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Walnut Consumption Increases Satiation but Has No Effect on Insulin Resistance or the Metabolic Profile Over a 4-day Period

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Obesity and diabetes have been associated with increased consumption of highly processed foods, and reduced consumption of whole grains and nuts. It has been proposed, mainly on the basis of observational studies, that nuts may provide superior satiation, may lead to reduced calorie consumption, and may decrease the risk of type 2 diabetes; but evidence from randomized, interventional studies is lacking. A total of 20 men and women with the metabolic syndrome participated in a randomized, double-blind, crossover study of walnut consumption. Subjects had two 4-day admissions to the clinical research center where they were fed an isocaloric diet. In addition, they consumed shakes for breakfast containing either walnuts or placebo (shakes were standardized for calories, carbohydrate, and fat content). Appetite, insulin resistance, and metabolic parameters were measured. We found an increased level of satiety (overall *P* value = 0.0079) and sense of fullness (*P* = 0.05) in prelunch questionnaires following the walnut breakfast as compared to the placebo breakfast, with the walnut effect achieving significance on day 3 and 4 (*P* = 0.02 and *P* = 0.03). We did not find any change in resting energy expenditure, hormones known to mediate satiety, or insulin resistance when comparing the walnut vs. placebo diet. Walnut consumption over 4 days increased satiety by day 3. Long-term studies are needed to confirm the physiologic role of walnuts, the duration of time needed for these effects to occur, and to elucidate the underlying mechanisms.

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INTRODUCTION

Obesity and diabetes are among the more significant public health epidemics of the twenty-first century around the world. Both obesity and diabetes have been associated with increased consumption of highly processed foods and reduced consumption of whole grains and nuts. Nuts, an important component of the Mediterranean diet, have been shown through several large epidemiological studies to have beneficial health effects. Nuts have been shown to reduce the risk of cardiovascular disease, sudden death, and diabetes mellitus (1–4). These benefits are thought to be due, in part, to improvements in lipid profile, inflammation, and endothelial function (5,6).

Walnuts, similar to other nuts, are rich in fat. Although they have a low content of saturated fatty acids and a high content of polyunsaturated fatty acids (PUFAs) (7), concerns have been raised that because of their high-fat content, increased walnut consumption would lead to weight gain. Epidemiological studies have shown a consistent inverse association between nut consumption and weight change (2,8–10). A randomized, crossover study recently found an increase in postmeal energy expenditure, but no difference in satiety between subjects fed walnuts vs. fat-rich dairy products. This was based on reported appetite satisfaction after one meal in an outpatient setting (11). Thus, although it has been proposed that whole foods, such as nuts, may provide superior satiation and lead to reduced calorie consumption (12), there have been no blinded and controlled feeding studies to evaluate the effects of walnuts on satiety. Similarly, no studies have been conducted where subjects are closely observed and fed over several days in a clinical research center. In addition, the effect of walnuts on energy expenditure remains unknown. Molecules important in regulating energy homeostasis, including pancreas, gut, and adipose tissue-derived peptides, have also not been studied in relation to walnut consumption. It would be reasonable to hypothesize that these molecules may provide a link between increased satiety and energy expenditure or the observed lower incidence of type 2 diabetes in habitual walnut consumers. Many of these effects would be of particular benefit to individuals with the metabolic syndrome, a condition associated with insulin resistance and increased risk of cardiovascular disease.

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We performed a randomized, double-blind, crossover study of nut consumption in men and women with the metabolic syndrome, who were studied during two randomly assigned, 4-day-long in-patient study periods in our institution's clinical research center. The primary aim of this study was to examine the effects of walnuts on satiety over a 4-day period in the context of a blinded, randomized, interventional trial. The secondary aims were to investigate the effects of walnut consumption on molecules that regulate appetite, as well as glucose tolerance and endocrine function.

