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Journal

Nutrition, Metabolism & Cardiovascular Diseases, 33(8)

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Publication Date

2023-08-01

DOI

10.1016/j.numecd.2023.05.013

Peer reviewed

Published in final edited form as:
Nutr Metab Cardiovasc Dis. 2023 August ; 33(8): 1529–1538. doi:10.1016/j.numecd.2023.05.013.

Effects of mixed nut consumption on LDL cholesterol, lipoprotein(a), and other cardiometabolic risk factors in overweight and obese adults

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Abstract

Background and aims: Elevated LDL-C, lipoprotein(a) [Lp(a)], and inflammation are associated with greater risk for atherosclerotic cardiovascular events. Consumption of individual nut types decreases these risk factors but knowledge about the effect of mixed nuts on Lp(a) is limited. The objective of this study was to determine the effects of consuming 42.5 g/day of mixed nuts on LDL-C, Lp(a), and inflammatory markers in individuals with overweight or obesity.

Methods and results: In a 16-week randomized control trial, 29 participants with overweight or obesity (BMI 25–40 kg/m^2) consumed either 42.5 g/day of mixed nuts (cashews, almonds, macadamia nuts, Brazil nuts, pecans, pistachios, walnuts, and peanuts) or 69 g/day isocaloric pretzels. Blood samples were collected at baseline, week 8, and week 16 for analysis on total cholesterol (TC), LDL-C, Lp(a), inflammation markers, glucose, insulin, adiponectin and liver function enzymes. No significant differences were seen in TC, LDL-C, HDL-C, Lp(a), or liver function enzymes between the two groups. Participants consuming mixed nuts had significantly lower body fat percentage and diastolic blood pressure, and higher adiponectin (all $P \leq 0.05$). C-reactive protein (CRP) and 8-oxo-deoxyguanosis (8-oxodG) showed non-significant decreasing

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Authors' contributions

Chelsea L. Nora: Conceptualization, Methodology, Formal analysis, Data curation, Writing original draft, and Visualization; **Liyue Zhang:** Investigation and Writing-reviewing and editing; **Robert J. Castro:** Investigation and Writing-reviewing and editing; **Amanda Marx:** Investigation and Project administration; **Hannah B. Carman:** Investigation; **Tiffany Lum:** Investigation and Writing-reviewing and editing; **Sotirios Tsimikas:** Conceptualization, Methodology, Investigation, Resources, and Writing-reviewing and editing; **Mee Young Hong:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing-reviewing and editing, Supervision and Funding acquisition.

Declaration of Competing Interest

The authors disclose no conflict of interest.

Clinical trial

The study was registered at clinicaltrials.gov (#NCT03375866).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2023.05.013.

trends and total antioxidant capacity (TAC) had a non-significant increasing trend in the mixed nut group.

Conclusion: Consumption of mixed nuts had no evidence of an effect on LDL-C or Lp(a) throughout the intervention. Notably, mixed nut consumption lowered body fat percentage without significant changes in body weight or BMI. Future studies with larger sample sizes investigating the changing trends of CRP, 8-oxodG, and TAC are warranted.

Clinical trial register: NCT03375866.

Keywords

Cardiovascular disease; Mixed nuts; LDL cholesterol; Lipoprotein(a) [Lp(a)]; Obesity; Lipid profiles; Inflammation

1. Introduction

Overweight and obesity are major public health concerns as they are major risk factors for the development of atherosclerosis [1], a major contributor to cardiovascular disease (CVD) [2]. In fact, there is a ten percent increase in the risk for atherosclerosis for every one-point increase in an individual's BMI above normal weight [1]. Additionally, obesity is associated with dyslipidemia [1], which contributes to atherosclerosis and higher risk of CVD. Atherosclerosis is a chronic inflammatory disease, which causes fatty-plaque formation to occur in the arterial walls [2]. Initiation of plaque formation occurs when the inner layer of the endothelial cells of the arterial wall is damaged from either hypertension, hypercholesterolemia, hyperglycemia, cigarette smoking, or unhealthy dietary habits [2]. Cholesterol is then deposited within the damaged endothelium by circulating cholesterol-rich apolipoprotein B (ApoB)-containing lipoprotein particles, e.g., low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), or lipoprotein(a) [Lp(a)], and it then becomes oxidized. Oxidation activates the endothelium to initiate an inflammatory response, thus causing the following cascade of events: monocyte infiltration, foam cell formation, plaque development, and ultimately, occlusion of the artery that decreases blood flow to major organs [2,3].

Along with elevated LDL-C, elevated Lp(a) levels may play a critical role in the progression of atherosclerosis. Lp(a) is an LDL variant and has an LDL-like particle in which ApoB is covalently bound to apolipoprotein(a) [Apo(a)] [4]. Serum concentration of Lp(a) vary between persons depending on genetic variation in *LPA* encoding apo(a) [4]. Assembly of apo(a) to a LDL-like particle is proposed to occur either on the surface of the hepatocyte or in circulation [4,5]. Several studies have shown that Lp(a) has pro-inflammatory oxidized phospholipids (OxPL) attached to apo(a) [6,7]. The presence of OxPLs potentially being taken up by the damaged endothelial cells could accelerate the development of atherosclerotic plaques [8].

Evidence from genetic and clinical studies has consistently demonstrated that elevated LDL-C (>130 mg/dL) and Lp(a) (>75 nM) are associated with a higher risk of CVD [9–12]. Previous data have suggested that reduction of LDL-C could reduce Lp(a) levels [4]. As such, most clinical trials have aimed to evaluate therapies that lower LDL-C, reasoning

that the reduction of circulating LDL particles would decrease apo(a) binding to LDL thus decreasing the formation of Lp(a) [13,14]. Since elevated LDL-C is primarily driven by unhealthy dietary habits, it would stand to reason that changes in diet could also be a form of preventative treatment.

