.6±2.8	17.2±2.7	17.7±2.8	15.0±3.1	17.9±2.9	17.7±1.6	18.4±1.6						
6 [2]	21.7±3.0	21.5±2.8	15.3±2.7	23.8±2.8	19.8±3.1	21.6±2.9	18.9±1.6	22.3±1.6						
Desire to Eat Sweet Foods									0.459	0.515	0.111	0.545	0.688	0.039
0	23.3±3.1	24.8±2.9	19.2±2.8	20.3±2.9	22.5±3.2	24.5±3.0	21.6±1.7	23.2±1.7						
2	22.3±3.1	18.2±2.9	19.0±2.8	24.3±2.9	18.5±3.2	21.4±3.0	19.9±1.7	21.3±1.7						
4	25.4±3.1	23.1±2.9	21.6±2.8	17.2±2.9	17.1±3.2	24.2±3.0	21.4±1.7	21.5±1.7						
6	23.0±3.1	23.9±2.9	19.6±2.8	21.7±2.9	22.7±3.2	28.0±3.0	21.7±1.7	24.5±1.7						

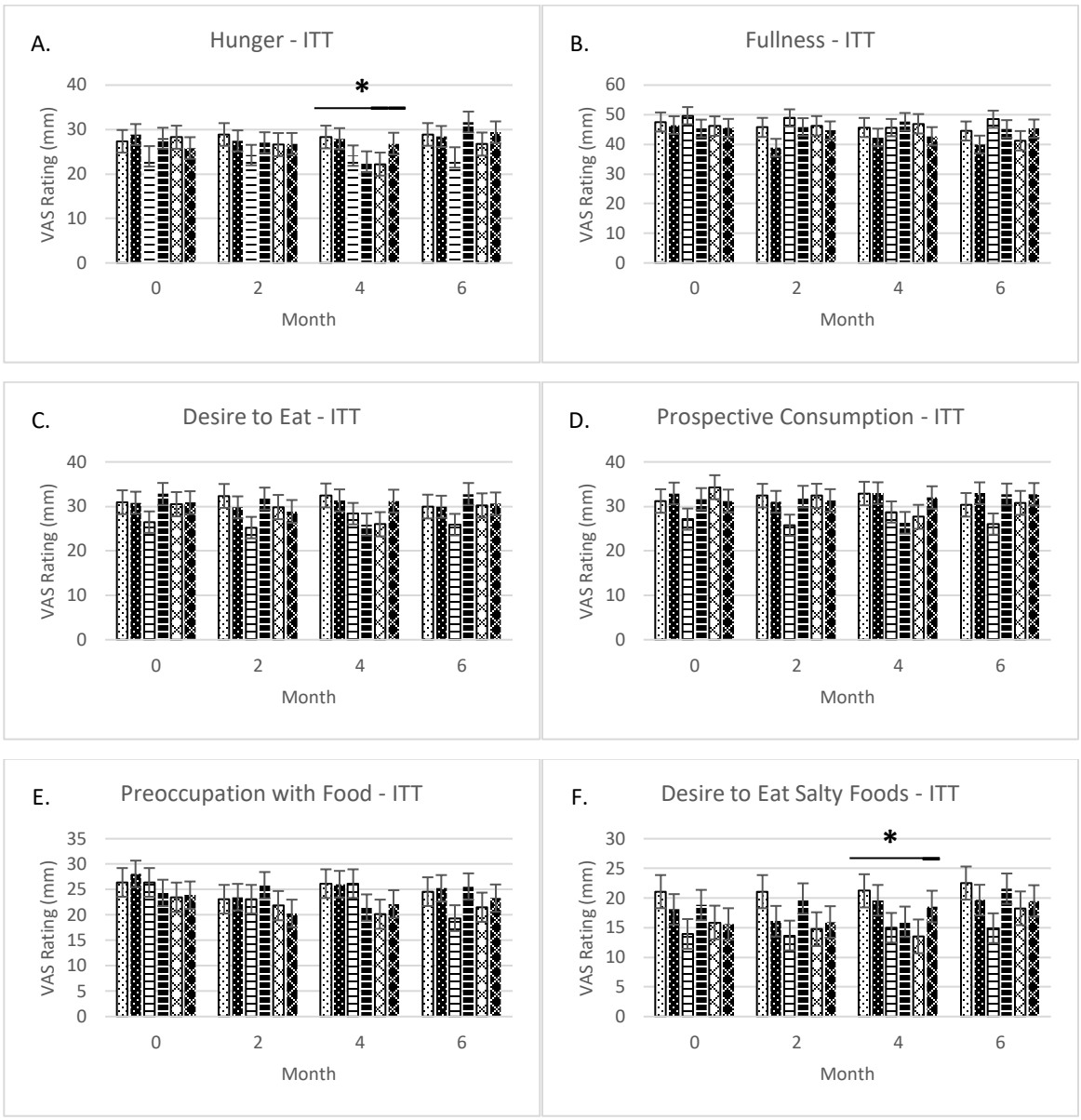
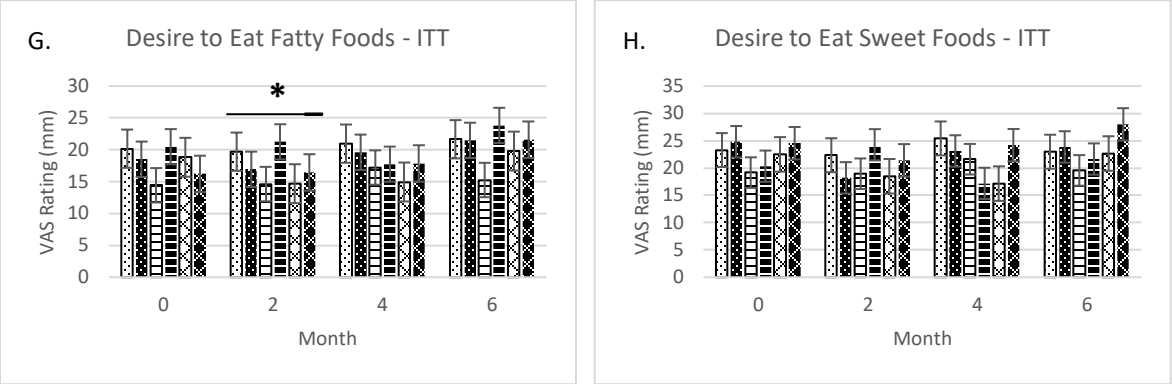


Figure 15. Average 24-hour appetite ratings at months 0, 2, 4, and 6 from the ITT analysis. A. Hunger, B. Fullness, C. Desire to Eat, D. Prospective Consumption, E. Preoccupation with food, F. desire to eat salty foods, G. desire to eat fatty foods, H. desire to eat sweet foods. *Significantly different from month 6 ($p<0.05$). There were no other significant differences in appetite ratings ($p>0.05$). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

Figure 15 continued.



4.8.2 Complier Analysis

There were no significant treatment, BFD, time, treatment*BFD, treatment*time, or treatment*BFD*time effects for participant's appetite ratings in the complier analysis (all $p>0.1$) (table 16) (figure 16).

Table 16. Average 24-hour appetite ratings at months 0, 2, 4, and 6 from the Complier analysis.

	High VAT		High SAT		High GF		Total		P-value					
	Control	Almond	Control	Almond	Control	Almond	Control	Almond						
Mean±SE	n=20	n=15	n=17	n=14	n=18	n=17	n=55	n=46	Tx	BFD	Time	Tx* BFD	Tx* Time	Tx* BFD* Time
Hunger									0.678	0.436	0.179	0.701	0.342	0.565
0	27.3±2.7	28.9±2.9	23.3±2.7	27.8±3.1	26.3±2.8	24.9±2.8	25.6±1.5	27.2±1.7						
2	28.5±2.7	26.9±2.9	23.6±2.7	25.7±3.1	26.6±2.8	23.7±2.8	26.2±1.5	25.4±1.7						
4	28.0±2.7	26.9±2.9	23.8±2.7	21.2±3.1	22.4±2.8	25.0±2.8	24.7±1.5	24.4±1.7						
6	28.3±2.7	26.6±2.9	21.9±2.7	29.8±3.1	26.2±2.8	27.8±2.8	25.5±1.5	28.1±1.7						
Fullness									0.252	0.41	0.319	0.721	0.73	0.427
0	47.1±3.7	44.4±3.8	50.1±3.6	44.5±4.2	47.2±3.8	45.3±3.7	48.1±2.0	44.8±2.3						
2	46.2±3.7	37.3±3.8	50.2±3.6	46.8±4.2	45.9±3.8	44.8±3.7	47.4±2.0	43.0±2.3						
4	46.2±3.7	41.7±3.8	44.2±3.6	48.5±4.2	46.7±3.8	42.5±3.7	45.7±2.0	44.2±2.3						
6	45.0±3.7	38.2±3.8	49.4±3.6	45.9±4.2	40.6±3.8	43.9±3.7	45.0±2.0	42.7±2.3						

Values are means ± SE. n=101. Complier Linear Mixed Model of main effects of Tx, BFD, time, and the interaction of Tx*time, Tx*BFD, and Tx*BFD*Time with Bonferroni post hoc comparisons when main effects were significant in SAS. Age was included as a covariate. Missing values were imputed with overall mean values.

Table 16 continued.

Desire to Eat									0.513	0.51	0.622	0.468	0.365	0.171
0	30.8±3.0	31.4±3.1	25.6±2.9	33.7±3.4	28.5±3.0	28.9±3.0	28.3±1.7	31.3±1.8						
2	32.0±3.0	29.4±3.1	24.0±2.9	31.0±3.4	29.7±3.0	26.8±3.0	28.5±1.7	29.1±1.8						
4	32.1±3.0	31.1±3.1	27.7±2.9	23.5±3.4	26.1±3.0	29.2±3.0	28.6±1.7	28.0±1.8						
6	29.3±3.0	28.9±3.1	23.6±2.9	32.1±3.4	29.9±3.0	28.9±3.0	27.6±1.7	30.0±1.8						
Prospective Consumption									0.378	0.36	0.554	0.431	0.427	0.172
0	31.4±2.9	33.5±3.1	26.3±2.9	33.3±3.4	33.3±3.0	29.6±3.0	30.3±1.6	32.1±1.8						
2	32.0±2.9	31.0±3.1	24.2±2.9	32.8±3.4	33.3±3.0	29.6±3.0	29.8±1.6	31.1±1.8						
4	32.6±2.9	33.3±3.1	28.2±2.9	25.5±3.4	27.9±3.0	30.6±3.0	29.5±1.6	29.8±1.8						
6	29.7±2.9	33.3±3.1	24.4±2.9	32.6±3.4	30.6±3.0	30.3±3.0	28.2±1.6	32.1±1.8						
Preoccupation with Food									0.343	0.305	0.588	0.485	0.408	0.771
0	26.6±3.2	26.4±3.3	19.9±3.1	24.4±3.6	21.1±3.3	21.6±3.2	22.5±1.8	24.2±2.0						
2	23.0±3.2	23.5±3.3	15.9±3.1	26.1±3.6	22.1±3.3	20.2±3.2	20.3±1.8	23.3±2.0						
4	26.3±3.2	25.4±3.3	20.3±3.1	20.1±3.6	19.9±3.3	21.1±3.2	22.1±1.7	22.2±2.0						
6	24.5±3.2	25.7±3.3	17.0±3.1	26.3±3.6	20.4±3.3	21.2±3.2	20.6±1.8	24.4±2.0						

Table 16 continued.

Desire to Eat Salt									0.92	0.238	0.217	0.351	0.936	0.446
0	21.2±3.0	16.7±3.1	13.4±2.9	19.4±3.4	13.6±3.1	13.7±3.0	16.1±1.7	16.6±1.8						
2	21.1±3.0	15.8±3.1	12.8±2.9	17.5±3.4	14.2±3.1	14.7±3.0	16.0±1.7	16.0±1.8						
4	21.2±3.0	18.6±3.1	15.1±2.9	13.0±3.4	13.3±3.1	16.7±3.0	16.5±1.7	16.1±1.8						
6	22.4±3.0	19.1±3.1	13.1±2.9	19.3±3.4	17.1±3.1	16.5±3.0	17.5±1.7	18.3±1.8						
Desire to Eat Fat									0.567	0.703	0.516	0.3	0.679	0.505
0	20.1±3.3	16.6±3.4	13.7±3.2	22.3±3.8	16.5±3.4	14.6±3.3	16.8±1.8	17.8±2.0						
2	19.5±3.3	17.0±3.4	13.9±3.2	22.0±3.8	14.3±3.4	15.6±3.3	15.9±1.8	18.2±2.0						
4	20.8±3.3	18.0±3.4	16.6±3.2	16.0±3.8	15.0±3.4	17.9±3.3	17.5±1.8	17.3±2.0						
6	21.3±3.3	19.0±3.4	12.7±3.2	21.6±3.8	18.7±3.4	18.4±3.3	17.6±1.8	19.7±2.0						
Desire to Eat Sweet									0.515	0.805	0.27	0.397	0.268	0.528
0	24.2±3.5	23.3±3.6	18.2±3.4	22.7±4.0	19.2±3.6	22.7±3.5	20.5±1.9	22.9±2.1						
2	22.6±3.5	17.3±3.6	18.5±3.4	24.7±4.0	17.7±3.6	20.1±3.5	19.6±1.9	20.7±2.1						
4	25.9±3.5	19.9±3.6	20.7±3.4	17.9±4.0	16.4±3.6	22.1±3.5	21.0±1.9	20.0±2.1						
6	23.0±3.5	22.9±3.6	17.1±3.4	23.8±4.0	21.1±3.6	26.1±3.5	20.4±1.9	24.3±2.1						

