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EFFECTS OF PISTACHIOS AS A NIGHTTIME SNACK COMPARED TO EDUCATION TO CONSUME 1-2 CARBOHYDRATE EXCHANGES ON GLYCEMIC CONTROL, CARDIOVASCULAR DISEASE RISK FACTORS, AND THE GUT MICROBIOTA IN ADULTS WITH PREDIABETES

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ABSTRACT

The aim of this dissertation was to investigate the glycemic, cardiometabolic, and gut microbial effects of consuming pistachios as a nighttime snack, compared to education to consume a 1-2 carbohydrate (CHO) exchanges (usual care: 15-30 g CHO), in adults with prediabetes. We conducted a randomized 2-period crossover trial with 12-week intervention periods and ≥ 4-week washout. The conditions were 57 g unsalted, pistachios [324 kcal (fat 26 g; CHO 16.04 g; protein 11.9 g; saturated fat: 3.2 g; monounsaturated fat: 13.9 g; polyunsaturated fat: 7.54 g; fiber: g; sodium: 3.4 mg)] and education to consume 1-2 CHO exchanges (15-30 g CHO)**.** Participants were instructed to consume the study foods after dinner, before bedtime, and consume no other calorie-containing food or drinks after the snack. Additionally, participants were instructed to avoid consuming any other pistachios, peanuts, or tree nuts for the duration of the study. The primary outcome was fasting plasma glucose (FPG) and secondary outcomes were insulin, hemoglobin A1c (HbA1c), insulin resistance (Homeostatic model assessment of insulin resistance [HOMA-IR]), lipids/lipoproteins, brachial blood pressure (BP), central BP, measures of arterial stiffness (pulse wave velocity [PWV]), diet quality, and the gut microbiota. Outcomes were assessed at the baseline and the end of each study period. Diet quality, measured by the Healthy Eating Index-2015 (HEI-2015), was assessed using dietary data collected with the Automated Self-Administered 24-recall system (ASA24). Fecal samples from study completers were analyzed using 16S rRNA sequencing to assess gut microbiota composition and diversity.

Sixty-six adults with prediabetes (40.9% female; [mean ± standard deviation]; age: 50.9 ± 11.6 years; BMI: 31.2 ± 4.0 kg/m^2; FPG: 106.2 ± 6.4 mg/dL) at screening were randomized. Fifty-one adults completed the study. There were no significant between-condition mean differences for FPG (pistachio vs. usual care: 1.0 mg/dL [95% CI: -0.7, 2.9 mg/dL]). Insulin was higher after the pistachio condition versus usual care (3.0 μIU/mL [95% CI: 1.2, 4.8 μIU/mL]).

HOMA-IR was higher after pistachio versus usual care (0.8 [95% CI: 0.3, 1.3]). PWV was lower after pistachios compared to usual care (-0.3 m/s [95%CI: -0.5, -0.0]). There were no between-condition mean differences for HbA1c, anthropometrics (weight, BMI, WC), lipid/lipoproteins, brachial BP, and central BP. The total HEI-2015 score was higher after the pistachio condition compared to usual care (5.6 points (95% CI: 0.2, 11.5]). Differences in total HEI scores were primarily driven by higher component scores for Seafood and Plant Protein (1.8 points [95% CI: 0.8, 2.8]) and Refined Grains (2.4 points [95% CI: 1.2, 3.6]) with pistachios. The pistachio condition resulted in higher consumption of total fat (3.9% kcals [95% CI: 0.2, 8.0]), monounsaturated fatty acids (2.2% kcals [95% CI: 0.4, 4.0]), fiber (5.0 g [95% CI: 1.1, 8.8]), potassium (487 mg [95% CI: 28, 946]), total protein (2.3 oz-eq [95% CI: 0.0, 4.6]), nuts and seeds (1.8 oz-eq [95% CI: 1.1, 2.6]), oils (12.2 g [95% CI: 1.4, 23.0]), and lower consumption of refined grains (1.5 oz-eq [95% CI: -2.8, -0.2]) versus usual care. Total energy intake was not different between conditions. There were no between condition mean differences in α- and β-microbial diversity. Following the pistachio condition, the bacteria genus *Roseburia* (Microbiome Multivariable Associations with Linear Models [MaAsLin] score = 2.1 $\pm$ 0.37 (SE); p = 0.00031) and NK4A214 group of *Oscillospiraceae* (MaAsLin score =1.0 $\pm$ 0.25; p = 0.045) were significantly enriched compared to the usual care condition.

In adults with prediabetes, consuming 57 g of unsalted pistachios as a nighttime snack did not alter FPG compared to the usual care condition. The pistachio condition resulted in higher fasting insulin and HOMA-IR versus usual care. However, diet quality was improved during the pistachio condition. Also, intake of fiber, monounsaturated fatty acids, and total protein was increased. Butyrate-producing bacteria (*Roseburia*) increased after the pistachio condition, which is associated with gut health. A lactic acid bacteria associated with obesity

(NK4A214 group of *Oscillospiraceae*) was also increased. Consuming pistachios as a nighttime snack compared to the usual care does not adversely affect FPG, increases insulin and insulin resistance, improves diet quality, and reduces arterial stiffness with beneficial changes to the gut microbiota. More research is needed determine the effect of nighttime pistachio consumption and the gut microbiota on glycemic in adults with prediabetes.

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ABBREVIATIONS

% kcals	Percent of total caloric intake
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
FBG	Fasting blood glucose
FPG	Fasting plasma glucose
FSG	Fasting serum glucose
HbA1c	Hemoglobin A1c/Glycated Hemoglobin
HDL-C	High-density lipoprotein cholesterol
HEI-2015	Healthy eating index-2015
HOMA-IR	Homeostatic model assessment for insulin resistance
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
LDL-C	Low-density lipoprotein cholesterol
MaAsLin	Microbiome Multivariate Association with Linear Models
MD	Mean Difference
MNT	Medical Nutrition Therapy
MUFA	Monounsaturated fatty acid
NHANES	National Health and Nutrition Examination Survey
OGTT	Oral glucose tolerance test
OR	Odds ratio
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
RD	Registered dietitian
RR	Relative risk
SBP	Systolic blood pressure

SCFA	Short-chain fatty acid
SFA	Saturated fatty acid
TIIDM	Type 2 diabetes mellitus
TC	Total cholesterol
TAG	Triglyceride
US	United States
USDA	United States Department of Agriculture
WMD	Weighted mean different

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Chapter 1:
Introduction

Cardiovascular disease (CVD) and diabetes are the 1st and 8th leading causes of death in the US, respectively (1). Despite considerable progress in reducing CVD mortality since 1980, rates have risen almost 20% from 2010 to 2020 (2). In addition, the number of adults diagnosed with diabetes has increased approximately 3% from 2001 to 2020 of which 90-95% have type 2 diabetes mellitus (TIIDM) (3). The growth in CVD and TIIDM highlights a concerning trajectory for the already substantial economic burden of these chronic diseases. Total costs for CVD are estimated at $216 billion each year (4). Estimates for TIIDM are even greater with annual costs of approximately $237 billion (4). Continued efforts are vital to improve health outcomes and reduce the economic burden of CVD and TIIDM in the US.

Adults with prediabetes are at increased risk of developing CVD and TIIDM. The American Diabetes Association (ADA) criteria for prediabetes includes a fasting plasma glucose (FPG) between 100 and 125 mg/dL, hemoglobin A1c > 5.6% and < 6.5%, and/or a blood glucose > 140 mg/dL and < 200 mg/dL following an oral glucose tolerance test (OGTT) (5). One in three US adults has prediabetes and approximately 80% are unaware they have it (3). Having prediabetes significantly increases the risk of incident CVD by up to 18% compared to those with normal glucose levels (6). The number of adults that progress from prediabetes to TIIDM is even more concerning with current estimates showing nearly 74% will develop TIIDM in their lifetime (7). Approximately 70% will make the transition from prediabetes to TIIDM within 10 years (7). Strategies that reduce glucose levels and/or reverse prediabetes are essential to attenuate progression to TIIDM and lower CVD risk.

Improving diet quality is among the primary treatment strategies for prediabetes. The ADA 2023 Standards of Medical Care in Diabetes recommendation for TIIDM prevention

includes intensive lifestyle therapy to achieve 7%-10% weight loss, 150 min/wk of exercise, and intake of a healthy dietary pattern (8). These treatment strategies are aimed at maintaining or reversing abnormal glucose measures (i.e., FPG, HbA1c, OGTT).

The American Heart Association (AHA) also emphasizes normal glucose levels among its 8 metrics for cardiovascular health which includes: a healthy diet, physical activity, avoid nicotine exposure, quality sleep, a healthy body weight, normal levels of blood lipids, normal blood glucose levels, and normal blood pressure (9). Treatment strategies for TIIDM and CVD identify diet and glucose control as primary targets for interventions that reduce disease risk. Importantly, interventions that improve diet quality also benefit multiple other healthy components such as glucose levels, weight, lipids/lipoproteins, blood pressure, and sleep. Despite this, the AHA reports that diet quality scores are low among US adults with only 1.8% (95% CI: 1.2%, 2.6%) having an ideal diet score indicated by higher intakes of fruits and vegetables, fish and shellfish, whole grains, nuts, seeds, and legumes and lower intake of sodium, sugar-sweetened beverages, processed meats, and saturated fat (Tsao et al. 2022). More than one in three adults have a poor diet (36.6% [95% CI: 32.8%, 40.6%]) and 61.6% (95% CI: 57.5%, 65.5%) have an intermediate level of diet quality. The poor adherence among US adults to a healthy dietary pattern underscores the need to expand available strategies targeting diet quality and prediabetes.

Morning hyperglycemia is an important target for adequate glucose control. Among individuals with impaired insulin response (i.e., prediabetes, TIIDM), excess hepatic glucose production in the early morning hours (from 3 a.m. and beyond) gives rise to hyperglycemia (O'Neal, et al. Teri & Luther, 2022). Morning glucose excursions of more than 50 mg/dL can result in higher daytime glucose levels (11). Such an effect makes it more difficult for

individuals to optimize their time in target glucose range (e.g., fasting glucose > 70 mg/dL < 100 mg/dL, postprandial glucose < 180 mg/dL) to attenuate progression to TIIDM (12). A common treatment strategy to lower morning hyperglycemia is to consume a snack before bedtime (13). A bedtime snack may reduce the gluconeogenic demand overnight and lessen the magnitude of postprandial glycemia that occurs with a prolonged fast (14). Because carbohydrates (CHO) are commonly used to manage glucose levels, a bedtime snack containing 15 g to 30 g of CHO is often recommended to patients with morning hyperglycemia (15). Health professionals (i.e., dietitian) may also provide education on how to track CHO intake at meals and snacks using the carbohydrate exchange method also called "CHO counting" (16). Although common in clinical practice, research supporting nighttime carbohydrate consumption to help regulate morning hyperglycemia is sparse.

Clinical trials show that consumption of pistachios as a snack can improve glycemic control and diet quality (17,18). Pistachios contain several components associated with health benefits including unsaturated fatty acids (mono- and polyunsaturated fatty acids), plant protein, fiber, non-nutritive bioactives, vitamins, and minerals (19). Consumption of pistachios has been shown to improve glycemic control, lipids/lipoproteins, and vascular health (20–22). These health benefits may be partially mediated by the gut microbiota since fecal microbial composition is a deterministic factor in glycemic response to foods (23). Furthermore, pistachios may improve diet quality as a source of plant protein and may displace other commonly consumed snacks with a less favorable fatty acid composition thereby lowering intakes of saturated fatty acids (18). While clinical trials support the efficacy of pistachio consumption on indicators of TIIDM and CVD, the effectiveness of pistachios in the management/treatment of prediabetes is limited.

The purpose of this dissertation research is to examine the effectiveness of nighttime pistachio consumption to improve fasting markers of glycemic control in adults with prediabetes over 12 weeks compared to a usual care condition. The current standard of clinical care (usual care) includes education to consume 1-2 carbohydrate exchanges as an evening snack to lower morning hyperglycemia. To evaluate effectiveness, the following outcomes were assessed in a randomized, 2-period, single-blinded, cross-over study: FPG, fasting measure of glycemic control (hemoglobin A1c, insulin, homeostatic model assessment of insulin resistance), lipids/lipoproteins, measures of vascular health (blood pressure, central blood pressure, arterial stiffening), and changes to the microbiota (diversity and composition).

Chapter 2:

Literature Review

2.1. Guidelines for prevention and treatment of cardiovascular disease (CVD) and Type II Diabetes Mellitus (TIIDM)

Current dietary recommendations for general health and chronic disease prevention (e.g., 2020-2025 Dietary Guidelines for Americans [DGAs]) state that a healthy dietary pattern should be followed throughout all life stages (24). Healthy dietary patterns are defined by intake of predominately nutrient dense foods and beverages within calorie limits (24). Therefore, recommended diets emphasize fruits, vegetables, whole grains, low fat or fat free dairy, plant proteins (nuts, seeds, soy, legumes), lean unprocessed animal proteins, and vegetable oils. Foods and beverages to limit (to meet specific recommendations) include those with added sugars (<10% kcals/d), saturated fat (<10% kcals/d), and excess sodium (<2,300 mg/d). These recommendations align with many non-government organizations with the goal of improving CVD and TIIDM health outcomes. The American Heart Association (AHA) guidance for a diet that optimizes CV health includes maximizing consumption of unsaturated fatty acids, healthy protein sources (i.e., seafood, legumes and nuts, low fat/fat free dairy), fruits and vegetables and minimizing added sugars, ultra-processed foods, high sodium foods, and tropical oils/fats (24,25). Similarly, the guidelines for prevention and treatment of TIIDM from the American Diabetes Association (ADA) recommend a healthy dietary pattern with the goal of maximizing the time within normal glucose levels (70-99 mg/dL fasting; 70-180 mg/dL post prandially) (ElSayed et al. 2023). A healthy dietary pattern according to the ADA is to consume a mostly plant-based diet with an emphasis on whole foods (e.g., fruits, nuts and seeds, whole grains,

vegetables) while limiting saturated fatty acids, added sugars, and highly processed foods (ElSayed et al., 2023). Current dietary guidance is consistent in emphasizing a healthy dietary pattern in addition to achieving a healthy weight, exercise, and meeting caloric needs to reduce risk of diet-related chronic diseases.

2.2 Diet quality as a measure of how diets align with current dietary recommendations

Diet quality indices are used to assess the healthfulness of dietary intake based on adherence to dietary guidelines (27). Some indices evaluate intake of foods and nutrients associated with risk for chronic diseases. Since many dietary patterns can be used to achieve a healthy diet, there are several indices that measure diet quality such as the Healthy Eating Index (HEI), alternative Healthy Eating Index, Mediterranean diet score, and Dietary Approaches to Stop Hypertension (DASH). Generally, lower diet quality scores represent lower diet quality and a higher chronic disease risk. For example, the HEI-2015 score ranges from 0-100 with higher scores given for greater consumption of total and whole fruits, total vegetables, greens and beans, seafood and plant proteins, total protein, dairy, fatty acid ratio (MUFA and PUFA:SFA) and whole grains whereas greater consumption of refined grains, sodium, added sugars, and SFA lower the HEI-2015 score. Higher diet quality scores are associated with improved health outcomes. Prospective cohort studies show that higher diet quality scores are associated with between 14–29% lower risk of CVD and 0.5–2.2 years greater CVD-free survival time (27). Likewise, higher diet quality scores are linked to a 10% to 29% lower risk for incident TIIDM compared to lower scores (28,29). Consequently, diet quality is considered a major modifiable risk factor for CVD and TIIDM and improving dietary patterns is a central component of prevention and treatment strategies (25,30).

Current diet quality scores among Americans fall short of a healthy dietary pattern. The average HEI-2015 score among all age groups is 59 out of 100 (24). Adults (ages 19-59 years) have a below average HEI-2015 score of 56 with average intakes for total vegetables, total fruits, total dairy, seafood, whole grains, and nuts and seeds below recommendations and added sugar intake above current recommendations. Older adults (≥ 60 years) have a slightly higher average HEI-2015 score of 63. However, most older adults exceed the recommendation for added sugars (males: 54%; females 58%), saturated fatty acids (males: 80%; females 77%), and sodium (males: 94%; females 72%). The current HEI-2015 scores among Americans show that current dietary patterns do not align with dietary guidelines. As such, interventions directed at addressing components of poor dietary patterns (e.g., low intake of nuts and seeds, whole, grains, seafood, high intakes of added sugars) are needed to improve diet quality.

2.3 Diet is a modifiable risk factor for the treatment of prediabetes

Prediabetes is among the major diet-related risk factors for CVD and TIIDM. Prediabetes is a condition in which blood glucose is elevated from abnormal carbohydrate metabolism. The glucose levels are above normal ranges but below the criteria for TIIDM (8). The glucose range for prediabetes includes a FPG between 100 and 125 mg/dL, glycated hemoglobin (hemoglobin A1c [HbA1c])) > 5.6% and < 6.5%, and/or a postprandial blood glucose > 140 mg/dL and < 200 mg/dL (ElSayed et al., 2023). Among the 1 in 3 US adults with prediabetes, approximately 74% will go on to develop TIIDM in their lifetime (31,32). While elevated glucose is commonly associated with diabetes, prediabetes also increases the risk of CVD by up to 18% (6). Prediabetes increases risk in both TIIDM and CVD because chronically elevated blood glucose may damage heart and blood vessels and is associated with other risk factors such as obesity,

dyslipidemia, and hypertension (ElSayed et al. 2023). Consequently, interventions targeting prediabetes improve risk for both TIIDM and CVD.

Improving diet quality is a practical and convenient treatment strategy for prediabetes. The ADA guidelines on lifestyle/behavior change for TIIDM prevention include intensive lifestyle/behavior change programs to achieve at least 7% weight loss by reducing caloric intake, achieving moderate to intense physical activity weekly, and improving diet quality through various healthy eating patterns (30). Sustaining weight loss and exercise pose substantial challenges for individuals, which has been outlined in the literature. For example, weight cycling is a term that specifically describes the failed attempts at sustained weight loss. Estimates show that more than half of the weight lost is regained within 2 years and more than 80% body weight is regained in 5 years (34). Similarly, exercise therapies for TIIDM treatment have a wide range of adherence with some estimates showing that between 10% - 80% of individuals sustain their exercise routine over long periods (35). The most common barriers of successful weight loss and continued exercise among those at all stages of TIIDM are a lack of motivation and injury. Overcoming these barriers may require substantial time and effort before committing to intensive lifestyle/behavior change programs as recommended by the ADA. Alternatively, commonly reported barriers to sustained dietary modifications include a dislike of foods included in meal plans and social influences (35). Addressing barriers involving food selection may be overcome with less intensive lifestyle/behavioral approaches. Moreover, convenient dietary modifications such as a healthful snack consumed each day has been shown to effectively improve diet quality (18). As such, improvements in diet quality can be achieved with small dietary changes and, thus, may be a more feasible treatment approach.

2.4 Education about carbohydrate (CHO) exchanges to improve abnormal glycemia

The general focus for dietary interventions that prevent or treat prediabetes is to limit the duration of abnormal glucose levels (12,16). Several dietary approaches are used by healthcare professionals to improve daily glycemic control such altering meal frequency, breakfast consumption, carbohydrate (CHO) distribution, and meal composition (36). Advice for improving glucose management independent of weight loss may focus on CHO intake since glucose levels are directly linked to the quantity and type (e.g., low vs. high glycemic index, nutrient dense, etc.) of CHO consumed. The ADA treatment strategies for abnormal glucose levels include individualized eating patterns that help to lower CHO intake, emphasize nutrient-dense CHO sources, and/or establish a consistent pattern of CHO timing and amount (ElSayed et al., 2023). Therefore, lowering or distributing CHO intake throughout the day is a common clinical approach to regulate abnormal glycemia.

The use of CHO counting, also referred to as "carbohydrate exchanges," is an effective tool to help individuals identify and distribute daily CHO intake. CHO counting is a technique whereby individuals are taught how to identify foods that contain CHO (37). A single CHO exchange is equal to 15 g CHOs. Education about how to use the CHO exchange method includes a listing of serving sizes for common foods that meet the 15 g CHO criteria. Individuals work with health professionals to distribute the number of CHO exchanges consumed at meals and snacks with meals having between 3-6 CHO exchanges and snacks between 1-2 CHO exchanges. The ADA and Academy of Nutrition and Dietetics, an organization that provides clinical practice guidelines for dietitians, recommends CHO exchanges as a method of tracking CHO intake at meals and snacks to address conditions of abnormal glucose levels (16). This recommendation is based on its effectiveness as a tool for reducing elevated blood glucose in

TIIDM. An RCT in adults with TIIDM reported lower HbA1c (−0.63% [95%CI: −1.03%, −0.18%]), a measure of longer-term glycemic control (~3 months), with education on CHO exchanges over 6 months compared to adults that received general health education (38). Similarly, CHO counting education lowered HbA1c 0.11% (95% CI: -0.78%, -0.44%) from baseline over 6 months in adults with TIIDM (39). This study also showed improved FPG (-18.00 mg/dL ± 7.0), 2-hour postprandial glucose (-21.8 mg/dL ± 29.0), and CVD risk markers SBP (-5.75 mm Hg ± 5.75), TC (-5.08 mg/dL ± 6.08), and LDL-C (-5.92 mg/dL ± 5.92) from baseline. The use of CHO counting is an effective tool for improving glycemic control among individuals with abnormal glucose levels.