Several clinical trial studies have consistently shown that nut consumption can reduce the risk factors for CVD. Nuts have a unique composition of unsaturated fats, soluble fibers, antioxidants, and phytochemicals, which have favorable effects on blood lipids and lipoproteins, such as triglycerides, total cholesterol (TC), LDL-C, and ApoB [15]. Previous studies have demonstrated that the individual composition of almonds, pistachios, walnuts, Brazil nuts, and macadamia nuts can significantly decrease LDL-C and TC, and increase HDL-C [16–20]. A few studies have demonstrated that nut consumption can decrease Lp(a). For example, 73 g/day of almond reduced Lp(a) in a cohort of hyperlipidemic patients [21]. Similarly, healthy adults consuming a pecan-enriched diet (72 g/day) [22] and hypercholesterolemic adults consuming a Mediterranean diet with walnuts (41–56 g/day) have experienced reduction in Lp(a) as well [23]. However, few studies have evaluated the effects of consuming mixed nuts on Lp(a) levels in populations with overweight or obesity, conditions that increase CVD risk [1].

The aim of the randomized controlled trial (RCT) was to examine the effects of mixed nut supplementation on Lp(a), lipid profiles, and inflammation. We hypothesized that diets supplemented with 42.5 g/day of mixed nuts would decrease levels of LDL-C, Lp(a), and inflammation markers in overweight and obese individuals.

2. Methods

2.1. Participants

Men and women with overweight or obesity (n = 34, BMI 25–40 kg/m^2), between the ages of 20–55 years, were recruited by posting fliers locally. Participants were eligible if they were: nonsmokers, not pregnant, not using any dietary supplements, had no known medical problems related to metabolic disorders or chronic inflammation, were not allergic to nuts or wheat, and were overweight or obese. The exclusion criteria included: cigarette smoker, pregnant, currently using dietary supplements, had a known medical condition related to metabolic disorders or chronic inflammation, had a nut or wheat allergy, were overweight or obese. Written informed consent was obtained from participants at the baseline laboratory visit.

2.2. Study design

Based on a previous human trial of nut consumption on LDL cholesterol [24], a power analysis (G*Power, Germany), with 70% power and an alpha-level of $P < 0.05$, indicated that significant differences would be detectable with a sample size of 14 subjects per group. Twenty-nine participants were randomly assigned to either consume the pretzel control (n = 15 [8M/7W], age: 26.8 ± 8.5 years) or mixed nut intervention (n = 14 (7M/7W), age: 28.3 ± 10.1 years) for 16 weeks. Participants were subject to 3 block randomizations, i.e., 3 subjects assigned to the mixed nut group then 3 subjects assigned to the pretzel group then back to

the mixed nut group and so on. Participants were blinded to what other variables they were being compared to and what other participants were doing. Subjects were only informed on their own snack. In order to avoid allocation bias, the lead investigator was the only one with the randomization sequence, and randomly assigned participants to their respective groups. The lead investigator did not meet the participants. Research assistants were in charge of informing participants which group they were in.

All participants made three laboratory visits while in a fasted state at baseline, week 8, and week 16. At baseline, a medical history was taken. At each visit, participants were asked to fill out a physical activity form and provide two 24-h recalls of food and beverage consumption for dietary analysis; in addition, participants fasting blood samples were collected by a venipuncture and anthropometric measurements were collected as well. This study protocol was approved by the San Diego State University Institutional Review Board and registered at ClinicalTrial.gov (#NCT03375866).

2.3. Diet intervention

Participants randomly assigned to the intervention group were provided with 42.5 g/day packets of mixed nuts consisting of 25.5 g of nut mixture (cashews, almonds, macadamia nuts, Brazil nuts, pecans) (Kirkland Signature, Issaquah, WA, USA), 5 g of pistachios, 5 g of walnuts, and 7 g of peanuts. The amount of mixed nuts provided, 42.5 g (1.5 oz), is approximately a small handful (1–2 oz) of nuts, which is approved as an FDA qualified health claim [25]. Participants in the control group were given pre-weighed packets (69 g/day) of unsalted pretzels (Snyder's, Charlotte, NC, USA). The nutrient composition of snacks was matched for sodium (mixed nuts 163 mg; pretzels 173 mg) and energy (253 kcal). The following macronutrient quantities varied respectively between pretzel versus mixed nut groups: carbohydrate (56.5 g, 9 g), protein (6.8 g, 7 g), and fat (0 g, 21 g). Participants were advised to consume one daily pre-weighted serving while maintaining their usual diet and physical activity. Compliance was monitored through email and phone reminders throughout the study. Diet intake was analyzed using the Food Processor Nutrition Analysis Software (ESHA Research, Salem, OR, USA).

2.4. Anthropometric and physiological measures

Body weight and height were measured using a weigh beam eye-level mechanical physician scale (Detecto, Webb City, MO, USA), and percent body fat was analyzed using a dual-energy X-ray absorptiometry (DXA; Prodigy, GE Healthcare, Chicago, IL, USA). Blood pressure (BP) was measured using an electronic BP monitor (Omron Healthcare, Kyoto, Japan). Waist and hip circumferences were measured using a tape measure and waist to hip (W/H) ratio was calculated. Anthropometric and physiological measures were collected at all three time points (baseline, week 8 and week 16).