METHODS AND PROCEDURES

Subjects

The study was conducted between March 2007 and November 2008 at Beth Israel Deaconess Medical Center's General Clinical Research Center. Participants were volunteers recruited from the community and were deemed eligible to participate if they were between the ages of 40 and 75 years and had the metabolic syndrome, as defined by the 2006 International Diabetes Federation criteria (13). Subjects were excluded if they had diabetes mellitus requiring pharmacotherapy, were pregnant or breastfeeding, and/or had anemia or malabsorption from any cause. Subjects with current alcoholism or drug abuse or use of medications that could interfere with the study, such as corticosteroids, growth hormone, or antiretroviral therapy were also excluded. In addition, individuals with any history of a nut allergy were excluded. These conditions were screened for by a detailed history and physical examination.

A total of 63 subjects were screened with an evaluation of medical history, physical examination, and laboratory parameters. Of these, 31 qualified to participate. Eleven subjects withdrew consent prior to randomization. The remaining 20 individuals participated in the study and were included in the per protocol screening analysis. A total of 15 subjects completed both arms of the study. The study was approved by the institutional review board of the Beth Israel Deaconess Medical Center and all subjects provided written informed consent to participate. The clinical trial registration number is NCT00525629.

Study design

The study was a randomized, double-blind, crossover study of walnut consumption. Eligible subjects were counseled by a research dietitian prior to starting the study and were asked to abstain from walnuts for 2 weeks prior to their first study visit. They also received standardized advice on the American Heart Association Therapeutic Lifestyle change diet and were asked to maintain a stable diet and exercise pattern throughout the study. After 2 weeks of washout, subjects were admitted for their first study visit at 9 PM prior to the first day of testing. Subjects were randomly assigned by a blinded statistician to either receive walnut-containing diet or placebo diet on the first visit. During both visits, subjects followed an identical isocaloric diet based on foods that they like to eat. Subjects could only eat food provided by the nutritionist. They were encouraged to eat the entire meal provided, and any food they did not eat was weighed back and accounted for in the metabolic kitchen. Following a baseline assessment and completion of appetite questionnaire on the first study day, subjects received a liquid breakfast meal. They were permitted to ambulate freely around the hospital during their stay, but were not permitted to exercise or leave the hospital. Subjects consumed a liquid breakfast each morning and had repeated assessment on the morning of the fourth day. Following completion of this assessment, subjects were discharged and asked to avoid walnuts for 1 month. After 1 month, they returned for a second, 4-day stay in the General Clinical Research Center.

Intervention

In order to allow for blinding of subjects and study staff, 48 g of walnuts were incorporated into a liquid meal with similar macronutrient composition. The walnut/placebo shakes were standardized for calories (walnuts 586 kcal vs. placebo 585 kcal), carbohydrate, and fat content. The walnut meal contained 6.58% protein, 46.05% fat, and 47.37% carbohydrates. The placebo meal contained 2.14% protein, 48.55% fat, and 49.31% carbohydrates. The walnut meal was rich in PUFA (22.82 g PUFA, 4.4 g monounsaturated fatty acid); whereas the placebo meal was rich in monounsaturated fatty acid (4.79 g PUFA, 24.01 g monounsaturated fatty acid). Remaining fat content consisted of small amounts of saturated fatty acids. The walnut meal contained 9.76 g of fiber whereas the placebo meal contained 7.21 g. The walnut-containing liquid meal contained 48 g of walnuts, 50 g of frozen mango, 50 g of frozen strawberries, 60 g of banana, 100 g of frozen berries, and 250 g of pineapple juice. The placebo liquid meal contained 32 g safflower oil, 60 g of frozen mango, 50 g of frozen strawberries, 80 g of banana, 100 g of frozen berries, 260 g of pineapple juice, and 40 drops of walnut flavoring. Walnut halves and pieces were used and blended with the other ingredients into a milk-shake consistency drink which was consumed cold for each morning meal. A pilot study in a group of 10 representative subjects demonstrated that subjects could not determine which shake contained walnuts. Subjects were fed an isocaloric diet for the 4 days of each visit. All food was weighed back to ensure compliance with the diet.