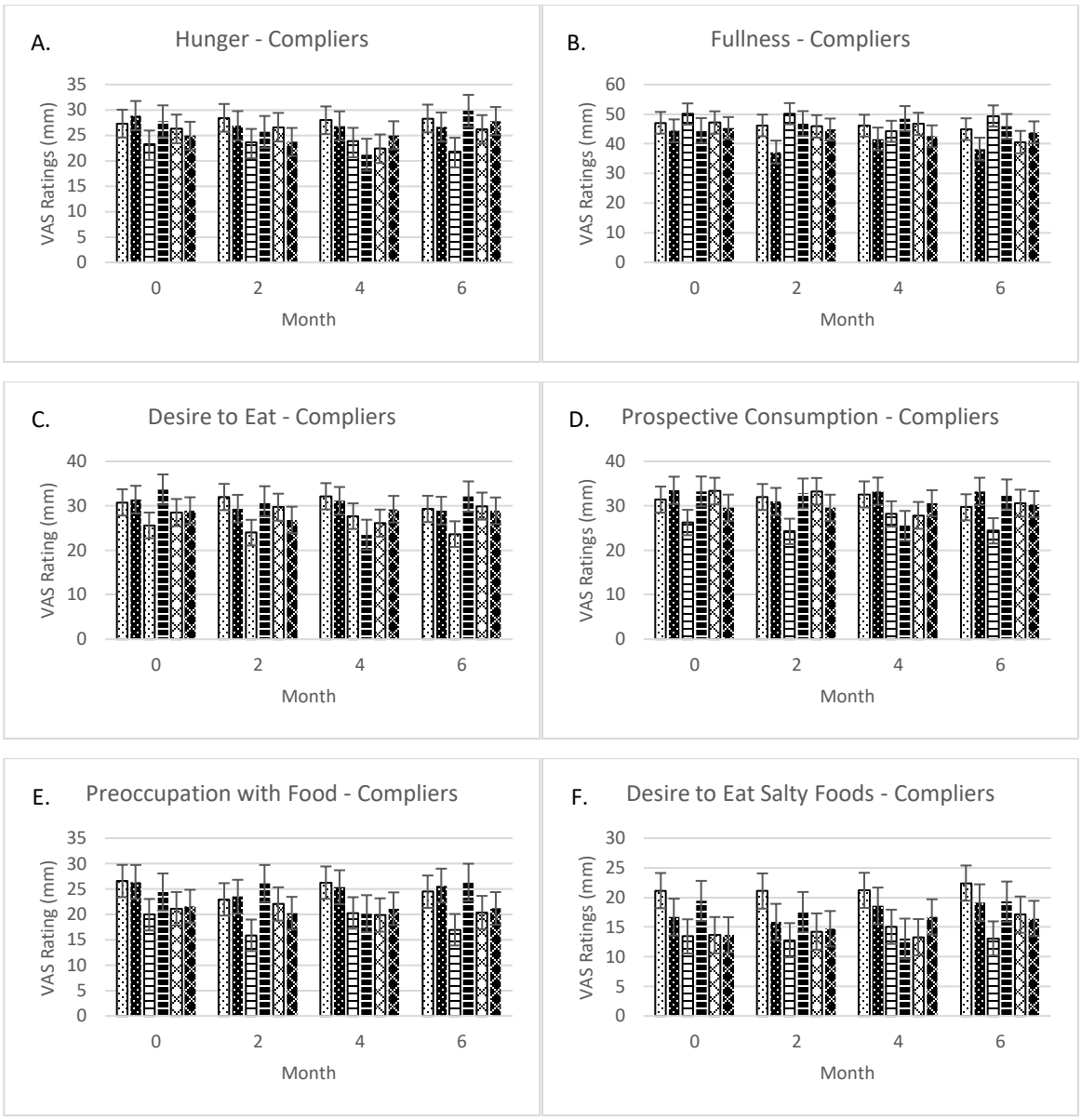
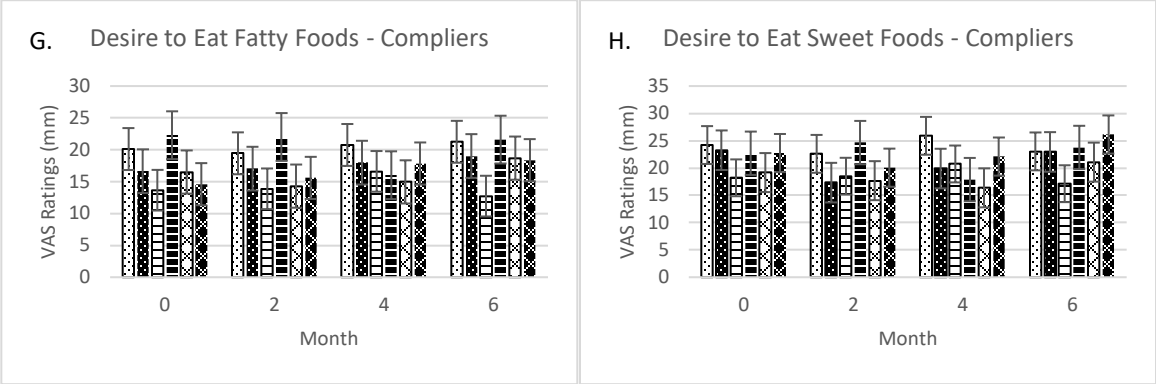


Figure 16. Average 24-hour appetite ratings at months 0, 2, 4, and 6 from the complier analysis. A. hunger ratings, B. fullness ratings, C. desire to eat ratings, D. prospective consumption ratings, E. preoccupation with food ratings, F. desire to eat salty foods, G. desire to eat fatty foods, H. desire to eat sweet foods. There were no significant differences in appetite ratings ($p>0.1$). Dotted bars=high VAT group, Horizontal striped bars=high SAT group, Diamond bars=high GF group. Lighter bar in each BFD=control treatment, darker bar=almond treatment.

Figure 16 continued.



4.9 Red Blood Cell Analysis

4.9.1 Nutrient Intake

Participants in the almond treatment group reported higher total MUFA (figure 17H), oleic acid (figure 17I), and linoleic acid intakes (figure 17K) ($p<0.05$), and total PUFA intake tended to be higher compared to participants in the control treatment group ($p=0.069$) (figure 17J) (table 17). Furthermore, a treatment*time analysis revealed participants in the almond treatment group reported higher total MUFA ($p<0.001$) and oleic acid ($p<0.001$) intakes at months 2 and 4, and higher total PUFA ($p=0.005$) and linoleic acid ($p=0.002$) intake at month 2 compared to participants in the control treatment. Within-subject analyses indicated participants in the almond treatment group had higher reported total MUFA ($p<0.001$) and oleic acid ($p<0.001$) intakes at months 2, 4, and 6 compared to month 0, and participants in the control treatment reported higher arachidonic acid intakes at month 4 compared to month 0 ($p=0.003$) (figure 17M). Saturated fat ($p=0.047$) (figure 17C) and myristic acid ($p=0.05$) (figure 17D) intakes were higher at month 2 compared to month 0, but this did not differ between treatments, or between treatments over time ($p>0.05$). There were significant treatment*time effects for total fat ($p=0.024$) (figure 17A), cholesterol ($p=0.028$) (figure 17B), and ALA ($p=0.037$) (figure 17L), but pairwise comparisons were not significant ($p>0.05$) (table 17).

Table 17. Nutrient intake from participants with RBC membrane fatty acid composition data.

Nutrient Intake	Month	Control	Almond	P-Value		
				Tx	Time	Tx*Time
		n=12	n=27			
Total Fat (g)	0	97±9	96±6	0.086	0.276	0.024
	2	76±9	102±6			
	4	81±9	100±6			
	6	78±9	99±6			
Cholesterol (mg)	0	244±49	371±32	0.541	0.126	0.028
	2	357±49	407±32			
	4	377±49	328±32			
	6	350±49	329±32			
Total Saturated Fatty Acids (g)	0 [1]	30±3	33±2	0.805	0.047	0.503
	2 [2]	25±3	28±2			
	4 [1,2]	29±3	28±2			
	6 [1,2]	29±3	27±2			
Myristic Acid (g)	0 [1]	3±0.3	3±0.2	0.921	0.05	0.957
	2 [2]	2±0.3	2±0.2			
	4 [1,2]	2±0.3	2±0.2			
	6 [1,2]	2±0.3	2±0.2			
Palmitic Acid (g)	0	16±2	18±1	0.571	0.127	0.27
	2	14±2	16±1			
	4	16±2	16±1			
	6	16±2	15±1			
Palmitoleic Acid (g)	0	1±0.2	1±0.1	0.359	0.668	0.581
	2	1±0.2	1±0.1			
	4	1±0.2	1±0.1			
	6	1±0.2	1±0.1			
Stearic Acid (g)	0	7±1	8±1	0.976	0.087	0.25
	2	6±1	7±1			
	4	7±1	7±1			
	6	7±1	7±1			

Nutrient intake from ASA24 totals of subgroup of participants whose RBC membrane fatty acid composition was analyzed. Means ± S.E. n=39 (Control n=12, Almond n=27). Linear mixed model of main effects of Tx, time, and the interaction of Tx*time with Bonferroni post hoc comparisons when main or interaction effects were significant in SAS. Missing values were imputed with overall means. *Significant difference from control treatment at same time point ($p<0.05$). Different number superscripts indicate differences between time points ($p<0.05$). Different lowercase letter superscripts indicate differences within a treatment over time ($p<0.05$).

Table 17 continued.

Total MUFA (g)	0	33±4	35±2[a]	0.005	0.55	<0.001
	2	25±4	42±2[b*]			
	4	29±4	42±2[b*]			
	6	31±4	41±2[b]			
Oleic Acid (g)	0	31±3	32±2[a]	0.004	0.52	<0.001
	2	23±3	40±2[b*]			
	4	27±3	40±2[b*]			
	6	28±3	39±2[b]			
Total PUFA (g)	0	21±2	21±2	0.069	0.486	0.005
	2	15±2	25±2*			
	4	19±2	22±2			
	6	21±2	23±2			
Linoleic Acid (g)	0	19±2	19±1	0.05	0.421	0.002
	2	13±2	22±1*			
	4	16±2	20±1			
	6	18±2	20±1			
ALA (g)	0	2±0.3	2±0.2	0.686	0.671	0.037
	2	1±0.3	2±0.2			
	4	2±0.3	2±0.2			
	6	2±0.3	2±0.2			
Arachidonic Acid (g)	0	0.1±0.03[a]	0.2±0.02	0.486	0.237	0.003
	2	0.2±0.03[a,b]	0.2±0.02			
	4	0.2±0.03[b]	0.2±0.02			
	6	0.2±0.03[a,b]	0.2±0.02			

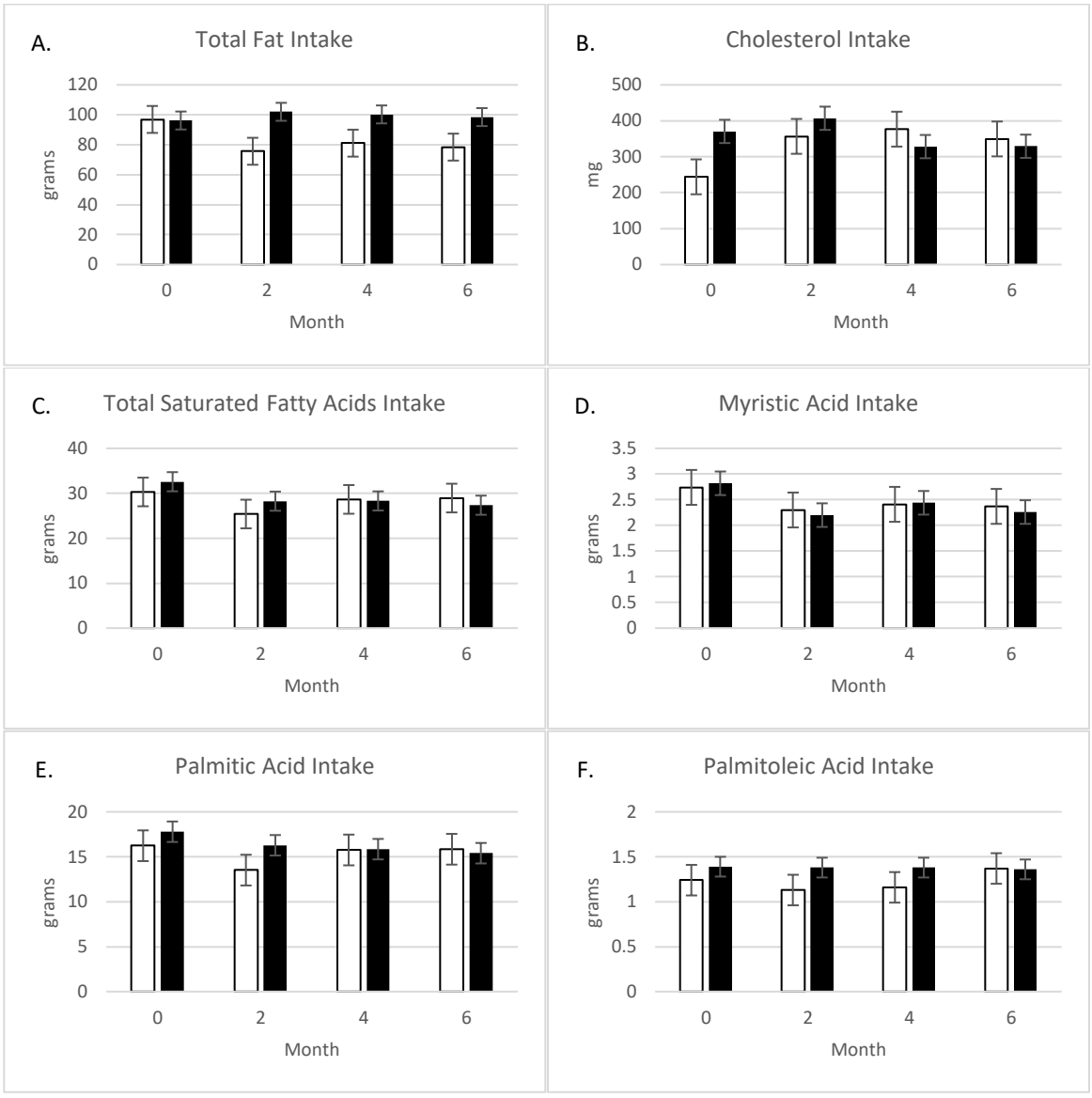
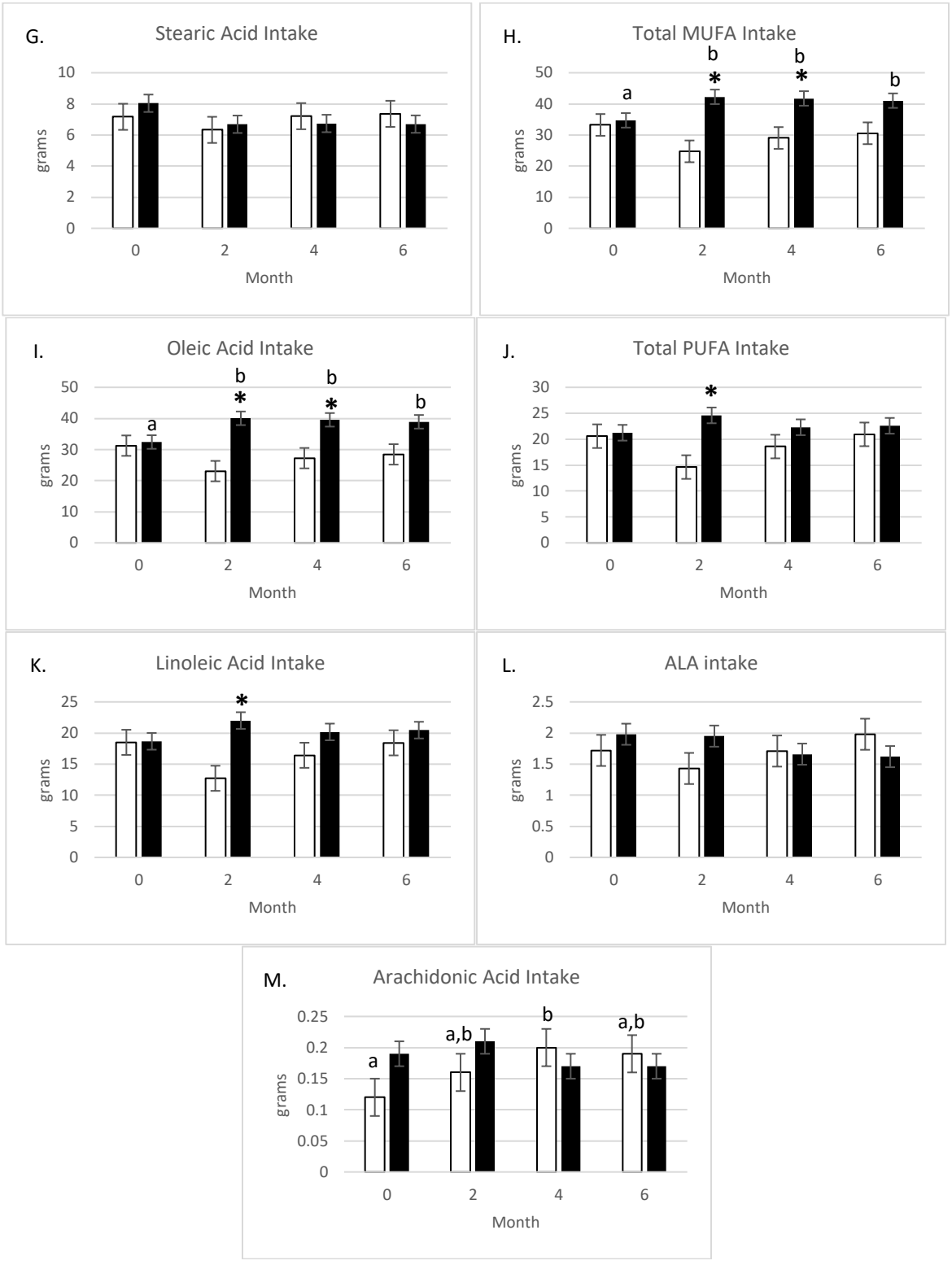