2.5. Biochemical analysis

The fasted blood samples were collected, and serum was separated by centrifugation at 1200×g for 10 min at 4 °C and stored at −80 °C for further analysis. Glucose was measured using the Glucose Liqui Color assay kit (EKF Diagnostics, Cardiff, UK). Glucose level was determined by the color intensity of the quinone complex according to the manufacturer's

instructions. Absorbance was read at 500 nm. Insulin was measured using a sandwich type enzyme-linked immunosorbent assay (ELISA) kit. Samples were incubated with the detection antibody and then 3,3′,5,5′-tetramethylbenzidine (TMB) substrate. A stop solution was added, and absorbance was read at 500 nm. Adiponectin was tested using an ELISA kit (RayBiotech, Norcross, GA, USA). Samples were incubated with biotinylated anti-human adiponectin antibodies, horseradish peroxidase (HRP) conjugated streptavidin, and then TMB substrate solution sequentially. The stop solution changed the color of the sample from blue to yellow and absorbance was read at 450 nm.

Triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were analyzed colorimetrically (EKF Diagnostics). LDL-C levels were calculated using the Friedewald equation (LDL-C = TC – HDL-C – (TG/5)) [26].

Lp(a) was measured using an ELISA kit that detected apo(a). The protocol used a Sandwich-ELISA detection method utilizing LPA4 (Millipore Sigma, Burlington, MA, USA) as the capture antibody, a biotin-labeled LPA4 detection antibody (Millipore Sigma), and Neutravidin (Thermo Fisher, Grand Island, NY, USA) with Lumiphos (Lumigen, Southfield, MI, USA) for fluorescence reading. This protocol was provided in collaboration with Dr. Sam Tsimikas at the University of California at San Diego.

Liver function enzymes, including aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), and gamma-glutamyl transferase (γ-GT), were analyzed using kits from EKF Diagnostics following the manufacturer's manual. AST and ALT activities were analyzed by measuring the rate of oxidation of NADH per minute, while LDH activity was tested by measuring the rate of reduction of NAD to NADH per minute. ALP activity was detected by the formation of 4-nitrophenol per minute. CK activity was tested by the presence of an antibody to CK-M monomer per minute. γ-GT activity was analyzed by the rate of liberation of 5-amino-2 nitrobenzoate per minute. Absorbance of AST, ALT, LDH, and CK were read at 340 nm, while ALP and γ-GT were read at 405 nm.

C-reactive protein (CRP) was analyzed using an ELISA kit (Immundiagnostik AG, Bensheim, Germany). Samples were incubated with coated antibodies and then peroxidase-labeled detection antibodies. Samples were tested for absorbance at 450 nm.

Total antioxidant capacity (TAC) was determined colorimetrically using a kit from Cayman Chemical Company (Ann Arbor, MI, USA). Trolox equivalents TAC was measured using the ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) decolorization method.

High mobility group box protein 1 (HMGB1) levels were determined using an ELISA kit (MyBioSource, San Diego, CA, USA). Serum samples were incubated with a biotin-conjugated antibody, an avidin conjugated HRP, and then a substrate solution. When mixing a stop solution to stop the color change, absorbance was read at 450 nm.

Eight-oxo-deoxyguanosine (8-oxodG) was measured using the ELISA kit II (Trevigen, Gaithersburg, MD, USA). An 8-OHdG monoclonal antibody binds competitively to 8-oxodG in serum samples. An HRP conjugate was used as a secondary antibody and

detection was performed with colorimetric substrate. Product formation is inversely proportional to the amount of 8-OHdG present in the sample.

2.6. Statistical analysis

Data was analyzed using SPSS version 24 (IBM, Armonk, NY, USA). Normality was assessed prior to analysis to determine possible outliers. Outliers were excluded from follow up analysis if they were 2.58 standard deviation (SD) units away from the mean. One cholesterol outlier was excluded from the analysis. Whether this outlier was included or not, it did not affect the statistical significance of the analysis. A mixed repeated measures ANOVA was used to determine if there were significant differences in treatment groups over time. If significant interactions were observed, follow-up Bonferroni post-hoc tests were performed. If data were not normally distributed, non-parametric tests were also applied. Lp(a), HDL-C and adiponectin data were analyzed using non-parametric tests (Mann-Whitney U test and Wilcoxon Rank Sum Test) between trials and over time, respectively. If basal values were significantly different following independent t-tests, an ANCOVA was performed using the baseline values as a covariate. Baseline values were different between trials in ALP and LDH measurements, therefore an ANCOVA was performed on them. Cohen's d values were calculated to determine the effect size. All results are expressed as means ± SDs. A P value ≤ 0.05 was considered statistically significant. Intra-assay of coefficient of variation (CV) for biochemical measures were calculated by dividing the standard deviation (SD) of a set of measurements by the mean of the set and multiplying by 100.

3. Results

3.1. Demographic

Of the 34 participants who passed the initial screening, 29 completed the study and their data was analyzed (Fig. 1). Fifteen were males and 14 were females. The mean age of the participants was 27.6 years. There were no significant differences in physical activity between groups (Supplemental Table 1). Non-completers dropped out of the study due to personal reasons (moving or non-compliance) and their data was not included in the analysis. Baseline characteristics did not significantly differ between treatment groups and therefore were not used as covariates in subsequent analyses.