Measurements

Subjects were weighed on the first day of each admission. Body composition was measured using Bioelectrical Impedence Analysis (Tanita, Arlington Heights, IL). Fullness, satiety, and hunger were assessed by a visual analog scale before breakfast and lunch on each day of both visits as previously described and validated for appetite research (14). Resting energy expenditure was measured using indirect calorimetry on the first and final day of each visit (V_{max} sensor).

Assessment of insulin resistance and laboratory parameters

On the first and last day of each visit, following a 12-h fast, subjects had a baseline blood draw after which they were requested to consume their liquid breakfast within 5 min. Repeat blood sampling was performed every 30 min for 3 h. Glucose and lipids (low-density lipoprotein, triglycerides, high-density lipoprotein, total cholesterol) were measured in the central clinical laboratory at Beth Israel Deaconess Medical Center using standard laboratory techniques. All other samples were stored as serum at −80 °C until assayed in duplicate. Samples for the same subject were run in the same assay. Standard techniques were used to measure insulin as previously described (15). Glucagon-like peptide 1 (GLP-1) was measured using enzyme-linked immunosorbent assay (ELISA) (LINCO, St Charles, MO) with sensitivity 2.0 pM/l, intra-assay coefficient of variation (CV) 6–9%, and interassay CV <1.0–13%. Glucose-dependent insulinotropic polypeptide was measured by ELISA (LINCO) with sensitivity 8.2 pg/ml, intra-assay CV 1.8–6.1%, and interassay CV 3.0–8.8%. Active ghrelin and total ghrelin were measured by ELISA (LINCO) as described previously (16). Leptin and adiponectin were measured using radioimmunoassays (LINCO) as described previously (17–19). Peptide YY (PYY) was measured by ELISA (LINCO), with sensitivity 1.4 pmol/l, intra-assay CV 0.86–5.78%, interassay CV 3.65–16.5%, and 100% crossreactivity for the full-length peptide (PYY1–36) and the truncated PYY3–36, both of which have biological activity. sCD40L was measured by ELISA (R&D System, Minneapolis, MN) with a sensitivity of 4.2 pg/ml, intra-assay CV 4.5–5.4%, and interassay CV 6.0–6.4%. Oxidized low-density lipoprotein was measured by ELISA (ALPCO Diagnostics, Salem, NH) with sensitivity 0.8 ng/ml, intra-assay CV 4.0–7.6%, and interassay CV 6.2–10.7%.

Statistical analysis

The results are presented as mean values ± s.e.m. SAS (version 9.1; SAS, Cary, NC) and SPSS (version 11.5; SPSS, Chicago, IL) was used for statistical analysis, and $P < 0.05$ (two-tailed) was considered statistically significant for all analyses. We used one-way ANOVA followed by the protected least significant-differences technique for continuous variables, and Fisher's Exact test for categorical variables, to compare subjects who completed the study with subjects who did not complete the study.

As a primary analysis, we used Wilcoxon's signed rank (paired tests), paired *t*-test and mixed model controlled for sequence to compare change under walnut-containing diet vs. a placebo diet (on-treatment analysis, $n = 15$ completing both visits) and Wilcoxon's rank sum test and mixed model controlled for sequence (independent-samples tests) as secondary analysis (intention-to-treat analysis, $n = 20$).

RESULTS

Twenty subjects were randomized and participated in the study. Eighteen completed at least one 4-day visit, and 15 subjects completed both study visits. Baseline demographic characteristics of the study sample are presented in Table 1. Completers vs. noncompleters had significantly lower high-density lipoprotein cholesterol (41.3 mg/dl vs. 58.6 mg/dl, $P = 0.02$) at baseline. All other baseline characteristics were not significantly different. Subjects' weight was not statistically different between the first day of each admission ($P = 0.33$). Similarly, there was no difference in macronutrient intake during either admission (96.01% target kcal/day on walnut diet, 97.73% target kcal/day on placebo diet, $P = 0.594$). There was no difference in any outcome between the total sample (all 18 subjects who completed at least 1 visit) and those who completed both visits. Only data for completers is shown.

