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EFFECTS OF PEANUTS AS A NIGHTTIME SNACK COMPARED TO A LOWER-FAT HIGHER-CARBOHYDRATE CONTROL ON GLYCEMIC CONTROL, CARDIOVASCULAR DISASE RISK FACTORS, AND THE GUT MICROBIOTA IN ADULTS WITH ELEVATED FASTING GLUCOSE

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ABSTRACT

The aim of this dissertation was to investigate the cardiometabolic effects of consuming peanuts as a nighttime snack, compared to a lower-fat higher-carbohydrate (LFHC) snack, in adults with elevated fasting glucose. In a randomized controlled, crossover, supplemental feeding study design, participants consumed the nighttime snacks for 6 weeks with ≥ 4-week compliance break between conditions. The conditions were 28.4 g dry roasted, unsalted, peanuts (164 kcal; 11% E carbohydrate, 17% E protein, and 73% E fat) and 6 whole grain crackers with one slice of low-fat American cheese (164 kcal; 53% E carbohydrate, 17% E protein, and 33% E fat). Participants were instructed to consume the study foods after dinner, before bedtime, and consume no other food or drinks containing calories after their snack. Additionally, participants were instructed to avoid consuming any other peanuts, or tree nuts, for the duration of the study. The primary outcome was fasting plasma glucose and secondary outcomes were weight, insulin, fructosamine, lipids/lipoproteins, brachial blood pressure (BP), central BP, measures of arterial stiffness [pulse wave velocity (PWV)], diet quality, and the gut microbiota with each of these outcomes being assessed at baseline and endpoint for both conditions. Diet quality, measured by the Healthy Eating Index-2015 (HEI-2015), was assessed using the Automated Self-Administered 24-recall system (ASA24). Fecal samples from all participants (n=50) were analyzed using 16S rRNA sequencing to assess gut microbiota composition and diversity while a subset of samples from responders (n=24) to the peanut condition (greatest reduction in fasting glucose) were analyzed using metatranscriptomics to assess bacterial gene expression.

Fifty adults with elevated fasting glucose (52% male; age: 42 ± 15 years; BMI: 28.3 ± 5.6 kg/m^2; fasting plasma glucose: 100 ± 8 mg/dL) completed the study. There were no significant between-condition mean differences for fasting plasma glucose (Peanut vs. LFHC -0.6 mg/dL; 95% CI: -2.7, 1.6; *P = 0.67*). There were no between-condition mean differences for weight,

fructosamine, lipid/lipoproteins, brachial BP, central BP, or PWV. Total energy intake was not different between conditions, but percent of energy from polyunsaturated and monounsaturated fatty acids was higher following the Peanut condition compared to the LFHC condition (1.9%; 95% CI: 0.0, 3.4; *P = 0.01* and 1.7%; 95% CI: 0.0, 3.4; *P = 0.04*). No between-condition mean difference for total HEI-2015 score was observed (Peanut vs. LFHC 3.6 points; 95% CI: -1.9, 9.0; *P = 0.19*). Examination of the HEI-2015 components showed that the added sugar (0.8 points; 95% CI: 0.0, 1.5; *P = 0.04*) and seafood/plant protein (2.0 points; 95% CI: 1.0, 2.9; *P < 0.01*) components were higher following the Peanut condition vs. the LFHC snack. The whole grain (-2.6 points; 95% CI: -3.8, -1.4; *P < 0.01*) component was lower with the Peanut snack vs. LFHC. No other HEI-2015 components were different between the conditions.

There were no between-condition differences in microbiota α- diversity or β-diversity. Following the Peanut condition, *Roseburia* [linear discriminant analysis score (LDA) = 3.1; *P = 0.035*)] and *Ruminococcaceae* (LDA = 2.8; *P = 0.037*) were significantly enriched compared to the LFHC snack. In the meta-transcriptomics exploratory analyses, a significant increase in expression of the K03518 (aerobic carbon-monoxide dehydrogenase small subunit) gene (LDA = 2.0; *P = 0.039*) was observed following peanut intake compared to baseline. Taxonomic contributor analyses showed *Roseburia Instinalis L1* contributed to the increase in K03518 gene expression (1.5% contribution in endpoint samples vs. no detectable contribution in baseline samples).

In adults with elevated fasting glucose, consuming 28 g of dry roasted, unsalted, peanuts as a nighttime snack did not alter fasting plasma glucose compared to a LFHC nighttime snack. Consumption of peanuts compared to the LFHC snack did not increase weight or total energy intake but was associated with improvements in the fatty acid profile of the diet. Peanuts improved seafood/plant protein and added sugar diet quality components but reduced the whole grain component compared to a LFHC control. Several SCFAs producing bacteria were enriched

following peanut intake and expression of a gene associated with fiber degradation and SCFAs production was increased. Consuming peanuts as a nighttime snack compared to a LFHC nighttime snack does not have adverse effects on fasting plasma glucose in adults with elevated fasting glucose and improves several aspects of dietary intake and quality with beneficial impacts on gut microbiota composition and functionality.

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ABBREVIATIONS

% E	Percent of energy
ADA	American Diabetes Association
AIX	Augmentation index
AP	Augmentation pressure
ASA-24	Automated self-administered 24-hour dietary assessment tool
ASV	Amplicon sequence variants
bDBP	Brachial diastolic blood pressure
BMI	Body mass index
BP	Blood pressure
bSBP	Brachial systolic blood pressure
cDBP	Central diastolic blood pressure
CDC	Centers for Disease Control and Prevention
CGM	Continuous glucose monitor
CHD	Coronary heart disease
CPM	Counts per million
CRP	C-reactive protein
cSBP	Central systolic blood pressure
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DPP	Diabetes Prevention Program
FMD	Flow mediated dilation
FPG	Fasting plasma glucose
HbA1c	Hemoglobin A1c
HDL-C	High-density lipoprotein cholesterol
HEI-2015	Healthy eating index-2015
HOMA-IR	Homeostatic model assessment for insulin resistance
HPFS	Health Professionals Follow-Up Study
HR	Hazard ratio
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
LDA	Linear discriminant analysis
LDL-C	Low-density lipoprotein cholesterol
LEfSe	Linear discriminant analysis effect size
LFHC	Lower-fat higher-carbohydrate
MGWAS	Metagenome-wide association study
MNT	Medical Nutrition Therapy

MUFA	Monounsaturated fatty acid
NHANES	National Health and Nutrition Examination Survey
NHS	Nurses' Health Study
NHSII	Nurses' Health Study II
OGTT	Oral glucose tolerance test
OR	Odds ratio
OTU	Operational taxonomic units
PAI-1	Plasminogen activator inhibitor-1
PCoA	Principal coordinates analysis
PLS-DA	Partial least squares discriminant analysis
PTT	Pulse transit time
PUFA	Polyunsaturated fatty acid
PWV	Pulse wave velocity
RCT	Randomized controlled trial
RDN	Registered dietitian nutritionist
ROC AUC	Area under the receiver operating characteristic curve
RR	Relative risk
SBP	Systolic blood pressure
SCFA	Short-chain fatty acid
SFA	Saturated fatty acid
T2DM	Type 2 diabetes mellitus
Tchol	Total cholesterol
TG	Triglyceride
US	United States
USDA	United States Department of Agriculture
WMD	Weighted mean difference

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Chapter 1

Introduction

Prediabetes affects approximately one in three adults in the United States (US), or an estimated 88 million individuals (1). In addition, prediabetes increases risk for type 2 diabetes mellitus (T2DM) (2,3) and cardiovascular disease (CVD) (4). Prediabetes is defined by elevated fasting glucose (≥100 mg/dL and ≤125 mg/L), impaired glucose tolerance (2-hour postprandial glucose level between 140 and 199 mg/dL following a 75g oral glucose tolerance test), or elevated hemoglobin A1c (HbA1c) (≥5.7% and ≤6.4%), all of which are potentially modifiable by dietary intervention. Consistent evidence suggests that higher diet quality is associated with a reduced risk of T2DM, and clinical trials show reduced incidence of T2DM with intensive lifestyle therapies (diet, exercise, weight loss, and behavioral modification) (5,6). However, specific dietary recommendations for adults with prediabetes were not issued until 2019 (7).

The current recommendations from the American Diabetes Association (ADA) for adults with prediabetes are focused on improving diet quality by Medical Nutrition Therapy (MNT) administered by a Registered Dietitian Nutritionist (RDN) (7). These recommendations focus on following an evidence-based healthy dietary pattern (e.g., Dietary Guidelines for Americans 2015-2020 Healthy Dietary Patterns, Mediterranean Diet, or low-fat diets). All of these dietary patterns are abundant in plant-based foods including fruits, vegetables, wholegrains, legumes, nuts, and seeds. At a population level, adherence to dietary recommendations in the US is low (8). Strategies to improve adherence to dietary recommendations and lower risk of diet-related cardiometabolic diseases are needed.

Accumulating evidence suggests eating occasions and/or meal timing contribute to glycemic control and cardiometabolic health (9,10); however, limited clinical research has

investigated interventions focused on timing of food consumption. Meal timing has shifted over the last 40-years with adults consuming a larger proportion of their daily energy from snacks [mostly nutrient poor and energy dense foods (i.e., chips, alcohol, sweets)] and consuming more snacks after dinner (11). Based on the low compliance to current dietary recommendations, changes in eating occasion/meal timing, and recommendations for nut consumption, a small dietary change such as replacing nuts for energy dense and nutrient poor snacks may improve cardiometabolic health.

There is consistent observational and experimental evidence supporting the relationship between tree nut consumption, reduced risk for T2DM (12), improvements in glycemic control (13), changes in the gut microbiota (14), and reduced CVD risk (15–17). Peanuts, although considered a legume, have a similar micro- and macronutrient profile to tree nuts, are included in the DGA nuts, seeds, and soy category, and are the most commonly consumed nut in the US (18).

Observational studies have demonstrated favorable associations between peanut consumption and both T2DM and CVD risk (17,19), but experimental studies have mainly focused on tree nuts with limited investigation of peanuts. Tree nuts have beneficial effects on CVD risk factors (i.e., blood pressure and lipids/lipoproteins) (15,16). Most clinical trials assessing tree nuts and glycemic control are in populations with T2DM. Postprandial feeding studies have demonstrated improvements in short-term glycemic control following peanut consumption (20,21), but the longer-term effects have not been established and no trials have evaluated the effect of peanuts on the gut microbiota.

The aim of this dissertation was to assess the effect of consuming peanuts (28 g/d) as a nighttime snack, compared to a lower-fat higher-carbohydrate (LFHC) control snack, on fasting plasma glucose, CVD risk factors and gut microbiota composition after six weeks in adults with elevated fasting glucose. The primary outcome was fasting plasma glucose. The secondary outcomes included weight, insulin, fructosamine, brachial blood pressure (BP), central BP,

arterial stiffness [pulse wave velocity (PWV)], lipids/lipoproteins, diet quality, and the gut microbiota.

Chapter 2

Literature Review

2.1 Homeostatic Regulation of Glucose

The American Diabetes Association (ADA) defines normal blood glucose levels in an individual without diabetes as < 100 mg/dL in the fasted state, hemoglobin A1c (HbA1c) < 5.7%, and 2-hour postprandial glucose level < 140 mg/dL following a 75g oral glucose tolerance test (OGTT) (22). Glucose homeostasis is regulated by multiple hormones with the two most important being insulin and glucagon. Together these hormones act to maintain normal blood glucose levels. Insulin is produced by the pancreatic β-cells following a meal (postprandial state) to promote glucose disappearance by signaling insulin-sensitive tissues (primarily skeletal muscle and adipose tissues) to increase uptake of glucose (23). Additionally, insulin stimulates glycogenesis (glycogen synthesis from glucose molecules) in the liver and inhibits the secretion of glucagon by pancreatic α-cells (23). Glucagon is the primary hormone regulating glucose in the fasted state. Glucagon has the primary role of stimulating hepatic glucose production through glycogenolysis (glycogen is catabolized for energy utilization) and gluconeogenesis (glucose production from non-carbohydrate precursors) (23).

2.2 Burden of Impaired Fasting Glucose

Prediabetes is an important clinical marker of increased risk for type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). The ADA defines prediabetes (**Table 2.1**) by the presence of impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or elevated HbA1c (24). A fasting plasma glucose (FPG) ≥ 100 and ≤ 125 mg/dL is the criterion for IFG. IGT is

defined by a 2-hour postprandial glucose level between 140 and 199 mg/dL following a 75g OGTT. HbA1c ≥ 5.7% and ≤ 6.4% also is a criterion for prediabetes (24). IFG is associated with dyslipidemia, hypertension, and obesity. Additionally, IFG is one of the five metabolic risk factors included in the National Institutes of Health cluster of risk factors used to diagnose Metabolic Syndrome. IFG significantly increases risk of T2DM and CVD, both of which are a major public health burden in the United States (25). The following sections will discuss the prevalence of IFG, CVD risk factors associated with IFG, morning hyperglycemia, and the relationship between IFG and both diet and the gut microbiota.

Fasting plasma glucose 100 mg/dL to 125 mg/dL (IFG)
OR
2-hour postprandial glucose during 75g OGTT 140 mg/dL to 199 mg/dL (IGT)
OR
HbA1c 5.7-6.4%

Figure **2-1**: Criteria defining prediabetes (24)

2.2.1 Prevalence of Prediabetes and Diabetes in the US

Based on the 2013-2016 National Health and Nutrition Examination Survey (NHANES) and 2018 US Census Bureau data, one in three, or approximately 88 million, adults in the US had prediabetes (1). Of these 88 million adults, only 15.3% were aware of their condition. Prediabetes does not impact all ages, races/ethnicities, or sexes equally. Of the estimated 88 million US adults with prediabetes in 2018, 47.1 million were female and 40.9 million were male. However, the estimated prevalence was greatest in men (38%) compared to women (31.2%) based on the 2013-2016 estimates. Adults between the ages of 45 and 64 years make up the greatest proportion of

the 88 million totaling 35.1 million, followed by 28.7 million individuals 18-44 years, and 24.2 million individuals ≥ 65 years. Individuals ≥ 65 years have the greatest prevalence (46.6%), followed by adults 45-64 years (41.7%), and adults 18-44 years (24.3%). A majority of the estimated individuals with prediabetes are non-Hispanic White (54.8 million), followed by Hispanic (14.6 million), non-Hispanic Black (11.4 million), and non-Hispanic Asian (5 million). All races and ethnicities had a similar prevalence of prediabetes between 33 and 37%.

Based on the 2013-2016 NHANES and 2018 US Census Bureau data, 34.1 million, or 13.1%, of US adults had diabetes (type 1 and T2DM). Cases of diabetes are further classified by diagnosed (26.8 million, or 10.5%) and undiagnosed (7.3 million, or 2.9%). Men had a greater number of cases and prevalence of diabetes (17.9 million and 14%) compared to women (16.2 million and 12%). Adults ≥ 65 years had the greatest prevalence (26.8%; 14.3 million cases), followed by adults 45-64 years (17.5%; 14.8 million cases), and adults 18-44 years (4.2%; 4.9 million cases) had the lowest prevalence. The greatest number of diabetes cases were in non-Hispanic White adults (19.5 million), followed by Hispanic adults (6.4 million), then non-Hispanic Black adults (5.2 million), and non-Hispanic Asian adults (2.3 million). Non-Hispanic Black adults had the greatest prevalence of diabetes (16.4%) compared to non-Hispanic Asian adults (14.9%), Hispanic adults (14.7%), and non-Hispanic White adults (11.9%).

2.2.2 Progression from Prediabetes to T2DM

Prediabetes significantly increases an individual's risk for developing T2DM throughout the lifespan with an annualized conversion rate of 5-10% (2). However, these estimates are variable across populations. The Centers for Disease Control and Prevention (CDC) estimates that 2% of the 88 million US adults with prediabetes will progress to T2DM each year. Blood glucose concentration, either 100-109 mg/dL or 110-125 mg/dL, is an important predictor for

progression to T2DM. Nichols et al. analyzed data from 5,452 subjects collected from 1994 to 2003 and found that 8.1% of subjects with a fasting glucose of 100-109 mg/dL and 24.3% of subjects with a fasting glucose of 110-125 mg/dL developed T2DM (26). Additionally, age is an important risk factor for progression from prediabetes to T2DM. The lifetime risk for progression from prediabetes to T2DM in adults ≥ 45 years is 74% (3). With the large proportion of US adults with prediabetes and continual progression from prediabetes to T2DM it is estimated that more than 54.9 million US adults will have diabetes by 2030 (27).

2.2.3 CVD Risk Factors Associated with Prediabetes

CVD is the leading cause of death and disability in the US and globally (28). Globally, cases of CVD and CVD deaths have increased from 1990 (cases: 271 million and deaths: 12.1 million) to 2019 (cases: 523 million and deaths: 18.6 million) (28). The total prevalence of CVD, including hypertension, for US adults ≥ 20 years based on 2013-2016 NHANES was 121.5 million (48%); the total CVD prevalence, excluding hypertension, was 24.3 million (9%) (29). The total CVD deaths for all ages in the US in 2017 was 859,125.

CVD disproportionally impacts adults with diabetes and prediabetes. Adults with diagnosed (HR: 2.36 [95% CI 2.20-2.76]) and undiagnosed diabetes (HR: 1.78 [95% CI 1.56-2.03]) have an increased risk for developing CVD (4). Adults with a fasting blood glucose concentration of 100-110 and 110-125 mg/dL have an 11% (HR: 1.11 [95% CI 1.04-1.08]) and 17% (HR: 1.18 [95% CI 1.08-1.26]) increased risk of developing CVD (4). Additionally, prediabetes is associated with major CVD risk factors (e.g., dyslipidemia, hypertension, and obesity).

Desirable levels of low-density lipoprotein cholesterol (LDL-C) are less than 100 mg/dL, high-density lipoprotein cholesterol (HDL-C) greater than 40 mg/dL, and triglycerides (TG) less

than 150 mg/dL (30). An estimated 30-60% of patients with T2DM have abnormal lipid/lipoprotein levels (31). Although similar estimates for the prevalence of dyslipidemia in patients with prediabetes are not available, they often have elevated LDL-C, low HDL-C, and elevated TG (32). A study of 613 adults (mean BMI ~28 ± 6 kg/m^2) in Saudi Arabia without diabetes (71% with normoglycemia and 31% with prediabetes) found that 65% individuals had dyslipidemia (33). Individuals with prediabetes had a mean LDL-C of 139 mg/dL compared to 119 mg/dL in adults with normoglycemia. Similarly, TG levels were higher in adults with prediabetes compared to those with normoglycemia (116 vs. 96 mg/dL).

Many adults with prediabetes have elevated brachial blood pressure (BP), or hypertension. The desirable systolic blood pressure (SBP) level is less than 120 mmHg and diastolic blood pressure (DBP) less than 80 mmHg. Hypertension is classified as: Stage 1 - a SBP of 130-139 mmHg or DBP of 80-89 mmHg and Stage 2 - a SBP ≥ 140 mmHg and DBP ≥ 90 mmHg (34). Individuals with prediabetes have a slightly greater prevalence of hypertension (60%) compared to individuals without prediabetes (57%) (35). The combination of prediabetes and hypertension significantly increases CVD risk (HR: 2.38; 95% CI 1.17-4.85) compared to individuals with normal glucose regulation and normal BP (35).

Central, or aortic, BP is a measure of the pressure the internal organs experience and is more predictive of preclinical organ damage, cardiovascular events, and mortality compared to brachial BP (36–38). Carotid-femoral pulse transit time and pulse wave velocity (PWV) is a measure of how quickly blood flows through the arteries based on the speed at which the pulse wave is transmitted through the vascular tree. Central BP and carotid-femoral PWV can be measured non-invasively, with carotid-femoral PWV being the gold standard (39). Unlike brachial BP, there are no clinical set points for central BP or carotid-femoral PWV in the US but elevated central BP and PWV are indicative of vascular damage and arterial stiffness (40). There

is clinical evidence suggesting adults with impaired glucose control and T2DM have increased central BP and carotid-femoral pulse transit time and pulse wave velocity (PWV) (41).

Body mass index (BMI) is associated with T2DM, prediabetes, and conversion to T2DM. A study assessing more than 37,000 adults estimated the relative risk for developing T2DM in individuals with a BMI 25-29.9 kg/m^2 was 1.5 (95% CI: 1.4, 1.6) and for individuals with a BMI 30-34.9 kg/m^2 was 2.5 (95% CI: 2.3, 2.6) (42). Adult men and women with prediabetes tend to have higher BMIs (men: 27.4 kg/m^2 and women: 25.7 kg/m^2) compared to individuals with normoglycemia (Men: 26.0 kg/m^2 and women: 22.9 kg/m^2). Additionally, adults with fasting glucose levels 110-125 mg/dL and a BMI $\geq$ 30 kg/m^2 have an annual conversion to T2DM rate > 4% compared to < 1% in individuals with a fasting blood glucose 100-110 mg/dL and BMI $\geq$30 kg/m^2 (43).

Prediabetes is associated with other CVD risk factors (e.g., inflammation and endothelial dysfunction [impaired vasodilation in blood vessels]) (44). C-reactive protein (CRP) and plasminogen activator inhibitor-1 (PAI-1) are inflammatory biomarkers positively associated with CVD risk and thrombosis (45,46). Individuals with prediabetes have higher CRP and PAI-1 compared to individuals with normoglycemia (44). Endothelial dysfunction is associated with IFG and is likely driven by increased insulin resistance. IFG and endothelial dysfunction, measured by flow mediated dilation (FMD), are positively associated (47). Important to note, a majority of data on endothelial dysfunction and IFG are from animal studies and further research is necessary to understand the relationship in humans.

2.2.4 The Dawn Phenomenon

The pathophysiology of prediabetes is a combination of altered endogenous glucose production and glucose disposal resulting from insulin resistance, reduced insulin sensitivity, and

pancreatic β-cell dysfunction (2). Insulin resistance is defined by impaired capacity of blood insulin concentrations to clear circulating glucose to maintain normal blood glucose concentrations and is driven by genetic and environmental factors (sedentary lifestyle, increased adiposity, and cigarette smoking) (48). Increased adiposity in the liver and muscular tissue impairs insulin signaling resulting in reduced glucose disposal, followed by increased insulin production from the pancreas to compensate for increased circulating glucose (49). Additionally, there is dysregulation of free fatty acid uptake, flux, and oxidation leading to reduced glycolysis and increased gluconeogenesis. Peripheral and hepatic tissues have reduced glucose uptake resulting from impaired insulin sensitivity. Lastly, pancreatic β-cells have a reduced ability to respond to increases in blood glucose concentrations.

Overnight blood glucose and plasma insulin concentrations remain constant with a slight increase in insulin between 3 to 7 am to blunt hepatic glucose production in adults with normoglycemia (50,51). Adults with prediabetes may have night-to-morning hyperglycemia or the dawn phenomenon. The exact mechanism is unknown but in adults with type 1 and T2DM it is likely driven by nighttime growth-hormone secretion and increased hepatic glucose production without compensatory insulin secretion (52). The prevalence of the dawn phenomenon in patients with prediabetes is unknown but for diabetes (type 1 and T2DM) is approximately 55% (53).

Adults with T2DM experience continued deterioration of glucose homeostasis if blood glucose is not controlled properly with lifestyle modifications and pharmacotherapies (54). HbA1c, a measure of long-term glucose control, continues to rise from the time of diabetes diagnosis, regardless of treatment (55). Individuals with greater HbA1c (>8%) have greater nocturnal glucose concentrations compared to those with lower HbA1c (<7.9%) and have significantly higher morning fasting glucose concentrations (dawn phenomenon) (55). Further research is necessary to determine the prevalence of the dawn phenomenon in adults with

prediabetes and whether this is a potential mechanism explaining elevated morning fasting glucose in adults with prediabetes.

2.2.5 Diet Quality

The diet quality of most US adults is poor based on the Healthy Eating Index-2015 (HEI-2015) score of 59 out of 100 (8). The HEI-2015 ranges from 0 to 100 and measures the adherence to the dietary recommendations from the United States Department of Agriculture (USDA) 2015-2020 Dietary Guidelines for Americans (56) . The HEI-2015 score is comprised of nine adequacy (greater intake equates to higher score) and four moderation (lower intake equates to higher score) components. The adequacy components are whole fruits, total fruits, green vegetables and beans, total vegetables, whole grains, dairy, seafood and plant proteins, total protein foods, and fatty acid ratio; and the moderation components are saturated fats, refined grains, sodium, and added sugar. The average HEI-2015 score for the US population ages 2 and older is 59 while the score for adults ages 19-30 is 56, ages 31-57 is 59, and ages 60 or greater is 63 (8).

Poor diet quality is the principal risk factor for mortality and in 2017 (57), 11 million global deaths were associated with dietary risk factors (low fruit and whole grain intake and high sodium intake) (58). Observational studies consistently demonstrate that higher diet quality is associated with lower risk of CVD, T2DM, and prediabetes (6,59–61). A study including 59,388 postmenopausal women followed for a median of 18.2 years, estimated that women with the highest diet quality (HEI-2015: 76.1-96.3) had an 18% lower risk [hazard ratio (HR) = 0.82; 95% confidence interval (CI): 0.76, 0.87] of all-cause mortality compared to women with the lowest diet quality (HEI-2015: 20.5-58.5) (62). Shan et al. assessed the association between diet quality and CVD in three prospective cohorts (n = 209,133) followed for 26-32 years and reported

significantly lower risk of CVD (HR = 0.83; 95% CI: 0.79, 0.86) when comparing the highest (HEI-2015: 75.5 ± 3.3) to lowest (HEI-2015: 50.8 ± 4.8) diet quality quintiles (63).

Increased diet quality is also associated with reduced risk of T2DM in adults. An analysis of 124,607 adults followed for more than 20 years demonstrated that a > 10% increase in diet quality [measured by the alternative healthy eating index-2010 (AHEI-2010)] over a 4-year period significantly reduced T2DM risk (HR = 0.84; 95% CI: 0.78, 0.90) (6). The evidence supporting the relationship between diet quality and prediabetes is limited. However, a recently published study used data from the China Health and Nutrition Survey to assess diet quality (Chinese Diet Balance Index-16) and its association with prediabetes in 7,693 adults with and without prediabetes (64). Individuals with unfavorable diet quality (high intake of salt and cereals and low intake of fish and vegetables) had a 45% increased risk [odds ratio (OR) = 1.45; 95% CI: 1.29, 1.63] of having prediabetes. Overall, increased diet quality is associated with reduced risk of chronic disease, but further research is necessary to characterize the relationship between diet quality and prediabetes.

Meal timing is an important dietary factor due to shifts in snacking over the last 40-years where a larger proportion of daily energy is being consumed as nighttime snacks in both men and women (11). Based on the 2013-2016 NHANES, the average number of eating occasions for adults ≥ 18 years was 5.7 and 89.6% of American's report four or more eating occasions per/day (65). Snacking represents 27% of all eating occasions and each snack is approximately 236 calories (65). Moreover, the average number of snacks consumed by US adults is 1.56/day equating to 368 calories/day from snacks (65). NHANES data from 22,305 men and 25,305 women collected between 1971 and 2010 reveal that 61-71% of men and women, who reported consuming dinner, reported having a snack after dinner (11). Additionally, adults who reported after dinner snacking consumed 15-16% of their daily energy needs from after dinner snacks (11). Lastly, nighttime snacks are often energy dense and nutrient poor foods like alcoholic and sugar-

sweetened beverages, savory snacks (e.g., chips), and candies (66). Thus, nighttime snacking is a contributor to poor diet quality and potentially intervening at this eating occasion may assist in improving diet quality.