2.5 Snacking is a critical component of a healthy dietary pattern

Food choices at snacks is an important factor for overall diet quality. Estimates from the National Health and Nutrition Examination Survey (NHANES) 2011-2018 show that between 17.9% and 23.2% of total energy is consumed as snacks among adults (40). The most recent trends show a growing percentage of energy is consumed as snacks with estimates between 3% to 5% from 1971 to 2010 (41). Most of the energy increase from snacks can be accounted for by a decrease in energy intake at meals. This shift is potentially concerning because diet quality is generally lower for snacks than meals. Diet quality scores from the NHANES 2011-2018 among those that consumed snacks were between 47.5 to 54.3 points whereas mealtime HEI scores ranged from 49.9 to 57.3 points (40). Moreover, the most common snack choice among US adults is “snacks and sweets” (i.e., cookies and pastries), which contribute a greater proportion of total energy (32% - 49%) when chosen as a snack. Given the contribution of energy from

snacking and current food choices, snacking behaviors have become an increasingly important factor in overall diet quality.

Food selection for snacking can have meaningful benefits on risk factors for chronic diseases. A recent review by Almoraie et al. found that food choices for snacks may exacerbate hypertension, glycemic control, and overweight/obesity when snacks are higher in energy, added sugars, and salt (42). Alternatively, nutrient dense snacks such as fruits, vegetables, nuts and seeds may improve chronic disease risk factors. While limited, studies examining snacking habits show that healthful food choices benefit risk factors for chronic diseases. A cross sectional analysis of working adults (n = 233) showed that dairy (milk, ice cream, yogurt), chips, crackers, popcorn, and desserts and sweets (cakes, pies, candy) contributed between 15% and 20% of energy from total daily snacking (43). When desserts and sweets were a greater percentage of total calories from snacks, diet quality decreased (HEI-2010: $\beta = -0.016$, $p = 0.024$) and BMI increased ($\beta = 0.04$; $p = 0.017$). Healthful snack choices were associated with improved diet quality such that each 10% increase in energy from fruit/fruit juices or nuts increased diet quality scores by 1.3 points and 1.6 points, respectively. These findings align with a cross-sectional analysis of NHANES (2001-2008) data that observed between 18.3% to 20.6% higher energy intakes and 14.8% to 21.8% higher added sugar consumption with snacking patterns that included cakes, cookies pastries, sweets, and milk desserts when compared to no snacks (44). When individuals consumed whole fruits as a snack, added sugars consumption was 20.5% lower and energy intake was similar compared to no snack. Taken together, healthful food options such as whole fruits, nuts, and fruit juices provide health benefits when compared other snack options (e.g., sweets, cakes, pastries, etc.) or no snacks.

2.5.1 Nighttime snacking to address morning hyperglycemia

Nighttime snacking has been used to help regulate elevation in morning fasting glucose. An estimated 3 in 5 Americans consume a snack after dinner but before bedtime otherwise known as a nighttime snack (41). Nighttime snacks account for nearly 15% of total energy intake, which constitutes the greatest proportion of daily energy intake from snacks consumed in a day. Among those with morning hyperglycemia, nighttime snacking has been a focus for dietary interventions (13). This focus primarily stems from hyperglycemia that occurs with endogenous glucose production and/or postprandial glucose excursions at morning meals. Excess hepatic glucose production in the early morning period can lead to hyperglycemia among individuals with impaired insulin excretion or response (i.e., prediabetes, TIIDM) (11,14). The excess rise in blood glucose coincides with reduced hepatic and/or peripheral insulin sensitivity. Chronic elevations in morning glucose resulting from excess hepatic glucose production and/or insulin insensitivity can ultimately lead to higher vascular complications if left untreated (12).

Morning hyperglycemia may also exacerbate postprandial glucose at meals following a fasting period. The incremental change in postprandial plasma glucose at lunch is nearly double when adults with TIIDM skip breakfast mimicking an extended fasting period (14). Moreover, morning glucose excursions of > 50 mg/dL can result in higher daytime glucose levels making target glucose ranges more difficult to achieve (11). As such, extending the fasting period to overcome morning fasting hyperglycemia can further exacerbate poor glycemic control. To attenuate the morning rise in glucose and reduce the fasting window, a common clinical practice is to recommend that patients consume a nighttime snack (13). A CHO-containing snack is generally suggested because daily glycemia is regulated by distributing CHOs evenly throughout the day (33). Health professionals (i.e., dietitian) may use techniques including CHO exchanges

to aid patients in identifying CHO-containing foods for their evening snack (15). However, the evidence supporting CHO-containing evening snacks to improve morning fasting glucose is mixed.

Several trials have examined differences in markers of morning glycemic control with CHO snacks. A randomized clinical trial (RCT) over 7-weeks among adults with TIIDM (n =24) consuming uncooked corn starch (0.3 g/kg of uncooked corn starch/d) versus a control (pectin: 0.06 g CHO/kg body weight) showed that a nighttime CHO snack did not alter fasting blood glucose levels whereas the control increased fasting glucose by 18 mg/dL (SE: 5.4 mg/dL) (45). This effect is likely not a function of meal timing alone (i.e., eating meals closer to bedtime), but relates to the quantity of CHO consumed. Continuous glucose monitoring of adults with TIIDM showed lower incremental area under the curve (AUC) between 11:00 pm - 8:00 am when 26.4 g CHO were consumed before bedtime versus 86.6 g CHO (147 ± 63 vs. 644 ± 156 mmol/L x min; $p < 0.01$) in a crossover clinical trial (Imai et al. 2017). Improvements in morning glucose with modest CHO consumption near bedtime have also been demonstrated in a crossover trial comparing 3 vs. 6 meals per day over 12 weeks (46). The 6 meals per day included a nighttime snack consisting of 10% of daily CHO (~150 kcals) whereas the interventions with 3 meals had no nighttime snack. Consuming 6 meals per day (10% CHO nighttime snack) resulted in approximately 4% lower HbA1c and -40 mg/dL 2-hr glucose concentration in response to an oral glucose tolerance test compared to 3 meals per day.

Trials investigating routine clinical measures of glycemic control do not show a consistent benefit. FBG is routinely assessed after an 8-12 hour fast. Fasting glucose levels in plasma (FPG) or serum (FSG) are used to approximate FBG. Abnormal FBG is used as a first sign of impaired glucose tolerance and FPG and hemoglobin A1c (HbA1c) are generally used as

methods for prediabetes and TIIDM screening or diagnosis (47). The effects of CHO snacking on FPG among the previously reviewed trials show no difference between the CHO interventions and non-CHO comparators (45,46,48–50). A 2-period crossover trial in among adults with TIIDM (n = 54) showed FPG was lowered by 19.2% and HbA1c by 0.04% from baseline after consuming 6 small meals throughout the day including an evening CHO snack (51). However, consuming 6 meals with CHO as an evening snack was no better than similar energy intake with only 2 meals per day suggesting that nighttime CHO consumption has similar effects on FPG and HbA1c (51). Other trials have examined evening CHO consumption on morning FBG and showed no benefits with CHO as an evening snack (52–55). Notably, these null findings are mostly limited to trials in which adults have a TIIDM diagnosis thus limiting their applicability as a method of prevention. Also, with the exception of a few trials (51,54), the number of included participants is generally low (n = 10 - 16) and interventions were between 1-3 days. Potential inferences about the effects of nighttime CHO are further confounded by the diversity in interventions between trials (e.g., cereal flakes, extend bar, corn starch, bread, crackers versus control [no snack, yogurt, cheese]). Taken together, more research is needed to better understand the effects of nighttime CHO snacking on FBG.

2.5.2. Nuts are an alternative to CHOs as a healthy nighttime snack

Nuts are a convenient and well-studied food with associations to improved health outcomes and diet quality (56). The health benefits of nuts are related to their nutrient composition. Nuts generally are low in SFA and higher in unsaturated fatty acids. Higher nut intake is associated with a dietary pattern that aligns with the DGAs and AHA recommendations to decrease SFA (and replace it with unsaturated fat), choose healthy sources of protein (mostly

plants), and choose minimally processed foods (9,57). Nuts are also high in fiber. Fiber is another component of a healthy dietary pattern and nutrient that can lower proatherogenic LDL-C to reduce CVD risk (58). A growing body of research also shows that fiber consumption supports a healthy gut microbial composition (59). Additionally, the gut microbiota help mediate glycemic response, which is described in more detail in section 2.7 (23,59). Nuts also contain plant protein, essential vitamins, minerals, and fatty acids that complement a nutrient-dense, healthy dietary pattern.

The DGAs include nuts as a core element of a healthy dietary pattern (24). Consumption of 5 oz-eq of nuts per week for a 2,000-calorie diet is recommended. Many other nations and institutions include nuts as a component of a healthy diet. The Canadian Cardiovascular Society (2016) recommends 30 g/d of nuts for dyslipidemia and CVD prevention with moderate quality of evidence (60). For TIIDM, nuts are specified as an essential component of a healthy diet for TIIDM prevention in an amount that does not increase total energy intake (61). Although many organizations suggest nuts are a component of a healthy diet, few specify which nuts confer health benefits and how they impact markers of chronic disease.

A systematic review and meta-analysis of 40 RCTs (n = 2,832) examined whether peanut and tree nuts beneficially affect markers of glycemic control among adults that are healthy or have prediabetes/TIIDM (20). Fasting insulin was lower (weighted mean difference [WMD]: −0.40 μIU/mL; 95% CI: −0.73, −0.07 μIU/mL; I^2 = 49.4%) when individuals consumed between 9 g/d - 128 g/d of mixed/individual nuts compared to comparator conditions devoid of nuts. Insulin resistance as measured by the homeostatic model assessment of insulin resistance (HOMA-IR) also improved with nut intake (WMD: −0.23; 95% CI: −0.40, −0.06; I^2 = 51.7%) versus nut devoid comparators. A subgroup analysis found that the benefits may be specific to

those with prediabetes. Insulin was reduced by a WMD of −3.86 μIU/mL (95% CI: −5.72, −2.0 μIU/mL) and HOMA-IR by −1.14 (95% CI: −2.0, −0.28; I^2 = 52.7%) among individuals with prediabetes whereas healthy individuals and those with TIIDM had no glycemic benefits.

2.6 Impact of pistachio intake on glycemic control

Pistachios may be unique among tree nuts/peanuts in their benefits to glycemic control. Tindall et al. performed a subgroup analysis on individual tree nuts or peanuts (i.e., almonds, hazelnuts, peanuts, walnuts, pecans, cashews) interventions and found that pistachios (between 40 and 128 g/d) were the only nut to lower fasting glucose (-5.18 mg/dL [95%CI: -8.76, -1.60]) among 7 RCTs over 1-6 months (20). Several recent systematic reviews and meta-analyses give support to the glycemic benefits of pistachio consumption. A review by Baghery et al. including 17 RCTs (n = 940) in healthy and unhealthy adults with risk factors for CVD and TIIDM found that the mean difference in fasting glucose was -3.62 mg/dL (95% CI: -6.45, -0.8 mg/dL) with 25 g/d - 128 g/d pistachios compared to nut devoid comparator conditions (62). The reduction in FBG was greater among individuals with chronic disease risk markers (MD: –5.1 mg/dL [95% CI, –9.14 to –1.05]) than in healthy individuals (MD: –3.75 mg/dL [95%CI, –5.34 to –2.16]). Other indicators of glycemic control also show benefits with pistachio consumption. A systematic review and meta-analysis of 8 RCTs (n = 535) found lower serum insulin (6 trials; n = 405): WMD –1.86 μIU/ml (95 % CI: –3·13, –0·59) and HOMA-IR (3 trials; n = 256: WMD −0.66 (95 % CI: −1.89, 0.58) after 4-24 weeks of pistachio consumption compared to control interventions (22). A systematic review and meta-analysis examined the odds (odds ratio [OR]) that pistachio interventions lower glycemic indicators compared to nut free comparator conditions (63). Consumption of 42-85 g/d of pistachios was associated with 1.7 times higher

odds (95% CI: 1.2-2.4) of a significantly lower blood glucose levels versus comparators. The greatest odds of having a lower fasting blood glucose were observed in one trial (17) among individuals with prediabetes in which the odds were 2.49 greater (95% CI: 1.24, 4.99). Collectively studies examining pistachio intake and glycemic control support a therapeutic benefit among those with and without risk factors for CVD and TIIDM.

2.6.1 Pistachio consumption and dyslipidemia, blood pressure, and vascular measures

The benefit of pistachio consumption may include other cardiometabolic markers. A 2019 network meta-analysis (trials = 34; n = 1677) examining the effect of tree nut consumption on lipids/lipoproteins reported that a pistachio-enriched diet lowered TC by -5%, LDL-C -5%, and triglycerides (TAGs) -10% compared to diets enriched with walnuts, hazelnuts, cashews (64). A meta-analysis by Baghery et al. (17 RCTs) reported that pistachio consumption reduced SBP (Mean Difference [MD]: –2.89 mmHg [95% CI, –4.11 to –1.67]), TAGs (MD: –16.76 mg/dL [95%CI, –16.89, –16.64]) and increased HDL-C (MD: 1.43 mg/dL [95%CI, 1.39, 1.47]) compared to nut free control conditions (62). Similarly, a meta-analysis of 11 RCTs showed lower SBP (WMD: -3.10 mmHg [95% CI: -5.35, -0.85]) with pistachio intake versus control conditions (65). Benefits were also observed for TC:HDL-C (WMD: -0.46 [95% CI: -0.76, -0.15; I^2 = 95%]) and LDL-C:HDL-C (WMD: -0.24 [95% CI: -0.38, -0.11; I^2 = 96%]), which suggests pistachios may alter lipids/lipoproteins to a less atherogenic profile.

2.7 The gut microbiota and glycemic control

A growing body of evidence shows that the gut microbiota is an important factor in mediating changes in TIIDM and CVD risk markers from dietary interventions. The gut microbiota refers to the bacteria, archaea, bacteriophages, eukaryotic viruses, and fungi that are

present in the gastrointestinal tract (59). While a healthy gut microbiota is generally unique to an individual, microbial characteristics may be considered health promoting such as high taxa diversity, high microbial gene richness, and stable microbiome functional cores (59). Changes in the nutrient composition (i.e., higher SFA intake, low fiber intake) or consumption of different foods (e.g., nuts and seeds) may alter these healthy gut characteristics, which can lead to decreased satiety, weight gain, and inflammation, all of which contribute to chronic disease pathogenesis (59).

The gut microbiota has been identified as a deterministic factor in the glycemic response to foods. The pioneering work by Zeevi et al. (2015) showed that gut microbial composition predicts an individual's glycemic response(23). Zeevi et al. developed a predictive algorithm for glycemic response to foods in a cohort of 800 individuals with prediabetes and TIIDM. The algorithm included changes in dietary intake, the microbiota, anthropometrics, exercise, and biometrics (e.g., FBG, lipids/lipoproteins, etc.). A separate cohort then participated in a parallel RCT in which 26 subjects consumed an individualized algorithm-based diet or an expert diet (dietitian). The algorithm-based diet resulted in lower postprandial glucose response and less fluctuations in overall blood glucose levels over 1 week (measured by continuous glucose monitor). Interestingly, the algorithm-based diets targeted at lowering postprandial hyperglycemia included foods more commonly associated with lower diet quality and poor glycemic control such as pizza and schnitzel (breaded and deep-fried meat). Berry et al. (2020) has since quantified the degree to which meal context, composition, and individual factors (e.g., biometrics, anthropometrics, microbiome) affect postprandial TAGs and glycemia in 1,002 twins and unrelated healthy adults (Berry et al. 2020). Their study showed high interindividual variation in fasting glucose (coefficient of variation [CV]: 68%) and TAG (CV: 103%). Gut

microbial composition accounted for 6.4% of the postprandial glucose response and 7.5% for TAG. The influence of the gut microbiota in these trials provides important context about the role of the gut microbiota in glycemic response and individual variability.

2.7.1 The gut microbiota and pistachio consumption

While the benefits of pistachio consumption on glycemic control are well established, only a few trials have examined the effect of pistachios on the gut microbiota (66,67). Generally healthy overweight adults consumed controlled diets with 42 g/d g/d of pistachios, 84 g/d of pistachios, or a standard western dietary pattern for 3 weeks each (68). Fecal samples analyzed using 16S rRNA sequencing showed that butyrate-producing bacteria was increased with pistachio consumption. Butyrate is produced from fiber degradation by gut microbes and used for colonocyte metabolism. Higher butyrate-producing bacteria are associated with improved gut health (59). Measures of microbial species diversity (α-diversity) and community diversity (β-diversity) were not different between conditions. A trial by Hernández-Alonso et al. examined effects of pistachio consumption on gut microbial metabolites in urine (66). Adults with prediabetes (n = 39) were provided 57 g/d of pistachios consumed as morning/afternoon snacks in controlled diets over 4 months or a similar diet in which most energy from pistachios was replaced by olive oil. Alterations to the gut microbiota were indirectly measured changes by microbial metabolites hippurate, p-cresol sulfate, dimethylamine (DMA), and trimethylamine N-oxide (TMAO). All four gut-derived metabolites were decreased in urine after pistachio consumption compared to control diets suggesting pistachios alter microbial production of these metabolites. Each metabolite has associations with chronic conditions related to chronic diseases. Hippurate is positively associated with metabolic diseases such as obesity, hypertension, insulin resistance and TIIDM (69,70). p-Cresol sulfate is a protein derived polyphenol that has been

shown to participate in endothelial dysfunction and leukocyte free radical production, which may lead to altered endothelial metabolism (71). Dimethylamine and trimethylamine N-oxide (TMAO) are molecules derived from choline, carnitine, or betaine, and are risk factors for CVD (72). Although pistachios are a choline-rich food, choline metabolites dimethylamine and TMAO were lower with the pistachio diet. These trials indicate that pistachio consumption affects the gut microbiome. However, major gaps remain about whether these changes are associated with changes in glycemic control.

2.8 Rationale for current research

The majority of US adults have a dietary pattern that falls short of current dietary recommendations, with most adults overconsuming saturated fatty acids (SFA), added sugars, and sodium (57). Moreover, most US adults do not meet current recommendations for nut consumption (5 oz-eq of nuts per week for a 2,000-calorie diet) (73). A greater focus on food selection for nighttime snacks is needed considering that energy from snacks increased from 1971-2010 and over half of snack related energy is consumed as nighttime snacks (41). The usual care for treatment of morning hyperglycemia among those with prediabetes and TIIDM is to consume between 1-2 CHO exchanges as an evening snack but current evidence for this approach is mixed (13). Alternatively, pistachios are the only tree nut, along with peanuts, that have been shown to improve markers of glycemic control in prediabetes and TIIDM (20). Given the unique effect of pistachios on glycemic control, this study is designed to evaluate the effect of nighttime pistachio consumption on fasting blood sugar levels, longer-term blood sugar control, and risk factors for CVD compared to a usual care condition involving education to consume 1-2 CHO exchanges as a nighttime snack (usual care).

Several systematic reviews and meta-analyses of clinical trials consistently show that pistachios improve measures of glycemic control (19,22,62–65,74,75). However, only 2 clinical trials have been conducted since 2016 and only one trial included adults with prediabetes. Examination of nighttime pistachio consumption compared with the current usual clinical care (1-2 CHO exchanges) among adults with prediabetes is needed to better understand glycemic effects of pistachio consumption. Moreover, investigating the effect of pistachios on the gut microbiota is needed to identify how pistachio consumption affects gut health.

In summary, this dissertation research was conducted to address knowledge gaps in the existing nighttime snacking, pistachio, and morning fasting glucose literature, with the overarching goal of identifying both glycemic control and cardiometabolic impacts of nighttime pistachio consumption and exploring the relationship with the gut microbiota.

2.9 Objectives and hypothesis

The purpose of this dissertation research is to examine the effect of nighttime pistachio consumption versus a usual care condition (education to consume 1-2 CHO exchanges) on fasting markers of glycemic control, cardiometabolic health, and the gut microbiota in adults with prediabetes. This study was a 2 period (12 weeks each) randomized crossover clinical trial. Participants were provided with 57 g/d of pistachios or education about CHO exchanges and how to select and purchase (with a gift card provided) 1-2 CHO exchanges to be consumed as a nighttime snack. The specific objectives and hypotheses of this study are:

Objectives:

1) Objective: To establish whether consuming 57 g/day of pistachios as an evening snack (after dinner and before sleep) improves glycemic control and risk factors for cardiometabolic disease compared with usual care (education to consume 1-2 exchanges of carbohydrate as an evening snack), in individuals with prediabetes.