3.2. Anthropometry

There were no significant differences in BMI, body weight, and W/H ratio between groups. However, body fat varied over the length of the intervention with significant increases observed between baseline and week 16 in the pretzel group, and significant decreases observed during the same time points in the mixed nuts group ($P \leq 0.05$; Cohen's d = 0.57) (Table 1). Body fat percentage was higher in the pretzel group compared to the mixed nut group at week 16 ($P \leq 0.05$).

The mixed nuts group had a non-significant tendency towards reductions in mean diastolic blood pressure (DBP) between baseline to week 8 ($P = 0.06$), while significant decreases were observed between baseline to week 16 ($P = 0.04$; Cohen's d = 0.49) (Table 1). The

pretzel group did not have any significant differences at any time point. Between the pretzel and the mixed nut groups, no significant differences for DBP were found at any time point.

3.3. Lipid profile and Lp(a)

There were no significant differences in TG, TC, HDL-C, or LDL-C between groups over the course of the intervention (Table 2). No significant differences were detected between the two treatment groups or over time on Lp(a) (Fig. 2A). Lp(a) values were adjusted to compare individual participants to their baseline levels. There were no significant differences between the participants consuming mixed nuts or pretzels (Fig. 2B).

3.4. Glucose, insulin, and adiponectin

Mixed nut consumption showed lower glucose level at week 8 ($P = 0.01$; Cohen's d = 0.78), while there were no significant changes with pretzel consumption (Table 2). There was no significant difference for insulin between groups over time. The mixed nuts group showed increased adiponectin level in week 16 ($P = 0.03$; Cohen's d = 0.56) while there were no significant changes in the pretzel group. At baseline, weeks 8 and 16, there were no significant differences between the pretzel and mixed nut groups for glucose and adiponectin.

3.5. Liver function enzymes

There were no significant changes between the groups for liver function enzymes (AST, ALT, ALP, LDH, CK, and γ-GT). There were no significant changes observed over time for either group (Table 2).

3.6. CRP, HMGB1, 8-oxodG, and total antioxidant capacity

Mixed nuts consumption resulted in a non-significant tendency towards lower CRP in contrast to an increasing non-significant tendency with pretzels ($P = 0.07$) (Table 3). There was a non-significant tendency of lower 8-oxodG with mixed nut consumption, but pretzels showed an increased non-significant tendency at week 8 ($P = 0.06$). There were no significant changes between the groups on HMGB1 over time. TAC had an increasing non-significant tendency with mixed nut consumption ($P = 0.06$) but no significant effect with pretzel consumption.

3.7. Dietary intake

Monounsaturated fatty acid (MUFA) ($P < 0.01$), polyunsaturated fatty acid (PUFA) ($P < 0.01$), and magnesium intakes ($P = 0.02$) were significantly higher in the mixed nut group compared to the pretzel group at week 8 and week 16, while no significant differences were observed between groups at baseline (Fig. 3). The MUFA and PUFA intakes were not significantly different between the baseline and week 8 or 16 for either group. There were no significant differences in Kcal, fat, protein, or fiber intakes between groups over the time (Supplemental Table 2).

4. Discussion

Many studies have examined the lipid profile responses to individual nuts but to our knowledge this is the first RCT that has assessed the effect of mixed nuts on both LDL-C and Lp(a) in overweight and obese adults. In the present study, mixed nut supplementation reduced some CVD risk factors such as body fat percentage, DBP, glucose, and adiponectin in obese and overweight adults. Additionally, supplementation showed a decreasing non-significant tendency on CRP and 8-oxodG, and an increasing non-significant tendency on TAC, but had no effect on plasma lipids, Lp(a), insulin, liver function enzymes, and HMGB1.

There are several potential reasons that could explain why there were no changes seen in LDL-C. Our study population were obese and overweight adults with no known metabolic disorders. Most nut-based clinical trials have shown the greatest reduction in LDL-C in hyperlipidemic populations [15,21,27]. These individuals have elevated lipid levels, thus detecting statistically significant reductions is easier. Studies have shown that only at higher doses of nut consumption (≥60 g/d) will reductions in LDL-C be observed [15,21,27]. Our participants only consumed 42.5 g/day of mixed nuts, which might have been below the threshold for observing differences.

High Lp(a) level is prevalent in 30–50% of the patients with the heterozygous form of familial hypercholesterolemia, which is linked to significantly higher LDL-C [28]. Lp(a) plasma concentration is affected by the FH mutation gene and located in LDL receptors [28]. However, limited evidence has demonstrated that circulating Lp(a) levels can be significantly influenced by lifestyle factors such as diet and physical activity [4]. There was a prevailing thought that dietary intervention did not affect Lp(a) concentrations [29]; and in the present study, there were no significant differences in Lp(a) concentrations in either group. These results are similar to that of a study done by Tindall et al. in that a diet with increased walnuts or vegetable oils and decreased saturated fat did not significantly change Lp(a) after 6-weeks [30]. Despite this, however, several studies highlighted by Enkhmaa et al. [29] have demonstrated that Lp(a) is in fact influenced by dietary interventions; albeit with inconsistencies. For example, previous studies have demonstrated that when dietary saturated fat is reduced, individuals may experience an increase in Lp(a) concentrations after 8-weeks [31]. Similarly, when saturated fat is reduced and replaced with MUFA or carbohydrates for 7-weeks, Lp(a) concentrations may increase as well [32]. However, a more recent study has shown Lp(a) can be reduced after 5-weeks with a reduction in saturated fat and increase in MUFA [33]. Moreover, almond supplementation (73 g/day) support the reduction of LDL-C and concomitant reduction of Lp(a) in hyperlipidemic individuals [21]. It has also been shown that a plant-based diet eaten ad-libitum for 4 weeks can substantially reduce Lp(a) concentrations by 16% [34]. Notably, LDL-C was reduced in the aforementioned studies [31e34]. These inconsistencies seen in Lp(a) outcomes may be due to the different methods of measuring Lp(a), where some research groups utilized vertical auto profiling [30,33], a method that has poor correlation to Lp(a) mass versus using an immunochemical assay [29]. Also, since circulating Lp(a) levels may be genetically determined [4,35], we suspect that inconsistencies may be related to genetic factors as well. For example, the interindividual range of Lp(a) concentrations can be a little as 0.1

