Pilot Study: The Synergistic Effect of Almond Consumption and Aerobic Activity on the Reduction of Cardiovascular Disease Risk in Sedentary Adults

by

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ABSTRACT

Cardiovascular disease is the leading cause of death in the world, responsible for 17.3 million deaths annually. Aerobic activity and almond ingestion have a cardio-protective effect against cardiovascular disease, however, the synergistic effect of both interventions is not known. This 8-week randomized, parallel, two-arm study examined the combined effect of daily almond ingestion (2.5 ounces) and brisk walking (10,000 steps per day) compared to ingestion of an isocaloric placebo (4 Tbsp cookie butter) and brisk walking (10,000 steps per day) in sedentary adults on various markers of cardiovascular health. The additive effect of the daily walking intervention with almond consumption resulted in significant differences in total cholesterol with a -11.0 ± 10.5 and +3.3 ± 15.8 mg/dL (p=0.043) change in the ALM and CON group respectively and LDL with a -11.5 ± 7.5 and +0.5 ± 13.7 mg/dL (p=0.025) change in the ALM and CON group respectively. There was a trend for TBARS to decrease in the ALM group versus the CON group (-0.2 ± 0.8 and +0.3 ± 0.6 nmol MDA/mL (p=0.099) respectively) with a large effect size of 0.304 but this did not reach statistical significance. There were no significant differences seen in markers of other plasma lipid profile measures, plasma inflammatory cytokines, or blood pressure regulation. Results suggest that the simple, cost-effective, and accessible intervention of daily brisk walking and almond consumption is an effective strategy to reduce cardiovascular disease risk in sedentary adults through improvements in cholesterol. This represents a pilot study due to the small sample size, therefore, additional studies are needed to determine the impact and mechanisms of this synergistic effect.

DEDICATION

Foremost, I dedicate this humble research work to God, the provider of everything, for bestowing the power of intellectual investigation and scientific acquisition.

I further dedicate this to my parents, Bill and Beth Schwab, for their unconditional love, support, and encouragement in all of my life adventures, to my siblings and family in the Abhá Kingdom, and to my Aunt Coral who ignited my passion for alternative medicine and nutrition at a formative age and set the stage for me in my career.

And ultimately, as a world citizen, I dedicate this to all of humanity as we advance together in the science of medicine.

"At whatever time highly-skilled physicians shall have developed the healing of illnesses by means of foods, and shall make provision for simple foods, and shall prohibit humankind from living as slaves to their lustful appetites, it is certain that the incidence of chronic and diversified illnesses will abate, and the general health of all mankind will be much improved. This is destined to come about."

Abdu'l-Bahá

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CHAPTER 1

INTRODUCTION

Overview

Cardiovascular disease (CVD) continues to remain the leading cause of death worldwide and within the United States.[1,2] Globally at present day, CVD accounts for 17.3 million deaths annually and this amount is anticipated to rise to greater than 23.6 million by 2030.[1] From 2010 to 2030 in the United States alone, total direct healthcare costs are estimated to triple and real indirect costs are projected to increase by 61 percent. Between the astronomical mortality rates and healthcare costs, it is apparent that this represents a significant public health issue. Effective prevention strategies are necessary to curb the mounting burden of CVD.[3]

Lifestyle modifications including physical activity and a cardio-protective diet represent the foundation for CVD prevention and treatment for all individuals even those on drug therapy.[4] Substantial evidence from epidemiological studies show an inverse relationship between physical activity and CVD risk with greater sedentary time associated with a 147% and 90% increase risk in CVD and cardiovascular mortality respectively.[5-9] Literature reveals even simply walking has a negative and dose-dependent association with CVD and all-cause mortality with increased distance, duration, pace, and energy expenditure.[10] The protective effect of aerobic activity against CVD stems from exercise enhancing vasodilation via heightened bioavailability of nitric oxide (NO).[11] Of various cardio-protective dietary factors, nuts have gained significant attention because they are rich in unsaturated fatty acids, vegetable proteins, minerals, vitamins, antioxidants, dietary fiber, and phytochemicals.[12] Large epidemiological studies have

consistently illustrated a causal association between nut ingestion and CVD rates and mortality across age, sex, various localities, and professions.[13-16] Furthermore, randomized control trials have shown that nuts have beneficial effects on numerous mediators of CVD including blood pressure, lipid profiles, various inflammatory, oxidative stress, and endothelial biomarkers.[17-21] Almonds in particular have been shown to dose-dependently reduce total cholesterol and low-density lipoprotein cholesterol (LDL) and lower cardiometabolic risk factors such as inflammatory markers, insulin resistance and secretion, and fasting and postprandial glucose insulin resistance.[22-31] The cardio-protective effect of almonds is attributable to their unique nutrient composition containing high levels of L-arginine, a precursor for NO which plays a chief role in endothelial function and CVD risk.[32-33]

To my knowledge, of the studies that have examined physical activity and almond consumption in relation to cardiovascular health, none have investigated the combined effects of each intervention on endothelium-dependent vasodilation in otherwise healthy sedentary adults. Due to the colossal mortality rates and healthcare costs from CVD and the lack of research combining exercise and diet interventions, the question of whether daily brisk walking coupled with almond consumption has an additive effect in reducing CVD risk therefore is greatly necessitated. If such a simple cost-effective diet and accessible exercise strategy is shown effective, the manageable incorporation of almonds into the daily diet may benefit a substantial portion of the population, which would otherwise be at increased risk of CVD.

Purpose of Study

The objective of this randomized parallel two-arm study was to examine the combined effects of daily almond ingestion (2.5 ounces) and brisk walking (10,000 steps per day) compared to ingestion of an isocaloric placebo (4 Tbsp of Speculoos Cookie Butter) and brisk walking (10,000 steps per day) for 8 weeks in otherwise healthy sedentary adults on various markers of cardiovascular health. It was anticipated that improvements would be observed in all cardiovascular health measures (decreases in blood pressure, C-reactive protein (CRP), oxidative stress, and lipids as well as increases in flow-mediated dilation (FMD) and total NO production).

Research Aim & Hypothesis

- Primary Aim:

  To examine the change in blood pressure regulation in otherwise healthy sedentary adults before and after daily almond consumption while on a walking program.

- Primary hypothesis:

  Daily almond ingestion along with brisk walking will act synergistically to improve blood pressure regulation as measured by FMD, total NO production, and blood pressure in otherwise healthy sedentary adults in comparison to brisk walking alone.

- Secondary Aim:

To examine the change in cardiovascular disease risk in otherwise healthy sedentary adults before and after daily almond consumption while on a walking program.

- Secondary hypothesis:

  Daily almond ingestion along with brisk walking will act synergistically to decrease CVD risk in otherwise healthy sedentary adults via an improvement in plasma lipid profile, plasma inflammatory cytokines, and oxidative stress biomarkers in comparison to brisk walking alone.

Definition of Terms

- **Atherosclerosis:** a condition where arteries build up with plaque resulting in the hardening and narrowing of arteries that in turn limits blood flow[34]
- **Vasodilation:** the widening of blood vessels
- **NO**: a soluble gas made from L-arginine in endothelial cells that aids in maintaining vascular equilibrium including dilator tone[35]
- **L-arginine:** an amino acid which is a precursor for NO so therefore is necessary in the conservation of proper arterial function[36]
- **FMD:** non-invasive technique assessing endothelial function via the dilation of the brachial artery in response to shear stress[37]
- **Cytokines:** proteins secreted by cells in the immune system that interact with other cells[38]
- **Oxidative stress:** an imbalance between reactive oxygen species creation and the ability for antioxidants to scavenge free radicals[39]

- **Nuts:** refer to the nine common tree nuts (i.e. almonds, Brazil nuts, cashews, hazel nuts, macadamias, pecans, pine nuts, pistachios, and walnuts) plus peanuts[40]
- **Phytochemicals:** naturally occurring bioactive compounds that play a protective role against chronic diseases[41]
- **Brisk Walking:** 40-60% of maximum capacity comparable to a 15 to 20-minute mile[42]

Limitations & Delimitations

Limitations:

Limitations of this study include use of a 3-day food record at the beginning and end of the study to assess estimated energy intakes, incomplete records of participant compliance with the daily study food ingestion (see Appendix A), uncertain compliance with walking intensity, a small pilot study sample size of 12 participants, a total study duration of 8 weeks which is a relatively short interval to influence some biomarkers of CVD risk, and inability to measure the effects of almonds on all inflammatory, oxidative, and endothelial biomarkers. There is also a potential for selection bias as the population for this study was self-selected. Additionally, antiseptic mouthwash was not controlled for and while participants met the inclusion criteria for being sedentary (sit > 8 hours a day), many were still physically active.

Delimitations:

Participants were restricted to only otherwise healthy sedentary (sit > 8 hours a day) men and postmenopausal women, aged 20-69 years of age. Therefore,

generalizability of study results is limited to individuals who similarly meet the aforementioned criteria and cannot be generalized to pre-menopausal women and non-sedentary individuals. Only women who were post-menopausal were included due to heightened CVD risk and to minimize confounding effects of physiological hormonal fluctuations.[43,44]

CHAPTER 2

REVIEW OF LITERATURE

Cardiovascular Disease

Definition

CVD refers to several heart and blood vessel conditions including coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis and pulmonary embolism.[45] Table 1 further describes the various CVD disorders.

Table 1. CVD Disorders

Types of CVD	Definition
Coronary Heart Disease	Disorder of blood vessels supplying the heart
Cerebrovascular Disease	Disorder of blood vessels supplying the brain
Peripheral Arterial Disease	Disorder of blood vessels supplying the extremities
Rheumatic Heart Disease	Cardiac muscle and value damage due to rheumatic fever
Congenital Heart Disease	Heart structure abnormalities from birth
Deep Vein Thrombosis & Pulmonary Embolism	Blood clots in veins of the legs (upon dislodgement can travel to lungs and heart)

Mortality & Costs

CVD is the leading cause of death in the world, responsible for 17.3 million deaths annually, as well as in the United States, accounting for approximately 1 in every 3 deaths or 787,000 deaths in 2011.[1] Along with mortality, CVD can inflict long-term disability on individuals due to complications of strokes, heart attacks, and heart failure.[46] Therefore, overall costs related to CVD are astronomical at an estimated $320.1 billion in the United States in 2011.[47] From this, $195.6 billion are related to direct costs such as physicians, healthcare services, and medication and $124.5 billion are related to indirect costs such as lost future productivity from premature mortality.[47] This constitutes 15% of

total health expenditures in 2011 which is more than any other major diagnostic group.[47] The projections are not promising as the American Heart Association anticipates that by 2030, 43.9% of the United States population will suffer from CVD with direct costs expected to increase to $918 billion and indirect costs expected to increase to $290 billion.[47] However, due to the numerous modifiable risk factors, over 200,000 annual deaths from heart disease and stroke are considered preventable in the United States.[48]

Risk Factors

Non-modifiable risk factors for CVD include age, sex, race, and family history.[49] Risk increases with age with approximately 80% of deaths due to CVD occurring in individuals ≥ 65 years of age.[50] For women, age becomes a risk factor at 55 years due to the fact that after menopause CVD risk increases because of a decrease in estrogen production.[51] Men have a greater risk of CVD than pre-menopausal women, however, once menopause has occurred, a woman's risk is similar to a man's.[49] Individuals with Asian or African ethnic descent, have increased risk for CVD than other racial groups.[49] This may be due to the fact that African American adults have among the greatest prevalence of hypertension globally and South Asians have a greater prevalence of diabetes compared to other populations.[47,52] Risk also increases if a first-degree blood relative has had a stroke or coronary heart disease before age 55, for a male relative, or age 65, for a female relative.[49]

Modifiable risk factors include hypertension, hypercholesterolemia, tobacco use (smoking or chewing), diabetes, obesity, excessive alcohol consumption, poor diet, physical inactivity, among others.[49] Hypertension represents the greatest risk factor for

stroke.[49] Living with diabetes makes one twice as likely to develop CVD than a non-diabetic, a high saturated fat diet is approximated to cause roughly 11% of stroke and 31% of coronary heart disease globally, and physical inactivity raises heart disease and stroke risk by 50%.[49] Other modifiable risk factors that increase CVD risk include low socio-economic status, chronic stress, anxiety and depression, social isolation, and specific treatments such as contraceptive pills and hormone replacement therapy.[49]

Pathophysiology

Atherosclerosis

Cardiovascular risk factors mentioned above increase the risk for atherosclerosis by damaging the vascular endothelium. Atherosclerosis, a condition of chronic inflammation of arteries, represents the chief cause of CVD.[53] The process of atherosclerosis includes 4 major sequential events that play a role in CVD: activation of the endothelium and inflammation; accumulation, retention, and alteration of lipoproteins and emergence of foam cells; development of complex plaques, augmentation of the necrotic core, fibrosis, thrombosis, and remodeling; and inducement of life-threatening events.[54]

Activation of the atherosclerotic process largely occurs when cardiovascular risk factors (i.e. hypertension, hypercholesterolemia, smoking, and diabetes) initiate and/or injure the endothelial cells lining blood vessels through mechanical, chemical, or immunological insult.[55] An inflammatory response cascade is then set in motion.[55] Endothelial cells start to produce cell surface adhesion molecules including but not limited to vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant

protein-1 (MCP-1) which attract monocytes, T-lymphocytes, neutrophils, and various other immune cells to attach to the endothelium and migrate into the sub-endothelial space where monocytes differentiate into macrophages.[53,54] As tight junctions become loose, lipoproteins, especially LDL, also move into the arterial wall where exposure to macrophage, endothelial, and smooth muscle cell oxidants cause oxidation.[53,56] Vascular smooth muscle cells (VSMC) and macrophages take up increasing amounts of oxidized lipoproteins which are then referred to as foam cells.[54] As VSMC, lipids, and other migrating cells accumulate in the sub-endothelial space an expanding lesion is created known as an atherosclerotic plaque that obtrudes the arterial lumen.[54] As the plaque increases in size, a fibrous cap is formed to cover it that acts as a protective barrier between prothrombotic plaque matter and platelets.[53,57] Apoptosis combined with phagocytic removal can curb plaque growth in inceptive lesions, however, this equal coupling does not occur in advanced lesions.[58] Buildup of necrotic cells stimulates the release of additional inflammatory cytokines such as IFN-γ causing inhibition of VSMC collagen development and matrix-dissolving enzymes such as matrix metalloproteases (MMPs) causing break down of collagen fibrils.[54,59] This leads to increased fragility of the plaque and fibrous cap and therefore greater susceptibility to plaque rupture.[54,59] Plaque rupture can result in thrombosis via the introduction of blood to tissue factors initiating coagulation and mobilizing platelets and subsequent tissue infarction thus ensuing CVD.[54,59]

Impaired NO Bioavailability

A central contributing factor to CVD is the impairment of NO bioavailability.

Many of the CVD risk factors mentioned above simulate processes that ultimately lead to this distinct manifestation of endothelial dysfunction.[60,61] Various factors contribute to reduced NO bioavailability including endothelial NO synthase (eNOS) uncoupling, oxidative stress, decreased eNOS activity and expression, arginase upregulation, and eNOS inhibitors such as asymmetric dimethylarginine (ADMA).[60-63]

NO is generated in numerous different cell types by NO synthase (NOS).[60] The three isoforms of NOS include neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) all of which use a number of cofactors along with L-arginine and oxygen in an electron transfer reaction to produce NO and L-citrulline.[60,64] For example, in a healthy blood vessel in response to shear stress and other stimuli, eNOS and cofactors such as reduced tetrahydrobiopterin (BH4) are activated causing the generation of NO and L-citrulline from L-arginine.[60] Then NO diffuses to the smooth muscle cells, binds to the reduced haem (Fe^{2+}) of soluble guanylyl cyclase (sGC) producing cyclic guanosine monophosphate (cGMP) which gives rise to vasodilation.[60] In an example of endothelial dysfunction, BH4 becomes oxidized to dihyrobiopterin (BH2) causing eNOS uncoupling.[60] NADPH electrons uncouple from L-arginine oxidation producing superoxide ($O_2^{\bullet-}$) instead of NO.[60,65,66] States of inflammation and oxidative stress activate oxidase enzymes which can react with NO to create peroxynitrite ($ONOO^-$) additionally curbing NO bioavailability.[60,66] Furthermore, the reactive oxygen and nitrogen species can oxide the haem in sGC desensitizing it to NO activation.[60] Inflammation also leads to upregulation of arginase which shares the same L-arginine substrate as NOS.[60] Therefore, substrate competition decreases L-arginine levels required for de novo NO synthesis and engenders a reduction in NOS activity.[60]

Decreased NO bioavailability can play a part in all stages of atherosclerosis progression.[61] In the beginning of this disease process, diminished NO can make the endothelium susceptible to heightened leukocyte diapedesis, increasing the likelihood of LDL oxidation in the sub-endothelium, allowing for uncurbed neointimal hyperplasia.[61] In the later stages, exacerbated platelet activation from reduced NO can result in thrombosis and stroke.[61] NO is a vital regulator of the vasculature and reduced bioavailability is involved in atherosclerotic vascular disease initiation, advancement, and future cardiovascular events.[67]

Biomarkers of CVD

A biomarker is regarded as a measurement of a pathological, physiological, or therapeutic response.[68,69] Cardiology biomarker investigation has mainly focused on circulating and imaging markers.[68] A plethora of biomarkers have been identified in relation to CVD but only the biomarkers pertaining to the pilot study will be addressed here as it is out of the scope of this thesis to include all established markers.

