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Short-term walnut consumption increases circulating total adiponectin and apolipoprotein A concentrations, but does not affect markers of inflammation or vascular injury in obese humans with the metabolic syndrome: data from a double-blinded, randomized, placebo-controlled study

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ABSTRACT

Long-term consumption of walnuts is associated with lower cardiovascular disease risk in epidemiological studies, possibly through improvements in lipid profile and endothelial function. It remains to be elucidated how soon after initiation of walnut consumption beneficial effects on lipid profile and biomarkers of inflammation or vascular injury can be observed. Fifteen obese subjects (9 men and 6 women; age, 58 ± 2.5 years; body mass index, 36.6 ± 1.7 kg/m^2) with the metabolic syndrome participated as inpatients in a randomized, double-blinded, placebo-controlled crossover study involving short-term placebo or walnut-enriched diet (48 g/d for 4 days). Apolipoproteins and markers of inflammation and vascular injury were measured before and after consumption of the experimental diets. Consumption of walnuts was associated with a statistically significant increase in serum apolipoprotein A concentrations (P = .03), but did not affect circulating levels of fetuin A, resistin, C-reactive protein, serum amyloid A, soluble intercellular adhesion molecules 1 and 3, soluble vascular cell adhesion protein 1, interleukins 6 and 8, tumor necrosis factor α, E-selectin, P-selectin, and thrombomodulin. Four days of walnut consumption (48 g/d) leads to mild increases in apolipoprotein A concentrations, changes that may precede and lead to the beneficial effects of walnuts on lipid profile in obese subjects with the metabolic syndrome.

KNA conducted research, analyzed the data, and wrote the paper. MTV and JPC conducted research. LLS and AMB contributed to the initial phase of this study. FM supervised analyses and wrote initial drafts of the paper, and CSM designed and supervised the study and wrote the paper. All authors have read and approved the final manuscript.

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1. Introduction

Cardiovascular disease (CVD) is a leading cause of death and disability in the United States [1]. Increased consumption of highly processed foods and reduced consumption of whole grains and nuts have been associated with elevated CVD risk [2]. Conversely, habitual nut consumption has been consistently associated with reduced CVD risk, regardless of the clinical end point used (that is, nonfatal myocardial infarction, fatal coronary incident, or sudden cardiac death) [3]. For instance, we have previously shown, in the context of a large epidemiological study, that during 54,656 person-years of follow-up, frequent nut consumption was inversely associated with total CVD risk [4]. Walnuts are the most popular type of nuts consumed [5]. The mechanisms responsible for the beneficial effects of dietary walnuts are not entirely clear, but favorable changes in blood lipid profile are likely to be involved [6]. We have observed that increasing nut consumption is significantly associated with a more favorable plasma lipid profile, including lower low-density lipoprotein cholesterol, non–high-density lipoprotein (HDL) cholesterol, total cholesterol, and apolipoprotein (apo) B-100 concentrations; but we did not observe significant associations with HDL cholesterol or inflammatory markers [4]. Furthermore, we have previously demonstrated that increased consumption of nuts is positively associated with plasma adiponectin concentrations in diabetic women [7].

These observational studies cannot address whether the beneficial effects of dietary walnuts on lipid profile are causal and/or whether they occur acutely or require long-term consumption. Moreover, the underlying mechanisms through which walnuts exert their beneficial effects remain to be elucidated. Limited evidence also indicates that relatively long-term walnut consumption improves endothelial function in hypercholesterolemic patients [8], an effect possibly linked to improvements in several inflammatory, oxidation, and vascular injury biomarkers [8-10]. Interestingly, recent studies have demonstrated that walnut-induced improvements in endothelial function [11] and antioxidant status [12] manifest acutely, even just after a single walnut-containing meal. There are currently no data available regarding the presence and/or the timing of any short-term effects of dietary walnuts on lipid profile and on markers of inflammation (eg, interleukins, tumor necrosis factor [TNF] α) and vascular injury (eg, selectins, intercellular adhesion molecule [ICAM], vascular cell adhesion molecule [VCAM]) in humans. Importantly, there are no studies on the effects of dietary walnuts on the levels of these biomarkers in patients with the metabolic syndrome that is characterized by endothelial dysfunction, systemic inflammation, and insulin resistance [13,14].

The aim of the present study was thus to evaluate the effect of short-term (4 days) walnut consumption on lipid profile and circulating markers of insulin resistance, inflammation, and vascular injury in obese subjects with the metabolic syndrome.