Hypothesis: I hypothesize that glycemic control will improve with pistachios compared to the usual care condition. Specifically, pistachios will lower morning FPG, insulin, insulin resistance (HOMA-IR), and glycated hemoglobin (HbA1c). Nighttime pistachio consumption will also improve cardiometabolic markers such as LDL-C, HDL-C, TAGs, peripheral blood pressure, central blood pressure, and markers of vascular health (i.e., pulse wave analysis and pulse wave velocity).

2) Objective: To evaluate changes in diet quality between daily nighttime pistachio intake and the usual care condition.

Hypothesis: Pistachio consumption will improve diet quality compared to the usual care condition as measured by the Healthy Eating Index-2015. These benefits will be driven by changes in scores for the following components that reflect the nutrient profile of pistachios: total protein, seafood and plant protein, fatty acid ratio (MUFA± PUFA/SFA), sodium, and total SFA intake SFA.

3) Objective: To assess changes in the profile of the gut bacterial community in response to pistachio consumption compared with usual care.

Hypothesis: I hypothesize that the bacterial community will shift to a healthier profile in quantity and diversity after the pistachio condition.

Chapter 3:
Effects of nighttime pistachio intake on glycemic control and cardiometabolic response compared to 1-2 carbohydrate exchanges in individuals with prediabetes

Abstract:

Background: Nut intake is associated with better glycemic control and lower risk of cardiovascular disease (CVD). It remains unclear if nut intake timing affects glycemic control and CVD risk factors. It is hypothesized that intake of pistachios as a nighttime snack may attenuate early morning endogenous glucose production resulting in lower fasting plasma glucose (FPG).

Objectives: To test this hypothesis, we assessed the effect of consuming 57 g/d of pistachios as a nighttime snack (i.e., after dinner and before sleep) for 12 weeks on markers of glycemic control, vascular health, lipids/lipoproteins and diet quality compared to 1-2 carbohydrate (CHO) exchanges (usual care) in individuals with elevated FPG.

Methods: A single-blind, 2-period, randomized crossover trial was conducted. Participants were provided 57 g/d of dry roasted unsalted pistachios (319 kcal; fat 26 g; CHO 16 g; protein 12 g; saturated fat 3.4 g; fiber 6 g) or education to consume 1-2 CHO exchanges (CHO 15-30 g) as a nighttime snack for 12 weeks. Primary FPG and secondary outcomes [hemoglobin A1c (HbA1c), insulin, HOMA-IR, lipids/lipoproteins, central and peripheral blood pressure, pulse wave velocity (PWV), and Healthy Eating Index-2015 (HEI-2015)] were measured at the beginning and end of each study period.

Results: 66 participants (40.9% female, 50.9 ± 11.6 years, 31.2 ± 4.0 kg/m^2, FPG 103.1 ± 9.9 mg/dL) were randomized and 51 participants completed the trial. No between-condition mean difference in FPG (-1.0 mg/dL [95% CI: -0.7, 2.9 mg/dL]) or HbA1c (0.0%; [95% CI: -0.0%, 0.0%]) was observed. Insulin was higher after the pistachio condition (3.0 μIU/mL [95% CI: 1.2,

4.8 μIU/mL]) compared with the usual care. HOMA-IR was higher after pistachio (0.8 [95% CI: 0.3, 1.3]) vs usual care. PWV was lower after pistachio (-0.3 m/s [95% CI: -0.5, -0.0 m/s]) compared to usual care. No-between condition effects were observed for lipids/lipoproteins, blood pressure, or vascular health. The HEI-2015 score was higher (5.6 points [95% CI: 0.2, 11.5 points]) following the pistachio condition vs. usual care. This was driven by higher scores for the seafood and plant proteins (1.8 points [95% CI: 0.8, 2.8 points]) and refined grains (2.4 points [95% CI: 1.2, 3.6 points]) components.

Conclusions: In individuals with prediabetes, intake of 57 g/d of pistachios as a nighttime snack did not improve FPG, lipids/lipoproteins, or blood pressure compared to the usual care control but increased diet quality and reduced arterial stiffness. Education to consume 1-2 CHO exchanges as a nighttime snack resulted in a reduction in insulin and insulin resistance compared to pistachios.

Introduction:

Prediabetes is defined as the presence of impaired fasting glucose (100 - 125 mg/dL FPG, 5.7% - 6.4% HBA1c) or impaired glucose tolerance (140 - 199 mg/dL) (12). Approximately 38% of US adults aged ≥ 18 years had prediabetes in 2017-2020 of which only 17.4% were aware they have it (31). The prevalence of prediabetes has increased by 3.5% from 2013 to 2020 (3). Estimates suggest that approximately 75% of adults with prediabetes go on to develop type 2 diabetes mellitus (TIIDM) in their lifetime (32). Moreover, 74% of adults with prediabetes at age 45 years will go on to develop TIIDM in the next 10 years (76). The presence of prediabetes increases the risk of other conditions with up to 18% higher risk of CVD compared to those with normal glycemia (6). Current treatment strategies for prediabetes involve intensive lifestyle and behavioral therapies such as sustained weight loss, medical nutrition therapy, and persistent moderate to intense physical activity (8). Intensive approaches may present substantial barriers and inhibit treatment success (36). As a result, there is a growing need for simple strategies that help to prevent or delay the development of prediabetes and TIIDM.

Dietary strategies to improve glycemic control can be an effective approach to prevent or delay further impairments in glycemic control. The American Diabetes Association (ADA) recommends improvements in diet quality with several dietary patterns (Mediterranean diet, DASH diet, etc.) (8). A common clinical recommendation for those with impaired fasting glucose is to consume a nighttime snack after dinner but before bedtime to address elevated fasting morning glucose sometimes referred to as the "dawn phenomenon" (O'Neal et al., Teri & Luther, 2022; Roach et al., 2022). Nighttime snack recommendations may include target quantities of carbohydrate (CHO)-containing foods since an even distribution of daily CHO consumption is used to reduce glycemic excursions. Both the ADA and the Academy of Nutrition and Dietetics include CHO counting, also called "CHO exchanges," as a method of

helping patients track daily CHO consumption (Academy of Nutrition and Dietetics, 2018; ElSayed, et al., 2023). Nighttime CHO intake has been shown to improve morning glucose excursions and daytime glycemic control (11,14). While daytime and postprandial glycemia is improved with nighttime CHO snacks, the effect on FPG is not well understood (48–50,54,77). More research is needed to better understand the effects of nighttime CHO snacking on morning fasting glucose measures.

Pistachios are a convenient and healthful snack shown to improve measures of glycemic control. An analysis of 7 randomized controlled trials (RCTs) found that, among other tree nuts (e.g., walnuts, cashews, almonds) and peanuts, pistachios were the only tree nut to improve fasting glucose (weighted mean difference: -5.18 mg/dL 95% CI: -8.76, -1.60) (20). Several other systematic reviews and meta-analyses report improved fasting blood glucose, insulin, HbA1c, and insulin resistance as measured the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) following pistachio interventions (22,62,63,65,75). Similar to other tree nuts and peanuts, pistachios contain several healthful components such as a high unsaturated to saturated fatty acid ratio, fiber, and essential nutrients. These components have been shown to improve lipids/lipoproteins, vascular health, and diet quality which underscores why nuts are part of current dietary recommendations (25,57,78). Despite this, few trials have examined how nighttime pistachio consumption affects morning fasting glycemic control.

To evaluate the effect of nighttime pistachios on morning fasting glycemic control, we conducted a randomized crossover trial in adults that met the criteria for prediabetes based on FPG. The consumption of 57 g/d (~2oz) of pistachios was compared to a usual care condition, which included education to consume 15-30g of CHO using the CHO counting technique. Both conditions were evaluated for their effect on markers of glycemic control, lipids/lipoproteins,

vascular health. It is hypothesized daily consumption of pistachios as a nighttime snack after dinner but before bed will improve FPG compared to the usual care condition.

Methods:

Trial Design:

A single-blind, 2-period, randomized crossover trial was conducted at the Pennsylvania State University between September 2019 and November 2022. Study activities were suspended in March of 2020 because of COVID-19. Research activities were resumed in March of 2021. Diet periods were approximately 12 weeks in duration and separated by a washout period of at least 4 weeks. Eligible individuals were randomly allocated in a 1-to-1 ratio to 2 randomization sequences generated using a computer-generated scheme (randomization.com) that contained blocks of 10 sequences. Randomization was performed after baseline testing was scheduled by an investigator not involved in data collection. The randomization code was held by the metabolic kitchen manager. The study coordinator involved in screening, data collection, and analysis was unaware of the randomization schedule until after trial completion. Blinding of participants was not possible because of the nature of the study conditions. This study is registered at clinicaltrials.gov (identifier: NCT04056208). The study protocol was approved by the Institutional Review Board of the Pennsylvania State University (University Park, PA). All participants provided written informed consent.

Participants:

Participants were recruited from State College, Pennsylvania and surrounding areas. Recruitment involved posting flyers in local businesses and university buildings as well as advertisements in local magazines and circulars. The study was also posted on Clinicaltrials.gov, StudyFinder (studyfinder.com), and our research group's webpage. Interested individuals were

telephone screened to assess potential eligibility. A follow-up clinic screening visit was then scheduled at the Pennsylvania State University Clinical Research Center to determine study eligibility. Measures of height, weight, blood pressure, waist circumference, and blood sampling were performed during the clinic screening appointment. Height was measured during the screening appointment without shoes. Weight measurements occurred with participants wearing light clothing and no shoes. Blood pressure was measured using a validated automated sphygmomanometer 3 times after a 5-minute rest (79); the mean of the last 2 measurements was used to determine eligibility. Nurses collected a fasting blood sample for a complete blood count, blood chemistry, and plasma glucose, which were assayed by a commercial laboratory (Quest Diagnostics Secaucus, NJ). Prior to the clinic screening, individuals were fasted (no food or drink except water) for at least 12 hours and avoided over-the-counter medications and alcohol for 48 hours.

Men and women aged 30–75 years with a BMI ≥25 and ≤45 kg/m^2 and elevated fasting plasma glucose ([FPG] ≥100 mg/dL and ≤125 mg/dL) measured at screening were eligible. Following the COVID-19 shutdown of research activities in March 2020, research was resumed in March 2021 after the PSU Dean of Health and Human Development, Campus Chancellors, the Senior Vice President for Research and the IRB approved research activities contingent upon a modified age inclusion criterion to 30–65 years. Age of inclusion was modified because available data at that time showed higher risk of adverse outcomes and severe COVID-19 for individuals above 65 years and vaccines were unavailable. This was the only eligibility criterion modified.

The exclusion criteria included a diabetes diagnosis (any type); systolic blood pressure (SBP) >160 mm Hg or diastolic blood pressure (DBP) > 100 mm Hg; use of antihypertensive,

lipid-lowering or glucose-lowering drugs, steroids or antibiotics in the previous month. In addition, eligible individuals did not have cardiovascular, liver or kidney disease, autoimmune disorders, or inflammatory conditions such as gastrointestinal disorders or rheumatoid arthritis. Other exclusion criteria were: use of supplements (psyllium, fish oil, soy lecithin, and phytoestrogens) and botanicals and not willing to discontinue use for the duration of the study; pregnancy, lactation or plans to become pregnant or have given birth in the past year; weight loss of ≥10% of body weight within the 6 months prior to enrolling in the study; or smoking or use of any tobacco products in past 6 months. In addition, shiftwork, an inability to consume a snack in the evening, allergy/intolerance/sensitivity to test foods (i.e., pistachios), consumption of >14 alcoholic drinks/week or not willing to avoid alcohol consumption for 48 hours prior to test visits were exclusion criteria. Individuals who donated whole blood within the previous 8-weeks, or those unwilling to refrain from donating blood during the study were also ineligible.

Intervention and Comparator:

During the pistachio condition, participants were provided with 57 g/d of dry roasted unsalted pistachios with education to consume them as an evening snack [324 kcal (fat: 26 g; carbohydrates: 16.04 g; protein: 11.9 g; saturated fat: 3.2 g; monounsaturated fat: 13.9 g; polyunsaturated fat: 7.54 g; fiber: g; sodium: 3.4 mg)]. During the control condition (usual care), participants were given education to consume 1-2 carbohydrate exchanges (15-30 g of carbohydrates) as an evening snack and provided with a gift card to a local grocery store, in approximately equal value to the pistachios, to purchase the snacks.

During both conditions a handout was provided including information about when to consume study foods, how much to consume, and to avoid consuming other calorie containing foods or beverages during and after the evening snack. The handout for the control condition

included information about carbohydrate exchanges, carbohydrate-containing food sources, and quantity of foods that met the 1-2 carbohydrate exchange criteria. This comparator was selected because carbohydrate exchanges are among the recommended approaches for achieving greater time in target glucose range and is not expected to negatively impact glucose control in those at risk for TIIDM (16,80) The education was conducted by the metabolic kitchen manager. For both conditions, participants were instructed to consume the snacks after dinner but before bedtime and avoid any other calorie-containing food or drink in the evening. Participants were asked to avoid any other tree nuts, peanuts, including nut butters, or additional pistachios not provided by the study team throughout the study.

During both conditions, participants met with the metabolic kitchen manager to receive pistachios or a gift card (depending on the condition) every two weeks. The monthly supply of pistachios was provided in daily 57 g portions. A single $30 gift card, approximately equivalent in value to the provided pistachios, was provided for each month of the usual care condition. In addition, adherence checks were conducted for both conditions, and re-education was provided when needed. Adherence was assessed using a daily adherence checklist completed by the participants at home. Questions about consumption of the study foods, if the study foods were consumed after dinner, a description of the snack (during the control condition), if other calorie-containing foods/beverages were consumed after study foods, if other peanuts/tree nuts were consumed, if changes in health or usual exercise habits occurred, and if any nonhabitual medications or supplements were taken were included in the checklist. Following the re-start of the study after the COVID-19 shutdown, the frequency of study visits was reduced to monthly for both conditions to limit in-person contact. Adherence checks were conducted biweekly by telephone. Adherence was calculated by dividing the number of days study foods were consumed

as directed (i.e., as an evening snack, without consuming any other food or drinks in the evening) by the number of days in each diet period.

Outcome Assessment:

Outcomes were measured on 2 separate consecutive days at baseline and end of each diet period (8 visits total). Participants were instructed to fast for 12 hrs prior to testing, avoid strenuous physical activity for 12 hrs, and refrain from drinking alcohol or taking over the counter medications for 48 hrs prior to each visit. Weight was measured on both days with a calibrated electronic scale while participants were wearing light clothing and no shoes. Blood was drawn at both visits to measure glucose, lipids and lipoproteins, and insulin. HbA1c was measured once at the beginning and end of each diet period. Vascular testing was performed on one of the two consecutive test days at each time-point.

Blood processing and assay methods:

Blood samples were drawn into three different collection vacutainers: serum separator, etheylenediaminetetraacetic acid (EDTA), and sodium fluoride (NaF)/potassium oxalate (KOx). Immediately following blood collection, tubes with NaF/KOx were inverted and centrifuged (1590 × g [± 90]) at room temperature for 15 mins. The plasma supernatant was aliquoted for storage. EDTA tubes containing whole blood were aliquoted after inverting 8 times. Serum separator tubes were left at room temperature to clot for 30 mins then centrifuged at 1590 × g (± 90) for 15 min. All samples were frozen at −80◦C upon collection before batch analysis at the end of the study. Glucose was measured in plasma samples. Glycosylated hemoglobin was measured in whole blood. Serum TC, LDL-C, HDL-C, and insulin were measured from serum separator tubes. Samples were assayed at the Pennsylvania State University Biomarker Core Lab (University Park, PA) using a Cobas c311 chemistry analyzer (Roche Diagnostics, Indianapolis,

IN) according to the manufacturer's instructions. Interassay coefficient of variation (CV) is estimated at <5% for all tests according to the manufacturers insert.

Vascular testing methods:

Vascular measures were performed with a SphygmoCor XCEL (AtCor Medical, Naperville, IL). Following a 5 min rest, peripheral and central blood pressure (BP) were measured in triplicate with participants resting in the seated position. Peripheral blood pressure and radial artery waveforms were measured with a cuff placed on the left arm. Central BP was calculated from measured peripheral BP and radial artery pressure waveform using a validated generalized transfer function. A heart rate of 75 beats per minute (BPM) was used as the adjustment for augmentation index. Immediately following the BP assessment, carotid-femoral pulse wave velocity (PWV) was measured with the SphygmoCor XCEL. Participants lay supine while a BP cuff was placed around the femur on the femoral artery. A tonometer was placed on the carotid artery. The carotid-femoral waveform was recorded for 10 seconds. Pulse wave analysis calculation divided the linear distance between the carotid and femoral sites by the transit time. The final 2 measures for BP and pulse wave analysis were averaged and used for the analyses.

Dietary assessment:

Dietary intake was assessed by nonrandom, participant-completed 24-h recalls prior to baseline and in the last week of each diet period. A total of four 24-h recalls were completed by each participant throughout the study with the Automated Self-Administered 24-Hour Dietary Assessment Tool (ASA24) (81). The diet recall tool was administered as recommended by the NCI Dietary Assessment Primer (82). Briefly, participants were provided with a unique username and password to access the ASA24 diet recall tool. The diet recall was to be completed

prior to study appointments. The Healthy Eating Index (HEI)-2015 was used to assess diet quality. HEI-2015 contains 13 components. Nine are "adequacy" components (whole grains, total fruits, dairy, etc.) and 4 are "moderation" components (saturated fats, sodium, refined grains, and sugars). Consuming more foods in the adequacy components increases the HEI score whereas lower consumption of foods in the moderation component reflects a higher score. The HEI-2015 was calculated using the Statistical Analysis System (SAS) code created by the NCI (83). Recalls were excluded if caloric intake was reported <600 or >4400 kcal/d for women and <650 or >5700 kcal/d for men. These cutoffs were derived from the NCI guidelines for reviewing and cleaning ASA24 data (84).

Statistical analyses:

The study was powered for the primary outcome, FPG. Based on prior research, to detect a 10 mg/dL difference (standard deviation 23.4 mg/dL) in FPG between conditions, 45 subjects were required (80% power; α=0.05) (17,85–89). Target enrollment was 59 individuals to ensure 45 subjects completed the protocol (assuming ~25% drop out rate). All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC). Data included all randomly assigned participants consistent with intent-to-treat principles. Normality of the residuals was assessed with univariate analysis (PROC UNIVARIATE). Skewed residuals were determined by distribution and normal probability plots (Q-Q plots). Variables with skewed residuals were logarithmically transformed (natural log) prior to analysis. Since weight, glucose, lipids, lipoproteins, and insulin were analyzed on two days at baseline and the end of each diet period, the average was calculated prior to analysis. Endpoint values were subtracted from baseline values to determine change from baseline. Data are presented as least-squares means ± standard error of the mean (SEM) unless otherwise stated. Between-condition effects for each outcome

variable were examined using the mixed-models procedure (PROC MIXED) at a predetermined α level of 0.05. Randomization sequence and condition were modeled as fixed effects. Participant nested within randomization sequence was modeled as a repeated effect to account for the crossover design. The baseline value was included as a covariate. Carryover effects and sex differences were determined by including randomization sequence, sex, and their interaction by condition (i.e., sex*condition, randomization*condition) in the model as fixed effects. In the primary analysis, the between-condition difference in mean values for each outcome was assessed with adjustment for the baseline value. Secondary analyses assessed within and between-condition change from baseline for all outcome variables. The covariance structure was determined using an optimized fit approach (lowest Bayesian information criterion), which varied depending upon the analysis being conducted.

Results:

Among the 286 individuals that completed a telephone screening, 66 participants were randomized (Figure 3-1). Baseline testing was conducted on 64 participants. In total, 51 participants completed the trial. The COVID-19 pandemic resulted in the suspension of human subject's research at Penn State University after 9 months of study activities. At that time, 23 participants were enrolled. Of these participants, 13 withdrew from the study, and 5 participants restarted diet period 1 and 5 participants restarted diet period 2 when study activities resumed. For the 13 participants that were withdrawn, 5 no longer met the age inclusion criterion (age > 65 years), 5 were lost to follow-up, and 3 did not meet the FPG criterion upon rescreening. After the study resumed following the COVID-19 shutdown, 43 participants were randomized. Of these participants, one was lost to follow up before starting diet period 1 and another was withdrawn

from the study before the end of diet period 1 because prescription of a lipid lowering medication was reported.

Randomized participants (27 women and 39 men) were on average 50.9 ± 11.6 years (mean ± standard deviation) with a BMI of 31.2 ± 4.0 kg/m^2 and FPG of 106.2 ± 6.4 mg/dL at screening (Table 3-1). Adherence was 91% as indicated by days in which participants reported eating study foods at the appropriate time during each condition. Adherence to study protocols (days consumed as directed/days in the diet period) was 90% during the pistachio condition and 93% during the usual care condition.

