

Effects of a high walnut and high cashew nut diet on selected markers of the metabolic syndrome: a controlled feeding trial

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We investigated the effects of a high walnut diet and a high unsalted cashew nut diet on selected markers of the metabolic syndrome. In a randomized, parallel, controlled study design, sixty-four subjects having the metabolic syndrome (twenty-nine men, thirty-five women) with a mean age of 45 (SD 10) years and who met the selection criteria were all fed a 3-week run-in control diet. Hereafter, participants were grouped according to gender and age and then randomized into three groups receiving a controlled feeding diet including walnuts, or unsalted cashew nuts or no nuts for 8 weeks. Subjects were required to have lunch at the metabolic ward of the Nutrition Department of the North-West University (Potchefstroom Campus). Both the walnut and the unsalted cashew nut intervention diets had no significant effect on the HDL-cholesterol, TAG, total cholesterol, LDL-cholesterol, serum fructosamine, serum high-sensitivity C-reactive protein, blood pressure and serum uric acid concentrations when compared to the control diet. Low baseline LDL-cholesterol concentrations in the cashew nut group may have masked a possible nut-related benefit. Plasma glucose concentrations increased significantly ($P=0.04$) in the cashew nut group compared to the control group. By contrast, serum fructosamine was unchanged in the cashew nut group while the control group had significantly increased ($P=0.04$) concentrations of this short-term marker of glycaemic control. Subjects displayed no improvement in the markers of the metabolic syndrome after following a walnut diet or a cashew nut diet compared to a control diet while maintaining body weight.

Metabolic syndrome: Hyperlipidaemia: Hypertension: Obesity: Lipids: Fatty acids: Diet

The National Cholesterol Education Program's Adult Treatment Panel III (ATP III) defines the metabolic syndrome by easily measured clinical parameters that include: increased abdominal circumference, elevated TAG, low HDL-cholesterol (HDL-C), elevated fasting blood glucose and/or elevated blood pressure (BP). Three of these five are required for diagnosis (National Cholesterol Education Program, 2001).

From the third National Health and Nutrition Examination Survey (NHANES III) (Ford, 2004) and the THUSA study (THUSA is the acronym for Transition and Health during Urbanisation in South Africa, and is also the Tswana word for "help") (Oosthuizen *et al.* 2002) it is evident that the increasing prevalence of the metabolic syndrome is a health problem not only for developed countries but also for developing countries.

This syndrome has been identified as a target for dietary therapies (Wirfalt *et al.* 2001) to reduce the risk of CVD (Shirai, 2004) and type 2 diabetes (Jimenez-Cruz *et al.*

2003). In lieu of the therapeutics of the metabolic syndrome various researchers have investigated different dietary approaches. Some of the recent dietary interventions range from caloric restriction (Wien *et al.* 2003; Muzio *et al.* 2005), type of diet, e.g. Mediterranean (Esposito *et al.* 2004; Michalsen *et al.* 2006), type and/or amount of dietary fat (Riccardi *et al.* 2004; Freire *et al.* 2005), amount of carbohydrates (Dansinger & Schaefer, 2006), increasing the intake of certain minerals (He *et al.* 2006) and increasing dietary intake of certain functional foods (e.g. nuts, grapefruit) (Pieters *et al.* 2005; Fujioka *et al.* 2006).

Riccardi *et al.* (2004) concluded that the diet for the treatment of the metabolic syndrome should be limited in the intake of saturated fat, while high-fibre/low-glycaemic index foods should be used without specific limitations (Giacco *et al.* 2000). Recently, in the Framingham Offspring cohort, the researchers found glycaemic index and glycaemic load (a measure of both carbohydrate quality and quantity) were

Abbreviations: ATP III, National Cholesterol Education Program's Adult Treatment Panel III; BP, blood pressure; %E, percentage of total energy; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; S-hs CRP, serum high-sensitivity C-reactive protein; TC, total cholesterol.

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positively associated with the prevalence of the metabolic syndrome (McKeown *et al.* 2004).