Circulating

LDL: Lipids are associated with a sound predictive potential for initial CVD events.[70] There is a well-established relationship between plasma LDL and total cholesterol and CVD risk as LDL cholesterol has a crucial role in the emergence and clinical indication of atherosclerosis.[71,72] Multiple clinical trials illustrate LDL reduction produces a dose-dependent decrease in CVD events.[68] Current CVD risk assessments and guidelines include LDL and/or total cholesterol as the foundation.[68,73] With the well-founded evidence and current practices, LDL, therefore, is an essential aspect of CVD risk

assessments in addition to pharmacological therapy targets.[68]

High-density lipoprotein (HDL): HDL is a lipoprotein that carries LDL cholesterol from the arteries back to the liver which breaks it down and then it is excreted from the body.[74] Substantial evidence illustrates the inverse and independent association of low HDL concentrations with increased CVD risk.[72,75,76] This is due to many cardioprotective mechanisms of HDL including endothelial dysfunction improvement, activation of macrophage cholesterol efflux, and anti-inflammatory, anti-apoptotic, and antioxidant properties.[77] Macrophage cholesterol efflux where excess cholesterol is removed from macrophages and returned to the liver as part of reverse liver transport is the most established property related to the cardioprotective effect of HDL.[77] As such a strong risk factor, HDL is routinely used in CVD risk estimations.[76,78]

Triglycerides: There is a long-established relationship between high triglyceride levels and CVD risk.[79] Triglycerides play a vital part in lipid metabolism. Although they are not directly atherogenic, they have a recognized association with atherogenic remnants and apolipoprotein C-III thus making them a valuable biomarker of CVD.[79]

CRP: CRP is an acute phase reactant and marker of inflammation mainly produced by the liver but also generated by vascular smooth muscle cells.[69,80] It represents one of the most well-studied inflammatory markers with a strong association to CVD. A wealth of evidence exists substantiating the high-sensitivity CRP (hsCRP) assay in CVD event prediction as an independent risk marker.[80,81] A graded and dose-dependent relationship has been demonstrated with controlling for other risk factors in many prospective and

nested case control experiments.[81]

Malondialdehyde (MDA): MDA represents a lipid peroxidation end product that is commonly measured as thiobarbituric acid (TBA) reactive substances (TBARS), which is an indicator of oxidative stress in CVD models.[82,83] Human and animal trials have linked oxidative stress via the TBARS assay to CVD risk, progression, and therapy response prediction.[82-85]

NO: Total amounts of nitrate plus nitrite known as the NOx test are commonly used in both animal and human studies.[86] Nitrite has been shown to be an indicator of CVD risk with decreased nitrite amounts associated with increased CVD risk.[87] This is largely due to the role NO plays as a vasodilator, regulating tone and structure of vasculature, and an antioxidant in lipid oxidation.[87-89] NO also curbs adhesion and aggregation of leukocytes and platelets, inhibits proliferation and migration of VSMCs, regulates microthrombi formation and vascular permeability, and exerts anti-inflammatory properties.[87,89] Because of this, reduced NO bioavailability promotes conditions related to CVD including hypertension and atherosclerosis.[89]

Imaging

FMD: FMD has been shown in numerous studies to be a strong predictor of CVD events in subjects with CVD, subjects with a high CVD risk, and in asymptomatic individuals.[90] FMD represents a direct evaluation of arterial function and therefore supplies important independent prognostic CVD data.[90]

Dietary Prevention

Dietary patterns

There have been several cardioprotective diets identified including vegetarian and Japanese diet patterns.[12] The dietary patterns that have been more thoroughly investigated, however, are the low-fat diet, the low-carbohydrate diet, the Mediterranean diet, and the Dietary Approach to Stop Hypertension (DASH) diet.[12,91]

A common clinical standard for CVD prevention is a consumption of a diet reduced in fat.[91] A low-fat diet is defined as total fat ingestion of 25-35% of total calories with a maximum for saturated and trans fat being 7-10% and less than 1% respectively.[91] It has been shown to significantly reduce CVD risk and may improve life span and quality.[91,92]

A low-carbohydrate diet is defined as 30-130 grams of carbohydrates daily.[91] In a recent systematic review and meta-analysis, a low-carbohydrate diet was demonstrated to have advantageous outcomes on key CVD risk factors with significant decreases in systolic and diastolic blood pressure, body weight, body mass index (BMI), triglycerides and a significant increase in HDL cholesterol.[91] A Mediterranean diet is distinguished by foods rich in fish and plant-based omega-3 fatty acids.[91] This dietary pattern has been linked with a low coronary heart disease risk in numerous studies and has been shown to lessen CVD event occurrence and is superior to a low-fat diet in decreasing triglycerides, increasing HDL, and enhancing insulin sensitivity.[91-93] The DASH diet emphasizes vegetables, fruits, whole grains, low-fat dairy, and lean protein sources while limiting saturated fat, sweets, and fatty meats.[91] It has been shown to lower systolic and diastolic blood pressure and therefore is recommended to prevent hypertension, a major CVD risk

factor.[91]

Individual food items

Specific foods in relation to CVD have been studied. For example, whole grain consumption lowers total and LDL cholesterol and fiber intake reduces LDL as well.[91,94,95] Fruit and vegetable consumption is associated with coronary heart disease risk reduction and lower blood pressure.[91,93,96-101] Other foods that have shown beneficial effects on CVD risk include nuts, low-fat milk and dairy products, green tea, chocolate, garlic, and red wine.[91,102,103] Because this work focuses on nuts, this particular food item will solely be expanded on in relation to CVD.

Nuts are nutrient dense foods because they are rich in protein, fiber, vitamins such as niacin, vitamin B-6, folic acid, and tocopherols, minerals such as potassium, calcium, and magnesium, phenolic compounds, phytosterols, and L-arginine.[91,104] Nuts have a distinct fatty acid profile being low in saturated fats (4-16%) and high in monounsaturated fats with a moderate amount of polyunsaturated fats such as omega-3.[104] This unique composition of fatty acids and nutrients account for many of their reported health benefits.[104]

Epidemiological studies have consistently demonstrated an inverse association between nut ingestion and fatal coronary heart disease (CHD) in men and women.[105,106] The major prospective studies include the Adventist Health Study, the Iowa Women's Study, the Nurses' Health Study, and the Physicians' Health Study which together show a 37% average lower risk of CHD death.[106] Each of these studies demonstrated a dose-dependent association between nut consumption and decreased risk of CHD.[106] The

Adventist Health Study additionally illustrated a significant decrease in nonfatal stroke risk whereas the Nurses' Health Study and Physicians' Health Study did not.[15] Nut intake has been shown by meta-analyses not only to be inversely associated with CHD incidence but also with all-cause mortality and CVD mortality.[18,107] One serving of nuts daily reduces all-cause mortality risk by 27% and a daily nut intake of 10 grams or more reduces CVD mortality by 17% compared to non-nut consumers.[104,108,109] Prospective studies have furthermore shown increased nut consumption decreases hypertension risk by 13%, 15%, and 18%.[105] Increased nut and seed consumption also has an inverse association with inflammatory markers such as CRP, fibrinogen, and interleukin-6.[19]

Randomized control trials exploring nut-enhanced diets versus control diets for 3 to 8 week durations clearly displayed nut ingestion ameliorates lipid levels in a dose-dependent fashion, especially among individuals with elevated LDL, lower BMI, or who adhere to a Western diet.[18] A pooled examination of 25 intervention studies with an average daily nut consumption of 67 grams showed mean total cholesterol reduction of 10.9 mg/dL (5.1% change), mean LDL reduction of 10.2 mg/dL (7.4% change), mean LDL to HDL ratio reduction of 0.22 (8.3% change), mean total cholesterol to HDL ratio reduction of 0.24 (5.6% change), and mean triglyceride reduction of 20.6 mg/dL in participants with triglycerides of 150 mg/dL or more.[91,18]

Nutritional supplements

Numerous supplements have been studied in regards to CVD but conclusive evidence has yet to be shown for the majority of them. Investigated supplements include the following: omega-3 and fish oil, phytosterols, antioxidants, vitamin D, magnesium,

homocysteine-reducing agents, Coenzyme Q10, and L-arginine.[91]

Almonds

Nutrient Composition

Almonds are a nutrient-rich food and an excellent source of manganese and vitamin E (i.e. containing > 20% of the US Daily Value), a good source of magnesium, riboflavin, copper, phosphorus, fiber, and protein (i.e. containing 10-20% of the US Daily Value) as well as being uniquely high in arginine.[110,111] In fact, almonds are deemed one of the richest alpha-tocopherol food sources.[112] Compared to other nuts, almonds have the highest amount of protein, fiber, riboflavin, niacin, vitamin E, and calcium.[112] Almonds have a high total lipid content ranging from 49-66% of weight within California-grown almonds and an unique fatty acid profile of approximately 13 grams of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), 1 gram of saturated fatty acids, and no cholesterol per one ounce serving.[110,111,113,114] The five predominant fatty acids in almonds are as follows in decreasing order: oleic acid (18:1), linoleic acid (18:2), palmitic (16:0), stearic (18:0), and palmitoleic (16:1) with the MUFA, oleic acid, making up 62-80% of total fatty acids.[113-115] Table 2 further delineates the nutrient composition and constitutes the most comprehensive cross-sectional data of almonds produced in California (see Appendix B for a full nutrient report).

Table 2. Nutrient Composition of Almonds Versus Cookie Butter

Nutrient	Unit	Almonds (2.5 oz)[a,b]	Cookie Butter (4 Tbsp)[a,c]
Macronutrients			
Water	g	3.13	-
Energy	kcal	410	360
Protein	g	14.99	4
Total fat	g	35.39	24
Carbohydrate	g	15.27	36
Dietary fiber	g	8.9	0
Total sugar	g	3.08	20
Minerals			
Calcium	mg	191	0
Iron	mg	2.63	0
Magnesium	mg	191	-
Phosphorous	mg	341	-
Potassium	mg	520	-
Sodium	mg	1	100
Zinc	mg	2.21	-
Copper	mg	0.73	-
Manganese	mg	1.545	-
Selenium	μg	3	-
Vitamins			
Thiamin	mg	0.145	-
Riboflavin	mg	0.807	-
Niacin	mg	2.564	-
Pantothenic	mg	0.335	-
Vitamin B6	mg	0.097	-
Total folate	μg	30	-
Vitamin A	IU	2.5	0
Vitamin E			
Total	mg	18.85	4
α-	mg	18.175	-
β-	mg	0.175	-
γ-	mg	0.45	-
δ-	mg	0.05	-
Lipids			
Saturated fats	g	2.695	4
	g	22.3625	-
18:1	g	22.18	-
	g	8.738	-
18:2	g	8.735	-
Phytosterols	mg	97.5	-
Amino acids			
Lysine	g	0.4025	-
Arginine	g	1.7475	-
Other			
Total			
	mg	1.75	-
Flavan-3-ols	mg	3.25	-
Flavanones	mg	0.5	-
Flavonols	mg	2.25	-
	mg	108.5	-
Total	mg	296.25	-

[a]Nutrient data obtained from USDA National Nutrient Database for Standard Reference, Release 28 (2015), slightly revised May 2016 [b]Nutrient data obtained from Chen et. al., 2006[110] [c]Nutrient data obtained from Food Processor Nutrition Analysis Software

Almonds additionally possess a variety of phytonutrients consisting of phenolic acids, phytosterols, and other polyphenolic compounds like flavonoids and proanthocyanidins (see Table 2).[110] Phytochemicals are bioactive plant compounds that have been shown to have anti-inflammatory, antioxidant, anti-thrombotic, antiviral, anti-microbial, anti-proliferative, chemopreventative (use of chemical agents either biological, synthetic, or natural for reversal, suppression, or inhibition of cancer), vasodilatory, and hypocholesterolemic properties.[116-119] Therefore, these compounds have been strongly associated with risk reductions of various chronic diseases including CVD where there has been an inverse association demonstrated with CHD and coronary mortality and an inverse correlation shown with total and LDL cholesterol levels and stroke incidence.[116] Thus far, almonds are the only food source found to provide flavanones and almonds, along with pistachios, are the only nuts that are known to have flavonols largely as isorhamnetin.[120]

As almonds are a natural product, the nutrient composition is anticipated to be variable, even within a single cultivar.[113] The nutrient make-up for almonds, particularly tocopherols, lipids, fatty acids, and phytonutrients, is primarily dependent on genotype as well as environment.[113] Factors such as geographical region of farming, cultivation methods, climate, soil composition, origin of water, irrigation, agronomic aids that differ between growing sites, harvest years, and kernel maturity all can influence the nutrient content.[113] Some phytonutrients are also affected by postharvest processing and storage of nuts.[117]

Energy Content

The United States Department of Agriculture (USDA) currently lists the energy value of almonds to be 164 kcal/28 g (1 oz serving).[114] This estimate is based on the Atwater factors which is a commonly used system to determine the energy content of foods.[121] However, sizable evidence indicates the Atwater factors may be a poor energy predictor for nuts and multiple studies confirm that Atwater factors whether general or specific lead to erroneous approximations of the metabolizable energy for almonds.[121,122] This is likely due to the digestibility coefficient, a main piece of the Atwater factors, of nuts differing from other foods.[121] A recent randomized, crossover controlled feeding study found the energy content of almonds to be 4.6 ± 0.8 kcal/g or 129 kcal/28 g serving.[121] A follow-up study confirmed these results.[118] Compared to the current Atwater factors applied to almonds of 6.0-6.1 kcal/g or 168-170 kcal/28 g serving, there is a large discrepancy present.[121] Current research shows that Atwater factors result in a 32% overestimation for energy content of almonds and, therefore, almonds actually have lower energy content than currently listed on Nutrition Facts labels.[121]

Bioaccessibility of Nutrients

Bioaccessibility refers to the relative portion of nutrients and phytochemicals "released" from a food matrix which are potentially accessible for absorption into the gastrointestinal tract.[123,124] The proportion of dietary components accessible for absorption is dependent on many factors including mastication and physical and chemical

food matrix properties (i.e. cell wall composition, structure, and strength, and inter-cell adhesion).[123,124]

Lipids, protein, & alpha-tocopherol

Lipids are enclosed within almond cell walls therefore making the number of cells on the tissue surface ruptured by mastication or mechanical methods the main factor in lipid bioaccessibility.[123] After mastication, largely only the initial layer of cells at the fractured area rupture, allowing the release of lipids.[125] Most almond cells stay intact thus inhibiting immediate bioaccessibility of intra-cellular lipids and nutrients in the early digestion phases.[126] This is illustrated by a recent study showing only 8.5% of intra-cellular lipids are released and that 89-92% of lipids remain within the tissue matrix and a second study showing similar results of approximately 10% of intra-cellular lipids being released after duodenal digestion.[126,127] Limited research has been done on protein and alpha-tocopherol bioaccessibility. A 2008 in vitro digestion study found that approximately 16.5% and 13.9% of vitamin E and protein were released following duodenal digestion, respectively.[127]

The documented mechanism of lipid encapsulation leading to severely limited bioaccessibility offers a reasonable explanation of the decreased metabolized energy content of almonds.[126] It also plausibly explains the decreased postprandial lipemic response of almonds and the sustained weight loss produced by an almond rich diet, both of which have advantageous implications for CVD risk.[126]

Skin phytochemicals

Phytochemicals are an emerging field of research and therefore bioaccessibility remains largely unknown.[117] The skins of almonds in particular contain large amounts of cell wall-bound phenolics as well as fiber.[128] The bioaccessibility of almond skin polyphenols over the various digestion phases, from the upper GI tract to the duodenum, has been demonstrated in multiple stimulated human digestion studies with a few human studies further substantiating acute bioavailability.[117,128,129] Table 3 delineates the amounts of phytochemicals released from almond skins within stimulated human digestion.[124]

A variety of factors have been well-documented to affect bioavailability and absorption rate of polyphenols including digestion enzymes, gut microbiota interaction, food matrix, and chemical structure.[128] A 2016 study suggests that the food matrix plays a significant role in both gastric and duodenal settings, specifically illustrating that the milk matrix significantly decreased phenolic acids and flavan-3-ols released from almond skins compared to water and homemade biscuits lowered bioavailability of flavonols.[128] The study also indicates that the innate fiber in almond skins may serve as a barrier for phytochemical bioaccessibility during digestion due to the complex carbohydrates within fiber directly influencing antioxidants.[128]

Table 3. Almond Skin Phenolic acid and Flavonoid Release During Digestion

Compound	NS G	NS G + D
Protocatechuic acid	34.4 (±5.6)	97.6 (±1.7)
p-Hydroxybenzoic acid	89.3 (±0.5)	93.3 (±0.5)
Catechin	89.8 (±0.6)	99.2 (±0.1)
Chlorogenic acid	85.1 (±0.2)	98.6 (±0.1)
Vanillic acid	68.7 (±2.4)	88.8 (±0.7)
Epicatechin	95.7 (±0.2)	97.6 (±0.2)
trans p-Coumaric acid	73.1 (±1.6)	86.2 (±1.9)
Eryodictiol-7-O-glucoside	95.3 (±0.3)	97.8 (±0.2)
Quercetin-3-O-rutinoside	25.1 (±4.7)	68.0 (±1.9)
Quercetin-3-O-galactoside	59.2 (±5.5)	79.1 (±2.6)
Quercetin-3-O-glucoside	86.2 (±1.2)	88.6 (±1.2)
Kaempferol-3-O-rutinoside	91.8 (±0.9)	98.9 (±0.1)
Naringenin-7-O-glucoside	76.6 (±0.8)	83.8 (±0.9)
Isorhamnetin-3-O-rutinoside	88.1 (±0.1)	93.2 (±0.2)
Kaempferol-3-O-glucoside	65.7 (±1.4)	67.4 (±2.2)
Isorhamnetin-3-O-glucoside	62.7 (±1.3)	66.8 (±1.7)
Eryodictiol	45.0 (±7.2)	96.3 (±0.5)
Quercetin	42.4 (±7.7)	53.5 (±4.2)
Naringenin	61.7 (±3.1)	74.9 (±1.4)
Kaempferol	28.4 (±4.4)	34.6 (±5.1)
Isorhamnetin	19.7 (±7.4)	25.1 (±7.0)

Values expressed as % released from initial amounts present in almond skins during stimulated human digestion. NS = natural skins; NS G = natural skins post in vitro gastric digestion; NS G + D = natural skins post in vitro gastric + duodenal digestion. Data obtained from Mandalari et. al., 2010[123]

Protective Role in CVD

Cholesterol reduction

The majority of studies conducted in people with hypercholesterolemia illustrate 1 to 4 ounces of daily almond ingestion results in significant decreases in total and LDL-C concentrations.[130] A common finding across studies shows a dose response where greater almond consumption is associated with larger cholesterol reductions.[130] Studies that have examined almond consumption in conjunction with a heart healthy diet substantiate the cholesterol-lowering effect in hypercholesterolemic individuals.[130]

This extensive research on the effects of almond ingestion on lipoprotein profiles has been translated into a U.S. Food and Drug administration health claim that almonds

can aid in the maintenance of normal cholesterol levels, especially in individuals with high cholesterol.[111] Appendix C reviews a summary of effects of almonds consumption on cholesterol from randomized controlled trials (RCTs) in more detail.