mg/dL and up to 300 mg/dL, which is a more than a 1000-fold range [35]. Population based studies have shown that individuals from sub-Saharan Africa have much higher Lp(a) concentrations compared to those from Europe and Asia largely due to the plasminogen gene kringle domain KIV repeat size polymorphism [35]. Other than the KIV repeat size polymorphism, there are other genetic variants of the *LPA gene* that also influence Lp(a) concentrations. Thus, genetic profiling will be necessary to better understand the mechanisms influencing Lp(a) concentrations. Further research is also required to elucidate the specific mechanisms that contribute to the reduction in Lp(a) concentrations with various dietary interventions such as differing macronutrient and fatty acid compositions.

Significant increases in PUFA and MUFA were observed in the mixed nut group at weeks 8 and 16 compared to the pretzel group. Almonds, cashews, pistachios and macadamia nuts have high MUFA content (>55%) while peanuts, pecans, Brazil nuts and walnuts have high PUFA content (>50%) [36]. MUFA and PUFA both have the ability to modulate plasma LDL-C through various mechanisms. PUFA decreases cholesterol via upregulation of the LDL receptor and by heightening CYP7 (cholesterol 7 alpha-hydroxylase) activity responsible for bile synthesis [37]. MUFA decreases LDL-C through reductions in CETP (elevated cholesteryl ester transfer protein) activity which positively correlates with VLDL-C and LDL-C [38]. Despite significant increases in MUFA and PUFA, reductions in LDL-C were not observed in the mixed nut group likely due to the lower intake of nuts compared to other similar studies that observed favorable changes in LDL-C [27].

Body fat was reduced in the mixed nut group and increased in the pretzel group during the intervention. Previous literature has suggested that nut consumption elevates fat oxidation which may provide a mechanism for attenuation of body fat accumulation over time. Specifically, acute consumption of 25–35 g walnuts was shown to increase postprandial fat oxidation when compared to a control diet in overweight and obese adults [39]. Other potential metabolic pathways may reduce adiposity with nut consumption, such as appetite control, replacement of unfavorable nutrients, better energy metabolism, or improving gut microbiome function [40]. Another possible mechanism of reduced adiposity with nut consumption is that nuts have been shown to be less bioaccessible thus providing less metabolizable energy than what has been predicted by the Atwater factors [41–44]. Human studies examining the mechanism of nut consumption on fat loss are warranted.

DBP was reduced in the mixed nut group throughout the intervention. This finding is consistent with a meta-analysis examining the effects of various nut consumption on BP. Similar to our findings, an all-nut intake had no significant effects on reducing systolic BP [45]. However, DBP was significantly lowered with both pistachio only and mixed nut consumption [45]. Similarly, a meta-analysis examining the effect of almond intake on blood pressure found that almond intake only reduces DBP [46]. A possible explanation for the reduction in only DBP could be that nuts may be improving peripheral vascular resistance rather than total peripheral resistance [46]. Increased intake of nuts has been associated with reductions in hypertension due to its array of macro- and micronutrients. Magnesium, potassium, calcium, dietary fiber, MUFA, and PUFA have been proposed to interact beneficially to reduce BP. More specifically, high MUFA and PUFA content plays a role in reducing serum levels of thromboxane 2, a vasoconstrictor influencing BP,

while magnesium stimulates vasodilation via production of nitric oxide and vasodilator prostacyclins [47]. However, only magnesium, MUFA, and PUFA demonstrated statistical significance in food intakes in this study, and it is likely the combination of these nutrients that collectively led to the reduction of BP.

The combination of fatty acids and bioactive components in nuts may play a role in regulating fasting blood glucose levels, but the exact mechanisms by which this occurs is unclear [48]. It is thought that nuts may be increasing insulin sensitivity thus resulting in increased glucose uptake and reduced fasting blood glucose level [48]. Alternatively, the effect nuts have may be through non-insulin mediated pathways, such as delayed gastric emptying, resulting in lower postprandial glucose excursions [48]. Our study found a significantly lower glucose level at week 8 in the mixed nut group and no significant difference for insulin between groups over time. These results align with the pistachio study's finding for both glucose and insulin [49]. It is unclear as to why the lower glucose did not persist to week 16. This may be due to confounding dietary factors or a transient effect although more studies are needed to clarify this. Interestingly, other studies have reported a similar outcome [50,51].

Adiponectin helps prevent vascular changes, glucose and lipid metabolism impairment, and insulin resistance. It is secreted from adipose tissues, goes into the injured arteries, and prevents the progression of atherogenic vascular changes [52]. Adiponectin plasma level decreases with the accumulation of body fat, especially visceral adipose tissue, although the mechanism is uncertain [52]. Adiponectin is also found in lower levels in diabetic and ischemic heart diseases patients [52]. This study found an increased adiponectin level at week 16 with mixed nuts consumption. The similar beneficial effect on adiponectin was found in the pistachio and walnut studies [53,54]. However, almond and mixed nuts studies, which were only 8 weeks in length, did not show increasing adiponectin levels [55,56].

