Almond Consumption During a Walking Intervention

in Relation to Heart Rate Recovery

by

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ABSTRACT

Objective: The purpose of this randomized parallel two-arm trial was to examine the effect that an intervention of combining daily almond consumption (2.5 ounces) with a walking program would have on heart rate recovery and resting heart rate when compared to the control group that consumed a placebo (cookie butter) in men and postmenopausal women, aged 20-69, in Phoenix, Arizona.

Design: 12 men and women from Phoenix, Arizona completed an 8-week walking study (step goal: 10,000 steps per day). Subjects were healthy yet sedentary, non-smokers, free from gluten or nut allergies, who had controlled blood pressure. At week 5, participants were randomized into one of two groups: ALM (2.5 oz of almonds daily for last 3 weeks of trial) or CON (4 tbsp of cookie butter daily for last 3 weeks of trial). Body weight, BMI, and percent body fat were measured using a stadiometer and Tanita at the screening visit. Resting heart rate, heart rate recovery, and anthropometric measurements were taken at weeks 0, 5, and 8.

Results: 8 weeks of walking 10,000 steps per day, with or without 3 weeks of almond consumption did not significantly improve heart rate recovery ($p=0.818$) or resting heart rate (0.968).

Conclusions: Almond consumption in combination with a walking intervention does not significantly improve heart rate recovery or resting heart rate.

DEDICATION

I would like to dedicate this work to my parents, Maureen and Robert McElaney. Thank you for teaching me the importance of nutrition, the importance of education, and for the support you have given me throughout this process.

I also dedicate this work to Peter, Becky, and Paul who have been supportive throughout this process as well.

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TABLE OF CONTENTS

CHAPTER Page

APPENDIX Page

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

Overview

Cardiovascular disease (CVD) remains the number one cause of death worldwide for both men and women. In the United States alone, 610,000 people die of heart disease every year. CVD is one of the many conditions linked to obesity, which has become a large concern over recent decades[25]. Poor diet and physical inactivity are two risk factors for heart disease. There is a dire need for a health intervention strategy that is simple to follow.

Walking is one of the easiest forms of exercises for Americans to implement in their daily lifestyle. Going for a brisk walk for 30 minutes per day can qualify a person to meet the physical activity guidelines[48]. Heart rate recovery (HRR) is a measure of physical fitness that is used to predict risk for CVD, as well as mortality. HRR has been shown to be directly related to cardiovascular health and physical fitness. Almonds and physical activity are both linked to reduced risk for CVD. Almond consumption has been related to improved athletic performance and endurance[106], weight loss and improved lipid profiles[1, 37]. Due to the evidence that HRR and resting heart rate (RHR) are predictive of CVD and metabolic syndrome[95], it would be beneficial to study whether the addition of almonds to the diet while following a walking intervention would improve HRR and RHR.

There is no research that specifically studies the consumption of almonds during exercise interventions. Given that these factors independently reduce the risk for CVD, it is important to see if the combination of the two interventions results in a greater improvement in HRR. It was expected that the walking protocol would improve fitness measures in both groups. Significant changes were expected to be related directly to almond intake versus the placebo intake. If this combination of interventions was shown to be effective, it could be used to create a simple prevention strategy against the largest cause of death around the globe.

Purpose of Study

The objective of this study was to examine the effect that an intervention of combining daily almond consumption (2.5 ounces) with a walking program would have on heart rate recovery when compared to the control group that consumed an iso-caloric placebo (cookie butter) while on the same walking program. For each group, the almonds or placebo butter were consumed as a daily snack to fuel their walking program. No studies have tested whether almond consumption has an effect on HRR.

Research Aim & Hypothesis

It was hypothesized that daily almond consumption would result in improved HRR measures in comparison to the control treatment. There is also no evidence in the literature that shows a relationship between almonds and resting heart rate. The secondary hypothesis was that there would be no relationship between almond consumption and resting heart rate in comparison to the control treatment.

Definition of Terms

Heart rate recovery: the decrease in heart rate immediately following exercise. Measured as the drop in heart rate (beats per minute) from the time exercise stops to one minute post-exercise. The higher the heart rate recovery, the better, as this means the heart rate returns to normal in a shorter period of time.

Resting heart rate: heart rate, measured in beats per minute, when a person is at complete rest.

Delimitations

Participants were sedentary men and postmenopausal women who sit for at least 8 hours a day. The results do not apply to children, premenopausal women, or active adults.

Limitations

This study included a self-feeding and self-monitoring protocol. The participants consumed the assigned snack on their own, and investigators did not directly observe the consumption of the snack or the walking. Compliance calendars were used to document the ingestion of the test foods, though not all participants followed instructions to mark off their calendar when they consumed their daily snack. The use of the step test instead of VO_2 max was also a limitation. The time period of 8 weeks may have been too short to allow participants to experience significant changes in heart rate recovery and resting heart rate. The sample size was too small to see any significant differences. A larger study using at least 50 subjects may be needed to attain adequate power to detect significant results.

Chapter 2

LITERATURE REVIEW

Nut Benefits. Nuts are an energy-dense snack that are rich in monounsaturated and polyunsaturated fats and other beneficial nutrients. Despite the high energy content, epidemiological studies indicate that regular consumption of nuts does not contribute to obesity or the onset of diabetes. On the contrary, nuts have actually been shown to aid in weight loss. Nuts act as an appetite suppressant and regulate fat absorption. They have a wide array of cardiovascular and metabolic benefits. With nuts being an easy snack to prepare, using nuts as a part of dietary intervention may support weight management and improve metabolic health[20].

Almond Nutrients. Among the many types of nuts, almonds are one of the most beneficial. They are rich in monounsaturated fats, fiber, vitamin E, magnesium, copper, phosphorus and phytonutrients. They are also rich in arginine and quercetin. Fiber consumption, in combination with the consumption of beneficial fats, results in a cholesterol-reducing effect. Other benefits specific to almonds are glucose homeostasis, reduction in inflammation and oxidative stress, and management of body weight. Evidence shows that almonds can be used to treat and manage high cholesterol, metabolic syndrome, and type 2 diabetes[62]. A study that replaced saturated fatty acids in the diet with almonds and then walnuts. After a 3-week period of daily almond ingestion (84 g/d), participants had a 7% reduction in total cholesterol and a 10% reduction in LDL cholesterol. The researchers cited the monounsaturated fats as the cholesterol-lowering mechanism[2]. The data from a study in which subjects ate 5 different meals with washout periods in between indicated that almonds decrease the glycemic and insulinemic responses to bread. The researches attributed this decrease to the antioxidant activity of almonds, which preserved protein thiols. Low protein thiols are a marker of oxidative stress. The results of this study indicate that lowering of postprandial glycemic and insulinemic responses following nut consumption may also contribute to the lower risk of CVD associated with almond consumption[58]. Frequent nut and seed consumption is linked with low levels of inflammatory markers, including C-reactive protein, IL-6 and fibrinogen. In a randomized control crossover feeding study, participants on a high-almond diet had 7.8% decrease in E-selectin than the control group, and 6.3% decrease in E-selectin than the low-almond group. E-selectin is an adhesion molecule, which when present indicates endothelial dysfunction. A decrease in E-selectin could lower inflammatory response, which could in turn decrease the risk for CVD. These investigators also contribute these results to the monounsaturated fatty acids in almonds[84]. Vitamin E, another component of almonds, stops lipid peroxidation. Lipid peroxidation reduces the capacity of cell membranes to maintain an equilibrated gradient of concentration, and increases membrane permeability and inflammation[36].

Modality of Almonds in Protection against Heart Disease. Though it is well-known that almonds are protective against heart disease, researchers are still working to understand mechanisms. A study of almond supplementation in rats found that the activation of HMG-CoA reductase in a tyloxapol model enhanced de novo cholesterol synthesis, which resulted in hypercholesterolemia. The tyloxapol was injected purposely to induce hyperlipidemia. One working theory is that the vitamin E and phytosterols in almonds are inhibitory against HMG-CoA reductase. Almond-induced hypertriglyceridemia may be controlled through actions on the enzymes lipoprotein lipase and lecithin-cholesterol acyltransferase, which in turn would result in the promotion of triglyceride clearance. These investigators also propose that almonds prevent endothelial dysfunction by inhibition of smooth muscle cell proliferation, prevention of vascular calcification, regulation of LDL-C oxidation in plaques by inhibiting the increase in serum GGT, the minimization of vascular fat accumulation by improving liver function and promoting reverse cholesterol transport, improving acetylcholine-induced aortic relaxation, restricting eNOS inhibition, and increasing serum NO. Overall, the findings of this study were that almonds inhibit de novo cholesterol synthesis, prevent hepatic damage, and restore vascular function. These are all ways that almond consumption may be playing a role in preventing cardiovascular disease and metabolic syndrome. Future studies may be able to link almonds to enhanced fitness measures based on factors such as serum NO[57]. A randomized parallel-group trial examined the effect of sixteen weeks of almond supplementation (2 oz per day) on insulin sensitivity and other cardiovascular risk factors in an adult population with prediabetes. The almond intervention was associated with lower fasting insulin levels in comparison to the control group. Fasting insulin is a marker of insulin resistance. The researchers propose that the high oleic acid content in the almonds may improve beta-cell efficiency, which results in improved insulin efficiency. This is beneficial for the prevention of CVD, as elevated fasting insulin and postprandial insulin levels along with impaired carbohydrate tolerance are associated with a higher risk of CVD[103]. A randomized crossover trial consisted of three 1-month diet phases, each separated by a 2-week washout period. The three phases were a control (muffin), a full dose of almonds (~73 g/d), and a half dose of almonds (~37 g/d) with a muffin. The full dose phase resulted in a 9.4% reduction in LDL from baseline. The half dose phase resulted in a 4.4% reduction in LDL from baseline. Based on this data, the researchers surmise that a dose response of 7 g of almonds per day results in a 1% reduction of LDL cholesterol, which equates to a 2% reduction in risk for coronary heart disease (CHD). The full dose of almonds was also associated with a decreased calculated risk of CHD based on total HDL cholesterol and blood pressure data[59].

Almonds and Vascular Function. Vascular function is another health component that can be improved by the consumption of almonds. A study at Tufts University studied the effect of almond consumption on vascular function in patients with coronary artery disease. This was a randomized control trial that issued participants either a diet that included almonds, or a control diet that did not include almonds. While the study examined outcomes such as flow-mediated dilation, blood pressure, oxidative stress, and blood lipids, fitness outcomes were not measured. Exercise was not a variable in this trial. In this particular study, almonds did not significantly impact vascular function. The addition of exercise in such a trial may result in a different impact on vascular function[18]. Nuts contain unsaturated fats, which have favorable effects on blood lipids, a marker that is used to predict cardiovascular risk. Nuts are also rich in arginine, which is the precursor for nitric oxide. Nitric oxide is a potent vasodilator and can inhibit platelet adhesion and aggregation. The anti-atherogenic effect of nuts may be related to the arginine-NO pathway[53]. The consumption of nuts is inversely associated with levels of inflammatory markers, CRP, IL-6 and fibrinogen. This is important for vascular function, as inflammation contributes to all phases of atherosclerotic disease. This includes initial recruitment of circulating leukocytes to inducing endothelial dysfunction and plaque rupture. Reducing the levels of inflammation by consuming nuts may prevent atherosclerotic disease[66]. A 4-week almond supplementation design was used in a study investigating whether almond supplementation can improve oxidative stress and cardiovascular risk factors. Participants consumed a daily snack of 50 g of almonds. Plasma triacylglycerol and HDL and LDL cholesterol remained unchanged after the 4 weeks. Plasma α-tocopherol corrected for LDL concentration increased significantly from 6.6±0.4 to 6.8±0.44 mmol α-tocopherol/mol LDL after the 50 g/d almond supplementation. Despite this improvement, there was no correlation between changes in LDL and α-tocopherol levels and measures of vascular function[19].