Antioxidation

As stated, almonds consist of a variety of antioxidants such as alpha-tocopherol and numerous phytonutrients.[111] Because of the collective action of antioxidants, almonds may enhance antioxidant defense capability through the action of scavenging radicals and/or up-regulating endogenous antioxidant systems.[111]

The majority of clinical trials substantiate that almond nutrients may safeguard vulnerable macromolecules against local and systematic oxidation in individuals with increased oxidative stress and enhance antioxidant defense.[111] Results have shown decreased fatty acid peroxidation and postprandial protein oxidative damage and increased serum glutathione peroxidase and superoxide dismutase action (see Appendix D).[111] It is important to note that such oxidative stress biomarker reductions could reflect amelioration of pathological conditions which can cause oxidant production.[111]

Anti-inflammation

The anti-inflammatory action of almonds has been illustrated by numerous studies remaining consistent with observational evidence that inflammation biomarkers are inversely associated with greater frequency of nut and seed ingestion.[111] A good deal of empirical evidence shows that almond interventions reduce biomarkers such as CRP, E-selectin, and interleukin-6.[111] The ability of almonds to modulate inflammation,

especially in individuals in a heightened inflammatory state are well-supported.[111] The specific underlying mechanisms of action for such effects calls for more investigation.[111]

Body weight control

Contrary to the energy density of almonds, the scientific body of evidence indicates almond consumption along with a healthy diet does not increase body weight and actually elicits a positive influence on body composition specifically in overweight and obese individuals.[130] Studies in normal weight individuals have shown daily almond ingestion improves satiety and may help to control cravings with no significant differences in total daily caloric intake, metabolic rate, or energy expenditure.[130]

Glucoregulation

Almonds have demonstrated neutral or favorable influence on postprandial plasma glucose and insulin responses in both healthy and hyperlipidemic subjects with normal control of blood glucose levels.[130] A few studies even illustrated almonds reduced glucose and insulin peaks both immediately and 2 hours postprandial compared to a meal with no almonds.[130] This emerging evidence suggest almonds may aid in lowering the risk of CVD as well as metabolic syndrome and Type 2 Diabetes via improved glucose regulation.[130]

Vascular function

There is currently very limited evidence on the effect of almonds on vascular function, in particular to FMD, and the few studies conducted show mixed results. Chen et al. demonstrated no advantageous effect on vascular reactivity following a chronic

National Cholesterol Education Program (NCEP) Step 1 diet with 85 g of almonds daily in patients with compromised endothelial function.[46] Choudhury et al. demonstrated 50 g of almonds daily enhanced FMD in healthy men compared to no almonds (3.6% vs. 2.9%).[131] Jamshed et al. showed 3 g/kg of almonds daily had a partial effect on vascular reactivity.[132] The differences in study design and subject characteristics may account for the inconsistent results. Table 5 summarizes the effect of almonds on vascular function.

Table 4. Effect of Almonds on Vascular Function

Reference	Subjects	Study Duration	Diet Intervention	Results
Chen et al., 2015[46]	27 F, 18 M, CAD patients	22 weeks	NCEP Step 1 diet absent nuts (CON) vs 85 g/day into the CON diet (ALM)	Vitamin E: 5.8% ↑ in ALM ($p \leq 0.05$) FMD, PAT, PWV: no change BP, CRP, TNF, E-selectin: no significant change VCAM-1: 5.3% ↓ trend ($p=0.064$)
Choudhury et al., 2014[131]	60 M, healthy middle-aged (20), healthy young (20), young with 2+ CV risk factors (20)	4 weeks	50 g almonds/day in 3 subject groups vs habitual diet (control)	Vitamin E: sig ↑ ($p < 0.05$) Plasma protein oxidation & nitrite: no change Diastolic BP: 6% ↓ in healthy middle-aged, 12% ↓ in healthy young, no change in young at risk and control Systolic BP: 6% ↓ in healthy middle-aged, 5% ↓ in healthy young, 4% ↓ in young at risk, no change in control FMD: significant ↑ in young groups and trend for ↑ in healthy middle-aged
Jamshed et al., 2014[132]	14 F/M Sprague-Dawley rats	4 weeks	3 studies in 3 groups of rats: 1) tyloxapol 2) a high-fat diet (HFD) 3) white-flour fructose (WFF). Group 3 in each study was fed 3 g/kg almonds/day	Vascular reactivity: partially restored isolated aortas, curbed HFD-induced endothelial dysfunction by decreasing inhibition of eNOS and encouraging release of NO

F = female, M = male, CAD = coronary artery disease, CV = cardiovascular, NCEP = National Cholesterol Education Program, FMD = flow-mediated dilation, PAT = peripheral arterial tonometry, PWV = pulse wave velocity, BP = blood pressure, CRP = C-reactive protein, TNF = tumor necrosis factor, VCAM = vascular cell adhesion molecules, ↓ = decrease, ↑ = increase

Mechanisms of Action

Fatty acid profile

The high unsaturated to saturated fatty acid ratio in almonds promotes a beneficial shift in the fatty acid profile of the diet when almond substitution occurs for high saturated fat foods.[111] A plausible mechanism for this effect is that unsaturated fats improve receptor-dependent LDL removal in the liver consequently decreasing LDL production.[133] Specific unsaturated fatty acid types may have slightly varied effects on the lipid profile. MUFA may be favorable to HDL increases while PUFA n-6 may be favorable to LDL decreases when isocalorically substituting carbohydrates.[133] Table 6 summarizes the varying lipid profile effects of fatty acids.[133]

Table 5. Lipid Profile Effect of Fatty Acid Classes

Fatty acids	LDL	HDL	Triglycerides
Saturated	↑	↑	- or ↑
Trans	↑	↓	↑
Cis monounsaturated	↓	- or ↑	↓
Polyunsaturated n-6	↓	- or ↓	↓
Polyunsaturated n-3	↑*	-	↓

↑ increase, ↓ decrease, - no change, *LDL composition altered to larger and less dense particles

Arginine content

Arginine plays a large role in lowering LDL by increasing bile acid output, cholesterol turnover, and steroid excretion and decreasing the pool of cholesterol.[134] It is also possible arginine increases glucagon which lowers cholesterol via a positive effect on the insulin to glucagon ratio.[134]

Phytosterol content

It has been well-documented that phytosterols lower LDL via reduced absorption and increased excretion of cholesterol by displacing cholesterol from micelles in the intestines and therefore minimizing the supply of absorbable cholesterol.[134] Other theorized mechanisms for the hypocholesterolemic action includes phytosterol interaction with intracellular enzymes allowing for heightened cholesterol transportation from plasma cell membranes to the endoplasmic reticulum for chylomicron packaging and decreased movement of very low density lipoproteins and chylomicron representative apolipoproteins.[134]

Fiber content

Viscous fiber in the diet has been clearly illustrated to have cholesterol-lowering mechanisms in which it augments excretion of cholesterol and bile acid and upregulates LDL receptors through enterohepatic circulation interference.[134] The mechanisms of insoluble fiber remain less clear. [134] The attributed mechanisms at present for insoluble fiber is that of increasing fecal bulk via long-chain polymers binding water and hydrating fecal matter and reducing intestinal transit time with an ensuing rise in satiation.[134]

Protein content

The partial replacement of carbohydrates with protein has demonstrated cardioprotective effects on LDL in healthy and hypercholesterolemic individuals.[134] This may be attributed to a higher protein and lower carbohydrate diet inhibiting VLDL release leading to a decrease in LDL, curbing de novo fatty acid synthesis, and increasing

fat oxidation.[134] Similarly, with phytosterols, lowered cholesterol levels upregulate LDL receptors and thus increase uptake of cholesterol into the liver.[134]

Alpha-tocopherol content

It has been well-documented that alpha-tocopherol reduces lipid peroxidation.[20] Mechanisms apart from antioxidants include protein kinase C inhibition leading to disruption of monocyte adhesion and multiplication of smooth muscle as well as downregulation of lipid homeostatic genes.[134]

Micronutrient content (Mg, Mn, Cu, Ca, Na:K)

There is no proven cause and effect relationship with magnesium and CVD due to present data mainly being epidemiological and conflicting randomized control trial (RCT) results.[134] However, an inverse association has been shown between magnesium and CAD, high blood pressure, and cardiac arrhythmias which are all cardiovascular risk factors.[134] There is little evidence on the relationship between manganese and CVD, however, there is enough promising results to warrant additional studies.[134] Research on copper and CVD continues to be controversial although an inverse association between copper levels and CVD risk factors, particularly sclerotic development, has been demonstrated.[134] The presence of calcium and potassium in addition to the absence of sodium may beneficially influence overall dietary quality and engender reduction in CVD risk factors, especially hypertension.[134]

Structure and properties

As mentioned previously, the lack of lipid bioaccessibility in almonds due to their cell wall structure and properties positively influence CVD risk factors.[134] This may likely be responsible for the postprandial hypolipidemic effect of almonds as well as for weight maintenance or loss demonstrated by an almond-rich diet.[134]

Aerobic Physical Activity

Recommendations

The Physical Activity Guidelines for Americans currently recommends inactivity should be avoided as any amount of physical activity (PA) produces some health advantages.[135] At least 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity aerobic activity per week or an equivalent combination is the recommendation for substantial health advantages in adults (18-64 years old).[135] It is specified that aerobic activity should be engaged in at least 10-minute bouts and ideally spread throughout the week.[135] For further and more sizable health benefits, 300 or 150 minutes of moderate and vigorous intensity, respectively, or an equivalent combination is recommended for adults.[135] Muscle strengthening activities encompassing all main muscle groups are recommended for adults at least 2 days a week.[135] It should be noted that moderate-intensity is defined as PA that raises one's breathing and heart rate to some extent (5 or 6 on a 10 point scale relative to one's capacity) while vigorous-intensity is defined as PA that raises one's breathing and heart rate to a great extent (7 or 8 on a 10 point scale relative to one's capacity).[135]

These recommendations are in line with the American Heart Association (AHA) and American College of Sports Medicine joint guidelines for otherwise healthy adults,

the AHA and American College of Cardiology joint guidelines for secondary prevention of CAD, and the AHA guidelines for primary prevention of stroke and CVD being 30 minutes of moderate PA most days of the week.[42,136] The AHA defines moderate PA as 40-60% of maximum capacity comparable to a 15 to 20-minute mile involving brisk walking.[42]

Protective Role in CVD

Strong evidence exists linking PA to CVD risk reduction.[42] Specifically, numerous studies have emphasized the pivotal role of walking in health promotion as interventions of walking improve several CVD risk factors.[137] A walking intervention is favorably suited to a PA prescription as it is low cost, low injury risk, and requires no special skills or resources.[42,137]

Epidemiological Evidence

Epidemiological evidence indicates slight increases in daily walking is better than nothing, with larger improvements extending greater CVD health advantages. Enhanced fitness, blood pressure, body composition, and lipid profiles constitute possible short-term benefits and lower CHD, coronary events, and mortality risk constitute long-term benefits.[138]

Observational Studies

Observational studies consistently illustrate walking associated with CVD endpoints in the long-term.[138] PA, including brisk walking, has been demonstrated to assist in CVD prevention with increased duration, distance, energy expenditure, frequency, and pace associated with larger risk reductions in a broadly dose-dependent

manner.[10,11,138] Such associations seem to be stronger for ischemic stroke in comparison with other outcomes like CHD or hemorrhagic stroke and multiple studies specifically suggest less subclinical atherosclerosis is associated with walking pace.[10,11] The associations of walking volume and intensity with decreased CVD risk are similar in both males and females supporting the widely held consensus that walking seems to have CVD health advantages in both healthy and patient populations regardless of age or sex.[138] Walking also has been shown to reduce risks for hypercholesterolemia, hypertension, diabetes mellitus, and possibly CHD similarly to iso-energetic expenditures of running.[139]

Intervention Studies

Intervention trials additionally support evidence from epidemiological and observational studies by demonstrating short-term benefits in clinical biomarkers and measurements. A recent systematic review and meta-analysis of randomized controlled trails on the effects of walking on CVD risk factors suggest that walking interventions improve aerobic capacity (3.04 mL/kg/min, 95% CI 2.48 to 3.60), systolic blood pressure (− 3.58 mm Hg, 95% CI −5.19 to −1.97), diastolic blood pressure (−1.54 mm Hg, 95% CI −2.83 to −0.26), weight (−1.37 kg, 95% CI −1.75 to −1.00), BMI (−0.53 kg/m^2, 95% CI −0.72 to −0.35), percentage body fat (−1.22%, 95% CI −1.70 to −0.73), and waist circumference (−1.51 cm, 95% CI −2.34 to −0.68).[137] However, there was no evidence of alterations in lipids (TC, HDL, LDL) or waist-to-hip ratio.[137] It is interesting to note that with a walking intervention, FMD has been demonstrated to improve in sedentary middle-aged and older men but not in postmenopausal women.[140,141]

Mechanisms of Action

PA provides many cardiac benefits including improvement in myocardial oxygen supply and contraction as well as decreases in sympathetic activity contributing to better electric stability of myocardial cells and vascular benefits which are detailed in Table 6.[142,143] Several CVD risk factors are also amended with regular PA such as blood pressure, adiposity, insulin sensitivity, and atherogenic lipoprotein levels.[142]

Table 6. Vascular Effects of Physical Activity[142]

Aorta	Aortic stiffness ↓ Aortic compliance ↑ (endurance training only)
Conduit Vessel	Endothelial vasodilation ↑ NO production ↑ Oxidative stress ↓
Resistance vessel & Microcirculation	Vasculogenesis by endothelial progenitor cells Sensitivity to adenosine ↑
Capillary bed	Capillary vessel formation ↑
Venous circulation	Venular capillaries ↑
Pulmonary artery	Endothelial function Pulmonary artery pressure ↓ (in chronic heart failure)

One of the key mechanisms of improved vascular health in response to regular PA is the augmentation of vascular NO.[144] PA is associated with greater flow-mediated shear stress which up-regulates eNOS via a complex scheme of intracellular regulation.[144] This up-regulation of eNOS by PA or increased shear stress is well-supported.[144] This increased activation of eNOS by PA has also been shown to be a main mechanism of endothelial progenitor cell mobilization from the bone marrow which contributes to vascular regeneration and repair.[144]

CHAPTER 3

METHODS

Participants

The initial enrollment goal for this study was 50 male participants between 45 and 60 years of age who were otherwise healthy and sedentary (e.g. fail to meet the Physical Activity Guidelines for Americans: 150 minutes/week of moderate-intensity activity, or 75 minutes/week of vigorous-intensity activity).[145] Due to initial low participant accrual, the study was expanded to otherwise healthy and sedentary (e.g. sit > 8 hours daily) men and postmenopausal women between 20 and 60 years of age. The sample size (n) of 50 participants was determined through colleague collaboration and a sample size calculator tool developed by David Schoenfeld of Harvard University. Recent studies examining differing effects of nuts on vascular reactivity provided data for the sample size calculations (see Appendix D).[146-150] An average difference of means and standard deviation in FMD of 2.5 $\pm$ 4.34 respectively was estimated using a significance level of P = 0.05 and power of 0.8. The calculation estimated n = 372. However, this was a pilot study and a n = 50 was decided to be appropriate as a sample size of n = 8-15 per group has been shown to be suitable for pilot studies to mirror large clinical trials.[151,152]

The inclusion criteria were as follows: otherwise healthy men and post-menopausal women (20-69 years of age) were considered eligible for the study if they routinely sit > 8 hours daily, believe they need to increase their physical activity, answered ‘no’ to all questions on the Physical Activity Readiness Questionnaire (PARQ), and have constant dosing of a non-nitrate vasoactive medication. The exclusion criteria were as follows: individuals who reported chronic disease (e.g. cancer, heart disease,

asthma, arthritis, and hypertension), individuals who were unwilling to participate in an 8-week walking intervention or comply with testing protocols, individuals with reported nut or gluten allergies, cigarette use within the past year, food allergies, and specific medication use (nitrate vasoactive hypertensive medications, nitroglycerin, beta-blockers, and calcium channel blockers), and individuals who measured as hypertensive at the test site (blood pressure > 140/90).

Participants were recruited from the Diocese of the East Valley, Arizona State University, Encanto Golf Course, the YCMA, religious groups, parking lots, and shopping areas in the Phoenix metropolitan area via listservs, emails, and flyers (see Appendix E). Interested participants were directed to complete an online pre-screening survey including the Physical Activity Readiness Questionnaire (see Appendix F). Sixty-four individuals responded to our pre-screening survey conducted through SurveyMonkey over a 2-month enrollment period. Of these, 15 individuals met the inclusion criteria and were invited to the downtown Phoenix campus of ASU to verify eligibility, provide informed consent, and complete a health history questionnaire (see Appendices G and H). One participant was excluded due to meeting the exclusion criteria (e.g. measured hypertensive at the test site) and two participants opted out before study completion, leaving 12 participants (see Figure 1).

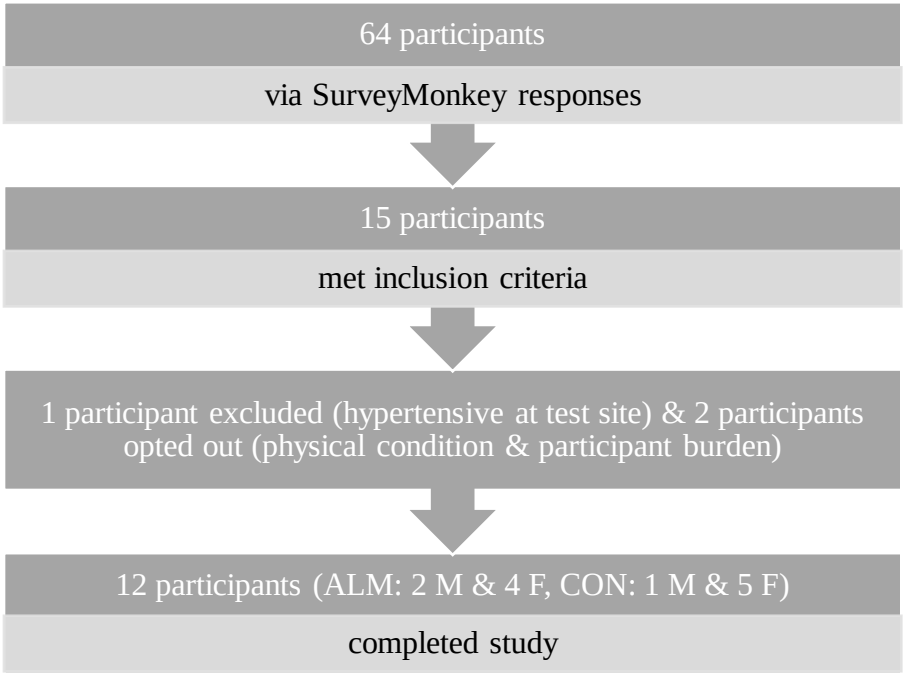

Figure 1. *Attrition Flow Chart*

Study Design

This pilot study represents a placebo-controlled, randomized parallel two-arm trial consisting of 12 participants. The study contained the following 2 groups: ALM Group consumed whole, raw almonds (2.5 oz daily) and briskly walked 10,000 steps per day and CON Group consumed a placebo (4 Tbsp of Speculoos Cookie Butter daily) and briskly walked 10,000 steps per day. The independent variable is represented by the type of nut product apportioned. The nut products included whole, raw almonds (2.5 oz equaling 400 calories) or isocaloric cookie (nut) butter (4 Tbsp equaling 360 calories; contains traces of tree nuts) (see Appendix I).[153] The raw whole almonds were provided by the Almond Board of California and were prepackaged into 2.5 oz servings.