Appetite

On all days of both placebo and walnut visits, the subjects reported no difference in their feelings of fullness, satiety, or hunger on prebreakfast questionnaires (data not shown). Subjects reported feeling more satiated prelunch on a walnut-containing diet (overall $P = 0.0079$) with the walnut effects achieving significance on day 3 and day 4 ($P = 0.02$ and $P = 0.03$, respectively) (Table 2). Subjects also had a significantly higher rate of feeling "full" during the walnut compared to placebo diet ($P = 0.05$) (Table 2 and Figure 1). There was a significant difference in reporting of feeling full between the two groups (Table 2) on day 3 ($P = 0.03$) and the difference was borderline on day 4 ($P = 0.1$).

Metabolic profile

Fasting glucose and insulin levels were measured on day 1 and day 4 of each visit. There was no significant difference of insulin

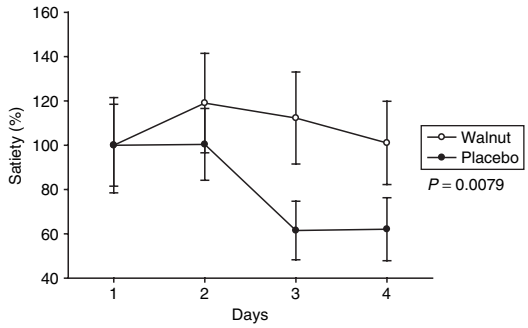

Figure 1 Satiety changes between day 1 and day 4 in response to either walnut or placebo breakfasts (last observation carried forward).

Table 1 Baseline characteristics of study subjects

	Total sample ($N = 20$)	Completed ($N = 15$)	Did not complete ($N = 5$)	P[a]
Age	59.0 ± 2.0	58.0 ± 2.5	62.0 ± 2.8	0.41
Height (cm)	169.1 ± 2.2	171.1 ± 2.5	163.3 ± 4.3	0.13
Weight (kg)	104.1 ± 3.6	106.2 ± 4.4	97.9 ± 5.4	0.33
BMI	37.0 ± 1.4	36.9 ± 1.7	37.1 ± 2.6	0.96
Waist circumference (cm)[b]	114.8 ± 2.3	117.0 ± 2.7	109.2 ± 3.8	0.14
Glucose (mg/dl)	89.9 ± 3.5	91.3 ± 4.4	85.4 ± 4.0	0.47
TC (mg/dl)	207.0 ± 11.8	208.5 ± 12.8	202.4 ± 30.1	0.83
LDL (mg/dl)	122.3 ± 10.7	124.6 ± 10.9	115.4 ± 29.8	0.72
Triglycerides (mg/dl)	199.2 ± 22.5	218.3 ± 26.1	142.0 ± 36.3	0.15
HDL (mg/dl)	45.7 ± 3.6	41.3 ± 3.1	58.6 ± 9.1	0.03
Systolic BP (mm Hg)	138.4 ± 3.0	138.9 ± 3.8	136.8 ± 4.7	0.78
Diastolic BP (mm Hg)	80.6 ± 2.0	81.5 ± 2.3	77.8 ± 4.2	0.43
Exercise (h/week)	4.1 ± 1.4	2.9 ± 1.1	7.8 ± 4.6	0.13
	N (%)	*N* (%)	*N* (%)	
Male	10 (50%)	9 (60%)	1 (20%)	0.30
White	15 (75%)	13 (87%)	2 (40%)	0.07
African American[c]	4 (21%)	2 (13%)	2 (50%)	0.18
BP medications[d]	4 (22%)	4 (27%)	0 (0%)	1
Cholesterol medications[d]	9 (50%)	6 (40%)	3 (100%)	0.21

Values are mean ± s.e.
BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol.
[a]*P* value is based on one-way ANOVA followed by the protected least significant-differences technique for continuous variables and Fisher's Exact test for categorical variables. [b]Information is only available for $n = 18$. [c]Information is only available for $n = 19$. [d]Information is only available for $n = 18$.