Figure 17. Nutrient intake from participants with RBC membrane fatty acid composition data at months 0, 2, 4, and 6. A. total fat intake, B. cholesterol intake, C. total saturated fatty acids intake, D. myristic acid intake, E. palmitic acid intake, F. palmitoleic acid intake, G. stearic acid intake, H. total MUFA itnake, I. oleic acid intake, J. total PUFA intake, k. linoleic acid intake, L. ALA intake, M. arachidonic acid intake. *Significant difference from control treatment at same time point ($p<0.05$). Different lowercase letter superscripts indicate differences within a treatment over time ($p<0.05$). There were no significant differences in total fat, cholesterol, total saturated fatty acids, myristic acid, palmitic acid, palmitoleic acid, stearic acid, or ALA intake ($p>0.05$). White bars=control treatment, black bars = almond treatment.

Figure 17 continued.



4.9.2 Membrane Fatty Acid Composition

There were significant main treatment effects, where participants in the control treatment group had higher composition of oleic acid (figure 18E), DHA (figure 18J), Oleic:Palmitic acid ratio (figure 18K), total MUFA (figure 18L), and MUFA:SFA ratio (figure 18N) in the RBC membrane compared to participants in the almond treatment group ($p<0.05$), however these did not differ over time, or between treatments at any time point ($p>0.05$) (table 18). These results are unexpected, since almonds are rich sources of oleic acid and MUFA, and participants reported higher intakes of these fatty acids. Participants in the almond treatment did have higher amounts of linoleic acid (figure 18F), ALA (figure 18G), and arachidonic acid (figure 18H) in the RBC membrane compared to the control treatment ($p<0.05$), although this did not differ over time, or between treatments at any time point ($p>0.05$). There were no significant time or treatment*time effects on fatty acid composition of the RBC membrane ($p>0.05$) (table 18).

Table 18. RBC membrane fatty acid composition

Fatty Acid	Month	Control	Almond	P-Value		
				Tx	Time	Tx*Time
		n=12	n=27			
Myristic Acid	0	0.38±0.05	0.22±0.03	0.158	0.338	0.227
	2	0.26±0.09	0.28±0.06			
	4	0.26±0.05	0.19±0.03			
	6	0.24±0.05	0.24±0.03			
Palmitic Acid	0	22.62±0.49	22.66±0.31	0.632	0.58	0.882
	2	22.80±0.56	22.46±0.36			
	4	22.17±0.48	22.32±0.32			
	6	23.05±0.52	22.60±0.36			
Palmitoleic Acid	0	0.66±0.05	0.57±0.03	0.489	0.309	0.538
	2	0.58±0.04	0.57±0.03			
	4	0.56±0.05	0.58±0.03			
	6	0.53±0.05	0.54±0.03			
Stearic Acid	0	17.47±0.45	17.64±0.28	0.444	0.991	0.854
	2	17.32±0.44	17.86±0.28			
	4	17.42±0.36	17.62±0.24			
	6	17.69±0.45	17.57±0.31			
Oleic Acid	0	16.03±0.47	15.11±0.30	<0.001	0.905	0.881
	2	16.14±0.41	15.06±0.26			
	4	16.14±0.39	15.38±0.26			
	6	16.48±0.49	15.13±0.34			
Linoleic Acid	0	11.16±0.69	12.40±0.44	0.025	0.844	0.808
	2	11.51±0.76	11.72±0.48			
	4	11.57±0.52	12.57±0.34			
	6	11.10±0.79	12.45±0.55			
ALA	0	0.23±0.03	0.3±0.02	0.002	0.74	0.95
	2	0.22±0.03	0.27±0.02			
	4	0.22±0.03	0.28±0.02			
	6	0.24±0.04	0.29±0.02			

RBC Membrane Fatty Acid composition, listed as percent of total membrane fatty acids. Means ± S.E. n=39 (Control n=12, Almond n=27). Linear mixed model of main effects of Tx, time, and the interaction of Tx*time with Bonferroni post hoc comparisons when main effects were significant in SAS. MUFA=monounsaturated fatty acids, SFA=saturated fatty acids

Table 18 continued.

Arachidonic Acid	0	22.30±0.72	22.66±0.46	0.068	0.645	0.74
	2	21.78±0.95	23.37±0.61			
	4	21.97±0.93	22.37±0.62			
	6	21.06±0.79	22.46±0.55			
EPA	0	0.48±0.04	0.50±0.03	0.596	0.984	0.878
	2	0.51±0.06	0.47±0.04			
	4	0.49±0.05	0.48±0.03			
	6	0.52±0.07	0.48±0.05			
DHA	0	8.66±0.72	7.95±0.46	0.048	0.898	0.996
	2	8.87±0.68	7.93±0.44			
	4	9.18±0.72	8.21±0.48			
	6	9.08±0.78	8.23±0.54			
Oleic/Palmitic Acid	0	0.72±0.03	0.67±0.02	0.0006	0.792	0.998
	2	0.72±0.03	0.68±0.02			
	4	0.73±0.02	0.69±0.02			
	6	0.72±0.03	0.67±0.02			
Total MUFA	0	16.70±0.47	15.68±0.30	<0.001	0.949	0.88
	2	16.73±0.42	15.63±0.27			
	4	16.70±0.40	15.96±0.26			
	6	17.02±0.49	15.67±0.34			
Total SFA	0	40.47±0.62	40.51±0.39	0.986	0.55	0.869
	2	40.38±0.75	40.61±0.48			
	4	39.86±0.54	40.13±0.36			
	6	40.98±0.68	40.41±0.47			
MUFA/SFA	0	0.41±0.01	0.39±0.01	0.001	0.782	0.986
	2	0.41±0.01	0.39±0.01			
	4	0.42±0.01	0.40±0.01			
	6	0.42±0.01	0.39±0.01			
UFA/SFA	0	1.48±0.04	1.47±0.03	0.963	0.511	0.739
	2	1.48±0.04	1.47±0.03			
	4	1.51±0.04	1.49±0.03			
	6	1.44±0.04	1.48±0.03			

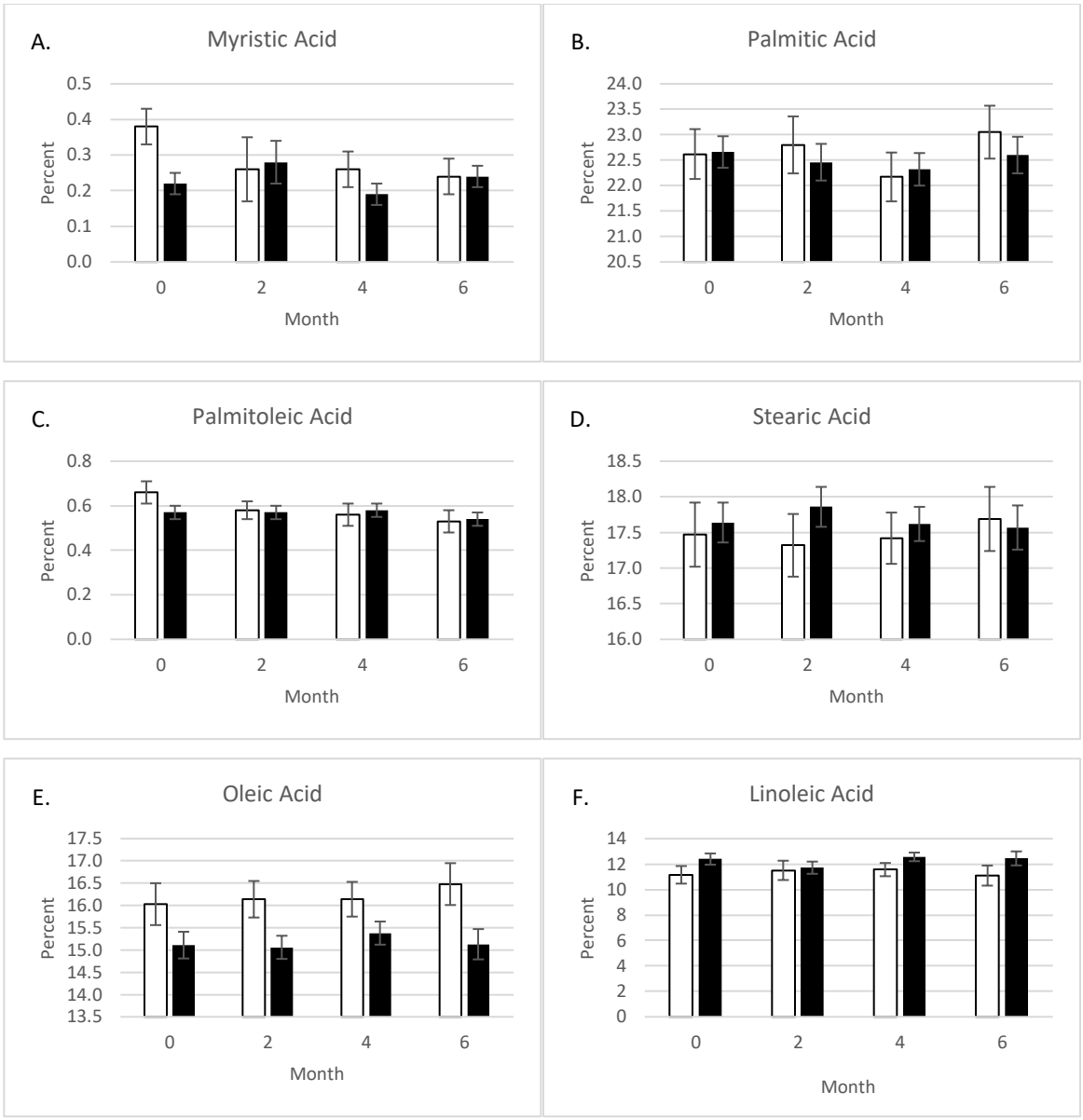
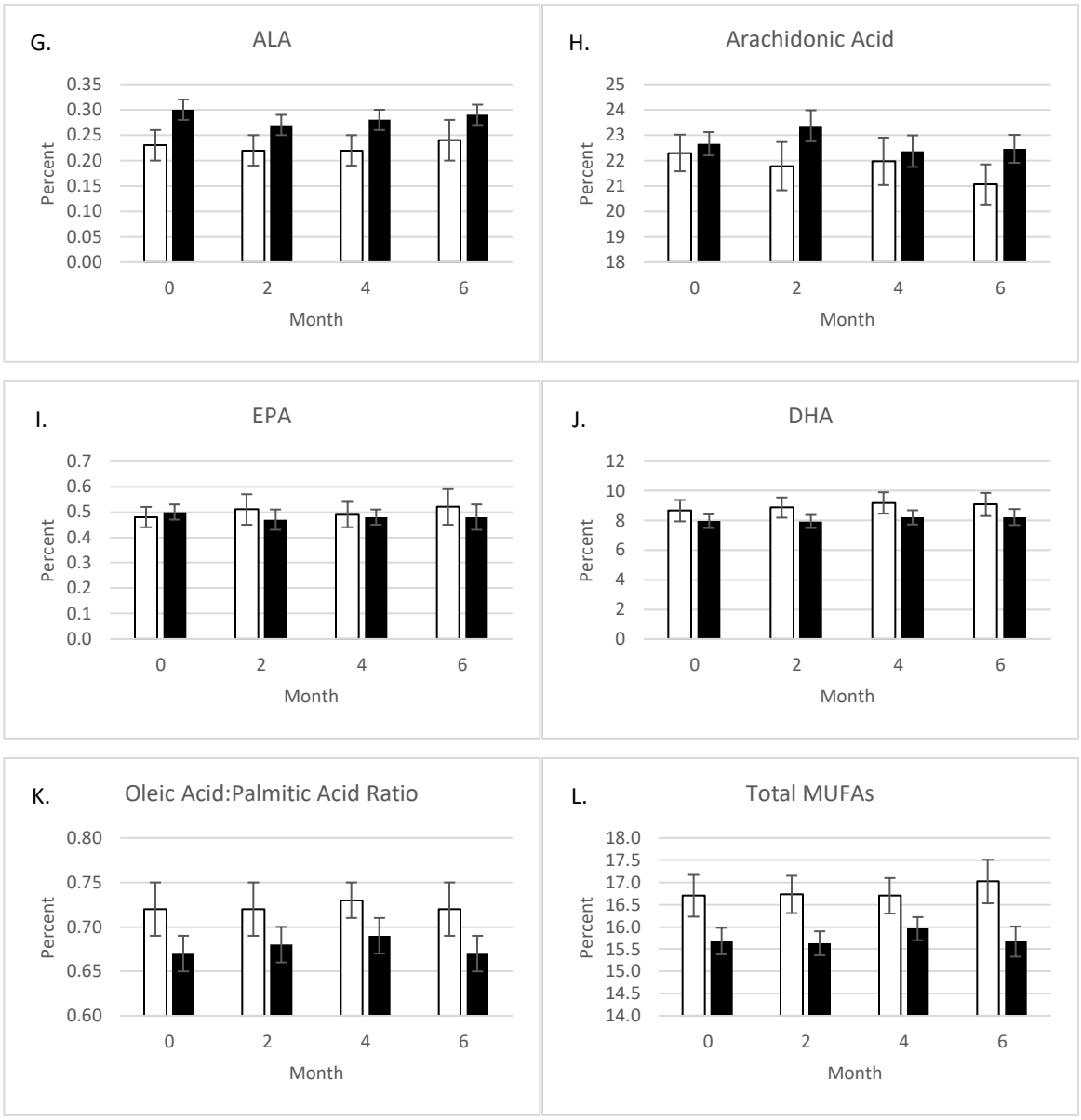


Figure 18. RBC membrane fatty acid composition at months 0, 2, 4, and 6. A. myristic acid, B. palmitic acid, C. palmitoleic acid, D. stearic acid, E. oleic acid, F. linoleic acid, G. ALA, H. arachidonic acid, I. EPA, J. DHA, K. Oleic Acid:Pamitc Acid Ratio, L. total MUFAs, M. total saturated fatty acids, N. total MUFA:total saturated fatty acid ratio, O. total unsaturated fatty acids:total saturated fatty acids ratio. Participants in the control treatment group had significant higher RBC membrane compositions of oleic acid, DHA, Oleic:Palmitic acid ratio, total MUFA, and MUFA:SFA ratio, and significant lower RBC membrane compositions of linoleic acid, ALA, and arachidonic acid compared to participants in the almond treatment group ($p<0.05$). There were no significant differences in RBC membrane composition over time, or between treatments over time ($p>0.05$). White bars=control treatment, Black bars=almond treatment. ALA=alpha-linolenic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, MUFA=monounsaturated fatty acids, SFA=saturated fatty acids, USA=unsaturated fatty acids.