Almonds and Weight Loss. The consumption of nuts has been linked to weight loss and weight maintenance in numerous studies. One randomized control trial studied the effects of a balanced diet that included almonds on weight and cardiovascular risk factors in overweight adult women. Women who consumed 50 g of almonds per day for three months had significantly greater weight loss than the women who followed a nut-free diet. The almond group also showed significant decreases in BMI, waist circumference, and waist to hip ratio. This group also showed improvements in triglyceride and total cholesterol levels[1]. Data from the 1994-1996 Continuing Survey of Food Intakes by Individuals by the USDA show that young persons and adults that consumed nuts had lower BMI compared to those who did not. The nut consumers had higher energy intakes, yet lower BMI. Multiple studies observed that subjects assigned a nut diet either maintained or lost weight, rather than gaining weight[52, 87]. In a randomized cross-over study, participants received a daily almond supplement (320 calories) for six months with no additional dietary advice. The daily almond consumption did not lead to significant weight gain[38]. A randomized, parallel-arm study in which test subjects consumed almonds as a snack for 4 weeks found that despite an additional 250 kcal/day in the subjects consuming almonds, daily energy intake was not significantly higher than at baseline, and weight did not change significantly between study groups[97]. Subjects typically compensate by reducing food intake from other sources. In a study by Hollis, et al., inefficiency in the absorption of almonds was documented[47]. In another study in which participants either consumed a low calorie diet including almonds or a low calorie diet including self-selected complex carbohydrates, the almond group experienced and sustained a greater weight reduction over a 24 week period than the complex carbohydrate group[104]. In both the Adventist Health Study and Nurses' Health Study, those who consumed more nuts tended to weigh less[53]. The nutrient profile of almonds may promote a feeling of satiety, which is beneficial to those attempting weight loss. The protein, fiber, and fat content of almonds allows individuals to feel full for longer periods of time.

Almonds and Exercise Performance. While there is a deficiency in the literature regarding almond consumption during a walking program, there are studies that have shown a relationship between almond consumption and improved exercise endurance and performance. One study examined the effect of almonds on elements of endurance exercise performance in trained athletes. Participants either consumed 75 g of almonds per day (ALM) or an isocaloric cookie (COK). Investigators measured carbohydrate and fat oxidation, energy expenditure, oxygen use, glucose levels, and biochemical markers. The consumption of 75 g of almonds per day resulted in increased cycling distance compared to the control group. It was also noted that ALM had lower oxygen consumption than COK during the 20-minute time trial. ALM also had lower blood free fatty acids and higher blood glucose post-exercise when compared to baseline. This indicates that almonds may assist athletes by mobilizing reserved carbohydrates instead of breaking down fat as a source of energy during training and intense exercise[107]. Untrained subjects in an exercise fatigue study were able to delay the sensation of exhaustion with almonds. The data from this study suggest that the ingestion of the fat from the almonds delayed the exhaustion to a greater extent than carbohydrate ingestion[3]. In a study where mice were given a placebo or quercetin for 7 days, the quercetin group had increased exercise capacity ($p < 0.05$). The mice with a 12.5 mg/kg dose increased treadmill run time by 36% and those with at 25 mg/kg dose increased treadmill run time by 37%. Mice fed 25 mg/kg of quercetin for 7 days increased their voluntary time ($p < 0.05$) and running distance ($p < 0.05$) on an exercise wheel in comparison to a placebo group[23]. There are few studies specifically regarding almonds and exercise performance, however the discussion in this review about the individual nutrients that are in almonds will shed some more light on the potential benefits of almond consumption on exercise performance.

Mastication of Almonds. To determine the serving size for the isocaloric placebo snack, it is important to review the available literature on the energy value of almonds. Studies have shown that the energy value from digested almonds is less than the value shown on labels or as calculated by the Atwater method. Mainly due to mastication, the digestibility of fat from almonds and other nuts is lower than fat in other foods that are digested in a more complete manner. Due to incomplete breakdown of almond particles during mastication, cell walls that encapsulate the lipids remain unbroken, resulting in a lower metabolizable energy content. The actual energy content derived from almonds after digestion was found to be 129 kcal per 28 g in comparison to the amount determined by the Atwater factors, which was 168 to 170 kcal per 28 g [15, 44, 79]. There is evidence to show that consuming almonds as a snack may be more beneficial than consuming them with a meal. Consuming almonds as a snack is an easy method to aid weight control, as they tend to result in a feeling of satiety and a lower level of hunger and desire to eat at the meal that follows the snack. Almonds also lower glucose responses postprandially. This effect was more prominent in the snack group when compared to those consuming almonds with a meal[97].

Weight loss and HRR. When the effects of a low calorie diet on post-exercise heart rate in obese men was studied, it was found that with weight loss, significant improvements in HRR were found at 90 seconds and 120 seconds post-exercise. The warm up consisted of a 5-minute stretching session, then two minutes on the bicycle ergometer at 0W. The workout started at 15 W, and then every minute 15 W was added gradually until the subject's capacity was reached. Blood glucose, triglycerides, and lipids also decreased significantly with weight loss. The author proposed that the exercise itself had a bigger impact on these measures than the fact that the participants lost weight. Improvements in HRR after exercise reflect improvements in the function of the vagus nerves and VO_2 max, which will in turn decrease the risk for cardiovascular disease[64].

Physical Activity Recommendations. The aerobic activity recommendations by the American College of Sports Medicine and Centers for Disease Control and Prevention are that adults between the ages of 18 and 65 should perform moderate-intensity physical activity for a minimum of 30 minutes five days a week, or vigorous aerobic exercise for a minimum of 20 minutes three days a week. Walking at a brisk pace for 30 minutes five times each week qualifies most Americans to meet these guidelines[48]. In terms of walking in relation to the physical activity guidelines, walking at an average pace for 30 minutes would equate to 2 miles or roughly 4,000 steps (The Walking Site). Only 22% of a sample of British men performed the minimum recommended amount of exercise. Not only did a small amount of participants exercise, but the amount of sedentary individuals increased with age. The number of subjects that exercised at least three times a week did not vary between age groups, which suggests that developing this schedule as a habit in or before early adulthood will raise the likelihood of continuing to do so into later adulthood. Increasing age results in the deterioration of health parameters, but the addition of exercise into a person's lifestyle can result in beneficial health changes and improve these parameters[9]. Meta-analyses of walking intervention studies have shown that walking alone can improve a number of cardiovascular risk factors. Interestingly, the majority of walking interventions have female participants. In a systematic review by Hanson et. al, 43% of the studies were for women only. 76% of the participants across 42 studies were female in this review[47]. This is a delimitation that can be addressed by recruiting men for future walking interventions. In the meta-analysis by Murphy, et al., the mean length of program was 34.9 weeks, with the mean volume of walking 188.8 minutes per week. Of 1,128 participants, 87.8% complied with the walking intervention[75]. Overall, studies in both meta-analyses showed an increase in VO_2 max. Statistically significant changes were found in resting heart rate, total cholesterol, 6-minute walk time, systolic blood pressure, and diastolic blood pressure. Participants also reported feeling less depressed and had a better quality of life. Decreases in BMI and body fat were apparent in both reviews[47, 75]. One particular study had participants in different groups performing different "bouts" of walking. The single-bout group did a 20 minute walk in one period. The accumulated-bout group did two separate 10-minute walking bouts in a day. Interestingly, the single-bout group had a greater reduction in heart rate than the accumulated-bout group[76].

Sedentary Activity Recommendations. While chronic disease risk is often defined by how much time a person spends doing physical activity, recent literature suggests that time spent sitting can also be indicative of risk. While guidelines about being more active are specific and quantitative, guidelines regarding sitting time tend to be very broad. It is suggested that people minimize their sitting time and to take breaks during long periods of sitting, but these guidelines are not specific[60]. While sedentary is often thought of as "not exercising," people who exercise may also engage in a great deal of sedentary activity throughout the day. Ekblom-Bak suggests that sedentary time should be defined as "the muscular inactivity rather than the absence of exercise"[32]. Guidelines were released in the British Journal of Sports Medicine that say desk-based workers should aim for at least 2 hours of standing time, and work their way up to 4 hours[13]. Participants from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) in the United States who wore an activity monitor for up to 7 days spent 57.7%, or 7.7 hours of their day participating in sedentary behaviors[73]. The results of a study in New Wales, Australia suggested that men who had at least 8 hours per day of sedentary activity had a higher incidence of all-cause mortality in comparison to men who had less than 3 hours per day of sedentary activity[82]. Another study in Australia found that higher sedentary time was adversely associated with waist circumference, plasma glucose, and triglycerides. However, if this sedentary time was paired with light-intensity activity, there were beneficial results in waist circumference and plasma glucose. Breaks in sedentary time were also associated beneficially with waist circumference, BMI, triglycerides, and 2-hour plasma glucose[50]. A larger study found that the successful maintenance of weight after a period of weight loss was associated with avoidance of watching television, which is considered the most common sedentary behavior[46]. If an individual is engaging in the recommended 30 minutes of activity per day, 5 days a week, but also sits for at least 8 hours per day, their step count may not add up to over 5,000 steps per day. For the average American who walks 2,000—3,000 steps per day, it could take a 4 mile walk to reach the 10,000 step goal.

Exercise Interventions and Physical Fitness. A study done between Arizona State University and Karolinska Institute found that the more steps participants took in a walking intervention, the greater the decline in systolic blood pressure and a greater linear decline in diastolic blood pressure, which slowed over time. Number of steps was also significantly related to changes in VO_{2max} over time. An important observation in this study was that participants were motivated in the beginning of the study, but over the course of the six months, the number of steps taken significantly decreased. The mean steps taken early in the study were 12,256 steps per day. This number decreased to 8,586 steps per day by the end of the six month period. This observation can be used to encourage investigators to check in with participants more often to increase compliance throughout interventions[94]. Another study followed women who were assigned to one of four treatment groups: aerobic walkers, brisk walkers, strollers, and sedentary controls. A linear, dose-response gradient across all walking groups was observed for both VO_2 max and heart rate[29]. Throughout the literature on walk studies, many of the study protocols involved using rate of perceived exertion throughout the exercise program[14, 64, 76, 95, 107]. This may be a useful tool to use to encourage participants to maintain a brisk pace throughout the 6-week walking intervention.