Table 2 Results of prelunch appetite questionnaires on days 1–4 for subjects who completed both visits (using LOCF)

	Walnut	Placebo	P^a	P^b	P^c
Full					0.05
Day 1	32.3 ± 6.5	23.6 ± 6.8	0.26	0.3	
Day 2	37.5 ± 8.3	24.6 ± 4.4	0.2	0.33	
Day 3	27.6 ± 6.8	14.6 ± 3.8	0.03	0.03	
Day 4	30.7 ± 5.1	22.3 ± 5.4	0.14	0.13	
Satiety					0.01
Day 1	38.5 ± 6.7	33 ± 7.3	0.56	0.9	
Day 2	45.7 ± 8.2	33.1 ± 5.3	0.25	0.37	
Day 3	43.1 ± 7.5	19.4 ± 4.4	0.01	0.02	
Day 4	38.8 ± 6.8	19.6 ± 4.7	0.02	0.03	
Hungry					0.17
Day 1	68.2 ± 6.9	63.5 ± 6.8	0.5	0.64	
Day 2	55.7 ± 8.2	59.4 ± 7.2	0.68	0.86	
Day 3	62.3 ± 7.7	68.9 ± 7.7	0.45	0.27	
Day 4	59.8 ± 7.2	52.7 ± 8.3	0.16	0.31	

Values are millimeters on a visual analog scale; mean ± s.e. are presented.
LOCF, last observation carried forward.
[a]*P* value is based on Wilcoxon signed rank test.[b]*P* value is based on paired *t*-test and mixed model controlled for sequence which have same results. [c]*P* value is based on repeated measures analysis to compare differences between walnut and placebo groups over 4 days of period.

levels between the two groups on both day 1 and day 4 (Table 3). Even though the baseline between-meal differences in area under curves of glucose (day 4) was significantly different, change of glucose between day 1 and day 4 were not significantly different (Table 4). There was no difference in homeostasis model assessment of insulin resistance on day 1 of both visits (data not shown). Similarly, after 4 days of a walnut-containing diet, insulin resistance was no different than the placebo diet (data not shown). We also performed glucose and insulin assessments every 30 min after walnut-containing and placebo breakfast. The difference between the area under the curve for mean glucose was similar between walnut and placebo on day 4 of each visit (Table 3). Forty percent of subjects were on statin treatment during the study. No subject had a change in their statin dose during the study period. There was no difference in low-density lipoprotein, high-density lipoprotein, total cholesterol, or triglyceride values on walnut-containing diet. There was also no difference in sCD40L between the two groups.

Molecules of energy homeostasis

We performed measurements of gut peptides following walnut and control meals on day 1 and day 4 of each visit. Levels of GLP-1, total ghrelin, and active ghrelin were unchanged between day 1 and day 4 (Table 4). We also performed measurements of adipokines on day 1 and day 4, but leptin and adiponectin levels were also unchanged. Even though the between-meal differences in area under curves of glucose-dependent insulinotropic polypeptide (day 1) and PYY (days 1 and 4, Table 3) were significantly different, the levels of glucose-dependent insulinotropic polypeptide, PYY were unchanged between day 1 and day 4 (Table 4).