The study was approved by the Arizona State University Institutional Review Board before it was initiated (see Appendix J). The study involved four visits to the test site at the ASU Nutrition Labs on the Phoenix Downtown Campus over the 8-week study period where anthropometrics, blood pressure, body composition, FMD, fitness step test and fasting blood samples were taken at each visit. Informed consent was administered at visit 1 where participants were apprised of the potential benefits (i.e. possible increased fitness level and FMD outcomes if desired) and risks (i.e. possible temporary nausea or faintness during the blood draw and possible injury with increased physical activity) of study participation. No conflicts of interest were involved in this study and confidentiality was maintained by assigning each participant an ID number and removing all patient identification factors from the data, keeping files in a locked file cabinet and password protecting all electronic files or computers housing files containing identifiable information, and blinding principal investigators and laboratory technicians.

Study Protocol

Visit 1 occurred one-week pre-trail where the participants visited the Arizona Biomedical Collaboration building for signed written consent, health history screening, gait, anthropometrics (i.e. height and weight), and body composition (Tanita) measurement. Participants were given the following: a compliance calendar with instructions to record daily steps from the pedometer and to check off when nut products (i.e. almonds or cookie butter) were consumed for trial weeks 6-8 (see Appendix K), a 3-day food record with instructions, and a pedometer with instructions to wear it each day for the entire trial. Participants were instructed to restrict their nut and seed consumption

(including almond milk) to 2 or less servings per week during the entire trial, excluding the study products. Participants were coached to continue their normal dietary and physical activity patterns (with the exception of the nut restriction) and not change from their usual routine.

At visit 2 (week 0 or trial baseline), pedometer data was downloaded and recorded, the 3-day food record was collected, and the following measures were completed: FMD, blood pressure, anthropometrics, body composition, YMCA Bench Step Test for Cardiovascular Fitness, and a fasting blood sample. Participants were coached to slowly increase step counts to 10,000 steps daily within the next 2 weeks and then asked to maintain this step count for the duration of the study. Trial weeks 3-5 represented the walking phase of the study and measurements demonstrate the benefits of solely walking for all study participants.

Visit 3 (week 5) involved downloading and recording pedometer data, assigning a second 3-day food record, dispensing nut products and providing instructions, repeating the measurements conducted at visit 2, and giving a $15 gift card. Participants were stratified by sex, age, height, weight, BMI, fat percentage, and baseline step count and randomly assigned (by coin toss) to receive either 30 individual 2.5 oz packages of raw whole almonds or four 14.1 oz jars of cookie (nut) butter. Trial weeks 6-8 represented the nut intervention phase of the study and measurements demonstrate the benefits of almond consumption in addition to walking for all study participants.

Visit 4 (week 8) involved downloading and recording pedometer data, collecting the second 3-day food record and compliance calendar, repeating the measurements conducted at visit 2, and giving a $15 gift card. All 4 visits took place at the ASU

Nutrition Labs within the Arizona Biomedical Collaboration (ABC) building on the Phoenix Downtown Campus and lasted approximately 45 minutes to 1 hour in length. Between all 4 site visits, emails were issued to follow-up, answer questions, and provide reminder instructions in regards to all dietary supplements restriction 3 days prior to their visit, strenuous exercise restriction 48 hours prior to their visit, and caffeine, alcohol, food and drink (except water) restriction 12 hours prior to their visit. See Figure 2 for complete overview of study protocol.

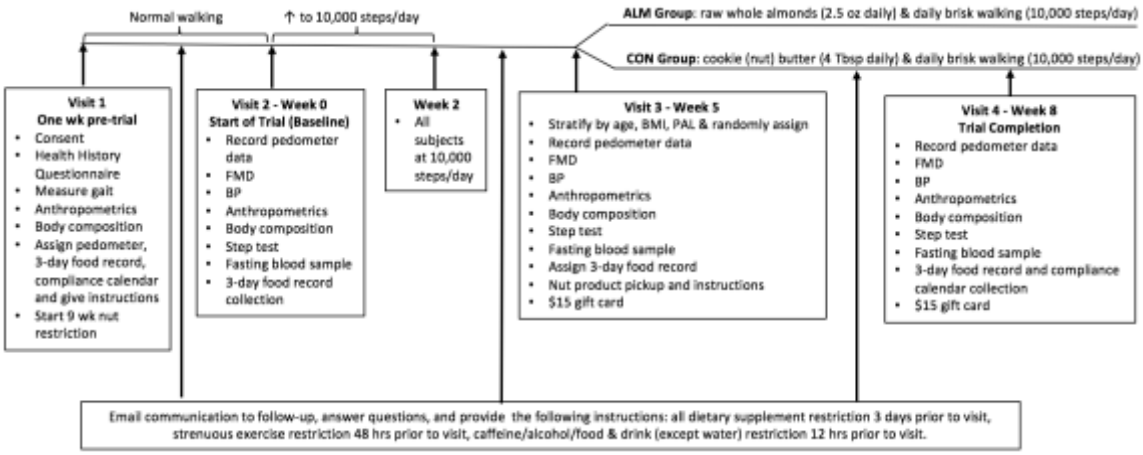

Figure 2. *Study Protocol Flow Chart*

Laboratory Analyses

FMD and blood pressure measures were conducted at baseline, week 5, and week 8 by a full-time School of Nutrition and Health Sciences trained, certified sonographer (Theresa Jorgensen RDCS, RDMS, RVT). FMD was performed using high resolution 2K and Doppler ultrasound (HDI 5000, ATL Philips Ultrasound). The cuff, appropriate to the size for the limb, was distally positioned to the ultrasound probe at the brachial artery. The cuff was then inflated to ≥ 50 mm Hg above systolic arterial pressure for 5 minutes

to generate a reactive hyperemic stimulus which is regarded as primarily endothelium mediated and NO dependent.[154] To ensure accuracy of measurement, probe location was recorded for each participant with repeated measures at the same site. To ensure proper probe location among tests, visible anatomical landmarks on the ultrasound were used (see Appendix L for detailed protocol).

Fasting venous blood draws were performed at baseline, week 5, and week 8. Trained staff (Ginger Hook RN, CDE and Veronica Zamora R.T. (R) (BD) ARRT) collected the blood samples using EDTA-coated vacutainers. Whole blood was centrifuged to remove the plasma. Plasma samples for total nitrate/nitrite analyses were pre-filtered using 30 molecular weight cut-off microcentrifuge filters according to the manufacturer's protocol (Millipore, Billerica, MA) following the Griess method (See appendix M; Cat. No. 780001, Cayman Chemical, Ann Arbor, MI). Thiobarbituric acid reactive substances (a measure of lipid peroxidation) was analyzed using a commercial colorimetric assay kit (Cat. No. 0801192, ZeptoMetrix Corporation, Buffalo, NY) following the manufacturer's instructions (See appendix N). Vitamin E analyses were performed by Dr. Kevin McGraw as a food study intervention compliance marker.

Anthropometrics (i.e. height and weight), body composition measures, and the YMCA Bench Step Test for Cardiovascular Fitness were performed at baseline, week 5, and week 8 for a separate study by a graduate researcher (Elizabeth McElaney MS, NASM – CPT, CES). As these data were collected for a separate study, the outcomes will not be included in this thesis.

Statistical Analyses

The statistical software Statistical Package for the Social Sciences (SPSS) was employed. A statistically significant p-value of ≤ 0.05 was used and data is presented as the mean $\pm$ SD. If normality was not attained using the Shapiro-Wilk test of normality, the data was transformed and the effect sizes were determined. Independent Samples T-tests were employed to compare variables between groups at baseline and week 5. Paired T-tests were used to compare changes within groups over the first 5 weeks. Analysis of Covariance (ANCOVA) controlling for baseline and change in steps was used to measure changes in variables between groups and multivariate analysis of variance (MANOVA) was used to measure changes in FMD between groups from baseline to week 8.

CHAPTER 4

RESULTS

Twelve participants (6 in ALM group and 6 in CON group) completed the pilot study. Verbal affirmation was received from all participants that daily nut products were consumed despite 8 of the 12 compliance calendars being incomplete and combined alpha-Tocopherol and gamma-Tocopherol measures showing no difference between groups for change with a mean of $+0.4 \pm 0.6$ μg/ml for the ALM group and a mean of $+0.5 \pm 0.6$ μg/ml for the CON group ($p = 0.470$) with an effect size of 0.067.

There were no differences between participants in each group at baseline as illustrated in Table 7. Mean participant age at baseline was 55.5 ± 8.6 y for the ALM group and 52.7 ± 10.9 y for the CON group. Additionally, there were no differences between participants in either group at week 5 as illustrated in Table 8.

Table 7. Participant Characteristics at Baseline

Variable[a]	ALM	CON	*P VALUE**
Subjects (M/F)	2 M / 4 F	1 M / 5 F	
Age (y)	55.5±8.6	52.7±10.9	0.629
Weight (kg)	77.2±11.7	72.8±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 (kg)	51.8±11.6	47.7±8.0	0.486
BMI (kg/m^2)	28.0±4.0	25.6±1.8	0.217
Heart Rate, rest (BPM)	73.8±6.7	72.5±11.1	0.806
Pedometer (steps)	8278±1657	7847±2672	0.744
METS (kcal·kg-1·wk-1)	33.2±20.8	71.7±52.8	0.128
Total nitrates and nitrites (µM/L)	56.6±37.7	57.8±26.4	0.948
Blood Pressure Systolic (mmHg)	120.0±6.5	121.3±17.6	0.865
Blood Pressure Diastolic (mmHg)	74.2±8.1	75.5±9.5	0.799
Total Cholesterol (mg/dL)	169.0±35.6	196.3±18.4	0.127
HDL (mg/dL)	52.2±8.9	56.9±14.2	0.504
LDL (mg/dL)	108.5±33.5	124.6±22.7	0.352
Triglycerides (mg/dL)	94.1±60.6	135.0±65.4	0.287
TBARS (nmol MDA/mL)	1.2±0.5	1.4±1.0	0.544
hsCRP (mg/L)	1.6±1.5	1.6±1.4	0.915

[a] Data is represented as Mean±SD. *p-value represents Independent Samples T-Test.

Table 8. Participant Characteristics at Week 5

Variable[a]	ALM	CON	*P VALUE**
Subjects (M/F)	2 M / 4 F	1 M / 5 F	
Weight (kg)	76.9±11.5	73.0±8.4	0.515
Body Fat (%)	34.1±8.3	33.5±8.9	0.909
Fat Free Mass (kg)	50.9±11.3	48.5±8.4	0.689
BMI (kg/m^2)	27.9±4.0	25.5±1.4	0.214
Heart Rate, rest (BPM)	77.0±7.1	72.7±5.7	0.272
Pedometer (steps)	9729±2068	10482±1348	0.472
Total nitrate and nitrites (µM/L)	37.9±13.8	34.8±15.3	0.720
Blood Pressure Systolic (mmHg)	117.5±6.9	124.7±9.8	0.173
Blood Pressure Diastolic (mmHg)	73.8±4.2	72.2±7.9	0.658
Total Cholesterol (mg/dL)	177.4±33.8	195.5±14.4	0.255
HDL (mg/dL)	55.5±8.4	57.4±16.7	0.807
LDL (mg/dL)	113.3±29.9	123.5±19.9	0.502
Triglycerides (mg/dL)	87.5±47.4	141.4±53.8	0.095
TBARS (nmol MDA/mL)	0.8±0.5	1.2±0.6	0.263
hsCRP (mg/L)	1.8±1.7	2.2±3.2	0.787

[a] Data is represented as Mean±SD. *p-value represents Independent Samples T-Test.

The walking intervention alone (week 0-5) resulted in a change in variables for step count of ALM and CON groups combined with a mean of 8063 ± 2132 steps at baseline and 10105 ± 1710 steps at week 5 (p=0.022) and total nitrates and nitrites ALM and CON groups combined with a mean of 57.2 ± 31.1 µM/L at baseline and 36.3 ± 14.0 µM/L at week 5 (p=0.052) as illustrated in Table 9. These two changes are not related when change was compared with correlation. All other variables including FMD, systolic and diastolic blood pressure, total cholesterol, HDL, LDL, triglycerides, TBARS, and hsCRP showed no significant change as displayed in Table 9.

Table 9. Impact of Walking Intervention Alone in All Participants

Variable[a]	Baseline	Week 5	*Paired t-test*
Pedometer (steps)	8063±2132	10105±1710	***0.022***
FMD (peak %)	6.7±4.6	7.2±3.5	*0.592*
Total nitrates and nitrites (μM/L)	57.2±31.1	36.3±14.0	***0.052***
Blood Pressure Systolic (mmHg)	120.7±12.7	121.1±8.9	*0.926*
Blood Pressure Diastolic (mmHg)	74.8±8.5	73.0±6.1	*0.408*
Total Cholesterol (mg/dL)	182.6±30.5	186.5±26.5	*0.338*
HDL (mg/dL)	54.5±11.6	56.5±12.7	*0.196*
LDL (mg/dL)	116.6±28.5	118.4±24.8	*0.602*
Triglycerides (mg/dL)	114.6±63.8	114.5±55.9	*0.986*
TBARS (nmol MDA/mL)	1.30±0.79	0.99±0.57	*0.181*
hsCRP (mg/L)	1.59±1.35	2.03±2.49	*0.260*

[a] Data is represented as Mean±SD; n = 12. Data was analyzed by paired t-tests. All data normally distributed.

The additive effect of the walking intervention with the nut product (week 5-8) resulted in significant differences in total cholesterol with a -11.0 ± 10.5 and +3.3 ± 15.8 mg/dL (p=0.043) change in the ALM and CON group respectively and LDL with a -11.5 ± 7.5 and +0.5 ± 13.7 mg/dL (p=0.025) change in the ALM and CON group respectively (see Table 10). These significant differences illustrate a 6.2% decrease of total cholesterol in the ALM group and a 10.2% decrease in LDL in the ALM group. There was a trend for TBARS to lower in the ALM group versus the CON group (-0.2 ± 0.8 and +0.3 ± 0.6 nmol MDA/mL (p=0.099) respectively) with a large effect size of 0.304 but this did not reach statistical significance (see Table 10). The significant difference in total cholesterol and LDL and the trend in TBARS as seen from week 5 to 8 is illustrated in Figures 3, 4, and 5 respectively. Additionally, there was a trend for steps to increase in the ALM group and a trend for steps to decrease in the CON group (+676 ± 1643 and -1416 ± 1569 steps (p=0.079)) with a large effect size of 0.304.

Table 10. Additive Effect of Walking + Snack

Variable[a]	Week 5[b]	Week 8[c]	Change	P-value (effect size)
Pedometer (steps)				
ALM	9729±2068	10404±1348	*+676±1643*	*0.079*
CON	10482±1348	9066±2347	*-1416±1569*	*(0.304)*
FMD (peak %)				
ALM	8.4±4.4	7.3±5.1	*+0.4±1.2*	*0.882*
CON	6.0±2.0	5.7±1.5	*-0.3±1.4*	*(0.003)*
Total nitrates and nitrites (µM/L)				
ALM	37.9±13.8	45.0±17.8	*+7.2±28.7*	*0.614*
CON	34.8 ±15.3	44.1±26.1	*+9.4±29.1*	*(0.033)*
Blood Pressure Systolic (mmHg)				
ALM	117.5±6.9	115.8±10.9	*-1.7±5.8*	*0.114*
CON	124.7±9.8	118.5±6.6	*-6.2±8.2*	*0.283*
Blood Pressure Diastolic (mmHg)				
ALM	73.8±4.2	72.3±9.0	*-1.5±7.6*	*0.843*
CON	72.2±7.9	74.8±8.5	*+2.7±5.9*	*0.005*
Cholesterol (mg/dL)				
ALM	177.4±33.8	166.4±34.7	*-11.0±10.5*	***0.043***
CON	195.5±14.4	198.8±20.7	*+3.3±15.8*	*(0.420)*
HDL (mg/dL)				
ALM	55.5±8.44	54.4±8.01	*-1.1±5.56*	*0.287*
CON	57.4±16.7	61.3±16.8	*+3.8±6.7*	*(0.140)*
LDL (mg/dL)				
ALM	113.3±29.9	101.8±30.2	*-11.5±7.5*	***0.025***
CON	123.5±19.9	124.0±24.3	*+0.5±13.7*	*(0.485)*
Triglycerides (mg/dL)				
ALM	87.5±47.4	105.5±81.7	*+18.0±38.9*	*0.136*
CON	141.4±53.8	147.7±69.1	*+6.3±29.1*	*(0.256)*
TBARS (nmol MDA/mL)				
ALM	0.8±0.5	0.6±0.4	*-0.2±0.8*	*0.099*
CON	1.2±0.6	1.4±0.7	*+0.3±0.6*	*(0.304)*
hsCRP (mg/L)				
ALM	1.8±1.8	1.7±1.7	*-0.1±0.4*	*0.388*
CON	2.2±3.2	1.1±1.3	*-1.1±2.0*	*(0.094)*

[a] Data is represented as Mean±SD; n = 6 per group with exception of FMD with n = 5 for the ALM group. P-value and effect size represent ANCOVA analyses controlling for baseline value and change in steps. All data normally distributed except for hsCRP which is near normal (could not be normalized because transformation worsened normality). Inverse transformation was used to normalize TBARS and removal of an outlier (2.8 SD away from mean) was used to normalize FMD.[b]Walking only; [c]Walking + almond or cookie butter.

Figure 3. *Significant Change in Total Cholesterol During Week 5-8*

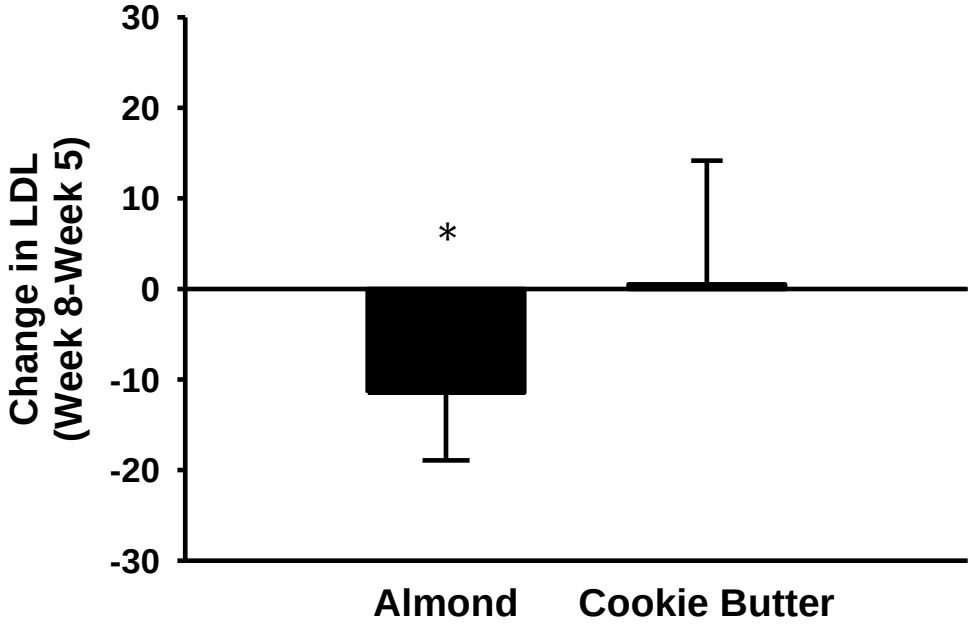

Figure 4. *Significant Change in LDL During week 5-8*

Figure 5. *Trend in Oxidative Stress Biomarker (TBARS) During week 5-8*

There were no significant differences seen in markers of the other plasma lipid profile measures, plasma inflammatory cytokines, blood pressure regulation, or step count as shown in Table 10. All data presented in Table 10 are normally distributed except for hsCRP. The biomarker hsCRP could not be normalized because transformation worsened normality. Inverse transformation was used to normalize TBARS and removal of an outlier (2.8 standard deviations away from the mean) was used to normalize FMD.