In a trial involving premenopausal obese women, the intervention group performed 4 or more hours of exercise per week. Significant improvements were shown in heart rate recovery at 1 minute post-exercise. At two minutes post-exercise, both the control group and intervention group had improved HRR. The intervention group also achieved modest weight loss, but the weight loss was not associated with improved HRR[14]. These findings are important when considering the management of obesity. With or without weight loss, implementing an exercise program can minimize the risk for cardiovascular disease.

A randomized exercise intervention study by Prakhinkit determined the effects of Buddhism-based walking meditation (BWM) and the traditional walking exercise (TWE) on depression, functional fitness, and vascular reactivity. Body composition was measured using a Tanita Body Composition Monitor, and heart rate was measured using a Polar heart rate monitor. A 6-minute walk test was used to assess cardiovascular endurance. Both TWE and BWM programs were performed 20 minutes each three times per week. In the second phase, the time increased to 30 minutes. Phase 1 was performed at a mild intensity and phase 2 was performed at a moderate intensity. Buddhist walking exercises involve swinging of the arms while voicing “Budd” and “Dha” with rhythmic arm swings. They were also instructed to practice mindfulness while walking. In phase 2, the participants increased their workload by holding water bottles in each hand while swinging the arms. At the end of this study, BMI and blood pressure decreased in both the TWE and BWM groups. In the BWM group, depression scores significantly decreased. Plasma cortisol concentrations in the BWM group also decreased. The mind and body connection that is incorporated into the Buddhist training method is thought to be effective in reducing depression and cardiovascular disease risk factors. Both exercise programs also resulted in an increase in plasma NO level. While Buddhist walking methods are not typically used in interventions, it is interesting to see that greater risk reduction resulted from using this method of exercise[83].

A study investigating the beneficial effects of exercise training in elderly people with coronary artery disease (CAD) implemented an intervention in which the exercise group completed a total of 36 exercise sessions over a period of 12 weeks, This included cardiopulmonary exercise training using a cycle ergometer, strengthening exercise training using free weights and weight machines, and balance training. Balance training consisted of exercises that involve standing, reaching, stepping, and single-limb exercises, among others. In the exercise group, HRR improved from a drop in heart rate in the first minute post-workout of 13.90/minute to 16.62/minute after training, which is a statistically significant improvement[18].

VO_2 Max and YMCA Step Test. The gold standard for measuring peak oxygen uptake is VO_2 max done using a treadmill. However, there are other methods to determine cardiorespiratory fitness that may be used in situations where using VO_2 max

is not possible or plausible. The YMCA 3-minute step test is a validated measure of cardiorespiratory fitness. It is only valid in subjects that properly perform the step test in its entirety, and do not finish early. If a participant terminates the test early, the test is not valid. This test entails having a subject step up and down on a 12” step for 3 minutes to the beat of a metronome set to 96 beats per minute. Heart rate recovery is measured one minute after the subject completes the 3 minute test[108]. It is suggested that the YMCA step test is used in studies where there subjects have a low exercise capacity[7]. Another study verified this information, by testing the Queen’s College step test in comparison to direct VO_2 peak measurement. The Queen’s College step test is similar to the YMCA step test. Participants step onto a step of 16.25” for 3 minutes at the rate of 24 cycles per minute following a metronome. The pulse is then measure from 5-20 seconds after completion of the exercise. The height of the step in the YMCA test is 12”, and the heart rate is measured at 60 seconds post-exercise. The QCT can be used to measure VO_2 peak in place of the direct measurement when direct measurement is not plausible[16]. A different study investigated the use of the 15-step climbing exercise test and compared it to a 6-minute walk test and VO_2 max test. The 15-step test, which is similar to the YMCA step test, showed to be a comparable test to the VO_2 max and 6-minute walk test. The authors felt confident that the 15-step climbing exercise test could be used in place of a VO_2 max test with patients who were intimidated by the VO_2 max, or who were unable to complete the latter due to medical conditions[86].

In a study done at the Kansas University Medical Center, investigators examined the ability of the YMCA submaximal exercise test protocol using a total body recumbent stepper (TBRS) to predict VO_2 peak. Participants were fitted with a Polar heart rate monitor for use during the test. Participants were instructed to maintain a pace of 100 steps per minute based on the YMCA protocol that was adapted for TBRS. Heart rate was measured throughout the test, and recorded until HR returned to baseline. VO_2 max was actually measured during a separate maximal exercise test for comparison. The researchers found that the YMCA submaximal exercise test using the TBRS can predict VO_2 max (r = 0.919) in a group of heterogeneous individuals with low to moderate risk for cardiovascular disease[8].

Heart Rate Recovery. Heart rate recovery refers to how much the heart rate falls in the first minute after exercise. The average HRR is a drop of 15-25 beats per minute. The quicker the recovery, the healthier the heart. The National Academy of Sports Medicine advises persons with a HRR of 12 beats per minute or fewer that they should not exercise without consulting a physician first[98]. The inability of the heart rate to appropriately decrease after exercise is linked to coronary heart disease and mortality. A study following a cohort of participants tested for cardiovascular risk factors found that the risk of death increased for every one beat decrease in HRR below 25 at one minute[26].

Metabolic syndrome is a set of factors that increase a person's risk for heart disease, diabetes, and stroke. The National Cholesterol Education Program's Adult Treatment Panel III report (ATP III) identified six components of metabolic syndrome that relate to CVD. These components are abdominal obesity, atherogenic dyslipidemia, raised blood pressure, insulin resistance and/or glucose intolerance, proinflammatory state, and prothrombotic state. Abdominal obesity is the form of obesity that is most strongly associated with metabolic syndrome. This is measured via waist circumference.

Atherogenic dyslipidemia is expressed through raised triglycerides and low levels of HDL cholesterol. Hypertension is often listed as a metabolic risk factor as it is often associated with obesity and insulin resistance. Insulin resistance often leads to glucose intolerance. Those with metabolic syndrome often present with high levels of C-reactive protein, which is a clinical marker of inflammation. Similarly, a prothrombotic state is characterized by increased plasma plasminogen activator inhibitor (PAI)-1 and fibrinogen[44]. A study by Sung, et al. studied the relationship between metabolic syndrome and heart rate recovery. HRR is indeed a factor that is associated with metabolic syndrome. In a study of almost 1,500 subjects, it was found that men have a lower HRR than women, and that smokers have a lower HRR than non-smokers. HRR was also found to be inversely correlated with age. It was apparent that those with metabolic syndrome had an impaired HRR (10.3±11.6 beats per minute) in comparison to those without (13.6±9.7 beats per minute). Those with metabolic syndrome also had higher RHR. Investigators noted that with an increasing number of criteria of metabolic syndrome present, there was a statistically significant difference in HRR. HRR is an indicator of vagal activity, which suggests there is a link between metabolic syndrome and impaired vagal activity. Delayed heart rate recovery was independently associated with metabolic syndrome after adjusting for resting heart rate, age, gender, and smoking. The implications of this study are that improving HRR through exercise can lower the chances of developing metabolic syndrome, which later leads to CVD. Also, patients who already have CVD can use exercise to improve HRR[95]. Another intervention examined the effectiveness on two aerobic exercise programs on the modification of metabolic syndrome components and its influence on cardiovascular risk in sedentary women.

Resting heart rate improved in the continuous exercise group, but not in the intermittent exercise or control group. A decrease was noted in the triglyceride/HDL ratio, implying there was an improvement in the peripheral response. This is important because it has been established that through the release of glucose, membrane surface transport, and intracellular flow of the substrate due to glycolysis, aerobic exercises increases peripheral insulin sensitivity. The TG/HDL ratio can be used as a low-cost insulin resistance marker[88].

In a trial with participants randomized into a placebo or omega-3 fatty acid supplementation group for two 4-month periods in a crossover design, resting heart rate and heart rate recovery at 1-minute post-exercise improved after omega-3 fatty acid supplementation. The improvement in RHR was from 73±13 to 68±13, and in HRR was -27±1- to -32±12 beats/min. Both of these changes were significant. These results suggest that a change of just 5 bpm could results in decreased risk for cardiovascular events[80]. Another study examined the change in HRR and PHR after 12 weeks of weight loss in participants who did not have CVD but had components of metabolic syndrome. There was a significant change in HRR, from -33.1±1.4 to -36.9±1.3 beats/min after weight loss[10]. These studies suggest that a reduction in HRR by 3-5 bpm can be meaningful, and achieved over a period of 3-4 months.

An interesting observation is that while men have a lower HRR than women, a study that explored HRR in children found that boys had a higher HRR than girls. This is expected to be linked to the statistic that more young boys are active than young girls. Resting heart rate increased with increasing heart rate recovery in these children. Those in the less fit (lower decrease in heart rate post-exercise) category had higher BMI and greater levels of triglycerides, LDL and total cholesterol. As HRR increased, HDL decreased. A positive relation was also observed between sedentary activities, such as watching TV and playing video games, and decreased fitness[91].

In a study that examined the relationship between step count and health outcomes, it was observed that those who accumulated ≥5,000 steps per day or more had lower body fat percentage, waist circumference, and a higher estimated VO_2 when compared with those who walked less than 5,000 steps. The study also noted that day-to-day walking behavior is often inconsistent. The researchers also reported that those who walked more were more frequently classified as “normal weight”[81]. A study investigating the effects of an 8-week, pedometer based physical activity intervention showed that the average step count for college employees participating in the study was 8565±3121 steps at baseline. At the end of the 8-week intervention, the average step count had increased to 10,538±3681 steps[22]. While the average step count for college employees based on the Croteau study is closer to the 10,000 step goal, a study looking at physical activity in adults over the age of 65 reported that the average step count at baseline amongst the 36 participants was just 2,992 steps per day. Over a 4-week period, the average step count increased to 3,670 steps per day. However, that increase was not sustained after a washout period in which the participants did not wear the pedometers. This suggests that wearing a pedometer may be a successful tool when it comes to increasing activity, but it would need to be worn on a regular basis to sustain the change[93].

Atherosclerosis. One cause of cardiovascular disease is atherosclerosis, which is prevalent in middle-aged men. Although little research has been done to analyze the relationship between almond consumption and atherosclerosis, there was a study that examines the effect dried plums have on the progression of atherosclerosis. Plums are in the same genus as almonds. The relationship may be one to examine in the future. The study on dried plums found that feeding plum powder to mice with atherosclerotic lesions reduced the area of the lesions[39]. An analysis of physical activity surveys found that there was no relationship between atherosclerosis and self-reported physical activity[42]. A study published in the Journal of the American College of Cardiology examined the effects of physical activity on early markers of atherosclerosis in pre-pubertal obese children. Some of these markers included flow-mediated dilation, BMI, body fat, V02 max. Children randomly assigned to an exercise group saw significant changes in blood pressure, BMI, abdominal fat, and V02 max at 3 months[35]. The Los Angeles Atherosclerosis Study examined the relationship between physical activity and the 3-year progression of carotid atherosclerosis. The progression of atherosclerosis was measured by carotid intima-media thickness. The thickness was increased nearly threefold in the sedentary group compared to the regular physically active group. Regularly physically active was defined as doing activities that cause sweating 3.5 times per week. The findings of this study suggest that someone increasing their activity from sedentary to moderate, or moderate to regular should slow the progression of carotid atherosclerosis[78].