Figure 3-1. Consort flow diagram

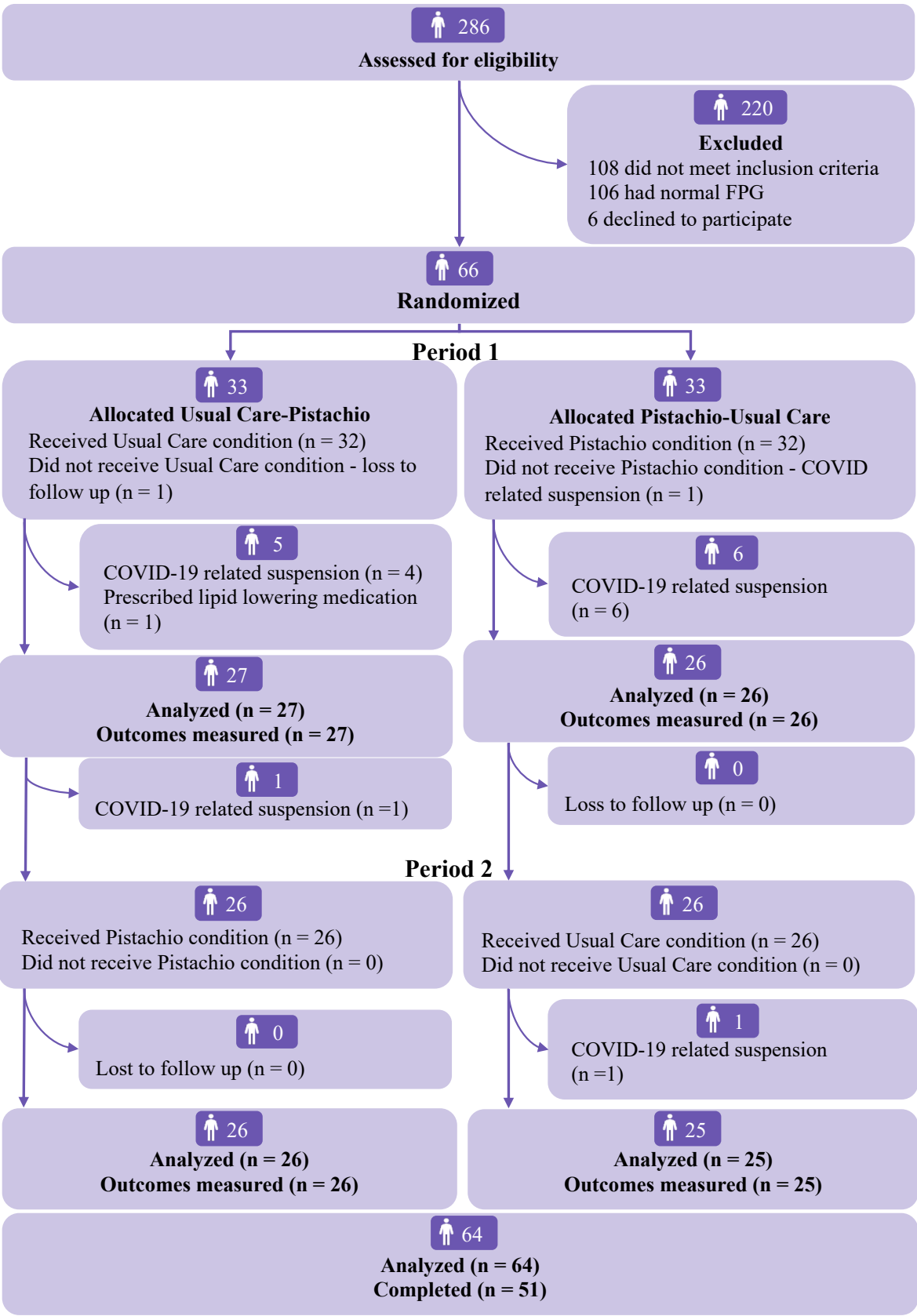


Table 3-1. Baseline characteristics of participants overall and by randomization sequence at screening (n = 66)[1]

Characteristic	Pistachio→Usual Care	Usual Care→Pistachio	Total
n (% female)	33 (30.0)	33 (48.4)	66 (40.9)
Age, y	51.9 (12.7)	51.2 (10.1)	50.9 (11.6)
Weight, kg	91.8 (15.0)	93.1 (16.5)	92.5 (15.6)
Height, m	1.71 (0.09)	1.72 (0.08)	1.72 (0.09)
BMI, kg/m^2	31.1 (3.7)	31.3 (4.4)	31.2 (4.0)
FPG, mg/dL	106.5 (6.8)	105.8 (6.0)	106.2 (6.4)
WC (men), cm	103.6 (11.8)	106.9 (13.0)	105.8 (12.3)
WC (women), cm	103.5 (8.5)	101.3 (12.0)	101.3 (10.3)

[1]Data are mean (standard deviation) unless otherwise stated. Usual care is defined as education to consume 1-2 carbohydrate (CHO) exchanges each night (15 g - 30 g CHO). BMI, Body mass index; y, years; kg, kilograms; m, meters; mg, milligrams; cm, centimeters; WC, waist circumference; FPG, Fasting plasma glucose.

There were no significant between-condition mean differences in FPG (Table 3-2). Insulin was 3.0 μIU/mL (95% CI: 1.2, 4.8 μIU/mL) higher after the pistachio condition compared to the usual care. Insulin resistance (HOMA-IR) was 0.8 units (95% CI: [95% CI: 0.3, 1.3 units]) higher after the pistachio condition compared to the usual care. HbA1c (% or μmol/L), weight, BMI, and waist circumference were not different between the conditions (Table 3-3). There were no between-condition mean differences observed for total cholesterol, LDL-C, HDL-C, or TAGs (Table 3-4).

PWV was lower after the pistachio condition compared to usual care (-0.3 m/s [95% CI: -0.5, -0.0 m/s]) (Table 3-5). Brachial SBP and DBP, central SBP and DBP, pulse pressure, augmentation index, heart rate, and pulse transit time were not significantly different between conditions. Central augmentation pressure was reduced during the pistachio (-1.7 mm Hg [95% CI: -2.9, -0.5 mm Hg]) and usual care (-1.7 mm Hg [95% CI: -2.9, -0.4 mm Hg]) conditions but not significantly different between conditions.

Table 3-2. Between condition mean differences and change from baseline for markers of glycemic control in adults with prediabetes (n = 64)[1]

	Pistachio			Usual Care			
Outcome	Baseline[2] (n = 58)	Endpoint[3] (n = 52)	Within condition difference[4]	Baseline[2] (n = 58)	Endpoint[3] (n = 52)	Within condition difference[4]	Between Condition Difference[4]
Glucose, mg/dL	103.3 ± 1.4	103.3 ± 0.8	1.0 (-0.7, 2.7)	102.5 ± 1.2	102.2 ± 0.8	-0.0 (-1.8, 1.6)	1.0 (-0.7, 2.9)
Insulin, µIU/mL	13.7 ± 1.2	15.0 ± 0.6	2.0* (0.7, 3.2)	12.2 ± 1.0	11.9 ± 0.6	-1.0 (-2.2, 0.2)	3.0* (1.2, 4.8)
HbA1c, µmol/L	9.9 ± 0.1	9.9 ± 0.0	-0.0 (-0.2, 0.0)	9.9 ± 0.1	10.0 ± 0.0	-0.1 (-0.2, 0.0)	-0.0 (-0.2, 0.1)
HbA1c, %	5.4 ± 0.0	5.4 ± 0.0	-0.0 (-0.0, 0.0)	5.4 ± 0.0	5.4 ± 0.0	-0.0 (-0.0, 0.0)	0.0 (-0.0, 0.0)
HOMA-IR	3.6 ± 0.4	3.9 ± 0.1	0.5* (0.1, 0.9)	3.1 ± 0.3	3.0 ± 0.1	-0.3 (-0.7, 0.0)	0.8* (0.3, 1.3)

[1]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 for within and between condition difference adjusted for baseline. Usual care is defined as education to consume 1-2 carbohydrate (CHO) exchanges each night (15 g - 30 g CHO). SE, standard error; 95% CI, 95% Confidence interval. HbA1c, Hemoglobin A1c; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

[2]Values are arithmetic means ± SE

[3]Values are least square mean ± SE

[4]Values are least square mean ± 95% CI

*Significantly different ($p < 0.05$)

Table 3-3. Between condition mean differences and change from baseline for anthropometrics in adults with prediabetes (n = 64)[1]

	Pistachio			Usual Care			
Outcome	Baseline[2] (n = 58)	Endpoint[3] (n = 52)	Within Condition Difference[4]	Baseline[2] (n = 58)	Endpoint[3] (n = 52)	Within Condition Difference[4]	Between-Condition Difference[4]
Weight, kg	93.9 ± 2.2	94.9 ± 0.3	0.7* (0.1, 1.4)	93.4 ± 2.0	94.1 + 0.3	-0.0 (-0.6, 0.6)	0.7 (-0.1, 1.7)
BMI, kg/m^2	31.6 ± 0.6	31.7 ± 0.1	0.2* (0.0, 0.4)	31.3 ± 0.5	31.5 ± 0.1	0.0 (-0.2, 0.2)	0.2 (-0.0, 0.5)
WC, cm	104.6 ± 1.6	104.5 ± 0.4	0.2 (-0.5, 1.1)	103.7 ± 1.4	104.7 ± 0.4	0.4 (-0.3 1.2)	-0.1 (-1.3, 1.0)

[1]Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the within- and the between-condition difference adjusted for baseline for each outcome measure. Usual care is defined as education to consume 1-2 carbohydrate exchanges (15 g - 30 g CHO). SE, standard error; 95% CI, 95% Confidence interval; BMI, Body mass index; WC, waist circumference.

[2]Values are arithmetic means ± SE

[3]Values are least square mean ± SE

*Significantly different ($p < 0.05$)

Total HEI-2015 score was 5.6 points (95% CI: 0.2, 10.9 points) higher after the pistachio condition compared to the usual care (Table 3-6). Between condition mean differences for HEI components showed higher scores for pistachio in seafood and plant proteins (1.8 points [95% CI: 0.8, 2.8]) and refined grains (2.4 points [95% CI: 1.2, 3.6 points]). There were no between condition mean differences among other HEI-2015 components.

Total energy intake was similar after both conditions (Table 3-7). The percentage of total calories (% kcals) from fat was higher after pistachio (3.9% [95% CI: 0.2%, 8.0%]) in part because MUFA intake was significantly higher after pistachio (2.2% [95% CI: 0.4%, 4.0%]). Fiber and potassium intake was higher after the pistachio condition compared to the usual care (5.0 g [95% CI: 1.1, 8.8 g]; -337 mg [95% CI: -665, -8.9 mg], respectively). Consumption of refined grains was significantly lower after pistachios compared to the usual care (-1.5 oz-eq [95% CI: -2.8, -0.2 oz-eq]). Pistachios increased the total protein foods consumed compared to the usual care condition (2.3 oz-eq [95% CI: 0.0, 4.6 oz-eq]). Nut and seed intake (combined food group) was higher (1.8 oz-eq [95% CI: 1.1, 2.6 oz-eq]) after the pistachio condition vs. usual care. Also, oil consumption was higher with pistachios (12.2 g [95% CI: 1.4, 23.0 g]) compared to usual care.

Table 3-4. Between condition mean differences and change from baseline for lipids/lipoproteins in adults with prediabetes (n = 64)[1]

	Pistachio			Usual Care			
Outcome	Baseline[2] (n = 58)	Endpoint[3] (n = 52)	Within Condition Difference[4]	Baseline[2] (n = 58)	Endpoint[3] (n = 52)	Within Condition Difference[4]	Between-Condition Difference[4]
Total Chol, mg/dL	199.7 ± 4.2	196.2 ± 2.4	-3.7 (-8.6, 1.0)	197.5 ± 5.2	199.1 ± 2.4	-0.8 (-5.6, 3.9)	-2.9 (-9.7, 3.9)
LDL-C, mg/dL	124.8 ± 3.2	121.4 ± 1.9	-3.4 (-7.4, 0.5)	122.2 ± 4.0	124.5 ± 1.9	-0.2 (-4.1, 3.7)	-3.0 (-8.6, 2.5)
HDL-C, mg/dL	50.9 ± 1.8	50.1 ± 0.5	-1.3* (-2.4, -0.2)	51.8 ± 1.7	50.8 ± 0.5	-0.7 (-1.8, 0.3)	0.6 (-2.1, 0.9)
TAG, mg/dL	117.4 ± 7.2	122.1 ± 5.2	4.5 (-5.9, 14.9)	114.9 ± 9.2	116.7 ± 5.2	-0.8 (-11.2, 9.6)	-5.3 (-9.4, 20.1)

[1]Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the within- and the between-condition difference adjusted for baseline for each outcome measure. Usual care is defined as education to consume 1-2 carbohydrate exchanges (15 g - 30 g CHO). SE, standard error; 95% CI, 95% Confidence interval; HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; Total Chol, total cholesterol; TAG, triglycerides.

[2]Values are arithmetic means ± SE

[3]Values are least square mean ± SE

[4]Values are least square mean ± 95% CI

*Significantly different ($p < 0.05$)

Table 3-5. Between condition mean differences and change from baseline for vascular measures in adults with prediabetes (n = 64)[1]

	Pistachio			Usual Care			
Outcome	Baseline[2] (n = 58)	Endpoint[3] (n = 52)	Within condition difference[4]	Baseline[2] (n = 58)	Endpoint[3] (n = 52)	Within condition difference[4]	Between-Condition Difference[4]
bSBP, mm Hg	127.6 ± 1.6	129.3 ± 1.2	1.6 (-0.8, 4.1)	127.0 ± 1.4	128.2 ± 1.2	0.5 (-2.0, 3.0)	1.1 (-2.4, 4.7)
bDBP, mm Hg	81.6 ± 1.0	82.3 ± 0.8	-0.8 (-0.8, 2.5)	80.8 ± 1.0	82.5 ± 0.8	1.1 (-0.5, 2.8)	-0.2 (-2.6, 2.1)
cSBP, mm Hg	117.8 ± 1.5	118.7 ± 1.2	1.8 (-0.6, 4.4)	115.5 ± 1.9	119.2 ± 1.2	2.3 (-0.2, 4.8)	-0.4 (-3.1, 2.2)
cDBP, mm Hg	82.0 ± 1.0	83.2 ± 0.7	1.2 (-0.3, 2.8)	81.6 ± 1.0	82.8 ± 0.7	0.7 (-0.8, 2.4)	0.4 (-1.8, 2.6)
PP, mm Hg	35.6 ± 0.8	35.9 ± 0.6	0.2 (-1.5, 1.1)	35.5 ± 0.8	35.8 ± 0.6	0.1 (-1.2, -1.4)	0.0 (-1.3, 1.5)
AP, mm Hg	11.9 ± 1.0	10.1 ± 0.6	-1.7* (-2.9, -0.5)	11.4 ± 1.0	10.2 ± 0.61	-1.7* (-2.9, -0.4)	-0.0 (-1.0, 0.9)
AIx5, %	23.6 ± 1.7	24.5 ± 1.0	0.6 (-1.4, 2.7)	23.9 ± 1.6	23.7 ± 1.0	-0.0 (-2.1, 2.0)	0.7 (-2.2. 3.7)
HR, bpm	63.9 ± 1.3[a]	63.2 ± 0.7b	-0.2 (-1.8, 1.2)	62.6 ± 1.4	63.7 ± 0.7	0.1 (-1.3, 1.7)	-0.4 (-2.6, 1.6)
PTT, ms	62.9 ± 1.0[a]	64.1 ± 0.7[b]	0.7 (-0.8, 2.2)	63.3 ± 0.7	63.3 ± 0.7	-0.1 (-1.6, 1.4)	-0.8 (-1.3, 3.0)
PWV, m/s	8.4 ± 0.1[a]	8.1 ± 0.1[b]	-0.1 (-0.4, 0.1)	8.1 ± 0.1	8.5 ± 0.1	0.1 (-0.0, 0.4)	-0.3* (-0.5, -0.0)

[1]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 for within and between condition difference adjusted for baseline. Usual care is defined as education to consume 1-2 carbohydrate (CHO) exchanges each night (15 g - 30 g CHO). SE, standard error; 95% CI, 95% Confidence interval. 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; PTT, pulse transit time; PWV, pulse wave velocity; mm Hg, millimeters of mercury

[2]Values are arithmetic means ± SE

[3]Values are least square mean ± SE

[4]Values are least square mean ± 95% CI

[5]Adjusted to a heart rate of 75 bpm

[a]n = 57; [b]n = 51

*Significantly different ($p < 0.05$)

Table 3-6. Between condition mean differences and change from baseline for the Healthy Eating Index-2015 score and individual components in adults with prediabetes (n = 61)[1]

		Pistachio			Usual care			
Component	Maximum Score	Baseline[2] (n = 56)	Endpoint[3] (n = 43)	Within-Condition Difference[4]	Baseline[2] (n = 49)	Endpoint[3] (n = 46)	Within-Condition Difference[4]	Between-Condition Difference[4]
Adequacy								
Total Fruits	5	1.7 ± 0.2	1.9 ± 0.3	0.3 (-0.2, 0.9)	1.4 ± 0.2	2.3 ± 0.3	0.7* (0.1, 1.3)	-0.4 (-1.3, 0.4)
Whole Fruits	5	2.0 ± 0.3	2.1 ± 0.3	0.1 (-0.5, 0.7)	1.7 ± 0.3	2.5 ± 0.3	0.5 (-0.1, 1.2)	-0.4 (-1.4, 0.5)
Total Vegetables	5	3.4 ± 0.2	3.7 ± 0.2	0.1 (-0.3, 0.6)	3.9 ± 0.2	3.4 ± 0.2	-0.1 (-0.6, 0.3)	0.3 (-0.3, 1.0)
Greens and Beans	5	2.3 ± 0.3	2.9 + 0.3	0.4 (-0.1, 1.1)	2.5 ± 0.3	2.8 + 0.3	0.3 (-0.3, 1.1)	-0.0 (-1.0, 1.0)
Whole Grains	10	2.6 ± 0.4	2.9 ± 0.5	-0.3 (-1.4, 0.7)	3.5 ± 0.5	2.7 + 0.5	-0.5 (-1.6, 0.5)	0.2 (-1.3, 1.8)
Dairy	10	5.5 ± 0.4	5.6 ± 0.4	-0.2 (-1.2, 0.6)	6.5 ± 0.4	5.7 ± 0.4	-0.1 (-1.1, 0.8)	-0.1 (-1.5, 1.2)
Total Protein Foods	5	4.3 ± 0.1	4.6 + 0.1	0.4* (0.2, 0.7)	4.0 ± 0.2	4.2 ± 0.1	0.0 (-0.3, 0.4)	0.4 (-0.0, 0.8)
Seafood and Plant Proteins	5	2.2 ± 0.3	3.8 ± 0.3	1.8* (1.1, 2.5)	1.9 ± 0.3	2.0 + 0.3	-0.0 (-0.7, 0.6)	1.8* (0.8, 2.8)
Fatty Acids	10	4.6 ± 0.4	6.2 ± 0.6	2.0* (0.7, 3.3)	3.9 ± 0.4	4.5 ± 0.6	0.4 (-0.8, 1.7)	1.6 (-0.1, 3.4)
Moderation								
Refined Grains	10	6.0 ± 0.5	7.4 ± 0.5	1.2* (0.1, 2.3)	6.2 ± 0.5	5.0 ± 0.5	-1.1* (-2.2, -0.1)	2.4* (1.2, 3.6)
Sodium	10	3.2 ± 0.4	3.6 ± 0.5	0.7 (-0.2, 1.8)	2.6 ± 0.4	2.8 + 0.5	0.0 (-1.0, 1.0)	0.7 (-0.7, 2.2)
Added Sugars	10	8.1 ± 0.3	8.8 ± 0.3	0.3 (-0.3, 1.0)	8.7 ± 0.2	8.5 ± 0.3	-0.0 (-0.7, 0.6)	0.3 (-0.6, 1.3)
Saturated Fatty Acids	10	4.9 ± 0.5	4.5 ± 0.5	0.3 (-0.8, 1.4)	4.1 ± 0.5	5.2 ± 0.5	0.9 (-1.7, 2.1)	-0.6 (-2.0, 0.6)
Total Score	100	51.2 ± 1.9	58.7 ± 1.8	7.4* (3.6, 11.2)	51.3 ± 2.1	52.5 ± 1.8	1.2 (-2.5, 5.1)	5.6* (0.2, 11.5)

[1]Baseline diet data was missing for 3 pre COVID-19 participants. 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 for within and between condition difference adjsuted for baseline. Usual care is defined as education to consume 1-2 carbohydrate (CHO) exchanges each night (15 g - 30 g CHO). SE, standard error; 95% CI, 95% Confidence interval

[2]Values are arithmetic means ± SE. At baseline, 2 and 9 recalls were not completed by participants for pistachio and usual care conditions, respectively.