Figure 18 continued.



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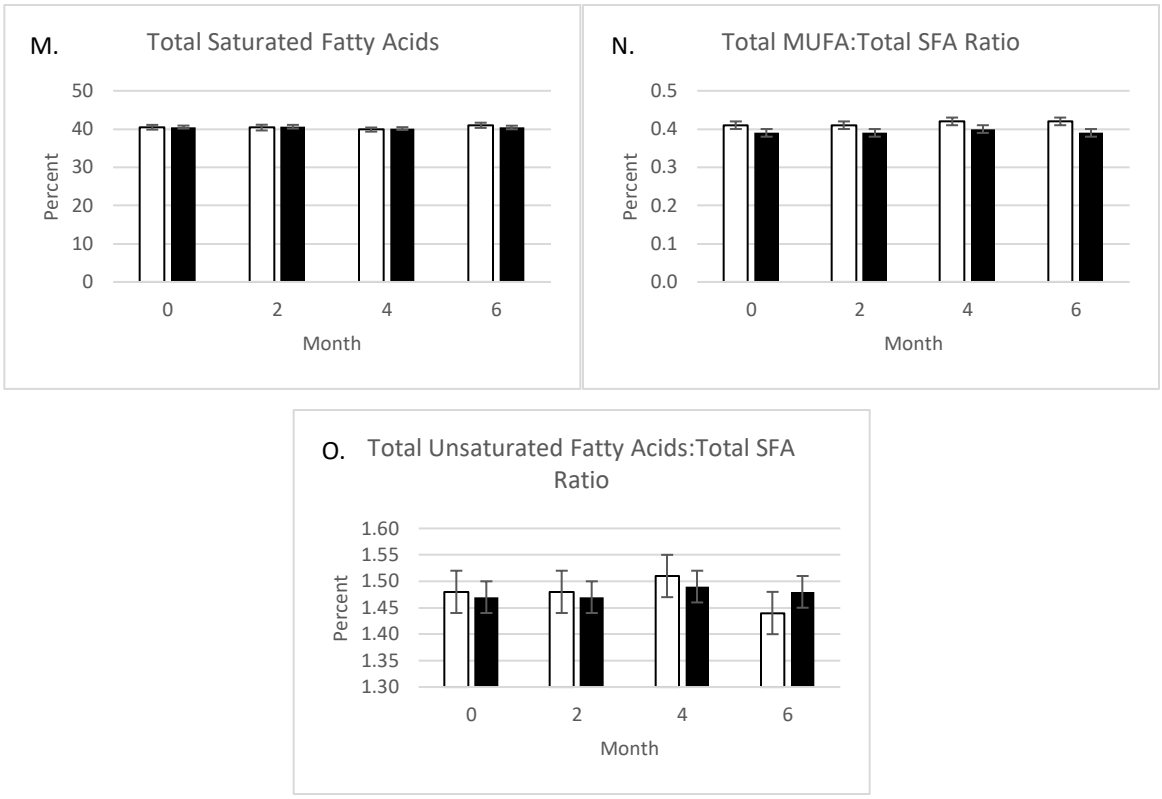

CHAPTER 5. DISCUSSION

Acute feeding trials indicate that almond consumption can lower the glycemic response to a meal (100), evoke a second meal effect (98), and help lower glycemia throughout the day, especially when they are consumed at breakfast or as an afternoon snack (98, 99). However, the literature is mixed with regard to the effect of almond consumption on HbA1c (100, 105), hampering the acceptance of a beneficial role for almond consumption on glycemic control. HbA1c is a reliable measure of long-term glycemic control. Why almond consumption improves postprandial glycemia, which contributes to HbA1c (9, 10), but not HbA1c, is unknown. Testing people with different body fat distributions may explain the inconsistent evidence on almond consumption on HbA1c. Different body fat distributions carry distinct risks for insulin resistance, independent of body weight, and thus may respond differently to dietary interventions. A large android visceral adipose tissue depot is consistently positively associated with insulin resistance, metabolic syndrome, T2DM, and CVD (19-21). Whether large amounts of android subcutaneous adipose tissue are associated with T2DM is disputed (25-30). A large gluteal femoral adipose tissue depot is not considered to be problematic and is consistently associated with insulin sensitivity (31-33). The purpose of this study was to determine whether body fat distribution plays a role in the physiological response to almond consumption and the mixed evidence of almond consumption on HbA1c. It was hypothesized that almond consumption would elicit a significant moderation of meal-stimulated glycemia, fasting glucose, insulin, HbA1c, triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, and calculated HOMA-IR, and improve HOMA-%β in individuals with high android visceral adiposity, which is associated with insulin resistance and T2DM (21). It was also hypothesized that there would be an intermediate effect in individuals with high android subcutaneous adiposity and a limited effect in individuals with high gluteal-femoral adiposity compared to control participants. Secondarily, it was hypothesized that substitution of almonds for other common snacks would decrease appetite and not promote weight gain and that almond consumption would improve total diet quality compared to individuals in the control condition.

We conducted a 6-month, randomized, controlled trial to address these hypotheses. One strength of this research design was grouping participants based on their body fat distribution. We used a combination of waist-to-hip circumference ratio and visceral adipose tissue ratio from

a DEXA scan. This allowed us to group participants with high or low android visceral adipose tissue, and high or low gluteal femoral adipose tissue into three groups: high android visceral adipose tissue, high android subcutaneous adipose tissue, and high gluteal femoral adipose tissue. Importantly, using the DEXA scan to determine body fat distribution allowed us to isolate masses of visceral adiposity and subcutaneous adiposity. These two fat masses are both located in the android region, but visceral adipose tissue is consistently associated with metabolic disease (19-21), while the effect of subcutaneous adipose tissue on metabolic disease is less certain (25-30). Furthermore, the DEXA scan allowed us to compare the effect of almond consumption on visceral and subcutaneous adipose tissue specifically, whereas other studies measure total abdominal fat or waist circumference (103, 105, 169, 170), which do not distinguish between visceral and subcutaneous adipose tissue depots. Since visceral adipose tissue is considered problematic, we could determine whether almonds decrease visceral adipose tissue to reduce the risk of insulin resistance and T2DM. Another strength of this study was the length of the intervention. This was a 6-month intervention which allowed a comparison of the chronic effects of almond consumption relative to a control customary diet. Previous studies on the effect of almond consumption on glycemia are often three months or less (98-101, 103). It is crucial to have an intervention length of at least three months to determine the effect of an intervention on HbA1c, since hemoglobin is attached to red blood cells, and red blood cells have a lifespan of about three months. An intervention of 6 months can help elucidate whether observed benefits from almond consumption in acute interventions are stable over a longer study period. Lastly, the inclusion of a control group was a strength of this study. Previous studies often compare almond consumption to a carbohydrate control, or substitute almonds for a single nutrient, which limits evaluation of the health effects of almonds to only the carbohydrate control or the nutrient they replaced in the diet. We recruited participants who regularly consumed breakfast and an afternoon snack of low nutrient density using a weighted nutrient density score (188) . This allowed us to broaden our conclusions to the health effects of almond consumption compared to a typical snack of low nutrient density.

We performed ITT and complier analyses on all outcomes. The ITT analysis included all participants who provided baseline data, including participants who dropped from the study, to draw unbiased conclusions about the effectiveness of the intervention (193). The complier analysis included only those who complied with the intervention determined using ASA24

dietary intake data. This would theoretically give us the true effect of the intervention when adherence to it was sufficient. However, compliance was documented using three-day, self-reported dietary intake data, which is notoriously unreliable (194), especially for underreporting snacks (195). Results from the ITT analysis are assigned greater weight, since there is limited confidence in the accuracy of self-reported compliance.

The mixed evidence of almond consumption on HbA1c led us to hypothesize almond consumption would improve HbA1c in participants with high VAT, moderately improve HbA1c in adults with high SAT, and have limited effect on HbA1c in adults with high GF compared to a control customary diet based on different body fat distribution risks for insulin resistance and T2DM. Almond consumption did not improve HbA1c in any body fat distribution group. We recruited participants with high android visceral adipose tissue, high android subcutaneous adipose tissue, or high gluteal femoral adipose tissue who had a BMI greater than 27 kg/m^2 to expand our population pool and because a BMI > 27 kg/m^2 is associated with higher risk factors for chronic disease and insulin resistance (196), especially with large amounts of visceral adipose tissue (21). However, participants had normal levels of HbA1c at baseline (<5.7%). Thus, there may have been limited capacity for HbA1c to decline. Previous studies have illustrated mixed effects of almond consumption on HbA1c when conducted in adults with prediabetes or T2DM and elevated HbA1c (100, 103, 105). Studies that assess the effect of nut consumption on HbA1c in adults with normal levels of HbA1c report an increase in HbA1c when interventions are less than three months, and no effect of nut consumption on HbA1c when interventions are longer than three months (197). Future studies should assess the effects of chronic almond consumption on HbA1c in adults with different body fat distributions with elevated HbA1c levels to clarify the role of almond consumption on glycemic control. Fasting and post-prandial glycemia contribute to HbA1c (9-11). However, HbA1c is the most clinically important endpoint to address because it is used to diagnose, treat, and monitor diabetes (4); is a reliable measure of long-term glycemic control; and correlates well with risk of complications from diabetes, such as cardiovascular disease and stroke, retinopathy, and microvascular complications (4, 6, 7). In contrast, other measures of glycemia are poor predictors (4, 6, 7).

Almond consumption has been reported to decrease postprandial glycemia (95-98) due to the high fiber content of almonds, which decreases the rate of gastric emptying and absorption of carbohydrates in the small intestine (114). However, there is mixed evidence on the effect of

almond consumption on fasting glucose and insulin. Studies reporting that almond consumption decreases fasting glucose, insulin, HOMA-IR and HOMA-%β are conducted in adults with prediabetes or T2DM who have elevated fasting glucose concentrations (103, 105, 197, 198). Studies in adults with normal fasting glucose concentrations report no effect of almond consumption on fasting glucose or insulin (99, 101, 102, 197). This was observed at various doses (38-100 g/day), intervention lengths (4-12 weeks), and whether weight loss occurred or not. Participants in this study who consumed almonds for 6 months did not have lower fasting or meal stimulated glucose or insulin, or have different HOMA-IR or HOMA-%β compared to participants in the control customary diet treatment. It was hypothesized that almonds would decrease fasting and meal stimulated glucose and insulin, and decrease HOMA-IR and increase HOMA-%β because of their high unsaturated fatty acid content. The high unsaturated fat content of almonds can increase the proportion of unsaturated fatty acids in cell membranes and thereby increase glycemic control. An increased ratio of unsaturated fatty acids to saturated fatty acids in cell membranes increases membrane fluidity and flexibility, which influences the effectiveness of glucose transport and can increase insulin sensitivity (199). However, evidence from analysis of the RBC membrane fatty acid composition indicated no differences in the total unsaturated fatty acids to saturated fatty acids ratio between treatment groups.

Almonds were expected to decrease blood lipid concentrations based on previous literature that reported almond consumption decreases LDL cholesterol (101, 131, 132, 134) and total cholesterol (101, 131, 134), especially in individuals with high VAT who have been reported to have elevated blood lipid concentrations compared to other BFD (42). While participants in the high VAT group did have higher triglyceride concentrations in the ITT and complier analyses compared to participants with high SAT and high GF, there was no effect of almond consumption on total cholesterol, LDL cholesterol, HDL cholesterol, or triglycerides in the ITT or complier analyses. Our results are similar to another 24-week study, where there were no differences in total or LDL cholesterol in adults who were overweight but with normal cholesterol concentrations who consumed their habitual diet with 15% (about 52 g) of energy from almonds every day (131). Almonds are reported to decrease total and LDL cholesterol because of their phytosterol and fiber content, which decrease cholesterol absorption and increase cholesterol excretion (114, 142-144). Studies indicating that almond consumption decreases LDL and total cholesterol recruit adults with elevated blood lipid concentrations (101,

131, 133, 135, 200), or assess almond consumption at doses greater than 60 g per day (101, 127). Other studies in adults with normal blood lipid concentrations and at doses less than 60 g per day find no effect of almond consumption on total or LDL cholesterol (102, 130). In our study, participants had normal total cholesterol, LDL cholesterol, and triglyceride concentrations, and consumed 42.5 g of almond per day. A 60 g or greater dose of almonds may be required to elicit an effect on serum lipid concentrations in adults with normal lipid concentrations (101, 201).

Epidemiological studies report that regular nut consumption is associated with a lower risk of weight gain over time compared to rare or no nut consumption (151, 152, 202) and a lower risk of becoming overweight or obese (151, 152), even though daily energy intake is higher in regular nut consumers (152, 203). In randomized controlled trials where almonds are added to the diet, there is less weight gain than expected (160, 204). Consistent with the literature, participants in this study who consumed 42.5 g of almonds every day for six months had higher daily energy intake compared to participants in the control treatment, but did not change body weight or body fat distribution as indicated by the ITT and complier analyses. However, total energy intake did not change over time.