2.2.6 The Gut Microbiota

The human gut microbiota is comprised of approximately 100 trillion (10^{14}) microorganisms consisting of viruses, bacteria, fungi, and protozoa (67,68). The composition of the gut microbiota is closely related to human health, largely reflective of overall diet quality, and can be modulated by small dietary changes (67,69). There is large inter-individual variability in the microorganisms present in the gut microbiota. However, an intestinal microbiota with sufficient richness and diversity of bacterial species and production of metabolites such as short-chain fatty acids (SCFA) is indicative of a healthy gut (70).

The bacteria present in the gut are necessary for the fermentation and degradation of non-digestible, or not fully digested, nutrients (e.g., fiber). Non-digestible fibers are degraded in the large intestine and generate SCFA. SCFA are carboxylic acids containing aliphatic tails with less than six carbons. Acetate, propionate, and butyrate are the most commonly produced SCFA from fiber degradation. SCFA are used as an energy source for colonocytes (primarily butyrate), are beneficial for epithelial barrier function, support epithelial cell proliferation, and have anti-inflammatory properties for the intestinal mucosa (71). Bacteria from the phyla Firmicutes mainly produce butyrate, while Bacteroidetes mainly produces propionate and acetate (71). More specifically, *Faecalibacterium prausnitzii, Clostridium leptum, Eubacterium rectale,* and *Roseburia spp* are the main butyrate producing bacteria, while *Bifidobacterium* and *Akkermansia muciniphilia* are the main acetate and propionate producing bacteria. SCFA are also important for

lowering the pH of the gut and are positively associated with glucose homeostasis, obesity, and CVD risk (72).

Differences in gut microbiota composition are apparent in adults with obesity compared to those without obesity. A recent analysis of 16S rRNA sequencing data from 4 cohorts of adults demonstrated lower alpha-diversity, measured by phylogenetic and Shannon diversity, in adults with higher BMI (73). The relationship between gut microbiota composition and obesity may be mediated by SCFA. SCFA regulate satiety and reduce appetite through the gut-brain axis evidenced by murine models demonstrating reduced energy intake and enhanced fat oxidation with butyrate intake (74). Moreover, free-fatty acid receptor-2 knockout mice fed an inulin supplemented diet had significant increases in peptide YY cell density and reduced energy intake. Similar to obesity, there is evidence of a relationship between gut dysbiosis and CVD (75). Observational studies suggest there is reduced richness and abundance in the gut bacterial community in subjects with hypertension, dyslipidemia, and insulin resistance compared to individuals free of disease (76,77).

2.2.6.1 The Gut Microbiota and Glycemic Control

Differences in microbiota composition in adults have been observed by glycemic status (normoglycemia, IFG, and diabetes). Individuals with elevated fasting glucose have significantly lower abundance of *Clostridium* and increased abundance of *Ruminocccus* compared to individuals with normoglycemia (78). *Clostridium* is an important butyrate producing bacteria and butyrate has been linked with insulin sensitivity and secretion (79). A recent population-based study evaluated the gut microbiota and dietary intake of 1726 Swedish adults without diabetes (80). The authors identified two dietary patterns (‘Health-conscious’ and ‘Sugar and High-Fat Dairy’) and found the ‘Health-conscious’ pattern was associated with lower prediabetes

prevalence and greater abundance of *Roseburia*. An analysis of two prospective cohorts (1996-2011) including 1,920 Chinese men and women assessed diet quality (Healthy Diet Score ranging from 8-40) and the gut microbiota (16S rRNA sequencing) (59). The highest quintile of diet quality was associated with greater alpha-diversity compared to the lowest group ($P = 0.03$) with greater relative abundance of *Bifidobacterium* (0.36% vs. 0.19%, $P = 0.0007$) and *Roseburia* (0.76% vs. 0.33%, $P = 0.0006$). Taken together, the gut microbiota can be modulated by diet quality and as such, is associated with glycemic control.

The composition of the microbiota is not only associated with glycemic status, T2DM risk, and diet quality, but may be directly related to postprandial glycemic response. In recent years, clinical trials have demonstrated that microbiota composition is predictive of postprandial glycemic response. The seminal trial was in a cohort of 800 healthy individuals followed for 7-days while wearing continuous glucose monitors (CGM) and consuming standardized meals (81). Additionally, fecal samples were collected at baseline and endpoint to assess the microbiota composition (16S rRNA sequencing). In this study, high inter-individual variability in postprandial glycemic responses, measured with CGM, to standardized meals consumed throughout the 7-day period were observed. Using this data [microbiota, meal content, blood parameters, and questionnaire (medical, lifestyle, and food frequency)] machine learning algorithm was developed that outperformed the standardized carbohydrate-counting method to predict postprandial glycemic response (r=0.68 vs. r=0.38, respectively). Korem et al. conducted a randomized, crossover, trial in 20 healthy subjects testing the effects of two types of bread [145g whole-grain sourdough or 110g white bread each morning (50g carbohydrates)] for one week with a two-week washout period (82). There were no significant differences in glycemic response or microbiota composition between the bread types. However, there was significant inter-individual variation in glycemic response to the different breads and microbiota data were used to independently predict individual glycemic response to the different breads [area under the

receiver operating characteristic curve (ROC AUC) = 0.83]. Similarly, Berry at al. conducted a clinical trial in 1,002 adult twins from the UK and a validation cohort of 100 adults from the US (83). The trial consisted of an in-lab postprandial challenge for 1-day followed by an at-home phase for 13-days with standardized meals. Researchers collected information on microbiota (16S rRNA sequencing), glucose (continuous glucose monitor), physical activity, sleep, triglycerides, C-peptide, genetics, and vascular health. There was large inter-individual variability to the standardized test meals for postprandial glucose and triglycerides. Individual microbiota characteristics influenced postprandial glucose (6.0% variance) but had a lesser impact than macronutrient composition (15.4% variance). A machine-learning model predicted postprandial glycemic response based on meal composition, habitual diet, meal context, anthropometry, genetics, microbiota, clinical, and biochemical parameters in the twins (r=0.77) and validation (r=0.75) cohort. These trials demonstrate that microbiota composition is associated with postprandial glycemic control and further assessment of microbiome functionality is necessary to understand how specific microbes are involved in glycemic control.

The gut microbiota is a novel therapeutic target for CVD and T2DM and research techniques to characterize the composition and functionality are rapidly evolving. Most studies evaluating the microbiota have employed 16S rRNA sequencing, like the studies discussed in this section, to characterize the microbes present. Specifically, this method is used to measure the gut microbiota composition and diversity (84,85). These studies have advanced the human microbiota field and provided important insight about the relationship with diet and disease. Nevertheless, this methodology does not provide information on the functionality of the gut bacteria present. A novel method, metatranscriptomics, allows for further evaluation of gene expression activity of the microbes present in the gut microbiome (84). This method has not been used to evaluate the effects of diet on the gut microbiome to date. Thus, employing 16S rRNA sequencing and metatranscriptomics in clinical trials evaluating dietary interventions for glycemic control will

provide novel information about changes in gene expression, functionality, and composition of the gut microbiota.

2.3 Treatment of Impaired Fasting Glucose

2.3.1 Lifestyle Recommendations

Nutrition therapy is a cornerstone for the prevention and treatment of prediabetes and T2DM. Although pharmacotherapy (e.g., metformin) is effective for preventing the onset of T2DM in adults with elevated fasting glucose (86), there is consistent evidence supporting the use of lifestyle therapies (nutrition, physical activity, and behavior change) for reducing fasting glucose and reducing incidence of T2DM in adults without diabetes (5). A landmark study, the Diabetes Prevention Program (DPP), evaluated three therapies (placebo, metformin, and intensive lifestyle therapy) in 3,234 adults with elevated fasting glucose (5,87,88). The placebo group received standard lifestyle information (instructed to increase physical activity and lose weight, follow the Food Guide Pyramid 14 and National Cholesterol Education Program Step 1 Diet) while the metformin group received the same standard lifestyle information + metformin (850 mg/day for one month followed by dose escalation to 2 x 850 mg/day). The intensive lifestyle therapy group received a 16-lesson curriculum about behavior modification, diet, and exercise with the goal of losing 7% of initial body weight (and maintaining the weight loss), increase exercise to 150 min/week, and following a healthy low-fat, low-calorie diet. After an average 2.8 years of follow-up there were 4.8 cases per 100-person years in the lifestyle group, 7.8 cases in the metformin group, and 11 cases in the placebo group (5). Lifestyle was more effective than both placebo and metformin at reducing incidence of diabetes (-58%; 95% CI: -48%, -56%) (5).

These findings were used to generate evidence-based nutrition recommendations for prediabetes that were issued by the ADA (7).

A consensus report about nutritional therapies for adults with prediabetes and diabetes was published by Evert and colleagues in 2019 (7). This was the first report from the ADA providing specific recommendations for individuals with prediabetes. The overarching recommendations for adults with prediabetes and overweight/obesity is referral to intensive lifestyle intervention programs. The programs should have an integrated medical nutrition therapy (MNT) component that is similar to the DPP or individualized to align with the patient's needs. The MNT program should be provided by a registered dietitian nutritionist (RDN) with the focus being a 7-10% reduction of initial weight (and maintaining the weight loss), participating in ≥ 150 min/week moderate-intensity physical activity, and improving eating habits. Improving eating habits includes altering the eating pattern and consuming whole foods instead of processed foods, eating more non-starchy vegetables, and reducing refined grains and added sugars. Low-fat, low-carbohydrate, and Mediterranean style eating patterns had the most robust data supporting prediabetes and T2DM prevention with the most common aspects of these patterns placing an emphasis on consumption of fruits, vegetables, nuts/seeds, lean protein, while avoiding refined grains and sugar sweetened beverages.

2.3.2 Treatment Recommendations for the Dawn Phenomenon

As stated in the above section, the dawn phenomenon affects more than 50% of adults with type 1 and T2DM and the prevalence in those with prediabetes is unknown. However, individuals without elevated fasting glucose may be impacted by the dawn phenomenon due to the presence of impaired glucose response and insulin resistance. Although the mechanisms causing the dawn phenomenon are not completely understood, researchers have evaluated many

therapies to reduce the nighttime hyperglycemic occurrences. Monnier et al. assessed the effect of 3 interventions on the dawn phenomenon in 248 adults with T2DM not currently prescribed insulin pharmacotherapy (89). The individuals were randomized into the following groups: diet (weight maintenance diet with 50% of energy from carbohydrates), insulin sensitizers, and insulin secretagogues with/without insulin sensitizers. The participants' glucose was assessed using CGM. None of the treatments had a significant effect on the dawn phenomenon. These results demonstrate the inability of current recommendations to ameliorate the effects of the dawn phenomenon in adults with T2DM. Although lifestyle modifications have not been effective in clinical trials, they are still recommended to lessen the impacts of the dawn phenomenon. The current lifestyle recommendations are to increase the protein to carbohydrate content of evening meals and engage in evening exercise (90).

Two short-term nighttime snacking trials have evaluated dietary interventions in adults with T2DM (91,92). The first study was a randomized controlled crossover trial that evaluated the effects of an Extend bar [30 g carbohydrate (5 g uncooked starch), 3 g protein, and 3 g protein) consumed before bedtime compared to a control snack bar (30 g carbohydrates, 3 g protein, and 3 g fat) in 28 adults (mean HbA1c: 8.21 ± 1.28%) for 3-days. Morning fasting glucose was significantly lower in the Extend bar group compared to control (114.2 ± 15.8 mg/dL vs. 158.49 ± 30.3 mg/dL) (92). Abbie et al. conducted a similar trial in adults with T2DM and tested the effects of three isocaloric nighttime snacks for 3-days using a randomized crossover design: two eggs (1.13 g carbohydrate, 10.67 g fat, and 12.38 g protein), two containers of low-fat yogurt (24.38 g carbohydrate, 0.33 g fat, and 12.38 g protein), and an isocaloric no snack condition. Fasting glucose was significantly lower following the egg condition compared to yogurt (129.6 ± 3.6 mg/dL vs. 136.8 ± 3.6 mg/dL) but not the no snack condition (91). These trials suggest nighttime snacks with less simple carbohydrates and a greater protein and fat content may improve morning fasting glucose in adults with T2DM. Currently, there is limited evidence for

lifestyle recommendations for the dawn phenomenon and no trials have evaluated the effects of nighttime snacks in adults with prediabetes. Therefore, further clinical trials are necessary to evaluate lifestyle therapies and nighttime snacking in adults with prediabetes.

2.4 Peanuts and Tree Nuts and CVD Risk Factors

2.4.1 Epidemiological Evidence for Tree Nuts, Peanuts, and CVD

Nuts are recommended in healthy, food-based eating patterns in the United States Department of Agriculture (USDA) Dietary Guidelines for American's 2020-2025 (8). Adults consuming 2,000 calories per day are recommended to consume 5-ounce equivalents/week (8). Tree nuts are nutrient dense foods low in saturated fatty acids (SFA) and rich in mono- and poly-unsaturated fatty acids (MUFA and PUFA) (**Table 2-1**). Peanuts, although considered a legume, have a similar macro- and micro-nutrient profile to tree nuts (**Table 2.1**). The USDA Dietary Guidelines for Americans includes peanuts in the nuts, seeds, and soy category. Additionally, peanuts are the most commonly consumed nut in the United States (18).

Table **2-1:** Nutrient Composition of Peanuts and Tree Nuts

Nut	Energy (kcal)	Fat, g	SFA, g	MUFA, g	PUFA, g	Protein, g	Carbs, g	Fiber, g
Peanut	166	14.1	2.2	7.4	2.8	6.9	6.0	2.4
Walnut	185	18.5	1.7	2.5	13.4	4.3	3.9	1.9
Pistachio	162	13	1.6	7.0	3.8	6.0	7.8	2.9
Cashew	163	13.1	2.6	7.7	2.2	4.3	9.2	0.9
Almond	170	14.9	1.2	9.4	3.7	5.9	6.0	3.1
Pecan	201	21.1	1.8	12.5	5.8	2.7	3.8	2.7

Per 28.35g (1-oz) serving. Data are from https://fdc.nal.usda.gov/index.html

Observational studies evaluating nut intake have demonstrated improvements in CVD, all-cause mortality, and T2DM risk in adults (17,19,93). The first longitudinal cohort to evaluate nut consumption and coronary heart disease (CHD) risk was the Adventist Health Study. This cohort included 31,208 healthy non-Hispanic White US adults who were followed for six years and nut intake was assessed with a 65-item food frequency questionnaire (FFQ). Adults consuming nuts ≥ 5 times/week, compared to adults consuming nuts < 1 time/week, had 48% lower risk for nonfatal myocardial infarction [relative risk (RR) = 0.52; 95% confidence interval (CI): 0.30, 0.87], 38% lower risk for fatal CHD (RR = 0.62; 95% CI: 0.44, 0.90), and 33% lower risk for fatal CHD determined by death certificate (RR = 0.67; 95% CI: 0.51, 0.88) (93). Importantly, the type of nut and amount consumed were not collected. A more recent observational study of three large cohorts (Health Professionals Follow-Up Study [HPFS], Nurses' Health Study [NHS], and Nurses' Health Study II [NHSII]) found similar associations between nuts and CVD risk (17). This study included 41,526 men from the HPFS followed for 26 years, 92,946 women from the NHSII followed for 22 years, and 76,364 women from the NHS followed for 32 years. The high nut consumption group (28 g ≥ 5 times/week), compared to the low nut consumption group (never or almost never), had lower pooled multivariate hazard ratio for CVD [hazard ratio (HR) = 0.86; 95% CI: 0.79, 0.93] and CHD (HR = 0.80; 95% CI: 0.72, 0.89). Additionally, consuming peanuts ≥ 2 times/week was associated with 15% lower risk for CHD (HR = 0.85; 95% CI: 0.79, 0.92) and 13% lower risk for CVD (HR = 0.87; 95% CI: 0.82, 0.93) when compared to the reference group (never, or almost, never consuming peanuts). Bao et al. conducted a similar study assessing the relationship between nut consumption and total and all cause-specific mortality with 42,498 men from the HPFS and 76,464 women from the NHS (19). The pooled multivariate hazard ratio for all-cause mortality ranged from 0.89 (95% CI: 0.86,

0.93) in those consuming nuts once per week to 0.83 (95% CI: 0.78, 0.88) in those consuming nuts ≥ 5 times/week, when compared to the reference group (never or rarely consuming nuts).

The first observational study evaluating the association between nut consumption and T2DM risk was conducted by Jiang et al. with the NHS cohort (12). The analyses included 83,818 women followed from 1980 to 1996. Women consuming 28 grams of nuts 1-4 times/week and ≥ 5 times/week had a 16 and 27% lower risk (RR = 0.84; 95% CI: 0.76, 0.93 and RR = 0.73; 95% CI: 0.60, 0.89) for developing T2DM when compared to the reference group (never/almost never consuming nuts). Consumption of peanut butter ≥ 5 times/week (~140 g peanuts/week) significantly reduced risk (RR: 0.79; 95% CI: 0.68, 0.91) compared to women who never/almost never consumed peanut butter. A recent systematic review and meta-analysis of observational studies assessing nut consumption and T2DM risk demonstrated no association between tree nut, peanut, and total nut consumption and T2DM risk (94). Becrra-Tomás et al. assessed 1004 studies and found 5 prospective cohort and 3 cross-sectional studies that met their criteria. The highest (>2 times/week) compared to lowest (never, almost never) nut consumption categories were not significantly different for T2DM risk [odds ratio (OR) = 0.91; 95% CI: 0.83, 1.01], but peanut butter consumption had an inverse association with T2DM incidence (RR = 0.87; 0.77, 0.98). Both cross-sectional and prospective cohort studies assessing walnuts and T2DM incidence demonstrated significantly lower risk (24-53%) in the highest (≥ 2 servings/week) vs. lowest (never, almost never) walnut consumption categories. However, the authors determined there were too few studies to conduct a meta-analysis on walnut studies individually. Collectively, this epidemiological evidence suggests tree nuts, peanuts, and peanut butter are associated with reduced risk for mortality, CVD, and T2DM.

2.4.2 Experimental Evidence for Tree Nuts, Peanuts, and Glycemic Control

There is consistent evidence suggesting that diets including peanuts and tree nuts benefit postprandial glucose response and longer-term glycemic control (13,20,21,95). A randomized, crossover trial in 11 healthy adults measured 30- and 60-minute postprandial glucose concentrations following two test meals: breakfast (bagel with butter and orange juice) and lunch (teriyaki chicken with rice, butter, and vegetables) (21). The test meals were consumed with three experimental conditions [1: a control condition; 2: 25 g apple cider vinegar pre-meal beverage (control was water and 1 tsp. saccharine); and 3: 25 g peanut butter substituted for butter on bagel, and 25g peanuts substituted for butter in chicken dish]. Peanut butter compared to butter for the breakfast meal reduced 60-minute postprandial glucose by 56% ($P < 0.05$) and non-significantly reduced 60-minute postprandial glucose by 50% ($P > 0.05$) when peanuts replaced butter in the lunch meal. A more recent trial explored the postprandial effects of adding 42.5 g of peanuts or peanut butter to a high carbohydrate breakfast (cream of wheat and orange juice) compared to a control group (cream of wheat and orange juice) in 15 women with overweight and obesity (mean BMI = 32.36 kg/m^2) (96). Additionally, postprandial glucose was measured after a standardized lunch (white bread and strawberry jam). The peanut butter meal significantly lowered the second meal postprandial glucose response by 18.7% [incremental area under the curve (IUAC) $P = 0.03$] and the peanut IUAC was 14.4% lower than control ($P = 0.48$). It is important to note, however, the breakfast test meals were not calorically matched, with the peanut and peanut butter meal being higher in calories compared to control (~590 vs. ~330 kcal).

Longer-term clinical trials have consistently demonstrated beneficial effects of tree nut and peanut consumption on glycemic control (13,95,97). However, most of the trials are in populations with diabetes and have varying study designs (nut dosage, length of trial, timing of nut consumption, composition of the diet/study meal). Viguiliouk et al. conducted a systematic

review and meta-analysis of randomized controlled trials exploring the effects of tree nuts on glycemic control in adults with diabetes (13). The authors evaluated 1491 trials and identified 12 trials (n = 450) that were ≥ 3-weeks, had a control group, examined tree nuts, and measured HbA1c, homeostatic model assessment for insulin resistance (HOMA-IR), fasting insulin, or fasting glucose. The nut intake ranged from 28-58 g/day with a median of ~56 g/day. Diets with tree nuts significantly lowered fasting glucose (-2.7 mg/dL; 95% CI: -4.9, -0.4 mg/dL) and HbA1c (-0.7%; 95% CI: -0.1, -0.03). Tindall et al. published a more recent systematic review and meta-analysis of randomized controlled trials evaluating the effects of peanuts and tree nut consumption in adults with and without diabetes (95). 1063 articles were assessed for eligibility and 40 (n = 2832) were included in the analyses. The included trials ranged from 1-12 months with a median nut intake of 52 g/day (range: 20-113 g/day). Tree and peanut consumption had favorable effects on HOMA-IR [weighted mean difference (WMD) = -0.23; 95% CI: -0.40, -0.06) and fasting insulin (WMD = -0.40 μIU/mL; 95% CI: -0.73, -0.07 μIU/mL). Collectively, these trials demonstrate the shorter-term effects of peanuts on glycemic control in healthy individuals and populations with obesity and longer-term effects of tree nut and peanut consumption in populations with/without diabetes. Importantly, further clinical trials are necessary to explore how longer-term peanut consumption impacts glycemic control in adults with prediabetes.

2.4.3 Experimental Evidence for Tree Nuts, Peanuts and Lipids/Lipoproteins

An evidence-based dietary recommendation for individuals with dyslipidemia or hypercholesterolemia is to replace SFA with MUFA and PUFA (8). Peanuts and tree nuts are an ideal food to replace SFA rich foods due to their high MUFA/PUFA and low SFA content. Short-term consumption of peanuts lowers postprandial lipid response in men with overweight and

obesity (98). A randomized, crossover, trial in 15 men (mean BMI: 31.4 kg/m^2) tested two isocaloric and macronutrient matched high-fat beverages with and without 85 g of ground peanuts. The Peanut condition had significantly lower 120- and 240-minute post meal triglycerides compared to control (188.9 vs. 197.5 and 189.9 vs. 197.3 mg/dL, respectively). A longer-term randomized, parallel trial in 151 healthy individuals evaluated the effects of peanuts (42 g/day) for 12-weeks and found no significant differences for serum lipids (96). However, when subjects were stratified by total cholesterol concentrations (>200 mg/dL [n = 24] and ≤ 200 mg/dL [n = 127]) there was a significant improvement in the hypercholesterolemic compared to normal cholesterol group (-12.2 ± 8.5 mg/dL vs. 5.5 ± 2.1 mg/dL, $P < 0.01$). Importantly, this trial did not include a control group. Many systematic reviews and meta-analyses have evaluated the effect of tree nuts on lipids/lipoproteins (16,99–101). Recently, Del-Gobbo et al. published a systematic review and meta-analysis of 61 randomized controlled trials including 2,582 participants and evaluated the effects of tree nut consumption on lipids/lipoproteins (16). Of the 61 included trials, more than 20 trials included individuals with metabolic syndrome, obesity, hypercholesterolemia, or diabetes. The median intervention length was 4-weeks (range: 3-26-weeks) and median nut intake was 56 g/d (range: 5-100 g/d). The authors calculated weighted mean differences for lipids for one serving/day (28.4 g). For each 28.4 g serving/d of tree nuts there was a significant reduction in cholesterol (-4.7 mg/dL; 95% CI: -5.3, -4.0 mg/dL), LDL-C (-4.8 mg/dL; 95% CI: -5.5, -4.2 mg/dL), and triglycerides (-2.2 mg/dL; 95% CI: -3.8, -0.5). Peanuts have pronounced effects on the postprandial lipid response and long-term consumption of tree nut enriched diets have significant lipid lowering effects when compared to control diets. However, many of the trials evaluating peanuts and tree nuts have altered other aspects of the diet and focused on populations with dyslipidemia. Therefore, a clinical trial testing the individual effect of longer-term peanut intake on lipids/lipoproteins in adults without dyslipidemia is needed.

2.4.4 Experimental Evidence for Tree Nuts, Peanuts and Vascular Measures

Recommended dietary patterns (i.e., Mediterranean and Dietary Approaches to Stop Hypertension [DASH] diets) including peanuts and tree nuts are beneficial for individuals with elevated BP (>120/80 mmHg). Jones et al. conducted a randomized, parallel trial (without a control group) testing the effect of 42.5 g of peanuts for 12-weeks in 151 healthy individuals and found that peanuts did not significantly improve BP (96). However, when participants were stratified by baseline BP (>120/80 mm Hg [n = 27] and ≤120/80 mm Hg [n = 124]) the elevated BP group had significant improvements in DBP compared to the normal BP group (-5.0 ± 1.7 mm Hg vs. 0.7 ± 0.6 mm Hg, $P < 0.01$). Only one systematic review and meta-analysis included randomized controlled trials assessing peanuts and tree nuts while the others included only tree nuts (15,16,102). Mohammadifard et al. included 21 trials (n = 1652) that assessed peanut, tree nut, and soy nut (range: 30-108 g) consumption for ≥ 2-weeks and found significant improvements for SBP (-1.29 mm Hg; 95% CI: -2.35, -0.22 mm Hg) in adults without T2DM (15). Recently, a systematic review evaluating only clinical trials testing almonds demonstrated improvements in blood pressure (102). Li et al. conducted a systematic review and dose-response meta-analysis of 15 randomized controlled trials (n = 853) evaluating almond consumption in primarily diseased populations (T2DM, coronary artery disease, hyperlipidemia, and overweight/obesity. The authors found a small, yet significant, reduction in brachial SBP (WMD = -0.90 mm Hg; 95% CI -1.74, -0.06 mm Hg).

However, a systematic review and meta-analysis of randomized controlled trials (details explained above) testing tree nuts did not find improvements in SBP [weighted mean difference (WMD) = 0.3 mm Hg; 95% CI: -0.8, 1.4] or DBP (WMD = 0.4; 95% CI: -0.8, 1.6) (16). These trials and meta-analyses demonstrate conflicting effects of peanut and tree nut consumption on brachial BP.

Trials evaluating the effect of peanut consumption on central blood pressure and arterial stiffness are limited. A recently published randomized controlled, crossover trial evaluated the effects of high-oleic peanuts (15% of energy) for 12-weeks in 61 adults and demonstrated improvements in arterial stiffness, measured by small artery elasticity, with a 10% increase ($P = 0.008$) following peanut consumption (103). However, there were no improvements in brachial blood pressure or large artery elasticity. Tindall et al. completed a randomized controlled, crossover, trial in adults at increased risk for CVD and examined the effects of diets (6-weeks) enriched with walnuts, a walnut fatty acid matched diet, and a diet where oleic acid replaced alpha-linolenic acid (104). The authors found that central DBP was significantly different for the walnut compared to oleic acid diet (-1.78 ± 1.0 mm Hg vs. 0.15 ± 0.7 mm Hg) and no differences in arterial stiffness were observed. Interestingly, when participants were stratified by BMI (overweight, obese, and morbidly obese) there was a significant reduction from baseline in central SBP for the walnut diet (-6 ± 2 mm Hg) and walnut fatty acid matched diet (-5 ± 2 mm Hg). A review of experimental studies exploring the impact of tree nut and peanut consumption on vascular health (PWV, artery elasticity, stiffness index, total peripheral resistance, and augmentation index) identified 16 studies with varying designs (short- and long-term controlled, parallel, and uncontrolled pre/post studies) (105). The authors found varying results with some trials reporting no changes, while some reported small improvements in different measures of vascular health and concluded that further research is necessary. Peanut and tree nut intake may improve vascular health in adults with elevated blood pressure and increased BMI, but further research is necessary to understand this relationship.