Heart Rate Monitors. Measuring heart rate manually is a possible delimitation depending on the experience of the investigator performing the test. Using a heart rate monitor is a simple, safe solution. Multiple studies in the past have used Polar watches to measure heart rate and calories burned based on anthropometrics. In a study examining energy expenditure during gait of patients with mucopolysaccharidosis (MPS), the Polar FT7 was used to determine said energy expenditure during a 50 meter walk. The watch was used to record initial heart rate, heart rate at the end of the walk, and total time of gait. The energy expenditure was provided based on anthropometric measurements and heart rate. In comparison to the control group, the MPS group had a 22% higher initial heart rate and 13% final heart rate, indicating that MPS patients have a decreased cardiopulmonary capacity and increased difficulty performing the walking task[72]. A study evaluating the effect of hydrogymnastics on the serum level of immunoglobin A in elderly women also used the Polar FT7 to measure the training intensity of the aerobic exercise to keep participants at 50-60% of their maximum heart rate[45]. Studies have been done to specifically test the accuracy of other models of the Polar watch. A study published in Medicine & Science in Sports Medicine examined the accuracy of the Polar S410 HRM for estimating energy expenditure (EE) during exercise when using both predicted and measured VO_2 max and HR max versus indirect calorimetry. The study found that the Polar S410 HRM provides a rough estimate of mean energy expenditure for all exercise modes, but did note that the mean EE in females was overestimated by 12%[20]. A different study published in the Journal of Sports Science and Medicine examined the accuracy of the Polar S810i and Sensewear Pro Armband for estimating energy expenditure during indoor rowing versus indirect calorimetry. Individual estimates of energy expenditure did vary. The results suggest that the Polar HRM may be accurate for estimating EE in obese or overweight adults, but that actual VO_2 max and HR max need to be entered into the watch, rather than using the watch calculations.

Additional benefits of using the SWA included ease of use and attachment, and little to no discomfort or interference in activity[2].

Polyunsaturated Fats. Polyunsaturated fatty acids are one of the components of almonds that may be associated with improvements in fitness measures. One study investigated the effect of PUFA on inflammation, reversal of muscle wasting, and functional status in chronic obstructive pulmonary disease (COPD). The results of this study showed that COPD patients had increased functional capacity after 8 weeks of PUFA supplementation in comparison to a placebo-controlled group. This was displayed through improvements in peak exercise capacity and submaximal endurance time. PUFA upregulate peroxisome proliferator activated receptors (PPARs), which have been shown to promote the uptake of circulating fatty acids by cells through the upregulation of the lipoprotein lipase gene. The receptors also control mitochondrial fatty acid import and β-oxidation. The increase in exercise capacity in this study may be a result of the PUFA-induced activation of PPARs. The dosage of PUFA in this study consisted of 3.4 g of active fatty acids, as well as a blend of stearidonic acid, gamma-linoleinic acid, alpha-linoleinic acid, eicosapentanoic acid, and docosahexanoic acid in a capsule. The placebo was also given in capsule form. This was consumed daily for 8 weeks. The increased exercise capacity shown in this study indicates that a daily supplementation of PUFA for 8 weeks is sufficient[11]. Improved heart rate recovery was also seen in two studies that involved the consumption of omega-3 PUFA[51, 69]. Further studies need to be done to determine whether PUFA consumption can improve resting heart rate and heart rate recovery in healthy individuals. It is worth noting that almond consumption may help those who are at risk for CVD or already have elevated resting heart rate when considering the past literature on PUFA[57].

Arginine. Arginine is another component found in almonds, that is a substrate for synthesis of nitric oxide. Arginine, along with quercetin, may help enhance the effectiveness of training on exercise performance through the up-regulation of mitochondrial biogenesis and oxygen sparing capacity and by facilitating oxygen delivery to skeletal muscle. A clinical trial showed that an arginine and antioxidant-containing supplement increased the anaerobic threshold in elderly cyclists, potentially indicating that L-arginine can improve exercise performance in the elderly population. This clinical trial consisted of just three weeks of L-arginine supplementation[18]. The researchers in another study suggest that arginine can effect insulin secretion and muscle glycogen synthesis. They also suggest that arginine and quercetin may contribute to reserving and using more carbohydrate and enhancing more effective oxygen utilization, which further results in improvements in exercise performance[107]. A study that aimed to determine the effects of oral L-arginine supplementation on physiological functions in soccer players did find that the supplementation significantly decreased blood pressure and increased VO_2 max, blood flow, femoral artery diameter, and urea levels. Other studies have shown that intravenous administration of L-arginine resulted in similar improvements. Short-term oral L-arginine supplementation has been shown to improve endothelial function in the femoral artery, reduced arterial blood pressure and increased femoral artery diameter, peak systolic velocity, and-diastolic velocity and VO_2 max. The researchers in this study suggest that L-arginine may have increased VO_2 max through an exercise-induced increase in cardio output via decreasing the ventricular afterload[62]. A six week trial examining the effect of oral L-arginine supplementation found that L-arginine enhanced endurance exercise tolerance. This was demonstrated through a reduction in heart rate and circulating lactate[26]. Yet another study determined that a single dose of arginine resulted in an increased time to exhaustion during maximal incremental exercise[106]. While L-arginine has been shown to improve VO_2 max and other exercise-related measures, it has not been shown to improve heart rate recovery time in any study to date.

Quercetin. Quercetin is another component of almonds that has been associated with lower risk of cardiovascular disease and diabetes mellitus, and may contribute to improved exercise performance. A six-week study showed that a daily 150 mg quercetin supplementation significantly reduced systolic blood pressure and plasma oxidized low density lipoprotein (LDL) concentrations. The subjects were overweight and had been identified as high-risk for CVD based on their phenotype. The researchers suggested that quercetin may be protective against CVD[30]. One study examined the effects of seven days of quercetin supplementation on maximal aerobic capacity and fatigue in healthy, yet untrained individuals. The seven day supplementation did result in a significant increase in VO_2 max, as well as a significant increase in ride time to fatigue[23]. Another trial tested the effects of two weeks of quercetin supplementation in untrained, adult males. This supplementation resulted in a significant improvement in a 12-minute treadmill time trial performance. The same study demonstrated that 100 mg/day of quercetin promoted skeletal muscle mRNA expression of genes involved in mitochondrial biogenesis[77]. A meta-analysis in 2011 reported that quercetin has a small but significant ergogenic effect on endurance performance[65]. In a study where mice were supplemented with quercetin during exercise sessions, they were found to have 78%

atherosclerotic plaque reduction in comparison to control mice (no exercise or quercetin) and 40% less atherosclerotic plaque formation in comparison to the control group that was supplemented with quercetin but did not exercise. This is important because atherosclerosis is a major cause of CVD[40]. Another study found quercetin supplementation in athletes may improve some performance indices. An 8-week daily supplementation of quercetin resulted in increased lean body mass, total body water, basal metabolic rate, and total energy expenditure[5]. A trial that supplemented rats with quercetin for 5 weeks found that heart rate and blood pressure were reduced in spontaneously hypertensive rats[28] . No such studies regarding quercetin and heart rate have been done with human subjects. There are numerous benefits of quercetin supplementation in relation to cardiovascular disease and exercise performance. The study in rats suggests that heart rate recovery may be improved by dietary quercetin intake.

Vitamin E. Vitamin E is an antioxidant that is found in almonds. Vitamin E supplementation has been shown to prevent exercise-induce lipid peroxidation and also inhibits enzyme leakage after exercise[89]. Vitamin E deficiency increases susceptibility to free radical damage, as shown in exercised rats[34]. Vitamin E prevents atherosclerosis by inhibiting the generation of oxidized LDL, reducing the formation and uptake of cholesteryl ester, decreasing the proliferation of smooth muscle cells, and by preventing inflammation as well as preventing the adhesion of monocytes and macrophages to the endothelium[85]. A 16-week exercise and vitamin E supplementation trial found that 16 weeks of moderate-intensity exercise and 16 weeks of vitamin E supplementation (either alone or combined) reduced lipid peroxidation induced by free radicals in older men and women. In both exercise groups, participants had decreases in weight and blood pressure, as well as increases in VO_2 max. Another study found that 10 daily injections of 100 mg/kg body weight of Vitamin E in rats resulted in improved functional recovery of heart rate[101]. Such studies have not been done in humans. Vitamin E has many benefits in relation to fitness measures, and may be beneficial in reducing heart rate recovery time.

There is evidence that walking and almond consumption independently improve cardiovascular health and fitness measures. There is a deficiency in the literature regarding a combination of both of these interventions to improve cardiovascular fitness.

Chapter 3

METHODS

Participants

Subject selection

Subjects were considered eligible for the study if they were sedentary (fail to meet Physical Activity Guidelines for Americans and/or spend 8 hours or more per day sitting,) and were healthy males or postmenopausal women with normal mobility between the ages of 20-49 who answer “no” to all of the questions on the Physical Activity Readiness Questionnaire (PARQ). Initially, the intent was to only include males between the ages of 40 and 69, as there is an increased risk for cardiovascular disease in that specific population[61]. However, after low response rate during the recruitment phase with the limited inclusion criteria, changes were made to the protocol. There is an age-related increase in CVD incidence and mortality in both sexes, but to a greater extent in women[61]. As sedentary adult men and postmenopausal women are at risk for metabolic syndrome and cardiovascular disease, it was determined that this population was suitable for this study[56, 92]. The actual age range of subjects who completed the study was 32-66. Premenopausal women have higher levels of estrogen. It is hypothesized that the estrogen is protective against atherosclerosis and cardiovascular disease, so the premenopausal population was excluded from the study[90]. The changes to the inclusion criteria were approved by the Arizona State University IRB. Exclusion criteria were as follows: high blood pressure and/or gluten or nut allergies and/or the unwillingness to participate in a six week walking intervention or comply with testing protocols.

Recruitment

A total of 13 participants were recruited on a rolling basis between January-May of 2016. The goal was to recruit 50 participants. Participants were recruited through the Diocese of the Easy Valley, the Downtown YMCA, and Arizona State University. Efforts were made to recruit through the VA, Arizona Hellenic Educational Progressive Association (AHEPA), the Masonic Lodge and the Tempe Elks Lodge. Many of these locations did not allow flyers to be posted or did not have members that fit the eligibility criteria. Listservs were obtained through these organizations, and flyers were distributed. Interested participants were directed to complete an online survey and PARQ. Those who qualified and were still interested in participating were contacted and recruited for screening. At the first visit, participants provided written consent. The Institutional Review Board at Arizona State University approved this study prior to recruitment. Changes to the protocol were approved by the IRB.

Study Design

This study was conducted as an 8-week randomized parallel two-arm trial. Enrolled participants were stratified by age, BMI, and activity level and randomly assigned to either the almond group (ALM) or control group (CON). Subjects were randomized via a randomized number generator (Appendix F).