CRP is an important inflammatory marker and an independent predictor of CVD and diabetes. 8-oxodG is a biomarker for oxidative stress and an indicator of DNA damage. Our study found that there was a non-significant tendency towards lower CRP and 8-oxodG, and an increasing non-significant tendency of TAC. Bioactive nutrients of nuts such as n–3 PUFA, antioxidants, vitamins, dietary fiber, L-arginine, and magnesium may play important roles in lowering inflammation, oxidative stress, and the risk of cardiovascular mortality and type 2 diabetes [57].

Nut consumption can help improve both obesity and dyslipidemia thus reducing the risk for CVD. Although our study did not show significant improvements in body weight, there was a reduction in body fat and an increase in lean body mass. Increasing lean mass and reducing fat mass lowers the risk of CVD [58]. Previous research has shown that a walnut-enriched diet and an almond-enriched diet can reduce body weight and improve lipids and blood pressure [59,60]. Reductions in body weight can also improve fasting blood glucose levels, which can help to reduce the risk for atherosclerosis and CVD. In the present study, mixed nuts reduced fasting blood glucose levels by week 8. Similar to the previous research, a pecan-enriched diet has been shown to reduce fasting blood glucose as well [60].

The strengths of this study provided new insight on effects of long-term mixed nut consumption in overweight and obese adults. Additionally, this study is the first RCT that has tested both LDL-C and Lp(a) as a function of mixed nut consumption. However, the small sample size of this study limits the generalizability of the results. Furthermore, this study lacked participants with a healthy body weight, which may also limit the generalizability of the findings. To address these limitations, future studies should aim to include larger sample sizes and participants with a healthy body weight. Additionally, future studies could focus on subjects with higher CVD risk factors, such as high LDL-C, to examine the role of mixed nut consumption on Lp(a).

In conclusion, this 16-week mixed nut intervention showed significant improvement on body fat percentage, DBP, glucose, and adiponectin, and beneficial non-significant tendencies on CRP, 8-oxodG, and TAC. However, no significant effects were found on LDL-C and Lp(a) levels over a 16-week dietary intervention in overweight or obese adults. Considering the ease of including mixed nuts into the diet, and the findings of this study with effect sizes, namely in body fat amount, DBP, glucose and adiponectin, the results might be clinically meaningful in health improvements for individuals with overweight or obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

This work was supported by the American Heart Association [Grant number 16GRNT31360007]. The funding source had no involvement in study design, collection, analysis and interpretation of data, writing of the report or decision to submit the article for publication.

Abbreviations:

8-oxodG	8-oxo-deoxyguanosine
ABTS	2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid
ALP	alkaline phosphatase
ALT	alanine transaminase
Apo(a)	apolipoprotein(a)
ApoB	apolipoprotein B
AST	aspartate aminotransferase
BP	blood pressure
CAD	coronary artery disease
CK	creatine kinase
CRP	c-reactive protein

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CVD	cardiovascular disease
DBP	diastolic blood pressure
DXA	dual-energy X-ray absorptiometry
ELISA	enzyme-linked immunosorbent assay
HDL-C	high-density lipoprotein cholesterol
HMGB1	high mobility group box protein 1
HRP	horseradish peroxidase
LDH	lactate dehydrogenase
LDL-C	low-density lipoprotein cholesterol
Lp(a)	lipoprotein(a)
MUFA	monounsaturated fatty acid
OxPL	oxidized phospholipids
PUFA	polyunsaturated fatty acid
RCT	randomized controlled trial
SD	standard deviation
TAC	total antioxidant capacity
TC	total cholesterol
TG	triglyceride
TMB	3,3′,5,5′-tetramethylbenzidine
VLDL-C	very low-density lipoprotein cholesterol
W/H	waist to hip
γ-GT	gamma-glutamyl transferase

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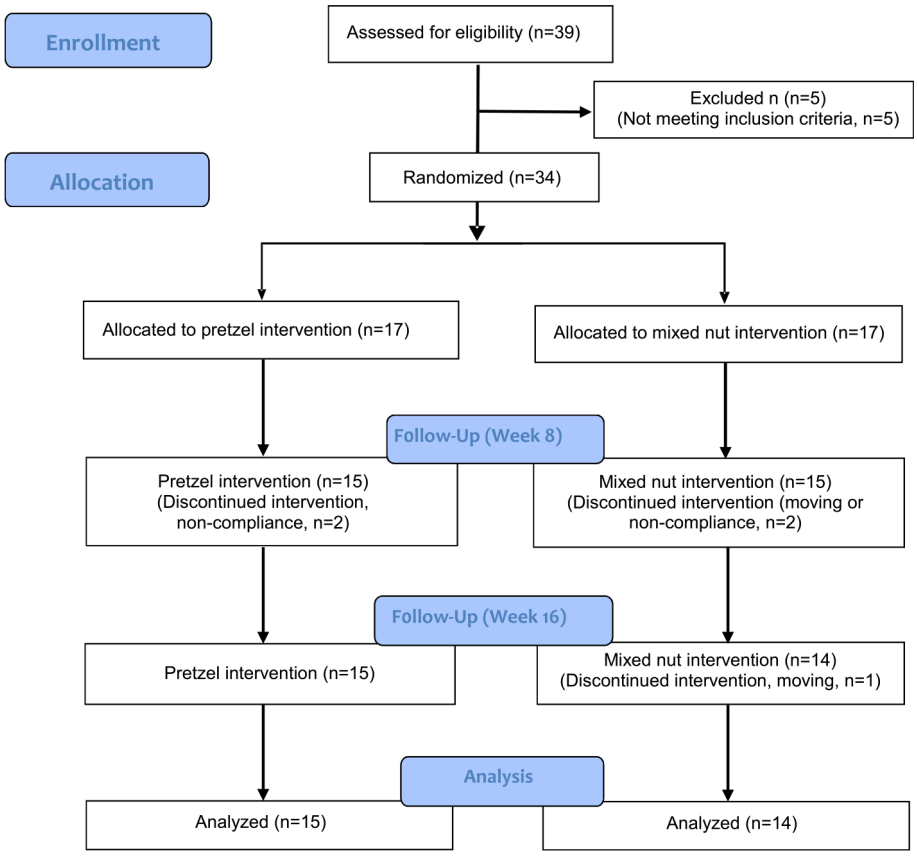