DISCUSSION

We demonstrate, herein, that a walnut-containing breakfast improves satiation over a 3–4-day period. It has been proposed, mostly on the basis of epidemiological studies, that whole foods, such as nuts and fiber, may provide superior satiation and lead to reduced calorie consumption (11,12). Casas-Agustench *et al.* recently performed a randomized, crossover trial comparing satiety between three high-fat meals administered once (11). One meal was high in PUFA derived from walnuts, another rich in monounsaturated fatty acid derived from olive oil, and the third meal was high in saturated fatty acid derived from dairy products. There was no difference in satiety between any of the groups. Interestingly, this study assessed satiety after only one meal, whereas our study found differences in satiety, which started achieving significance after 3 days of walnut treatment. Importantly, although subjects in the prior study were instructed to follow an isocaloric diet at home, we provided the meals in a blinded and randomized manner, as well as in the controlled environment of an in-patient setting where diet and activity could be closely monitored. A possible explanation for the differing results of these studies is that the mechanism by which walnuts increase satiety may not manifest in an extremely short term basis and are difficult to assess in an uncontrolled environment. It is also important to note that in our study, the mastication step of walnut consumption is eliminated. Mastication has been shown in almonds to have important consequences on satiety, meal fat availability, and postprandial and gastrointestional hormone stimulation (20). But, because our study is a randomized controlled trial, we expect that we would not see a difference based on this elimination. If anything, because mastication has

Table 3 Biomarkers (paired results) for subjects who completed both visits

	Day 1				Day 4			
Area under curve	Walnut	Placebo	P^a	P^b	Walnut	Placebo	P^a	P^b
Glucose	18,332 ± 1,091	20,540 ± 1,230	0.01	0.10[c]	17,576 ± 834.1	18,332 ± 1,091	0.12	0.009[c]
Insulin	9,533 ± 1,182	10,756 ± 1,078	0.30	0.25	11,533 ± 1,127	11,935 ± 1,118	0.56	0.58
FFA	80.79 ± 5.2	78.1 ± 3.8	0.60	0.48	68.14 ± 4.2	67.2 ± 4	0.93	0.71
AGh	35,712 ± 5,429	35,077 ± 4,626	0.46	0.82	29,020 ± 5,202	25,478 ± 3,902	0.33	0.28
TGh	160,619 ± 6,708	162,588 ± 6,005	0.76	0.63	142,714 ± 7,916	140,390 ± 8,820	0.90	0.72
GLP	723.06 ± 81.43	755.76 ± 72.34	0.54	0.15[d]	791.45 ± 75.74	811.31 ± 90.47	0.95	0.89[d]
GIP	40,188 ± 4,379	49,657 ± 6,031	0.01	0.01[c]	35,643 ± 3,678	45,517 ± 6,580	0.06	0.10[c]
PYY	19,231 ± 2,191	16,254 ± 1,518	0.01	0.003[c]	19,626 ± 2,632	15,688 ± 1,639	0.02	0.02[c]
Baseline level								
Glucose (mg/dl)	97.43 ± 3.85	101.64 ± 3.74	0.16	0.17	97.79 ± 3.08	97.43 ± 3.851	0.80	0.87
Insulin (μU/ml)	18.25 ± 1.88	19.82 ± 2.55	0.52	0.50	22.58 ± 2.49	27.44 ± 3.46	0.30	0.18
FFA (mmol/l)	0.7 ± 0.06	0.73 ± 0.05	0.50	0.50	0.46 ± 0.04	0.52 ± 0.05	0.05	0.06
AGh (pg/ml)	253.49 ± 38.61	250.68 ± 36.43	0.81	0.89	193.32 ± 36.79	181.17 ± 27.96	0.43	0.61
TGh (pg/ml)	955.79 ± 48.23	956.2 ± 49.1	0.39	0.99	838.84 ± 54.83	829.97 ± 61.11	1.00	0.80
GLP (pmol/ml)	3.44 ± 0.36	3.46 ± 0.29	0.95	0.50[d]	3.44 ± 0.322	3.32 ± 0.343	0.46	0.72[d]
GIP (pg/ml)	55.04 ± 9.5	69.27 ± 11.74	0.13	0.29	46.66 ± 6.05	52.26 ± 9.59	0.79	0.43
PYY (pg/ml)	56.6 ± 7.61	70.58 ± 14.33	0.68	0.38	52.57 ± 9.82	52.62 ± 8.49	0.85	0.99
Leptin (ng/ml)	28.38 ± 5.42	27.01 ± 5.23	0.25	0.30	26.75 ± 5.03	28.32 ± 6.40	0.93	0.51
Adiponectin (μg/ml)	3.96 ± 0.51	3.81 ± 0.46	0.30	0.28	3.48 ± 0.46	3.56 ± 0.44	0.36	0.53
oxLDL (ng/ml)					398.01 ± 65.06	414.92 ± 54.65	0.90	0.41
sCD40L (ng/ml)					1.37 ± 0.47	1.43 ± 0.46	0.72	0.93