2.4.5 Experimental Evidence for Tree Nuts, Peanuts and Gut Microbiota

As noted, diet significantly impacts the composition and functionality of the gut microbiota. Observational data assessing the relationship between peanuts and tree nuts and the gut microbiome are limited but several clinical trials have explored the effects of nuts on the gut microbiota. Recent evidence suggests tree nuts, walnuts and almonds in particular, have beneficial effects on the gut microbiota (e.g., increased abundance of short-chain fatty acid producing bacteria and increased diversity) but the results are inconsistent and there are limited data on peanuts (14,106). Two similar systematic reviews were published in 2020 with the same randomized controlled trials included except for one (14,106). Both of these systematic reviews found significant increases in the abundance of *Lachnospira*, *Clostridium*, *Dialister*, and *Roseburia* following consumption of tree nuts (range: 33-99 g/day) for 3 days to 24 weeks.

Although these systematic reviews have established the beneficial impact of tree nuts on the composition of the gut microbiota, there are no studies assessing peanut intake in populations with prediabetes. These trials provide convincing evidence for a beneficial shift in the abundance of SCFA producing bacteria, but these data do not assess the functionality. Peanuts, because of their similar macro- and micronutrient profile to tree nuts, may improve the abundance of SCFA producing bacteria. Investigation using both 16S rRNA sequencing and metatranscriptomics is necessary to evaluate alterations in microbiota composition and gene expression to determine if there are any shifts in the abundance/diversity of the bacteria and assess functional changes associated with peanut intake.

2.5 Rationale for Current Research

Prediabetes, or IFG, impacts more than one third of US adults and increases risk for developing T2DM and CVD (1). However, dietary interventions for adults with prediabetes are not well-established. The ADA recently published their first report on nutritional recommendations for adults with prediabetes (7). These recommendations focus on dietary patterns to reduce incidence and manage T2DM with a common component of many of the patterns being nut intake. Tree nuts have well-established glucoregulatory benefits in adults with T2DM, but the research is limited in populations with prediabetes and the longer-term effect of peanuts on glycemic control are unknown.

Adults with T2DM and prediabetes often have elevated morning fasting glucose (dawn phenomenon). Currently, there are no dietary recommendations from authoritative organizations for controlling morning hyperglycemia. However, some evidence suggests that higher-fat and higher-protein snacks, a healthier alternative to the commonly consumed energy dense and nutrient poor snack (e.g., chips), in the evening may improve morning fasting glucose in adults with T2DM, but the effects in adults with prediabetes are unknown.

The gut microbiota is associated with glycemic control, modulated by dietary changes, and recent trials have established beneficial effects of tree nuts on the gut microbiota. Until recently, methods for assessing gut microbiota provided compositional data but new methodologies assess functionality of the present microbes. There is limited evidence for the effect of peanuts on the gut microbiota. Additionally, no clinical nutrition trials have employed metatranscriptomics to evaluate changes in gene expression to assess functional changes of the gut microbiome.

Peanuts, a legume, have similar macro- and micronutrient profiles compared to tree nuts and are the most commonly consumed nut in the United States. However, nut research has

focused primarily on tree nuts and the effects on glycemic control, cardiovascular health, and the microbiota are largely unknown. This dissertation presents findings from a randomized, controlled crossover trial that examined the glycemic, vascular, lipid/lipoprotein, diet quality, and microbiota effects of one ounce of dry roasted, unsalted peanuts as a nighttime snack compared to a lower-fat higher-carbohydrate snack in adults with elevated fasting glucose in central Pennsylvania. The primary outcome for this study was fasting plasma glucose. Secondary outcomes included insulin, fructosamine, weight, lipids/lipoproteins, vascular health, arterial stiffness, diet intake/quality, and changes in the gut microbiota.

2.6 Hypotheses and Aims

Aim 1: To determine the effect of nighttime peanut consumption, in adult men and women with elevated fasting glucose, on fasting plasma glucose, insulin, fructosamine, weight, lipids/lipoproteins, blood pressure, arterial stiffness, dietary intake, and diet quality compared to an isocaloric lower-fat higher-carbohydrate control snack.

Hypothesis: Participants consuming peanuts for 6-weeks will have improvements in fasting plasma glucose, lipids/lipoproteins, insulin, fructosamine, blood pressure, arterial stiffness, the gut microbiome, dietary intake, and diet quality compared to an isocaloric lower-fat higher-carbohydrate control snack.

Aim 2: To determine the effect of nighttime peanut consumption (one ounce) for 6-weeks, in adult men and women with elevated fasting glucose, on microbiota composition and

diversity using 16S rRNA sequencing and identify compositional and functional changes using metatranscriptomics.

Hypothesis: Participants consuming peanuts for 6-weeks will have improvements in gut microbiota composition, and increased abundance of SCFA producing bacteria compared to an isocaloric lower-fat higher-carbohydrate control snack.

Hypothesis: Participants consuming peanuts for 6-weeks will have increased expression of genes associated with SCFA production.

Chapter 3

Peanuts as a Nighttime Snack do not Affect Fasting Glucose in Adults with Elevated Fasting Glucose Concentrations: A Randomized Crossover Trial (107)

Abstract

Background: The glycemic effects of peanuts are not well-studied and no trials have been conducted in adults with elevated fasting plasma glucose (FPG). Furthermore, intake of peanuts as a nighttime snack, an eating occasion affecting FPG, has not been examined.

Objective: The aim was to determine the effect of consuming 28 g/d of peanuts as a nighttime snack for 6-weeks on glycemic control and cardiovascular disease (CVD) risk factors, compared to an isocaloric lower-fat higher-carbohydrate (LFHC) snack (whole grain crackers and low-fat cheese), in adults with elevated FPG.

Methods: In a randomized crossover trial, 50 adults (FPG 100 ± 8 mg/dL) consumed dry roasted, unsalted, peanuts (164 kcal; 11% E carbohydrate, 17% E protein, and 73% E fat) or a LFHC (164 kcal; 53% E carbohydrate, 17% E protein, and 33% E fat) as a nightly snack (after dinner and before bedtime) for 6-wk with a 4-wk washout period. Primary (FPG) and secondary endpoints (Healthy Eating Index-2015 (HEI-2015), weight, insulin, fructosamine, lipids/lipoproteins, central and peripheral blood pressure, and pulse wave velocity) were evaluated at the beginning and end of each condition. Linear mixed models were used for data analysis.

Results: FPG was not different between peanuts vs. LFHC (endpoint mean difference -0.6 mg/dL; 95% CI -2.7, 1.6; $P = 0.67$). There were no between-condition effects for secondary cardiometabolic endpoints. The HEI-2015 score was not different between the conditions (3.6

points; $P = 0.19$), although seafood/plant protein (2.0 points; $P < 0.01$) and added sugar (0.8 points; $P = 0.04$) components were improved following peanut intake. The whole grain component was lower with peanuts vs. LFHC (-2.6 points; $P < 0.01$).

Conclusion: In adults with elevated FPG, peanuts as a nighttime snack (28 g/d) did not affect FPG vs. an isocaloric LFHC snack after 6-weeks.

3.1 Introduction

In 2018, approximately 88 million (34.5%) U.S. adults had prediabetes (1). For adults with prediabetes at age 45 years, the lifetime risk for progression to type 2 diabetes mellitus (T2DM) is 74% (95% CI 67.6 – 80.5%) (3). Individuals with prediabetes have a higher risk (HR: 1.18 [95% CI 1.08-1.26]) of developing cardiovascular disease (CVD) (108) and present with more CVD risk factors (hypertension, inflammation, dyslipidemia, and obesity) compared to individuals with normoglycemia (109). Intensive lifestyle therapies (i.e., weight loss, medical nutrition therapy, and physical activity) are recommended for all adults with prediabetes and T2DM, but compliance is often suboptimal. Simple strategies to improve fasting blood glucose and delay or prevent T2DM onset and CVD in individuals with prediabetes are needed.

Observational evidence suggests that habitual peanut and tree nut consumption (4 servings of 28.4 g/week) is associated with a significantly lower incidence of T2DM (RR: 0.87 [95% CI: 0.81, 0.94]), fatal ischemic heart disease (RR: 0.76 [95% CI: 0.69, 0.84]), and non-fatal ischemic heart disease (RR: 0.78 [95% CI: 0.67, 0.92]) (110). These findings are supported by randomized controlled trials showing improvements in glycemic outcomes with peanut and tree nut intake in healthy individuals and those with prediabetes or T2DM (13,95,111). Few studies have examined the effect of peanuts on glycemic outcomes (20,97,98,112,113), and no study has been conducted in individuals with prediabetes. In addition, limited research has examined whether nighttime nut intake affects glucose control.

The dawn phenomenon affects approximately 50% of individuals with type 1 and type 2 diabetes and is the primary cause of elevated fasting blood glucose, although the etiology is not well understood (90). The dawn phenomenon is characterized by an increase in blood glucose concentrations between 2 and 8 am, and is caused by dysregulated compensatory insulin secretion in response to hepatic glucose production at “dawn”. Dietary strategies (i.e., reducing

carbohydrate to protein ratio of nighttime snacks) are suggested to attenuate the Dawn Phenomenon, but limited empirical evidence is available. In two clinical trials, consumption of evening snacks containing primarily complex carbohydrates or a higher protein/fat to carbohydrate ratio for 3 nights improved fasting glucose in adults with T2DM (91,92). Investigation of how healthy evening snacks affect fasting glucose in individuals with prediabetes is warranted.

We conducted a randomized controlled trial in individuals with elevated fasting glucose to evaluate the effect of consuming 28 g/d of peanuts, as a nighttime snack, compared to an isocaloric lower-fat higher-carbohydrate snack (LFHC), on fasting plasma glucose and risk factors for CVD. We hypothesized that consuming peanuts as an evening snack, after dinner and before bedtime, would lower fasting plasma glucose compared to an isocaloric LFHC in adults with elevated fasting glucose.

3.2 Methods

3.2.1 Trial Design

A single-blind, 2-period, randomized crossover controlled clinical trial was conducted at the Pennsylvania State University between October 2018 to January 2020 (**Figure 3-1**). Eligible individuals were randomized immediately prior to baseline testing in a one-to-one ratio to two randomization sequences generated using a computer-generated scheme (randomization.com) by an investigator that was not involved in data collection. The randomization code was held by the Metabolic Kitchen Manager; the Study Coordinator who conducted screening, enrollment, and data collection was unaware of participants' randomization until data collection was completed. Participants were not blinded to the randomization because of the nature of the study. Participants

consumed the following evening snacks for 6-weeks: 1) 28 g/day of dry-roasted unsalted peanuts; 2) an isocaloric LFHC snack. Participants had a minimum 4-week break between the diet periods (median break 28 days; range 28-41 days). Outcomes were measured at baseline and the end of each diet period. The Institutional Review Board of the Pennsylvania State University (University Park, PA) approved the protocol. Written informed consent was obtained from all participants before screening for this study. This trial is registered at ClinicalTrials.gov identifier NCT03654651.

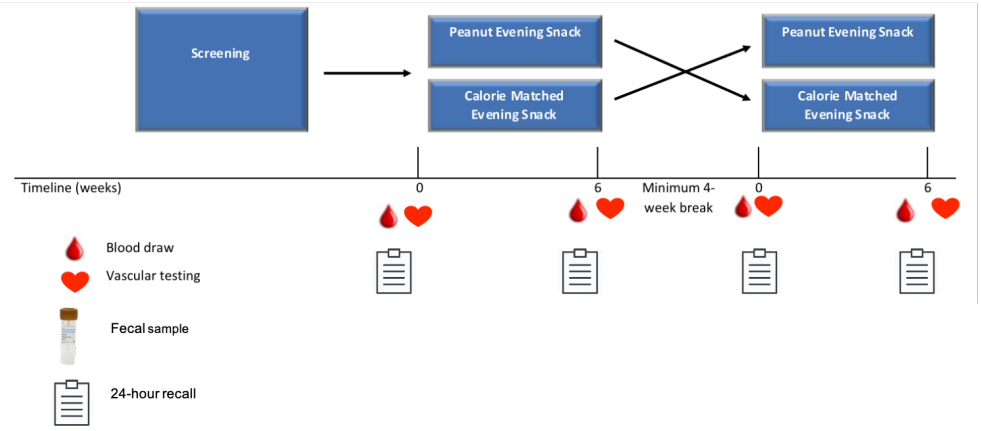

Figure **3-1**: Study flow chart

3.2.2 Participants

Participants were recruited between November 2018 to September 2019 from the State College, Pennsylvania area. Recruitment was conducted using clinicaltrials.gov and StudyFinder (studyfinder.com) and our research group's webpage. Advertisements were posted on university and local businesses' bulletin boards, listservs, and in local newspapers and circulars. Men and women aged 18-75 years, who had an elevated fasting plasma glucose (≥100 mg/dL and ≤125 mg/dL) measured at screening, were non-smokers, and had a BMI ≥20 and ≤40 kg/m^2 were eligible. Exclusion criteria were diagnosed diabetes or fasting plasma glucose >126 mg/dL;

systolic blood pressure (BP) >160 mmHg, diastolic BP >100 mmHg; taking glucose, lipid, or blood-pressure lowering medications; or taking antibiotics ≤6-weeks prior to enrollment. Individuals with a history of CVD, stroke, liver, kidney, autoimmune disease or inflammatory conditions were excluded. Participants taking supplements (psyllium, fish oil, soy lecithin, and phytoestrogens) and botanicals were excluded unless willing to abstain during the course of the study. Finally, pregnant or lactating women, individuals consuming >14 alcoholic beverages/week or those who had lost ≥10% of their body weight in the previous 6-months were excluded.

A telephone screening was conducted to assess medical history and lifestyle to determine eligibility for a clinical screening visit. Eligible participants were scheduled for an in-person screening at the Pennsylvania State University Clinical Research Center (CRC). Each participant fasted for 12-hours (no food or drink except for water) and avoided alcohol and over the counter medications for 48-hours. During this visit, weight and height (without shoes) was measured. Blood pressure (BP) was measured (validated automated sphygmomanometer following a 5-min rest) three times (114). A mean of the last two of the three BP measurements was used to determine eligibility. Pre-menopausal women provided a urine sample for a pregnancy test. Finally, nurses collected a fasting blood sample for a complete blood count, blood chemistry, and plasma glucose assayed by a commercial laboratory (Quest Diagnostics; Pittsburgh, PA).

3.2.3 Intervention

The experimental snack was 28 g/d (one ounce) of dry-roasted, unsalted, skinless peanuts (164 kcal [fat: 14g and 73% E; carbohydrates: 5g and 11% E; protein: 7g and 17% E; saturated fat: 2.2g; fiber: 2.4g; sodium: 5mg]) and the comparison LFHC snack was six low-sodium whole grain crackers (28g) and one slice (19g) of low-fat pre-packaged American cheese (165 kcal [fat:

6g and 33% E; carbohydrates: 22g and 54% E; protein: 7g and 17% E; saturated fat: 2.0g; fiber: 3.0g; sodium: 270mg]). This comparator was selected because it represents a snack choice that may be recommended for blood glucose control (low glycemic index) and therefore would not be expected to worsen blood glucose control in this cohort at risk for type 2 diabetes. In addition, the LFHC snack had a similar saturated fat, fiber, and protein content to the peanuts. Participants were asked to consume the study food after dinner, but before bedtime, and avoid any other food or drink containing calories in the evening. A specific time for intake of the evening snack was not given because of the inter-individual as well as daily variability in dinner time and bedtime. Non-prescriptive timing mimics real-world application and increases generalizability. Additionally, participants were asked to avoid any other peanuts or tree nuts, including nut butters, throughout the entirety of the study. Study food was provided every 14-days by the Metabolic Kitchen Manager.

Adherence was assessed bi-weekly by the Metabolic Kitchen Manger based on daily adherence checklists completed by the participants that included questions about consumption of the study foods, if the study foods were consumed after dinner, if other calorie containing foods/beverages were consumed after the study food, if other peanuts/tree nuts were consumed, if any changes in health status or usual exercise occurred, and if any non-habitual medications were taken. Adherence was calculated by dividing the total number of days the participants consumed the study food by the total number of days in the diet period. Additionally, the percentage of study days the participant consumed the study food as directed (i.e., as an evening snack, without consuming any other food or drinks in the evening) was calculated.

3.2.4 Outcomes

Testing was conducted on two separate consecutive days at baseline and the end of each diet period for a total of eight study visits throughout the study. For 12-hours prior to the testing visits participants fasted and avoided strenuous physical activity and for 48 hours prior participants were asked to refrain from drinking alcohol or taking over-the-counter medications. Each participant confirmed they had followed these protocols prior to beginning data collection. On both test days weight was measured using a calibrated electronic scale while participants wore light clothing and no shoes, and a fasting blood draw was taken for analysis of glucose, lipids and lipoproteins, insulin, and fructosamine. On one of the test days, vascular testing was performed.

3.2.4.1 Blood Collection and Assay Methods

Blood samples were drawn into serum separator and sodium fluoride/potassium oxalate tubes. The sodium fluoride/potassium oxalate tube was centrifuged immediately for 15 minutes. Blood drawn into the serum separator tube was allowed to clot for 30 minutes and then centrifuged for 15 minutes. Serum samples were used to measure total cholesterol, low-density lipoprotein cholesterol (LDL-C; direct measurement), high-density lipoprotein cholesterol (HDL-C) and triglycerides. Plasma was used to measure glucose. All samples were frozen at -80 degrees Celsius upon collection and analyzed in one batch at the end of the study. Serum samples were assayed for lipids/lipoproteins, insulin, and fructosamine at the Pennsylvania State University Biomarker Core Lab (University Park, PA) using a Cobas c311 chemistry analyzer (Roche Diagnostics, Basel, Switzerland) according to manufacturer instructions. In addition, plasma was analyzed for glucose at the Biomarker Core Lab using the Cobas c311 chemistry analyzer.

3.2.4.2 Vascular Testing Methods

Following a 5-minute seated rest, a SphygmoCor ECEL (AtCor Medical, Sydney, Australia) was used to assess peripheral and central blood pressure in the seated position. A cuff was placed on the left arm for measurement of peripheral blood pressure and radial artery waveforms. A validated generalized transfer function was used to calculate central blood pressure from peripheral blood pressure and the radial artery pressure waveform. Augmentation index (AIX) was adjusted to a heart rate of 75 beats per minute. Three measurements were taken and the last two results were averaged and used for the analyses.

Immediately following the blood pressure assessment, carotid-femoral PWV was measured using the SphygmoCor XCEL (AtCor Medical, Sydney, Australia) while participants were in the supine position. A tonometer was placed on the carotid artery and a blood pressure cuff was placed on the femoral artery. A 10-second recording of the carotid-femoral waveform was taken and PWV was calculated by dividing the linear distance between the carotid and femoral sites by the transit time using the SphygmoCor XCEL (AtCor Medical, Sydney Australia). On each test day, three PWV measurements were obtained and the average of the last two was used for analysis.

3.2.4.3 Dietary Assessment Methods

Dietary intake was assessed by non-random, participant-completed 24-hour recalls prior to baseline and in the last week of each diet period; each participant completed a total of four 24-hour recalls throughout the study. The Automated Self-Administered 24-hour Dietary Assessment Tool (National Cancer Institute) was used and administered as recommended by the National Cancer Institute Dietary Assessment Primer (115). Briefly, participants were emailed a

unique username and password prior to baseline and endpoint data collection visits and asked to complete the 24-hour recall prior to their study visit. Recall data were reviewed and cleaned to ensure the study snacks were reported correctly and consistently. Diet quality was assessed using the Healthy Eating Index (HEI)-2015, which consists of thirteen components including nine 'adequacy' components (whole grains, total fruits, dairy, etc.) and four 'moderation' components (saturated fats, sodium, refined grains and sugars). A higher adequacy score reflects higher intake whereas, a higher moderation score reflects a lower intake. The HEI-2015 was calculated using the SAS code created by the National Cancer Institute (116). Dietary recalls where energy intake was <600 or >4400 kcal/day for women and <650 or >5700 kcal/day for men were excluded from the analyses based on the National Cancer Institute guidelines for reviewing and cleaning ASA24 data (117).

3.2.5 Statistical Analyses

Sample size calculations indicated that completion of 45 participants would provide 80% power *(*$P < 0.05$) to detect a minimum 10 mg/dL difference in fasting glucose between the conditions (standard deviation of 23.4 mg/dL) based on previous studies (92,113,118). Fasting plasma glucose is the primary outcome. All other outcomes are secondary.

All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc.). All data collected from randomized participants were included in data analyses consistent with intent-to-treat principles. Univariate analysis (PROC UNIVARIATE) was used to assess normality of the residuals for each variable based on the distribution and normal probability plots (Q-Q plots). In the instance of skewed residuals, the variable was logarithmically transformed. Weight, glucose, lipids and lipoproteins, and insulin values taken on the two test days at each timepoint were averaged for analysis. Change from baseline for each diet period was calculated by

subtracting the endpoint value from the baseline value. Data are presented as least-squares means ± standard error of the means unless otherwise stated.

The effect of each condition on outcome variables was examined using the mixed-methods procedure (PROC MIXED) at a predetermined α level of 0.05. Subject nested within condition was modeled to account for the repeated-measures crossover design, and baseline value was included as a covariate. Sex and randomization sequence were included as fixed effects to assess sex and carryover effects. There were neither sex nor carryover effects based on nonsignificant *Condition x Sex* and *Condition x Sequence* interactions for each outcome variable; therefore, sex and sequence were removed from the final model. The primary analyses assessed between-diet differences in endpoint means for each outcome variable. Additionally, change from baseline was evaluated using the mixed-methods procedure for each condition. The covariance structure for the models was based on optimizing fit statistics (lowest Bayesian Information Criterion) and varied depending upon which analysis was being conducted. Exploratory analyses were conducted to assess endpoint-mean differences and change from baseline for each condition by eating occasion (breakfast, brunch, lunch, dinner/supper, snack, drinks, and supplements).

3.3 Results

Fifty-one adults were randomized out of the 267 individuals that completed a telephone screening with further details in the flow chart (**Figure 3-2**). Of the fifty-one individuals randomized, fifty (female n=25) aged 42 ± 15 years with a BMI of 28.3 ± 5.6 kg/m^2 and fasting plasma glucose of 100 ± 8 mg/dL completed the study. Participant characteristics were comparable between the randomization sequences at baseline (**Table 3-1**). On average, participants reported eating their study food on 87% of study days. Additionally, on 88% of study days, participants reported consuming the study food as directed (i.e., after dinner and before

bedtime), although slightly greater adherence was observed during the LFHC condition vs. the Peanut condition (89% compared to 87%). One subject withdrew without consuming any study food due to personal reasons. The fifty individuals who provided endpoint data were included in all analyses, unless otherwise stated.

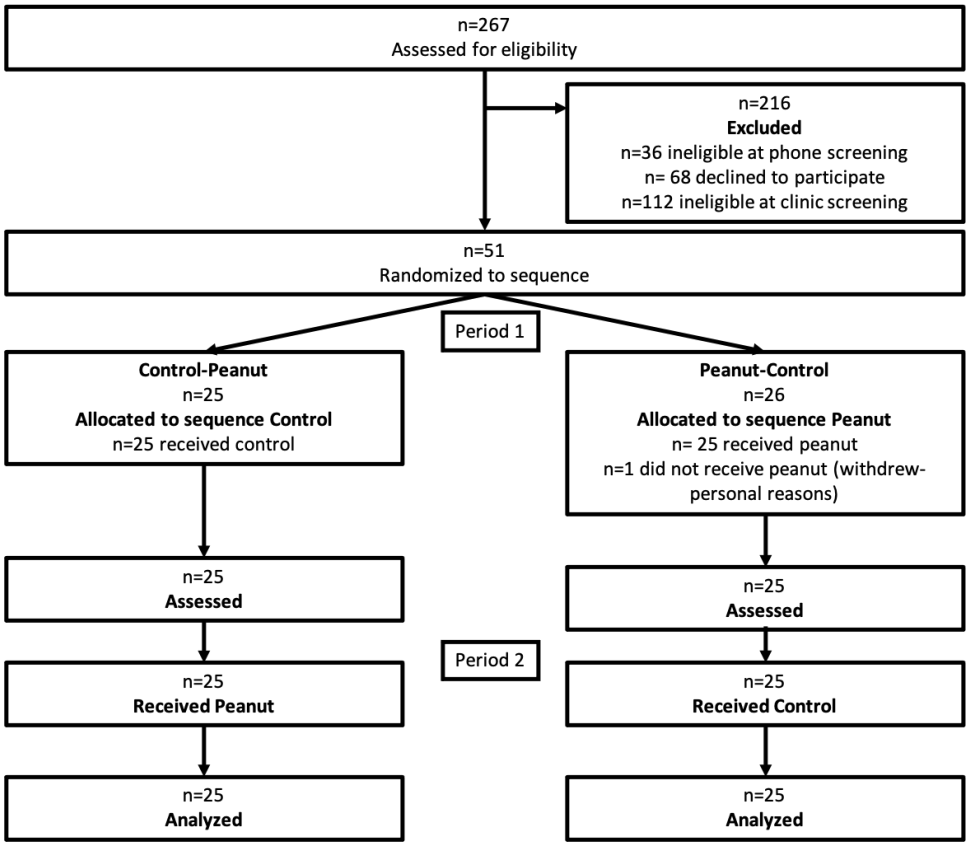

Figure **3-2**: Consort diagram

No significant between-condition effects were observed for fasting plasma glucose (Peanut vs. LFHC mean difference -0.6 mg/dL; 95% CI -2.7, 1.6; P = 0.67), insulin (0.45 µIU/mL; 95% CI -1.2, 2.1; P = 0.60), or fructosamine (0.7 µmol/L; 95% CI -4.8, 3.4; P = 0.74) (**Table 3-2**). In addition, no differences were observed between the conditions for lipids and lipoproteins, weight, peripheral or central blood pressure or measures of arterial stiffness.

Total energy intake (kcal) was similar for both conditions (Peanut vs. LFHC mean difference -43 kcal; 95% CI -251, 236; P = 0.71). Percent of energy from monounsaturated and polyunsaturated fatty acid was higher following peanut intake compared to the LFHC snack (1.7%; 95% CI 0.0, 3.4; P = 0.04 and 1.9%; 95% CI 0.0, 3.4; P = 0.01); no other significant differences were observed for nutrient intakes (**Table 3-3**). Intake of total protein foods (includes nuts) and oil-containing foods (includes nuts) was significantly higher with peanut intake compared to the LFHC snack (mean difference: 1.9 oz-eq [95% CI 0.1, 3.7]; P = 0.04 and 8.3 g [1.9, 14.6]; P = 0.01). Whole grain consumption was significantly lower for the Peanut condition compared to LFHC (-1.2 oz-eq; 95% CI -1.6, -0.8; P < 0.01). No differences in intake of vegetables, total grains or refined grains, fruit or dairy were observed between the conditions.