Although FMD increased in the ALM group from baseline to week 8, there were no significant differences seen in FMD from baseline with a mean of 6.9 ± 6.1 and 6.3 ±

2.9 to week 8 with a mean of 7.3 ± 5.1 and 5.7 ± 1.5 in the ALM and CON group respectively (p=0.382, effect size = 0.077).

CHAPTER 5

DISCUSSION

This pilot study represents a randomized, parallel, two-arm study comparing the combined effect of daily almond ingestion (2.5 ounces) and brisk walking (10,000 steps per day) versus the combined ingestion of an isocaloric placebo (4 Tbsp cookie butter) and brisk walking (10,000 steps per day) in sedentary adults. The possible synergistic effect of almond ingestion in conjunction with a walking intervention on CVD markers was examined. The main biomarkers investigated were FMD (peak %), total nitrates and nitrites (µM/L), blood pressure (mmHg), total cholesterol (mg/dL), HDL (mg/dL), LDL (mg/dL), triglycerides (mg/dL), TBARS (nmol MDA/mL), and hsCRP (mg/L).

The additive effect of the daily walking intervention with almond consumption resulted in significant differences in total cholesterol and LDL. There was a trend for TBARS to decrease in the ALM group versus the CON group with a large effect size of 0.304 but this did not reach statistical significance. There were no significant differences seen in FMD, total nitrates and nitrites, blood pressure, HDL, triglycerides, and hsCRP. Additionally, there was a trend for steps to increase in the ALM group and a trend for steps to decrease in the CON group with a large effect size of 0.304.

Due to the small sample size (n=12) and lower statistical power, statistical findings may be misleading as the dietary associations with CVD risk markers might have been blunted. There is also the possibility that only large differences could be detected in measures as opposed to being able to detect small effects had a larger sample size been recruited. Therefore, the trend for TBARS with a large effect size suggests a moderate to high practical significance. The trend in step count to increase in the ALM

and decrease in the CON group could reflect the inevitable unpredictable participant compliance.

The results of significant differences in total cholesterol and LDL and no significant difference in HDL and triglycerides are consistent with the plethora of well-established empirical evidence of the effects of almond ingestion.[155] A recent meta-analysis of RCTs including normolipidemic, hyperlipidemic, prediabetic and/or diabetic, and obese individuals concluded that almond ingestion reduces total cholesterol with a strong trend to lower LDL but HDL and triglycerides remain largely unaffected.[156] However, some studies have conversely shown improvements in HDL and triglycerides.[26,157] The inclusion of postmenopausal women may have confounded HDL results. Estrogen is believed to have cardio-protective effects by preserving vascular health and function specifically in regards to protecting the flexibility of blood vessels to adapt to blood flow.[44] Endogenous estrogen declines with menopause, therefore, increasing CVD risk indicated by a substantial rise in LDL in the perimenopause period and continuing up to the age of 60 years at least.[158] Interestingly, prospective and cross-sectional evidence demonstrate only a slight decrease in HDL at the time of menopause.[158] HDL levels in females carry on to be higher than males for 30 years after menopause at the minimum and thus may have impacted results of this study.[158] The significant difference in LDL is in agreement with dose response findings in previous studies of a 1% decrease in LDL for each 7-8 g of almonds.[25] This pilot study demonstrated a 10.2% decrease in LDL in the ALM group vs the CON per 70.88 g/d of almonds or a 1% decrease in LDL for each 7 g of almonds.

While there is epidemiological evidence that indicates a MUFA and tocopherols-enriched diet lowers blood pressure and clinical evidence that specifically an almond-enriched diet can reduce blood pressure, the study results did not reflect this effect.[131] However, additional research corroborates the results of a lack of significant blood pressure change.[146] In the present pilot study a lack of change in blood pressure is expected due to the following: participants were not extremely sedentary even though they met the inclusion criteria, participants only had a 25% increase in steps from baseline to week 5 and that more or less sustained until week 8, and NOx and FMD had no significant changes. Almond ingestion has been demonstrated to influence some but not all inflammatory and oxidative stress markers which are correspond with the mixed results of a trend for TBARS to decrease in the ALM group and no significant difference in hsCRP.[111,131] It is supported that antioxidant effects of almonds may be seen in individuals with heightened oxidative stress.[111] Furthermore, TBARS as a biomarker of oxidative stress is questionable as this measure has low repeatability, poor reproducibility, and lacks specificity and full validation data in biological fluids.[157]

Almonds being rich in arginine and alpha-tocopherol are anticipated to increase NO bioavailability and FMD in theory.[131] However, the result of no significant difference in total plasma nitrates and nitrites is consistent with recent studies.[146,131] The presence of additional eNOS-independent origins of serum nitrate and nitrites may likely explain the results.[160] NO concentrations are not solely dictated by endogenous NO by way of NO synthases.[160] Exogenous sources including the diet (i.e. vegetables), cigarette smoke, and medications (i.e. nitrovasodilators) and additional endogenous sources including oral and intestinal bacteria (i.e. nitrate reductase enzymes) also contribute to the NO storage

pool.[87,160] Therefore, dietary and environmental factors as well as the limitation of not controlling for antiseptic mouthwash which reduces oral nitrite production (90%, $P < 0.001$) and serum nitrite concentrations (25%, $P = 0.0001$) may have skewed total nitrates and nitrites results.[161] Measuring NO is challenging as the free radical has a 1 microsecond to 2 milliseconds of half-life and a diffusion reach of approximately 0.1 μm.[87] Because of this, the metabolic products of NO (nitrite and nitrate) are commonly used as accepted surrogate markers due to their longer half-lives of 11-42 minutes and 5-8 hours respectively.[87] However, recent research refute that nitrate and/or NOx are beneficial markers and suggest NOx, an assay of the total amount of nitrates and nitrites, is inaccurate in acute NO testing.[162,163] The findings show NOx concentrations remain unchanged with pharmacological alteration of the L-arginine: NO pathway, sensitivity of serum nitrite levels indicate acute alterations in regional eNOS activity, acetylcholine-induced blood flow reactions and serum nitrite augmentation are nearly solely NOS mediated, and serum nitrite concentrations are vasodilator-inactive.[163] Accordingly, there is strong evidence for nitrite specificity as a marker for NO production as it more accurately reflects acute eNOS activity fluctuations in the human forearm circulation.[161] Hence, nitrite alone has been suggested as a NO marker.[163] NOx over solely nitrite was employed as a biomarker in this study due to the fasted state of the participants making it unlikely to be able to detect measurable levels of plasma nitrite with a half-life of 11-42 minutes.[87] The questionable accuracy of NOx used also may have influenced data and explain the lack of significant change for total nitrates and nitrites. Even though as much as 70% of plasma nitrite is from eNOS, additionally dietary and environmental factors may have influenced total nitrates and nitrites results.[160]

Again, it would be predicted in theory that almond consumption increases FMD but no significant difference was shown.[131] Very few studies have examined almond consumption with the outcome measure of FMD but those that have report mixed results (i.e. no effect, partial effect, full effect).[46,131,132] There are many factors that may have confounded such results including food, drugs, temperature, sympathetic stimuli, etc.[164] Subjects were instructed to avoid all dietary supplements, strenuous exercise, and caffeine, food, and drink (except water) 3 days, 48 hours, and 12 hours prior to visits, however, patient noncompliance is always a possibility.[164] Current guidelines recommend all vasoactive drugs be withheld a minimum of four half-lives prior to testing if possible, however, there is evidence that may suggest otherwise.[164] Gokce et. al. demonstrated that healthy subjects randomized to a single oral dose of felodipine, enalapril, metoprolol, or a placebo and CAD patients on prescribed antianginal and/or antihypertensive drugs, all common non-nitrate vasoactive medications, resulted in no significant effect on brachial artery dilation.[165] In light of this evidence and greater methodology practicality, participants on a constant dose of non-nitrate vasoactive drugs were included in the study. FMD results may have been confounded by not withholding all vasoactive drugs four half-lives prior to testing and/or may have been confounded if participants were noncompliant with constant dosing of non-nitrate vasoactive drugs during the entirety of the study. Additionally, significant improvements in FMD are seen when parallel-group study designs have 40-60 subjects typically with baseline mean FMD measures for asymptomatic adults being 6.1 ± 3.2.[166,167] In a study of such size, an absolute FMD change of 1.5-2% is the minimal statiscially significant improvement required to be able

to detect a significant change with intervention.[166] Therefore, the FMD results may have been blunted due to the small sample size (n = 12) and lower statistical power.

Overall, almond ingestion had an advantageous effect over walking. The impact of a 25% increase in steps during the walking intervention alone in all participants had no effect on any CVD biomarkers with the exception of a decrease in total nitrates and nitrites. This change in variable was not expected and may be due to the possibility that study participants had diets high in L-arginine (i.e. seeds and nuts) prior to study initiation. A nut restriction commenced with study initiation. So, if participants went from high nut and seed consumption to little to no consumption this could explain the change in this variable. A 25% increase in steps does not represent a substantial increase which therefore may have limited the effectiveness of the walking intervention alone.

Participant compliance for the walking intervention alone is demonstrated by the 25% increase in step count by participants of which the compliance calendars support as well. Verbal affirmations, compliance calendars, and tocopherols measures were employed to gauge participant compliance for the almond intervention in addition to the walking intervention. While 8 of the 12 compliance calendars were incomplete, verbal affirmation was received from all participants that daily nut products were consumed. The combined alpha-Tocopherol and gamma-Tocopherol measures showed no difference between groups for change, with a mean of $+0.4 \pm 0.6$ μg/ml for the ALM group and a mean of $+0.5 \pm 0.6$ μg/ml for the CON group ($p = 0.470$) which illustrates compliance in both groups. The fat in the cookie butter is from palm and canola oil. Both of these oils contain between approximately 16 to 18 mg per 100 g of vitamin E, therefore, the placebo of 4 tablespoons of cookie butter can be estimated to contain 4 mg of vitamin

E.[114] Even though the 2.5 oz almond intervention contained 18.85 mg of vitamin E, around 16.5% of vitamin E is released following duodenal digestion whereas up to 90% of vitamin E is absorbed from oils.[127,168,169] Hence, the two food interventions provide comparable amounts of vitamin E. For this reason, the cookie butter used constitutes an ideal placebo controlling for vitamin E.

The current study had many limitations as stated previously but it also had many strengths. While the pilot study did not represent the most ideal and controlled conditions, it did reproduce real-world circumstances with home-prepared foods as the background diet and used almonds as the supplemental food intervention which are easily available and commonly consumed by the public in a daily dose (2.5 oz) that is reasonable for realistic implementation.

CHAPTER 6

CONCLUSIONS & RECOMMENDATIONS

Conclusions

Total cholesterol and LDL cholesterol improve favorably for the ALM versus CON group with statistically significant differences seen, in addition to a trend of improvement in MDA with a large effect size indicating possible significance if the study had been adequately powered. Hypothesis 1 being that daily almond ingestion along with brisk walking will act synergistically to improve blood pressure regulation was not supported as there was no evidence of a statistically significant improvement in FMD, total NO production, or systolic and diastolic blood pressure. Hypothesis 2 being that daily almond ingestion along with brisk walking will act synergistically to decrease CVD risk was partially supported as there was evidence of statistically significant improvements in total cholesterol and LDL but not HDL or triglycerides, a non-statistically significant trend of improvement in the oxidative stress biomarker MDA but no evidence of statistically significant improvement of the plasma inflammatory cytokine hsCRP.

In the present study it has been shown that an almond-enriched diet improves lipid profiles and may potentially ameliorate oxidative stress while daily brisk walking alone had no positive effect on CVD risk in otherwise healthy sedentary adults. This simple, cost-effective, and manageable diet strategy of the incorporation of 2.5 oz of almonds in the daily diet can benefit a substantial portion of the population which would otherwise be at increased risk of CVD.

Recommendations

Further research that additionally explores the topic at hand is recommended to improve understanding of the influence of almonds on CVD risk, particularly adding to the body of knowledge on the effects of almonds and mechanism of action on lipids and oxidative stress. It is suggested to include additional measurements in regard to LDL particle size and oxidative stress. While the walking intervention alone showed no effect, sole examination of a similar almond intervention, possibly with the addition of almond butter, is proposed. Recruitment of a larger and more focused sample (i.e. only males and/or only middle-aged individuals) may be necessary to capture stronger conclusions that are adequately powered. A 3-week almond intervention interval is a rather short period to influence some CVD risk biomarkers so employment of a longer duration of an almond intervention is recommended.

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APPENDIX A

3-DAY FOOD RECORD SHEET

3-Day Dietary Food Log Instructions

1. Fill in the 3-day dietary food log over the span of 3 consecutive days including 2 week days and 1 weekend days.

2. Please record ALL food and drinks consumed throughout each 24-hour period. Even if it is a handful of chips or a few pieces of candy.

3. Be as specific as you can about the food type and amounts.

4. If the food is a combination food (i.e. a sandwich), include all contents of the meal including any condiments or sauces consumed and the amount of each ingredient.

5. Record the amount in the way that it is ordinarily measured or by using the portion size worksheet attached.

6. Include the brand name when applicable or if the meal is not prepared by you, be as specific as possible with the preparation methods used (i.e. fried, baked, sautéed in oil).

7. Please be honest and be precise.

Sample Log

Time	Food Item	Amount	Description
e.g. 9am	*e.g. banana*	*Give as tsp, tbsp, cups, oz, weight, or portion*	*Include type of food, brand name, or restaurant*
6:30 am	English Muffin	1 Muffin	Whole wheat, Thomas
6:30 am	Banana	1 Medium Fruit	
6:30 am	Peanut butter	2 tbsp	Low-fat JIFF
9:30 am	Yogurt	6 oz	Low-fat Vanilla, Dannon
9:30 am	Granola	½ C	Nature Valley
12:30 pm	Spinach	3 C	
12:30 pm	Tomato	½ C	
12:30 pm	Tofu	3 oz	Firm
12:30 pm	Balsamic Vinaigrette	2 tbsp	Kraft

ID#

Date

3-Day Food Log (Day 1)

Time	Food Item	Amount	Description
e.g. 9am	*e.g. banana*	*Give as tsp, tbsp, cups, oz, weight, or portion*	*Include type of food, brand name, or restaurant*

3-Day Food Log (Day 2)

ID#

Date

Time	Food Item	Amount	Description
e.g. 9am	*e.g. banana*	*Give as tsp, tbsp, cups, oz, weight, or portion*	*Include type of food, brand name, or restaurant*

3-Day Food Log (Day 3)

ID#

Date

Time	Food Item	Amount	Description
e.g. 9am	*e.g. banana*	*Give as tsp, tbsp, cups, oz, weight, or portion*	*Include type of food, brand name, or restaurant*

APPENDIX B

FULL ALMOND NUTRIENT REPORT

Nutrient	Unit	1Value per 100 g	Data points	Std. Error	2.5 oz = 70.88g
Proximates					
Water	g	4.41	90	0.219	3.13
Energy	kcal	579	--	--	410
Energy	kJ	2423	--	--	1717
Protein	g	21.15	91	0.117	14.99
Total lipid (fat)	g	49.93	90	0.286	35.39
Ash	g	2.97	90	0.04	2.1
Carbohydrate, by difference	g	21.55	--	--	15.27
Fiber, total dietary	g	12.5	89	0.295	8.9
Sugars, total	g	4.35	--	--	3.08
Sucrose	g	3.95	93	0.212	2.8
Glucose (dextrose)	g	0.17	93	0.035	0.12
Fructose	g	0.11	93	0.04	0.08
Lactose	g	0	93	0	0
Maltose	g	0.04	93	0.02	0.03
Galactose	g	0.07	88	0.021	0.05
Starch	g	0.72	4	0.101	0.51
Minerals					
Calcium, Ca	mg	269	89	7.668	191
Iron, Fe	mg	3.71	89	0.139	2.63
Magnesium, Mg	mg	270	88	1.371	191
Phosphorus, P	mg	481	88	6.308	341
Potassium, K	mg	733	88	16.386	520
Sodium, Na	mg	1	85	0.467	1
Zinc, Zn	mg	3.12	86	0.062	2.21
Copper, Cu	mg	1.031	86	0.05	0.731
Manganese, Mn	mg	2.179	88	0.075	1.544
Selenium, Se	µg	4.1	26	2.447	2.9
Vitamins					
Vitamin C, total ascorbic acid	mg	0	49	0	0
Thiamin	mg	0.205	85	0.009	0.145
Riboflavin	mg	1.138	88	0.115	0.807
Niacin	mg	3.618	88	0.15	2.564
Pantothenic acid	mg	0.471	88	0.025	0.334
Vitamin B-6	mg	0.137	47	0.006	0.097
Folate, total	µg	44	41	6.482	31
Folic acid	µg	0	--	--	0
Folate, food	µg	44	41	6.482	31
Folate, DFE	µg	44	--	--	31
Choline, total	mg	52.1	--	--	36.9
Betaine	mg	0.5	5	0.051	0.4
Vitamin B-12	µg	0	--	--	0
Vitamin B-12, added	µg	0	--	--	0
Vitamin A, RAE	µg	0	--	--	0
Retinol	µg	0	--	--	0
Carotene, beta	µg	1	26	0.676	1
Carotene, alpha	µg	0	9	0	0
Cryptoxanthin, beta	µg	0	8	0	0
Vitamin A, IU	IU	2	--	--	1
Lycopene	µg	0	8	0	0
Lutein + zeaxanthin	µg	1	8	0	1