Independent variables: All participants were instructed to increase their daily step count gradually over the period of two weeks to reach 10,000 steps daily. Participants received an Omron pedometer and instructions on how to use the pedometer. For one week prior to the start of the intervention, the subjects recorded their daily steps and the daily average was determined at Visit 2. The participants received instructions to limit nut consumption to only those provided to them during the study, with the allowance of two additional servings per week. The participants were randomized into two groups using a random number generator, and were entered into either the almond group (ALM) or the control group (CON). At the baseline visit (week 1 of the intervention), participants were encouraged to increase step counts by 1000-2000 steps weekly to reach 10,000 steps within two weeks and to maintain this level of walking for the remainder of the 8-week trial. Participants randomized to the almond group were instructed to consume 2.5 oz almonds daily (~58 whole kernels or 413 kcal), which were distributed to them in 2.5 oz serving packages at Visit 3. Participants randomized to the control group were instructed to consume 4 tablespoons of an isocaloric cookie butter daily. A tablespoon measuring tool and 4 jars of cookie butter were distributed to these participants at Visit 3. Participants were instructed to consume their almonds or cookie butter as a daily snack. They were also instructed not to alter their typical diet patterns throughout the study (Appendix F).

Dependent variables: Outcome measures were assessed at baseline and trial weeks 5 and 8 and included anthropometrics, fitness test (step test), blood pressure, body composition, flow-mediated dilation, and fasting blood samples. On test days, participants arrived fasted for 12 hours (no food or drink except water) and refrained from strenuous exercise for the prior 48 hours and alcohol and caffeine for the prior 24 hours. Use of antihistamines and vitamin C supplementation were restricted for the 5-day period prior to testing. Emails were sent out to participants as reminders prior to each visit, which included instructions about restrictions. A blood sample was drawn at each visit. All blood draws were performed by a registered nurse, and flow mediated dilation was measured by a trained stenographer. Data from participants' pedometers were downloaded upon each visit (Appendix F).

Statistical analyses: All data are expressed as the mean ± SD. SPSS version 23 was used to perform statistical analyses. Baseline variables for subjects in each group were compared by t-tests and changes in variables between and within groups were assessed by one-way analysis of covariance. A p-value of ≤ 0.05 will be considered statistically significant. The Shapiro-Wilk test was used to check normality.

Chapter 4

DATA & RESULTS

Recruitment for this randomized control trial took place from January to May 2016. The total number of survey responses during this time period was 64. Out of the 64 responses, 21 individuals were eligible for the study, and 15 respondents enrolled in the study. The 6 eligible participants who did not enroll did not respond to emails regarding scheduling an initial consent meeting. One participant dropped out during the initial week of the trial. A second participant was disqualified due to high blood pressure readings. Another participant dropped out at week 4 due to an ankle injury sustained during a hike. The remaining 12 participants at week 5 were stratified by age, height, weight, BMI, body fat percentage, and baseline step count, and randomized to the intervention (ALM) or control (CON) group. This resulted in 6 subjects in the ALM group and 6 subjects in the CON group and these subjects were included in the data analysis **(Table 1)**. Participants reported 100% compliance on their calendars.

Table 1. Baseline characteristics of the two study groups: almond (ALM) and control (CON)

	ALM	CON	P value[a]
N (M/F)	2/4	1/5	
Age (y)	55.5±8.6	52.7±10.9	0.629
Weight (kg)	77.2±11.7	72.6±8.7	0.482
Height (cm)	166.2±10.6	168.5±7.3	0.667
Body Fat (%)	33.1±8.3	34.3±9.4	0.817
Fat Free Mass	51.8±11.6	47.7±8.0	0.486
BMI (kg/m^2)	28.0±4.0	25.6±1.8	0.204
METS	33.2±20.8	71.7±52.8	0.128

Data are the mean±SD. [a]Independent t-test analysis.

The increase in steps from week 0 to week 5 was significant (p=0.025), with the average increase in steps being 2,043 steps. The change overall for the whole 8 weeks was not significant (p=0.090), with the change in steps from baseline to week 8 being 1,672 steps **(Table 2)**.

Table 2. Pre- and post-intervention step count data for participants consuming almonds daily (ALM) or a placebo daily (CON)

	ALM n=6	CON n=6	P value[a] [η^2_p][b]
Baseline	8278±1657	7848±2673	
Week 1	8298±1722	8917±2090	
Week 2	8575±1996	9193±1945	
Week 3	10217±2152	9055±1806	
Week 4	9639±2268	10295±1420	
Week 5	9729±2068	10482±1348	
Change (week 0 to week 5)	±1450±2378	±2635±2981	0.025 [0.408]
Week 6	9968±2005	10034±2244	
Week 7	9865±1165	10286±678	
Week 8	10404±1845	9066±2347	
Change (week 0 to week 8)	±2126±2418	±1219±3628	0.090 [0.261]

Data are the mean±SD. [a]Repeated Measures ANOVA for change over time. [b]Effect size: partial eta-squared η^2_p. The change in step counts at week 5 or week 8 did not differ between groups.

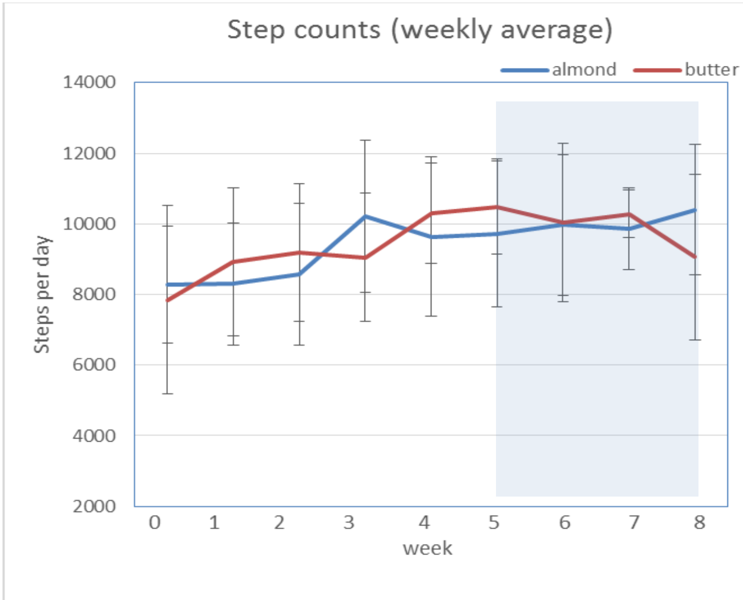

Figure 1. Changes in weekly step count among participants in both the ALM and CON groups.

An independent t-test showed that the change in resting heart rate (RHR) from week 5 to week 8 was not significantly different between ALM and CON (p = 0.968). The change in peak heart rate (PHR) from week 5 to week 8 was not significantly different between ALM and CON (p=0.665). The change in heart rate recovery (HRR) from week 5 to week 8 was not significantly different between ALM and CON (p=0.818)**(Table 3)**. The Shapiro-Wilk test was used to check normality. Data were normally distributed with the exception of Heart Rate Recovery at week 5. After a log transformation and square root transformation, the numbers stayed similar, so the original data was used in the analyses.

RHR did not begin to decrease until week 5 for both ALM and CON. The decrease from week 5 to week 8 was not significantly different between groups (p = 0.968) **(Table 3, *Figure 4*)**. For PHR, the ALM group decreased across the 8 weeks consistently, although the decrease was not significant. The PHR for CON increased until week 5, and then began to decrease from week 5 to week 8**(Table 3)**. These changes were not significant between groups (p=0.665). Similar trends occurred with HRR. HRR increased, though not significantly, from week 0 to week 5, then decreased from week 5 to week 8 in both the ALM and CON groups, but there was no significant difference between groups (p=0.818) **(*Figure 3, Figure 4*)**. Interestingly, during the walking intervention, PHR is the only heart rate measure that decreased, while HRR and RHR increased **(Figure 3)**. After the dietary intervention, all three measures decreased, though not significantly **(*Figure 2, Figure 4*)**.

Table 3. Pre- and post-intervention data and change data for participants consuming almonds daily (ALM) or a placebo daily (CON)

	ALM n=6	CON n=6	P value[a] [η^2_p]
Resting heart rate			
Baseline	73.8±6.7	72.5±11.1	
Week 5	77.0±7.1	72.7±5.7	
Change (baseline to week 5)	+1.7±8.6		
Week 8	76.2±3.4	72.0±6.8	
Change (week 5 to week 8)	-0.8±8.6	-0.7±4.5	0.968 [0.000]
Heart rate recovery			
Baseline	99.7±11.0	81.0±21.8	
Week 5	97.8±3.5	85.5±15.5	
Change (baseline to week 5)	+1.3±13.5		
Week 8	94.0±9.7	83.0±12.6	
Change (week 5 to week 8)	-3.8±11.3	-2.5±8.0	0.818 [0.006]
Peak heart rate			
Baseline	154.2±7.6	142.8±23.1	

Week 5	150.0±8.2	145.8±17.9	
Change (baseline to week 5)	-0.6±9.8		
Week 8	147.3±12.1	144.3±20.5	
Change (week 5 to week 8)	-2.7±5.3	-1.5±3.6	0.665 [0.002]

Data are the mean±SD. [a]One-way analysis of covariance for change by group controlling for pre-intervention value. [b]Partial eta-squared η^2_p.

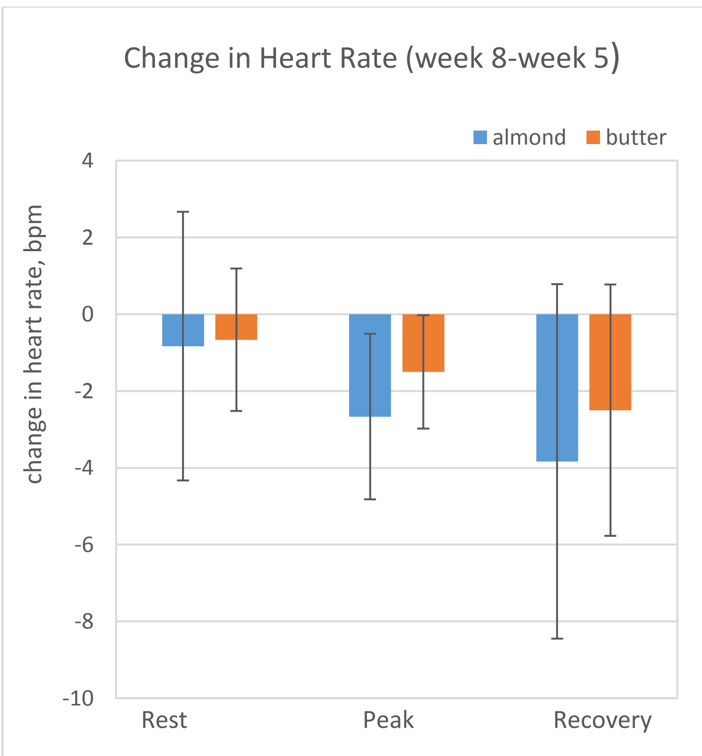

Figure 2**.** Changes in heart rate (rest, peak, and recovery) in beats per minute from week 5 to week 8 for ALM and CON.