2. Materials and methods

2.1. Subjects and study design

Fifteen obese subjects (9 men; 13 white; age, 58 ± 2.5 years; body mass index, 36.6 ± 1.7 kg/m^2; waist circumference, 117 ± 2.7 cm) with the metabolic syndrome, as defined by the 2006 International Diabetes Federation criteria [15], were enrolled in a randomized, double-blinded, placebo-controlled crossover study of short-term walnut or placebo consumption. The details of the study design have been previously described [16]. The aims of this article were the focus of a supplemental competitive grant application that was submitted and funded after the original study had been published. Briefly, 2 different isocaloric diets, one with 48 g of walnuts daily, incorporated into a liquid meal to allow for blinding of the subjects, and one without walnuts (placebo), but otherwise with the same macronutrient composition, were administered for 4 days in a randomized, double-blinded fashion during 2 different inpatient visits in the General Clinical Research Center. The two 4-day inpatient visits were spaced 1 month apart to achieve adequate washout. All subjects were asked to avoid walnut intake for 3 weeks before randomization and throughout the washout period. Before starting the study, subjects were evaluated using questionnaires on whether they could distinguish the walnut vs the placebo diet; and we found that they could not. Blood samples were collected before and after 4 days of diet consumption (walnut or placebo), following 12 hours of fasting; and serum was prepared and stored in –80°C until analyses. The study was approved by the Institutional Review Board of the Beth Israel Deaconess Medical Center, and all subjects gave written informed consent before participating in the study.

2.2. Biochemical measurements

Commercially available immunoassays were used to determine the concentrations of total and high–molecular weight (HMW) adiponectin (Multimeric Adiponectin ELISA; Alpco, Salem, NH), resistin (Resistin ELISA; Alpco) [17], and fetuin-A (Human Fetuin-A ELISA; BioVendor, Candler, NC) in serum. To measure the HMW fraction of adiponectin, samples were pretreated with Protease II (Alpco, Salem, NH), which degrades the low– and medium–molecular weight isoforms of adiponectin, for 20 minutes at 37°C. The serum concentrations of granulocyte macrophage colony-stimulating factor, interferon-γ, interleukin (IL) 1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, and TNF-α were determined with the MSD Human Ultra-Sensitive Proinflammatory 9-plex electrochemiluminescent (ECL) assay (MSD: Meso Scale Discovery, Gaithersburg, MD); thrombomodulin, ICAM-3, E-selectin, and P-selectin levels were measured with the MSD Vascular Injury Panel-I ECL assay; and serum amyloid A (SAA), C-reactive protein (CRP), VCAM-1, and ICAM-1 concentrations were determined with the MSD Vascular Injury Panel-II ECL assay [18]. Samples were run as duplicates, and measurements with coefficient of variation less than 15% were considered acceptable. Apolipoproteins A and B were analyzed with the automated Cobas c311 chemical analyzer (Roche, Indianapolis, IN).

2.3. Statistical analysis

Statistical analysis was performed with Stata version 11.2 (Stata, College Station, TX). Normality was examined with the Shapiro-Wilks statistic. Most variables were skewed; hence, nonparametric tests were used, and the results are presented

as median and 25th-75th quartile. Comparisons between the first and last day of each diet (walnut and placebo) were made using the Wilcoxon signed rank test. Comparisons across conditions were also performed with the Wilcoxon signed rank test because of the crossover design of the study. All tests were 2-sided, and the α criterion was set at the .05 level.

3. Results

Total adiponectin concentration measured using the Alpco assay used herein increased significantly after 4 days of walnut consumption from 3.42 (2.49-4.78) μg/mL to 3.93 (1.97-4.31) μg/mL (P = .03), but HMW adiponectin or the HMW to total adiponectin ratio did not change (Table 1). Apolipoprotein A concentration increased significantly after the walnut diet from 113.00 (108.90-121.60) mg/dL to 115.10 (91.90-125.30) mg/dL (P = .038), whereas apo A concentration decreased significantly after the placebo diet from 114.60 (102.10-133.60) mg/dL to 106.50 (91.10-119.30) mg/dL (P = .003). Apolipoprotein B concentration and the apo A/apo B ratio did not change significantly (Table 2). The circulating concentrations of fetuin A and resistin did not change significantly with walnut consumption (Table 1). The concentrations of CRP, SAA, soluble ICAM-1 and ICAM-3, soluble VCAM-1, IL-6, IL-8, TNF-α, E-selectin, P-selectin, and thrombomodulin did not change with 4 days of walnut consumption (Table 2). The levels of granulocyte macrophage colony-stimulating factor, interferon-γ, IL-1β, IL-2, IL-10, and IL-12p70 were not detectable in our samples.