An increase in feelings of satiety after almond consumption that can promote decreased energy intake is reported to have the largest effect from nut consumption on managing body weight (160). Acute studies report that almond consumption affects feelings of appetite post-prandially (98, 99, 205, 206), but there are no studies that report any difference in day-long appetite ratings after chronic almond consumption (99, 102, 159). Similarly, there were no differences in appetite ratings in the ITT or complier analysis between participants in the almond and control treatment groups.

Almonds, despite being an energy dense food, help manage body weight because, in part, their energy is inefficiently absorbed. Atwater factors, used to calculate the metabolizable energy of a food, overestimate the energy of almonds by 19% in participants who consumed 42 g of whole roasted almonds in a controlled diet for 9 days (153). Mastication ruptures and fractures the cell walls in the almond, leading to the lipids stored within the cells to become bioaccessible and bioavailable. Cells that are not fractured from mastication may remain intact through the gastrointestinal tract and are excreted, along with their lipids, in the feces. A previous study quantified the lipids in the feces to be 21.1 ± 14.4 g in those consuming an almond rich diet, compared to 2.8 ± 1.5 g in those consuming a control diet without nuts (158). The internal

structure of the almond cell may also decrease lipid bioaccessibility (207). Protein bodies that cover the surface of oil bodies within the almond cells stabilize the oil body and protect it from lipolysis (207), which also contributes to decreased bioavailability of the lipids from almonds. Almonds may also help manage body weight by increasing energy expenditure. Previous studies with other nuts report increases in resting energy expenditure or thermic effect of food with nut consumption (208-211), both of which contribute to total energy expenditure. However studies with almonds report no effect of almond consumption on energy expenditure compared to the control customary diet treatment (160, 204), although one study did report that 14% of the energy from almonds was dissipated by energy expenditure (160). While metabolizable energy and energy expenditure were not measured in this study, they do provide plausible mechanisms for the lack of effect of almond consumption on body weight.

The effects of almond consumption on body composition are mixed, but may mimic changes in body weight. In studies where almonds did not change body weight, there were also no effects of almond consumption on fat mass (135), fat free mass (135), abdominal fat (135), and percent body fat (103) compared to participants in the control groups or compared to baseline values. In studies where almond consumption decreased body weight, in contrast, there were concomitant reductions in truncal fat, VAT, body fat percentage, and fat mass compared to baseline or participants in the stipulated control group (102, 105, 170). Participants in our study did not change total fat mass or total fat mass percentage in the ITT or complier analysis, or change total lean mass in the ITT analysis, which is similar to previous studies where there was no change in fat mass when body weight was stable (103, 135). Participants in the almond, High SAT group increased total lean mass compared to participants in the control, High SAT group in the complier analysis, despite no weight loss. However, this finding should be interpreted with caution as the complier analysis was not adequately powered. Almonds may increase total lean mass by contributing protein to the diet, which enhances energy expenditure and fat loss (212). In the ITT analysis, participants with high SAT who consumed almonds for 6 months decreased android fat mass percentage and increased android lean mass percentage. Android lean mass percentage trended toward significance in the complier analysis, but the effect on android fat mass percentage was no longer significant. The effects of almond consumption on android fat and lean mass percentages are similar to those reported by Dhillon et al (102), where healthy adults decreased truncal fat mass percentage and increased truncal lean mass percentage.

However, this study was a weight loss study where participants consumed an almond enriched (15% of energy from almonds) or nut free energy restricted diet for 12 weeks. Despite the differences in almond dose, length of intervention, and energy intake, the similar outcomes provide confidence that the effect of almond consumption on android fat and lean mass percentage is real and robust. In the ITT analysis, participants with High SAT who consumed almonds tended to gain less android VAT mass compared to participants with High SAT on the control customary diet treatment. However, this effect was no longer significant in the complier analysis. While consumption of almonds slightly decreased VAT mass in participants with High SAT, participants in the control, High SAT group gained VAT mass. It is unclear why participants in the control, High SAT group had a large increase in VAT mass over the 6-month intervention. There are limited studies assessing the effect of almond consumption on VAT mass directly, and results are inconsistent (102, 134, 184, 213). Another study that reported an effect of almond consumption on VAT similarly found that participants in the control group gained VAT mass over the intervention, but almond consumption prevented the gain of VAT mass (213). This study was 20 weeks long, and participants in the control group also gained slightly more body weight compared to participants in the almond group, although this difference was not significant (213). Another study reported that when participants consumed almonds as a preload to a meal for 16 weeks, VAT slightly decreased compared to when participants consumed almonds as a snack and compared to participants on the control treatment, who slightly increased VAT (184). Another study where participants consumed almonds as morning or afternoon snacks for 8 weeks reported no effect on VAT mass compared to participants on the control treatment (135). Thus, consuming almonds with meals as opposed to snacks may moderate VAT mass. Furthermore, each study that reported an effect of almond consumption on VAT mass was 16 weeks or greater. Future studies, especially 16 weeks or longer, should clarify the role of almond consumption on VAT mass, especially when consumed with meals. Whether the effect of almond consumption on VAT mass is limited to those with High SAT should also be elucidated. Previous studies did not classify participants based on BFD, and we cannot compare our participants VAT and SAT mass with other studies due to different methods of measuring body composition.

It was hypothesized that almond consumption would yield the most improvements in body weight, body composition, and blood biochemistries in those with high VAT because of the

association between high VAT and insulin resistance and T2DM (19-21). Despite participants in the high VAT group having more VAT mass, and normal but higher concentrations of glucose, insulin, HOMA-IR, and triglycerides in the ITT analysis, and higher insulin and triglycerides in the complier analysis compared to other BFD, consuming almonds did not significantly change any outcome compared to participants in the control, high VAT group. Large amounts of VAT in the android region is considered problematic because it has a high turnover rate, which increases the concentration of FFA transported to the liver because VAT drains into the portal vein (34, 35). As a result, VAT increases hepatic lipid content (37), hepatic gluconeogenesis (38), and VLDL production (36). This contributes to a state of insulin resistance. VAT may have a causal role in insulin resistance, however debates on putative mechanisms are ongoing (22, 23). Reducing VAT mass can help decrease risk for insulin resistance and chronic disease. In a 16 week caloric restriction-induced weight loss study in adults with obesity, improvements in insulin sensitivity were positively correlated with a decrease in VAT mass, but not a decrease in fat mass or SAT mass (214). Decreasing VAT mass may be required to decrease glucose, insulin, and blood lipid concentrations in adults with high VAT. In addition to participants having normal blood biochemistries, there may have been no effect of almond consumption on fasting or meal stimulated glucose, insulin, HOAM-IR, HOMA-%B, HbA1c, or fasting lipid concentrations because VAT mass did not change.

Almond consumption may help decrease android fat mass and prevent the gain of visceral adipose tissue because of their high MUFA content. A randomized, crossover, controlled feeding study in adults with central obesity reported that when participants consumed isocaloric diets high in MUFA (from canola oil and high oleic acid canola oil), there was a decrease android fat mass in men, but not women, over four weeks of the controlled diet compared to when participants consumed diets high in PUFA (from a blend of flax and safflower oil) (171). High MUFA diets may decrease android adiposity due to increased fatty acid oxidation rates and lipolysis due to activation of PPAR-delta and alpha receptors (172) and increased energy expenditure (174). There is also evidence that MUFA may preferentially deposit in SAT, whereas saturated fatty acids preferentially deposit in VAT (171, 175, 176).

Both epidemiological and randomized controlled trials report that almond consumption increases total diet quality, measured by the Healthy Eating Index (HEI). Data from the NHANES 2001-2010 survey reported that total diet quality, measured by HEI-2010 score, was

15% higher in almond consumers compared to non-consumers (215). Additionally, every HEI-2010 score category was higher in almond consumers compared to non-consumers, including categories that are recommended to be consumed in moderation (215). Randomized controlled trials also report higher diet quality, measured by HEI-2010, in adults who incorporated almonds into their diet for three weeks compared to when no almonds were consumed in the diet (182). When almonds were incorporated into the diet, whole fruit score was lower, and total protein score, seafood and plant protein score, fatty acid score, and empty calorie score was higher, and sodium score tended to be higher in adults who incorporated almonds into their diet compared to when no almonds were consumed (182). In our study, participants in the almond treatment group had higher seafood and plant protein scores at months 2, 4 and 6, as expected, and higher dietary fatty acid scores at months 2, 4, and 6 compared to participants on the control customary diet treatment in the ITT and complier analyses. Because nuts are included in the seafood and plant protein score, higher scores in participants in the almond treatment group were expected. Higher fatty acid scores are also not surprising, since the nutrient intake of regular nut consumers matches the nutrient of the nuts (180), and fatty acid score measured the proportion of PUFA and MUFA compared to saturated fatty acids in the diet. However, while total HEI score was higher in participants in the almond treatment group at months 2, 4, and 6 compared to month 0 in the ITT and complier analyses, total HEI score was not significantly higher compared to participants in the control treatment. This is likely a power issue, as only 39 and 32 participants were included in the ITT and complier analyses, respectively, due to a technical error where 95 participants had their baseline dietary intake data recorded after the intervention started, and thus did not reflect their habitual diet before the intervention. Despite the higher values from baseline in participants in the almond treatment group, the total HEI score was still lower than the average American total HEI score (59/100) for most participants throughout the study (216).

An objective measure of compliance in long-term free-feeding trials is important to reliably assess whether the intervention was implemented effectively so the hypotheses were truly tested. We collected samples of RBC every two months during the study to objectively document compliance to the intervention by assessing the fatty acid composition of the RBC membranes. Because the lifespan of RBC is about 120 days (217), this provides an objective long-term measure of compliance (218). However, only a subset of participants had RBC samples where the RBC membrane fatty acid composition could be analyzed. The plasma was

not removed from the blood samples of 95 participants and were frozen as whole blood. Freezing and thawing lyses the RBC, mixing the lipids from the RBC membrane with the lipids from the plasma. Plasma lipids reflect short term dietary intake (219), thus the fatty acid analysis would be biased by what they recently ate prior to blood collection, and would not be a reliable measure of long term compliance.

Theoretically, the fatty acid composition of the RBC would mirror the fatty acid composition of the almonds in those who complied with the analysis, specifically having higher proportions of oleic acid, linoleic acid, monounsaturated fatty acids, and lower amounts of saturated fatty acids (SFA). However, participants in the almond treatment had significantly lower percent oleic acid, oleic/palmitic acid ratio, total MUFA, and MUFA/SFA ratio in their RBC membranes compared to the control treatment participants, although this did not differ between treatments at any time point. These results are unexpected based on the high oleic acid content of almonds, and because participants in the almond treatment reported higher intakes of total MUFA, oleic acid, linoleic acid, and total PUFA compared to participants in the control customary diet treatment group. Previous studies report that participants who consumed a high MUFA diet for three weeks had increased proportions of oleic acid in the RBC membrane (220, 221), but there was no correlation between dietary sources of saturated fatty acids and RBC membrane fatty acid composition (220). These were controlled dietary interventions (220, 221), but other studies report changes when the balance of the diet was not controlled. In one trial, supplementation of 45 grams of olive oil into the diet for 2 months resulted in a significant increase in the proportion of oleic acid in the RBC membrane, whereas there was no difference in the RBC membrane fatty acids in those who did not change their diet in the control treatment (222). Another study in children and adolescents who consumed hazelnuts, which are also high in MUFA, for 8 weeks reported an increased proportion of total MUFA, oleic acid, and the MUFA/SFA ratio in the RBC membrane compared to children and adolescents on the control treatment who consumed no hazelnuts (223). However, another study in adults with T2DM who consumed 30 g of walnuts for one year had significantly elevated ALA levels in the RBC membrane after three months of consumption, but the value at 1 year was lower than at 3 months (224). The authors concluded that this indicated compliance was best in the first three month of the intervention (224). Thus, previous studies report that oleic acid in the RBC membrane correlates with dietary intake, however findings are from controlled feedings trials, or short-term

dietary interventions. Although there was no difference in RBC membrane fatty acid composition between participants in the almond and control treatment at any time point in our trial, the habitual diets were not controlled for. The lack of effect of almond consumption on the RBC membrane fatty acid composition could be due to low power, as a previous study assessing the effects of a dietary intervention on RBC membrane fatty acids reported that 20 participants per group was needed to detect a difference in fatty acid composition with 80% power (225). Our study only had 12 participants in the control treatment, and 27 participants in the almond treatment with samples that could be analyzed. It could also indicate poor compliance in the sample tested, however we feel this is not the case because compliance based on dietary recalls was high and participants were incentivized to comply with the intervention through the promise of increased compensation. The composition of the RBC membrane may not have matched differences in nutrient intake due to how the fatty acids in the membrane were measured. Fatty acids were measured as percent of total composition. Thus, increases in multiple fatty acids could dilute their proportions in the RBC membrane (219). Further clarification of the role of absolute changes in fatty acid composition in the RBC membrane is required.

This study has limitations. Because of the processing error, self-reported dietary intake was used to document compliance to the intervention, which is notoriously biased and inaccurate (194), especially for underreporting snacks (195). Alternatively, participants may have stated that they consumed almonds in their dietary records because they knew that was expected of them, but failed to do so. There were not enough RBC samples to objectively assess compliance based on membrane fatty acid composition with enough power. However, it is not clear if the RBC membrane fatty acid composition is a method to reliably document compliance with a single dietary change (inclusion of almonds). Metabolomics is an objective measure of compliance that has been used previously, in combination with self-reported dietary intake data to document compliance to an almond intervention. However, this method requires additional expertise and is more expensive (226). Another limitation of this study was the use of participants with normal biochemistries. Much of the literature that reports a decrease in glycemia and lipids with almond consumption are in adults with elevated concentrations. Recruiting participants with elevated glycemia or lipemia would have provided better insights on whether the physiological effect of almond consumption differs between BFD. Lastly, although DEXA is a valid way of measuring visceral adipose tissue to group participants based on BFD, its accuracy decreases with

increasing levels of visceral adiposity (227). MRI or CT are the gold standard for quantifying visceral adipose tissue and may have provided a more accurate assessment of VAT mass (228).

CHAPTER 6. CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Summary and Conclusions

Participants with different body fat distributions who consumed 42.5 g of almonds with breakfast and as an afternoon snack every day did not change HbA1c, fasting glucose, insulin, HOMA-IR, HOMA-%β, blood lipid concentrations, or meal stimulated glucose or insulin compared to participants who continued their habitual breakfast and afternoon snack routines for the 6-month intervention. However, participants had normal blood biochemistry values at baseline which may explain the lack of effect. Future studies should test people with elevated blood biochemistry values to determine whether almonds have an effect on these indices, and if this differs between adults with different body fat distributions. Participants who consumed almonds every day for 6 months in this study did not change body weight, nor did almond consumption have an effect on appetite ratings over the 6-month intervention compared participants in the control treatment group. However, participants with high subcutaneous adipose tissue who consumed almonds every day for 6 months decreased android fat mass percentage, increased android lean mass percentage, and tended to decrease visceral adipose tissue mass compared to participants with high subcutaneous adipose tissue in the control treatment group. Consuming almonds increased total diet quality over the 6-month intervention. Thus, almonds can improve body composition in adults with large amounts of subcutaneous adipose tissue, however testing people with different body fat distributions may not explain the mixed evidence on the effect of almond consumption on HbA1c.