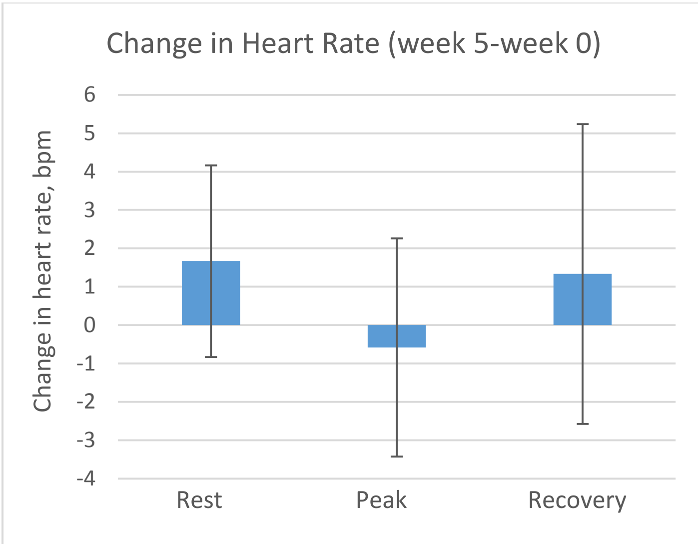

***Figure 3*.** Changes in heart rate (rest, peak, and recovery) in beats per minute from week 0 to week 5 for all participants.

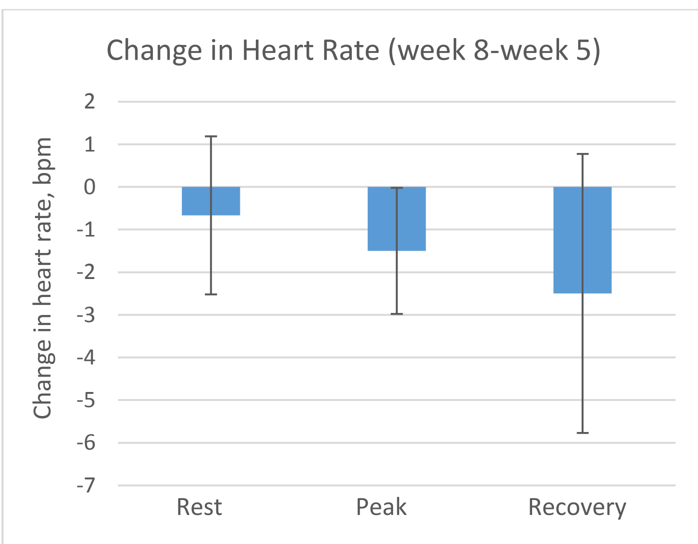

***Figure 4*.** Changes in heart rate (rest, peak, and recovery) in beats per minute from week 5 to week 8 for all participants.

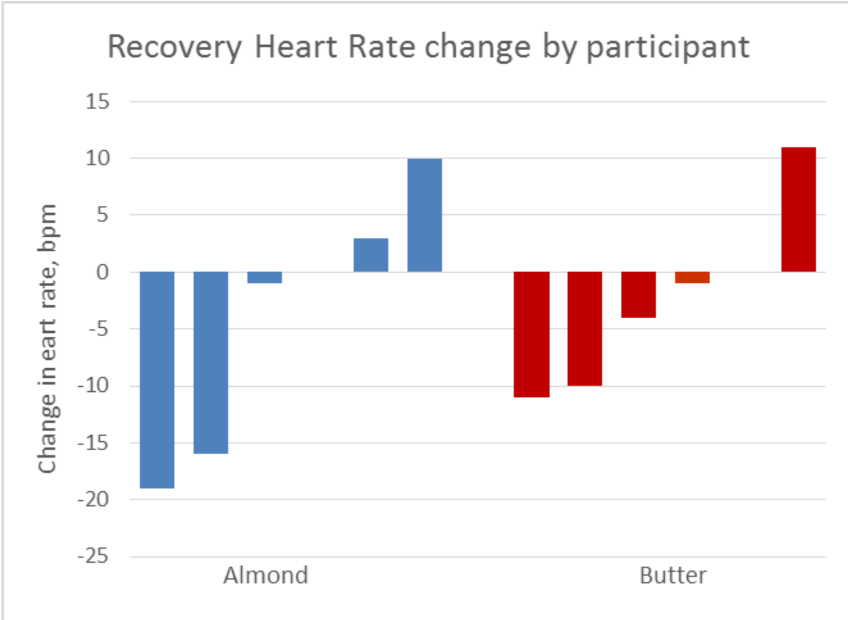

Figure 5. Changes in heart rate recovery in beats per minute for each individual participant over the course of the 8 weeks.

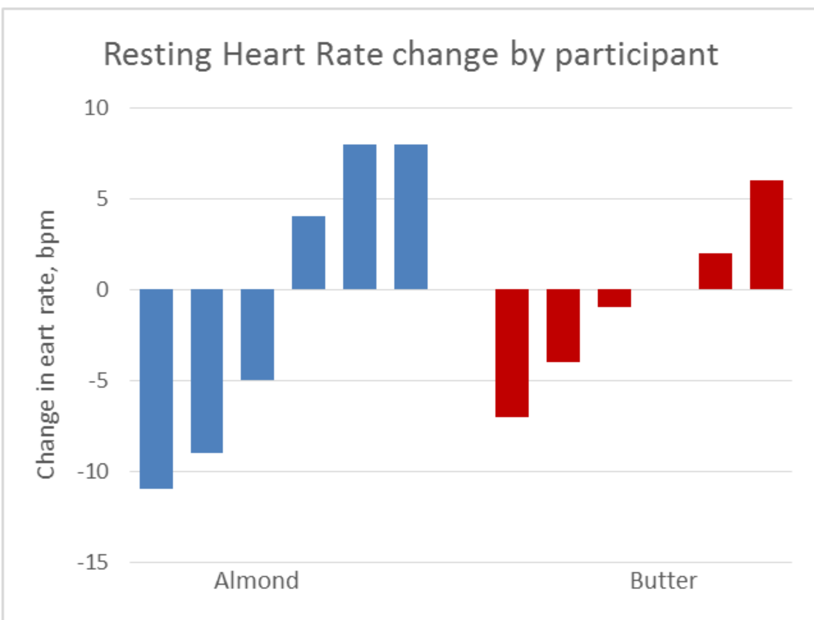

Figure 6. Changes in resting heart rate in beats per minute for each individual participant over the course of the 8 weeks.

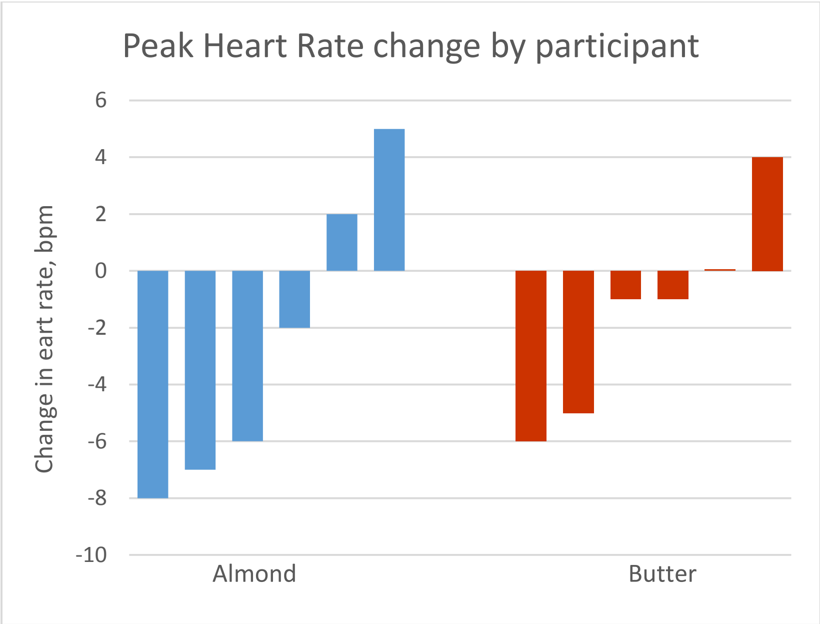

Figure 7. Changes in heart rate peak in beats per minute for each individual participant over the course of the 8 weeks.

Although neither weight loss nor body fat loss were part of the hypothesis, these data were examined. The change in weight was not significant (p=0.674). The mean weight of ALM increased from 77.1± kg to 77.6 kg over the course of the 8 weeks, while the mean weight of CON had a slight increase from 72.8 kg to 72.9 kg over the course of the 8 weeks. The change in body fat was significant (p=0.023), as shown by a slight increase in body fat percentage demonstrated by the overall group. The mean body fat of ALM increased from 33.1% to 34.4% over the course of the 8 weeks, though this change was not significant. The mean body fat of CON decreased from 34.3% to 33.5% over the course of the 8 weeks, though this change was not significant **(Table 4)**.

Table 4. Pre- and post-intervention weight and body fat data for participants consuming almonds daily (ALM) or a placebo daily (CON)

	ALM n=6	CON n=6
Weight (kg)		
Baseline	77.2±11.7	72.8±8.7
Week 5	76.9±11.5	73.0±8.4
Week 8	77.6±11.2	72.9±8.7
Body fat (%)		
Baseline	33.1±8.3	34.3±9.4
Week 5	34.1±8.3	33.5±8.9
Week 8	34.5±8.5	33.5±8.3

Data are the mean±SD.

Table 5. Pre- and post-intervention YMCA step test rating data for participants consuming almonds daily (ALM) or a placebo daily (CON)

Participant No.	Baseline	Week 5	Week 8	Group
1	Above Average	Above Average	Above Average	ALM
2	Excellent	Excellent	Excellent	CON
3	Above Average	Good	Excellent	CON
4	Good	Average	Good	ALM
6	Above Average	Excellent	Good	ALM
7	Good	Good	Excellent	ALM
9	Excellent	Excellent	Excellent	CON
10	Excellent	Excellent	Excellent	CON
12	Above Average	Good	Good	ALM
13	Above Average	Good	Good	ALM
14	Above Average	Good	Good	CON
15	Excellent	Excellent	Excellent	CON

Ratings from guidelines published by the YMCA.

Chapter 5

DISCUSSION

In this 8-week randomized parallel two-arm trial, 8 weeks of increasing the daily step count to a goal of 10,000 steps per day and 3 weeks of consumption of 2.5 oz of almonds did not significantly change heart rate recovery or resting heart rate as compared to a placebo (4 tbsp of cookie butter per day). Participants did increase their step counts over the course of the 8 weeks, as confirmed by the data collected from their pedometers.

Although the changes in heart rate recovery were not statistically significant, 6 of the 12 participants did move up a ranking on the age-adjusted standards based on guidelines published by the YMCA **(Appendix A, Table 5)**. Notably, all of the participants started out with a heart rate recovery rating between “above average” and “excellent.” In future studies, researchers may want to include a certain heart rate recovery rating in their inclusion criteria. The almond group achieved a change of 3-5 bmp in their HRR, which is meaningful, and can move someone up on the ranking by YMCA standards. Four participants in the ALM group and two participants in the CON group moved up a category in the YMCA ranking system (i.e. “good” to “excellent” or “above average” to “good”) from week 0 to week 8. Three participants in the ALM group moved up a ranking in the walking intervention from week 0 to week 5, and two moved up a ranking in the dietary intervention from week 5 to week 8. In the control group, one participant moved up a ranking during the walking intervention from week 0 to week 5, while another dropped down a ranking. The participant who dropped down a ranking moved back up to their baseline ranking after the dietary intervention. One participant in the CON group moved up a ranking in the dietary intervention from week 5 to week 8 **(Table 5)**.