[3]Values are least square mean ± SE. At endpoint, 15 and 12 recalls were not completed by participants for pistachio and usual care conditions, respectively.

[4]Values are least square mean ± 95% CI

*Significantly different ($p < 0.05$)

Discussion:

This study demonstrated that consuming 57 g/d of unsalted pistachios as a nighttime snack for 12 weeks did not improve FPG compared to education to consume 1-2 CHO exchanges in adults with prediabetes. Insulin levels and HOMA-IR were higher after the pistachio condition versus the usual care (1-2 CHO exchanges) suggesting greater insulin was needed to achieve similar FPG levels and/or cells were less insulin sensitive. However, in the context of the lack of change in HbA1c, the clinical relevance of observed differences in insulin and HOMA-IR remains unclear. Alternatively, pistachio consumption reduced arterial stiffness (PWV) suggesting improved vascular health. Moreover, pistachios improved diet quality scores compared to the usual care condition driven by higher component scores for seafood and plant proteins and refined grains. Taken together, intake of 57 g/d of pistachios as a nighttime snack as part of habitual diets results in beneficial shifts in vascular health and dietary patterns without affecting long term glucose control when compared to education to consume 1-2 CHO exchanges. The effects on lipids/lipoproteins, peripheral BP, and central BP were similar between conditions.

Several factors may have contributed to the lack of improvement in short term glycemic control in our trial. This trial was powered to detect a 10% difference in FPG ($\pm$ 10 mg/dL with 100 mg/dL) which is larger than differences observed among other clinical trials and systematic reviews on pistachio consumption. The larger difference in FPG was used to account for a greater portion of day-to-day variability in FPG among adults with prediabetes (~16%) (90,91).

Although FPG was similar between conditions, insulin and insulin resistance were worse with pistachio consumption compared to usual care condition. One contributor may have been an increase in weight (0.7 kg [95% CI: 0.1, 1.4 kg]) and BMI (0.2 kg/m^2 [95% CI: 0.0, 0.4 kg/m^2]) during the pistachio condition. Weight gain is a well-established risk factor for TIIDM and CVD and even relatively small changes in weight can have a meaningful impact on chronic disease trajectory (92). Moreover, insulin resistance has a direct, incremental association with increasing BMI that further contributes to chronic disease progression (93). Reported energy intake increased approximately 150 kcals/d during the pistachio condition but was not significantly higher than baseline. The pistachios contributed ~300 kcals and it is likely that compensatory reductions in energy intake did not occur. A better account of the compensatory behaviors that occur when supplementing habitual diets may help clarify differences among trials examining pistachios and glycemic control.

Our findings are incongruent with other clinical trials that examined the glycemic effects of pistachios consumption. A similar trial by Hernandez-Alonso et al. compared controlled diets with 57 g/d of pistachios as morning/afternoon snacks versus a control diet (predominantly olive oil) in adults with prediabetes (n = 54) over 4 months (17). Comparing the changes from baseline, pistachios improved FPG (-5.17 mg/dL vs. 6.7 mg/dL; $p < 0.001$), plasma insulin (-2.04 µIU/mL vs. 2.51 µIU/mL; $p < 0.001$), and HOMA-IR (-0.69 vs. 0.97; $p < 0.001$) versus control. However, longer-term glycemic control (HbA1c) was similar between diets, which aligns with our findings. A parallel trial in adults with TIIDM (n = 100) treated with oral agents (e.g., metformin, sulfonylureas) reported lower HbA1c (-0.19% [95% CI: -0.35%, -0.02%]) when supplementing 75 g of mixed nuts (including pistachios) versus a muffin (CHO snack) into diets intended to reduce cholesterol after 3 months (94). A crossover trial in adults with TIIDM (n =

30) taking anti-hyperglycemic medications and statins showed reduced fructosamine (228.5 μmol/L vs 233.5 μmol/L), a measure of glucose control over 1-3 weeks, after the pistachio intervention (20% kcals) versus education to consume a dietary pattern consistent with AHA Therapeutic Lifestyle Change diet (87). In contrast, two trials found no differences when comparing pistachios (40 g/d shelled or 10-20% kcals) with education to consume heart healthy diets (AHA Therapeutic Lifestyle and Change, STEP I diet) in adults with dyslipidemia (86). These trials suggest that the effects of pistachios may be influenced by the presence of impaired glycemia. However, several meta-analytical studies among adults with risk factors for TIIDM/CVD and healthy adults show a consistent benefit to glycemic control with pistachio consumption versus conditions without pistachios (22,62,63,65,75). Notably, effect estimates are small for FBG (< 8 mg/dL), insulin (< 5 μIU/mL), HOMA-IR (<2 units), and HbA1c (< 3%) and are likely not detectable with small clinical trials.

The effects of nighttime snacking on impaired morning fasting glucose remains unclear. Based on NHANES 2013–2016, the average US adult (≥18 y) consumes an average of 236 kcals on each snacking occasion (95). Nighttime snacks account for nearly 15% of total caloric intake (41). Dyer-Parziale et al. observed lower capillary morning FBG after nighttime consumption of a fiber rich Extend bar [30 g carbohydrate (5 g uncooked cornstarch), 3 g protein, and 3 g fat] compared with an isocaloric bar absent uncooked cornstarch (114.2 mg/dL vs. 158.5 mg/dL; $p < 0.0001$]) in patients with TIIDM after 3 days (96). In our trial, participants consumed more total daily fiber during the pistachio condition but no benefit on FPG was observed. Whether participants chose fiber-rich nighttime snacks during the usual care condition is not known since study foods were self-selected and diet recalls only occurred at baseline and endpoints. Abbie et al. reported glycemic benefits in adults with TIIDM consuming nighttime snacks in a short-term

(3 days) crossover trial that compared 2 eggs to a calorie- and protein-matched yogurt (97). The egg snack resulted in lower FBG (129.6 mg/dL ± 3.6 vs. 136.8 mg/dL ± 3.6) and insulin (111 μIU/mL ± 52 vs. 128 μIU/mL ± 56) compared to yogurt. A longer-term (6 weeks) crossover trial by Sapp et al. found no differences in FPG or insulin between 27 g of peanuts versus a calorie matched snack (crackers and cheese) consumed as an evening snack in adults with elevated FPG (54). This trial was hypothesized to decrease baseline FPG levels but average FPG at baseline was lower than the inclusion criteria. Daily variation in morning fasting glucose is a potential contributor to the differences in screening and baseline glucose measures and likely impacted their findings. More research is needed to better understand the relationship between nighttime snacking and morning glycemia.

Supplementing habitual diets with pistachios may be an effective strategy to better align dietary patterns with national guidelines. The 2020–2025 Dietary Guidelines for Americans recommend consuming 5 oz-eq/wk of nuts (2000 kcal/d diet) as part of a healthy dietary pattern (DGAs). Our trial showed higher HEI-2015 scores (5.6 points [95% CI: 0.2, 11.5 points]) when supplementing habitual diets with 57 g (~ 2 oz) pistachios compared to education to consume 1-2 CHO exchanges. Sheridan et al. provided adults (n = 15) with hypercholesterolemia 2-3 oz of pistachios and education to replace calories from fat over 4 weeks (98). Compared to habitual diets (no intervention), supplementing pistachios resulted in a -2.7% (95% CI: -5.4%, -0.10%) mean difference (MD) in calories from SFA and higher fiber intake (MD: 15 g [95% CI: 8.4g, 22 g]). Similarly, providing healthy women (n = 60) with 44 g/d of pistachios mid-morning over 12 weeks was shown to increase total protein, total fat, MUFA, and PUFA intake compared to diets higher in CHOs (99). Our trial observed similar changes in fiber, total protein, total fat. Moreover, improvements in nutrition composition translated to increases in total HEI-2015,

seafood and plant protein, and refined grain scores. Similar effects in diet quality were seen in adults with overweight/obesity provided 42 g/d of pistachios plus counseling to reduce caloric intake 500-1000kcals compared to counseling alone (18). Although biometrics (e.g., insulin, FPG, LDL-C, TC, TAGs,) and total HEI-2015 diet quality scores were not different between conditions, pistachio supplementation increased total HEI score by 10 points from baseline (60.9 points vs. 70.3 points; $p \leq 0.001$). Component scores that reflect supplementation of pistachios to habitual diets were also improved such as added sugar (8.4 vs. 9.2; $p \leq 0.05$), saturated fats (5.4 vs. 7.4; $p \leq 0.05$), and seafood and plant protein (3.9 vs. 4.5; $p \leq 0.05$) scores. This trial also showed the fatty acid ratio component score was significantly higher with pistachio versus weight loss counseling (6.9 points vs. 5.0 points; $p \leq 0.05$). We observed significant increases from baseline during the pistachio condition of the total HEI-2015 score (7.4 points [95% CI: 3.6, 11.2 points]), refined grains (1.2 points [95% CI: 0.1, 2.3 points]), fatty acid ratio (2.0 points [95% CI: 0.7, 3.3 points), seafood and plant protein (1.8 points [95% CI: 1.1, 2.5 points]), and total protein (0.4 points [95% CI: 0.2, 0.7 points]) with supplementation of pistachios to habitual diets. Whether these benefits to diet quality with pistachio supplementation translate to improvements in longer-term health outcomes needs further investigation.

Supplementing pistachios in habitual diets may be a simple strategy for improving vascular health. Impaired glucose tolerance and TIIDM are well established risk factors for diseases of the heart and vasculature (26). Nut consumption has been shown improve vascular health such as blood pressure, flow mediated dilation, and vascular stiffness (100). Effects of pistachios on blood pressure and the vasculature are mixed. Kasliwal et al. observed lower brachial PWV (-0.28 m/s) with 80g /d pistachios compared to education to consume a higher CHO heart healthy diet (86). Sauder et al. observed lower ambulatory BP (-3.5 mm Hg, p =

0.046) when heart healthy, controlled diets contained 20% kcals from pistachios versus foods higher in CHO (101). Significant reductions from baseline for resting systolic (-9.0 mm Hg) and diastolic (-5 mm Hg) blood pressure have been observed with 42 g/d pistachio supplementation, but weight loss was a significant component of the study interventions and may influence effects on BP (18). Several other trials show no effects of pistachio consumption on blood pressure or vascular health with 50 g/d - 75 g/d of pistachios with TIIDM or impaired glucose tolerance over 4-16 weeks (17,87,88,94). We observed lower PWV (-0.3 m/s [95% CI: -0.5, -0.0 m/s]) with pistachios compared to the usual care condition. While within condition mean differences were not significant, changes from baseline during the pistachio condition tended to lower PWV (8.4 m/s to 8.1 m/s) whereas PWV increased during the usual care (8.1 m/s to 8.5 m/s). Despite this, PWV in our populations falls below the clinical cut point of 10 m/s carotid femoral PWV where CVD risk increases (age 63 and 4% risk of CVD) (102). Additional work is needed to determine how more modest changes in PWV effect CVD risk in those with prediabetes and what aspects of pistachio consumption (i.e., changes in diet quality, nutrients, bioactives) influence vascular health.

The strengths of this study are the design, measures of short- and longer-term glycemic control, and method of dietary assessment. The crossover design included baseline measures for each condition allowing for change from baseline estimates for mean differences between conditions. Measures of morning fasting glycemic control are corroborated by the longer-term measures of glycemic control including HbA1c. Measures of diet quality and nutrient composition were collected using the validated ASA24, which allows for the identification of small yet important differences in dietary intake between conditions.

This trial has potential limitations. The COVID-19 pandemic resulted in trial suspension and lowering of the inclusion criteria to ≤ 65 years. Since risk of TIIDM increases with age, we do not know how pistachios alter glycemic control in a population where TIIDM risk is higher. The study protocol was single blinded since participants were aware of the conditions. The nature of each condition did not allow for blinding of participants. While single blinding may introduce bias, both study investigators and those involved in the analysis were blinded for data collection and analysis. Another limitation is the limited dietary control. While the conditions in this trial more closely represents simple strategies for prediabetes, we cannot account for the effects of other diet and lifestyle factors. The degree of impaired FPG may also be considered a limitation since inclusion was assessed by a single day of fasting glucose. For dietary assessments, a single nonrandom 24-hr recall at the beginning and end of each diet period may have impacted reporting. Also, these recalls occurred on only 1 day at the beginning and end of each intervention which may not reflect longer-term dietary habits. Finally, several risk indicators were assessed in this trial which may inflate the risk of type I error for secondary endpoints.

Conclusion:

In summary, this trial showed that 57 g/d of pistachios as an evening snack did not lower FPG and the levels of FPG were maintained with higher levels of insulin when compared to education to consume 15 g – 30 g CHO. However, pistachios improved arterial stiffness, diet quality, and the nutrient profile when compared to the usual care condition. These findings suggest that pistachios are a convenient nighttime snack that improves diet quality and nutrient composition compared to the current dietary recommendations provided to those with

prediabetes. Further research is needed to identify simple and effective strategies for prediabetes treatment.

Chapter 4:

Effects of pistachios on gut microbial diversity and composition compared to education to consume 1-2 carbohydrate exchanges (15-30 g) over 12 weeks in adults with prediabetes.

Abstract:

Background: Pistachios have glucoregulatory effects and contain relatively high amounts of insoluble and small amounts of soluble dietary fibers, a primary food of gut microbiota. Therefore, with the objective of identifying any link between gut microbiota and the glucoregulatory effects of pistachios, we examined the effect of pistachio on gut microbiota composition.

Objectives: The aim was to examine the effect of 57 g/d of pistachios for 12-wks, compared to education to consume 1-2 carbohydrate (CHO) exchanges (usual care), on gut microbiota composition including α- and β-diversity and taxonomic enrichment.

Methods: In a randomized 2 period crossover trial, fecal samples were available from 51 adults that completed the trial (37.2% female; 49.7 ± 10.7 y; BMI 31.5 ± 4.1 kg/m2; plasma glucose 102.4 ± 10.7 mg/dL). Participants consumed 57 g/d of unsalted pistachios (324 kcal (fat: 26 g and % kcals; CHO: 16.04 g and % kcals; protein: 11.9 g and % kcals; saturated fat: 3.2 g; monounsaturated fat: 13.9 g; polyunsaturated fat: 7.54 g; fiber: g; sodium: 3.4 mg)] or received education to consume 1-2 CHO exchanges (15-30 g CHO) as a nighttime snack for 12-wks each ($\geq$ 4-wk washout period before the crossover). Gut bacterial composition was measured using 16S rRNA sequencing.

Results: There were no between condition differences in α- and β-diversity. Following the pistachio condition, *Roseburia* (Microbiome Multivariable Associations with Linear Models score [MaAsLin] = 2.1 $\pm$ 0.37 (SE); p = 0.00031) and NK4A214 group of *Oscillospiraceae* (MaAsLin = 1.0 $\pm$ 0.25; p = 0.045) were significantly enriched compared to the usual care condition. *Roseburia* increased (MaAsLin = 1.55 $\pm$ 0.35; p = 0.033) during the pistachio

condition from baseline. There were no significant changes from baseline in the usual care condition.

Conclusion: An increased abundance of *Roseburia* and NK4A214 group of *Oscillospiraceae* was observed after consumption of 57 g/d of pistachios compared to the usual care. These findings suggest that pistachios induce gut microbial modulation of butyrate producing *Roseburia.*

Introduction

Approximately 1 in 3 US adults have prediabetes (103). Prediabetes is a condition of impaired glucose tolerance measured by elevated fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), or postprandial glucose that does not meet the classification for type 2 diabetes (TIIDM) (104). Diet is a major aspect of management and treatment strategies for prediabetes and TIIDM (105). Recent evidence shows that the gut microbiota also plays a role in glycemic control and is influenced by glycemic status (23,106). Diet and the gut microbiota are directly linked since dietary interventions affect microbial composition (59). While diet and the gut microbiota are interdependent factors that influence glycemic control, limited research has examined the relationship between diet and gut microbiota in adults with prediabetes.

Tree nut and peanut consumption has been shown to exert modulatory effects on the gut microbiota across the lifespan (≥ 3 years) (107). Tree nuts and peanuts contain unsaturated fats, fiber, phytochemicals, vitamins, and minerals that support a beneficial microbial composition and favor metabolic health (i.e., lower cholesterol, inflammation, insulin resistance) (59). The health benefits are partly mediated by microbial metabolites that exert physiological effects such as short chain fatty acids (SCFAs; acetate, butyrate, propionate) (59,107,108). Consumption of pistachios has been shown to have a strong positive effect on butyrate-producing gut bacteria in generally healthy adults with overweight (67). Enrichment of butyrate-producing bacteria favors metabolic pathways that support colonic metabolism (109). Moreover, a host genetic-driven increase in microbial butyrate production is associated with improved postprandial insulin response after an oral glucose tolerance test in normoglycemic adults (110). Several studies support the glucomodulatory effects of pistachio consumption in healthy adults and those with prediabetes, TIIDM, and CVD risk factors (20,22,62). While pistachio consumption improves glycemic indicators and results in beneficial shifts in the gut microbiota, it's unclear if the

glycemic effects are driven by the microbial changes. Identifying the gut microbial shifts with pistachio consumption may provide new targets for therapies that improve glycemic control in individuals with impaired glucose tolerance (i.e., prediabetes).

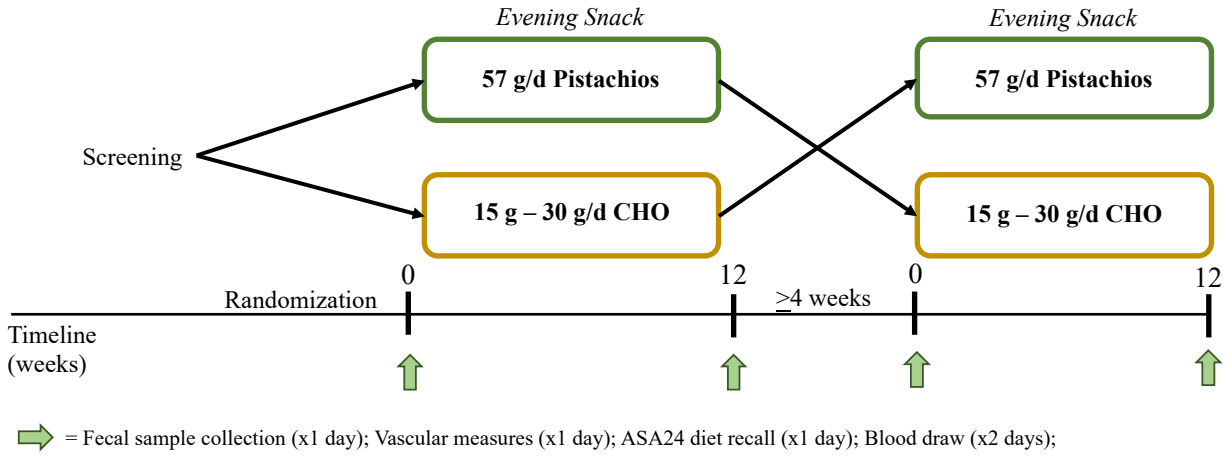
The objective of this pre-specified secondary analysis of fecal samples from the previously conducted crossover trial in adults with prediabetes was to evaluate gut microbial changes when comparing nighttime pistachio consumption (57 g/d) with education to consume 1-2 carbohydrate (CHO) exchanges (usual care) for 12 weeks. The usual care condition reflects common clinical guidance to address morning elevated FPG (13). The gut microbiota was assessed by 16S rRNA sequencing to compare alpha- and beta-diversity and relative abundance. We hypothesized that pistachio consumption would promote beneficial shifts in microbial diversity when compared to the usual care condition.

Methods

Samples were collected as part of the clinical trial described in Chapter 3. The research design is depicted in Figure 4-1. Briefly, we conducted a randomized, crossover trial consisting of two 12-week dietary conditions in which participants were provided 57 g pistachios or educated to consume 1-2 carbohydrate exchanges (usual care). Participants were asked to consume the foods as a nighttime snack (after dinner but before bedtime) and have no other calorie-containing foods or beverages after the nighttime snack. There was a 4-wk washout period between conditions. In addition, they were asked to avoid peanuts and tree nuts throughout the trial while all other aspects of the diet remained unchanged. The results from a planned secondary analysis, 16S rRNA sequencing on samples from individuals that completed

the trial to assess fecal bacterial diversity, are presented here.

Figure 4-1. Study flow diagram



Compliance was monitored using daily questionnaires, and overall compliance with the study protocol was 91% (93% during the usual care and 90% during the pistachio condition). The protocol for this study was approved by the Pennsylvania State University (University Park, Pennsylvania) Institutional Review Board. Written informed consent was obtained from all participants prior to study screening. This trial is registered at ClinicalTrials.gov (identifier: NCT04056208).