6.2 Future Directions

- How does body fat distribution alter the physiological response to chronic almond consumption in adults with elevated HbA1c?
    - Previous studies assessing the effect of almond consumption on HbA1c were tested in adults with T2DM (100, 103) or prediabetes (105), who have elevated HbA1c. Our study directly recruited participants of a certain body weight and body fat distribution, not based on HbA1c status. As a result, the participants in this study on average had healthy levels of HbA1c, so there limited room for improvement. In a 2014 systematic review

and meta-analysis assessing the effects of tree nut consumption on glycemic control in adults with T2DM, there was a significant decrease in HbA1c with tree nut consumption (198). However, a similar 2019 systematic review and meta-analysis reported no effect of nut consumption on HbA1c in individuals with T2DM, and an increase in HbA1c in healthy adults (197). This suggests that healthy adults and adults with T2DM who have elevated HbA1c may respond differently to interventions. Whether body fat distribution plays a role in decreasing HbA1c in those who have elevated levels would address the discrepancies in almond consumption on HbA1c in the current literature in adults with elevated HbA1c. Understanding if almonds can be incorporated into the diet to help decrease HbA1c in those with elevated levels is more relevant for managing T2DM.

- Do people with different body fat distributions respond differently to an almond enriched hypocaloric diet?
    - Many studies that report a positive effect of almond consumption on body composition are also weight loss studies (102, 105, 170), whereas studies where weight loss does not occur do not report improvements in body composition (103, 135, 169). Specifically, the effect of almond consumption on visceral adipose tissue is limited and inconsistent (102, 135, 184). In this study, there was only an effect of almond consumption on visceral adiposity in those with high SAT in the ITT analysis. VAT is quickly mobilized and oxidized in early weight loss (229), and there is evidence that MUFA, which are abundant in almonds, preferentially deposit in SAT compared to VAT (171, 175, 176). Visceral adipose tissue is considered the most problematic adipose tissue depot (23, 230, 231), and strategies to decrease its mass may help decrease risk for chronic disease. Thus, whether an almond enriched hypocaloric diet decreases VAT, and whether the amount of VAT loss varies depending on BFD needs to be explored further.
- Does liking of almonds change over the course of 6 months?
    - The liking of a food is one of the primary reasons for its ingestion. Liking of a food may increase, decrease, or stay the same after repeated exposure (232) . Regular consumption of a familiar food may reduce its pleasantness, also known as producing a monotony effect, while regular consumption of a novel food may increase liking due to reduction of neophobia (233). Almonds may be susceptible to a monotony effect because they are not a staple food. However, they may be resistant to monotony effects if they are

incorporated into meals or combined with other foods to increase variety of the eating occasion. There is preliminary evidence to support that almonds are resistant to monotony effects in some people (102), however it may be dose dependent. This study could not confirm or refute these findings because the palatability of almonds at month 6 compared to month 0 was not tested in this study. Almonds only exert their health effects if they are consumed on a regular basis. If almonds are resistant to monotony, this may promote regular consumption to exert their health effects. However, if almonds are susceptible to monotony effects, strategies to reduce their susceptibility are warranted to promote regular consumption.

- What are the mechanisms by which timing of almond consumption improves glycemia and body composition?
    - In this study, almonds were consumed with breakfast and as an afternoon snack due to previous studies indicating those as the optimal time to consume almonds to have the most beneficial effect on glycemic control (98, 99). While almonds at breakfast may exert a second meal effect to attenuate glycemia throughout the day, the mechanism for almonds consumed as a snack is unknown. Consuming almonds as snacks may decrease gastric emptying and reduce the rate of carbohydrate release and absorption from previous meals to decrease glycemia. Similarly, increased eating frequency may delay gastric emptying from subsequent meals to decrease glycemia (234). Future studies should confirm the timing effect on glycemia, and assess the rate and onset of gastric emptying and glycemia when almonds are consumed as a snack compared to a control snack and no snack in combination with a three meal per day eating pattern.

      Another study assessing the effect of timing on almond consumption reported that consuming almonds as a preload before meals decreased visceral adipose tissue compared to consumption of a control preload or when almonds were consumed as a snack (184). Almonds may decrease energy intake during a meal because they are highly satiating (98, 102). Previous studies report that higher energy intake per eating occasion may lead to an increase in VAT (235). Thus, the satiating properties of almonds and their influence on dietary intake may be one reason for the effect of timing of almond consumption on VAT mass. Future studies should confirm the timing effect on VAT

mass, and assess the effect of an almond preload compared to a control or no preload for at least three months on energy intake from that meal, appetite effects, and VAT mass.

- How can almond consumption be assessed to document compliance?
  - In this study, compliance to the intervention was assessed using dietary intake data from ASA24. While frequently used, this is notoriously biased by participants underreporting either consciously or unconsciously. Biomarkers of almond intake are a more reliable measure of documenting compliance. In this study, the fatty acid composition of the red blood cell membranes were analyzed in a subset of participants; however, the composition of the red blood cells membrane during the 6-month intervention did not reflect expected changes in fatty acid composition based on the fatty acid composition of almonds. This method of compliance, while objective, can also be indicative of fatty acid composition of the overall diet. Whether this can serve as a reliable biomarker of compliance or is too susceptible to shifts in overall diet can be explored in future studies.
- Are almonds unique in their potential effects?
  - In this study, participants consuming almonds were compared to participants consuming a snack of low nutrient density. However, almonds are often compared to a high carbohydrate control snack, or replace a single nutrient source in randomized controlled trials, which limits the health effects of almonds versus the carbohydrate snack or the nutrient they replace in the diet. The control group in this study allowed us to broaden our conclusions about the effects of almonds versus a typically consumed snack of low nutrient density. Future studies could similarly use a weighted nutrient density score to compare almonds to a typically consumed snack of low nutrient density, or compare almonds to snacks/foods with similar nutrient density. This would help to understand if the effects of almond consumption are unique to almonds, or if the effects stem from what they replace in the diet. Furthermore, whether other types of nuts exert similar effects as almonds can be investigated. While nuts typically are more similar than different in their potential effects, there are studies that report differences in the health effects of nuts (129, 197).

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APPENDIX A. RECRUITMENT MATERIALS

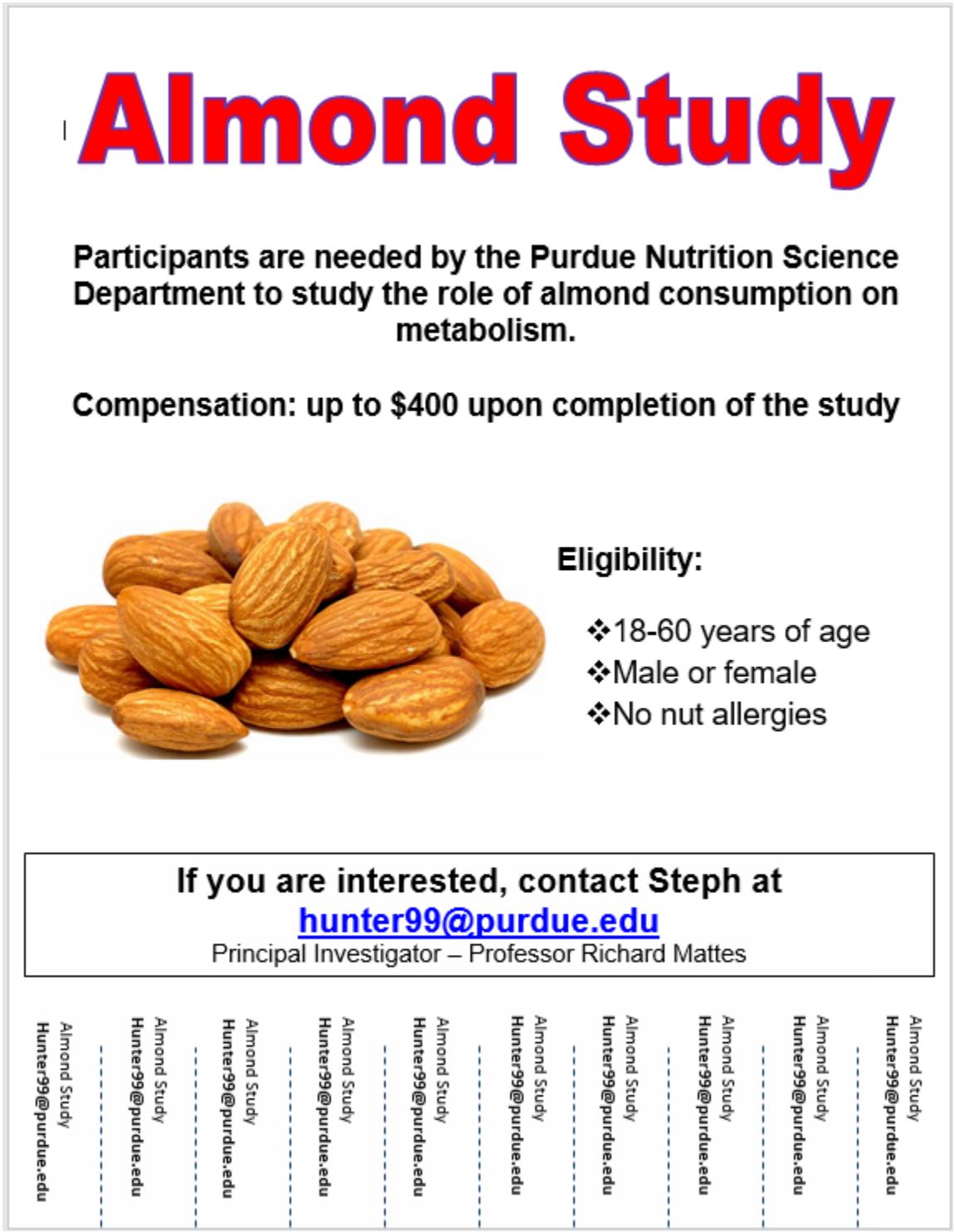


Figure A.1 Purdue University recruitment flyer for all body fat distributions.

Almond Study

Participants are needed by the Purdue Nutrition Science Department to study the role of almond consumption on metabolism.

Compensation: up to $400 upon completion of the study

Eligibility:

- ❖18-60 years of age
- ❖Male or female
- ❖BMI>27
- ❖Tend to gain weight in their hips
- ❖No nut allergies

If you are interested, contact Steph at
hunter99@purdue.edu
Principal Investigator – Professor Richard Mattes

Almond Study Hunter99@purdue.edu

Figure A.2 Purdue University recruitment flyer when participants with high GF only were needed for participation.

Almond Study

The Department of Medicine at Indiana University
(Principal Investigator: Robert Considine)
is seeking participants for a study on the effect
of almond consumption on metabolism.

Compensation: up to $400 upon completion of the study

Eligibility:

- ❖18-60 years of age
- ❖Women and Men
- ❖No nut allergies

If you are interested, please email or call
diabres@iu.edu
317-274-7679

Almond Study
diabsres@iu.edu
317-274-7679

Figure A.3 IUSM recruitment flyer for all participants.

Almond Study

Do you have a pear-shaped body type? Did you know this body fat distribution may be metabolically beneficial?

The Department of Medicine at Indiana University (Principal Investigator: Robert Considine)
is seeking participants with larger hips and buttocks for a study of the effect
of almond consumption on metabolism.

Compensation: up to $400 upon completion of the study

Eligibility:

- 18-60 years of age
- Women and Men
- No nut allergies

If you are interested, please email or call
sawainsc@iu.edu
317-274-1554

Almond Study
sawainsc@iu.edu
317-274-1554

Figure A.4 IUSM recruitment flyer when participants with high GF only were needed for participation.

APPENDIX B. CONSENT FORMS

Purdue University Consent form

RESEARCH PARTICIPANT CONSENT FORM

Almond consumption and metabolism

Principle Investigator: Richard D Mattes, MPH, PhD, RD

Department of Nutrition Science

Purdue University

Key Information

Please take time to review this information carefully. This is a research study. Your participation in this study is voluntary which means that you may choose not to participate at any time without penalty or loss of benefits to which you are otherwise entitled. You may ask questions to the researchers about the study whenever you would like. If you decide to take part in the study, you will be asked to sign this form, be sure you understand what you will do and any possible risks or benefits.

• This randomized controlled trial is looking to understand how almonds incorporated into the diet at specific times long-term may benefit a person's health, including their body composition, blood glucose and insulin, and blood lipid levels.

• This research study will last 6 months. Visits occur once every two weeks and vary in length from about 20 minutes to about 4.5 hours.

• Potential risks of participating in this study include pain, bruising, infection, or fainting during blood collections, and a small amount of radiation exposure. There are no expected benefits from participating in this study.

What is the purpose of this study?

This study will examine the effects of almonds consumed as a breakfast and snack by adults with different body fat distributions on indices of carbohydrate and lipid metabolism. We would like to enroll 120 people in this study.

What will I do if I choose to be in this study?

This study requires you to visit the laboratory (Room 226 of Stone Hall, Purdue University) every 2 weeks for 6 months. There are three groups to this study, which will be defined by primary fat depot: Group 1: High truncal visceral fat (high internal body fat in the abdominal area); Group 2: High truncal subcutaneous fat (high fat just under the skin in the abdominal area); and Group 3:

High gluteo-femoral fat (high fat in the hip area). To determine your group placement, your total body fat and body fat distribution will be determined by Dual Energy X-ray Absorptiometry (DEXA) using a sensitive and validated technique for defining fat depots. After your group placement has been confirmed, you will be randomly assigned to a food intervention group: almonds or no almonds. Those in the almond group will consume 42 g of almonds, half at breakfast and half as an afternoon snack (provided) daily for 6 months, but will be prohibited from consuming any other nuts or nut products. Those in the no almond group will be prohibited from consuming any nuts or nut products throughout the study. They will continue their daily breakfast and afternoon snacking routine for 6 months. Those in the no almond group will be compensated with almonds upon completion of the study. Throughout the 6-month study period, multiple assessments will be performed at fixed intervals (as described below).