In the United States, the average BMI is 26.6 for an adult male and 26.5 for an adult woman[49]. In this study, the ALM group had a mean BMI of 28.0 at baseline and CON group had a mean BMI of 25.6 at baseline. Based on BMI, the participants did represent the typical American population.

A review of the literature suggests that the daily step count for Americans is highly variable, with healthy adults taking anywhere between approximately 4,000 and 18,000 steps per day. Studies included in the review typically considered ≤5,000 steps per day to be sedentary[100]. The ALM group in this study started with a baseline mean step count of 8,278 steps, and the CON group started with a baseline mean step count of 7,848 steps per day. The population in this study is not representative of the average American when it comes to step count. Walking 10,000 steps a day would be nearly double the average step count for sedentary Americans, but for our participants, 10,000 steps was just 2,000-3,000 more steps than they started with. Many of our participants were college employees. Their baseline step count is consistent with another pedometer-based study which used college employees as participants[22]. It is possible that college employees do not sit as much throughout the day in comparison to the average American. In future studies, it may be beneficial to use more stringent guidelines to define participants as "sedentary". Based on the step test guidelines by the YMCA, all of our participants were ranked "above average" to "excellent" at baseline, meaning that they were seen as moderately fit to fit based on heart rate recovery[108].

Past trials investigating the effect of dietary and weight loss interventions on heart rate recovery saw significant change after interventions lasting 12-16 weeks[10, 18, 101]. It may be the case that longer interventions are necessary to see significant change in HRR. Our physical intervention lasted 8 weeks, and our dietary intervention lasted just 3 weeks.

In future studies, a different placebo may be recommended to ensure that no ingredients from the cookie butter were affecting results (such as coconut oil). Many studies in the literature assign the control group to a "habitual diet" void of nuts rather than using a placebo[19, 103] Another study used a muffin in the control phase of the trial[59]. The daily serving of 2.5 oz of almonds is sufficient for future studies, as it represents a realistic serving to recommend to the general public as an intervention. Despite consuming an additional 400 calories per day, no significant weight was gained as a result of the diet change. This may be a result of the increase in physical activity, or compensating by eating less throughout the day. Future studies should exclude participants who have "good" or "excellent" heart rate recovery ratings at baseline. Future studies should also either exclude participants with a higher step count at baseline or have the participants walk a certain number of steps above their baseline step count rather than aiming for 10,000. The participants in this study were more active than the typical person. As most of the participants were fairly fit at baseline according to the YMCA guidelines, the VO_2 max test could have been used in this study. The YMCA 3-minute step test was used in order to reduce the burden on participants.

Although the participants reported 100% compliance to both almond consumption and walking 10,000 steps per day, it is quite plausible that the additional steps they were taking were not at a “moderate” or “vigorous” pace. In future studies, it would be optimal to have participants perform their exercise under the supervision of the investigators, so that intensity and heart rate can consistently be monitored during the exercise. In addition to monitoring the exercise, participants could also consume the almonds during their visit to ensure compliance. An average walking pace is about a mile in 15 minutes. A mile is typically between 1,760 and 2,640 steps[99]. Based on this information, the participants in this study were only adding about one mile, or 15 minutes of exercise into their routine. If the subject pool was more representative of the American population, increasing their step count to 10,000 steps per day would be closer to an additional 4 miles, or roughly one hour of exercise into their routine per day. This would be more in line with the Physical Activity Guideline. These additional steps were taken on a daily basis, participants in this trial were likely averaging about 105 additional minutes of exercise above their baseline activity level. If this was vigorous activity, they would meet the physical activity guidelines of at least 60 minutes per week of vigorous intensity exercise. However, if this was moderate-intensity exercise, as the participants were directed to perform, 105 minutes falls short of the guidelines for moderate-intensity exercise[48]. The guidelines do not include suggestions for low-intensity exercise. Although one would need to perform low-intensity activity for longer periods of time to be comparable to the guidelines for moderate and vigorous activity, this does not mean that low-intensity exercise should be excluded from the guidelines. This is something to consider in future revisions to the physical activity guidelines.

Strengths. Participants were screened for a number of exclusion criteria. These included having chronic diseases, gluten and other food allergies, physical ailments, smoking, physical ailments that would interfere with the ability to participate in a walking program, high activity level and high blood pressure. Inclusion criteria included willingness to travel to campus on four occasions, willingness to consume nut products daily, answering "no" to all questions on the PARQ, and sitting more than 8 hours per day. Since pedometer data was downloaded through the Omron program to a PC, adherence to the walking program was monitored. Additionally, participants were randomized, which prevented participants from knowing what treatment they were receiving.

Limitations. This trial had several limitations. The intended target population was sedentary individuals. Based on the sedentary guidelines, the subjects were considered unfit. However, based on the step test data, the participants in this study were already above average fitness level at baseline. The average step count at baseline was 8,609 steps per day for all participants. It is quite possible that although we instructed participants to walk at a moderate pace, their additional steps may have been more of a "stroll" or low-intensity activity. Perhaps the level of intensity was not high enough to see a change in physical fitness. This should be a consideration in future studies. The 8-week intervention was short in comparison to trials that included a dietary or weight loss intervention of 12-16 weeks[10, 18, 101]. Subtle changes in heart rate measures were not seen until week 5, which suggests that it may take longer to see significant changes in heart rate recovery and resting heart rate. Despite the goal to enroll 50 participants in the trial, only 12 completed the study. The initial goal was to enroll 50 males between the ages of 40-69 in the study, but due to lack of responses to enrollment surveys, the IRB protocol was changed to allow men between the ages of 20-69, and postmenopausal women to enroll in the study.

Conclusion. This randomized control trial suggests that the daily consumption of 2.5 oz of almonds for 3 weeks in conjunction with an 8 week walking program does not lead to changes in heart rate recovery or resting heart rate. Future trials on this topic would benefit from having strict guidelines on the definition of "sedentary", a larger sample size, a longer trial, having participants perform exercise and consume almonds under supervision, and using the VO_2 max test rather than the YMCA step test.

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APPENDIX A

IRB APPROVAL

Instructions and Notes:

- Depending on the nature of what you are doing, some sections may not be applicable to your research. If so mark as "NA".
- When you write a protocol, keep an electronic copy. You will need to modify this copy when making changes.

1 Protocol Title

Include the full protocol title: Nut Consumption during a Walking Intervention

2 IRB Review History

If you have submitted this protocol for review by an external IRB, provide the previous study identification number and provide details of the review including the IRB name, date of review, and IRB contact information.

NA

3 Background and Objectives

Provide the scientific or scholarly background for, rationale for, and significance of the research based on the existing literature and how will it add to existing knowledge.

- Describe the purpose, specific aims, or objectives.
- State the hypotheses to be tested.
- Describe the relevant prior experience and gaps in current knowledge.
- Describe any relevant preliminary data.

Background: Cardiovascular disease (CVD) remains the number one cause of death worldwide for both men and women. In the United States alone, 610,000 people die of heart disease every year. CVD is one of the many conditions linked to obesity, which has become a large concern over recent decades. Poor diet and physical inactivity are two risk factors for heart disease. There is a dire need for a health intervention strategy that is simple to follow. Walking is one of the easiest forms of exercises for Americans to implement in their daily lives. Going for a brisk walk for 30 minutes per day can qualify a person to meet the physical activity guidelines,[1] and much evidence has demonstrated the benefits of physical activity to reduce risk for CVD. Almond consumption is also linked with reduced risk for CVD, perhaps because almond ingestion is linked to improved athletic performance and endurance, [2] weight loss,[3] and improved lipid profiles.[3,4] Given that these factors independently reduce the risk for CVD, it would be valuable to study whether the addition of almonds to the diet while following a walking intervention would improve CVD risk by augmenting exercise-induced improvements in blood vessel function and fitness level. There is no research that specifically studied the consumption of almonds during exercise interventions, but it is important to see if the combination of the two interventions results in greater benefits than exercise alone.

This study will measure flow mediated dilation (FMD) to assess vascular health and heart rate recovery (HRR) following exercise to assess physical fitness. FMD is a major indicator of cardiovascular health,[5] and FMD improves with physical activity.[6] HRR has been shown to predict risk for CVD, as well as mortality.[7] HRR, the decrease in heart rate immediately following exercise, is usually measured as HRR 60 seconds post-exercise and HRR 120 seconds post-exercise.

Objectives: The objective of this study is to examine the combined impact of daily almond consumption (2.5 ounces) with a walking program on FMD and HRR as compared to the control intervention (3 tablespoons cookie butter) in sedentary ~~middle-aged men~~adults.

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4 Inclusion and Exclusion Criteria

APPENDIX B

COMPLIANCE CALENDAR

ASU Walking Intervention ID#

Please record your step count each day of the trial. **When you receive your nut product (study weeks 6-8) please check each day that these foods are consumed.** Your scheduled study visits are stated below.

January

Sun	Mon	Tue	Wed	Thu	Fri	Sat
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24/31	25	26	27	28	29	30

February

Sun	Mon	Tue	Wed	Thu	Fri	Sat
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29					

March

Sun	Mon	Tue	Wed	Thu	Fri	Sat
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

April

Sun	Mon	Tue	Wed	Thu	Fri	Sat
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

Study visit #2: ________
Study visit #3: ________
Study Visit #4: ________

Please fast overnight and avoid heavy exercise the day prior to testing

INSTRUCTIONS

1. Please post this calendar on your refrigerator at home and record daily step counts during the trial.
2. There are 3 visits to the nutrition labs for testing (these are marked on the calendar). On the day prior to testing, you will need to restrict exercise and fast overnight (no food or beverage with the exception of water for 10 hours. Please do not consume any dietary supplements or vitamins the 3 days prior to testing.
3. Please email Liz (emcelane@asu.edu) if you have questions/concerns.
4. Professor Carol Johnston's phone number is 602-827-2265 if you need to contact her.

APPENDIX C

HEALTH HISTORY QUESTIONNAIRE

Health History Questionnaire

ID#______________

Age:________

Gender (please circle): Female Male

Smoked cigarettes in the past year?

(please circle): Yes No

To be completed by investigator	
	Height:______ft. ______in.
	Weight:__________lbs.
	Waist:________in.

1. Are you taking any medications regularly? (including aspirin, steroids, thyroid meds, etc.) Y N

   If yes, what medications and how often?

2. Do you currently take supplements? (vitamins, minerals, herbs, etc.) Y N

   If yes, what supplements and how often?