4. Discussion

Observational epidemiological studies have been remarkably consistent in demonstrating a lower risk of CVD [3,19] and more favorable outcomes in patients with diabetes and the metabolic syndrome associated with increased nut consumption [20,21]. We have previously demonstrated that frequent nut consumption is inversely associated with age-adjusted CVD risk in the setting of a large epidemiological study with 54 656 person-years of follow-up [4]. In addition, even after adjustment for conventional CVD risk factors, consumption of at least 5 servings per week of nuts or peanut butter remained significantly associated with a lower risk of CVD by almost 50% (serving size, 28 g [1 oz] for nuts and 16 g [1 tablespoon] for peanut butter) [4].

Recent studies have proposed that the various adiponectin multimers have different target tissues and/or different biological effects [22]. The HMW isoform may mediate the majority of adiponectin's effects on the liver [23], endothelial cells [24], and probably also skeletal muscle [25], whereas the trimers and full-length monomeric forms are responsible for other actions in various tissues [26]. Moreover, the HMW isoform of adiponectin is considered to be responsible for its proinflammatory actions, whereas the low–molecular weight isoform is responsible for its anti-inflammatory ones [27]. Lack of an effect of walnuts to alter HMW adiponectin may explain the lack of its effect to alter inflammatory markers. Although the above underscore the need to consider adiponectin isoforms when studying its actions and functions, we have previously shown that, in terms of in vivo whole-body insulin sensitivity, total adiponectin and HMW adiponectin are comparably good predictors without any major difference in their predictive value [17]. In addition to the improvement of insulin sensitivity, adiponectin has also been proposed to have cardioprotective [28] and antineoplastic [29] properties.

In this interventional study, we demonstrate that short-term walnut consumption led to a statistically significant increase in the concentration of circulating total adiponectin by approximately 15%, whereas it did not affect the HMW isoform and/or resistin or fetuin-A levels. This is in agreement with previous long-term observational studies reporting that nut consumption is associated with higher adiponectin concentrations [7]. In contrast, despite demonstrating a similar trend, we failed to show statistically significant changes in adiponectin, measured using a Linco assay (Linco Diagnostics, Decatur, IL), after short-term walnut administration [16]. Adiponectin is an adipocyte-secreted insulin sensitizer that improves insulin sensitivity and decreases inflammation [30]. Thus, the observed increase of adiponectin concentration that was not very pronounced and that achieved significance only when measured using one of the available assays that apparently has the highest discriminatory ability might reflect an initial phase of preclinical improvement of insulin sensitivity. Total adiponectin concentrations are not significantly different in predicting insulin resistance compared with HMW adiponectin [17], with higher adiponectin levels being inversely associated with risk for developing diabetes later in life [31]. These initial findings on adiponectin levels need to be studied further.

Table 1 – Serum concentrations of adiponectin, fetuin-A, and resistin at baseline and after 4 days of either placebo or walnut consumption

	Placebo			Walnut		
	Day 1	Day 4	*P*	Day 1	Day 4	*P*
Total adiponectin (μg/mL)	3.27 (2.37-4.92)	3.06 (2.04-4.51)	.16	3.42 (2.49-4.78)	3.93 (1.97-4.31)	.03
HMW adiponectin (μg/mL)	1.73 (0.58-2.27)	1.42 (0.32-2.32)	.01	1.82 (0.49-2.53)	1.49 (0.66-1.97)	.11
% HMW adiponectin	45.8 (26.2-52.3)	40.1 (19.7-53.5)	.38	50.9 (21.1-58.5)	36.7 (29.1-55.1)	.86
Fetuin A (μg/mL)	261.03 (237.70-71.59)	265.40 (205.04-442.99)	.91	264.51 (191.94-335.04)	258.25 (235.41-332.90)	.28
Resistin (ng/mL)	4.39 (2.34-6.97)	4.34 (2.83-6.00)	.43	3.27 (2.50-5.62)	4.91 (3.04-6.83)	.05

Results displayed as median and 25th to 75th interquartile range (n = 15).