Table **3-1**: Baseline characteristics of study participants overall and by randomization sequence (n=50)[1]

Characteristic	Peanut-LFHC	LFHC-Peanut	Total
n (% female)	24 (50%)	26 (46%)	50
Age, y	40 ± 15	43 ± 15	42 ± 15
Weight, kg	85.0 ± 21.1	82.7 ± 16.5	83.8 ± 18.7
Height, m	1.72 ± 0.1	1.71 ± 0.1	1.72 ± 0.1
BMI, kg/m^2	28.5 ± 6.0	28.2 ± 5.2	28.3 ± 5.6
Glucose, mg/dL	99 ± 8	101 ± 8	100 ± 8

[1]Values are mean and SD unless otherwise stated

The HEI-2015 score was not significantly different between the conditions (mean difference 3.6 points; 95% CI -1.9, 9.0; P = 0.19). Compared with the LFHC snack, the seafood/plant protein (2.0 points; 95% CI 1.0, 2.9; P < 0.01) and added sugar (0.8 points; 95% CI 0.0, 1.5; P = 0.04) components were higher with Peanut intake. The whole grain component score

was higher (mean difference 2.6 points; 95% CI 1.4, 3.8; P < 0.01) following the LFHC snack vs. peanuts. No other significant between-condition differences were observed for HEI components (**Table 3-4**).

The total calories from snacks were not significantly different between conditions (mean difference -37 kcal; 95% CI -165, 92; P = 0.41). Additionally, caloric intake at all other eating occasions (i.e., breakfast, lunch dinner, supper, and drinks) was not significantly different between conditions. Following both conditions, no change in energy, nutrient, or food group intake for snacking occasions was observed (**Table 3-5 to 3-9**).

Table **3-2**: Between-condition mean differences and change from baseline for glycemic, lipid, lipoprotein, vascular, and anthropometric outcomes in adults with elevated fasting glucose concentrations (n=50)[1]

	Peanut			LFHC				
Outcome	Baseline[2]	Endpoint	Within-condition difference[3]	Baseline[2]	Endpoint	Within-condition difference[3]	Between- condition effect[3]	Between-condition p-value
Glucose, mg/dL (Primary Outcome)	100 ± 1.0	99.2 ± 0.9	-0.9 (-2.7, 0.9)	100 ± 1.2	100 ± 0.9	-0.4 (-2.2, 1.4)	-0.6 (-2.7, 1.6)	0.67
Insulin, uIU/mL	11.35 ± 1.14	11.34 ± 0.59	-0.2 (-1.5, 1.0)	11.93 ± 1.34	10.89 ± 0.59	-0.8 (-2.1, 0.5)	0.45 (-1.2, 2.1)	0.6
Fructosamine, umol/L	228 ± 2.2	224 ± 1.7	-4.0 (-5.1, 5.4)	229 ± 2.6	225 ± 1.7	-3.8 (-5.1, 5.4)	0.7 (-4.8, 3.4)	0.74
TChol, mg/dL	191 ± 5	192 ± 2	0.1 (-4.9, 5.1)	194 ± 5	190 ± 2	-2.7 (-7.8, 2.3)	2.2 (-3.8, 8.2)	0.46
LDL-C, mg/dL	122 ± 4.1	126 ± 2.7	2.4 (-2.1, 6.9)	125 ± 4.2	122 ± 2.3	-2.3 (-6.8, 2.2)	4.3 (-1.4, 9.8)	0.13
HDL-C, mg/dL	53.2 ± 2.1	54.1 ± 0.6	0.6 (-0.6, 1.8)	53.8 ± 2.0	54.1 ± 0.8	0.6 (-1.6, 2.3)	0.0 (-2.3, 2.3)	0.99
TG, mg/dL	122 ± 11	103 ± 3	-17.0 (-29.1, -4.8)*	115 ± 9	111 ± 4	-5.7 (-17.1, 5.7)	-7.7 (-18.3, 2.8)	0.15
Weight, kg	83.9 ± 2.6	84.1 ± 0.2	0.2 (-0.1, 1.5)	83.8 ± 2.6	83.9 ± 0.2	0.0 (-0.5, 0.5)	0.2 (-0.3, 0.7)	0.51
bSBP, mmHg	125 ± 1.9	125 ± 1.3	0.4 (-2.5, 3.3)	125 ± 1.8	125 ± 1.3	-0.3 (-3.1, 2.6)	0.7 (-3.1, 4.6)	0.7
bDBP, mmHg	79 ± 1.3	80 ± 0.9	0.5 (-1.5, 2.5)	80 ± 1.3	80 ± 0.9	-0.0 (-2.0, 2.0)	0.4 (-2.1, 2.9)	0.73
cSBP, mmHg	114 ± 1.6	114 ± 1.1	0.6 (-1.9, 3.1)	114 ± 1.8	113 ± 1.1	-0.4 (-2.9, 2.1)	1.0 (-2.4, 4.4)	0.54
cDBP, mmHg	80 ± 1.3	81 ± 0.9	0.5 (-1.5, 2.6)	81 ± 1.4	80 ± 0.9	-0.4 (-2.4, 1.6)	0.8 (-1.7, 3.3)	0.54
AP, mmHg	6 ± 3.0	7 ± 0.5	0.3 (-0.8, 1.4)	7 ± 0.8	7 ± 0.5	-0.0 (-1.1, 1.1)	0.3 (-1.3, 1.9)	0.72
AIX[4], %	16 ± 3.0	16 ± 1.6	-0.4 (-3.7, 2.9)	18 ± 2.6	17 ± 1.6	0.2 (-4.0, 4.3)	-1.4 (-5.9, 3.3)	0.55
PTT, ms	64 ± 1.3	62 ± 0.8	-1.2 (-2.7, 0.3)	63 ± 1.3	63 ± 0.8	-0.2 (-1.7, 1.3)	-0.9 (-3.0, 1.1)	0.36
PWV, m/s	6.8 ± 0.2	7.2 ± 0.1	0.3 (0.1, 0.6)*	7.0 ± 0.2	7.0 ± 0.1	0.1 (-0.1, 0.3)	0.2 (-0.1, 0.5)	0.19

[1]Data are presented as least squares means and SE unless otherwise stated; Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the effect of the conditions on each outcome measure, the between-condition p-values represent the main effect of condition. The MIXED procedure was also used to assess change from baseline. AIX: augmentation index; AP: augmentation pressure; bDBP: brachial diastolic blood pressure; bSBP: brachial systolic blood

pressure; cDBP: central diastolic blood pressure; cSBP: central systolic blood pressure; HDL-C: high-density lipoprotein cholesterol LDL-C: low-density lipoprotein cholesterol; PTT: pulse transit time; PWV: pulse wave velocity; TChol: total cholesterol; TG: triglycerides.

[2]Values are mean and SE.

[3]Values are least squares mean effect estimate and 95% CI.

[4]Adjusted to a heart rate of 75 bpm

[*]Indicates within-condition significant difference ($p < 0.05$)

Table **3-3**: Between-condition mean differences and change from baseline for nutrient and food group intake in adults with elevated fasting glucose concentrations (n=46)[1]

	Peanut			LFHC				
Outcome	Baseline[2]	Endpoint	Within-condition difference[3]	Baseline[2]	Endpoint	Within-condition difference[3]	Between condition effect[3]	Between condition p-value
Energy, kcal	2090 ± 113	2036 ± 89	-60 (-304, 184)	2075 ± 114	2078 ± 88	-8 (-197, 244)	-43 (-251, 236)	0.71
Protein, g	94 ± 6	91 ± 5	-0.7 (-14.0, 12.6)	91 ± 6	90 ± 5	-0.1 (-13.4, 13.2)	1.1 (-14.6, 16.7)	0.89
Protein, E %	18.3 ± 1	18.4 ± 1	0.7 (-1.5,3.0)	17.5 ± 1	17.7 ± 1	0.4 (-1.2, 1.9)	0.6 (-1.7, 2.9)	0.59
Carbohydrate, g	232 ± 15	211 ± 11	-21.2(-50.8, 8.4)	232 ± 15	228 ± 11	-4.8 (-34.4, 24.8)	-17.1 (-44.7, 10.4)	0.22
Carbohydrate, E %	44.4 ± 2	41.4 ± 1	-3.2 (-6.7, 0.2)	44.7 ± 1	44.4 ± 1	-0.2 (-3.4, 3.0)	-3.0 (-7.4, 1.4)	0.17
Total fat, g	90 ± 7	93 ± 6	1.4 (-15.0, 16.8)	88 ± 5	90 ± 6	1.0 (-14.4, 16.4)	3.1 (-13.6, 19.8)	0.71
Fat, E %	38.6 ± 1	41.0 ± 1	2.0 (-1.2, 5.1)	38.2 ± 1	38.3 ± 1	-0.0 (-3.2, 2.8)	2.6 (-1.3, 6.6)	0.19
SFA, g	32 ± 3	29 ± 3	-2.9 (-9.8, 3,9)	29 ± 2	33 ± 3	3.1 (-3.8, 9.9)	-3.4 (-11.9, 5.2)	0.43
SFA, E %	13.6 ± 1	12.8 ± 1	-1.1 (-2.9, 0.7)	12.8 ± 1	13.6 ± 1	0.8 (-1.0, 2.7)	-0.8 (-2.8, 1.2)	0.41
MUFA, g	30 ± 2	33 ± 2	2.9 (-2.5, 8.2)	30 ± 2	31 ± 2	0.8 (-4.5, 6.2)	2.7 (-3.0, 8.3)	0.35
MUFA, E %	13.1 ± 1	14.8 ± 1	1.8 (0.0, 3.2)*	13.0 ± 1	13.1 ± 1	0.1 (-1.2, 1.5)	1.7 (0.0, 3.4)	0.04
PUFA, g	21 ± 2	23 ± 2	1.9 (-2.9, 6.7)	22 ± 2	19 ± 2	-3.3 (-8.1, 1.5)	4.1 (-0.0, 8.2)	0.05
PUFA, E %	8.8 ± 1	10.2 ± 1	1.4 (-0.0, 2.7)	9.4 ± 1	8.3 ± 1	-1.2 (-2.5, 0.0)	1.9 (0.0, 3.4)	0.01
Fiber, g	22 ± 2	19 ± 1	-1.9 (-5.3, 1.5)	21 ± 2	21 ± 1	-0.5 (-3.9, 2.9)	-1.2 (-4.0, 1.7)	0.42
Potassium, mg	2831 ± 144	2772 ± 136	26 (-291, 343)	2774 ± 144	2787 ± 136	59 (-258, 337)	-15 (-335, 305)	0.93
Sodium, mg	3752 ± 224	3678 ± 196	-63 (-564, 439)	3892 ± 263	3827 ± 196	50 (-451,552)	-148 (-677, 380)	0.57
Total vegetables (c-eq)	2.1 ± 0.1	1.9 ± 0.2	-0.2 (-0.7, 0.4)	1.9 ± 0.2	2.1 ± 0.2	0.1 (-0.4, 0.7)	-0.1 (-0.7, 0.4)	0.62
Dark green vegetables (c-eq)	0.4 ± 0.1	0.4 ± 0.1	-0.0 (-0.3, 0.2)	0.5 ± 0.1	0.4 ± 0.1	-0.1 (-0.3, 0.2)	0.0 (-0.2, 0.3)	0.69
Red/orange vegetables (c-eq)	0.5 ± 0.1	0.6 ± 0.1	0.1 (-0.1, 0.3)	0.5 ± 0.1	0.5 ± 0.1	0.0 (-0.1, 0.2)	0.1 (-0.1, 0.3)	0.27

Starchy vegetables (c-eq)	0.5 ± 0.1	0.3 ± 0.1	-0.2 (-0.6, 0.1)	0.3 ± 0.1	0.4 ± 0.1	0.0 (-0.3, 0.4)	-0.0 (-0.4, 0.4)	0.93
Total grains (oz-eq)	5.9 ± 0.5	6.1 ± 0.5	-0.1 (-1.4, 1.2)	6.9 ± 0.5	6.7 ± 0.5	-0.2 (-1.5, 1.1)	-0.6 (-1.9, 0.7)	0.37
Whole grains (oz-eq)	1.1 ± 0.1	0.6 ± 0.2	-0.3 (-0.8, 0.2)	1.4 ± 0.2	1.8 ± 0.2	0.5 (0.0, 1.0)*	-1.2 (-1.6, -0.8)	<0.01
Refined grains (oz-eq)	5.0 ± 0.5	5.4 ± 0.5	0.2 (-1.0, 1.5)	5.5 ± 0.5	4.8 ± 0.5	-0.7 (-2.0, 0.6)	0.6 (-0.7, 1.9)	0.34
Total fruit (c-eq)	0.9 ± 0.1	1.1 ± 0.2	0.2 (-0.2, 0.6)	1.1 ± 0.2	1.1 ± 0.2	-0.0 (-0.4, 0.4)	0.0 (-0.4, 0.5)	0.84
Total dairy products (c-eq)	1.9 ± 0.3	1.6 ± 0.2	-0.3 (-0.9, 0.3)	2.0 ± 0.2	2.0 ± 0.4	-0.0 (-0.8, 0.7)	0.0 (-0.1, 0.2)	0.32
Total protein foods (oz-eq)	6.6 ± 0.7	7.9 ± 0.6	1.6 (-0.1, 3.2)	6.5 ± 0.7	6.0 ± 0.6	-0.5 (-2.2, 1.2)	1.9 (0.1, 3.7)	0.04
Oils (g)	26.8 ± 3.3	30.7 ± 2.3	4.6 (-3.9, 13.1)	29.2 ± 3.4	22.4 ± 2.3	-7.6 (-16.9, 0.9)	8.3 (1.9, 14.6)	0.01

[1]Data are presented as least squares means and SE unless otherwise stated; Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the effect of the conditions on each outcome measure, the between-condition p-values represent the main effect of condition. The MIXED procedure was also used to assess change from baseline. MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

[2]Values are mean and SE.

[3]Values are least squares mean effect estimate and 95% CI

*Indicates within-condition significant difference ($p < 0.05$)

Table **3-4**: Between-condition mean differences and change from baseline for the Healthy Eating Index (HEI) score and individual components in adults with elevated fasting glucose concentrations (n=46)[1]

	Peanut			LFHC				
Outcome	Baseline[2]	Endpoint	Within-condition difference[3]	Baseline[2]	Endpoint	Within-condition difference[3]	Between condition effect[3]	Between condition p-value
HEI 2015 total score[4]	53.2 ± 1.7	56.5 ± 1.9	3.6 (-1.1, 8.2)	56.9 ± 2.1	52.9 ± 1.9	-2.9 (-7.5, 1.8)	3.6 (-1.9, 9.0)	0.19
Adequacy Components								
Total vegetables[5]	3.7 ± 0.2	3.5 ± 0.3	-0.2 (-0.9, 0.4)	3.7 ± 0.2	3.5 ± 0.3	-0.1 (-0.8, 0.5)	-0.0 (-0.6, 0.6)	0.94
Greens and beans[5]	2.3 ± 0.3	2.8 ± 0.4	0.4 (-0.5, 1.3)	3.2 ± 0.3	2.2 ± 0.4	-0.7 (-1.6, 0.2)	0.5 (-0.5, 1.6)	0.32
Total fruits[5]	2.3 ± 0.3	2.3 ± 0.3	-0.0 (-0.7, 0.7)	2.6 ± 0.3	2.3 ± 0.3	-0.2 (-0.9, 0.5)	-0.0 (-0.9, 0.9)	0.96
Whole fruits[5]	2.7 ± 0.3	2.4 ± 0.3	-0.2 (-1.0, 0.5)	2.9 ± 0.3	2.7 ± 0.3	-0.1 (-0.8, 0.7)	-0.3 (-1.3, 0.7)	0.52
Whole grains[6]	3.1 ± 0.4	2.4 ± 0.5	-0.5 (-1.8, 0.7)	4.2 ± 0.6	5.0 ± 0.5	1.0 (-0.2, 2.2)	-2.6 (-3.8, -1.4)	<0.01
Total dairy[6]	5.8 ± 0.4	6.0 ± 0.4	0.0 (-1.1, 1.1)	6.8 ± 0.5	5.9 ± 0.4	-0.8 (-1.9, 0.3)	0.1 (-1.0, 1.2)	0.83
Total protein foods[5]	4.0 ± 0.2	4.7 ± 0.2	0.8 (0.3, 1.2)*	4.2 ± 0.2	4.2 ± 0.2	0.1 (-0.4, 0.6)	0.5 (-0.0, 1.0)	0.06
Seafood and plant proteins[5]	2.7 ± 0.3	3.8 ± 0.3	1.2 (0.3, 2.1)*	2.4 ± 0.3	1.8 ± 0.3	-0.6 (-1.5, 0.3)	2.0 (1.0, 2.9)	<0.01
Fatty acid ratio[6]	4.2 ± 0.5	5.1 ± 0.5	0.8 (-0.5, 2.2)	4.7 ± 0.5	3.6 ± 0.5	-1.0 (-2.3, 0.4)	1.5 (-0.1, 3.1)	0.07
Moderation Components								
Sodium[6]	3.2 ± 0.5	4.0 ± 0.5	0.8 (-0.5, 2.1)	2.8 ± 0.5	3.3 ± 0.5	0.5 (-0.8, 1.9)	0.7 (-0.7, 2.0)	0.31
Refined grains[6]	6.9 ± 0.5	6.4 ± 0.5	-0.5 (-1.8, 0.8)	6.2 ± 0.6	7.3 ± 0.5	1.1 (-0.3, 2.5)	-0.9 (-2.1, 0.3)	0.12
Saturated fats[6]	4.3 ± 0.5	5.4 ± 0.5	1.0 (-0.1, 2.2)	4.6 ± 0.5	4.3 ± 0.5	-0.2 (-1.4, 1.0)	1.1 (-0.4, 2.5)	0.14
Added sugars[6]	8.0 ± 0.4	8.9 ± 0.3	0.8 (0.1, 1.5)*	8.6 ± 0.3	8.1 ± 0.3	-0.4 (-1.1, 0.3)	0.8 (0.0, 1.5)	0.04

[1]Data are presented as least squares means and SE unless otherwise stated; Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the effect of the conditions on each outcome measure, the between-condition p-values represent the main effect of condition. The MIXED procedure was also used to assess change from baseline. HEI: healthy eating index.

[2]Values are mean and SE.

[3]Values are least squares mean effect estimate and 95% CI

[*]Indicates within-condition significant difference ($p < 0.05$)

[4]Maximum total score 100

[5]Maximum score 5

[6]Maximum score 10

3.4 Discussion

The present study demonstrated that consuming 28 g/d of dry roasted, unsalted, peanuts as an evening snack for 6-weeks did not change fasting plasma glucose compared to an isocaloric lower-fat higher-carbohydrate snack in participants with elevated fasting glucose. In addition, neither peanuts, nor the lower-fat higher-carbohydrate snack significantly improved insulin, fructosamine, lipids, lipoproteins, vascular measures, or weight. Consumption of peanuts did not change overall diet quality compared to the LFHC snack, but did increase intake of protein from the seafood and plant sources category. Furthermore, percent of energy intake from polyunsaturated and monounsaturated fatty acids, oil-containing foods and total protein foods were greater with peanut intake. Collectively, in individuals with elevated fasting glucose, consumption of 28 g/d of peanuts as a nighttime snack, compared to an isocaloric lower-fat higher-carbohydrate nighttime snack, improved several components of diet quality without changing fasting plasma glucose, fructosamine or CVD risk factors after 6 weeks.

To our knowledge, this is the first clinical trial to examine evening snacking in adults with elevated fasting glucose without T2DM. Dyer-Parizale (92) demonstrated significantly lower morning fasting glucose (114.2 mg/dL vs 158.5 mg/dL [$P < 0.0001$]) after nighttime consumption of an Extend bar (30 g carbohydrate [5 g uncooked cornstarch], 3 g protein, and 3 g fat) compared to an isocaloric, macronutrient matched, snack without cornstarch in patients with T2DM after 3 days. Similarly, Abbie et al. compared the effects of nighttime snacks high in protein and fat (egg) and carbohydrate (yogurt), as well as no snack on morning fasting glucose and glucose measured by continuous glucose monitor in patients with T2DM. Morning fasting glucose and average CGM glucose were significantly lower in the high protein group (129.6 and

136.8 mg/dL) compared to yogurt group (136.8 and 147.6 mg/dL) (91). Our study compared similar nighttime snacks, but we did not observe comparable effects on fasting blood glucose. The discordance may be because our study population did not have T2DM. In addition, our study had a longer study duration, which may have resulted in short-term acute effects similar to those observed in the aforementioned studies being missed.

Nuts (peanuts and tree nuts) have established short- and longer-term glucoregulatory properties. Several clinical trials show peanuts improve postprandial glucose response. Liu et al. conducted a clinical trial in men with overweight and obesity and demonstrated 4-hour postprandial glucose was lower following a high-saturated fat beverage with 85 g of peanuts compared to an isocaloric, macronutrient matched, high-saturated fat beverage without peanuts (98). Furthermore, in a study where 42.5 g of peanuts or peanut butter was added to breakfast, improvements in postprandial (240-490 min) glucose were observed in women with obesity compared to a high-carbohydrate control breakfast (20). Peanuts contain 49.3 g/100 g of fat (24.6 g is monounsaturated fatty acids) and likely improve postprandial glucose response by delaying gastric emptying, which delays carbohydrate absorption following a meal. Therefore, it is plausible that intake of meals containing peanuts improves post-prandial glucose homeostasis without affecting fasting glucose. However, the current study was not designed to assess short-term glycemic response and is not directly comparable to theses postprandial trials.

A recent systematic review and meta-analysis of 40 randomized controlled trials with a median duration of 12-weeks, showed that nut intake (tree and peanuts; median dose 52 g/day) improved fasting insulin (weighted mean difference: -0.40 μIU/mL; 95% CI -0.73, -0.07), homeostasis model assessment of insulin resistance (weighted mean difference: -0.23; 95% CI -0.40, -0.06); fasting glucose was unaffected (weighted mean difference: -0.52 mg/dL; 95% CI -1.43 0.38) (95). However, heterogeneity existed in the interventions tested, particularly how the study foods were consumed, and only four studies examined peanuts intake, which limits the

direct comparability to our study. A recent 12-week parallel arm randomized controlled trial conducted in China evaluated the effect of peanuts (56 g/day [28g 1-hr before lunch and 28g 1-hr before dinner]) compared to an isocaloric white rice bar in participants with metabolic syndrome or at risk for metabolic syndrome (97). The Peanut condition non-significantly reduced fasting glucose from 100 mg/dL (95% CI: 97.7, 102.1) to 94 mg/dL (95% CI: 91.8, 96.3) with similar reductions observed for the white rice bar. It is possible that the dose tested (i.e., 28 g/d) in our trial and the study duration were not sufficient to detect changes in glycemic control.

Consistent evidence shows that tree nuts and peanuts improve lipids and lipoproteins. A systematic review of 23 trials concluded that consuming 50-100 g of peanuts and tree nuts 5-times/week, as a part of a heart-healthy diet, lowers total cholesterol levels 2-16% and LDL-C 2-19% (100). Del Gobbo et al. conducted a systematic review and meta-analysis of 61 trials and reported a dose-response reduction per-serving/day of peanuts and tree nuts for total cholesterol (-4.7 mg/dL), LDL-C (-4.8 mg/dL), and triglycerides (-2.2 mg/dL) (16). The response for LDL-C was non-linear with greater reductions observed in studies that provided >60 g/day of nuts. Many of the trials included in this systematic review evaluated tree nuts, in subjects with hyperlipidemia, and had greater median nut intake than our study. We likely did not observe significant improvements in lipids and lipoproteins because our participants were normolipidemic at baseline, the dose of peanuts tested may have been insufficient to affect lipids and lipoproteins, and peanuts, because of their fatty acid profile, may have different lipid-lowering effects compared to some tree nuts.

The 2020-2025 (8) and 2015-2020 (119) Dietary Guidelines recommend consuming 5 oz-eq/week of nuts, for those consuming 2,000 kcal/day, as part of a healthy dietary pattern. In our study, participants were instructed to consume 14 oz-eq/week and we did not observe an increase in diet quality measured by the HEI-2015 (3.6 [95% CI: -1.9, 9.0; P = 0.19]) with peanut vs. the LFHC snack. Baseline diet quality in our sample was reflective of average U.S. diet quality (58

for adults 18-64 years and 64 for adults 65+ years) (120). The HEI-2015 changes we observed were driven by significant improvements in seafood/plant protein and non-significant improvements in total protein and fatty acids, which is expected based on the composition of peanuts. Therefore, the observed dietary changes following both conditions reflect the intervention, suggesting that the participants' background diets did not change. Our findings suggest that intake of peanuts as a nighttime snack is a relatively simple dietary strategy to improve the fatty acid profile of the diet and protein intake from plants/seafood in individuals with elevated fasting glucose. This dietary intervention together with other small changes may significantly improve overall dietary quality and benefit health (121).

Based on NHANES 2013-2016, the average US adult (≥18 years) engages in 1.56 snacking occasions per day, with an average of 236 kcal/snack; therefore snacking contributes approximately 370 kcal/day to the U.S. diet (65). Our participants consumed a similar number of calories from snacks at baseline. Calorie, nutrient and food group intake from snacks were unchanged following both conditions and no significant between condition differences were observed. Additionally, we did not observe any compensatory eating behaviors (i.e., consuming more, or less, calories at other eating occasions) for either condition. These findings demonstrate that the addition of peanuts as a nighttime snack does not have adverse effects on total, or snacking, caloric intake and does not promote compensatory eating behaviors.

The strengths of this study are the design, glycemic control measures, and dietary assessment. The crossover design with baseline measures for each condition allows for the change from baseline to be calculated for each condition and determination of mean differences between the treatments. Assessing both fasting blood glucose and fructosamine provides information about short- and longer-term glycemic control. The dietary assessment using ASA-24 generated information about small, yet important, dietary changes that may not have been detected using other dietary assessment methods. Limitations of this study include the single-

blinded protocol, participant screening procedures, lack of no-snack condition, and non-random dietary assessment. The participants were not blinded to the intervention allocation, which may introduce bias; however, double-blinding was not feasible given the study design. All outcome measures have a low risk for bias from the single-blind protocol and were collected/analyzed by blinded study personnel. We enrolled subjects based on a single fasting plasma glucose measure ≥100 mg/dL at screening. However, due to day-to-day variability, some individuals had baseline glucose levels <100 mg/dL. We did not include a no-snack condition and therefore it remains unclear whether no nighttime snack is superior to nighttime snacking for fasting plasma glucose control. Non-random 24-hour recalls were administered and participants were aware of the diet assessment days, which may have impacted reporting. Finally, the risk of type 1 statistical errors is inflated by of the number of analyses conducted for the secondary endpoints.