Specific Procedures

Baseline visit

a. You will arrive at the laboratory having fasted overnight. A trained phlebotomist will place a catheter (flexible needle) in your arm and a baseline sample will be collected. Prior to each draw, a small amount (1-2 ml) of sterile saline (salt solution) will be washed through the catheter to ensure it is clear. Then you will consume a chocolate drink (8 oz.) within 10 minutes. Additional blood samples will be taken at 10, 20, 30, 60, 120, and 180 minutes after consuming the beverage. All samples will be drawn from the catheter. Using the samples, we will measure insulin, GLP-1, GIP, glucose, C-peptide, HcA1c, a lipid panel, and triglycerides.
b. Your total body fat will be measured using dual-energy x-ray absorptiometry (DEXA) by a qualified technician. You will have scans covering 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 (about 15 to 20 minutes).
c. Your body weight will be measured in a hospital gown on a Scaletronix clinical scale.
d. Compliance to the almond intervention will be measured by using one of the blood samples.
e. At the end of this visit, you will be given:
    i. A link to a web-based "Appetite Questionnaire", where you will be asked to record your hourly appetite ratings during waking hours for 24 hours.
    ii. A link to the web-based "Automated Self-Administered 24-hour dietary recall" system, in which you will be asked to record dietary intake for three non-consecutive days that include two weekdays and one weekend day (specific days to be determined).

Every 2 weeks

a. Your body weight will be measured in a hospital gown on a Scaletronix clinical scale.

Months 2 and 4:

a. Compliance to the almond intervention will be measured by analysis of a single 5 ml blood sample obtained from an arm vein.
b. At the end of these visits, you will be given:
    i. A link to a web-based "Appetite Questionnaire", where you will be asked to record your hourly appetite ratings during waking hours for 24 hours.
    ii. A link to the web-based "Automated Self-Administered 24-hour dietary recall" system, in which you will be asked to record dietary intake for three non-consecutive days that include two weekdays and one weekend day (specific days to be determined).

Month 6

a. All measurements at baseline will be repeated.

How long will I be in the study?

In total, your participation in this study requires 6 months and includes visits once every two weeks. 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**: 4 – 4 ½ hours
    ii. **Two-week weigh-ins:** 20 minutes
    iii. **Months 2 and 4:** 20 minutes
    iv. **Final Visit:** 4-4 ½ hours

What are the possible risks or discomforts?

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.

The dual-energy x-ray absorptiometry measurement will expose you to a small amount of radiation of approximately 0.5 mrem. For comparison, exposure from a single chest x-rays is 6 mrem, a pelvis and hip scan is 65 mrem, and a CT pelvis scan is 1000 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 exposure limit for the public from 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. In this study, the total amount of radiation you will be exposed to is about 1 mrem if all sessions are completed in 6 months.

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.

Breach of confidentiality is always a risk with data, but we will take precautions to minimize this risk as described in the confidentiality section.

Conduct of this study is supported by a grant from the Almond Board of California.

Are there any potential 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 of diabetes.

Will I receive payment or other incentive?

You will receive a total payment of $400 as compensation for satisfactory completion of the full study. You will be guaranteed to receive a payment of $350 with an additional $50 if plasma vitamin E tests confirm that you were compliant with eating or not eating the almonds (depending on your group assignment – measured by analysis of your blood samples) throughout the trial. If you do not meet pre-set eligibility criteria during the baseline screening assessment, a payment of $15 will be made. A payment of $10 will be made for each completed week of study should you withdraw or be withdrawn from the study.

In order to receive payments, you must provide your Social Security Number to the Business office. International students will be required to complete additional payment procedures via the Purdue Glacier system, which may have tax consequences.

Are there costs to me for participation?

There are no anticipated costs to participate in this research.

What happens if I become injured or ill because I took part in this study?

If you feel you have been injured due to participation in this study, please contact Richard Mattes at 494-6192, email mattes@purdue.edu. 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.

Conflict of Interest Disclosure

The Almond Board of California is supporting this study and will be providing the almonds.

The following disclosure is made to give you an opportunity to decide if this relationship will affect your willingness to participate in the research study.

Will information about me and my participation be kept confidential?

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, after confidentiality measures have been taken (destroying key linking ID numbers and data), all of your research records may be reviewed by The Almond Board of California and by departments at Indiana University and Purdue University responsible for regulatory and research oversight. This study is funding by the Almond Board of California.

What are my rights if I take part in this study?

Your participation in this study is voluntary. You may choose not to participate or, if you agree to participate, you can withdraw your participation at any time without penalty or loss of benefits to which you are otherwise entitled.

Who can I contact if I have questions about the study?

If you have questions, comments or concerns about this research project, you can talk to one of the researchers. Please contact Richard Mattes at 494-0662, email (mattes@purdue.edu) or Judy George at 494-6192, email (georgej@purdue.edu).

If you have questions about your rights while taking part in the study or have concerns about the treatment of research participants, please call the Human Research Protection Program at (765) 494-5942, email (irb@purdue.edu)or write to:

Human Research Protection Program - Purdue University
Ernest C. Young Hall, Room 1032
155 S. Grant St.,
West Lafayette, IN 47907-2114

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 study, and my questions have been answered. I am prepared to participate in the research study described above. I will be offered a copy of this consent form after I sign it.

_____ _____

Participant's Signature Date

_____

Participant's Name

_____ _____

Researcher's Signature Date

IUSM Consent Form

INDIANA UNIVERSITY INFORMED CONSENT STATEMENT FOR
Project Title: Almond Consumption and Metabolism
Principal Investigator: Robert V. Considine, Ph.D.

You are invited to participate in a research study entitled, "Almond Consumption and Metabolism." This study is being conducted by Dr. Robert Considine in the Department of Medicine at Indiana University and Dr. Richard Mattes, in the Department of Nutrition Science at Purdue University

STUDY PURPOSE

This study will examine the effects of almonds consumed as a breakfast and snack on the amount of sugar and fat in the blood of adults with different body fat distributions. This study is supported by a grant from the Almond Board of California to Drs. Mattes and Considine. The Almond Board will supply the almonds for this study.

NUMBER OF PEOPLE TAKING PART IN THE STUDY

If you agree to participate, you will be one of approximately 120 subjects in total. Locally at Indiana University, approximately 100 subjects will be enrolled.

PROCEDURES FOR THE STUDY

This study requires you to visit the Indiana CTSI Clinical Research Center every 2 weeks for 6 months. There are three groups with different amounts and distribution of body fat who will be studied. To determine which group you will be in, your total body fat and body fat distribution will be determined by Dual Energy X-ray Absorptiometry (DEXA). After your group placement has been confirmed, you will be randomly assigned to a food intervention group: almonds or no almonds. Those in the almond group will consume 0.75 ounces of almonds (about 21-30 almonds) at breakfast and 0.75 ounces (about 21-30 almonds) as an afternoon snack daily for 6 months. Those receiving almonds will also be prohibited from consuming any other nuts or nut products during the study. The other group that does not receive the almonds will be prohibited

from consuming almonds and other nuts or nut products during the study, and will continue their daily breakfast and afternoon snacking routine for 6 months. Those in the no almond group will be given almonds upon completion of the study. Throughout the 6-month study period, multiple assessments will be done at fixed intervals (as described below).

Specific Procedures

Screening visit

Your waist and hips will be measured using a tape measure

Your body weight and height will be measured

Your total body fat will be measured using dual-energy x-ray absorptiometry (DEXA) by a qualified technician. You will have scans covering 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 (about 15 to 20 minutes).

Baseline visit

You will arrive at the laboratory following an overnight fast. A trained phlebotomist will place a catheter (flexible needle) in your arm and a blood sample will be collected. Prior to each blood draw, a small amount (1-2 ml) of sterile saline (salt solution) will be washed through the catheter to ensure it is clear. Then you will consume a chocolate drink (8 oz.) within 10 minutes. Additional blood samples will be taken at 10, 20, 30, 60, 120, and 180 minutes after consuming the beverage. All samples will be drawn from the catheter. Using the samples, we will measure hormones and metabolites that indicate how your body digests the chocolate drink.

Your body weight and height will be measured.

A baseline blood sample will be taken to use in determining your compliance with eating almonds during the study. This will be taken through the catheter described above and does not require an additional needle stick.

At the end of this visit, you will be given:

A link to a web-based “Appetite Questionnaire”, where you will be asked to record your hourly appetite ratings during waking hours for 24 hours.

A link to the web-based "Automated Self-Administered 24-hour dietary recall" system, in which you will be asked to record dietary intake for three non-consecutive days that include two weekdays and one weekend day (specific days to be determined).

Every 2 weeks

a. Your body weight will be measured. Months 2 and 4:

Compliance with eating the almonds will be measured by analysis of a single 5 ml blood sample obtained from an arm vein.

At the end of these visits, you will be given:

i. A link to a web-based "Appetite Questionnaire", where you will be asked to record your hourly appetite ratings during waking hours for 24 hours. ii. A link to the web-based "Automated Self-Administered 24-hour dietary recall" system, in which you will be asked to record dietary intake for three non-consecutive days that include two weekdays and one weekend day (specific days to be determined).

Month 6

a. All measurements done at the screening and baseline visits will be repeated.

In total, your participation in this study requires 6 months and includes visits once every two weeks. You will be required to attend:

UAll testing visits which will be conducted Uat the Indiana CTSI Clinical Research Center (550 N. University Blvd, 5PthP floor, Indianapolis, IN)

The length of each visit is:

Screening visit: 30 minutes

Two-week weigh-ins: 20 minutes

Baseline visit: 4 – 4 ½ hours

Months 2 and 4: 20 minutes

Final Visit: 4-4 ½ hours

RISKS OF TAKING PART IN THE STUDY

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.

Your participation in this research study involves exposure to radiation using a device that is used for patient care. The benefit from the radiation that patients receive for medical care typically outweighs the risk, because it allows a doctor to provide appropriate medical care; however, the additional radiation "dose" you receive for research purposes does not benefit you personally. Everyone is exposed to "background" radiation (e.g. radon gas in our homes, radiation from space, uranium in soil, etc.) and the radiation dose varies, depending upon where you live. Individuals who live in certain areas of the country may actually receive radiation doses that are higher than the average; however, individuals who live in those areas have not shown an increased risk of health effects (cancer and/or leukemia) above the average for the US population. The radiation dose you will receive in one year from this study is less than the average annual "background" dose received by a member of the US population. We cannot say with absolute certainty that there is no risk from the radiation dose in this study. While there is no evidence that any risk exists for humans exposed to such low levels, it is assumed that the risks rise with lifetime accumulated dose from all sources of ionizing radiation, including the doses you receive from medical procedures and the environment. The calculated effective dose resulting from your participation in this study is available upon request.

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.

There is a small but possible risk of loss of confidentiality. To reduce this risk the investigators have established rules and procedures to limit the possibility that your personal medical information will be obtained by others not associated with this study.

BENEFITS OF TAKING PART IN THE STUDY:

You will not benefit personally by taking part in this study.

ALTERNATIVES TO TAKING PART IN THE STUDY:

You are welcome to decline to take part in this study.

CONFIDENTIALITY

We will keep your personal information confidential. We cannot guarantee absolute confidentiality. Your identity will be held in confidence in reports in which the study may be published. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include: the study investigator and his/her research associates, the IU Institutional Review Board or its designees, the study sponsor, and (as allowed by law) the Office for Human Research Protections (OHRP), who may need access to your research records.

COSTS

Taking part in this study will not lead to added costs to you or your insurance company, except possible costs described in the section entitled 'Compensation for Injury', below.

PAYMENT

You will receive a total payment of $400 for satisfactory completion of the full study. You are guaranteed to receive a payment of $350 with an additional $50 if plasma vitamin E tests confirm that you were compliant with eating or not eating the almonds (depending on your group assignment – measured by analysis of your blood samples) throughout the trial. Payments will be made by check, which should be received approximately 2 weeks after study completion. If you do not meet pre-set eligibility criteria during the baseline screening assessment, you will receive a payment of $15. If you withdraw, or are withdrawn from the study by study personnel prior to completion, you will receive a payment of $10 for each week you completed.

VOLUNTARY NATURE OF STUDY

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. Your decision whether or not to participate in this study will not affect your current or future relations with the Indiana University School of Medicine and IU Health.

Your participation may be terminated by Dr. Considine without regard to your consent in the following circumstances: you do not cooperate with the study procedures or you miss any scheduled study sessions. Your participation may also be terminated if Dr. Considine or other study investigators believe that your continued participation will be bad for your health, or if the study investigators believe that the data obtained are limited in quality.

COMPENSATION FOR INJURY

In the event of physical injury resulting from your participation in this research, necessary medical treatment will be provided to you and billed as part of your medical expenses. Costs not covered by your health care insurer will be your responsibility. Also, it is your responsibility to determine the extent of your health care coverage. There is no program in place for other monetary compensation for such injuries. However, you are not giving up any legal rights or benefits to which you are otherwise entitled. If you are injured, you should immediately notify Robert V Considine at (317) 278-2389.

CONTACTS FOR QUESTIONS OR PROBLEMS

If I have questions regarding the study I can reach Robert V. Considine at (317) 278-2389.
If you cannot reach the researcher during regular business hours (i.e. 8:00AM-5:00PM), please call the IU Human Subjects Office at (317)278-3458 or (800)696-2949.
For questions about your rights as a research participant or to discuss problems, complaints or concerns about a research study, or to obtain information, or offer input, contact the IU Human Subjects Office at (317) 278-3458 or (800) 696-2949.
I may write to the Principal Investigator at any time and request that I be withdrawn from the study, and that my data be destroyed.

CONSENT

I have been given an opportunity to ask questions about this study; answers to such questions (if any) have been satisfactory.
The information in the study records will be kept confidential and will be made available only to persons conducting the study unless I specifically give my permission in writing to do otherwise. If the results of this study are published, I will not be identified.

In consideration of all of the above, I give my consent to participate in this research study. I may drop out of or be withdrawn from the study without fear of changing the investigator's interest or the quality of medical care which I may seek or receive in the future from the doctors participating in the study.

I acknowledge receipt of a copy of this informed consent statement.

SUBJECT'S PRINTED NAME:_____
(please print)

SUBJECT'S SIGNATURE:_____ DATE:_____
(must be dated by subject)

PRINTED NAME OF PERSON
OBTAINING CONSENT_____

SIGNATURE OF PERSON
OBTAINING CONSENT_____ DATE:_____

APPENDIX C. ALMOND NUTRIENT COMPOSITION

Almond nutrient composition. Covance Laboratories, Madison, WI 53704.