Figure 1.
CONSORT flow diagram of participant selection.

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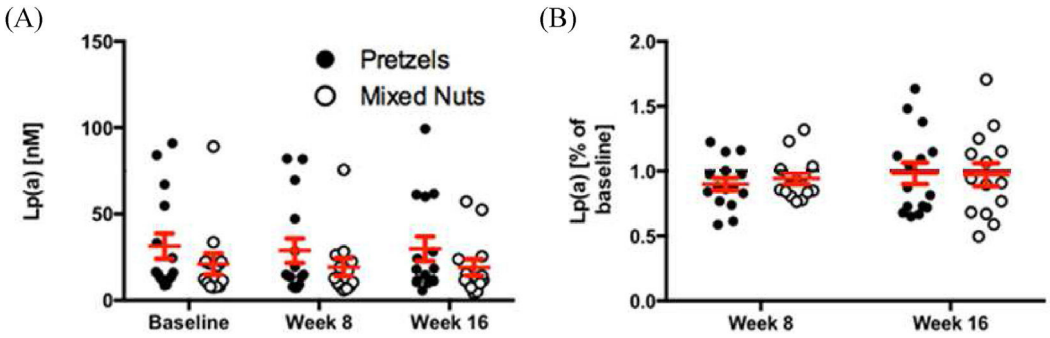

Figure 2.

(A) Lp(a) absolute values (nM) of participants at baseline, week 8, and week 16. Closed circles represent participants in the pretzels group and the open circles represent participants in the mixed nut group. (B) Lp(a) levels compared to baseline. Solid horizontal line represents 100%. Data are represented as means ± SDs.

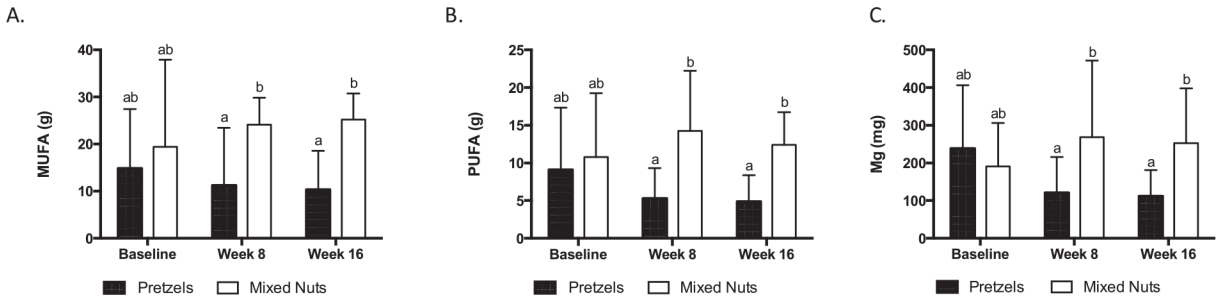

Figure 3.

MUFA (A), PUFA (B), and Mg (C) intakes in overweight and obese adults at baseline, week 8, and week 16. Data are represented as means ± SDs. Different letters denote statistical significance at $P \leq 0.05$. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Mg, magnesium.

Table 1

Effects of pretzels or mixed nut consumption on body weight, LBM, BMI, BP, W/H ratio, and body fat percentage at baseline, week 8, and week 16.

		Pretzels		Mixed Nuts		
	Baseline	**Week 8**	**Week 16**	**Baseline**	**Week 8**	**Week 16**
Body weight (kg)	89.8 ± 16.0	89.9 ± 16.4	90.0 ± 16.3	89.9 ± 18.3	89.6 ± 19.1	90.1 ± 19.0
LBM (kg)	61.3 ± 10.5[a]	60.9 ± 10.3[a,b]	60.3 ± 10.2[b]	61.5 ± 8.70[a]	62.2 ± 8.40[a,c]	63.0 ± 8.78[c]
BMI (kg/m^2)	30.0 ± 5.02	30.0 ± 4.98	30.0 ± 4.78	31.3 ± 5.36	31.2 ± 5.56	31.4 ± 5.60
SBP (mm Hg)	125.7 ± 7.86	126.7 ± 6.37	124.0 ± 7.57	130.1 ± 9.89	129.9 ± 11.9	133.6 ± 10.7
DBP (mm Hg)	81.7 ± 6.51[a,b]	80.7 ± 9.37[a,b]	79.8 ± 5.65[a,b]	82.0 ± 11.3[a]	78.6 ± 11.5[a,b]	78.9 ± 10.7[b]
W/H ratio	0.84 ± 0.08	0.84 ± 0.08	0.85 ± 0.07	0.85 ± 0.06	0.85 ± 0.06	0.86 ± 0.07
Body Fat, %	38.7 ± 10.5[a]	39.1 ± 10.3[a,b]	39.7 ± 10.2[b]	38.5 ± 8.70[a]	37.8 ± 8.40[a,c]	37.0 ± 8.78[c]

Values are expressed as means ± standard deviations (SDs). Data within rows with different superscript letters are statistically different at $P \leq 0.05$.
BMI: body mass index; DBP, diastolic blood pressure; LBM: lean body mass; SBP, systolic blood pressure; and W/H ratio, waist to hip ratio.