In summary, we demonstrated that 28 g/d of dry roasted, unsalted, peanuts as a nighttime snack did not affect fasting plasma glucose, lipids/lipoproteins, vascular health, or weight in subjects with elevated fasting glucose compared to a lower-fat higher-carbohydrate snack. However, this relatively low-calorie dietary intervention resulted in a greater percentage of energy from polyunsaturated and monounsaturated fatty acids being consumed, as well as higher intake of oil-containing foods, and total protein from plant/seafood sources. These findings suggest that peanuts are a suitable nighttime snack for those with elevated fasting glucose and, importantly, do not have adverse effects on fasting plasma glucose. Further research is necessary to elucidate the effect of larger doses of peanuts as an evening snack on glycemic control in individuals with prediabetes

Supplementary Data

Table **3-5**: Between-condition mean differences and change from baseline for nutrient and food group intake at breakfast in adults with elevated fasting glucose concentrations (n=41)[1]

	Peanut			LFHC			
Outcome	Baseline[2]	Endpoint	Within-condition difference[3]	Baseline[2]	Endpoint	Within-condition difference[3]	Between-condition p-value
Energy, kcal	439 ± 74	537 ± 43	86 (-2, 174)	468 ± 84	591 ± 45	**140 (48, 231)**	0.4
Protein, g	15.8 ± 4.1	18.9 ± 2.4	0.6 (-4.2, 5.4)	20.9 ± 4.6	23.8 ± 2.5	**5.4 (0.4, 10.9)**	0.17
Carbohydrate, g	53.8 ± 7.7	72.8 ± 7.0	18.4 (4.3, 32.5)	56.6 ± 10.3	75.0 ± 7.4	**20.6 (5.7, 35.6)**	0.83
Fat, g	18.8 ± 4.3	19.7 ± 2.4	0.8 (-4.0, 5.6)	18.8 ± 4.2	22.8 ± 2.5	3.9 (-1.1, 8.9)	0.37
SFA, g	7.4 ± 2.0	7.6 ± 1.1	0.3 (-1.8, 2.5)	7.2 ± 1.8	8.6 ± 1.1	1.3 (-0.9, 3.6)	0.51
MUFA, g	6.6 ± 1.4	6.4 ± 0.9	0.1 (-1.7, 1.9)	5.9 ± 1.2	7.6 ± 0.9	1.3 (-0.6, 3.2)	0.34
PUFA, g	3.3 ± 0.6	4.6 ± 0.7	0.8 (-0.6, 2.2)	4.3 ± 1.0	4.5 ± 0.7	0.7 (-0.8, 2.2)	0.9
Fiber, g	2.9 ± 0.4	4.6 ± 0.7	0.8 (-0.6, 2.2)	4.8 ± 1.1	6.4 ± 0.7	**2.6 (1.2, 4.1)**	0.08
Total vegetable, c-eq	0.1 ± 0.0	0.2 ± 0.1	0.0 (-0.2, 0.3)	0.2 ± 0.1	0.3 ± 0.1	0.2 (-0.0, 0.4)	0.37
Total grain, oz-eq	1.6 ± 0.2	2.1 ± 0.3	**0.7 (0.1, 1.3)**	1.3 ± 0.3	2.0 ± 0.3	0.6 (-0.0, 1.2)	0.79
Whole grain, oz-eq	0.3 ± 0.1	0.6 ± 0.2	0.3 (-0.0, 0.6)	0.3 ± 0.1	0.6 ± 0.2	0.3 (-0.0, 0.7)	0.86
Refined grain, oz-eq	1.3 ± 0.2	1.5 ± 0.2	0.4 (-0.1, .8)	1.0 ± 0.3	1.4 ± 0.2	0.3 (-0.2, 0.7)	0.77
Total fruit, c-eq	0.2 ± 0.1	0.4 ± 0.1	0.0 (-0.3, 0.2)	0.6 ± 0.1	0.6 ± 0.1	0.2 (-0.0, 0.5)	0.17
Total dairy, c-eq	0.6 ± 0.3	0.5 ± 0.1	-0.2 (-0.4, 0.0)	0.6 ± 0.1	0.8 ± 0.1	0.1 (-0.1, 0.4)	0.07
Total protein, oz-eq	0.6 ± 0.2	1.2 ± 0.3	0.2 (-0.4, 0.9)	1.4 ± 0.4	1.2 ± 0.3	0.2 (-0.5, 0.9)	0.97
Oils, g	3.1 ± 0.9	4.2 ± 1.5	0.5 (-2.4, 3.5)	4.2 ± 1.1	5.2 ± 0.6	1.5 (-1.7, 4.6)	0.66

[1]Data are presented as least squares means and SE unless otherwise stated; Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the effect of the conditions on each outcome measure, the between-condition p-values represent the main effect of condition. The MIXED procedure was also used to assess change from baseline, within-condition significant differences are denoted with bold text ($p<0.05$). MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

[2]Values are mean and SE.

[3]Values are least squares mean effect estimate and 95% CI

Table **3-6**: Between-condition mean differences and change from baseline for nutrient and food group intake at lunch in adults with elevated fasting glucose concentrations (n=41)[1]

	Peanut			LFHC			
Outcome	Baseline[2]	Endpoint	Within-condition difference[3]	Baseline[2]	Endpoint	Within-condition difference[3]	Between-condition p-value
Energy, kcal	568 ± 74	691 ± 68	87 (-50, 224)	650 ± 132	800 ± 76	**197 (43, 350)**	0.29
Protein, g	26.0 ± 4.0	29.7 ± 3.5	1.6 (-5.4, 8.6)	30.7 ± 5.9	40.9 ± 3.9	**12.8 (4.9, 20.6)**	0.04
Carbohydrate, g	60.2 ± 7.0	72.9 ± 8.5	10.5 (-6.8, 27.8)	63.6 ± 14.0	81.1 ± 9.5	18.6 (-0.7, 37.9)	0.53
Fat, g	25.8 ± 4.9	32.2 ± 4.2	4.5 (-4.1, 13.1)	31.1 ± 6.6	35.4 ± 4.7	7.7 (-1.9, 17.2)	0.62
SFA, g	7.8 ± 1.6	11.1 ± 1.4	2.3 (-0.6, 5.2)	10.5 ± 2.2	10.6 ± 1.6	1.8 (-1.4, 5.1)	0.84
MUFA, g	8.5 ± 1.6	10.9 ± 1.3	2.0 (-0.7, 4.7)	9.6 ± 1.8	11.3 ± 1.5	2.4 (-0.6, 5.4)	0.85
PUFA, g	7.4 ± 1.8	7.4 ± 1.8	-0.3 (-4.0, 3.3)	8.6 ± 2.2	10.4 ± 2.0	2.6 (-1.4, 6.7)	0.28
Fiber, g	6.6 ± 1.1	6.3 ± 0.9	-0.1 (-1.9, 1.7)	6.1 ± 1.0	7.3 ± 1.0	0.8 (-1.2, 2.9)	0.5
Total vegetable, c-eq	0.5 ± 0.1	0.8 ± 0.2	0.2 (-0.1, 0.6)	0.8 ± 0.2	0.9 ± 0.2	0.3 (-0.1, .7)	0.73
Total grain, oz-eq	1.6 ± 0.3	1.9 ± 0.4	0.2 (-0.7, 1.0)	2.0 ± 0.4	2.8 ± 0.5	**1.0 (0.1, 2.0)**	0.16
Whole grain, oz-eq	0.4 ± 0.2	0.3 ± 0.1	0.0 (-0.3, 0.2)	0.2 ± 0.1	0.3 ± 0.1	-0.1 (-0.3, 0.2)	0.97
Refined grain, oz-eq	1.2 ± 0.2	1.7 ± 0.4	0.2 (-0.6, 1.1)	1.8 ± 0.4	2.5 ± 0.5	**1.1 (0.1, 2.0)**	0.21
Total fruit, c-eq	0.4 ± 0.1	0.4 ± 0.1	0.1 (-0.1, 0.3)	0.3 ± 0.1	0.3 ± 0.1	-0.1 (-0.3, 0.1)	0.38
Total dairy, c-eq	0.5 ± 0.1	0.9 ± 0.1	**0.3 (0.0, 0.6)**	0.8 ± 0.2	0.6 ± 0.2	0.0 (-0.3, 0.3)	0.14
Total protein, oz-eq	2.0 ± 0.4	1.9 ± 0.4	-0.3 (-1.2, 0.5)	2.4 ± 0.7	3.6 ± 0.5	**1.4 (0.4, 2.3)**	**0.01**
Oils, g	10.6 ± 3.1	9.9 ± 2.6	-1.2 (-6.5, 4.1)	12.3 ± 3.9	14.2 ± 2.9	3.1 (-2.8, 9.0)	0.27

[1]Data are presented as least squares means and SE unless otherwise stated; Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the effect of the conditions on each outcome measure, the between-condition p-values represent the main effect of condition. The MIXED procedure

was also used to assess change from baseline, within-condition significant differences are denoted with bold text ($p<0.05$). MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

[2]Values are mean and SE.

[3]Values are least squares mean effect estimate and 95% CI

Table **3-7**: Between-condition mean differences and change from baseline for nutrient and food group intake at dinner in adults with elevated fasting glucose concentrations (n=45)[1]

	Peanut			LFHC			
Outcome	Baseline[2]	Endpoint	Within-condition difference[3]	Baseline[2]	Endpoint	Within-condition difference[3]	Between-condition p-value
Energy, kcal	632 ± 72	828 ± 107	189 (-27, 405)	669 ± 102	937 ± 116	**298 (64, 532)**	0.49
Protein, g	29.5 ± 4.1	43.5 ± 6.0	12 (-0.2, 24.2)	35.2 ± 5.8	46.6 ± 6.5	**15.1 (2.0, 28.3)**	0.73
Carbohydrate, g	59.2 ± 7.4	75.3 ± 9.6	11.7 (-7.7, 31.1)	7.8 ± 14.3	91.1 ± 10.3	**27.5 (6.7, 48.4)**	0.27
Fat, g	31.2 ± 4.1	40.4 ± 6.0	11.6 (-0.5, 23.7)	26.9 ± 3.9	42.2 ± 6.5	**13.4 (0.3, 26.5)**	0.84
SFA, g	10.9 ± 1.6	13.7 ± 2.4	3.6 (-1.2, 8.5)	9.2 ± 1.6	15.2 ± 2.6	5.2 (-0.1, 10.5)	0.67
MUFA, g	10.9 ± 1.5	14.0 ± 1.9	**4.1 (0.2, 7.9)**	9.2 ± 1.4	14.3 ± 2.1	**4.4 (0.2, 8.5)**	0.91
PUFA, g	7.0 ± 1.0	9.9 ± 1.4	**3.4 (0.6, 6.2)**	6.1 ± 0.9	9.0 ± 1.5	2.6 (-0.5, 5.5)	0.65
Fiber, g	5.6 ± 0.9	7.6 ± 0.7	0.8 (-0.6, 2.2)	8.3 ± 1.9	8.8 ± 0.7	**2.0 (0.5, 3.5)**	0.24
Total vegetable, c-eq	0.8 ± 0.2	1.2 ± 0.2	0.3 (-0.0, 0.7)	1.0 ± 0.2	1.3 ± 0.2	**0.4 (0.0, 0.7)**	0.87
Total grain, oz-eq	1.6 ± 0.2	2.5 ± 0.3	0.6 (-0.0, 1.7)	2.4 ± 0.5	2.8 ± 0.3	**0.9 (0.3, 1.5)**	0.46
Whole grain, oz-eq	0.2 ± 0.1	0.2 ± 0.1	0.1 (-0.1, 0.2)	0.1 ± 0.1	0.3 ± 0.1	0.1 (-0.1, 0.3)	0.64
Refined grain, oz-eq	1.4 ± 0.2	2.3 ± 0.3	0.5 (-0.1, 1.1)	2.3 ± 0.5	2.5 ± 0.3	**0.8 (0.1, 1.4)**	0.6
Total fruit, c-eq	0.1 ± 0.1	0.1 ± 0.1	0.0 (-0.2, 0.1)	0.2 ± 0.1	0.2 ± 0.1	0.0 (-0.1, 0.2)	0.54
Total dairy, c-eq	0.5 ± 0.1	0.5 ± 0.1	0.0 (-0.2, 0.3)	0.5 ± 0.1	0.7 ± 0.1	0.2 (-0.1, 0.5)	0.31
Total protein, oz-eq	2.4 ± 0.5	3.7 ± 0.7	1.3 (-0.1, 2.7)	2.5 ± 0.4	3.7 ± 0.7	1.3 (-0.1, 2.8)	0.97
Oils, g	9.1 ± 1.8	11.5 ± 2.0	3.0 (-1.0, 7.1)	8.1 ± 1.5	12.2 ± 2.1	3.7 (-0.6, 8.0)	0.82

[1]Data are presented as least squares means and SE unless otherwise stated; Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the effect of the conditions on each outcome measure, the between-condition p-values represent the main effect of condition. The MIXED procedure

was also used to assess change from baseline, within-condition significant differences are denoted with bold text ($p<0.05$). MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

[2]Values are mean and SE.

[3]Values are least squares mean effect estimate and 95% CI

Table **3-8**: Between-condition mean differences and change from baseline for nutrient and food group intake for snacks in adults with elevated fasting glucose concentrations (n=41)[1]

	Peanut			LFHC			
Outcome	Baseline[2]	Endpoint	Within-condition difference[3]	Baseline[2]	Endpoint	Within-condition difference[3]	Between-condition p-value
Energy, kcal	420 ± 77	510 ± 59	41 (-607, 688)	457 ± 101	381 ± 75	-47 (-844, 749)	0.41
Protein, g	16.5 ± 4.6	19.8 ± 2.7	1.2 (-28.6, 31.1)	17.8 ± 4.6	12.8 ± 3.4	-3.4 (-40.1, 33.3)	0.36
Carbohydrate, g	49.0 ± 9.1	55.7 ± 8.0	5.2 (-85.3, 95.8)	43.3 ± 8.7	50.7 ± 9.9	4.6 (-104.6, 113.7)	0.76
Fat, g	18.5 ± 4.0	23.1 ± 2.7	1.7 (-30.8, 34.2)	25.4 ± 6.7	15.9 ± 3.5	-6.6 (-46.7, 33.6)	0.36
SFA, g	6.8 ± 1.9	14.1 ± 3.4	0.3 (-13.5, 14.1)	7.3 ± 2.0	2.6 ± 3.7	-2.0 (-18.9, 14.9)	0.22
MUFA, g	6.0 ± 1.2	9.0 ± 1.0	1.1 (-10.6, 12.9)	9.6 ± 2.8	5.0 ± 1.4	-2.8 (-17.2, 11.6)	0.27
PUFA, g	4.1 ± 0.8	6.2 ± 1.2	-0.1 (-10.8, 10.5)	7.0 ± 1.9	4.1 ± 1.4	-0.9 (-13.6, 11.7)	0.45
Fiber, g	4.6 ± 1.2	5.5 ± 1.4	0.2 (-7.3, 7.6)	5.3 ± 1.3	5.0 ± 1.6	-0.1 (-9.8, 9.5)	0.87
Total vegetable, c-eq	0.3 ± 0.1	0.2 ± 0.1	-0.2 (-0.7, 0.3)	0.4 ± 0.1	0.0 ± 0.1	-0.2 (-0.8, 0.3)	0.38
Total grain, oz-eq	1.2 ± 0.3	1.5 ± 0.3	0.3 (-2.8, 3.5)	0.8 ± 0.2	1.7 ± 0.4	0.6 (-3.2, 4.4)	0.74
Whole grain, oz-eq	0.2 ± 0.1	0.5 ± 0.2	0.3 (-1.8, 2.4)	0.2 ± 0.1	0.9 ± 0.2	0.8 (-1.7, 3.2)	0.32
Refined grain, oz-eq	1.1 ± 0.3	1.0 ± 0.3	0.0 (-2.6, 2.7)	0.6 ± 0.2	0.7 ± 0.3	-0.2 (-3.4, 3.0)	0.62
Total fruit, c-eq	0.3 ± 0.1	0.5 ± 0.2	0.1 (-2.0, 2.3)	0.4 ± 0.1	0.4 ± 0.2	0.1 (-2.4, 2.5)	0.79
Total dairy, c-eq	0.6 ± 0.3	1.0 ± 0.3	0.0 (-2.1, 2.1)	0.5 ± 0.2	0.4 ± 0.3	0.0 (-2.4, 2.4)	0.36
Total protein, oz-eq	0.9 ± 0.2	1.4 ± 0.2	0.1 (-2.5, 2.7)	1.8 ± 0.7	0.6 ± 0.3	-0.6 (-3.7, 2.6)	0.25
Oils, g	5.3 ± 1.2	7.7 ± 1.2	-0.9 (-15.2, 13.4)	11.7 ± 3.9	3.8 ± 1.5	-4.2 (-21.7, 13.4)	0.3

[1]Data are presented as least squares means and SE unless otherwise stated; Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the effect of the conditions on each outcome measure, the between-condition p-values represent the main effect of condition. The MIXED procedure

was also used to assess change from baseline, within-condition significant differences are denoted with bold text ($p<0.05$). MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

[2]Values are mean and SE.

[3]Values are least squares mean effect estimate and 95% CI; Δ endpoint value – baseline.

Table **3-9**: Between-condition mean differences and change from baseline for nutrient and food group intake for beverages in adults with elevated fasting glucose concentrations (n=29)[1]

	Peanut			LFHC			
Outcome	Baseline[2]	Endpoint	Within-condition difference[3]	Baseline[2]	Endpoint	Within-condition difference[3]	Between-condition p-value
Energy, kcal	302 ± 105	123 ± 45	-152 (-267, -37)	258 ± 130	160 ± 53	-115 (-250, 119)	0.5
Protein, g	13.2 ± 5.3	4.3 ± 2.1	-7.6 (-13.1, -2.1)	12.2 ± 6.6	6.6 ± 2.6	-5.2 (-11.9, 1.4)	0.43
Carbohydrate, g	36.1 ± 13.8	16.7 ± 6.6	-13.7 (-30.6, 3.2)	23.6 ± 13.2	20.1 ± 8.0	-10.4 (-30.9, 10.1)	0.7
Fat, g	12.2 ± 4.4	3.3 ± 1.9	-9.1 (-14.0, -4.2)	13.6 ± 6.5	6.1 ± 2.2	-6.3 (-12.0, -0.5)	0.26
SFA, g	4.0 ± 1.7	1.2 ± 0.6	-2.9 (-4.5, -1.3)	4.5 ± 2.5	1.6 ± 0.7	-2.5 (-4.2, -0.8)	0.4
MUFA, g	4.1 ± 1.5	0.9 ± 0.5	-3.3 (-4.4, -2.1)	4.6 ± 2.1	1.6 ± 0.6	-2.5 (-4.1, -1.0)	0.4
PUFA, g	3.2 ± 1.1	0.7 ± 0.6	-2.5 (-4.1, -1.0)	3.6 ± 1.7	1.9 ± 0.7	-1.4 (-3.3, 0.4)	0.17
Fiber, g	3.4 ± 1.7	0.7 ± 0.4	-1.9 (-3.1, -0.8)	2.4 ± 1.6	1.5 ± 0.6	-1.1 (-2.6, 0.4)	0.32
Total vegetable, c-eq	0.3 ± 0.2	0.1 ± 0.1	-0.2 (-0.4, 0.1)	0.2 ± 0.1	0.2 ± 0.1	0.0 (-0.4, 0.3)	0.41
Total grain, oz-eq	0.9 ± 0.4	0.4 ± 0.3	-0.3 (-1.0, 0.4)	0.4 ± 0.2	0.5 ± 0.3	-0.2 (-0.9, 0.6)	0.56
Whole grain, oz-eq	0.1 ± 0.1	0.2 ± 0.1	0.1 (-0.2, 0.4)	0.0 ± 0.0	0.2 ± 0.1	0.1 (-0.2, 0.5)	0.78
Refined grain, oz-eq	0.8 ± 0.4	0.2 ± 0.2	0.4 (-0.8, 0.0)	0.4 ± 0.2	0.4 ± 0.2	-0.3 (-0.8, 0.2)	0.42
Total fruit, c-eq	0.2 ± 0.1	0.1 ± 0.1	0.0 (-0.2, 0.1)	0.2 ± 0.2	0.1 ± 0.1	-0.1 (-0.3, 0.2)	0.91
Total dairy[2,4], c-eq	0.3 ± 0.1	<0.01 ± <0.01	-	0.3 ± 0.2	<0.01 ± <0.01	-	-
Total protein, oz-eq	0.9 ± 0.4	0.4 ± 0.2	-0.5 (-0.9, -0.1)	1.1 ± 0.6	0.5 ± 0.2	-0.4 (-0.9, 0.1)	0.82
Oils, g	3.6 ± 1.3	0.9 ± 0.5	-3.1 (-4.3, -1.9)	4.7 ± 2.1	1.5 ± 0.6	-2.5 (-4.2, -0.9)	0.51

[1]Data are presented as least squares means and SE unless otherwise stated; Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the effect of the conditions on each outcome measure, the between-condition p-values represent the main effect of condition. The MIXED procedure

was also used to assess change from baseline, within-condition significant differences are denoted with bold text ($p<0.05$). MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

[2]Values are mean and SE.

[3]Values are least squares mean effect estimate and 95% CI; Δ endpoint value – baseline.

[4]Endpoint intake too low; data did not converge.

Chapter 4

Peanuts as a Nighttime Snack Enrich Butyrate Producing Bacteria Compared to an Isocaloric Lower-Fat Higher-Carbohydrate Snack in Adults with Elevated Fasting Glucose: A Randomized Crossover Trial

Abstract

Objectives: To assess the effect of consuming 28 g/d of peanuts as a nighttime snack for 6-weeks, compared to an isocaloric lower-fat higher-carbohydrate (LFHC) snack, on gut microbiota composition in adults with elevated fasting glucose. Further, to identify functional and compositional differences in primary endpoint responders using metatranscriptomics and assess the relationship between cardiovascular disease (CVD) risk factors and microbial diversity.

Methods: In a randomized, crossover trial, 50 adults (48% female; 42 ± 15 y; BMI 28.3 ± 5.6 kg/m^2; glucose 100 ± 8 mg/dL) consumed 28g/d of dry roasted, unsalted, peanuts (164 kcal; 11% E carbohydrate, 17% E protein, 73% E fat, and 2.4g fiber) or a LFHC snack [6 whole grain crackers and one slice of pre-packaged low-fat cheese (164 kcal; 53% E carbohydrate, 17% E protein, 33% E fat, and 3g fiber)] for 6-wk with a 4-wk washout period. Fecal samples and CVD risk factors (glucose, insulin, fructosamine, and lipids/lipoproteins) were collected at the baseline and endpoint of each period. Gut microbiota composition was measured using 16S rRNA sequencing. Exploratory metatranscriptomic analyses were conducted on baseline and endpoint samples from subjects with the greatest reduction in fasting glucose (primary endpoint responders) following the Peanut condition (n=24), to measure gene expression related to microbial metabolic pathways. The NUGEN library preparation method was used to generate cDNA. Kraken2 and Emapper were used for taxonomic and functional gene annotation,

respectively. Additionally, correlations between microbial diversity and CVD risk factors were assessed.

Results: No between-condition differences in α- or β-diversity were observed. Following peanut intake, *Roseburia* and *Ruminococcaceae* were significantly enriched [Linear discriminant analysis score (LDA) = 3.1 and 2.8; $P = 0.035$ and $P = 0.037$)] compared to LFHC. Metatranscriptomics demonstrated enrichment of the K03518 (aerobic carbon-monoxide dehydrogenase small subunit) gene following peanut intake (LDA = 2.0; $P = 0.039$) and *Roseburia intestinalis L1-82* was identified as a contributor to the increased expression. No correlations were observed between endpoint glucose and measures of α-diversity for either condition.

Conclusion: Enrichment of *roseburia* was observed following consumption of 28 g/d of peanuts in adults with elevated fasting glucose. Metatranscriptomics revealed enrichment of the K03518 gene, which is associated with short chain fatty acid production and degradation of β-mannans. These results suggest peanut intake enriches a known butyrate producer and the increased expression of a gene implicated in butyrate production adds further support for peanut-induced gut microbiome modulation.

4.1 Introduction

The prevalence of prediabetes and type 2 diabetes in United States adults has increased in recent years with an estimated 88 and 34.2 million cases in 2018 (1). Recent evidence suggests gut microbiota play a role in glycemic control (122) and microbial composition varies by glycemic status (123). Dietary intake impacts gut microbiota composition and dietary compounds, like fiber, are degraded by gut bacteria producing metabolites that affect host health (69). However, limited clinical research has been conducted to examine the relationship between diet and microbiota composition and functionality in adults with elevated fasting glucose.

Peanuts and tree nuts are recommended as part of healthy dietary patterns and are rich in unsaturated fatty acids and fiber (8,14). Tree nuts beneficially impact gut microbiota by increasing diversity and abundance of short-chain fatty acid (SCFA) producing bacteria (14). At present, no studies have examined the effect of peanuts on gut microbiota composition. Furthermore, in all of the prior analyses investigating the effects of tree nuts on the gut microbiota, 16S rRNA sequencing has been. Next generation sequencing techniques such as metatranscriptomics assesses bacterial gene expression as an indicator of microbiota functionality. Investigation of the effect of peanuts, a commonly consumed nut, on gut microbiota composition as well as bacterial gene expression will contribute to understanding dietary modulation of the gut microbiome.

The objective of this pre-specified secondary analysis was to evaluate the effect of nighttime peanut consumption (28 g) for 6-weeks on gut microbiota composition, assessed by 16S rRNA sequencing, compared to a lower-fat higher-carbohydrate snack in individuals with elevated fasting glucose. Exploratory metatranscriptomics analyses were conducted to identify changes in bacterial gene expression following peanut consumption in subjects with the greatest reduction in fasting plasma glucose (primary endpoint responders). In addition, exploratory analyses were conducted to examine the correlation between microbial diversity and risk factors

for cardiometabolic diseases. We hypothesized that peanut consumption would promote beneficial shifts in the gut microbial community and increase the relative abundance of SCFA producing bacteria compared with the lower-fat, higher-carbohydrate (LFHC) snack.

4.2 Methods

4.2.1 Study Design

The details of the trial design and glycemic, lipid/lipoprotein, vascular, and diet-related endpoints are reported elsewhere (107).The results from a planned secondary analysis, 16S rRNA sequencing on all samples to assess fecal bacterial diversity, are presented here. Additionally, results from exploratory metatranscriptomics analyses in a subset of participants that responded to the Peanut condition defined by the greatest reduction in fasting plasma glucose from baseline to the end of the condition (6 weeks) are presented. Briefly, we conducted a randomized, crossover, trial consisting of two 6-week dietary interventions (a Peanut condition and a lower-fat higher-carbohydrate condition [LFHC]) with a 4-week compliance break (median break 28 days; range 28-41 days. The conditions (Peanut vs. LFHC) were energy (~160 kcal) and protein (7 g) matched but varied in total fat (14 g vs. 6 g) and carbohydrate (6 g vs 22 g) content. Participants were provided the snack and instructed to consume them in the evening, after dinner, and before bedtime. In addition, they were asked to avoid peanuts and tree nuts throughout the trial while all other aspects of the diet remained unchanged. Compliance was monitored using daily questionnaires and overall compliance with the study protocol was 88%. The protocol for this study was approved by the Pennsylvania State University (University Park, Pennsylvania) Institutional Review Board. Written informed consent was completed by all participants prior to screening for this study. This trial is registered at ClinicalTrials.gov identifier NCT03654651.

4.2.2 Participants

Study participants were recruited from the State College, Pennsylvania area between November 2018 and September 2019. A preliminary telephone screening was conducted with interested participants to determine potential eligibility. Potentially eligible individuals attended a clinic screening appointment for final determination of eligibility based on the inclusion/exclusion criteria. Eligible participants were healthy women and men aged 18-75 y with elevated fasting plasma glucose (≥100 mg/dL and ≤125 mg/dL), taking no lipid, glucose, or blood-pressure lowering medications; or taking antibiotics within 6-weeks prior to enrollment. Study samples were collected at the Penn State University Clinical Research Center (CRC).