Values are mean ± s.e.
AGh, active ghrelin; FFA, free fatty acids; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; oxLDL, oxidized low-density lipoprotein; PYY, peptide YY; TGh, total ghrelin.
[a]*P* value is based on Wilcoxon signed rank test. [b]*P* value is based on paired *t*-test and mixed model controlled for sequence. [c]*P* value is based on mixed model controlled for sequence, and baseline level. [d]*P* value is based on log-transformed data.

been shown to increase satiety, removing this step would have resulted in our satiation results being less significant.

Interestingly, the same epidemiological evidence that suggests walnuts improve satiation also suggests that they may lead to reduced calorie consumption (10). This theory is supported by epidemiology and interventional studies, which demonstrate a consistent inverse association between nut consumption and weight change (10,11). Some of the satiation effects of walnuts are thought to be due, in part, to a compensatory reduction in energy consumption. This accounts for up to 75% of the calories contained in the nuts so that when nuts are added to the diet, without any other intervention, the resulting weight gain is far less than expected (21). We could not assess caloric intake because, by study design, subjects were instructed to consume all food provided to them to maintain an isocaloric diet for the duration of the study. We did not find any resting energy expenditure changes on day 4 of admission; we only report an effect of walnuts on satiety on the third and fourth days of walnut-enriched breakfast. We then hypothesized that peripherally secreted hormones such as leptin, adiponectin, or recently discovered gut peptides may underlie the observed effect of walnut consumption. Several gut peptides such as GLP-1, PYY, and glucose-dependent insulinotropic polypeptide are increased with meal consumption, and act to slow gastric emptying, stimulate insulin secretion, inhibit glucagon secretion, and control body weight (22,23). Recent evidence indicates that meal content may have a direct effect on the secretion of gut peptides such as GLP-1 from intestinal L cells through duodenal taste receptors (24). We found no change in levels of GLP-1 and/or total or active ghrelin. Ghrelin is a hormone secreted by the P/D1 cells that line the fundus of the human stomach and epsilon cells of the pancreas, which stimulate hunger (25). Ghrelin increases food intake by an action exerted at the level of the hypothalamus (26). If the satiation caused by walnuts was due to changes in gut hormones, we would have expected a decreased level of active ghrelin in the walnut group during the time frame of our study. Our data does not support this hypothesis, but perhaps frequent sampling or more long-term studies may help to elucidate this further. Further, we did not see any changes in the levels of leptin or adiponectin after the 4 days of treatment. A finding consistent with the more long-term role of these adipokines is the regulation of energy homeostasis. It is possible that there is an undiscovered protein, either peripherally or centrally derived,

Table 4 Change in biomarkers after 4 days of treatment with walnut or placebo (data based on subjects who completed both visits)