Table 2 – Serum concentrations of inflammatory markers, markers of vascular injury, and apolipoproteins at baseline and after 4 days of either placebo or walnut consumption

	Placebo			Walnut		
	Day 1	Day 4	P	Day 1	Day 4	P
CRP (μg/mL)	2.21 (0.52-5.85)	1.23 (0.27-3.95)	.02	1.82 (1.09-5.08)	2.01 (0.43-3.86)	.39
SAA (μg/mL)	1.63 (1.48-4.28)	1.31 (1.01-3.34)	.01	1.68 (1.09-2.29)	1.47 (1.13-2.33)	.07
sICAM-1 (ng/mL)	184.61 (144.72-270.29)	172.32 (150.24-256.88)	.69	177.27 (149.47-256.76)	185.10 (158.05-264.03)	.11
sICAM-3 (ng/mL)	0.51 (0.45-0.67)	0.56 (0.36-0.73)	.82	0.54 (0.41-0.64)	0.59 (0.42-0.67)	.73
sVCAM-1 (ng/mL)	293.57 (209.75-402.94)	277.08 (238.54-404.56)	.91	279.27 (206.40-422.31)	285.49 (236.47-432.57)	.14
IL-6 (pg/mL)	1.50 (0.92-2.41)	2.10 (1.63-3.65)	.03	1.57 (1.13-2.58)	1.78 (1.34-2.78)	.02
IL-8 (pg/mL)	6.69 (3.02-11.00)	6.21 (3.10-16.46)	.36	5.70 (3.46-8.46)	5.58 (3.34-12.80)	.97
TNF-α (pg/mL)	7.76 (5.48-10.38)	7.59 (5.77-9.08)	.77	6.57 (4.92-8.23)	7.22 (5.75-11.04)	.14
E-selectin (ng/mL)	3.03 (1.91-4.77)	2.86 (1.64-4.17)	.42	3.20 (1.91-3.91)	2.91 (1.65-4.09)	.57
P-selectin (ng/mL)	13.16 (10.42-16.83)	14.17 (9.03-16.53)	.77	14.78 (11.38-16.65)	13.85 (9.75-17.15)	.82
Thrombomodulin-1 (ng/mL)	0.57 (0.44-0.65)	0.53 (0.42-0.63)	.11	0.53 (0.46-0.69)	0.59 (0.39-0.68)	.73
Apo A (mg/dL)	114.60 (102.10-133.60)	106.50 (91.10-119.30)	<.01	113.00 (108.90-121.60)	115.10 (91.90-125.30)	.03
Apo B (mg/dL)	74.80 (66.60-107.90)	70.30 (59.70-101.30)	.28	82.20 (70.00-114.00)	78.30 (63.40-100.40)	.21
Apo A/Apo B	0.73 (0.55-0.87)	0.77 (0.48-0.87)	.17	0.73 (0.54-0.96)	0.79 (0.53-0.99)	.28

Results displayed as median and 25th to 75th interquartile range (n = 15).

We also studied several other adipokines that have been proposed to be markers of vascular health [32]. Resistin is an adipose tissue–derived proinflammatory cytokine that directly activates endothelial cells, inducing the release of various chemokines, and is associated with insulin resistance, inflammation, and CVD [33]. Fetuin-A is a liver-derived molecule that directly modulates insulin resistance and regulates the production of endogenous inflammatory cytokines and adipocytokines [34]. All these hormones contribute toward the regulation of insulin sensitivity and endothelial function and ultimately regulate the balance between cardiovascular health and disease. Because levels of resistin and fetuin A remained unchanged, we propose that the increase of adiponectin concentration described herein is the first observed effect of walnut consumption that ultimately leads to the long-term improvement of the cardiovascular risk profile that is associated with walnut consumption [6].

Our data are consistent with the results of a long-term interventional study in women with polycystic ovary syndrome, another insulin-resistance state, in which 6 weeks of walnut consumption improved the insulin response to an oral glucose tolerance test by 26% [35]. The results that we present herein support the notion that changes in adiponectin levels occur even with short-term walnut consumption and might precede the improvement of insulin resistance and, by extension, diabetes [36,37], CVD [38], and cancer [29,39-42]. There is also mounting evidence suggesting that adiponectin has anti-inflammatory properties relevant to vascular function [32]. For instance, adiponectin in physiological concentrations has been shown to dose-dependently inhibit TNF-α–induced cell adhesion and expression of VCAM-1, E-selectin, and ICAM-1 in human aortic endothelial cells [43]. Nevertheless, we found no changes in the circulating levels of these latter biomarkers in serum.