4.2.3 Glycemic Control, Lipids, Lipoproteins, and Vascular Outcomes

Baseline and endpoint measures were collected for all outcomes. Blood samples were collected on two consecutive days at each baseline and endpoint visit. Fasting plasma glucose and serum fructosamine, insulin, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides were analyzed at the Pennsylvania State University Biomarker Core Lab (University Park, PA). Vascular health was assessed on one day at each baseline and endpoint visit with a SphygmoCor XCEL (AtCor Medical, Sydney, Australia). Additional details about experimental methodologies used are published elsewhere (107).

4.2.4 Fecal Sample Collection

Two fecal samples were collected from a single defecation ≤48-hours before day-2 testing at each baseline and endpoint visit using a collection kit (Ziploc bags, cooler, icepack, non-latex gloves, a long-handled spoon, a stool collection hat, and 2-30mL Para-Pak Clean Vials; Meridian Bioscience, Cincinnati, Ohio). Participants were instructed to store the fecal samples in their freezer until they transported them in a cooler with an icepack to the CRC. Samples were stored at -80°C until analysis. All analyses were done by Wright Labs, LLC (Huntingdon, Pennsylvania).

4.2.5 16S rRNA Methods

4.2.5.1 DNA Extraction and Quantification

DNA was extracted from samples using the DNeasy PowerSoil kit according to the manufacturer's protocol (Qiagen, Frederick, Maryland) and eluted using 50 μL of DNase/RNase free water. Following extraction, samples were quantified using an Invitrogen Qubit 4 Fluorometer and 1X Qubit dsDNA High Sensitivity Assay Kit (ThermoFisher Scientific, Waltham, Massachusetts).

4.2.5.2 PCR Amplification

All 16S rRNA illumina-tag PCR reactions were performed on DNA extracts per the Earth Microbiome Project's protocol (124). PCR products were pooled, and gel purified on a 2% agarose gel using the QIAquick Gel Purification Kit (Qiagen, Frederick, Maryland, USA). Prior to sequencing, the purified pool was quality checked using an Agilent 2100 BioAnalyzer and

Agilent DNA High Sensitivity DNA kit (Agilent Technologies, Santa Clara, California). The purified pool was stored at -20˚C and then sequenced at Wright Labs, LLC (Huntingdon, Pennsylvania) using an Illumina MiSeq v2 chemistry with paired-end 250 base pair reads.

4.2.5.3 Bioinformatic Analysis Procedures

Raw data were imported into QIIME2 for processing and analyses (125). Initial quality in the form of Phred q scores were determined using QIIME2, while cumulative expected error for each position was determined with VSEARCH (126). Based on these quality data, forward reads were truncated at a length of 197 and reverse reads were truncated at a length of 200, with a maximum expected error of 0.5 for both within QIIME2's implementation of the DADA2 pipeline (127). Additionally, QIIME2's DADA2 pipeline was used to merge forward and reverse reads, remove chimeras, and assign the remaining sequences to amplicon sequence variants (ASVs).

Representative sequences were used to determine taxonomic information for the ASVs, using a Naive Bayes classifier as implemented in QIIME2's "qiime feature-classifier classify-sklearn" command, with a pre-trained Silva 132 database containing 515F/806R sequences (128). Additionally, representative sequences were used to create a rooted phylogenetic tree using MAFFT (129) and FastTree2 (130) through QIIME2's "qiime phylogeny align-to-tree-mafft-fasttree" command.

ASVs identified as Mitochondria or Chloroplasts were removed because they likely represented eukaryotic contamination, instead of true bacterial signals. Samples with fewer than 1,000 sequences remaining after filtration were removed from the ASV table.

4.2.5.4 *α-diversity Analysis*

α-diversity was calculated by subsampling the ASV table at 10 different depths, ranging from 800 to 8000 sequences, for the Faith's Phylogenetic Diversity (131), Observed features, and Pielou's Evenness metrics (132). Twenty iterations were performed at each depth to obtain average α-diversity values for the different metrics. A rarefaction plot was created with the results of this subsampling to confirm that diversity approached an asymptote and slope decreased as depth increased. Averages for the greatest depth were used to see if any of the α-diversity metrics differed significantly between condition and timepoint (Kruskal-Wallis, $p \leq 0.05$)

4.2.5.5 *β-diversity Analysis*

β-diversity analyses were conducted after the ASV table had first undergone cumulative sum scaling normalization to mitigate differences between samples based on sequencing depth (133). Distances between samples were calculated using the Weighted Unifrac metric based on the normalized table and rooted tree (134). The resulting distance matrix was visualized as a Principal Coordinates Analysis (PCoA) plot. Statistical differences between sample groupings based on condition and timepoint were evaluated as well (PERMANOVA, $p \leq 0.05$). Adonis tests were used with numerical and categorical metadata to see if they significantly ($p \leq 0.05$) explained variation among the bacterial communities based on the Weighted Unifrac distance matrix. Partial Least Squares Discriminant Analysis (PLS-DA) was performed using the mixOmics package in R to assess distinct clustering according to timepoint with the normalized table for samples from the Peanut condition only (135).

4.2.5.6 Differential Feature Analysis

Linear discriminant analysis effect size (LEfSe) was conducted to identify taxa that had significantly different abundances between Peanut and LFHC conditions and based on timepoint within the same condition (136). The ASV table was collapsed to level 7 (species) using Qiime2. Counts per million (CPM) normalization and initial table formatting was done thru R.

4.2.6 Metatranscriptomics Methods

4.2.6.1 Selection of Subset

In a subset of participants (n=24) with the greatest reduction in fasting plasma glucose during the Peanut condition (primary endpoint responders) metatranscriptomics sequencing was performed on baseline and endpoint samples.

4.2.6.2 RNA Extraction, Quantification, and Metatranscriptomic Library Preparation

RNA was extracted from samples using the ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research, Irvine, California) according to the manufacturer's protocol and eluted using 50 uL of DNase/RNase free water. After extraction, samples were quantified using an Invitrogen Qubit 4 Fluorometer and RNA HS Assay Kit (ThermoFisher Scientific, Waltham, Massachusetts). Metatranscriptomic libraries were prepared using RNA extracts and the NEB Ultra II RNA Library Prep kit (New England BioLabs, Ipswich, Massachusetts). Libraries were quality checked using an Agilent 2100 Bioanalyzer and DNA High Sensitivity kit and then pooled in an equimolar ratio. The pool was gel purified using a 2% agarose gel and the Qiagen

QIAquick gel extraction kit (Qiagen, Germantown, Maryland). Following purification, the pool was sequenced at UC Davis Genome Center (Davis, California) using an Illumina Hiseq 4000 to produce 2x150 bp reads.

4.2.6.3 Bioinformatic Analysis Procedures

Based on initial quality, fastp was used to filter the data with the last 3 bases being trimmed for the reverse reads and a sliding window of 4 with a minimum average Phred q score of 28 being used for both forward and reverse (137). Sequences shorter than 90 bp were discarded.

The remaining sequences were then annotated using Kraken2 with a version of its standard database that included fungi in addition to the standard libraries (138). A table of species level annotations was created for use with downstream analyses, with *Homo sapiens* being excluded to avoid human contamination impacting results.

Sequences classified as *Homo sapiens* were removed, and the remaining classified sequences were dereplicated with VSEARCH (126). Emapper v2.0 (139) was run on the dereplicated sequences with the EggNOG 5.0 database (140). Hits against Kegg Orthologs were then used, along with the abundances of the sequence they aligned to, to create a table of gene abundances. A second table with hits for Kegg Orthologs split by contributor (Kraken2's taxonomic assignment for the matching sequence) was also created.

4.2.6.4 α-diversity Analysis

α-diversity was calculated by subsampling the table at 10 different depths, ranging from 66000 to 660000 sequences for observed species and 910 to 9100 for observed genes. 20 iterations were performed at each depth to obtain average α-diversity values for the different

metrics (Observed Features and Pielou's Evenness) (132,141). Averages for the greatest depth were used to see if any of the α-diversity metrics differed significantly based on timepoint (Kruskal-Wallis, $p \leq 0.05$).

4.2.6.5 β-diversity Analysis

β-diversity analyses were conducted after the tables had first undergone CPM normalization to mitigate differences between samples based on sequencing depth. Distances between samples were calculated using the Bray-Curtis distance metric (142). The resulting distance matrix was visualized as a PCoA plot. Statistical differences between sample groupings based on timepoint were evaluated as well (PERMANOVA, $p \leq 0.05$). Adonis tests were used with numerical and categorical metadata to examine whether these variables significantly ($p \leq 0.05$) explained any of the variation in the gene expression based on the Bray-Curtis distance matrices. PLS-DA was performed using the mixOmics package in R to assess distinct clustering of expressed genes according to timepoint with the normalized tables (135).

4.2.6.6 Differential feature analysis

Biomarker analysis was performed using LEfSe to identify genes that had significantly different abundances based on timepoint (136). The table was normalized with the counts per million method. Only genes identified as having significantly differential abundance (Kruskal-Wallis, $p \leq 0.05$) with a log(LDA) score of at least 2.0 were considered to be enriched.

4.2.6.7 Contribution analysis

Taxonomic contributors to active gene expression were determined by assessing all sequences identified from a gene and the contribution values (relative abundance) were calculated by dividing the number of sequences per taxa by the total number of sequences from that gene. The contributions table was first CPM-normalized. Contributing taxa for the enriched gene of interest (K03518) were then determined using a custom Python script to select only rows containing those genes and then reformatting the resulting table so that genes were columns and taxa were rows.

4.2.6.8 Correlation Analysis

Spearman correlations were calculated between endpoint fasting serum lipids/lipoproteins and plasma glucose, fructosamine, insulin, BMI, and α-diversity metrics based on data for samples present in all three datasets (ASVs, genes, and species). Fasting plasma glucose, insulin, and serum lipid/lipoproteins values were an average of two samples collected on two consecutive days at endpoint visits. Fructosamine values were from a single sample collected during day one of each endpoint visit. Two samples without α-diversity values were not included because the maximum rarefaction depth was set higher than their total species counts.

4.3 Results

4.3.1 Participants

A total of 51 participants were enrolled, 51 were randomized, and 50 completed the study (**Figure 4-1**). One participant withdrew prior to receiving study food due to personal reasons.

Fecal samples were available for all 50 participants at all timepoints. 16S rRNA sequencing was completed for all 50 participants. Baseline characteristics are presented in (**Table 4-1**). Metatranscriptomics sequencing was completed on baseline and endpoint samples from a subset of primary endpoint responders (based on the greatest reduction in plasma fasting glucose) for the Peanut condition only (n=24). Participants included in the metatranscriptomics sequencing (**Table 4-2**) were similar to the total sample having elevated fasting glucose (101 ± 9 mg/dL) and BMI (28.7 ± 5.6 kg/m^2).

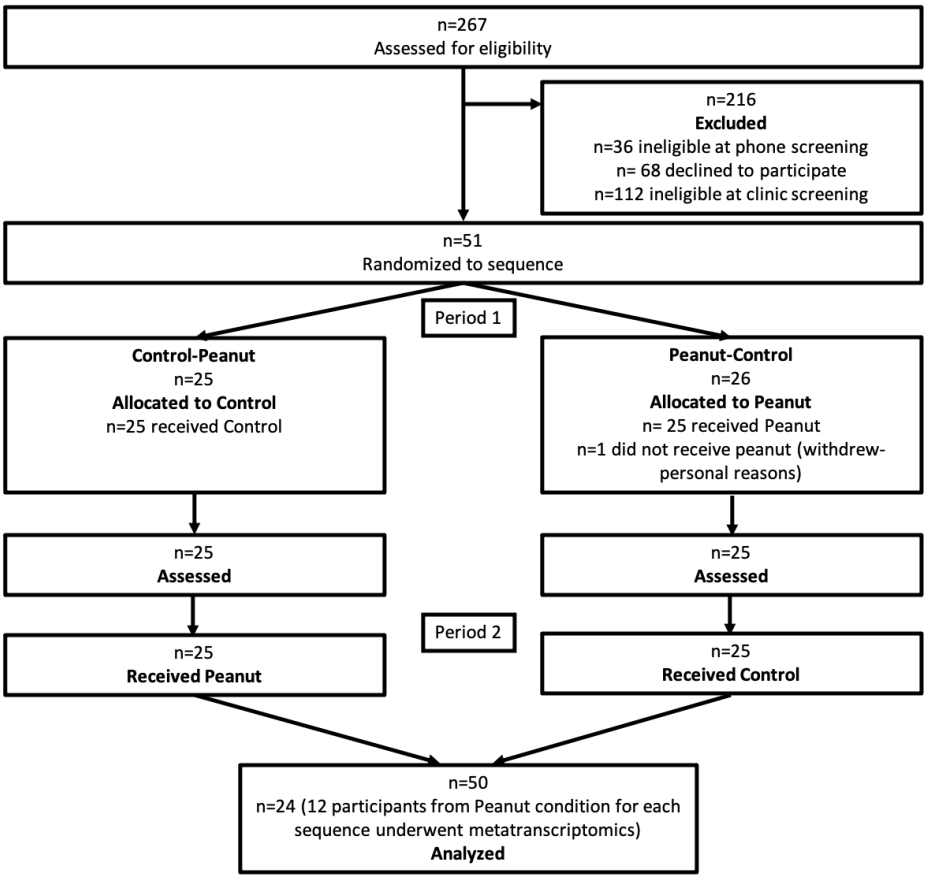

Figure **4-1**: Consort diagram

4.3.2 16S rRNA

4.3.2.1 Sequencing Results

16S rRNA gene PCR amplification of the hypervariable V4 region was successfully completed on all samples. High-quality sequencing data were obtained from 198 fecal samples. After quality filtering, sequence counts ranged from 1,013 to 59,445 sequences per sample. A total of 7,970,680 sequences were obtained after quality filtering, merging, and chimera removal.

Table **4-1**: Baseline characteristics of study participants overall and by randomization sequence (n=50)[1]

Characteristic	Peanut-LFHC	LFHC-Peanut	Total
n (% female)	24 (50%)	26 (46%)	50
Age, y	40 ± 15	43 ± 15	42 ± 15
Weight, kg	85.0 ± 21.1	82.7 ± 16.5	83.8 ± 18.7
Height, m	1.72 ± 0.1	1.71 ± 0.1	1.72 ± 0.1
BMI, kg/m^2	28.5 ± 6.0	28.2 ± 5.2	28.3 ± 5.6
Glucose, mg/dL	99 ± 8	101 ± 8	100 ± 8

[1]Values are mean and SD unless otherwise stated

Table **4-2**: Baseline characteristics of study participants overall and by randomization sequence for metatranscriptomics subset (n=24)[1]

	Peanut		
Characteristic	First Period	Second Period	Total
n (% female)	12 (42%)	12 (50%)	24
Age, y	36 ± 14	46 ± 16	41 ± 15
Weight, kg	86.6 ± 20.6	83.4 ± 13.2	85.0 ± 17.0
Height, m	1.74 ± 0.1	1.71 ± 0.1	1.72 ± 0.1
BMI, kg/m^2	28.6 ± 6.5	28.8 ± 4.9	28.7 ± 5.6
Glucose, mg/dL	101 ± 8	101 ± 11	101 ± 9

[1]Values are mean and SD unless otherwise stated

4.3.2.2 α- and β-diversity

The composition of the bacterial communities at the phyla level was not significantly different between conditions (*P > 0.05*). Firmicutes, Bacteroidetes, and Actinobacteria were the most abundant bacteria phyla in the baseline and endpoint samples for both conditions (**Figure 4-2, supplementary**).

No significant between-condition differences were observed in α-diversity assessed by Faith's Phylogenetic Diversity (*P=0.905*) (**Figure 4-3, Panel A**), Observed Features (*P=0.749*) (**Figure 4-3, Panel B**), and Pielou's Evenness (*P=0.823*) (**Figure 4-3, Panel C**) following the conditions. Faith's Phylogenetic Diversity [median (inter quartile range)] at the baseline [Peanut: 9.8 (2.9); LFHC: 9.8 (3.0)] and endpoint [Peanut: 10.4 (2.9); LFHC: 10.6 (3.2)] of each condition was similar (**Figure 4-3, Panel A**). Observed Features [median (inter quartile range)] at the baseline [Peanut: 131.2 (48.6); LFHC: 132.3 (51.0)] and endpoint [Peanut: 133.2 (44.0); LFHC: 142.9 (49.7)] of each condition was similar (**Figure 4-3, Panel B**). Peilou's Evenness [median (inter quartile range)] at the baseline [Peanut: 0.76 (0.07); LFHC: 0.77 (0.07)] and endpoint [Peanut: 0.79 (0.08); LFHC: 0.75 (0.09)] of each condition was similar (**Figure 4-3, Panel C**).

β-diversity was unchanged following both conditions. Samples did not significantly cluster based on timepoint for the Peanut or LFHC conditions (PERMANOVA, *P = 0.853*).

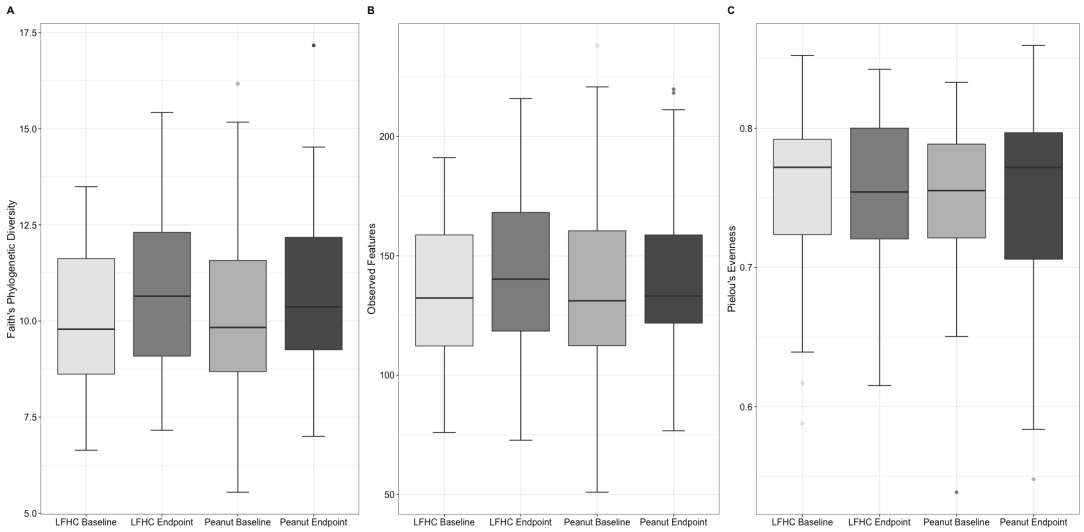

Figure **4-3**: Between-condition differences in α-diversity for the Peanut and LFHC condition in adults with elevated fasting glucose concentrations (n=50).

Panel A: α-diversity values based on the Faith's Phylogenetic Diversity Metric and presented with a boxplot. Panel B: α-diversity values based on the Observed Features metric and presented with a boxplot. Panel C: α-diversity values based on Pielou's Evenness metric and presented with a boxplot.

4.3.2.3 Taxonomic Biomarker Results

LEfSe plot (LDA ≥ 2) comparisons between conditions revealed several taxa that were differentially abundant [**Figure 4-4 and Table 4-3 (supplementary)**]. *Roseburia* (LDA = 3.1 and $P = 0.035$) and *Ruminococcaceae* (LDA = 2.8 and $P = 0.037$) were significantly more abundant following the Peanut condition vs. the LFHC condition. Within-condition comparisons showed *Roseburia* (LDA = 2.9 and $P = 0.026$) and *Ruminococcaceae* (LDA = 2.4 and $P = 0.007$) were significantly more abundant following the Peanut condition vs. baseline. Additionally, *Ruminococcaceae* (LDA = 2.7 and $P = 0.036$) was significantly more abundant following the LFHC condition vs. baseline.

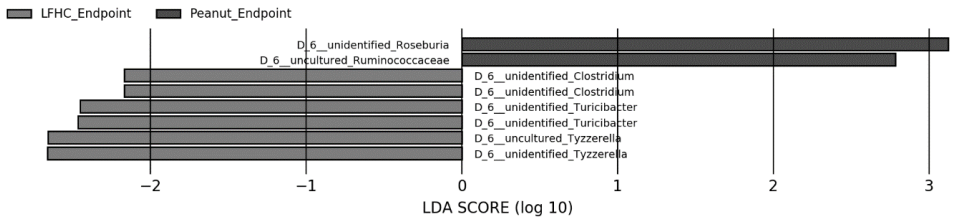

Figure **4-4**: Between-condition comparisons in bacteria enrichment in adults with elevated fasting glucose concentrations (n=50).

LefSe plots displaying significantly enriched ($p < .05$, LDA > 2) taxa between LFHC and Peanut conditions. LDA scores are displayed on the x-axis and quantify the strength of enrichment within each respective categorical group. LefSe, linear discriminant analysis effect size; LDA, linear discriminant analysis.

4.3.3 Metatranscriptomics Subset

4.3.3.1 Sequencing Results

Metatranscriptomics sequencing was successfully completed on all 48 samples. Overall, 47 out of 48 samples had greater than 500,000 sequences remaining after quality filtering, with a range of 602,685 to 31,624,379 sequences per sample. One sample was omitted from all downstream analyses due to yielding 264,431 raw sequences. A total of 399,270,945 sequences were obtained after quality filtering. The microbiota diversity and composition, assessed by 16S rRNA sequencing, of the participants in the metatranscriptomics subset was similar to the whole cohort (**Table 4-4, supplementary**)

4.3.3.2 α- and β-diversity

No significant shifts in α-diversity occurred following the Peanut condition for active composition (Observed Features: $P = 0.590$; Pielou's Evenness: $P = 0.818$) (**Figure 4-5, Panel A and C**) or genes (Observed Features: $P = 0.455$; Pielou's Evenness: $P = 0.869$) vs. baseline (**Figure 4-5, Panel B and D**).

β-diversity was unchanged following the Peanut condition. Samples did not significantly cluster based on timepoint for the active composition (PERMANOVA, $P = 0.998$) or genes (PERMANOVA, $P = 0.590$) datasets. However, large intra-individual shifts in gene expression were observed between baseline and endpoint for the same subject (**Figure 4-6**). Additionally, PLS-DA of genes revealed consistent differences between timepoints (**Figure 4-7**).

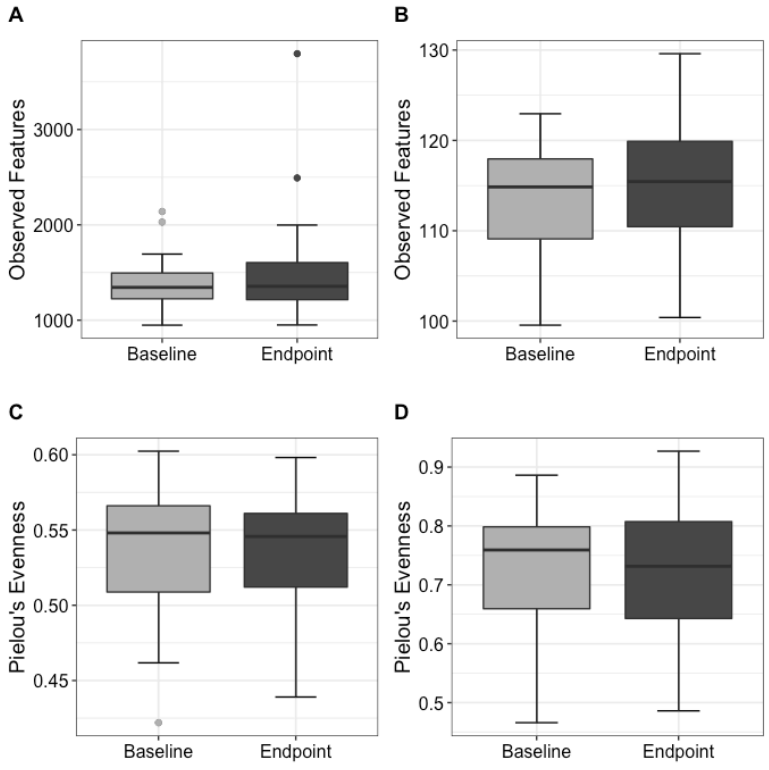

Figure **4-5**: Between-condition comparisons in bacteria enrichment in adults with elevated fasting glucose concentrations (n=50).

Panel A: Within-condition differences in α-diversity for the Peanut condition based on Observed Features metric with boxplots for metatranscriptomics compositional data. Panel B: Within-condition differences in α-diversity for the Peanut condition based on Observed Features metric with boxplots for gene data. Panel C: Within-condition differences in α-diversity for the Peanut condition based on Pielou's Evenness metric with boxplots for metatranscriptomics compositional data. Panel D: Within-condition differences in α-diversity for the Peanut condition based on Pielou's Evenness metric with boxplots for metatranscriptomics gene data.

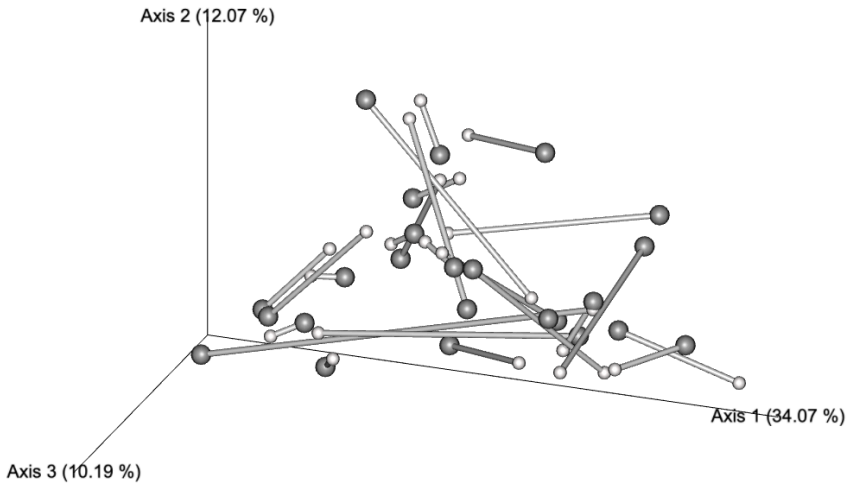

Figure **4-6**: Inter-individual changes in β-diversity in response to the Peanut condition in adults with the greatest reduction in fasting glucose (n=24).

PCoA plot based on the Bray-Curtis matrix for the genes dataset. Light spheres represent baseline and dark spheres represent endpoint. PCoA, principal coordinate analysis.