Nutrient	Unit	1Value per 100 g	Data points	Std. Error	2.5 oz = 70.88g
Vitamin E (alpha-tocopherol)	mg	25.63	90	0.29	18.17
Vitamin E, added	mg	0	--	--	0
Tocopherol, beta	mg	0.23	90	0.071	0.16
Tocopherol, gamma	mg	0.64	90	0.07	0.45
Tocopherol, delta	mg	0.07	88	0.042	0.05
Vitamin D (D2 + D3)	µg	0	--	--	0
Vitamin D	IU	0	--	--	0
Vitamin K (phylloquinone)	µg	0	8	0	0
Lipids					
Fatty acids, total saturated	g	3.802	--	--	2.695
4:00	g	0	--	--	0
6:00	g	0	--	--	0
8:00	g	0	4	0	0
10:00	g	0	68	0	0
12:00	g	0	67	0	0
13:00	g	0	4	0	0
14:00	g	0.003	90	0.002	0.002
15:00	g	0	76	0	0
16:00	g	3.083	90	0.03	2.185
17:00	g	0.004	88	0.002	0.003
18:00	g	0.704	90	0.027	0.499
20:00	g	0.007	90	0.003	0.005
22:00	g	0.001	82	0	0.001
24:00:00	g	0	4	0	0
Fatty acids, total monounsaturated	g	31.551	--	--	22.362
14:01	g	0	71	0	0
15:01	g	0	66	0	0
16:1 undifferentiated	g	0.239	90	0.004	0.169
16:1 c	g	0.227	28	--	0.161
16:1 t	g	0.012	28	--	0.009
17:01	g	0.013	84	0.009	0.009
18:1 undifferentiated	g	31.294	90	0.284	22.18
18:1 c	g	31.294	33	--	22.18
18:1 t	g	0	47	0	0
20:01	g	0.005	85	0.003	0.004
22:1 undifferentiated	g	0	4	0	0
24:1 c	g	0	4	0	0
Fatty acids, total polyunsaturated	g	12.329	--	--	8.738
18:2 undifferentiated	g	12.324	90	0.177	8.735
18:2 n-6 c,c	g	12.32	61	0.269	8.732
18:2 CLAs	g	0.002	7	0.001	0.001
18:2 t not further defined	g	0.003	58	0.002	0.002
18:3 undifferentiated	g	0.003	90	0.002	0.002
18:3 n-3 c,c,c (ALA)	g	0.003	67	0.001	0.002
18:3 n-6 c,c,c	g	0	67	0	0
18:04	g	0	1	--	0
20:2 n-6 c,c	g	0.002	80	0.001	0.001

Nutrient	Unit	1Value per 100 g	Data points	Std. Error	2.5 oz = 70.88g
20:3 undifferentiated	g	0	56	0	0
20:4 undifferentiated	g	0	4	0	0
20:5 n-3 (EPA)	g	0	4	--	0
22:5 n-3 (DPA)	g	0	4	--	0
22:6 n-3 (DHA)	g	0	4	0	0
Fatty acids, total trans	g	0.015	--	--	0.011
Fatty acids, total trans-monoenoic	g	0.012	--	--	0.009
Cholesterol	mg	0	2	--	0
Stigmasterol	mg	4	82	0.75	3
Campesterol	mg	5	82	0.458	4
Beta-sitosterol	mg	130	68	6.608	92
Amino Acids					
Tryptophan	g	0.211	--	--	0.15
Threonine	g	0.601	--	--	0.426
Isoleucine	g	0.751	--	--	0.532
Leucine	g	1.473	--	--	1.044
Lysine	g	0.568	--	--	0.403
Methionine	g	0.157	--	--	0.111
Cystine	g	0.215	--	--	0.152
Phenylalanine	g	1.132	--	--	0.802
Tyrosine	g	0.45	--	--	0.319
Valine	g	0.855	--	--	0.606
Arginine	g	2.465	--	--	1.747
Histidine	g	0.539	--	--	0.382
Alanine	g	0.999	--	--	0.708
Aspartic acid	g	2.639	--	--	1.87
Glutamic acid	g	6.206	--	--	4.399
Glycine	g	1.429	--	--	1.013
Proline	g	0.969	--	--	0.687
Serine	g	0.912	--	--	0.646
Other					
Alcohol, ethyl	g	0	--	--	0
Caffeine	mg	0	--	--	0
Theobromine	mg	0	--	--	0
Flavonoids					
Anthocyanidins					
Cyanidin	mg	2.46	8	0.58	1.74
Petunidin	mg	0	8	0	0
Delphinidin	mg	0	8	0	0
Malvidin	mg	0	8	0	0
Pelargonidin	mg	0	8	0	0
Peonidin	mg	0	8	0	0
Flavan-3-ols					
(+)-Catechin	mg	1.3	12	0.33	0.9
(-)-Epigallocatechin	mg	2.6	3	0.31	1.8
(-)-Epicatechin	mg	0.6	12	0.1	0.4
(-)-Epicatechin 3-gallate	mg	0	4	0	0
(-)-Epigallocatechin 3-gallate	mg	0	2	--	0
(+)-Gallocatechin	mg	0	4	0	0
Flavanones					

Nutrient	Unit	1Value per 100 g	Data points	Std. Error	2.5 oz = 70.88g
Eriodictyol	mg	0.2	8	0.06	0.2
Hesperetin	mg	0	4	0	0
Naringenin	mg	0.4	51	0.05	0.3
Flavones					
Apigenin	mg	0	8	0	0
Luteolin	mg	0	4	0	0
Flavonols					
Isorhamnetin	mg	2.6	47	0.27	1.9
Kaempferol	mg	0.4	47	0.04	0.3
Myricetin	mg	0	8	0	0
Quercetin	mg	0.4	16	0.11	0.3
Isoflavones					
Daidzein	mg	0	2	--	0
Genistein	mg	0	2	--	0
Glycitein	mg	0	1	--	0
Total isoflavones	mg	0.01	2	--	0.01
Formononetin	mg	0	1	--	0
Coumestrol	mg	0.02	1	--	0.01
Proanthocyanidin					
Proanthocyanidin dimers	mg	9.3	17	3.16	6.6
Proanthocyanidin trimers	mg	7.6	17	2.71	5.4
Proanthocyanidin 4-6mers	mg	27.4	17	12.67	19.4
Proanthocyanidin 7-10mers	mg	28.2	17	10.93	20
Proanthocyanidin polymers (>10mers)	mg	80.3	8	28.09	56.9

Nutrient data obtained from the USDA National Nutrient Database for Standard Reference, Release 28 (2015), slightly revised May 2016

APPENDIX C[A]

SUMMARY OF EFFECTS OF ALMONDS ON CHOLESTEROL

Table 2. Summary of study design and duration, almond dose and baseline blood lipids

References	Study design		Dose (g/d)		Control food/ diet		Duration (weeks)		TC		LDL-C		HDL-C		TAG			
	P	X	≥45	<45	Pr	NPr	<12	≥12	BL*	EOT	BL*	EOT	BL*	EOT	BL*	EOT	TC: HDL-C	LDL-C: HDL-C
Abazarfard *et al.*[9]	✓		✓			✓		✓	N.O.	✓	N.O.	✓	O	✓	N.O.	✓	✓	–
Berryman *et al.*[10]		✓		✓	✓		✓		N.O.	✓	N.O.	✓	O	✓	O	✓	✓	✓
Cohen & Johnston[24]	✓			✓	✓			✓	O	✓	O	✓	–	–	N.O.	✓	–	–
Damasceno *et al.*[25]		✓	✓		✓		✓		N.O.	✓	N.O.	✓	O	✓	O	✓	–	✓
Foster *et al.*[11]	✓		✓			✓		✓	O	✓	N.O.	✓	O	✓	O	✓	✓	–
Jenkins *et al.* stratum 1[26]		✓		✓	✓		✓		N.O.	✓	N.O.	✓	O	✓	N.O.	✓	✓	✓
Jenkins *et al.* stratum 2[26]		✓	✓		✓		✓		N.O.	✓	N.O.	✓	O	✓	O	✓	✓	✓
Jia *et al.* stratum 1[13]	✓		✓		✓		✓		O	✓	–	–	–	–	O	✓	–	–
Jia *et al.* stratum 2[13]	✓		✓		✓		✓		O	✓	–	–	–	–	O	✓	–	–
Kurlandsky & Stote stratum 1[14]	✓		✓			✓	✓		N.O.	✓	N.O.	✓	O	✓	O	✓	–	–
Kurlandsky & Stote stratum 2[14]	✓		✓			✓	✓		O	✓	N.O.	✓	O	✓	O	✓	–	–
Li *et al.*[27]		✓	✓		✓		✓		N.O.	✓	N.O.	✓	O	✓	O	✓	–	✓
Lovejoy *et al.* stratum 1[28]		✓	✓		✓		✓		O	✓	N.O.	✓	O	✓	–	✓	✓	✓
Lovejoy *et al.* stratum 2[28]		✓	✓		✓		✓		O	✓	N.O.	✓	O	✓	–	✓	✓	✓
Ruisinger *et al.*[17]	✓		✓			✓	✓		O	✓	N.O.	✓	O	✓	N.O.	✓	–	–
Sabaté *et al.* stratum 1[18]		✓		✓	✓		✓		N.O.	✓	N.O.	✓	O	✓	O	✓	–	✓
Sabaté *et al.* stratum 2[18]		✓	✓		✓		✓		N.O.	✓	N.O.	✓	O	✓	O	✓	–	✓
Spiller *et al.* stratum 1[15]	✓		✓		✓		✓		N.O.	✓	N.O.	✓	–†	–†	–†	–†	–	–
Spiller *et al.* stratum 2[15]	✓		✓		✓		✓		N.O.	✓	N.O.	✓	–†	–†	–†	–†	–	–
Sweazea *et al.*[12]	✓			✓		✓		✓	O	✓	N.O.	✓	O	✓	N.O.	✓	–	–
Tamizifar *et al.*[31]		✓		✓		✓	✓		N.O.	✓	N.O.	✓	N.O.	✓	N.O.	✓	✓	–
Tan & Mattes stratum 1[32]	✓			✓		✓	✓		O	✓	O	✓	O	✓	O	✓	–	–
Tan & Mattes stratum 2[32]	✓			✓		✓	✓		O	✓	O	✓	O	✓	O	✓	–	–
Tan & Mattes stratum 3[32]	✓			✓		✓	✓		O	✓	O	✓	O	✓	O	✓	–	–
Tan & Mattes stratum 4[32]	✓			✓		✓	✓		O	✓	O	✓	O	✓	O	✓	–	–
Wien *et al.*[29]	✓		✓			✓		✓	N.O.	✓	N.O.	✓	N.O.	✓	N.O.	✓	–	✓
Wien *et al.*[30]	✓		✓			✓		✓	N.O.	✓	N.O.	✓	O	✓	O	✓	✓	–
Total	17	10	17	10	14	13	21	6	14 N.O. 13 O	27	20 N.O. 5 O	25	2 N.O. 20 O	22	7 N.O. 16 O	25	9	10

TC, total cholesterol; LDL-C; LDL-cholesterol; HDL-C; HDL-cholesterol; P, parallel; X, crossover; Pr, provided; NPr, not provided; BL, baseline; EOT, end of treatment; N.O., not optimal; O, optimal; –, not reported.
* Mean baseline TC, LDL-C, HDL-C and TAG were categorised as O or N.O., based on the targets established in the National Cholesterol Education Program Adult Treatment Panel III guidelines (i.e. optimal blood lipid levels were defined as: TC < 5·17 mmol/l; LDL-C < 2·59 mmol/l; HDL-C ≥ 1·03 mmol/l; TAG < 69 mmol/l).
† In the study by Spiller *et al.* strata 1 and 2[15], HDL-C and TAG levels were assessed at BL and at EOT; however, values were presented only in figure form, with no measures of variability. The results related to HDL-C and TAG could not be included in the meta-analyses.

Table 3. Effects of almonds on blood lipid levels: results of meta-analyses of randomised controlled trials*‡

		TC (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TAG (mmol/l)	TC:HDL-C	LDL-C:HDL-C
All strata		*n* 27 −0·153 (−0·235, −0·070) *P* < 0·001	*n* 25 −0·124 (−0·196, −0·051) *P* = 0·001	*n* 22 −0·017 (−0·043, 0·009) *P* = 0·207	*n* 25 −0·067 (−0·132, −0·002) *P* = 0·042	*n* 9 −0·207 (−0·382, −0·052) *P* = 0·009	*n* 10 −0·089 (−0·209, 0·031) *P* = 0·145
Almond dose (g/d)	≥45	*n* 17 −0·212 (−0·315, −0·108) *P* < 0·001	*n* 15 −0·132 (−0·209, −0·054) *P* = 0·001	*n* 13 −0·020 (−0·050, 0·010) *P* = 0·188	*n* 15 −0·071 (−0·159, 0·017) *P* = 0·114	*n* 6 −0·173 (−0·382, 0·037) *P* = 0·106	*n* 7 −0·065 (−0·230, 0·101) *P* = 0·445
	<45	*n* 10 −0·039 (−0·188, 0·109) *P* = 0·605	*n* 10 −0·060 (−0·223, 0·103) *P* = 0·470	*n* 9 −0·008 (−0·071, 0·055) *P* = 0·808	*n* 10 −0·064 (−0·162, 0·034) *P* = 0·199	*n* 3 −0·260 (−0·404, −0·116) *P* < 0·001	*n* 3 −0·186 (−0·279, −0·094) *P* < 0·001
Baseline lipid level	Not optimal	*n* 14 −0·271 (−0·394, −0·148) *P* < 0·001	*n* 20 −0·158 (−0·238, −0·078) *P* < 0·001	*n* 2; NA	*n* 7 −0·189 (−0·447, 0·069) *P* = 0·151	NA	NA
	Optimal	*n* 13 −0·044 (−0·125, 0·038) *P* = 0·294	*n* 5 0·100 (−0·064, 0·265) *P* = 0·232	*n* 20 0·003 (−0·016, 0·021) *P* = 0·773	*n* 16 −0·034 (−0·075, 0·007) *P* = 0·100	NA	NA
Study design	Crossover	*n* 10 −0·182 (−0·261, −0·103) *P* < 0·001	*n* 10 −0·205 (−0·316, −0·094) *P* < 0·001	*n* 10 −0·017 (−0·066, 0·031) *P* = 0·485	*n* 10 −0·017 (−0·112, 0·077) *P* = 0·723	*n* 6 −0·147 (−0·320, 0·026) *P* = 0·095	*n* 9 −0·138 (−0·210, −0·066) *P* < 0·001
	Parallel	*n* 17 −0·135 (−0·268, −0·002) *P* = 0·047	*n* 15 −0·048 (−0·117, 0·022) *P* = 0·178	*n* 12 −0·014 (−0·052, 0·023) *P* = 0·456	*n* 15 −0·111 (−0·204, −0·017) *P* = 0·020	*n* 3 −0·336 (−0·683, 0·021) *P* = 0·065	*n* 1; NA
Control food/diet	Provided	*n* 14 −0·147 (−0·241, −0·053) *P* = 0·002	*n* 12 −0·155 (−0·241, −0·069) *P* < 0·001	*n* 9 0·015 (−0·008, 0·038) *P* = 0·189	*n* 12 −0·062 (−0·132, 0·008) *P* = 0·081	*n* 5 −0·100 (−0·266, 0·067) *P* = 0·241	*n* 9 −0·138 (−0·210, −0·066) *P* < 0·001
	Not provided	*n* 13 −0·152 (−0·293, −0·011) *P* = 0·034	*n* 13 −0·093 (−0·193, 0·007) *P* = 0·068	*n* 13 −0·028 (−0·069, 0·014) *P* = 0·188	*n* 13 −0·085 (−0·188, 0·018) *P* = 0·106	*n* 3 −0·386 (−0·688, −0·084) *P* = 0·012	*n* 1; NA
Duration (weeks)	<12	*n* 21 −0·141 (−0·210, −0·072) *P* < 0·001	*n* 19 −0·151 (−0·231, −0·071) *P* < 0·001	*n* 19 −0·013 (−0·048, 0·022) *P* = 0·468	*n* 19 −0·046 (−0·100, 0·009) *P* = 0·101	*n* 6 −0·147 (−0·320, 0·026) *P* = 0·095	*n* 9 −0·138 (−0·210, −0·066) *P* < 0·001
	≥12	*n* 6 −0·169 (−0·520, 0·182) *P* = 0·346	*n* 6 −0·031 (−0·142, 0·079) *P* = 0·576	*n* 5 −0·027 (−0·100, 0·046) *P* = 0·467	*n* 6 −0·155 (−0·416, 0·106) *P* = 0·245	*n* 3 −0·336 (−0·683, 0·021) *P* = 0·065	*n* 1; NA

TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; NA, not applicable.
* For each meta-analysis, the results indicate (in vertical order from top to bottom): *n* (the number of strata), the pooled effect/point estimate, the 95 % CI, and the statistical significance of the point estimate. Subgroup analyses were conducted if there were at least three or more strata.
† An assessment of publication bias was conducted for each lipid parameter, but only for the meta-analysis that included all strata. Publication bias was not identified for TC, LDL-C, HDL-C or TAG. For the ratio of TC:HDL-C, two studies were found to be missing to the right of the pooled effect, and with the missing studies imputed, the pooled effect was −0·120 (95 % CI −0·289, 0·050). For the ratio of LDL-C:HDL-C, two studies were found to be missing to the right of the pooled effect, and with the missing studies imputed, the pooled effect was −0·059 (95 % CI −0·175, 0·056).