	Walnut	Placebo	P^a	P^b
Area under the curve				
Glucose	−785.09 ± 657.60	−2,208.21 ± 456.59	0.09	0.16[c]
Insulin	2,000.64 ± 812.30	1,178.89 ± 854.01	0.25	0.37
FFA	−12.65 ± 5.64	−10.87 ± 3.28	0.93	0.71
AGh	−6,691.38 ± 3,055.74	−9,598.96 ± 3,953.04	0.76	0.47
TGh	−17,904.84 ± 3,969.25	−22,198.79 ± 7,133.54	0.50	0.57
GLP	68.39 ± 24.02	55.55 ± 61.58	0.63	0.83
GIP	−4,544.81 ± 3,815.86	−4,139.68 ± 4,540.23	0.84	0.72[c]
PYY	395.87 ± 1,288.87	−566.11 ± 1,168.63	0.80	0.75[c]
Baseline level				
Glucose (mg/dl)	−0.86 ± 1.70	−4.21 ± 1.70	0.17	0.14
Insulin (μU/ml)	4.33 ± 2.49	7.62 ± 2.93	0.49	0.33
FFA (mmol/l)	−0.23 ± 0.06	−0.20 ± 0.05	0.82	0.59
AGh (pg/ml)	−60.18 ± 19.44	−69.51 ± 32.40	0.50	0.74
TGh (pg/ml)	−116.95 ± 41.83	−126.24 ± 37.30	0.86	0.86
GLP (pmol/ml)	0.00 ± 0.14	−0.14 ± 0.16	0.71	0.57
GIP (pg/ml)	−8.38 ± 8.29	−17.01 ± 8.55	0.27	0.52
PYY (pg/ml)	−4.03 ± 6.90	−17.96 ± 16.09	0.64	0.39
Leptin (ng/ml)	−1.62 ± 1.07	1.31 ± 2.84	0.37	0.56
Adiponectin (μg/ml)	−0.48 ± 0.17	−0.26 ± 0.11	0.11	0.21

Values are mean ± s.e.
AGh, active ghrelin; FFA, free fatty acids; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; PYY, peptide YY; TGh, total ghrelin.
[a]*P* value is based on Wilcoxon signed rank test. [b]*P* value is based on paired *t*-test and mixed model controlled for sequence. [c]*P* value is based on mixed model controlled for sequence, sex, and baseline level.

which mediates the induced satiety. Alternatively, the fatty acid composition of walnuts could, by acting centrally, alter appetite, as previously suggested (27). It is important to note, that the satiation effects could also have been noted because of differences in food variables such as fiber (walnut shake 9.76 g vs. placebo 7.21 g). Further investigation is needed. Thus, based on our analysis, short term satiety from walnut consumption does not appear to be mediated through gut hormones and/or the adipocyte secreted hormones, leptin, and adiponectin.

Walnuts have been shown to have beneficial effects in diabetic patients and those with the metabolic syndrome. A large prospective cohort found that increased nut intake is associated with decreased risk of type 2 diabetes (3). A recent randomized interventional study showed that diets containing walnuts result in significantly lower fasting insulin levels at 1 year (28). In contrast, in a randomized controlled study of 8 weeks duration, a high walnut content diet did not result in any benefit in metabolic parameters, or any of the markers of the metabolic syndrome (29). Another randomized study showed that diets containing 30 g of walnuts improve the lipid profile in diabetic subjects after 6 months, in addition to showing a trend toward reduced body fat (6). And, importantly, its been shown that compared to a low-fat diet, a Mediterranean diet enhanced with one serving of nuts daily lead to a reduction in the overall prevalence of the metabolic syndrome (30). Our study was of significantly shorter duration, only 4 days of a walnut-containing diet, and is consistent with the prior literature by not demonstrating a large change in metabolic or hormonal measures within a few days, including changes in insulin resistance, fasting glucose, or lipid levels. In 2007, Estruch *et al.* showed that 3 months of a diet high nuts (30 g/day) showed significant improvement in cardiovascular risk factors, including fasting glucose and insulin sensitivity, in older men and women (31). In the future, more long-term studies need to be performed to completely understand both the exact timing and the specific long-term effects and/or benefits of walnuts on patients with type 2 diabetes mellitus and/or the metabolic syndrome.

In conclusion, this randomized, crossover study found that satiation is increased within 3–4 days of a walnut-containing diet. We did not detect effects on body weight, insulin resistance, or metabolic hormone levels. Long-term studies are needed to further elucidate the underlying mechanisms and the physiologic role of walnuts on these outcomes.

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DISCLOSURE

The authors declared no conflict of interest.

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