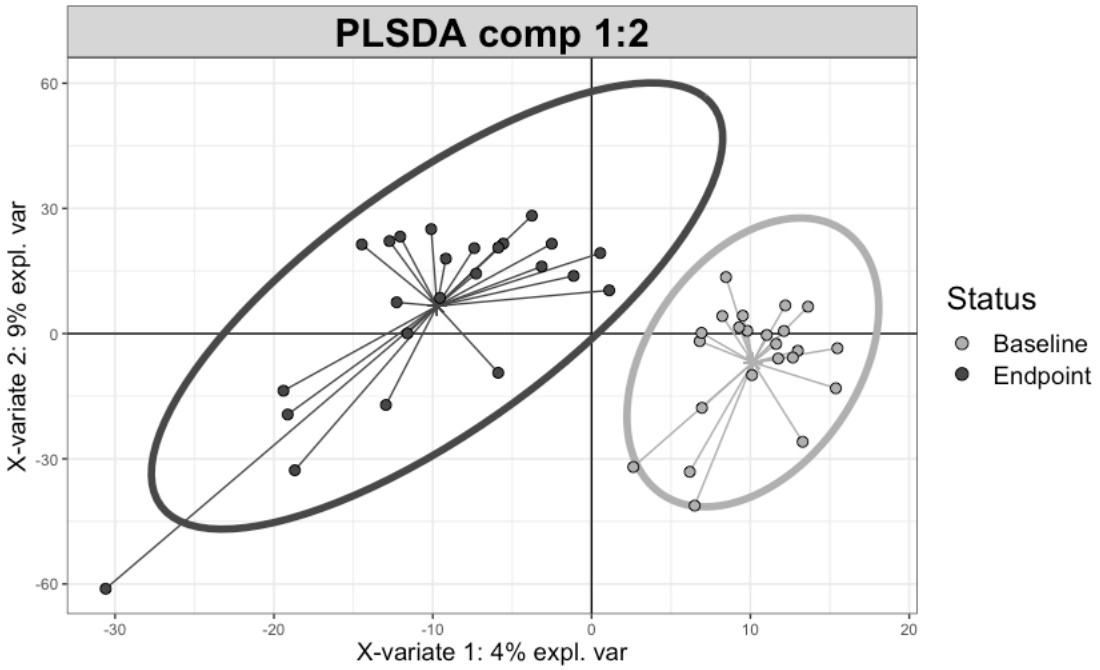

Figure **4-7**: Within-condition changes in β-diversity in response to the Peanut condition in adults with the greatest reduction in fasting glucose (n=24).

PLS-DA plot of baseline and endpoint samples based on cpm-normalized expression of detected genes, ellipses represent 95% CI. PLS-DA, partial least squares discriminant analysis.

4.3.3.3 Taxonomic Biomarker Results

Multiple genes were significantly enriched at baseline compared to the end of the Peanut condition [**Figure 4-8 and Table 4-5 (supplementary)**]. Following the Peanut condition, the K03518 (aerobic carbon-monoxide dehydrogenase small subunit) gene was enriched (LDA = 2.0 and $P = 0.039$) vs. baseline. The top two contributors to K03518, *Blautia* sp. SC05B48 (39.7–53.0% of sequences identified as K03518) and *Faecalibacterium prausnitzii* (5.2–8.9% of sequences identified as K03518), were the same at baseline and following the Peanut condition. *Roseburia intestinalis L1-82*, represented 1.5% of the relative contribution in the endpoint samples with no detectable relative contribution in the baseline samples [**Table 4-6 and Figure 4-9 (supplementary)**].

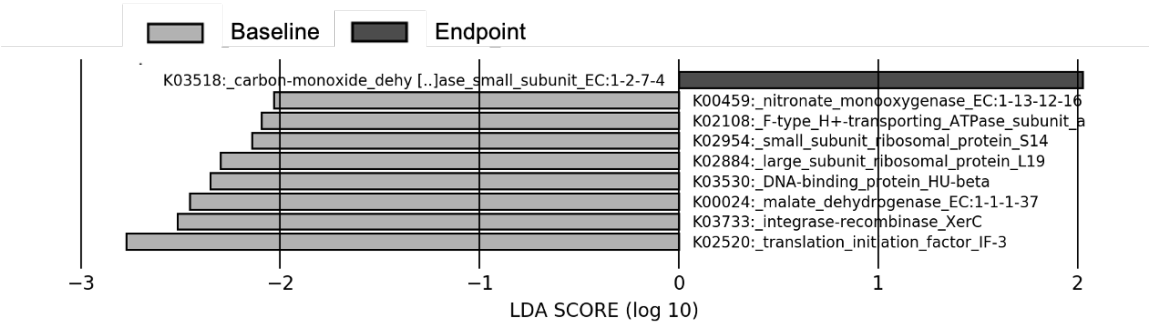

Figure **4-8**: Within-condition comparisons of enriched genes for the Peanut condition in adults with the greatest reduction in fasting glucose (n=24).

LefSe plots displaying significantly enriched ($p < .05$, LDA > 2) genes. LDA scores are displayed on the x-axis and quantify the strength of enrichment within each respective categorical group. LefSe, linear discriminant analysis effect size; LDA, linear discriminant analysis.

Table **4-6**: Relative taxonomic contributors to K03518 gene expression for the Peanut condition in adults with the greatest reduction in fasting glucose (n=24)[1]

Baseline K03518		Endpoint K03518	
Contributor	Relative Contribution	Contributor	Relative Contribution
Blautia sp SC05B48	39.70%	Blautia sp SC05B48	53.00%
Faecalibacterium prausnitzii	8.90%	Faecalibacterium prausnitzii	5.20%
Parabacteroides distasonis	6.70%	Unclassified Lachnospiraceae	3.20%
Unclassified Parabacteroides	4.70%	Parabacteroides distasonis	2.80%
Unclassified Oscillospiraceae	3.60%	Sulfurospirillum sp UCH001	2.70%
Parolsenella catena	3.00%	Unclassified Desulfovibrionaceae	2.40%
Pseudodesulfovibrio sp SRB007	2.20%	Unclassified Parabacteroides	1.80%
Desulfovibrio piger	2.10%	Unclassified Alphaproteobacteria	1.60%
Butyricimonas faecalis	2.10%	Anaerostipes hadrus	1.60%
Unclassified Alphaproteobacteria	1.80%	Roseburia intestinalis L1	1.50%
Other	25.20%	Other	24.20%

[1]Relative contribution values determined by assessing all sequences identified from a gene and the values (relative abundance) calculated by dividing the number of sequences per taxa by the total number of sequences from that gene

4.3.3.4 Correlations with Cardiometabolic Risk Factors

There were no significant correlations between 16S rRNA α-diversity measures and endpoint glucose for either condition. Similarly, endpoint glucose was not related to α-diversity metrics based on metatranscriptomics (**Table 4-7, supplementary**). A significant positive relationship was observed between observed ASVs and total cholesterol following Peanut condition (r = 0.303; $P = 0.039$). Following the Peanut condition, observed genes were negatively associated with total cholesterol (r = -0.457; $P = 0.032$) and positively associated (r = 0.441; $P = 0.035$) with LDL-cholesterol. No other significant correlations were observed between α-diversity metrics and insulin, fructosamine, BMI or lipids/lipoproteins.

4.4 Discussion

To the best of our knowledge, this is the first clinical trial to assess the effects of peanut consumption as a nighttime snack on gut microbiota composition and bacterial gene expression. In this trial, there was no difference in α- or β-diversity between the Peanut and LFHC condition but following intake of peanuts (28.4 g/day for 6-weeks), the abundance of *Roseburia* and *Ruminococcaceae* increased compared to the LFHC snack. Moreover, metatranscriptomics analysis in a subset of participants that had reduced fasting plasma glucose (primary endpoint) following peanut intake revealed enrichment of the K03518 (aerobic carbon-monoxide dehydrogenase small subunit) gene. *Roseburia intestinalis L1*-82, an important butyrate producer, was identified as a contributor to the increase expression of K03518. Therefore, consuming 28.4 g of peanuts as a nighttime snack for 6-weeks, compared to a LFHC snack, altered gut microbiota composition by increasing the abundance of fiber degrading SCFA producers. In alignment, peanut intake increased expression of a gene involved in SCFA production.

Neither condition significantly altered microbial α-diversity measured by Faith's PD score, Observed Features, or Pielou's Evenness. These measures summarize the diversity of the gut microbial community by assessing the number of taxonomic groups (richness) and distribution of the groups (evenness) (143). Thus, our results suggest that 28 g/d of peanuts for 6-weeks does not affect richness and evenness of the gut microbial community. One clinical trial that assessed almond consumption (56 g/day), compared to a high carbohydrate snack, for 8-weeks in adults with elevated fasting glucose showed significant within-condition improvements in α-diversity but no between-condition differences (144). However, a number of other previous clinical trials assessing tree nut intake (almonds, walnuts, and pistachios) have demonstrated no change in α-diversity (145–147). Therefore, our findings are in agreement with previously published trials.

We observed no significant shifts in β-diversity, which measures the variation in bacterial communities between samples, in our study. Evidence supporting significant shifts in β-diversity following nut consumption are mixed. Four trials assessing walnuts (57-99 g/day for 6-weeks), almonds (42.5-56.7 g/day for 3-8-weeks), and pistachios (42.5-85 g/day for 18-days) did not show shifts in β-diversity (146,148–150). In contrast, clinical trials investigating the effect of walnut intake (~42 g/day for 3-8 weeks) in healthy populations showed significant effects on β-diversity (145,147). The differences in findings across these studies is likely driven by the composition of the comparison diets and variation in the sample sizes, which may reduce statistical power. We studied a lower dose of nuts, than the aforementioned trials, and only observed small shifts in dietary intake in response to the intervention, which likely explains the lack of variance in bacterial communities observed.

Our trial demonstrated significant enrichment of two bacteria, *Roseburia* and *Ruminococcaceae,* following the Peanut condition relative to the LFHC condition. The enrichment of *Ruminococcaceae* following the Peanut condition is congruent with findings by

Choo et al, who reported increases in the relative abundance of *Ruminococcaceae* following 56 g/day of almonds for 8-weeks in adults with elevated fasting glucose compared to a higher carbohydrate snack (144). Similarly, Holscher et al. reported significant increases in the relative abundance of *Roseburia* following almond consumption in healthy adults compared to a no almond control group (149). *Roseburia* and *Ruminococcaceae* are two of the main butyrate-producing bacteria (151). Increased abundance of SCFA producing bacteria following peanut consumption in our trial suggests that peanuts may increase the production of SCFA.

Metatranscriptomics analyses in responders (greatest reduction in fasting glucose) to the Peanut condition revealed significant enrichment of K03518, in the endpoint samples, which is a gene involved with butyrate production (152,153). The top taxonomic contributors to expression of the K03518 gene were the same (*Blautia* sp. SC05B48 and *Faecalibacterium prausnitzii)* in both baseline and endpoint samples. Of note, *Roseburia intestinalis L1-82*, represented 1.5% of the relative contribution in the endpoint samples compared to no detectable relative contribution in the baseline samples. *Faecalibacterium prausnitzii* is involved with butyrate production (154). Since butyrate is a beneficial metabolite for intestinal homeostasis (epithelial cell fuel source and important for immune function), an increased expression of the K03518 gene could indicate increased butyrate production. This suggests that intake of 28 g/d of peanuts for 6 weeks may cause a shift in microbial community function that are beneficial to human hosts.

These metatranscriptomics findings are congruent with the 16S rRNA sequencing results, which demonstrated increased abundance of *Roseburia* following the Peanut condition vs. the LFHC condition. Therefore, it is plausible that the Peanut condition increased both the abundance and beneficial activity of *Roseburia.* However, it should be noted that this was an exploratory analysis in a small subset of responders from Peanut condition and the lack of comparison to a control condition increases the risk of regression to the mean and/or temporal changes confounded by other factors.

We did not observe any significant correlations for measures of α-diversity and endpoint glucose for the LFHC and Peanut condition in the whole cohort. Additionally, there was no relationship between endpoint glucose and α-diversity measures for bacterial composition or genes in the subset of responders. We likely did not observe any relationships between glucose and α-diversity due to small sample size and lack of significant effect of the conditions on glycemic outcomes (107). Interestingly, there was a positive correlation between observed ASVs and total cholesterol following the Peanut condition, suggesting higher total cholesterol following peanut intake was associated with higher α-diversity. A recent trial evaluated the microbiota in adults (n=1141) with and without dyslipidemia and demonstrated reduced α-diversity in those with dyslipidemia and α-diversity was inversely associated with triglyceride concentrations (155). Our trial included only 50 participants who were primarily normolipidemic. Thus, these exploratory findings should be interpreted cautiously. Observed species in the metatranscriptomic subset was positively associated with LDL-cholesterol and observed genes were negatively associated with total cholesterol. The LDL-cholesterol relationship is congruent with the total cholesterol correlation from the whole cohort, but the genes and total cholesterol relationship could indicate that greater microbiota functionality (measured by the number of genes) was associated with lower total cholesterol. We did not observe enrichment of genes associated with lipid metabolism to support these relationships. Additionally, correlational data are purely descriptive and hypothesis generating. Further research is necessary to establish the relationship between lipid metabolism and the microbiota.

This study enrolled participants with elevated fasting glucose. Recent studies have demonstrated that there are differences in the gut microbiota based on glycemic status (78) and microbiota composition explains a portion of the variation in postprandial glycemic response (83). A metagenome-wide association study (MGWAS) demonstrated that gut microbial markers may be useful for classifying T2DM (156). The authors developed metagenomic linkage groups

(MLGs) based on microbiota data that had consistent abundances of bacteria and taxonomic assignments to compare control and T2DM samples. Enriched MLGs in the controls tended to have butyrate-producing bacteria like *Faecalibacterium prausnitzii* and *Roseburia intestinalis.* The PREDICT 1 Trial assessed microbiota composition and postprandial glycemic response to standardized meals in 1102 healthy individuals from the UK and US. The authors found that the microbiota composition explained 6.4%, a relatively small percentage, of the variation in postprandial glucose. In our trial, intake of peanuts for 6-weeks did not affect markers of glycemic control measured in the fasting state, although shifts in microbiota composition were observed (enrichment of *Faecalibacterium prausnitzii* and *Roseburia intestinalis)*. It is plausible that longer-term glycemic control is less influenced by microbiota composition than postprandial glycemic control, although this requires further investigation; adults with elevated fasting glucose have greater postprandial hyperglycemia compared to adults with normoglycemia (157). Future studies are necessary to evaluate the role of the gut microbiome in longer-term glycemic control.

The strengths of this study are the trial design, compliance to the dietary protocol, and the use of metatranscriptomics to assess the microbiome. The randomized crossover controlled clinical trial design provided sufficient data to assess between-condition and within condition differences and only altered one aspect of the participant's diet. Participants were compliant with the dietary protocol as evidenced by the 88% adherence to the diet condition as illustrated by increases in seafood/plant protein and percent of energy from mono- and poly-unsaturated fatty acids in the Peanut condition and increased intake of whole grains for the LFHC condition (107). Additionally, the dietary conditions were matched as best as possible for fiber (Peanut: 2.4g and LFHC: 3g), a major contributor to microbiota composition (158). Metatranscriptomics is a novel method to assess changes in bacterial gene expression and analyze functional changes in the gut microbiome, but no previous clinical nutrition trials have used this method. Limitations included the small subset used for metatranscriptomics and some participants not having elevated fasting glucose at baseline. Changes in bacterial gene expression, measured by metatranscriptomics, was

an exploratory outcome and only used a subset of responders to the Peanut condition. A single fasting plasma glucose measure was used at screening to determine eligibility and some participants did not have elevated fasting glucose during their initial baseline visit.

In conclusion, this study was designed to evaluate how peanuts as a nighttime snack affects gut microbiota composition compared to an isocaloric LFHC snack in adults with elevated fasting glucose. We observed enrichment of SCFA producing bacteria, *Roseburia* and *Ruminococcaceae,* following the Peanut condition compared to the LFHC. In exploratory metatranscriptomics analyses, enrichment of the K03518 gene was observed following peanut intake; *Roseburia intestinalis L1-82* was identified a taxonomic contributor to increased K03518 gene expression. Collectively, these findings suggest that intake of 28 g of peanuts for 6-weeks alters microbiota composition by increasing the relative abundance of SCFA producers and the active gene expression of these taxa, which is indicative of greater functionality. Further analyses are required to confirm these exploratory observations.

Supplementary Data

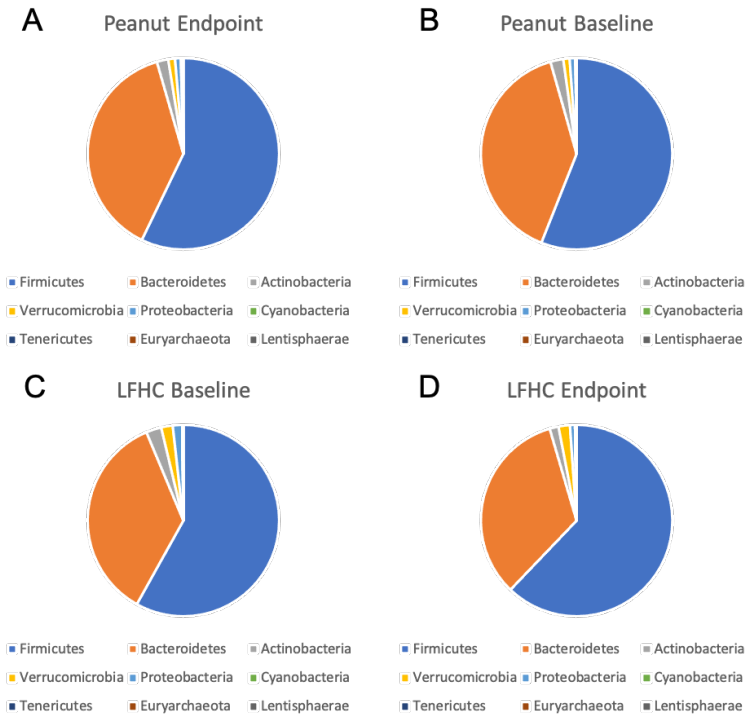

Figure **4-2**: Mean relative abundance of bacteria phyla for Peanut baseline (Panel A), Peanut endpoint (Panel B), LFHC baseline (Panel C), and LFHC endpoint (Panel D) samples (*n*=50)

Table **4-3**: Between-condition comparisons in bacteria enrichment in adults with the elevated fasting glucose (n=24)[1]

Enriched bacteria [2]	Condition	LDA score	p-value
D_6 Ruminococcaceae	Peanut	2.8	0.037
D_6 Roseburia sp	Peanut	3.1	0.035
D_6 unidentified Tyzzerella	LFHC	2.7	0.032
D_6 uncultured Tyzzerella	LFHC	2.7	0.032
D_6 unidentified Turicibacter	LFHC	2.5	0.019
D_6 unidentified Turicibacter	LFHC	2.4	0.026
D_6 unidentified Clostridium	LFHC	2.2	0.028
D_6 unidentified Clostridium	LFHC	2.2	0.028

[1]LEfSe enrichment plot values for enriched taxa between-conditions. LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size

[2]D_6 indicates this bacteria has been identified down to the species level.

Table **4-4**: Peanut baseline and endpoint α-diversity metrics for the whole cohort[1] (n=50) and metatranscriptomics subset[2] (n=24)

	Whole cohort[1]		Metatranscriptomics	
	Baseline	Endpoint	Baseline	Endpoint
Faiths PD	9.8 (2.9)	10.4 (2.9)	9.4 (2.9)	10.403 (2.4)
Observed Features	131.2 (48.2)	133.2 (37.0)	126.3 (57.6)	134.2 (41.8)
Pielou's Evenness	0.76 (0.07)	0.77 (0.09)	0.77 (0.07)	0.74 (0.1)

[1]Data presented as medians and interquartile ranges. Faiths PD, Faiths phylogenetic diversity.

Table **4-5**: Within-condition comparison of enriched genes for the Peanut condition in adults with the greatest reduction in fasting glucose (n=24)[1]

Gene	Timepoint	LDA score	p-value
K02108: F-type H+-transporting ATPase subunit a	Baseline	2.1	0.003
K02520: translation initiation factor IF-3	Baseline	2.8	0.043
K00024: malate dehydrogenase EC:1-1-1-37	Baseline	2.5	0.012
K02884: large subunit ribosomal protein L19	Baseline	2.3	0.039
K00459: nitronate monooxygenase EC:1-13-12-16	Baseline	2	0.032
K03733: integrase-recombinase XerC	Baseline	2.5	0.027
K02954: small subunit ribosomal protein S14	Baseline	2.1	0.045
K03530: DNA-binding protein HU-beta	Baseline	2.4	0.033
K03518: carbon-monoxide dehydrogenase small subunit	Endpoint	2	0.039

[1]LEfSe enrichment plot values for enriched genes within the Peanut condition based on the metatranscriptomics subset. LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size

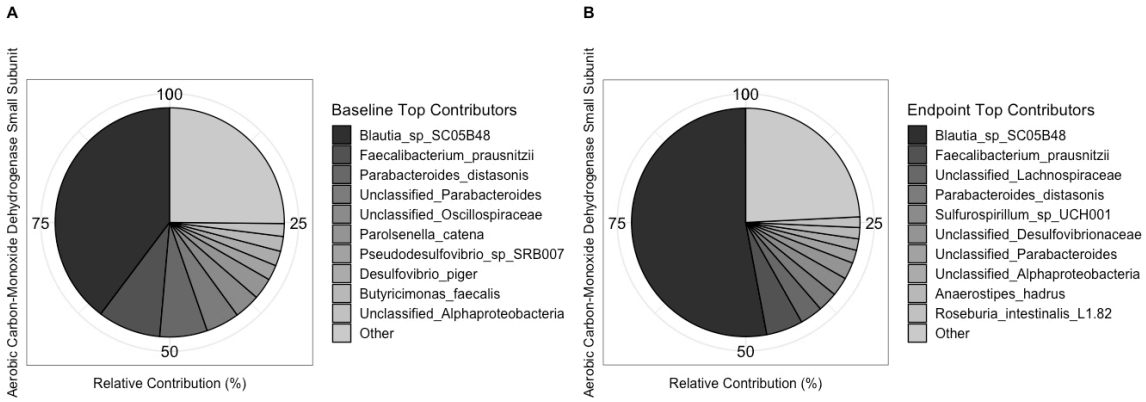

Figure **4-9**: Within-condition comparisons in relative contributions for the K013518 gene for the Peanut condition in adults with elevated fasting glucose concentrations (n=24). Peanut baseline (A), peanut endpoint (B), and values are percentages.

Table **4-7**: Spearman correlations between α-diversity metrics and endpoint glucose the whole cohort[1] (n=50) and metatranscriptomics subset[2] (n=24)

Whole Cohort[1]	Microbiome metric	Endpoint	*r*	*P*
	LFHC Endpoint Observed ASVs	LFHC Endpoint Glucose	0.277	0.057
	LFHC Endpoint Faith ASVs	LFHC Endpoint Glucose	0.246	0.092
	LFHC Endpoint Evenness ASVs	LFHC Endpoint Glucose	0.149	0.311
	Peanut Endpoint Faith ASVs	Peanut Endpoint Glucose	0.073	0.628
	Peanut Endpoint Observed ASVs	Peanut Endpoint Glucose	0.014	0.927
	Peanut Endpoint Evenness ASVs	Peanut Endpoint Glucose	0.01	0.949
Metatranscriptomic Subset [2]			*r*	*P*
	Peanut Endpoint Evenness ASVs	Peanut Endpoint Glucose	0.415	0.062
	Peanut Endpoint Observed ASVs	Peanut Endpoint Glucose	0.154	0.505
	Peanut Endpoint Faith ASVs	Peanut Endpoint Glucose	0.125	0.59
	Peanut Endpoint Observed Genes	Peanut Endpoint Glucose	0.326	0.138
	Peanut Endpoint Evenness Genes	Peanut Endpoint Glucose	0.294	0.185
	Peanut Endpoint Observed Species	Peanut Endpoint Glucose	0.066	0.764

[1]Spearman correlation coefficient to assess the association between endpoint glucose following both conditions and measures of α-diversity. ASVs, amplicon sequence variants.

[2]Spearman correlation coefficient to assess the association between endpoint fasting glucose following the Peanut condition and measures of α-diversity. ASVs, amplicon sequence variants.

Chapter 5

Conclusions, Limitations, and Future Directions

The overarching aim of this dissertation was to investigate the effects of nighttime peanut consumption on fasting plasma glucose, cardiovascular disease (CVD) risk factors, diet quality, gut microbiota composition and, as an exploratory component, assess bacterial gene expression and gut microbiome functionality. Specifically, the aims were (1) to determine the effects of 28.4 g of dry roasted, unsalted, peanuts as a nighttime snack for 6-weeks compared to an isocaloric lower-fat higher-carbohydrate (LFHC) snack on fasting plasma glucose, insulin, fructosamine, lipids/lipoproteins, brachial blood pressure, central blood pressure, arterial stiffness, and diet quality in adults with elevated fasting glucose, (2) to assess the effects of the dietary conditions on gut microbiota composition, and (3) to explore the effects of peanuts on gut bacterial gene expression in a subset of participants that had the greatest reduction in fasting glucose following the Peanut condition.

The data presented in this dissertation add novel evidence about the effects of longer-term, daily peanut consumption on glycemic control, CVD risk factors, diet quality, and gut microbiota. Tree nut consumption has been reported to improve glycemic control and CVD risk factors, as well as modify gut microbiota composition in adults. This 6-week randomized, crossover, supplemental feeding trial in adults with elevated fasting plasma glucose demonstrates that peanut consumption does not change glycemic control compared to a lower-fat higher-carbohydrate (LFHC) control snack. However, peanut consumption improved several aspects of diet quality, modestly changed microbiota composition, and exploratory analyses showed potential shifts in microbiome functionality. Taken together, both nighttime snacks did not have adverse effects on glycemic control and did not increase risk for diabetes or CVD in adults with

elevated fasting glucose. Therefore, peanuts or whole grain crackers and cheese may be healthy nighttime snack options for adults with elevated fasting glucose in the evening.

Our study had specific limitations that are explained in the thesis. Briefly, the limitations were the screening protocol, non-random dietary assessment, single-blinded design, and the small subset of participants used for the exploratory analysis. First, participants were eligible for this trial based on a single fasting plasma glucose ≥ 100 mg/dL. However, due to day-to-day variability in glucose concentrations some participants did not have elevated fasting glucose at their baseline visit. Secondly, the study protocol required participants to complete non-random 24-hour dietary recalls at baseline and endpoint for each condition. which may have affected dietary reporting. Thirdly, we employed a single-blinded study, in which the study coordinator was blinded to the participants' randomization but the participants were aware of the study food being provided. Awareness of the study food may have introduced bias, but double blinding was not feasible. The final limitation was the use of a small subset for the metatranscriptomics analyses, and the results must be interpreted with caution. In addition to these limitations there is risk for potential confounding in dietary clinical trials. We conducted a well-designed randomized, controlled trial, but participants may have consumed the assigned study foods differently throughout the trial or made changes knowing the study foods they would be consuming during the next condition. A potential example of this was the increase in whole grain component score during the LFHC condition which was potentially influenced by the reduced whole grain components score during the Peanut condition. However, it is important to note that any confounding likely had small to no effect on our primary outcome.