Fecal sample collection

Two fecal samples were collected from a single defecation at baseline and endpoint of each condition. Samples were collected on the second day of clinical testing or within 48 hours prior to day 2 testing. Participants were provided with a collection kit (Ziploc bags, cooler, icepack, non-latex gloves, a long-handled spoon, a stool collection hat, and 2x 30 mL Para-Pak Clean Vials; Meridian Bioscience, Cincinnati, Ohio). Participants were instructed to store fecal samples in their freezer (< 48-h prior to study visit) and transport the samples to the Clinical Research Center using the provided cooler and ice packs. Upon arrival, samples were stored at -

80ºC until analysis. All 16S rRNA analyses were completed at Wright Labs, LLC (Huntingdon, Pennsylvania).

DNA extraction and quantification

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

All 16S rRNA Illumina-tag polymerase chain reaction (PCR) was performed on DNA extracts as described by the Earth Microbiome Project's protocol (111). PCR products were pooled, and 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 after gel purification on a 2% agarose gel with the QIAquick Gel Purification Kit (Qiagen, Frederick, Maryland). The purified pool was sequenced at Wright Labs, LLC using an Illumina MiSeq v2 chemistry with paired-end 250 base pair reads.

Bioinformatic Analysis Procedures

Raw data were imported into QIIME2 for processing and analyses (112). Initial quality indicated by Phred q scores were determined using QIIME2 and cumulative expected error for each position was determined with VSEARCH (113). Using these quality data, forward reads were truncated at a base length of 170 and reverse reads were truncated at a base length of 150. The maximum expected error was 0.5 for both within QIIME2's implementation of the DADA2 pipeline (114). QIIME2's DADA2 pipeline was also 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 138 database containing 515F/806R sequences (115). Representative sequences were also used to create a rooted phylogenetic tree using MAFFT and FastTree through Qiime 2's "qiime phylogeny align-to-tree-mafft-fasttree" command (116,117).

ASVs identified as Mitochondria or Chloroplasts were removed on the basis that they likely represented eukaryotic contamination rather than a true bacterial signal. Samples with fewer than 1,000 sequences remaining after that filtration were removed from the ASV table.

Statistical Analysis:

α-diversity analysis

Alpha diversity was calculated by subsampling the ASV table at 10 different depths for Faith's Phylogenetic Diversity, Observed ASVs, and Pielou's Evenness metrics (118–120). To obtain average alpha diversity values, 20 iterations were performed at each. Results from the subsampling were used to create a rarefaction plot and confirm that diversity approached an asymptote and slope decreased as depth increased.

The α-diversity analyses were conducted using SAS (version 9.4; SAS Institute Inc). PROC UNIVARIATE was used to determine normality of the residuals for each metric based on the distribution and normal probability plots (Q-Q plots). Metrics were logarithmically (natural log) transformed in the instance of skewed residuals. The effect of each condition on Faith's Phylogenetic Diversity, Observed Features, and Pielou's Evenness metrics was assessed with the mixed-models procedure (PROC MIXED). Baseline measures were included as a covariate. Subjects were nested within condition and included as a repeated effect in the model to account for the crossover design. Carryover effects and sex differences were determined by including

randomization sequence, sex, and their interaction by condition (i.e., sex*condition, randomization*condition) in the model as fixed effects. There were no significant sex or randomization effects thus, were omitted from the final model.

β-diversity analysis:

Beta diversity was tested after cumulative sum scaling normalization of ASV tables to mitigate differences between samples based on sequencing depth (121). Distances between samples were calculated using the Weighted Unifrac metric (122). Weighted Unifrac metrics were based on the normalized table and rooted tree. A Principal Coordinates Analysis plot was used to visualize the resulting distance matrix. A 3-dimensional Principal Coordinates Analysis (PCoA) plot in the Qiime2 visual interface visualized the resulting distance matrix (data not reported). The "vegan" R package was used to calculate average distances to median for each condition and timepoint within the distance matrix to confirm PERMANOVA (Adonis) was appropriate to run. The effects of condition and time on sample clustering were run with the pairwise "Adonis" R package. Participant was used as the strata variable using the Weighted UniFrac distance matrix.

Taxonomic comparisons:

Biomarker analysis was performed using Microbiome Multivariable Associations with Linear Models (MaAsLin) score to identify taxa that had significantly different abundances based on Baseline vs. Endpoint within the same condition (123). The ASV table was collapsed to level 7 (species) and normalized with the counts per million methods. Only taxa identified as having significantly differential abundance (Kruskal-Wallis, $p \leq 0.05$) with a MaAsLin score of at least 2.0 were considered to be enriched. The "mixOmics" package in R was used to determine multilevel PLSDA to assess distinct clustering between timepoints based on expressed genes with the normalized tables while accounting for the repeated measures design (122).

Results:

A total of 66 participants were randomized and 51 participants completed the study (Figure 3-1). Thirteen participants were withdrawn from the trial because the COVID-19 pandemic resulted in study activities being suspended. One participant was lost to follow up prior to receiving study foods and another was withdrawn prior to the end of diet period 1 due to starting a lipid lowering medication. Fecal samples were available for 50 participants at all timepoints; one participant provided fecal samples at three of the timepoints. 16S rRNA sequencing was completed on all available samples (n = 203). Baseline characteristics for completers are presented in (Table 4-1).

Table 4-1. Baseline characteristics of study completers overall and by randomization sequence (n = 51)[1]

Characteristic	Pistachio→Usual Care	Usual Care→Pistachio	Total
n (% female)	25 (20)	26 (50)	51 (37.2)
Age, y	50.7 (10.6)	50.5 (10.7)	49.7 (10.7)
Weight, kg	93.1 (13.0)	94.7 (18.9)	94.0 (16.1)
Height, m	1.72 (0.07)	1.72 (0.09)	1.73 (0.08)
BMI, kg/m2	31.2 (3.5)	31.6 (4.6)	31.5 (4.1)
Glucose, mg/dL	102.9 (13.4)	101.7 (7.6)	102.4 (10.7)
WC (men), cm	102.4 (10.8)	109.0 (12.6)	105.9 (11.8)
WC (women), cm	104.5 (8.0)	99.8 (11.8)	99.9 (10.3)

[1]Data are mean (standard deviation) unless otherwise stated. Usual care is defined as education to consume 1-2 carbohydrate (CHO) exchanges each night (15 g - 30 g CHO). BMI, Body mass index; y, years; kg, kilograms; m, meters; mg, milligrams; cm, centimeters; WC, waist circumference; FPG, Fasting plasma glucose.

16S rRNA gene PCR amplification of the hypervariable V4 region was successfully completed on all samples. High-quality sequencing data were obtained from 199 fecal samples.

The relative abundance of bacterial phyla between conditions is depicted in Figure 4-2. No significant between-condition differences were observed for α-diversity assessed by Faith's Phylogenetic Diversity (p = 0. 7066) (Figure 4-3), Observed Features (p = 0.9044) (Figure 4-4),

and Pielou's Evenness (p = 0.9452) (Fig. 4-5). β-diversity was unchanged following both conditions. Samples did not significantly cluster based on timepoint for pistachio and usual care conditions (Table 4-2). The shift in weighted unifrac distance observed for each participant at each timepoint throughout the trial is depicted in Figure 4-6. Points are distributed across the X-axis by each of the 4 respective timepoints within the study with baseline diet period 1 as the reference. A higher value on the Y-axis is indicative of an increased shift in weighted unifrac distance relative to each respective individual's first sampling timepoint. There were no significant differences in the shift in weighted unifrac distance when compared to the reference for each sequence.

Figure 4-2. Mean relative abundance (% total) of bacterial phyla for baseline and endpoint timepoints (n = 51)

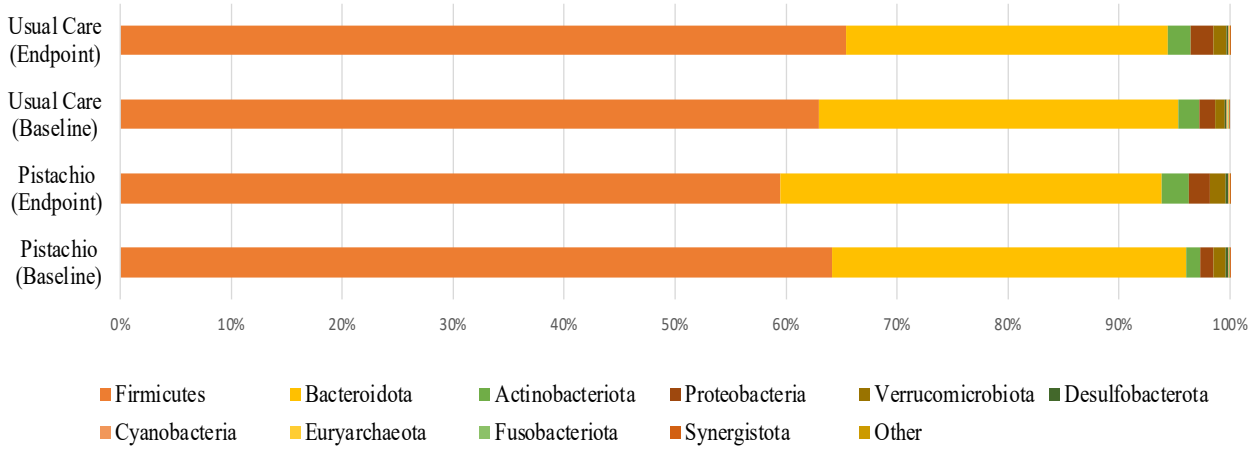
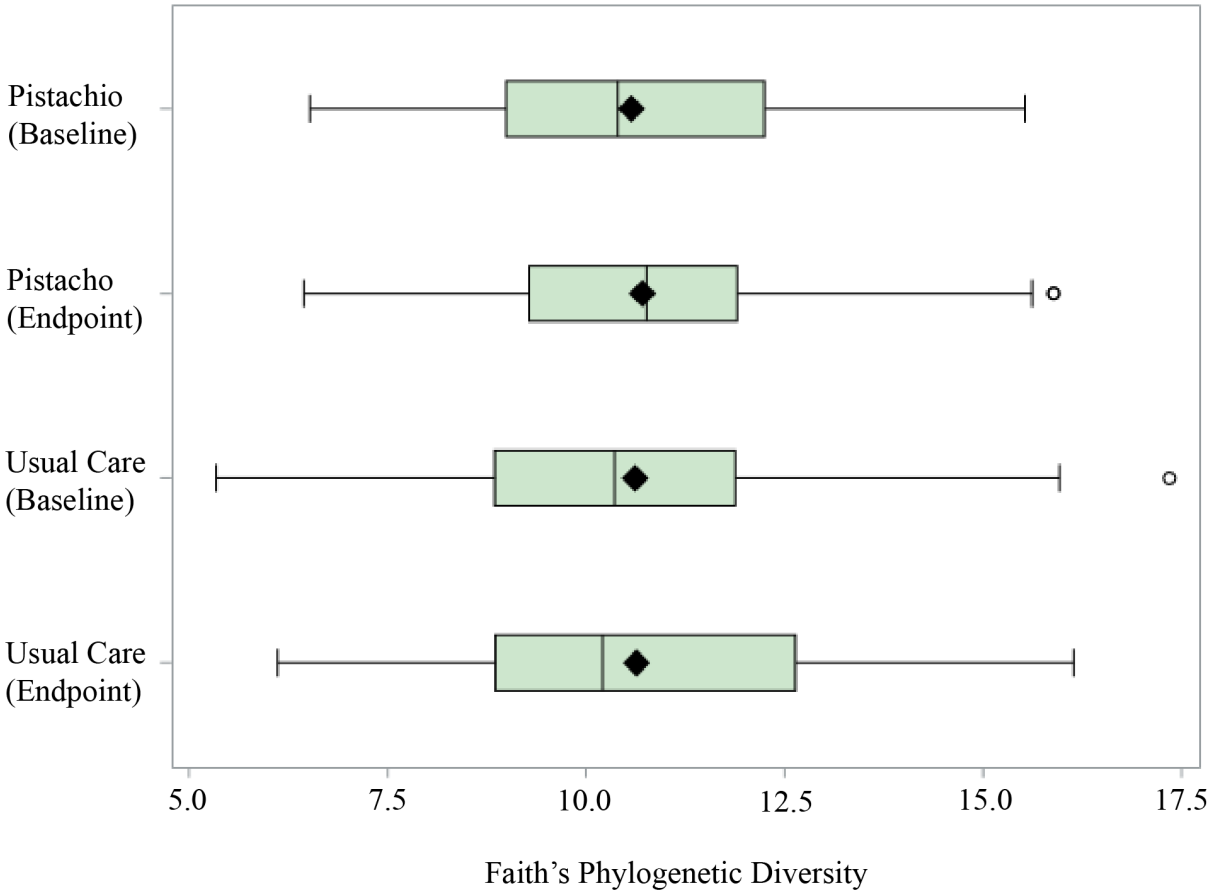
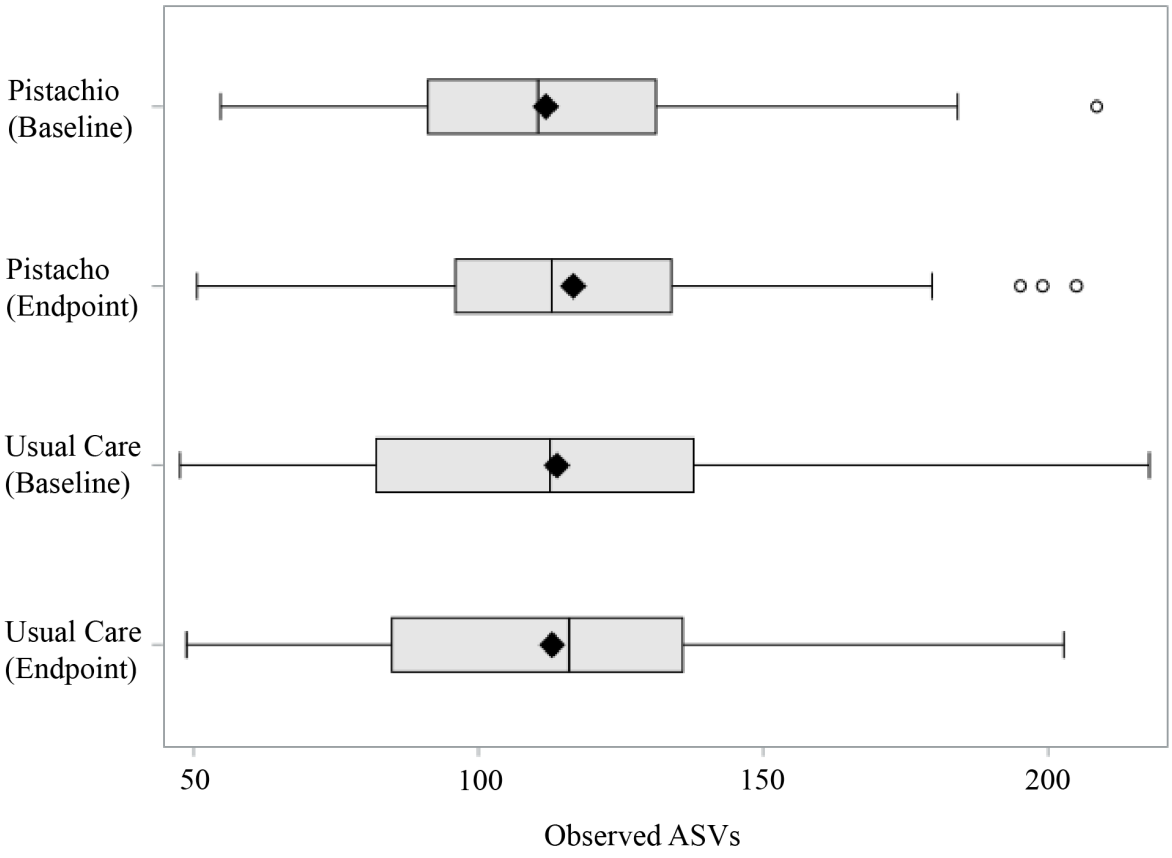


Figure 4-3. Between condition differences in α-diversity values based on the Faith's Phylogenetic Diversity for the pistachio and usual care condition in adults with prediabetes (n = 51)[1]



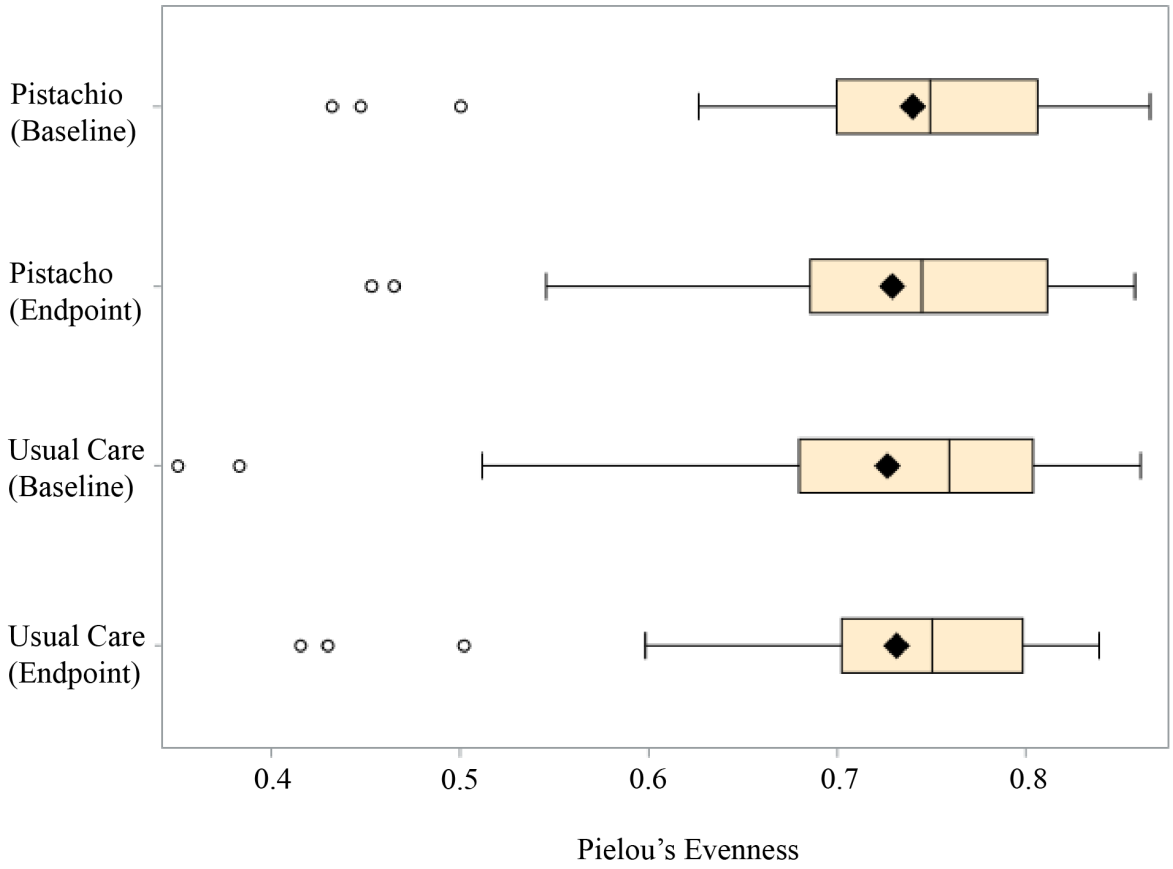
[1]Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the effect of the conditions on each outcome measure for within and between condition difference adjusted for baseline. Usual care is defined as education to consume 1-2 carbohydrate (CHO) exchanges each night (15 g - 30 g CHO).