Per 42.5 g: 270.3 Calories, 198.5 Calories from fat, 22.06 g total fat, 14.3 g monounsaturated fatty acids, 5.1 g polyunsaturated fatty acids, 8.8 g total carbohydrates, 0.9 g soluble fiber, 4.3 g insoluble fiber, 5.1 g total fiber, 1.7 g total sugar, 9.1 g protein

Per 100 g serving: 636 Calories, 467 Calories from fat, 51.9 g total fat, 33.7 g monounsaturated fatty acids, 12.0 g polyunsaturated fatty acids, 20.8 g Total Carbohydrates, 2.08 g soluble fiber, 9.99 g insoluble fiber, 12.1 g total fiber, 4.1 g total sugar, 21.4 g protein

APPENDIX D. WEIGHTED NUTRIENT DENSITY SCORE ALGORITHM

[(1.40*Protein g per 100 kcal/50 g) + (3.13*Fiber g per 100 kcal/25 g) + (1.00*Calcium mg per 100 kcal/1000 mg) + (2.51*Unsaturated fat g per 100 kcal/44 g) + (0.37*Vitamin C mg per 100 kcal/60 mg) – 2.95*Saturated fat g per 100 kcal/20 g) – (0.52*Added sugars g per 100 kcal/50 g) – (1.34*Sodium mg per 100 kcal/2400 mg)] * 100

APPENDIX E. CHOCOLATE ENSURE DRINK NUTRIENT COMPOSITION

Ensure Original Milk Chocolate Nutrition Shake, Abbott Laboratories, Chicago, IL.

Per 8 oz bottle: 220 Calories, 6g total fat, 1g saturated fat, 0g trans-fat, 2g polyunsaturated fat, 3g monounsaturated fat, <5 mg cholesterol, 210 mg sodium, 33 g total carbohydrate, 1g dietary fiber, 15 g total sugars, 14g added sugars, 9g protein, 60 mcg folic acid.

APPENDIX F. GOLDBERG FORMULA

$$EI_{rep}:BMR > PAL \times \exp\left[s.d._{min} \times \frac{(S/100)}{\sqrt{n}}\right]$$

$$EI_{rep}:BMR < PAL \times \exp\left[s.d._{max} \times \frac{(S/100)}{\sqrt{n}}\right] \qquad S = \sqrt{\frac{CV_{wEI}^2}{d} + CV_{wB}^2 + CV_{tP}^2}$$

PAL=1.55

CV_{WEI}=23

CV2wb=8.5

CV2tP=15

D=12

N=134

S=184752

s.d.max=3 (99% confidence interval)

1.47753>EIrep:BMR<1.626019

APPENDIX G. APPETITE QUESTIONNAIRE

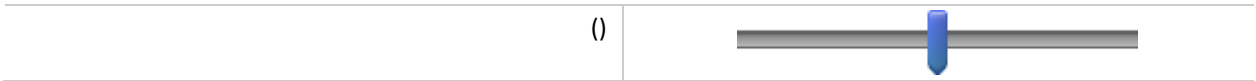
Appetite Questionnaire - Almond Study

Participant Number

_____

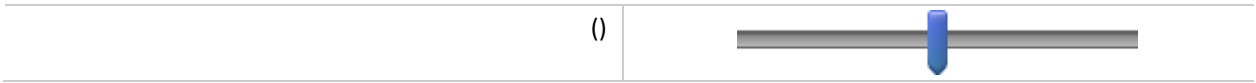
How strong is your feeling of hunger?

Not at all Extremely



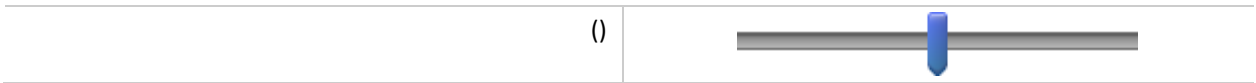
How strong is your feeling of fullness?

Not at all Extremely



How strong is your desire to eat?

Not at all Extremely



How much food could you eat right now?

Not at all An extremely large amount

()	

How strong is your preoccupation with food?

Not at all Extremely

()	

How strong is your desire to eat something salty?

Not at all Extremely

()	

How strong is your desire to eat something fatty?

Not at all Extremely

()	

How strong is your desire to eat something sweet?

Not at all Extremely

()	

APPENDIX H. HEI RUBRIC

TOTAL_SCORE =_TOTALVEG +_GREEN_AND_BEAN + TOTALFRUIT
+_WHOLEFRUIT +_WHOLEGRAIN +_TOTALDAIRY +_TOTPROT + SEAPLANT_PROT
+_FATTYACID +_SODIUM + REFINEDGRAIN + SFAT + ADDSUG

APPENDIX I. HEI -2015 COMPONENTS AND SCORING STANDARDS

Table H.1. HEI-2015 Components and Scoring Standards, produced by NCI

Component	Max points	Standard for maximum score	Standard for minimum score of zero
Adequacy:			
Total Fruits[2]	5	≥0.8 cup equiv. per 1,000 kcal	No Fruits
Whole Fruits[3]	5	≥0.4 cup equiv. per 1,000 kcal	No Whole Fruits
Total Vegetables[4]	5	≥1.1 cup equiv. per 1,000 kcal	No Vegetables
Greens and Beans[4]	5	≥0.2 cup equiv. per 1,000 kcal	No Greens and Beans
Whole Grains	10	≥1.5 oz equiv. per 1,000 kcal	No Whole Grains
Dairy[5]	10	≥1.3 cup equiv. per 1,000 kcal	No Dairy
Total Protein Foods[4]	5	≥2.5 oz equiv. per 1,000 kcal	No Protein Foods
Seafood and Plant Proteins[6]	5	≥0.8 oz equiv. per 1,000 kcal	No Seafood or Plant Proteins
Fatty Acids[7]	10	(PUFAs + MUFAs)/SFAs ≥2.5	(PUFAs + MUFAs)/SFAs ≤1.2
Moderation:			
Refined Grains	10	≤1.8 oz equiv. per 1,000 kcal	≥4.3 oz equiv. per 1,000 kcal
Sodium	10	≤1.1 gram per 1,000 kcal	≥2.0 grams per 1,000 kcal
Added Sugars	10	≤6.5% of energy	≥26% of energy
Saturated Fats	10	≤8% of energy	≥16% of energy

1: Intakes between the minimum and maximum standards are scored proportionately.
2: Includes 100% fruit juice.
3: Includes all forms except juice.
4: Includes legumes (beans and peas).
5: Includes all milk products, such as fluid milk, yogurt, and cheese, and fortified soy beverages.
6: Includes seafood, nuts, seeds, soy products (other than beverages), and legumes (beans and peas).
7: Ratio of poly- and monounsaturated fatty acids (PUFAs and MUFAs) to saturated fatty acids (SFAs).

VITA

Stephanie R. Hunter

700 W. State Street, Stone Hall G-7, West Lafayette, IN 47907

hunter99@purdue.edu

Education

2016-present Candidate for Doctor of Philosophy
College of Health and Human Sciences, Purdue University, West Lafayette, IN
Interdepartmental Nutrition Program, emphasis in Ingestive Behavior
Dissertation: The role of almonds consumed as a breakfast and snack by adults with different body fat distributions on indices of carbohydrate and lipid metabolism
Mentor: Richard Mattes

2012-2016 Bachelor of Science
College of Science and Engineering, Winona State University, Winona, MN
Major: Cell and Molecular Biology, Minor: Biochemistry, GPA: 4.0, Summa Cum Laude
Capstone: Reduced Acidity Cranberry Improves Glycemic Response in Persons with Metabolic Syndrome; Characterization of Vanilla Taste and Olfactory Preferences
Advisor: Ted Wilson

Publications

Peer Reviewed Journal Articles

1. **Hunter S.R.**, Reister E.J., Cheon E., Mattes R.D. Low Calorie Sweeteners Differ in Their Physiological Effects in Humans. *Nutrients* 2019, *11*, 2717. https://doi.org/10.3390/nu11112717
2. Li M., George J., **Hunter S.R.**, Hamaker B.R., Mattes R.D., Ferruzzi M.G. Potato product form impacts in vitro starch digestibility and glucose transport but only modestly

impacts 24h blood glucose response in humans. Food & Function. 2019, 10, 1846-1855. https://doi.org/10.1039/C8FO02530D

3. Mattes R.D., **Hunter S.R.**, Higgins K.A. Sensory, gastric, and enteroendocrine effects of carbohydrates, fat, and protein on appetite. Current Opinion in Endocrine and Metabolic Research. 2019, 4, 14-20. https://doi.org/10.1016/j.coemr.2018.09.002.

Book Chapters

1. **Hunter S.R.**, Mattes R.D. The Role of Eating Frequency and Snacking on Energy Intake and BMI. In: Meiselman HL, editor. Handbook of Eating and Drinking: Interdisciplinary Perspectives. Cham: Springer International Publishing 2019; p. 1-21. https://doi.org/10.1007/978-3-319-75388-1_115-1
2. McArthur B.M., Higgins K.A., **Hunter S.R.**, Mattes R.D. The energetics of nut consumption: Oral processing, appetite, and energy balance. In: Sabate J., Salvado JS, Cesarettin A. (eds). Health Benefits of Nuts and Dried Fruit. CRC Press/Taylor & Francis 2018; p.317-332. https://doi.org/10.1201/9781315173337

Other Publications

1. **Hunter S.R**., Fanzo J. Plenary lecture: Eating our way through the anthropocene: the challenges, risks and ethics of actions. Physiology & Behavior. 2020:113050. doi: https://doi.org/10.1016/j.physbeh.2020.113050.
2. **Hunter S.R.,** Gibb R., Rubenstein G. Session 6: Future directions. Physiology & Behavior. 2020;224:113038. doi: https://doi.org/10.1016/j.physbeh.2020.113038.
3. Kant A., Mattes R., Slavin J., **Hunter S.R**. Session 5 discussion: Snacking. Physiol Behav. 2018;193(Pt B):288-90. Epub 2018/05/22. https://doi.org/10.1016/j.physbeh.2018.05.019

Manuscripts in Progress

1. Cheon E., Reister E.J., **Hunter, S.R.,** Mattes R.D. Sweet Hedonics and Ingestive Behavior. *In Review,* Journal of the Academy of Nutrition and Dietetics.

Awards and Certificates

2020 Recipient of the Bilsland Dissertation Fellowship

Purdue University Office of Interdisciplinary Graduate Program. Award accompanied by a $7,692.30 stipend.

2020 Certificate of Foundations in College Teaching

Center for Instructional Excellence, Purdue University

2019 Certificate of Practice in College Teaching
Center for Instructional Excellence, Purdue University

2019 Certificate of Completion, Applied Management Principles program (mini-MBA)
Krannert School of Management, Purdue University

2018 Certificate of Excellence in Interdisciplinary Research
Office of Interdisciplinary Graduate Programs Spring Reception, Purdue University

2016 Recipient of the Lynn Fellowship
Purdue University Graduate School. Award accompanied by a $19,510 stipend.

Teaching Experience

2019 Graduate Teaching Assistant, Purdue University
NUTR 53400: Human Sensory Systems and Food Evaluation, 9 students (advanced level undergraduate and graduate course)
Instructor of Record: Richard Mattes
Responsibilities: My primary role as the TA was to lead the lab section of the course. This entailed giving an introduction to each lab and describing the methods used in sensory research, walking through the procedure with the students, answering questions and assisting students throughout the lab period, and grading and providing feedback on lab reports each week. I also assisted in the lecture session of the course and gave lectures on Time-Intensity and Hedonics.

Presentations

Hunter, S.R., Considine, R.V., and Mattes, R.D. The effects of long-term almond consumption in adults with different body fat distributions on anthropometric characteristics and HbA1c. American Society for Nutrition, Nutrition 2020, online, June 2020.

Hunter, S.R. Is cultured meat an environmentally ethical and acceptable alternative to conventional meat? The Ethics of Eating: Promoting Personal and Global Choices Poster Session, Purdue University, October 2019.

Hunter, S.R., Considine, R.V., and Mattes, R.D. The role of almonds consumed at breakfast and as an afternoon snack by adults with different body fat depots on indices of carbohydrate and lipid metabolism. Health and Disease: Science, Technology, Culture and Policy Research Poster Session, Purdue University, March 2019.

Hunter, S.R. Calorie for calorie, dietary fat restriction results in more body fat loss than carbohydrate restriction in people with obesity. Ingestive Behavior Research Center Journal Club, October 2018.

Hunter, S.R., Mattes, R.D., Ferruzzi, M. Effect of phenolic acids and resistant starch in potato products on post-prandial glycemia and appetite. Office of Interdisciplinary Graduate Program Spring Reception Poster Session, Purdue University, May 2018.

Hunter, S.R. The effects of nutrition knowledge on food label use. A review of the literature. Ingestive Behavior Research Center Journal Club, January 2018.

Hunter, S.R., and Mattes, R.D. Effects of phytochemicals in potato products on post-prandial glycemia. Interdepartmental Nutrition Program Day Poster Session, Purdue University, March 2017.

Dahl, R.A., Bolstad, K.T., Googins, S.L., **Hunter, S.R.**, Mackay, K.E., Vorsa, N., Limburg, P.J., and Wilson, T. Reduced Acidity Cranberry Improves Glycemic Response in Persons with Metabolic Syndrome. Experimental Biology Poster Session, Boston, MA, April 2015.

Nelson, H.N., Lange, K.M., **Hunter, S.R.**, O'Bert, A.E., Wilson, T. Characterization of Vanilla Taste Preference. Experimental Biology Poster Session, Boston, MA, April 2015.

O'Bert, A.E., Lange, K.M., Nelson, H.N., **Hunter, S.R.**, Connell, J.M., Kluver, I.L., Villari, T.Y., Wilson, T. Characterization of Vanilla Olfactory Preference. Experimental Biology Poster Session, Boston, MA, April 2015.

Service Activities

Leadership in Professional Organizations and Committees

2019 Session Chair, 'The Ethics of Eating – Promoting Personal and Global Choices', Ingestive Behavior Research Center Conference, Purdue University

2018-2020 Member, Ingestive Behavior Research Center Executive Board, Purdue University

2018-2020 President, Ingestive Behavior Graduate Student Association Purdue University

2017-2018 President Elect, Ingestive Behavior Graduate Student Association Purdue University

2017-2019 Treasurer, Nutrition Sciences Graduate Student Organization Purdue University

2016-2017 Social Chair, Ingestive Behavior Graduate Student Association Purdue University

Educational Presentations in the Community

2019 Science in Schools – The Jelly Bean Test

2019 4-H Conference – Techniques in Descriptive Analysis

Other Service Activities

2017-2020 Prospective Student Host, Interdepartmental Nutrition Program, Purdue University

Additional Work Experience

2013-2016 Supply Quality Intern

Sargento Foods, Inc., Plymouth, WI

Primary roles were to create, distribute, file, and maintain various reports, documents, and programs essential to the operation and efficiency of the Procurement and Supply Quality departments. Also led a project obtaining authenticity documents for product claims.

2012-2016 Supplemental to Instruction Leader

Tutoring Services

Winona State University, Winona, MN

Led study sessions for Anatomy and Physiology classes with high failure risk to help students better understand lecture material and learn study techniques. Also held one-on-one tutor sessions for Anatomy, Physiology, Chemistry, Nutrition, and Microbiology to help students individually. I was responsible for working with professors and tutoring staff to provide an excellent learning environment for students.

2014-2016 Anatomy and Physiology Lab Supervisor

Winona State University, Winona, MN

Responsible for sitting in the Anatomy Lab to make sure rules and regulations were being followed and offered assistance with students' questions.

References

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