The findings presented provide novel information describing the effect of nighttime peanut consumption in adults with elevated fasting plasma glucose, but further research is necessary to: (1) examine the effect of peanuts on glycemic control in a cohort with prediabetes, (2) determine whether nighttime snack avoidance is superior to an evening snack (healthy or

unhealthy), (3) assess both conditions using metatranscriptomics, (4) measure microbiota-derived metabolites using metabolomics in response to dietary intervention in adults with prediabetes and lastly (5) evaluate the effect of peanuts as a pre-meal snack on longer-term glycemic control. Conducting a study in a population with prediabetes would require a different screening protocol using two fasting plasma glucose measurements of ≥100 mg/dL taken on two separate days or a single HbA1c measurement of ≥ 5.7%. We did not observe a significant reduction in fasting plasma glucose (-0.6 mg/dL, $P = 0.67$), although in a cohort with prediabetes that has higher baseline glucose/impaired glycemic control greater reductions in fasting plasma glucose may be achievable. It is also of clinical relevance to determine if avoidance of snacks in the evening is superior, or has differential effects, to healthy snacks. Due to the cost of metatranscriptomic analyses, these were only conducted on a smaller subset of primary endpoint responders to the Peanut condition. To confirm the observed changes were caused by peanut intake and not regression to mean or confounding, further analyses involving the control condition are warranted. However, we observed compositional changes following the Peanut condition vs. the LFHC condition that aligned with changes in the expressed gene profile observed in the exploratory metatranscriptomics analyses. Thus, a potential next step to further understand the functional and health implications of the microbiota changes observed is to assess changes in plasma- and fecal-derived microbiota metabolites e.g. short-chain fatty acids. Based on previous data demonstrating improvements in postprandial glycemic control following peanut consumption and the lack of significant effects on morning fasting glucose in our trial a potential follow-up study should assess peanuts as a pre-meal snack over a 3-month period. A controlled-feeding trial with peanuts as a pre-meal snack compared to an average American diet for 3-months in adults with prediabetes while assessing HbA1c and glucose with continuous glucose monitor will assess whether peanuts have effects on shorter-term and longer-term postprandial glycemic control and risk for diabetes.

In the present study we demonstrated that consumption of 28.4 g dry roasted, unsalted, peanuts for 6-weeks as a nighttime snack did not affect glycemic control or CVD risk factors in adults with elevated fasting plasma glucose compared to an isocaloric LFHC snack. We observed improvements in several aspects of dietary intake and diet quality, which at a population level may lower risk of disease due to the low diet quality of US adults and previous observational studies showing adults with higher diet quality have fewer markers of chronic disease (hyperglycemia, high blood pressure, obesity, and hypercholesterolemia) than those with poor diet quality (159). However, this study's sample size may have been too small and the duration too short to detect benefits from small shifts in diet quality. Improvements in the dietary endpoints are congruent with the current guidelines for healthy eating patterns that recommend 5 oz-eq/week of nuts, seeds, or soy products; participants in our study consumed 7 oz-eq/week. Moreover, the present study demonstrated several modest improvements in gut microbiota composition and potentially beneficial shifts in microbiome functionality. Further research is needed to confirm the observed changes in microbiome functionality.

Appendix A

Participant telephone informed consent

CONSENT FOR RESEARCH

The Pennsylvania State University

Title of Project: The role of the microbiome in blood glucose regulation in response to peanut consumption

Principal Investigator:

Name: Dr. Kristina Petersen
Address: 320 Chandlee Lab
Telephone: 814-863-8622

Subject's Printed Name: _____

1. Why is this research study being done?
We are asking you to participate in this research to evaluate the effects of nighttime peanut consumption (i.e., after dinner and before sleep) on fasting blood sugar levels, longer-term blood sugar control, and risk factors for cardiovascular disease. We will also be investigating how peanut consumption affects gut health and how this relates to blood sugar control.

2. What will happen in this research study?
I am going to first read a description of the study to you, and then ask you some questions about your medical history and lifestyle. If any of the questions make you uncomfortable, you can stop the phone interview at any time. If you don't understand something, please ask questions at any time. If you qualify for the study after we complete the phone screening, we will discuss further details of your participation and set up an appointment for a clinic screening appointment.

3. What are the risks and possible discomforts from being in this research study?
You may experience minor discomfort when being asked questions about your personal medical history. If this occurs, please alert study personnel. There is a risk of loss of confidentiality if your information or your identity is obtained by someone other than the investigators, but precautions will be taken to prevent this from happening. The confidentiality of your electronic data created by you or by the researchers will be maintained to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed. Your information will be identified with a code, your name will not be kept with data collected during the study.

4. What are the possible benefits from being in this research study?
The proposed study will investigate whether evening consumption of peanuts improves blood sugar control and risk factors for cardiovascular disease. It will also explore how peanut change gut health and how this relates to blood sugar control. This study will provide evidence for a strategy that could be used to improve blood sugar control in people with elevated blood sugar levels.

5. What other options are available instead of being in this research study?
You may decide not to participate in this research.

6. How long will you take part in this research study?
The phone screening will take approximately 15 minutes.

7. How will your privacy and confidentiality be protected if you decide to take part in this research study?
Efforts will be made to limit the use and sharing of your personal research information to people who have a need to review this information. If you do not qualify for the study, the general reason why you did not qualify for the study will be recorded under an assigned screening study ID#. Documents containing information gathered during the telephone screening will be stored in a locked cabinet in 317 Chandlee lab or in a password protected file on a secure network. If you do qualify for the study, information gathered during the telephone screening such as names, addresses, telephone numbers, email addresses, date of birth, and visit dates will be linked to data via a code break list. Once enrolled in the study, all data collection will be performed under an assigned study ID# that is known only to the principal investigators, study coordinators and CRC staff (for purposes of blood collection and safety).

In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared. A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

We will do our best to keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may find out about your participation in this research study. For example, the following people/groups may check and copy records about this research.

- The Office for Human Research Protections in the U. S. Department of Health and Human Services
- U.S. Food and Drug Administration
- The research study sponsor, The Peanut Institute
- The Institutional Review Board (a committee that reviews and approves research studies) and
- The Office for Research Protections.

Some of these records could contain information that personally identifies you. Reasonable efforts will be made to keep the personal information in your research record private. However, absolute confidentiality cannot be guaranteed.

8. What happens if you are injured as a result of taking part in this research study?
In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By verbally consenting, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

9. Who is paying for this research study?
The funding for this study is provided by The Peanut Institute. However, the funding source will not be involved in data analysis. They will have the right to review all publications before submission, however there are no contractual agreements that allow them to have influence on, or restrict, the publication of results.

10. What are your rights if you take part in this research study?

Taking part in this research study is voluntary.
- You do not have to be in this research.
- If you choose to be in this research, you have the right to stop at any time.
- If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.
- If you stop being in the research, already collected data may not be removed from the study database.

During the course of the research you will be provided with any new information that may affect your health, welfare or your decision to continue participating in this research.

11. If you have questions or concerns about this research study, whom should you call?

Please call the head of the research study (principal investigator), Dr. Kristina Petersen, at 814-863-8622 if you:
- Have questions, complaints or concerns about the research.
- Believe you may have been harmed by being in the research study.

You may also contact the Office for Research Protections at (814) 865-1775, ORProtections@psu.edu if you:
- Have questions regarding your rights as a person in a research study.
- Have concerns or general questions about the research.
- You may also call this number if you cannot reach the research team or wish to offer input or to talk to someone else about any concerns related to the research.

<u>INFORMED CONSENT TO TAKE PART IN RESEARCH</u>

Do you consent to take part in this telephone screening?
Tell the researcher your decision regarding whether or not to participate.

Appendix B

Participant informed consent

CONSENT FOR RESEARCH

The Pennsylvania State University

Title of Project: Peanut consumption, blood sugar control, and gut health

Principal Investigator:

Name: Dr. Kristina Petersen
Address: 320 Chandlee Lab
Telephone: 814-863-8622

Subject's Printed Name: _____

We are asking you to be in a research study. This form gives you information about the research.

Whether or not you take part is up to you. You can choose not to take part. You can agree to take part and later change your mind. Your decision will not be held against you.

Please ask questions about anything that is unclear to you and take your time to make your choice.

1. Why is this research study being done?

We are asking you to participate in this research to evaluate the effects of nighttime peanut consumption (i.e., after dinner and before sleep) on fasting blood sugar levels, longer-term blood sugar control, and risk factors for cardiovascular disease. We will also be investigating how peanut consumption affects gut health and how this relates to blood sugar control.

This research is being done because elevated fasting blood sugar levels (or prediabetes) affects approximately 84 million adults in the US and therefore strategies are required to improve blood sugar control. Based on previous evidence, nighttime peanut consumption may improve fasting blood sugar levels, although no study has directly tested this. This study will be the first to examine the effect of evening peanut consumption on fasting blood sugar levels, risk factors for cardiovascular disease, and gut health.

Approximately 45 people will take part in this research study conducted at the Pennsylvania State University, University Park Campus, PA.

2. What will happen in this research study?

General overview of the study

If you agree to participate in this study, your participation will last approximately 16 weeks in total. There are two treatment phases each lasting 6 weeks and separated by an approximate 4-week break.

The two treatment periods will include: 1) evening (i.e., after dinner and before sleep) consumption of one-ounce of peanuts; 2) evening (i.e., after dinner and before sleep) consumption of a calorie matched snack. The calorie matched snack will be whole wheat crackers and spread (e.g. margarine, cream cheese). These foods will be provided to you biweekly. You will follow your normal self-selected diet, although the treatment foods provided must be consumed as an evening snack and no other foods/beverages (except water) can be consumed after your evening meal.

Over the course of the study you will receive both study foods; the order you receive the foods will be randomly assigned and your treatment order may be different from that of other participants. This assignment is done in a way similar to flipping a coin – we use a computer program to assign the order of treatments that you will receive.

During each treatment period, you will be required to avoid peanut or tree nut consumption other than what is provided to you. You will be required to pick-up your study foods from the metabolic kitchen on the Penn State University campus biweekly. In addition, you will be required to complete a log of your daily treatment intake. This is not a weight loss study, so you must try to keep your body weight and physical activity level constant throughout the entire study.

Procedures to be followed

Screening:

If you decide to participate in the study and are considered eligible after the telephone screening, you will be further screened for eligibility during a visit to the Clinical Research Center (CRC) at Penn State. The screening clinic visit will consist of filling out forms (informed consent, medical history, personal information); measuring height and weight so that body mass index (BMI) can be calculated; and measuring blood pressure to determine eligibility. If your blood pressure is >140/90 at screening you will require written approval from your Primary Care Physician to enroll in the study. If, after these measurements, it is determined you are still eligible to continue in the research, a blood sample will be taken from a forearm or hand vein and a complete blood count, including liver and kidney function and a blood fat panel will be performed (approximately 19 ml of blood or 1.25 tablespoons will be taken). You will feel a small pinch or discomfort when the needle is inserted. If the initial blood draw is unsuccessful it may need to be repeated, with your permission. If you take thyroid medicine, and do not have a current lab test (within 6 months), we will draw 3.5 ml (0.2 Tbsp) more blood to conduct a thyroid test. If you are female of child bearing potential, you will be given a urine pregnancy test. You will be contacted within 3-5 days with the results of the screening blood sample. A clinician at the CRC will review all of the screening data and based on this the research team will determine your eligibility. If you are eligible for the study, you will be contacted to schedule your start date and baseline data collection appointments. There will be no charge for the screening blood work or measurements and you will get these results. If you agree to continue your participation in this study, you will agree to check with the study staff before participating in any other research studies; the study coordinator will determine if it is alright for you to participate.

Baseline and endpoint testing

At the beginning and end of each treatment period you will have your weight measured. If you are female of child bearing potential, you will be given a urine pregnancy test.

Blood sampling:

You cannot consume any food or drinks except for water for 12 hours, and cannot drink alcohol during the 48 hours prior to having your blood taken.

In addition to the blood taken at screening, blood samples will also be taken on two consecutive days at the beginning and end of each treatment period for a total of 8 times. After a 12 hour fast (consumption of no food or drink except water), a blood sample will be taken from your arm. If the initial blood draw is unsuccessful it may need to be repeated, with your permission. Approximately 60 ml (about 4 tablespoons) of blood will be collected at each endpoint over the two days (30 ml or 2 tablespoons each day). Therefore, over the ~16 week study, blood will be taken 8 times with a total amount of approximately 240 ml. A typical American Red Cross blood donation is 1 pint (473 ml). Blood samples will be frozen and analyzed at the end of the study (when all subjects have completed). The results of the study will only be available at the end of the study (which may take up to 3 years). Your blood may be tested for the following: blood fats (total cholesterol, LDL- cholesterol, HDL-cholesterol, triglycerides), blood sugar (glucose and fructosamine), and insulin. No personal information will be kept with any sample – only ID# assignments and only the Primary Investigators and the Study Coordinator will have access to the ID# assignments with the study files.

Measures of vascular health:

Pulse wave analysis (PWA) and Pulse Wave Velocity (PWV):

You will undergo a test that measures your blood pressure and pulse wave forms at the beginning and end of each treatment period. The PWA measurement is very similar to a routine blood pressure measurement. Prior to the measurement, you will be asked to sit quietly with your feet flat on the floor for at least 5 minutes. A blood pressure cuff will be placed on your upper arm. The cuff will inflate, then deflate for 5 seconds, and then partially re-inflate. It is important that you remain still during this measurement. The procedure will be repeated twice, for a total of 3 measurements. Repeated measurements are used to increase accuracy. For the PWV measurement, we will ask you to lay flat on a hospital bed without a pillow. A blood pressure cuff will be placed on your upper leg. We will gently place a hand-held sensor against an artery in your neck. This will measure the pressure waves of the blood in your artery. Once a good waveform is obtained, the blood pressure cuff on your leg will inflate to measure the pressure waveforms in that artery. Having these simultaneous measurements allows the device to calculate the speed at which blood is traveling through your arteries. The PWV test will also be performed three times.

Fecal collection:

At the beginning and end of each treatment period you will be asked to collect a fecal sample (~50 g). You will be provided with a stool sample kit and detailed instructions for collection of a clean sample. The amount and number of different bacteria will be measured in your stool samples as a measure of your gut microbiome.

Dietary intake:

You will be asked to complete a 24-hour dietary recall at the beginning and end of each diet period; a total of 4 recalls. You will complete these recalls using an online system (Automated Self-Administered 24-Hour (ASA24®) Dietary Assessment Tool). Study staff will provide you with a login code and you will be asked to provide information about the foods, beverages, and supplements

you consumed during the previous day. You will have the option of completing this dietary recall at home or at your visit.

3. What are the risks and possible discomforts from being in this research study?

Study Treatments

You will be asked to report any food allergies during the telephone screening, however it is possible that an unknown food allergy may manifest during the study. This is most likely to occur within the first week of a treatment phase since the same foods will be repeated daily. It is unlikely that you will experience any discomfort with the addition of peanuts or the calorie matched snack to your diet. However, you may have an unknown sensitivity to this amount of peanuts or the calorie matched snack that may cause you to experience GI (stomach) upset such as bloating, diarrhea, or gas. You should report any adverse reactions to study personnel.

Food preparation

All foods will be prepared according to accepted standards of sanitation and provisions are made to ensure the safety of foods provided for off-site consumption. However, it is possible that incorrect food handling during shipping, storage or preparation, if not detected, could result in food-borne illness. Every effort will be made to safeguard against this possibility. To date, no food related contamination or illnesses have occurred.

Blood Sampling

Blood draws often cause mild pain, swelling or bleeding. There may be some bruising (blood under the surface of the skin), which will be minimized by pressing on the site after the needle is removed. There is also a slight chance of infection, dizziness or fainting. These risks will be minimized and most likely eliminated by having trained medical staff draw the blood in a clinical setting using sterile supplies. If dizziness or fainting occurs, the symptoms will be alleviated by having you lie flat with your feet raised. The medical staff will ask that you remain at the clinic until your blood pressure has been checked and you are cleared from any further risk.

Pulse Wave Analysis (PWA) and Pulse Wave Velocity

There are no known risks associated with these measurements. The sensation of pressure from the blood pressure cuff or hand-held probe may be uncomfortable. There is a possibility for red blotching or mild bruising (petechiae) appearing on the skin above and below the location of the blood pressure cuff. Studies indicate that petechiae are rare (occurring in less than ½ of 1% of patients) and it is typically not uncomfortable and does not require treatment.

Loss of Confidentiality

Your participation in this research is confidential. However, there is always a potential for loss of confidentiality despite our best efforts. To prevent this from occurring all records are coded with a unique ID number and no names are used. Records containing names or other identifying

information are kept under lock at the PI's research office. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical records. In the event of publication of this research, no personal identifying information will be disclosed.

Stool collection

You may experience some level of embarrassment or discomfort from being asked to collect stool samples. However, you will be provided with detailed instructions on how to collect the samples within the comfort of your own home, and at your convenience, to help reduce any concerns you may have.

4. What are the possible benefits from being in this research study?
4a. What are the possible benefits to you?

Participants will receive their screening laboratory results, including a complete blood count, interpretation of liver and kidney function, and blood lipid values, at no cost.

4b. What are the possible benefits to others?

The proposed study will investigate whether evening consumption of peanuts improves blood sugar control and risk factors for cardiovascular disease. It will also explore how peanut change gut health and how this relates to blood sugar control. This study will provide evidence for a strategy that could be used to improve blood sugar control in people with elevated blood sugar levels.

5. What other options are available instead of being in this research study?

You may decide not to participate in this research.

6. How long will you take part in this research study?

If you agree to take part, it will take you about 16 weeks to complete this research study. There are two treatment periods each lasting 6 weeks, separated by a ≥ 4 week break. You will be expected to pick up the test foods biweekly at the diet center on campus. At the beginning and end of each treatment period, endpoint data collection will occur (8 visits total).

Total time for study visits, after the initial screening is approximately 6 hours. Times may vary and females will require an additional 5 minutes for a urine pregnancy test at baseline and the end of each diet period. The following is an estimate of the amount of time you will spend in study activities:

Screening appointment: Forms, blood pressure, weight, height, blood draw – 45-60 minutes (pregnancy testing: females only – 5 minutes)

Beginning of treatment period 1 and 2

- Day 1: blood draw, weight, PWA, PWV – 60 minutes (pregnancy testing: females only – 5 minutes)
- Day 2: blood draw – 30 minutes

End of treatment period 1 and 2:

- Day 1: blood draw, weight, PWA, PWV – 60 minutes
  (pregnancy testing: females only – 5 minutes)
- Day 2: blood draw – 30 minutes

Picking up food, completing stool sample collections, and 24-hour dietary recalls: ~ 5 hours

Total time for clinic and diet center visits from baseline to the end of the study ~11 hours

7. How will your privacy and confidentiality be protected if you decide to take part in this research study?

Efforts will be made to limit the use and sharing of your personal research information to people who have a need to review this information.

- A list that matches your name with your code number will be kept in a locked file or password protected file in the PI's research office.
- Your research records will be labeled with a unique ID number and will be kept locked at the PI's research office. All records associated with your participation in the study will be subject to the usual confidentiality standards applicable to medical records.
- Your research samples will be labeled with your unique ID number and will be stored in locked freezers at the CRC and in 318 Chandlee Lab. They will be maintained until three years after the date from when the study is published, and then destroyed unless you give permission for us to keep your blood samples for future use (see end of document).

  For research specimens sent to other laboratories or facilities for analysis, no personal identifiable information will be used. Samples will be labelled only with ID numbers. Blood samples will be sent to Quest diagnostics, Pittsburgh, PA. Fecal samples will be sent to Wright Labs LLC, Huntingdon PA.

In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

We will do our best to keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may find out about your participation in this research study. For example, the following people/groups may check and copy records about this research.

- The Office for Human Research Protections in the U. S. Department of Health and Human Services
- U.S. Food and Drug Administration
- The research study sponsor, The Peanut Institute
- The Institutional Review Board (a committee that reviews and approves research studies) and
- The Office for Research Protections.

Some of these records could contain information that personally identifies you. Reasonable efforts will be made to keep the personal information in your research record private. However, absolute confidentiality cannot be guaranteed.

8. What happens if you are injured as a result of taking part in this research study?

In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

9. Will you be paid or receive credit to take part in this research study?

For your participation in this study you will receive monetary compensation of $400.00, prorated as follows and paid at the completion of your participation in the study. If you drop out of the study for any reason before its completion, the following compensation will be provided:

Completion of first treatment period and endpoint testing= $100
Completion of second treatment period and endpoint testing= $300
Total for completion of the study = $400
If you are a Penn State employee, you will be asked to provide you name and Penn State ID number and payment will be provided by direct deposit via the payroll system. If you are not a Penn State employee, you will be paid by check and your Social Security Number must be collected for tax reporting purposes. The compensation that you receive for participation in this study is taxable income.

Total payments within one calendar year that exceed $600 will require the University to report these payments to the IRS annually. This may require you to claim the compensation that you receive for participation in this study as taxable income.

If determined that specific work or visa laws conflict, participants may not be eligible for compensation. Participants can discuss this with the research team for more information.

10. Who is paying for this research study?

The funding for this study is provided by The Peanut Institute. However, the funding source will not be involved in data analysis. They will have the right to review all publications before submission, however there are no contractual agreements that allow them to have influence on, or restrict, the publication of results.

11. What are your rights if you take part in this research study?

Taking part in this research study is voluntary.

- You do not have to be in this research.
- If you choose to be in this research, you have the right to stop at any time.
- If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.
- If you stop being in the research, already collected data may not be removed from the study database.

The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include non-compliance with the study protocol (consuming treatment foods) or study visits (attending clinic visits).

During the course of the research you will be provided with any new information that may affect your health, welfare or your decision to continue participating in this research.

12. If you have questions or concerns about this research study, whom should you call?

Please call the head of the research study (principal investigator), Dr. Kristina Petersen, at 814-863-8622 if you:

- Have questions, complaints or concerns about the research.
- Believe you may have been harmed by being in the research study.

You may also contact the Office for Research Protections at (814) 865-1775, ORProtections@psu.edu if you:

- Have questions regarding your rights as a person in a research study.
- Have concerns or general questions about the research.
- You may also call this number if you cannot reach the research team or wish to offer input or to talk to someone else about any concerns related to the research.

INFORMED CONSENT TO TAKE PART IN RESEARCH

Signature of Person Obtaining Informed Consent

Your signature below means that you have explained the research to the subject or subject representative and have answered any questions he/she has about the research.

_____	_____	_____
Signature of person who explained this research	Date	Printed Name

(Only approved investigators for this research may explain the research and obtain informed consent.)

Signature of Person Giving Informed Consent

Before making the decision about being in this research you should have:

- Discussed this research study with an investigator,
- Read the information in this form, and
- Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

Signature of Subject

By signing this consent form, you indicate that you voluntarily choose to be in this research and agree to allow your information to be used and shared as described above.

_____	_____	_____
Signature of Subject	Date	Printed Name

Optional part(s) of the study
In addition to the main part of the research study, there is another part of the research. You can be in the main part of the research without agreeing to be in this optional part.

Optional Storage of Tissue for Future Research
In the main part of this study, we are collecting blood samples and fecal samples from you. If you agree, the researchers would like to store leftover sample(s) for future research.

- These future studies may be helpful in understanding cardiovascular disease and diabetes.
- It is unlikely that these studies will have a direct benefit to you.
- Neither your doctor nor you will receive results of these future research tests, nor will the results be put in your health record.
- Sometimes tissue is used for genetic research about diseases that are passed on in families. Even if your samples are used for this kind of research, the results will not be put in your health record.

Your leftover samples will be labeled with a code number that will be linked to a master list accessible only to the PI and research coordinator. This list will be destroyed 3 years after publication of the study results. These samples will be stored in a locked freezer in a locked office of the PI's.

- The length of time they will be used is unknown.
- You will be free to change your mind at any time before the master list is destroyed (approximately 3 years after publication of the study results) at which point we will no longer be able to identify your samples.
- You should contact the principal investigator if you wish to withdraw your permission for your blood samples or fecal samples to be used for future research. If it is still possible to identify your samples, any unused samples will be destroyed and not used for future research studies.

You should initial below to indicate what you want regarding the storage of your leftover blood samples and fecal samples for future research studies.

a. Your sample*s* may be stored and used for future research studies to learn about diabetes and cardiovascular disease prevention.

_____ Yes _____ No

c. Your samples may be shared with other investigators/groups without any identifying information.

_____ Yes _____ No

Do we have permission to keep your personal information and contact you about your interest in participating in future studies for Dr. Kris-Etherton, Dr. Petersen and their collaborators?

_____ Yes _____ No

Signature of Person Obtaining Informed Consent

Your signature below means that you have explained the optional part(s) of the research to the subject or subject representative and have answered any questions he/she has about the research.

_____	_____	_____
Signature of person who explained this research	Date	Printed Name

Signature of Person Giving Informed Consent

Signature of Subject

By signing below, you indicate that you have read the information written above and have indicated your choices for the optional part(s) of the research study.

_____	_____	_____
Signature of Subject	Date	Printed Name

Appendix C

Participant compliance sheet

	Day: **Date:**	**Day:** **Date:**	**Day:** **Date:**	**Day:** **Date:**	**Day:** **Date:**	**Day:** **Date:**	**Day:** **Date:**
Did you eat your study food today?	o YES o NO	o YES o NO	o YES o NO	o YES o NO	o YES o NO	o YES o NO	o YES o NO
Did you eat your study food in the evening (i.e. after dinner but before sleeping)?	o YES o NO	o YES o NO	o YES o NO	o YES o NO	o YES o NO	o YES o NO	o YES o NO
Did you eat or drink any non-study foods in the evening (i.e. after dinner but before sleeping)? *Excluding water*	o YES o NO *If yes describe:*	o YES o NO *If yes describe:*	o YES o NO *If yes describe:*	o YES o NO *If yes describe:*	o YES o NO *If yes describe:*	o YES o NO *If yes describe:*	o YES o NO *If yes describe:*
Did you take any medications or supplements other than those already on file? *Please also record any change in dosage of your pre-approved medications*	o NO o YES *if yes:* Description: Amount/dose: Reason:	o NO o YES *if yes:* Description: Amount/dose: Reason:	o NO o YES *if yes:* Description: Amount/dose: Reason:	o NO o YES *if yes:* Description: Amount/dose: Reason:	o NO o YES *if yes:* Description: Amount/dose: Reason:	o NO o YES *if yes:* Description: Amount/dose: Reason:	o NO o YES *if yes:* Description: Amount/dose: Reason:

Has there been any change in your health (i.e. feel as if you are getting a cold, flu, stomachache, etc.)? If so, has your eating changed as a result? Please describe any changes.							
Have you maintained your current level of physical activity? If not, please describe							
Other comments							

REMINDERS: You **MUST** eat your study food **EVERY DAY** in the **EVENING**. Do not consume any other ***peanuts or tree nuts***, including peanut butter and other nut butters. ***Do not consume any other foods or drinks after dinner*** (excluding plain water, non-caloric beverages, tea or coffee without milk/cream/sweetener)

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Education

The Pennsylvania State University, PhD, Nutritional Sciences	2021
Lipscomb University, MS, Exercise and Nutritional Sciences	2018
The Pennsylvania State University, BS, Nutritional Sciences	2016

Publications

Sapp PA, Kris-Etherton PM, and Petersen KS. (2021). Peanuts as a nighttime snack do not affect fasting glucose in adults with elevated fasting glucose: A randomized, crossover trial. *The Journal of nutrition.* **Under review.**

Sapp PA, Petersen KS, Kris-Etherton PM. (2021). 'Dietary fat: the good, the bad, and what is best?', in Wilson T et al. (eds.) *Nutrition Guide for Physicians and Related Health Professionals, Third Edition.* Switzerland: Springer Nature. **In Preparation.**

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Awards

Penny M. Kris-Etherton Doctoral Award Fund (2020-2021)
Nutritional Sciences Graduate Educational Enhancement Endowment (2020-2021)
Fellowship of Excellence in Graduate Recruiting (2018-2019)