Figure 4-4. Between condition differences in α-diversity based on Observed ASVs for the pistachio and usual care condition in adults with prediabetes (n = 51)[1]



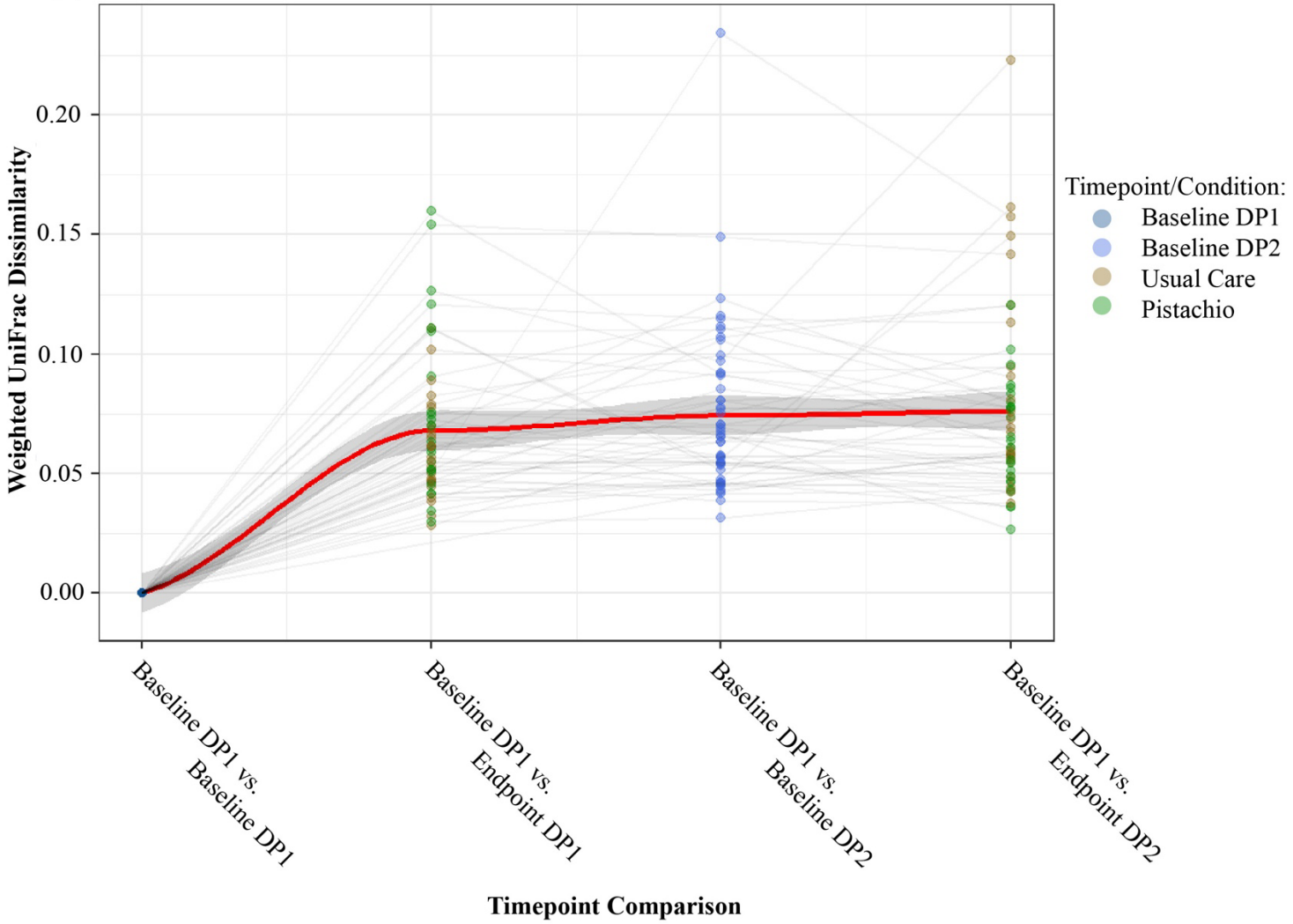
[1]Statistical analyses were performed with SAS version 9.4 (SAS Institute). The MIXED procedure was used to determine the effect of the conditions on each outcome measure for within and between condition difference adjusted for baseline. Usual care is defined as education to consume 1-2 carbohydrate (CHO) exchanges each night (15 g - 30 g CHO). ASVs, Amplicon sequence variants.

Figure 4-5. Between condition differences in α-diversity values based on the Pielou's Evenness for the pistachio and usual care condition in adults with prediabetes (n = 51)[1]



[1]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 for within and between condition difference adjusted for baseline. Usual care is defined as education to consume 1-2 carbohydrate (CHO) exchanges each night (15 g - 30 g CHO).

Figure 4-6. The shift in weighted unifrac distance (β-diversity) observed by each participant over the course of the study (n = 49)



Weighted UniFrac distance was determined using pairwise Adonis R package with participant as the strata variable. A higher value on the Y-axis is indicative of an increased shift in weighted unifrac distance relative to each respective individual's first sampling time point. The usual care condition refers to education to consume 1-2 CHO exchanges as a nighttime snack. CHO, Carbohydrates; CPM, Counts per million; DP, Diet period; (B), Baseline; (E), Endpoint

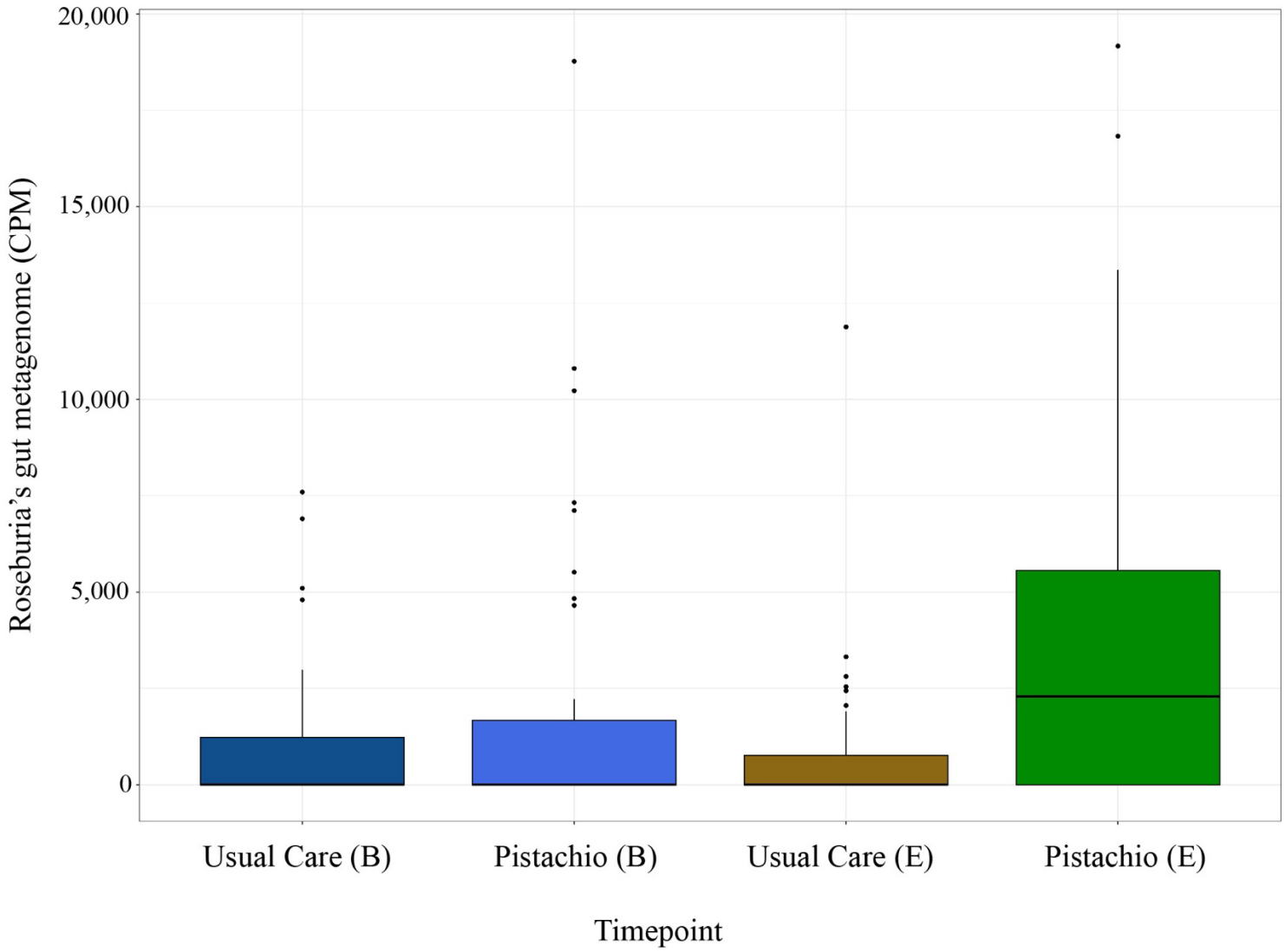
Table 4-2. Adonis test results between pairwise timepoint comparisons within and between conditions[1]

Comparison	Df	SumOfSqs	R2	F	Pr(>F)
Usual Care (B) vs. Usual Care (E)	1	0.00012282	0.00011768	0.01176935	0.993
Pistachio (B) vs. Pistachio (E)	1	0.00116776	0.0015098	0.14818423	0.653
Pistachio (B) vs. Usual Care (B)	1	0.00496147	0.00521271	0.52400261	0.193
Pistachio (E) vs. Usual Care (E)	1	0.00672014	0.00767415	0.7578832	0.059

[1]The effects of condition and time on sample clustering were run with the pairwise "Adonis" R package. Participant was used as the strata variable using the Weighted UniFrac distance matrix. (B), Baseline; (E), Endpoint.

Roseburia was enriched after the pistachio condition (Figure 4-7) compared to the usual care (MaAsLin coefficient = 2.1 $\pm$ 0.37 (SE); p = 0.00031). The relative abundance of NK4A214 group of *Oscillospiraceae* (MaAsLin = 1.0 $\pm$ 0.25; p = 0.045) increased with pistachios versus the usual care condition. Enrichment of *Roseburia* occurred during the pistachio condition (MaAsLin = 1.55 $\pm$ 0.35; p = 0.033) from baseline. There were no differences from baseline during the usual care condition. No other taxa were significantly enriched between or within conditions.

Figure 4-7. Relative abundance of Rosburia at each timepoint



Samples included = 98. Relative abundance was determined by Microbiome Multivariable Associations with Linear Models (MaAsLin) analysis. The usual care condition refers to education to consume 1-2 CHO exchanges as a nighttime snack. CHO, Carbohydrates; CPM, Counts per million; (B), Baseline; (E), Endpoint

Discussion:

In this study, we evaluated the gut bacterial shifts following the consumption of 57 g of pistachios as a nighttime snack for 12-wks compared to education to consume 1-2 CHO exchanges (15-30 g CHO). Our findings suggest that bacterial enrichment occurs with small dietary modification such as nighttime intake of pistachio. Pistachio consumption resulted in enrichment of butyrate producing *Roseburia*. Enrichment of NK4A214 group of *Oscillospiraceae* also occurred with pistachio when compared to the usual care condition. Neither the pistachio or usual care condition affected α- and β-microbial diversity. These findings suggest that intake of 57 g/d of pistachios for 12 weeks enriches bacteria associated with gut health in adults with prediabetes without affecting microbial richness and evenness.

Our trial observed increases in *Roseburia* with pistachio consumption, which is a major butyrate-producing bacteria (109). Butyrate is a primary fuel source for colonocytes and associated with reduced inflammation, cell barrier function, and cell turnover (Louis & Flint, 2017). The increases in *Roseburia* after the pistachio condition suggest that production of SCFA is increased favoring gut health in this context.

The enrichment of *Roseburia* in our trial is consistent with findings from other RCTs that included peanuts or tree nuts. Choo et al. observed increased relative abundance of *Roseburia* with 56 g/d of almonds for 8-wks in adults with elevated fasting glucose compared to a higher CHO snack (124). Similarly, Sapp et al. observed increased abundance of *Roseburia* following peanut consumption (27 g/d) compared to a lower fat higher CHO nighttime snack over 6 weeks (54). Holscher et al. observed increased relative abundance of *Roseburia* ($p < 0.05$) with almond consumption (42 g/d g/d) versus no almonds for 3 weeks (Holscher, et al., 2018). Notably, *Roseburia* is enriched with lower doses of peanuts/tree nuts. This is important considering that nuts are energy dense and may increase total energy consumption in the absence of

compensatory dietary behaviors. This potential concern is highlighted in dietary recommendations for nuts. Nuts are part of a healthy diet but should not contribute to excess energy intake (60). Given this, more work is needed understand the functional implications of *Roseburia* enrichment with nut intake.

The biological relevance of increased NK4A214 group of *Oscillospiraceae* with pistachio versus the usual care condition is unclear. Burakova et al. showed that 2 weeks of supplementing a probiotic containing lactic acid bacterial cultures reduced NK4A214 group of *Oscillospiraceae* in adults with obesity (126). Changes in weight were not reported thus cannot be compared with our study. The only other study we were able to identify was a preprint of an observational study that reported higher abundance of NK4A214 group of *Oscillospiraceae* in Mexican adults with obesity compared to Mexican adults without obesity (127). However, the enrichment of NK4A214 group of *Oscillospiraceae* may be a function of age considering that NK4A214 group of *Oscillospiraceae* was enriched in children without obesity compared to children with obesity. The age-dependent differences in NK4A214 group of *Oscillospiraceae* and association with body weight can only be speculated at this point. We observed an increase in body weight (0.8 kg (95% CI: 0.1, 1.4 kg) and BMI 0.2 kg/m^2 (0.0, 0.5 kg/m^2) during the pistachio condition but no mean differences between conditions. The increase in body weight during pistachio coincided with enrichment of NK4A214 group of *Oscillospiraceae* when compared with the usual care condition. Notably, our study population had an average BMI of 31.5 kg/m^2 ($\pm$ 4.5) and elevated FPG, which differs on NK4A214 group of *Oscillospiraceae*. Whether pistachios, weight gain, glycemic status, or caloric intake influenced the enrichment of NK4A214 group of *Oscillospiraceae* needs further investigation.

Our trial observed resistance to alterations in α- and β-microbial diversity. However, a trend towards a difference in beta-diversity was observed when comparing pistachio and usual care endpoint ($p = 0.059$). This suggests that supplementing habitual diets with nighttime snacks has the potential to create dissimilar gut microbial communities. Another trial examining microbial changes with pistachio consumption also observed resistance to alterations in composition. Ukhanova et al. performed gel electrophoresis-based DNA profiling with samples from a controlled feeding trial enriched with 42 g/d and 84 g/d of pistachios over 3 weeks (67). DNA profiling found that mean lactic acid bacteria were reduced ($p = 0.02$) after 19 days compared to baseline but bifidobacteria was unaffected. Operational taxonomic units were identified with 16S rRNA sequencing and showed that α-diversity (Chao-1) was unaffected by pistachio consumption. β-diversity (mean unifrac distance) showed strong effects with pistachios, specifically in reducing Firmicutes and *Clostridium sp.*, but was not significantly different from baseline. Our trial observed increased abundance of bacteria in the Firmicutes and Clostridia taxa with pistachio consumption but no differences in β-diversity. The relative stability between conditions in α- and β-microbial diversity occurred despite significantly higher fiber consumption with pistachios (5.0 g [95% CI: 1.1 g, 8.8 g]). Microbial stability may reflect the dietary conditions since small changes (i.e., supplementing pistachios, education to consume 15-30 g CHO) were introduced into habitual diets. Also, pistachio intake may select for metabolic functions present across phyla making α- and β-microbial diversity more difficult to identify. Investigation of the metagenomic differences between conditions may help better understand the effects of pistachio consumption on functional properties of the gut microbiota.

Trials examining shifts in α- and β-microbial diversity following nut consumption are mixed. An RCT in adults with prediabetes observed significant shifts in microbial composition

($p = 0.011$) and increased bacterial richness, evenness, and diversity ($p \leq 0.01$) with almonds (56 g/d) compared to an isocaloric high CHO control for 8 weeks (124). Notably, the fiber content of the interventions was not matched (7.3 g vs. 1.6 g) and it is unknown how this affected total fiber intake. Sapp et al. matched fiber content of the study foods (28 g/d peanuts vs. high CHO cheese and crackers) which contained about 3 g fiber each and observed no differences in α- and β-microbial diversity between conditions over 6 weeks (54). Total fiber intake in this study was similar between groups (−1.2 g [95% CI: −4.0, 1.7 g]). Similarly, β-diversity was unaffected in 4 trials that assessed walnuts (57 - 99 g/d for 6-wks), almonds (42.5-56.7 g/d for 3-8-wks), and pistachios (42.5, 85 g/d for 18-d) (Dhillon et al., 2019; Holscher, et al., 2018; Tindall et al., 2020; Ukhanova et al., 2014). However, two RCTs investigating the effect of walnut intake (~42 g/d for 3 and 8 wks) in healthy populations showed significant effects on b-diversity (Bamberger et al., 2018; Holscher, et al., 2018). Our trial did not match fiber between conditions and observed no differences in α- and β-diversity. The different findings across studies are likely driven by differences in comparator diets and sample sizes which influence statistical power.

Recent studies have demonstrated that glycemia is altered by the gut microbiota. Zeevi et al. used the gut microbiota to predict individual glycemic response to foods in a cohort of 800 individuals with prediabetes and TIIDM (23). A separate cohort 26 participants consumed an individualized algorithm-based diet which resulted in lower postprandial glucose response and less fluctuations in overall blood glucose levels over 2 weeks. Interestingly, lower postprandial glucose was achieved with foods more commonly associated with lower diet quality and poor glycemic control such as pizza and schnitzel (breaded and deep-fried meat). Berry et al. have since quantified the degree to which meal context, composition, and individual factors (e.g., biometrics, anthropometrics, microbiome) affect postprandial TAGs and glycemia in 1,002 twins

and unrelated healthy adults (132). It was reported that gut microbial composition accounted for 6.4% of the postprandial glucose variation. Assessing whether changes in the gut microbiota predicts the responsiveness to the conditions in our trial (i.e., who had improved FPG with pistachios) will help to better understand the microbiota mediated effects.

The strengths of this study are the trial design, compliance to the dietary protocol, diet assessment, and dietary conditions. The randomized crossover clinical trial design allowed for assessment of between-condition and within condition differences. Compliance was very good with an average adherence to study protocols being 91%. Participants reported dietary intake at the beginning and end of each diet period which reflected the differences in dietary interventions. Fiber intake was higher after the pistachio condition which provides a plausible contributor to microbiota effects. Moreover, most of the observed changes in the microbiota are consistent with fiber related effects.

Conclusions:

In conclusion, we observed increased abundance of a SCFA producer, *Roseburia,* and lactic acid bacteria, NK4A214 group of *Oscillospiraceae* following the pistachio condition. Roseburia was enriched with pistachios. Collectively, these findings suggest that implementing a simple dietary intervention, 57 g/d of pistachios as a nighttime snack for 12 weeks, alters microbiota composition in adults with prediabetes. Pistachios increased the relative abundance of butyrate-producing bacteria, which is indicative of greater functionality. The NK4A214 group of *Oscillospiraceae* was also increased with pistachios which requires further investigation. Additional research is needed to determine the clinical implications of these microbial effects on markers of chronic disease.

Chapter 5:
Conclusions, Limitations, and Future Directions

The overall goal of this dissertation was to investigate the effects of nighttime pistachio consumption on FPG, cardiovascular disease (CVD) risk factors, diet quality, and the gut microbiota composition in adults with prediabetes.

Specifically, the aims were:

(1) to determine the effects of 57 g of unsalted pistachios as a nighttime snack for 12-weeks compared to education to consume 1-2 carbohydrate exchanges (usual care) as a nighttime snack on FPG, HbA1c, insulin, and insulin resistance (HOMA-IR) in adults with prediabetes;

(2) to examine the effects of pistachios on lipids/lipoproteins, brachial blood pressure, central blood pressure, arterial stiffness, and diet quality;

(3) to explore the effects of pistachio consumption on gut bacterial diversity (α- and β-diversity) and composition compared to the usual care condition.

The data presented in this dissertation add novel evidence about the effects of daily pistachio consumption on glycemic control, CVD risk factors, diet quality, and the gut microbiota. Several clinical trials and meta-analytical studies show that tree nut and peanut consumption improve glycemic control and CVD risk factors (22,62,63). Among tree nuts and peanuts, pistachios may be unique in their ability to improve markers of glycemic control (20). Moreover, better glycemic control may be mediated by the gut microbiota.

This 12-week randomized, crossover, supplemental feeding trial in adults with prediabetes demonstrates that FPG and HbA1c are not improved with pistachio consumption when compared to the usual care condition. Instead, the usual care condition had lower insulin and insulin resistance (HOMA-IR) after 12-weeks compared to pistachios. Pistachio

consumption benefited several other indicators of disease. Arterial stiffness (PWV) was lower with pistachios than the usual care condition. Diet quality was also improved with pistachios when compared to the usual care. Pistachios increased *Roseburia* bacteria, which is associated with a favorable gut microbial composition and gastrointestinal health. However, enrichment of NK4A214 group of *Oscillospiraceae*, a bacterial group previously reported to be present in adults with obesity, was higher during the pistachio condition. Taken together, pistachios intake did not improve glycemic control compared to a condition representing the usual care. Intake of pistachios as part of usual intake improved diet quality, which may help individuals with prediabetes consume a dietary pattern that more closely aligns with dietary guidelines. Pistachios improved arterial stiffness and the abundance of microbiota that support gut health. These findings provide support that supplementing pistachios may be a simple approach to improve indicators of chronic disease among individuals with prediabetes.