Study name	Baseline TC	Almond intake	Control food/diet	Study design	Statistics for each study: Difference in means	Lower limit	Upper limit	P
Spiller *et al.* stratum 2[17]	Not optimal	100 g	Provided	Parallel	-1.060	-1.883	-0.237	0.012
Abazarfard *et al.*[9]	Not optimal	50 g	Not provided	Parallel	-0.920	-1.277	-0.563	0.000
Spiller *et al.* stratum 1[17]	Not optimal	100 g	Provided	Parallel	-0.470	-1.055	0.115	0.115
Wien *et al.*[23]	Not optimal	60 g	Not provided	Parallel	-0.430	-0.742	-0.118	0.007
Tamizifar *et al.*[21]	Not optimal	25 g	Not provided	Crossover	-0.410	-0.607	-0.213	0.000
Li *et al.*[25]	Not optimal	56 g	Provided	Crossover	-0.300	-0.547	-0.053	0.017
Jia *et al.* stratum 1[15]	Optimal	84 g	Provided	Parallel	-0.250	-0.638	0.138	0.206
Sabaté *et al.* stratum 2[18]	Not optimal	68 g	Provided	Crossover	-0.240	-0.567	0.087	0.150
Jenkins *et al.* stratum 2[20]	Not optimal	73 g	Provided	Crossover	-0.230	-0.488	0.028	0.081
Jenkins *et al.* stratum 1[20]	Not optimal	37 g	Provided	Crossover	-0.190	-0.448	0.068	0.150
Ruisinger *et al.*[27]	Optimal	100 g	Not provided	Parallel	-0.190	-0.463	0.083	0.173
Damasceno *et al.*[19]	Not optimal	50 to 75 g	Provided	Crossover	-0.185	-0.458	0.087	0.182
Jia *et al.* stratum 2[15]	Optimal	168 g	Provided	Parallel	-0.180	-0.551	0.191	0.342
Wien *et al.*[28]	Not optimal	84 g	Not provided	Parallel	-0.150	-0.505	0.205	0.408
Berryman *et al.*[11]	Not optimal	43 g	Provided	Crossover	-0.130	-0.252	-0.008	0.036
Kurlandsky & Stote stratum 2[14]	Optimal	60 g	Not provided	Parallel	-0.120	-0.262	0.022	0.097
Lovejoy *et al.* stratum 2[26]	Optimal	57 to 113 g	Provided	Crossover	-0.060	-0.306	0.186	0.633
Foster *et al.*[16]	Optimal	56 g	Not provided	Parallel	-0.050	-0.287	0.187	0.680
Sabaté *et al.* stratum 1[18]	Not optimal	34 g	Provided	Crossover	-0.050	-0.377	0.277	0.764
Tan & Mattes stratum 3[22]	Optimal	43 g	Not provided	Parallel	-0.020	-0.426	0.386	0.923
Tan & Mattes stratum 4[22]	Optimal	43 g	Not provided	Parallel	-0.020	-0.439	0.399	0.925
Lovejoy *et al.* stratum 1[26]	Optimal	57 to 113 g	Provided	Crossover	0.000	-0.246	0.246	1.000
Kurlandsky & Stote stratum 1[14]	Not optimal	60 g	Not provided	Parallel	0.020	-0.112	0.152	0.766
Tan & Mattes stratum 1[22]	Optimal	43 g	Not provided	Parallel	0.150	-0.197	0.497	0.396
Tan & Mattes stratum 2[22]	Optimal	43 g	Not provided	Parallel	0.170	-0.289	0.629	0.468
Sweazea *et al.*[10]	Optimal	30.7 to 43 g	Not provided	Parallel	0.190	-0.310	0.690	0.456
Cohen & Johnston[24]	Optimal	20 g	Provided	Parallel	0.400	0.037	0.763	0.031
					-0.153	-0.235	-0.070	0.000

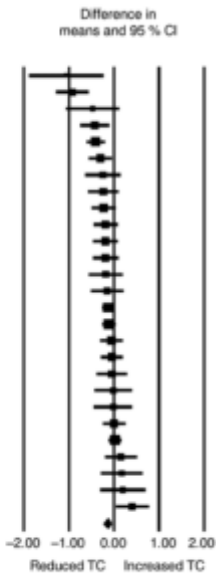


Fig. 2. Effect of almond consumption on total cholesterol (TC).

Study name	Baseline LDL-C	Almond intake	Control food/diet	Study design	Statistics for each study: Difference in means	Lower limit	Upper limit	P
Spiller *et al.* stratum 2[17]	Not optimal	100 g	Provided	Parallel	-0.854	-1.610	-0.098	0.027
Tamizifar *et al.*[21]	Not optimal	25 g	Not provided	Crossover	-0.569	-0.748	-0.390	0.000
Spiller *et al.* stratum 1[17]	Not optimal	100 g	Provided	Parallel	-0.414	-0.895	0.067	0.092
Li *et al.*[25]	Not optimal	56 g	Provided	Crossover	-0.400	-0.663	-0.137	0.003
Wien *et al.*[23]	Not optimal	60 g	Not provided	Parallel	-0.286	-0.560	-0.012	0.041
Sabaté *et al.* stratum 2[18]	Not optimal	68 g	Provided	Crossover	-0.260	-0.621	0.101	0.158
Damasceno *et al.*[19]	Not optimal	50-75 g	Provided	Crossover	-0.247	-0.419	-0.076	0.005
Jenkins *et al.* stratum 2[20]	Not optimal	73 g	Provided	Crossover	-0.210	-0.427	0.007	0.057
Ruisinger *et al.*[27]	Not optimal	100 g	Not provided	Parallel	-0.190	-0.405	0.025	0.084
Berryman *et al.*[11]	Not optimal	43 g	Provided	Crossover	-0.140	-0.236	-0.044	0.004
Jenkins *et al.* stratum 1[20]	Not optimal	37 g	Provided	Crossover	-0.120	-0.337	0.097	0.278
Wien *et al.*[28]	Not optimal	84 g	Not provided	Parallel	-0.104	-0.331	0.123	0.369
Foster *et al.*[16]	Not optimal	56 g	Not provided	Parallel	-0.077	-0.263	0.109	0.418
Lovejoy *et al.* stratum 2[26]	Not optimal	57 to 113 g	Provided	Crossover	-0.070	-0.245	0.105	0.434
Kurlandsky & Stote stratum 1[14]	Not optimal	60 g	Not provided	Parallel	-0.070	-0.172	0.032	0.179
Sabaté *et al.* stratum 1[18]	Not optimal	34 g	Provided	Crossover	-0.040	-0.401	0.321	0.828
Kurlandsky & Stote stratum 2[14]	Not optimal	60 g	Not provided	Parallel	-0.030	-0.157	0.097	0.643
Lovejoy *et al.* stratum 1[26]	Not optimal	57 to 113 g	Provided	Crossover	0.000	-0.175	0.175	1.000
Tan & Mattes stratum 4[22]	Optimal	43 g	Not provided	Parallel	0.005	-0.366	0.376	0.979
Abazarfard *et al.*[9]	Not optimal	50 g	Not provided	Parallel	0.025	0.009	0.041	0.002
Tan & Mattes stratum 3[22]	Optimal	43 g	Not provided	Parallel	0.033	-0.307	0.373	0.849
Tan & Mattes stratum 1[22]	Optimal	43 g	Not provided	Parallel	0.054	-0.283	0.391	0.753
Sweazea *et al.*[10]	Not optimal	30.7 to 43 g	Not provided	Parallel	0.120	-0.353	0.593	0.619
Tan & Mattes stratum 2[22]	Optimal	43 g	Not provided	Parallel	0.196	-0.188	0.580	0.317
Cohen & Johnston[24]	Optimal	20 g	Provided	Parallel	0.300	-0.138	0.738	0.179
					-0.124	-0.196	-0.051	0.001

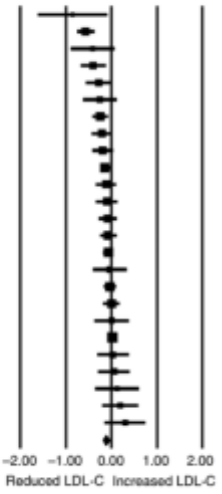


Fig. 3. Effect of almond consumption on LDL-cholesterol (LDL-C).

Study name	Baseline HDL-C	Almond intake	Control food/diet	Study design	Statistics for each study			
					Difference in means	Lower limit	Upper limit	*P*
Sweazea *et al.*[10]	Optimal	30.7 to 43 g	Not provided	Parallel	0.070	-0.014	0.154	0.104
Foster *et al.*[16]	Optimal	56 g	Not provided	Parallel	0.060	-0.058	0.178	0.321
Ruisinger *et al.*[27]	Optimal	100 g	Not provided	Parallel	0.060	-0.065	0.185	0.347
Jenkins *et al.* stratum 2[20]	Optimal	73 g	Provided	Crossover	0.040	-0.110	0.190	0.600
Kurlandsky & Stote stratum 1[14]	Optimal	60 g	Not provided	Parallel	0.040	-0.013	0.093	0.142
Berryman *et al.*[11]	Optimal	43 g	Provided	Crossover	0.040	0.009	0.071	0.012
Jenkins *et al.* stratum 1[20]	Optimal	37 g	Provided	Crossover	0.020	-0.119	0.159	0.778
Sabaté *et al.* stratum 2[18]	Optimal	68 g	Provided	Crossover	0.020	-0.066	0.106	0.648
Tan & Mattes stratum 1[22]	Optimal	43 g	Not provided	Parallel	0.020	-0.202	0.242	0.860
Tan & Mattes stratum 3[22]	Optimal	43 g	Not provided	Parallel	0.010	-0.192	0.212	0.923
Li *et al.*[25]	Optimal	56 g	Provided	Crossover	0.000	-0.261	0.261	1.000
Abazarfard *et al.*[9]	Optimal	50 g	Not provided	Parallel	-0.009	-0.015	-0.003	0.003
Sabaté *et al.* stratum 1[18]	Optimal	34 g	Provided	Crossover	-0.010	-0.096	0.076	0.820
Tan & Mattes stratum 2[22]	Optimal	43 g	Not provided	Parallel	-0.020	-0.153	0.113	0.769
Damasceno *et al.*[19]	Optimal	50 to 75 g	Provided	Crossover	-0.023	-0.128	0.082	0.669
Lovejoy *et al.* stratum 1[26]	Optimal	57 to 113 g	Provided	Crossover	-0.030	-0.100	0.040	0.404
Lovejoy *et al.* stratum 2[26]	Optimal	57 to 113 g	Provided	Crossover	-0.040	-0.110	0.030	0.266
Tan & Mattes stratum 4[22]	Optimal	43 g	Not provided	Parallel	-0.040	-0.188	0.108	0.596
Kurlandsky & Stote stratum 2[14]	Optimal	60 g	Not provided	Parallel	-0.070	-0.139	-0.001	0.048
Wien *et al.*[23]	Optimal	60 g	Not provided	Parallel	-0.080	-0.191	0.031	0.156
Tamizifar *et al.*[21]	Not optimal	25 g	Not provided	Crossover	-0.130	-0.179	-0.081	0.000
Wien *et al.*[28]	Not optimal	84 g	Not provided	Parallel	-0.180	-0.271	-0.089	0.000
					-0.017	-0.043	0.009	0.207

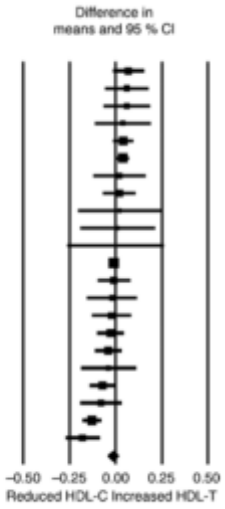


Fig. 4. Effect of almond consumption on HDL-cholesterol (HDL-C).

Study name	Baseline TAG	Almond intake	Control food/diet	Study design	Statistics for each study			
					Difference in means	Lower limit	Upper limit	*P*
Abazarfard *et al.*[9]	Not optimal	50 g	Not provided	Parallel	-0.720	-0.996	-0.444	0.000
Ruisinger *et al.*[27]	Not optimal	100 g	Not provided	Parallel	-0.320	-0.723	0.083	0.120
Tan & Mattes stratum 4[22]	Optimal	43 g	Not provided	Parallel	-0.250	-0.503	0.003	0.052
Jenkins *et al.* stratum 1[20]	Not optimal	37 g	Provided	Crossover	-0.220	-0.419	-0.021	0.030
Cohen & Johnston[24]	Not optimal	20 g	Provided	Parallel	-0.200	-1.119	0.719	0.670
Jenkins *et al.* stratum 2[20]	Optimal	73 g	Provided	Crossover	-0.160	-0.359	0.039	0.114
Jia *et al.* stratum 1[15]	Optimal	84 g	Provided	Parallel	-0.140	-0.501	0.221	0.448
Tan & Mattes stratum 3[22]	Optimal	43 g	Not provided	Parallel	-0.130	-0.499	0.239	0.490
Li *et al.*[25]	Optimal	56 g	Provided	Crossover	-0.100	-0.347	0.147	0.427
Sweazea *et al.*[10]	Not optimal	30.7 to 43 g	Not provided	Parallel	-0.100	-0.411	0.211	0.529
Kurlandsky & Stote stratum 1[14]	Optimal	60 g	Not provided	Parallel	-0.090	-0.221	0.041	0.177
Tan & Mattes stratum 2[22]	Optimal	43 g	Not provided	Parallel	-0.080	-0.368	0.208	0.586
Berryman *et al.*[11]	Optimal	43 g	Provided	Crossover	-0.080	-0.213	0.053	0.238
Jia *et al.* stratum 2[15]	Optimal	168 g	Provided	Parallel	-0.080	-0.401	0.241	0.625
Sabaté *et al.* stratum 2[18]	Optimal	68 g	Provided	Crossover	-0.050	-0.377	0.277	0.764
Wien *et al.*[23]	Optimal	60 g	Not provided	Parallel	-0.030	-0.253	0.193	0.792
Kurlandsky & Stote stratum 2[14]	Optimal	60 g	Not provided	Parallel	-0.010	-0.068	0.048	0.736
Sabaté *et al.* stratum 1[18]	Optimal	34 g	Provided	Crossover	0.000	-0.327	0.327	1.000
Wien *et al.*[28]	Not optimal	84 g	Not provided	Parallel	0.000	-0.341	0.341	1.000
Foster *et al.*[16]	Optimal	56 g	Not provided	Parallel	0.070	-0.118	0.258	0.464
Tan & Mattes stratum 1[22]	Optimal	43 g	Not provided	Parallel	0.090	-0.232	0.412	0.584
Lovejoy *et al.* stratum 2[26]	NR	57 to 113 g	Provided	Crossover	0.090	-0.227	0.407	0.578
Lovejoy *et al.* stratum 1[26]	NR	57 to 113 g	Provided	Crossover	0.100	-0.217	0.417	0.536
Damasceno *et al.*[19]	Optimal	50 to 75 g	Provided	Crossover	0.149	-0.052	0.349	0.147
Tamizifar *et al.*[21]	Not optimal	25 g	Not provided	Crossover	0.190	0.003	0.377	0.047
					-0.067	-0.132	-0.002	0.042

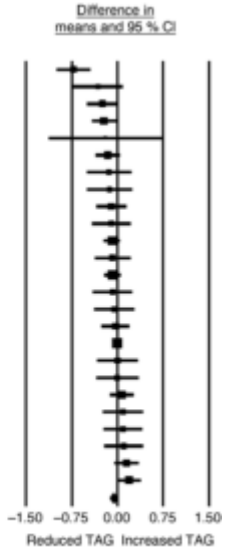


Fig. 5. Effect of almond consumption on TAG.

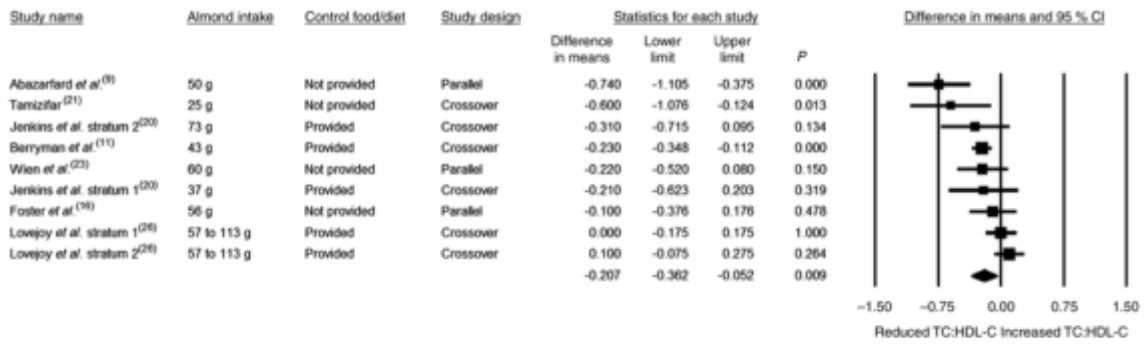


Fig. 6. Effect of almond consumption on the ratio of total cholesterol:HDL-cholesterol (TC:HDL-C).

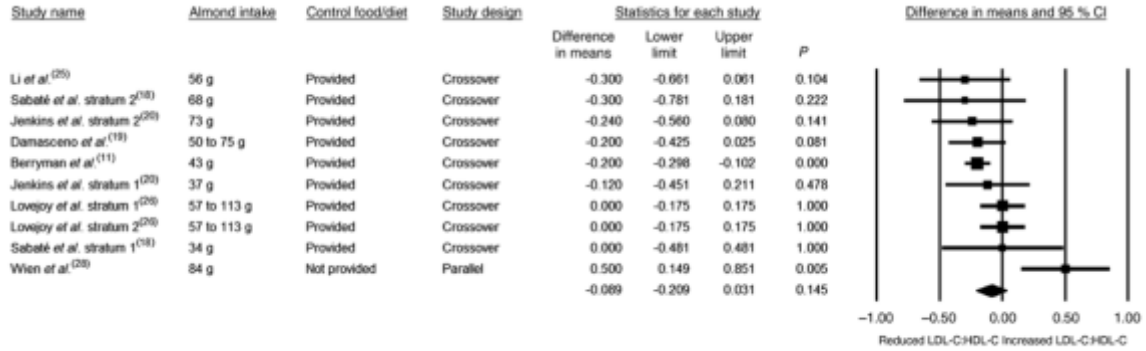


Fig. 7. Effect of almond consumption on the ratio of LDL-cholesterol:HDL-cholesterol (LDL-C:HDL-C).

[A] Data obtained from Musa-Veloso K, Paulionis L, Poon T, Lee HY. The effects of almond consumption on fasting blood lipid levels: a systematic review and meta-analysis of randomised controlled trials. Journal of Nutritional Science. 2016;5.