It has also been suggested that favorable changes in lipid profile, which follow long-term consumption of nuts such as walnuts, could also account, at least in part, for the cardioprotective effect of walnut consumption [9,19,44]. In a recent meta-analysis, 3 to 6 weeks of walnut consumption has been demonstrated to increase HDL cholesterol concentration and decrease total and low-density lipoprotein cholesterol and triglyceride concentrations [44]. Circulating apo A represents the HDL fraction of lipoproteins and has been demonstrated to be a powerful predictor of CVD risk [45]. In this study, we demonstrated that walnut consumption for 4 days leads to a small, but statistically significant, increase in apo A concentration, which is consistent with the previously reported long-term results [19,44]. Thus, this is the first study to demonstrate that the beneficial effects of walnut consumption on the lipid profile are evident even within the first 4 days of walnut consumption.

Furthermore, recent large-scale cross-sectional studies have put forth the hypothesis that favorable changes in inflammatory markers relating to atherosclerosis, such as reductions in CRP and IL-6, could also contribute to the observed reduction in CVD risk associated with nut consumption [46]. Smaller interventional studies have demonstrated that, besides its hypocholesterolemic effect, relatively prolonged walnut consumption (40-65 g/d for 4-6 weeks) also favorably alters several inflammatory and vascular injury biomarkers, such as CRP, VCAM-1, ICAM-1, and E-selectin [8,10], and improves endothelial function in hypercholesterolemic subjects [8]. Remarkably, an improvement in endothelial function by approximately 25% was also apparent even after a 40-g single walnut-containing meal; but this effect was largely independent of changes in oxidative stress, inflammatory, and vascular health biomarkers [11]. Consistent with these observations, we observed no changes in a large array of circulating markers of inflammation and vascular injury after 4 days of walnut consumption. Because the daily amount of walnuts consumed was rather similar in all the previous studies as well as the present one (40-65 g/d) [8,10,11], the different results between studies of short-term and long-term walnut consumption are likely due to the length of the dietary intervention (≤4 days as opposed to ≥4 weeks). Differences in baseline lipid profile and extent of endothelial dysfunction could also be responsible for these discrepant results because the walnut-induced improvement in endothelial function has

only been demonstrated in hypercholesterolemic and not in normocholesterolemic subjects [11]. Only 4 of our subjects were hypercholesterolemic, and this may partly account for the lack of an effect of dietary walnuts on circulating markers of inflammation and vascular injury. It is possible that walnut consumption may be inducing changes in the expression of proinflammatory genes at intracellular sites (eg, reduces TNF-α and IL-6 messenger RNA in peripheral blood mononuclear cells) that do not manifest as changes in the concentrations of these biomarkers in serum [47]. Alternatively, it is also possible that more than 4 days are needed for the beneficial effects of the intervention and/or increased adiponectin concentration to manifest. This remains to be examined by longer, dose-response studies in the future.

Major strengths of our study include its crossover, randomized, placebo-controlled, and double-blinded design. Laboratory assays were performed using state-of-the-art instruments by blinded technicians unaware of the hypotheses underlying the study. The major limitation is the lack of any hard clinical measurements of endothelial dysfunction and vascular health, but 4 days would be too early to detect such changes. Furthermore, we only evaluated the circulating levels of inflammatory and vascular injury biomarkers; and these may not necessarily reflect changes in biological function. Furthermore, the specific nutrients in walnuts responsible for the observed beneficial effects remain unknown. Walnuts contain a wide array of nutrients with relevance to cardiovascular health. They are particularly rich sources of the polyunsaturated fatty acids linoleic and α-linolenic acids, and also contain high amounts of dietary fiber, arginine-rich protein, potassium, copper, and magnesium, as well as antioxidant vitamin E and other compounds with biological activity such as flavonoids, other polyphenols, and sterols [19,48]. It is thus likely that the effects of walnut consumption on CVD risk stem from the combined actions of more than one nutrient on many biological functions. It is also possible that their effect on markers studied herein could be mediated through changes in appetite and short-term caloric intake, but these possibilities could not be assessed directly and/or through multivariable adjustment in this study because of limitations imposed by the data set.

In conclusion, the results of this randomized, double-blinded, placebo-controlled, crossover study suggest that short-term consumption of walnuts (48 g/d for 4 days) improves lipid profile by increasing apo A concentration, suggesting that walnuts exert their beneficial effect on lipid metabolism even within 4 days of consumption. The apparent increase in the circulating concentration of the endogenous insulin sensitizer, adiponectin, suggests that adiponectin might be the link to the long-term beneficial effects of walnut consumption on CVD, insulin resistance, and neoplasia. Longer-term studies could provide further insight into the mechanisms through which dietary walnuts exert their beneficial actions.

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Conflict of Interest

The authors have no conflicts of interest.

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