Our study has limitations previously discussed in detail. 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. Participants were eligible for this trial based on a single FPG $\geq$ 100 mg/dL. While diagnosis of TIIDM requires at least two measures impaired fasting glucose, prediabetes does not. Thus, daily variation in fasting glucose measures is not accounted for in the determination of prediabetes. Mean FPG at baseline in our trial was 102.2 $\pm$ 1.3 (SE) mg/dL but some individuals had a FPG below 100 mg/dL at baseline. Blinding was a limitation because of the nature of the conditions. Participants were provided either pistachios or education to consume 1-2 carbohydrate exchanges and were aware of which condition they received. Finally, the COVID-19 pandemic resulted in a change in our age of inclusion ($\leq$ 65 years) limiting, in part, the interpretation of our findings.

This trial helped to identify future directions that, if addressed, would advance our understanding about the effects pistachio intake on glycemic control. Of note, future research should account for variability in fasting glucose measures. Unlike TIIDM, the identification of prediabetes only requires one measure of impaired FPG, HbA1c, or OGTT. The intraindividual daily variation and measurement error (coefficient of variation) in FPG is subject to a degree of inconsistency. Previous work suggests that intraindividual day-to-day variability can be as high as 16% and Quest Lab diagnostics includes the potential for $\pm$ 10 mg/dL variability in FPG (Mooy et al., 1996; Ollerton et al., 1999; personal communication with the local medical sciences representative). Therefore, it is possible that an individual with a FPG of 100 mg/dL may test up to 16 mg/dL $\pm$ 10 mg/dL above or below initial levels. The variability in FPG may lead to misidentification of prediabetes especially among those near the bounds of the prediabetes range. Our trial may have been influenced by this effect since some individuals were below our FPG inclusion criteria at their starting clinical visits. While it is tempting to dismiss FPG as a reliable indicator of prediabetes, FPG provides a convenient, inexpensive, routine clinical measure that often leads to additional testing for TIIDM screening (HbA1c or OGTT). Instead, attention should be given to the other markers that might enhance the reliability of FPG as an indicator of prediabetes. The ADA provides guidance on factors that may be used to determine whether screening for prediabetes should be performed (104). Risk factors such as overweight, obesity, family history is sufficient to screen for prediabetes. Our trial included older adults with a BMI $\geq$ 25 kg/m^2 to account for other factors associated with fasting glucose impairment (104). BMI and age did not completely account for the daily variation and coefficient of variation. As such, there is a need to identify factors that improve the reliability of FPG for prediabetes screening. Individual and/or combinations of TIIDM and CVD risk factors

may be used in predictive modeling (receiver under the area of the standard operating curve) to test individual and combinations of risk factors (e.g., obesity, lipids/lipoproteins, BMI, etc.). This evidence would help to better understand factors associated with prediabetes, maintain cost effectiveness, and may provide evidence for earlier intervention in those with prediabetes.

The magnitude of TIIDM and CVD risk with impaired glucose tolerance may have also influenced our findings. The ADA Standards of Diabetes Care 2023 note the disproportionate increase in TIIDM and CVD as FPG approaches 125 mg/dL. As such, those with a FPG closer to 100 mg/dL are at a much lower risk for TIIDM and CVD than those closer to 125 mg/dL. Higher glucose levels also signify a greater degree of glucose impairment. The distinction in glucose impairment is highlighted when comparing the ADA (US) and European guidelines for prediabetes. The ADA criteria for prediabetes is a FPG of 100-125 mg/dL whereas the World Health Organization classification includes higher glucose thresholds such as a FPG 110 – 125 mg/dL (104,133). Higher degrees of glucose impairment may be more sensitive to interventions that delay or improve impaired glucose tolerance. The concentration-dependent magnitude of effect is observed for other CVD and TIIDM risk factors. For example, those with higher concentrations of LDL-C have a greater reduction with interventions that reduce LDL-C. Moreover, trials in which participants had higher baseline FPG (mean FPG: 108 mg/dL – 116 mg/dL) show that supplementing pistachios reduces FPG (17). Since our trial included individuals with a FPG closer to 100 mg/dL, it is possible that study conditions were not sufficient to reduce FPG. An exploratory analysis that stratifies participants by the ADA and European guidelines is not feasible for our trials since power would decrease due to the limited number of participants that meet the European prediabetes criteria. Pooling data from this trial and previous trials performed by Dr. Kris-Etherton and Dr. Petersen would likely provide

enough information to delineate whether intervention effectiveness is influenced by baseline level of glucose impairment. Also, pooling previous trials may be used to identify a sufficient cutoff for single-day FPG at screening to ensure baseline FPG meets prediabetes criteria. Such an analysis would inform future research to better identify impaired glucose tolerance for interventions that prevent TIIDM onset.

Future trials examining insulin and HOMA-IR should interpret fasting glycemic control based on these markers with caution. Insulin is often used as a metric for determination of glucose control. HOMA-IR uses both insulin and FPG as a metric of insulin resistance. Elevations in either glucose or insulin will increase HOMA-IR since the numerator relies on the relationship between glucose and insulin. However, insulin measured in blood does not entirely account for its ability to control glucose levels. Insulin works to drive glucose and other nutrients from the blood into the cells of the body. It would be expected that the pattern of peripheral (i.e., capillary or veinous glucose) insulin in blood would parallel the pattern of blood glucose, but this is not the case. A study by Walker et al. showed that elevations (above steady state) in glucose persists for up to 3 hours before returning to baseline levels (134). Insulin elevations measured in blood occur between 20-60 mins suggesting that insulin action goes unmeasured in external compartments. As such, fasting insulin levels may not adequately account for glucose intolerance in the early stages of impairment (e.g., prediabetes) where insulin is not chronically elevated. This fact is a primary contributor to the limitations of measuring fasting insulin resistance (HOMA-IR) which is described in more detail by Park et al. (135). Notably, assessment of HOMA-IR using FPG, or fasting insulin may represent hepatic insulin resistance since fasting glucose levels are primarily managed by gluconeogenic metabolism. While the change in postprandial insulin may better reflect the degree of glucose intolerance, the postprandial insulin

response is influenced by a high degree of individual variability, individual food responses, meal timing/frequency, and microbial composition (23,132).

Another important consideration for fasting glycemia is the shifts in peak morning FPG and fasting insulin from hepatic glucose production. A shift in fasting glycemic peak refers to the time it takes for an insulin bolus to be released into the blood and subsequent insulin response. The peak insulin response is influenced by meal/snack timing (i.e., nighttime snack) and diurnal factors. Diurnal insulin sensitivity (i.e., insulin peak) is disrupted by several factors including obesity, TIIDM, and/or poor sleeping habits (136). Given this, shifts in glycemic peaks may appear to capture hyperglycemia, but may represent influential factors such as the differences in the timing of the nighttime snack, severity of disease, body composition, and/or sleep habits. The degree to which diurnal glucose levels are altered in prediabetes and whether nighttime snacks alter the patterns of diurnal glucose regulation has not yet been explored. Research is needed to better understand the expected patterns of glucose control in adults with prediabetes and the context in which nighttime snacks improve indicators of health.

We observed shifts in the gut microbiota with pistachio consumption. *Roseburia* and NK4A214 group of *Oscillospiraceae* were increased, which indicates two different effects with pistachio consumption. The increase in *Roseburia* suggests increased production of SCFA (specifically butyrate), which promotes gut health. The NK4A214 group of *Oscillospiraceae*, bacteria associated with obesity, was also increased with pistachio consumption (126,127). Weight was not different between groups but there was a significant increase weight and BMI in the pistachio condition. This finding suggests that pistachio consumption may influence enrichment of NK4A214 group of *Oscillospiraceae*, but the nature of this relationship is unclear. The enrichment of NK4A214 group of *Oscillospiraceae* did not occur during the pistachio

condition despite an increase in weight and BMI. However, within condition effects are subject to confounding and may not represent true effects. As such, further investigation is warranted to determine whether pistachios increased NK4A214 group of *Oscillospiraceae* directly and contributed to weight gain. Another plausible scenario is that supplementing pistachios increased weight then altered the gut microbiota. Including dietary (i.e., total fiber consumption, energy intake) and anthropometric (i.e., weight, BMI, WC) variables in a secondary analysis may shed light on the nature of this relationship. Furthermore, investigation of the association between NK4A214 group of *Oscillospiraceae* and/or weight on insulin and other markers of glycemic control is needed.

Finally, the observed effects on weight and insulin within the pistachio condition may provide insight into metabolic health of study participants. It is well understood that, under normal conditions, excess energy intake leads to weight gain and/or increased adiposity. Although weight gain is a contributor to chronic disease incidence, increased adiposity is a natural metabolic consequence of excessive energy intake and representative of normal homeostatic function (137). Homeostasis can be contrasted with conditions of obesity where excess energy is not allocated to energy storage tissues (e.g., adipocytes) but accumulates in alternative tissues (e.g., muscle, mesentery, liver) (138). This dysmetabolic state can occur when adipocytes are resistant to insulin signaling, which would lead to an increase in circulating lipids and lipid accumulation in alternative tissues. Our trial observed increased insulin, weight, and BMI (adiposity) during the pistachio condition all of which are typically associated with adverse outcomes. However, this metabolic profile suggests normal homeostatic function and may indicate that our participants were not in a dysmetabolic state during excess energy intake. Moreover, it may be concluded that elevations in insulin resistance (HOMA-IR) observed during

the pistachio condition did not occur in adipocytes. Investigation as to why our participants had elevated FPG can then be focused on the aptly named "triumvirate" of TIIDM pathogenesis: impaired insulin secretion, increased hepatic glucose production, decreased glucose uptake (i.e., muscle) (139). Other biomarkers associated with impaired glucose tolerance may identify the components that led to the classification of prediabetes in our population. For example, changes in waist circumference, TAG:HDL-C ratio, c-peptide (released with insulin), and HOMA-B (beta cell function indicator) can provide insights into factors that lead to the early stages of elevated FPG. Given that our data includes some of these other factors involved in glucose homeostasis, further examination of our data is warranted to better understand impairments in glucose metabolism.

In conclusion, this dissertation research incorporates numerous scientific domains including the assessment of the effect of simple dietary conditions for prediabetes, assessment of intermediate biomarkers, diet quality and nutrition composition, and fecal microbial metabolites. This research comprehensively addresses pertinent nutritional sciences research to address chronic disease conditions and inform clinical practice. The findings described herein provide data to address the burden of TIIDM and CVD with simple dietary strategies. Moreover, we provide evidence that helps to better understand the role of the gut microbiota and its relationship with diet interventions. Further research is warranted to delineate the role of pistachio consumption on the gut microbiota and markers of chronic disease in adults with prediabetes.

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Appendix A.

CONSENT FOR RESEARCH
The Pennsylvania State University

Title of Project: The effect of pistachios on blood sugar control, heart and gut health
Principal Investigator:

Name: Dr. Penny Kris-Etherton
Address: 319 Chandlee Lab
Telephone: 814-863-2923

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 and there will be no penalty or loss of benefits to which you are entitled.

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 pistachio nut 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 pistachio consumption affects gut health and how this relates to blood sugar control. 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?
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. Prior to the screening appointment you will need to fast for 12 hours (no food, you may drink water), and avoid alcohol and over-the-counter medications for 48 hours.

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 as required by applicable law and to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed.

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

4a. What are the possible benefits to you?

You will not benefit from this research study.

4b. What are the possible benefits to others?

The proposed study will investigate whether evening consumption of pistachios improves blood sugar control and risk factors for cardiovascular disease. It will also explore how pistachios 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 study.

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

If you agree to take part, it will take you about 15 minutes to complete this telephone screening. If you are eligible following the telephone screening, you will be asked to attend the Clinical Research Center for a clinic screening appointment on one occasion.

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

7a. What happens to the information collected for the research?

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. Reasonable efforts will be made to keep the personal information in your research record private. However, absolute confidentiality cannot be guaranteed.

A list that matches your name with your code number will be kept in a locked file (in 317 Chandlee Lab) or password protected file.

Your research records will be labeled with your code number and will be kept in a safe area in 317 Chandlee Lab.

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

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
The research study sponsor, The American Pistachio Growers
The Institutional Review Board (a committee that reviews and approves research studies) and Penn State's Office for Research Protections.
U.S. Food and Drug Administration

8. What are the costs of taking part in this research study?

8a. What will you have to pay for if you take part in this research study?

Not applicable

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

It is possible that you could experience complications or injuries as a result of being in this research study. If you experience a side effect or injury and emergency medical treatment is required, seek treatment immediately at any medical facility. If you experience a side effect or injury and you believe that emergency treatment is not necessary, you should contact the principal investigator listed on the first page of this consent form as soon as possible. You should also let any health care provider who treats you know that you are in a 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 agreeing to participate in this study, 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.

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

You will not receive any payment or compensation for doing the telephone screening or clinic screening appointment.

10. Who is paying for this research study?

The institution and investigators are receiving a grant from The American Pistachio Growers to support this research.

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.

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 protocols or ineligibility to participate in the study.
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. Penny Kris-Etherton 814 863 2923 if you:

- Have questions, complaints or concerns about the research, including questions about compensation.
- 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, IRB-ORP@psu.edumailto: if you:

Have questions regarding your rights as a person in a research study.
Have concerns, complaints, 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.

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.

INFORMED CONSENT TO TAKE PART IN RESEARCH

Do you consent to take part in this telephone screening?

Tell the researcher your decision regarding whether or not to participate in the research.

CONSENT FOR RESEARCH
The Pennsylvania State University

Title of Project: The effect of pistachios on blood sugar control, heart and gut health
Principal Investigator:

Name: Dr. Penny Kris-Etherton
Address: 319 Chandlee Lab
Telephone: 814-863-2923

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 pistachio nut 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 pistachio 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 U.S. and therefore strategies are required to improve blood sugar control. Based on previous evidence, nighttime pistachio 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 pistachio 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?

<u>General overview of the study</u>

If you agree to participate in this study, your participation will last approximately 28 weeks in total. There are two diet conditions each lasting approximately 12 weeks and separated by an approximate 4-week break. The two conditions will include: 1) evening (i.e., after dinner and before sleep) consumption of 57 g (~2 oz.) of pistachios; 2) usual care, which is advice to consume 1-2 exchanges of carbohydrate in evening (i.e., after dinner and before sleep). During the pistachio condition you will be provided with the pistachios. During the usual care condition, you will receive a handout that provides information about carbohydrate exchanges and food choices that contain 1-2 exchanges of carbohydrate. You will also receive a grocery voucher worth $30 at each monthly appointment ($90 total for a 12 – week period) that you should use to purchase the evening snack foods. During both diet conditions, you will visit the metabolic kitchen monthly for provision of pistachios or grocery vouchers (depending on the condition you are in). Compliance monitoring and education will be perfirned bi-weekly by phone. You will follow your normal self-selected diet, although the pistachios or recommended carbohydrate containing foods 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 undergo both conditions; the order you receive the conditions will be randomly assigned and your condition 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 condition, you will be required to avoid peanut or tree nut consumption (including nut butters) other than what is provided to you. In addition, you will be required to complete a log of your daily evening 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 (medical history, personal information); measuring height and weight so that body mass index (BMI) can be calculated; measuring waist circumference, and 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 childbearing 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 and waist circumference measured. If you are female of childbearing 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 ~28-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, fructosamine, HbA1c), insulin, and markers of inflammation. No personal information will be kept with any sample – only ID# assignments and only the Primary Investigator, co-investigator and study 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 pistachios or the recommended carbohydrate containing foods. However, you may have an unknown sensitivity to this amount of pistachios or the carbohydrate containing foods 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
The pistachios 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 pistachios improves blood sugar control and risk factors for heart disease. It will also explore how pistachios 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 28 weeks to complete this research study. There are two treatment periods each lasting approximately 12 weeks, separated by a ≥ 4-week break. You will be expected to attend the diet center on campus monthly for compliance monitoring and provision of the pistachios. Additionally, you will be expected to participate in biweekly compliance telephone calls with study staff during each diet period. 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, waist circumference, blood draw – 45-60 minutes

(pregnancy testing: females only – 5 minutes)

Beginning of treatment period 1 and 2

- Day 1: blood draw, weight, waist circumference, 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, waist circumference, PWA, PWV – 60 minutes

(pregnancy testing: females only – 5 minutes)

- Day 2: blood draw – 30 minutes

Attending compliance visits, taking bi-weekly compliance telephone calls, completing stool sample collections, and 24-hour dietary recalls: ~ 10 hours

Total time for clinic and diet center visits from baseline to the end of the study ~17 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.

An electronic list that matches your name with your code number will be saved in a secure, password-protected folder where only approved study personnel will have access.

Your research records will be labeled with a unique ID number and will be kept locked at the PI's research office. All electronic records will be saved to a secure, password-protected folder only accessible by approved study personnel. 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 American Pistachio Growers
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.

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 $510.00, prorated as follows and paid at the end 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= $150
Completion of second treatment period and endpoint testing= $360
Total for completion of the study = $510
If you are a Penn State employee, you will be asked to provide your 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 American Pistachio Growers. The funder will not be involved in data collection or 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. Penny Kris-Etherton, at 814-863-2923 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.ed 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 heart 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, co-investigator and research team. 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 heart 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, her 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 B.

EVENING SNACK: CARBOHYDRATE COUNTING

What is carbohydrate counting?

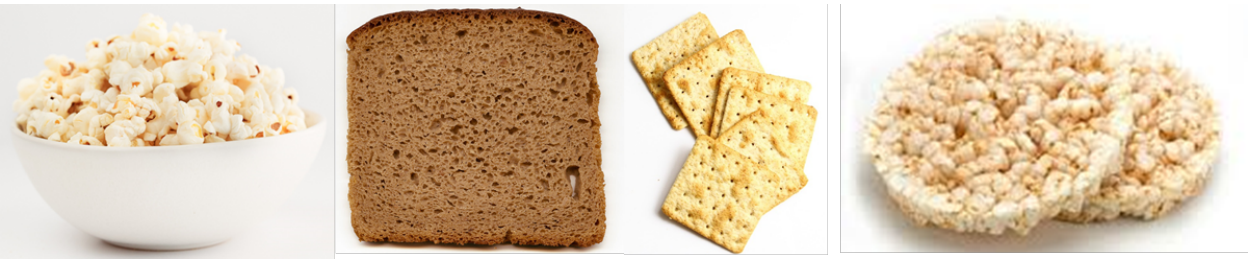
Carbohydrate counting is a way to plan your meals and snacks. It could help you manage your blood glucose (sugar). Carbohydrates, or carbs, are one of three main energy sources in food. The other two are protein and fat. Counting carbohydrates, especially when selecting your evening snack, can help you to reduce your fasting blood sugar levels.

Using carbohydrate countering to control fasting blood sugar levels

This sheet provides information about how to count carbohydrates and select evening snacks that contain 1-2 carbohydrate exchanges (15-30 g). In general, 1-2 carbohydrate exchanges are recommended for snacks for blood glucose control.

Choosing an evening snack

Below is a list of snacks that contain 1 carbohydrate exchange. Each evening (i.e., after dinner but before sleeping) you should select one (or up to 2 of these) as your evening snack. You should not consume any other caloric foods or beverages in the evening.






Evening snacks containing 1 carbohydrate exchange (15 g of carbohydrate)

Evening snacks choices	Serving suggestions *(if desired)*
1 slice of whole wheat bread	- Plain or toasted - Spread with margarine, low-fat/fat free cream cheese, jam or jelly (1 tablespoon = 1 carbohydrate exchange) - Top with a slice of cheese
½ whole wheat English muffin	- Plain or toasted - Spread with margarine, low-fat/fat free cream cheese, jam or jelly (1 tablespoon = 1 carbohydrate exchange) - Top with a slice of cheese
Crackers 6 saltines-type 6 round-butter type 2-5 (3/4 oz.) whole-wheat regular 2-5 (3/4 oz.) whole-wheat lower fat or crisp bread	- Plain - Spread with margarine, low-fat cream cheese, jam or jelly (1 tablespoon = 1 carbohydrate exchange) - Top with a slice of cheese - Top with hummus (1/3 cup = 1 carbohydrate exchange)
3 Graham crackers	Plain
8 Animal crackers	Plain
3 cups of popcorn (popped)	Plain
¾ oz. pretzels	Plain
¾ oz. Matzoh	Plain
2 rice cakes	- Plain - Spread with margarine, low-fat cream cheese, jam or jelly (1

	tablespoon = 1 carbohydrate exchange) - Top with a slice of cheese - Top with hummus (1/3 cup = 1 carbohydrate exchange)
Snack chips 15–20 (3⁄4 oz.) fat--free or baked (tortilla, potato), baked pita chips 9-13 (3/4 oz.) regular (tortilla, potato)	Plain

Adapted from the "All About Carbohydrate Counting Resource" by the American Diabetes Association

CURRICULUM VITAE

Terrence M. Riley RD

PUBLICATIONS IN REFERRED JOURNALS

Kris-Etherton, PM. Sapp, PS. **Riley, TM**. Davis, K. Hart, T. Lawler, O. The Dynamic Interplay of Healthy Lifestyle Behaviors for Cardiovascular Health. Current Atherosclerosis Reports. 24, 969-980. DOI: https://doi.org/10.1007/s11883-022-01068-w

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