3. Has a doctor ever told you that you have any of the following conditions?

Heart disease?	Y	N	Thyroid problems?	Y	N
Kidney disease?	Y	N	Cancer?	Y	N
Liver disease?	Y	N	High blood pressure?	Y	N
Type 2 Diabetes?	Y	N			

Gluten, nut, or other food allergy? Y N

(if yes, what foods?)____

If you have other chronic conditions, please list: ____

4. Are you willing to participate in an 8-week long walking program and record your step count each day during the study ? Y N

5. Are you willing to provide blood samples from an arm vein on 3 occasions during the study? Y N

6. Are you willing to travel to the ASU Downtown Phoenix campus on 4 separate occasions? Y N

Please turn over ⟶

APPENDIX D

CONSENT FORM

CONSENT FORM

Title of research study: Nut Consumption during a Walking Intervention

Investigators: Drs. Karen Sweazea and Carol Johnston, ASU Nutrition Professors

Why am I being invited to take part in a research study?

We invite you to take part in this research study because you are a healthy male or post-menopausal woman (20 - 69 years old), willing to participate in an 8-week walking program, and willing to eat nuts daily during the trial.

Why is this research being done?

The purpose of this research is to determine if daily nut consumption augments the cardiovascular benefits of a walking program in sedentary men.

How long will the research last?

We expect individuals to spend about 8 weeks participating in the proposed activities.

How many people will be studied?

We expect 50 men and women will participate in this research study.

What happens if I say yes, I want to be in this research?

You will be a participant in this study for 8-9 weeks. You will be asked to restrict nut consumption to 2 or less times weekly during the study (excluding the study products). Your participation includes four visits to the research laboratory: visit 1 – consenting, health history screening, and discussion of study instructions including diet recording and pedometer use; visits 2 – to measure blood pressure and blood vessel function, to collect information on body weight, to collect a blood sample, and to conduct a step test to measure fitness level; visit 3 – to repeat the measurements conducted at visit 2 and to dispense the nut products which you are to consume daily; and visit 4 - to perform final measurements (repeating the same measurements taken at visits 2 and 3). The length of each visit is about 45 minutes. At visit 1, you will be given a compliance calendar to record daily steps from the pedometer and to check off when the nut products are taken (trial weeks 6-8). You will be given a pedometer at visit 1 which you will wear each day for the entire trial. You will be coached to slowly increase step counts to 10,000 steps daily early in the trial. You will be asked to maintain this step count for the duration of the study.

For the blood sampling, you will need to fast overnight for 12 hours (no food or beverage with the exception of water). Approximately 2 tablespoons of blood will be collected by a trained phlebotomist at each of these visits. You will be asked to abstain from all dietary supplements for 3 days prior to testing (e.g., visits 2, 3, and 4); caffeine for 12 hours prior to testing; exercise for 12 hours prior to testing. At visit 3, you will be randomly assigned (by coin toss) to receive one of two nut products: almonds (2.5 ounces equaling 400 calories) or nut butter (3 tablespoons equaling 360 calories). These products are to be consumed as a mid-morning snack each day for study weeks 6-8. At study visits 2, 3, and 4 you will complete a step test (stepping up and down on a platform for 3 minutes) to evaluate your fitness level. You will also have a blood pressure cuff inflated on your lower arm, which will be left for 5 minutes then released. Using an ultrasound machine we will examine your blood vessel function. During the research process, you will interact with the research team, consisting of the investigators, a registered nurse, and a sonographer. The contact information of the investigators will be provided to you. All measurements, as well as the processing of the blood sample, will be done at the Arizona Biomedical Collaborative laboratory (ABC) on the ASU downtown campus in Phoenix. The research trial is expected to last from January to April 2016.

APPENDIX E

YMCA STEP TEST PROTOCOL

YMCA Bench Step Test for Cardiovascular Fitness

Testing for cardiovascular fitness can be costly, time consuming, and also require elaborate equipment. Luckily there is an easy Do-It-Yourself assessment that can easily be completed at home.

The YMCA 3-minute Bench Step Test is based on how quickly your heart rate recovers following a short bout of exercise.

Below are the essentials to perform the test on your own:

- 12-inch tall step, bench, or box (as close to 12 inches as you can find)
- Stopwatch, timer, or clock with a secondhand
- Metronome (free – www.metronomeonline.com)
- Heart rate monitor (optional)
- Partner to assist with cadence and form (optional)

Procedures:

1. Set the metronome to 96 beats per minute and turn the volume up loud enough that you can hear each beat.
2. Stand facing your step.
3. When ready to begin start the stopwatch or timer and begin stepping on and off the step to the metronome beat following a cadence of up, up, down, down.
4. Continue for 3 minutes.
5. As soon as you reach 3 minutes, stop immediately and sit down on your step.
6. Perform a manual pulse reading and count the number of beats for an entire 60 seconds - http://www.webmd.com/heart/taking-a-pulse-heart-rate - If wearing a heart rate monitor record your heart rate 1 minute from when you sit down.
7. Record your pulse when you have reached 1 minute and then locate your score on the rating scale below.

Results: Age-adjusted standards based on guidelines published by YMCA.

Ratings for Women, Based on Age

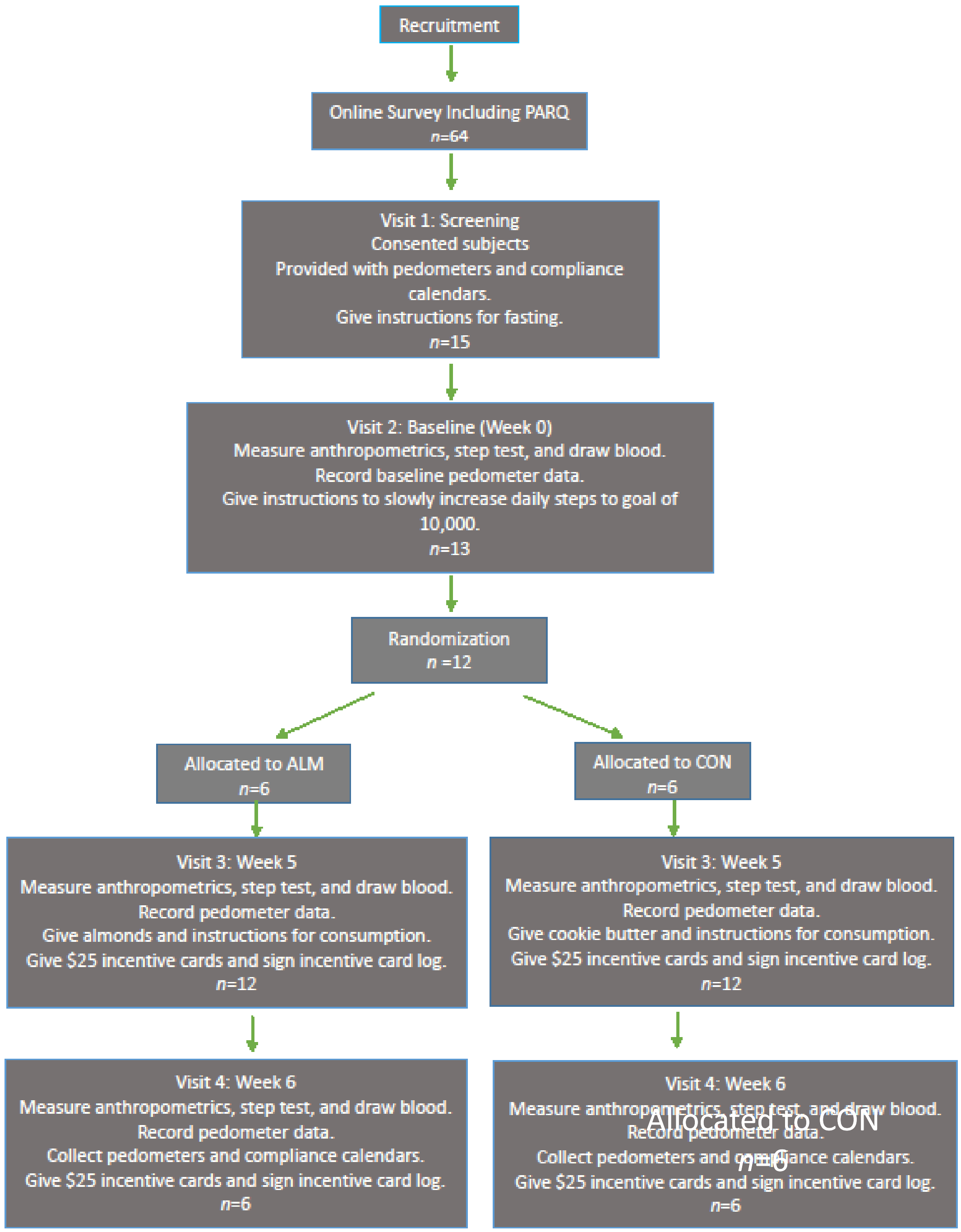
	18-25	26-35	36-45	46-55	56-65	65+
Excellent	52-81	58-80	51-84	63-91	60-92	70-92
Good	85-93	85-92	89-96	95-101	97-103	96-101
Above Average	96-102	95-101	100-104	104-110	106-111	104-111
Average	104-110	104-110	107-112	113-118	113-118	116-121
Below Average	113-120	113-119	115-120	120-124	119-127	123-126
Poor	122-131	122-129	124-132	126-132	129-135	128-133
Very Poor	135-169	134-171	137-169	137-171	141-174	135-155

Ratings for Men, Based on Age

	18-25	26-35	36-45	46-55	56-65	65+
Excellent	50-76	51-76	49-76	56-82	60-77	59-81
Good	79-84	79-85	80-88	87-93	86-94	87-92
Above Average	88-93	88-94	92-88	95-101	97-100	94-102
Average	95-100	96-102	100-105	103-111	103-109	104-110
Below Average	102-107	104-110	108-113	113-119	111-117	114-118
Poor	111-119	114-121	116-124	121-126	119-128	121-126
Very Poor	124-157	126-161	130-163	131-159	131-154	130-151

APPENDIX F

Study Design Flow Chart


Recruitment
Online Survey Including PARQ
n=64
Visit 1: Screening
Consented subjects
Provided with pedometers and compliance calendars.
Give instructions for fasting.
n=15
Visit 2: Baseline (Week 0)
Measure anthropometrics, step test, and draw blood.
Record baseline pedometer data.
Give instructions to slowly increase daily steps to goal of 10,000.
n=13
Randomization
n =12
Allocated to ALM
n=6
Allocated to CON
n=6
Visit 3: Week 5
Measure anthropometrics, step test, and draw blood.
Record pedometer data.
Give almonds and instructions for consumption.
Give $25 incentive cards and sign incentive card log.
n=12
Visit 3: Week 5
Measure anthropometrics, step test, and draw blood.
Record pedometer data.
Give cookie butter and instructions for consumption.
Give $25 incentive cards and sign incentive card log.
n=12
Visit 4: Week 6
Measure anthropometrics, step test, and draw blood.
Record pedometer data.
Collect pedometers and compliance calendars.
Give $25 incentive cards and sign incentive card log.
n=6
Visit 4: Week 6
Measure anthropometrics, step test, and draw blood.
Record pedometer data.
Collect pedometers and compliance calendars.
Give $25 incentive cards and sign incentive card log.
n=6
Allocated to CON
n=6