APPENDIX D

SUMMARY OF EFFECTS OF ALMONDS ON OXIDATIVE STRESS

Table 4. Effect of Almonds on Oxidative Stress in Clinical Interventions

ref	subjects	study design/duration	diet intervention	results
Jambazian et al., 2005[90]	8 F, 8 M, healthy	crossover, controlled, 4 weeks	28 or 58 g/day almonds vs baseline diet	plasma α-tocopherol concentrations: 13.7 and 18.7% ↑
Jenkins et al., 2008[47]	12 F, 15 M, hyperlipidemic	crossover, 3 months	36.5 or 73 g/day almonds vs baseline diet	for 73 g/day almonds, serum MDA: 19% ↓ urinary isoprostanes: 15% ↓
Jalali-Kharabadi et al., 2010[48]	30 M, healthy	parallel, 4 weeks	60 g/day almonds vs baseline diet	LDL oxidizability: 30% ↓ (prolonged lag time)
Chen et al., 2011[49]	20 subjects, T2DM	crossover, 4 weeks	~60 g/day almonds vs NCEP Step 2 diet	circulating oxidized LDL-C: ↓ serum protein carbonyls content: ↓
Hyson et al., 2002[38]	12 F, 10 M, healthy	crossover, 6 weeks	66 g whole almonds and 35 g almond oil vs baseline diet	LDL oxidizability: no change
Jenkins et al., 2006[35]	8 F, 7 M, healthy	crossover, controlled feeding, 10 weeks	60 g almonds added to composite meal vs parboiled rice and potatoes	increased postprandial serum protein thiol concentrations (15 mmol/L) were 50% greater than rice and potatoes (10 mmol/L)
Li et al., 2007[34]	60 subjects, smokers	crossover, 4 weeks	84 g/day almonds vs baseline diet	lymphocyte DNA strand breaks: ↓ urinary 8-hydroxy-deoxyguanosine: ↓ activities of serum superoxide dismutase: ↑ activities of glutathione peroxidase: ↑

APPENDIX E

SAMPLE SIZE CALCULATION

Author	Year	FMD	N per grp	Calculated n per grp	Age range	Subject state	Test
Chen et al.	2015	.6 ± 3.5	25	536	61.8 ± 8.6	Proven CAD (M/F)	Randomized control crossover trial (almonds)
West et al.	2012	.83 ± 3.3	27	250	48 ± 1.5	Elevated LDL (≥ 2.86 mmol/L); otherwise healthy/nonsmokers (M/F)	Randomized crossover controlled-feeding study (pistachios)
Kasliwal et al.	2015	2.5 ± 5.5	27	77	39.8 ± 8.1	Mild dyslipidemia and no DM/CVD (M/F)	Open label, randomized parallel group study (pistachios)
West et al.	2010	2.1 ± 3.8	12	53	49.3 ± 1.7	hypercholesterolemic (M/F)	Randomized, crossover study (walnut/flax oil)
Orem et al.	2013	6.6 ± 5.6	21	13	44.6 ± 10.4	hypercholesterolemic (M/F)	Double control sandwich model (hazelnuts)
Average		2.5 ± 4.34	23	186	48.7 ± 6.06		

All data represented as means ±SD; FMD = flow-mediated dilation; CAD = coronary artery disease; M = male. F = female; LDL = low density lipoprotein; DM = diabetes mellitus; CVD = cardiovascular disease

APPENDIX F

RECRUITMENT ADVERTISEMENTS

Step into 2016!

ASU is starting a research trial to examine the impact of nuts on exercise performance

THE NUTRITION PROGRAM AT ASU IS RECRUITING HEALTHY MEN AND POST-MENOPAUSAL WOMEN (20-69 y of age) TO EXAMINE WHETHER NUT CONSUMPTION ENHANCES THE HEALTH EFFECTS OF WALKING

Participation will include:

- Traveling to the ASU's Downtown campus on four test days over an 8-week period (lasting about 45 minutes each).
- Initiating a walking program with the goal of walking 10,000 steps per day after a few weeks.
- During the trial, you will record your step count daily and ingest the nut or nut product daily during study weeks 6-8.
- Testing will include fitness assessments and cardiovascular testing.

You will receive $50 in Target gift cards INTERESTED?? Please visit our recruitment site:
https://www.surveymonkey.com/r/ASUNutStudy

Can nuts enhance the health effects of walking?

The ASU Nutrition Program is recruiting men and post-menopausal women (ages 20-69) to examine whether nut consumption enhances the health effects of walking. Study participation includes an 8-week walking program with the goal of walking 10,000 steps daily after several weeks. Nut consumption would take place during study weeks 6-8. Testing will include fitness assessments and cardiovascular testing.

Participation in this study is voluntary. Participants will receive test foods and $50 in Target gift cards during the study.

For more information or to apply for the study, please visit our recruitment site:
https://www.surveymonkey.com/r/ASUNutStudy

APPENDIX G

SURVEYMONKEY SCREENING SURVEY

Exit this survey

Nuts and Health

Thank you for your interest in this research study conducted by Drs. Carol Johnston and Karen Sweazea, ASU nutrition professors, and Nutrition Master's students, Elizabeth McElaney and Emily Schwab. This survey will ask questions to determine if you may qualify for this study. You may quit the survey at any time if you do not want to continue answering questions. If you complete the survey, you will be contacted via email and told whether you qualify for the study. If you wish to continue to participate in the recruitment process, you can reply to the email. If you have any questions, please contact Dr. Johnston at carol.johnston@asu.edu or Dr. Sweazea at karen.sweazea@asu.edu. Information collected from this survey may be used in research reports in aggregate form only.

Thank you for your interest in research conducted in the School of Nutrition and Health Promotion.

* 1. Please provide your email address

* 2. Please select your gender

- ○ Male
- ○ Female

* 3. What is your age?

Age (years)

* 4. Please enter your height and weight

Height (inches)

Weight (pounds)

* 5. If female, are you post-menopausal?

- ○ Yes
- ○ No
- ○ not female

* 6. Are you generally healthy and free of chronic disease? (e.g., cancer, heart disease, asthma, arthritis, and/or hypertension)

- ○ Yes
- ○ No
- ○ Unsure

* 7. Do you have a nut, gluten, or other food allergy?

- ◯ Yes
- ◯ No
- ◯ If 'yes' please specify

* 8. Are you interested in undertaking an 8-week long walking program with the goal of walking 10,000 steps daily after a few weeks? (You will be provided with a pedometer to monitor your daily step counts.)

- ◯ Yes
- ◯ No

* 9. Do you have any physical ailments that would interfere with your ability to participate in a walking program?

- ◯ Yes
- ◯ No

* 10. Over a 7-day period, how many minutes do you engage in moderate intensity exercise?

* 11. Over a 7-day period, how many minutes do you engage in vigorous intensity exercise?

12. During the workweek, how many hours per day do you typically sit? (e.g., working on computer, reading, doing paperwork, playing games, watching TV or movies, in the car, etc.)

* 13. Are you willing to consume the study nuts or nut products daily during study weeks 6-8?

- ◯ Yes
- ◯ No

* 14. Do you smoke?

- ◯ Yes
- ◯ No

* 15. Are you willing to meet with investigators at the Nutrition Laboratory on the downtown Phoenix campus for ~45 minutes on four occasions to undergo a fitness test and cardiovascular test? You will be compensated for your time at the end of visits 3 and 4 target gift cards ($25 + $25).

- ◯ Yes
- ◯ No

* 16. Answer yes or no to the following questions.

- Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
- Do you feel pain in your chest when you do physical activity?
- In the past month, have you had chest pain when you were not doing physical activity?
- Do you lose your balance because of dizziness or do you ever lose consciousness?
- Do you have a bone or joint problem that could be made worse by a change in your physical activity?
- Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
- Do you know of any other reason why you should not do physical activity?

◯ Yes

◯ No

Thank you for your interest in ASU research.

Done

APPENDIX H

INFORMED CONSENT

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.

What happens if I say yes, but I change my mind later?

You can leave the research at any time it will not be held against you. If you decide to leave the research, there will be no adverse consequences, but please let the investigators know of your intentions.

Please turn over →

Is there any way being in this study could be bad for me?

You may feel temporarily nauseous or faint during the blood draw. Blood draws are performed by a registered nurse or a trained phlebotomist who are experienced in handling these issues. The measurement of blood vessel function entails placing a blood pressure cuff on your arm and pumping air into the cuff to cut off blood circulation. You will feel pressure on your arm for 5 minutes. In other research, participants ranked the pain associated with this procedure a '2' on a 10-point scale. A trained sonographer (ultrasound technician) will perform this procedure. You will initiate a walking program for this trial, and as with any increase in physical activity there is a possibility of injury. To qualify for the trial, you passed a validated screening tool indicating that you are at low risk for adverse events from physical activity. Taking part in this research study may lead to added costs and time commitments. You may need to pay the city meter for curbside parking (costs should be about $1.50 per visit).

Will being in this study help me any way?

You may improve your fitness level by participating in this trial since you will increase your physical activity level by walking 10,000 steps daily. Importantly, your participation will help the scientists advance knowledge regarding nut supplementation for improving cardiovascular health.

What happens to the information collected for the research?

Efforts will be made to limit the use and disclosure of your personal information, including research study data, to people who have a need to review this information. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the Institutional Review Board at Arizona State University and other representatives of this organization. All data and blood samples collected during this study will be identified only by a number assigned to you and stored in a secure setting in the ABC laboratory.

What else do I need to know?

If you agree to participate in the study, written consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury. You will receive a $25 at 3 and 4 ($50 total).

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the research team at Arizona State University. You may email or call the investigators [Carol.Johnston@asu.edu (602)827-2265 or Karen.Sweazea@asu.edu (480)965-6025] to express any concerns.

This research has been reviewed and approved by the Bioscience Institutional Review Board at ASU. You may talk to them at (480) 965-6788 or research.integrity@asu.edu if:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research participant.
- You want to get information or provide input about this research.

Your signature documents your permission to take part in this research.

Signature of participant	Date
Printed name of participant	Email or phone#
Signature of person obtaining consent	Date

APPENDIX I

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 →

7. Would you be able to restrict all nuts and nut product consumption to twice weekly during the 8-week study (aside from that pertaining to the study) for 3 weeks? Y N

8. Aside from the nut restriction, will you be willing to maintain your normal diet and eating habits during the trial? Y N

9. Do you follow a specific diet? (weight loss/gain, vegetarian, low-fat, etc.) Y N

If yes, please explain _____

10. Are you able to follow this pre-visit protocol for visits 2, 3, and 4: abstain from dietary supplements for 3 days, abstain from caffeine for 12 hours, abstain from heavy exercise for 12 hours prior to testing, and fast overnight (12 h)? Y N

11. Please circle the total time you spend in each category for an average week.

Light activities such as: slow walking, golf, easy swimming, gardening, etc.

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Moderate activities such as: moderate walking, cycling, swimming, weight lifting, etc.

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Vigorous activities such as: fast walking, jogging, cycling, heavy/intense weight lifting, etc.

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

12. Are you currently training to compete in a sport? Y N

If yes, please explain your training schedule:

_____

13. Please describe any other medical conditions or situations that may affect your ability to participate in this research trial (i.e., infections, travel, work deadlines, etc.).

_____

_____

APPENDIX J

COOKIE BUTTER & ALMOND NUTRITION LABEL COMPARISON

Almond Nutrition Facts

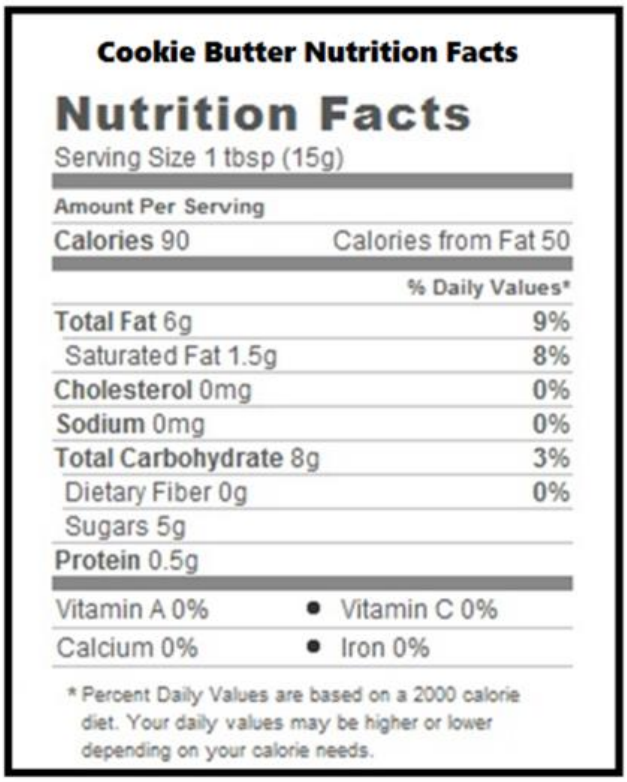
Serving Size 1 ounce (28g)
or about 23 almonds

Amount Per Serving
Calories 160 Calories from Fat 120

	% Daily Value*
Total Fat 14g	22%
Saturated Fat 1g	5%
Polyunsaturated Fat 3.5g	
Monounsaturated Fat 9g	
Cholesterol 0mg	0%
Sodium 0mg	0%
Potassium 200mg	6%
Total Carbohydrate 6g	2%
Dietary Fiber 3g	12%
Sugars 1g	
Protein 6g	

Vitamin A 0%	•	Vitamin C 0%
Calcium 8%	•	Iron 6%
Vitamin E 35%	•	Folate 4%
Magnesium 20%	•	Phosphorus 15%

*Percent daily values are based on a 2,000 calorie diet.



Cookie Butter Nutrition Facts

Nutrition Facts

Serving Size 1 tbsp (15g)

Amount Per Serving

Calories 90	Calories from Fat 50
	% Daily Values*
Total Fat 6g	9%
Saturated Fat 1.5g	8%
Cholesterol 0mg	0%
Sodium 0mg	0%
Total Carbohydrate 8g	3%
Dietary Fiber 0g	0%
Sugars 5g	
Protein 0.5g	

Vitamin A 0%	•	Vitamin C 0%
Calcium 0%	•	Iron 0%

* Percent Daily Values are based on a 2000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

APPENDIX K

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.

[1]Haskell, W. L., Lee, I. M., Pate, R. R., Powell, K. E., Blair, S. N., Franklin, B. A., et al. (2007). Physical activity and public health: Updated recommendation for adults from the american college of sports medicine and the american heart association. Circulation, 116(9), 1081-1093.
[2]Ye, M., Fu, J., Zhou, L., Gao, H., Fan, C., Shao, J., et al. (2014). The effect of almond consumption on elements of endurance exercise performance in trained athletes. Journal of the International Society of Sports Nutrition, 11, 18-2783-11-18. eCollection 2014.
[3]Abazarfard, Z., Salehi, M., & Keshavarzi, S. (2014). The effect of almonds on anthropometric measurements and lipid profile in overweight and obese females in a weight reduction program: A randomized controlled clinical trial. Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences, 19(5), 457-464.
[4]Foster, G. D., Shantz, K. L., Vander Veur, S. S., Oliver, T. L., Lent, M. R., Virus, A., et al. (2012). A randomized trial of the effects of an almond-enriched, hypocaloric diet in the treatment of obesity. The American Journal of Clinical Nutrition, 96(2), 249-254.
[5]Versari D, Daghini E, Virdis A, Ghiadoni L, Taddei S. Endothelial dysfunction as a target for prevention of cardiovascular disease. Diabetes Care. 32(Suppl 2): S314-S321, 2009.
[6]McKechnie R, Rubenfire M, Mosca L. Association between self-reported physical activity and vascular reactivity in postmenopausal women. Atherosclerosis. 2001 Dec;159(2):483-90.
[7]Sung, J., Choi, Y. H., & Park, J. B. (2006). Metabolic syndrome is associated with delayed heart rate recovery after exercise. Journal of Korean Medical Science, 21(4), 621-626.

4 Inclusion and Exclusion Criteria

APPENDIX L

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 M

FLOW-MEDIATED DILATION PROTOCOL

Endothelium-dependent dilation of the brachial artery will be measured by B-mode ultrasound (Terason u3300+^{TM}, Burlington MA) using guidelines set forth by the Brachial Artery Reactivity Task Force. Participants will be asked to lie quietly for 15 minutes on the ultrasound table before baseline images are obtained for 30 seconds from the participant's non-dominant arm. Simultaneous ultrasound images (B-mode) and Doppler waveforms will always be recorded. Subsequently, an appropriately sized blood pressure cuff will be wrapped around the patient's forearm. After the baseline images are acquired, the blood pressure cuff will be rapidly inflated using a rapid-cuff inflator (Hokanson) on the participant's forearm to >200mmHg for 5 minutes. Thirty seconds prior to cuff release, imaging of the brachial artery will commence. At 5 minutes following cuff inflation, the cuff will be rapidly deflated and arterial images will be recorded for up to 5 minutes. Images that are obtained will be analyzed by a blinded researcher using a previously validated, brachial artery edge-detection software. Intra-class correlation coefficients in our lab for baseline and peak diameter are 0.994 and 0.995 respectively (Chronbach $\alpha = 0.976$).

APPENDIX N

NITRIC OXIDE ASSAY PROTOCOL

1. Add 200 µl of water or Assay Buffer to the blank wells. Do not add any other reagents to these wells.

2. Add up to 80 µl of sample or sample dilutions to the wells in a pattern you choose. The final volume must be adjusted to 80 µl using the Assay Buffer solution. *NOTE: Plasma samples should be assayed with no more than 40 µl when undiluted samples are used (Samples which have been diluted 1:2 or greater can use up to 80 µl in the assay). Caution should be taken when pipetting plasma samples to ensure no bubbles enter to the well as this will lead to erroneous results.*

3. Add 10 µl of the Enzyme Cofactor Mixture (Item No. 780012) to each of the wells (standards and unknowns).

4. Add 10 µl of the Nitrate Reductase Mixture (Item No. 780010) to each of the wells (standards and unknowns).

5. Cover the plate with the plate cover and incubate at room temperature for one hour. *NOTE: This incubation time should be increased to two hours when assaying tissue culture medium, and increased to three hours when assaying plasma or tissue nitrate + nitrite concentrations. It is not necessary to shake the plate during incubation.*

6. After the required incubation time, add 50 µl of Griess Reagent R1 (Item No. 780018) to each of the wells (standards and unknowns).

7. Immediately add 50 µl of Griess Reagent R2 (Item No. 780020) to each of the wells (standards and unknowns).

8. Allow the color to develop for 10 minutes at room temperature. It is not necessary to cover the plate. *NOTE: The 10 minute incubation is optimal for color development. However, if the plate has been left to develop for longer time periods the data is still*

valid, provided the Griess Reagents have been added to the standard curve and unknowns at the same time. Developing the standard curve at the same time as the unknowns ensures the presence of an accurate control.

9. Read the absorbance at 540 nm or 550 nm using a plate reader.

APPENDIX O

TBARS ASSAY PROTOCOL

Allow all reagents to reach room temperature before use. SDS Solution will take at least one hour if stored at 2-8°C. Heating the SDS Solution at 37˚C briefly will redissolve precipitated SDS. SDS Solution can then be stored at room temperature.

Step 1: Collect EDTA plasma (lavender top Vacutainer®).

Step 2: Label two sets of disposable microcentrifuge tube with the standard number or sample identification. Poke a hole in the lid of each tube.

Step 3: Add 30 µl sample or standard (prepare malondialdehyde standard curve as described in the protocol) to properly labeled tube.

Step 4: Add 30 µl SDS Solution to each tube and swirl to mix.

Step 5: Add 750 ml TBA/Buffer Reagent forcefully down the side of each tube.

Step 6: Incubate each tube at 95ºC for 60 min.

Step 7: Remove from incubation and cool to room temperature in an ice bath for 10 min.

Step 8: Centrifuge samples at 3000 rpm for 15 min.

Step 9: Remove supernatant from samples for analysis.

Step 10: Aliquot 150 µl of each standard or sample to duplicate wells in a 96-well plate and read absorbance of supernatants at 532 nm.