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Table 2

Effects of pretzel or mixed nut consumption on biomarkers (lipids, glucose, insulin, adiponectin, and liver function enzymes) at baseline, week 8 and week 16.

		Pretzels		Mixed Nuts		
	Baseline	**Week 8**	**Week 16**	**Baseline**	**Week 8**	**Week 16**
TG (mg/dL)	131.65 ± 11.13	125.90 ± 8.37	128.34 ± 7.53	135.56 ± 8.77	136.80 ± 10.59	132.79 ± 7.72
TC (mg/dL)	175.81 ± 25.96	173.39 ± 13.60	178.35 ± 23.38	168.82 ± 21.84	183.11 ± 23.10	181.31 ± 24.39
HDL-C (mg/dL)	51.89 ± 2.46	50.83 ± 2.19	50.79 ± 1.97	52.10 ± 1.66	49.85 ± 2.40	52.63 ± 2.18
LDL-C (mg/dL)	99.72 ± 22.26	101.96 ± 20.25	104.93 ± 24.32	90.68 ± 21.31	107.23 ± 29.95	106.72 ± 34.95
Glucose (mmol/L)	5.37 ± 0.69[a]	5.04 ± 0.69[a,b]	5.05 ± 0.48[a,b]	5.36 ± 0.86[a]	4.81 ± 0.78[b]	5.01 ± 0.52[ab]
Insulin (mIU/L)	17.36 ± 7.81	18.13 ± 7.78	18.3 ± 10.34	16.45 ± 13.48	16.41 ± 14.55	15.68 ± 10.5
Adiponectin (μg/mL)	28.89 ± 43.41[a,b]	35.49 ± 41.86[a,b]	26.53 ± 32.76[a,b]	21.58 ± 35.69[a]	26.08 ± 29.28[a]	45.09 ± 74.82[b]
AST (U/L)	15.17 ± 3.00	13.17 ± 3.56	13.56 ± 4.03	14.95 ± 2.80	15.27 ± 4.20	16.70 ± 4.20
ALT (U/L)	11.82 ± 6.63	11.25 ± 3.74	12.15 ± 9.14	10.11 ± 2.43	13.02 ± 5.51	12.16 ± 4.60
ALP (U/L)	53.44 ± 11.71	52.76 ± 8.90	52.81 ± 10.49	46.22 ± 4.86	47.01 ± 6.10	44.95 ± 6.65
LDH (U/L)	98.88 ± 23.28	126.57 ± 68.60	106.50 ± 23.94	124.46 ± 22.96	122.27 ± 25.96	111.44 ± 6.66
CK (U/L)	39.61 ± 17.42	42.54 ± 19.26	47.71 ± 22.32	46.06 ± 21.42	44.13 ± 20.45	56.19 ± 40.09
γ-GT (U/L)	6.32 ± 1.68	6.63 ± 1.86	6.52 ± 1.45	7.15 ± 1.88	7.40 ± 2.66	7.40 ± 2.79

Values are expressed as means ± SDs. Data within rows with different superscript letters are statistically different at $P \leq 0.05$. TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol, AST, aspartate aminotransferase; ALT, alanine transaminase; ALP: alkaline phosphatase; LDH, lactate dehydrogenase; CK, creatine kinase; and γ-GT, gamma-glutamyl transferase. Intra-assay CV of TG was 7.59, TC 5.79, HDL-C 5.10, glucose 2.69, insulin 3.63, adiponectin 8.91, AST 6.28, ALT 7.56, ALP 5.30, LDH 5.23, CK 7.10, and r-GT 7.88.

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Table 3

Effects of pretzel or mixed nut consumption on TAC, CRP, HMGB1, and 8-oxodG at baseline, week 8, and week 16.

		Pretzels		Mixed Nuts		
	Baseline	**Week 8**	**Week 16**	**Baseline**	**Week 8**	**Week 16**
TAC (mmol/L)	0.39 ± 0.26	0.36 ± 0.30	0.46 ± 0.26	0.40 ± 0.32	0.51 ± 0.23	0.53 ± 0.20
CRP (ng/mL)	7.01 ± 4.28	7.32 ± 6.24	8.53 ± 6.69	7.99 ± 5.74	7.58 ± 5.58	6.83 ± 3.50
HMGB1 (pg/mL)	2.65 ± 1.26	2.73 ± 1.53	2.43 ± 1.35	2.71 ± 1.32	2.47 ± 1.44	2.85 ± 1.23
8-oxodG (ng/mL)	182.69 ± 33.14	188.93 ± 34.54	175.27 ± 27.25	177.76 ± 32.57	160.71 ± 24.10	161.23 ± 21.11

Values are expressed as means ± SDs. Data within rows with different superscript letters are statistically different at $P \leq 0.05$. TAC, total antioxidant capacity; CRP, c-reactive protein; HMGB1, high mobility group box protein 1; and 8-oxodG, 8-oxo-deoxyguanosine. Intra-assay CV of TAC was 5.26, CRP 8.35, HMGB1 9.13, and 8-oxodG 8.20.