Epidemiological findings indicate that frequent nut consumption offers protection from fatal and non-fatal CHD events (Sabate, 1993). Nuts have a low glycaemic index (Foster-Powell *et al.* 2002) and are a rich source of protein, unsaturated fatty acids (MUFA and PUFA), vitamin E, B₆, folic acid and niacin, fibre, magnesium, potassium, arginine, phytosterols and other phytochemical compounds (such as polyphenols and ellagic acid) (Dreher *et al.* 1996). The protective effects of nuts are mediated through several mechanisms. Specifically, the health benefits of walnuts include lowering cholesterol, increasing the ratio of HDL-C to total cholesterol (TC), decreasing TAG and increasing HDL-C, reducing inflammation and improving arterial function in patients with type 2 diabetes and hyperlipidaemia (Ros *et al.* 2004; Tapsell *et al.* 2004; Zhao *et al.* 2004; Zibaenezhad *et al.* 2005). Besides our recent contribution (Pieters *et al.* 2005), there is a scarcity of literature on the effects of cashew nut interventions.

In fact, to date there are no other studies that investigated the effects of nuts on either markers of the metabolic syndrome or using subjects with metabolic syndrome. Therefore, the primary objective of the present study was to determine the effects of a high walnut diet and a high unsalted cashew nut diet on markers of this syndrome compared to a control diet. In this contribution we report the effects on the following markers: serum lipids (TC, LDL-cholesterol (LDL-C), HDL-C and TAG), serum fructosamine, plasma glucose, BP, serum uric acid and serum high-sensitivity C-reactive protein (S-hs CRP).

Subjects and methods

Subjects

All participants gave written informed consent and the study was approved by the Ethics Committee of the Potchefstroom Campus of the North-West University. Sixty-eight White/Caucasian volunteers with the metabolic syndrome were recruited mainly from the Potchefstroom Campus of the North-West University and surrounding areas in Potchefstroom, South Africa. According to the power calculations providing 80%

power at 5% significance based on LDL-C data from several studies (Chisholm *et al.* 1998; Jenkins *et al.* 2002; Lovejoy *et al.* 2002; Sabate *et al.* 2003; Ros *et al.* 2004), twenty subjects per treatment group were needed to detect a decrease of 15% (0.6 mmol/l) which might reduce the risk of CVD by more than 20% (Moruise *et al.* 2006). The ATP III criteria for the diagnosis of the metabolic syndrome was used. In this regard, the metabolic syndrome was defined as the presence of three or more of the following criteria (Ford *et al.* 2002): abdominal obesity (waist circumference >88 cm for women or >102 cm for men); fasting TAG \geq 1.7 mmol/l; HDL-C \leq 1.0 mmol/l for men, HDL-C \leq 1.3 mmol/l for women; BP \geq 130/85 mmHg (the use of anti-hypertensive medication was also an indication of high BP) and fasting glucose \geq 6.1 mmol/l (fasting finger prick blood glucose concentrations were measured with a SureStep™ blood glucose meter (Lifescan Inc., Milpitas, CA, USA), using Fine Point lancets, and SureStep™ test strips (code 11).

Additional inclusion criteria included subjects being able to comply with controlled feeding conditions; being willing and able to eat walnuts and cashew nuts, and participants had to be older than 21 and younger than 65 years. Pregnancy or lactation, thiazide (>25 mg/d) and β -blocker (non-specific, β_1 and β_2) use, subjects having nut allergies and diagnosed diabetes formed part of the exclusion criteria.

Study design

A randomized, controlled, parallel, study design was used. The study protocol consisted of a 3-week run-in period during which the subjects consumed a control diet (percentage of total energy (%E) intake from protein-carbohydrate-fat = 20:47:33; Table 1). After the run-in period participants were grouped according to gender and age and then into three groups by randomly drawing numbers from a hat. Group one received walnuts (*n* 21), group two received unsalted cashew nuts (*n* 21), while group three continued with the control diet without any nuts or nut-based ingredients (*n* 22). Furthermore, these three intervention diets were followed for 8 weeks. Due to practical reasons the study was divided into three cohorts distributed over a 1-year period. All the food was provided to the participants for the duration

Table 1. Planned and analysed composition of diets as well as the habitual diets

Nutrients, glycaemic index and glycaemic load	Habitual diet prior to dietary intervention (<i>n</i> 64)	Walnut diet (<i>n</i> 21)		Cashew nut diet (<i>n</i> 21)		Control diet (<i>n</i> 22)	
	FFQ†	Planned†	Analysed‡	Planned†	Analysed‡	Planned†	Analysed‡
Protein (% of energy)	15.1	15.7	17.5	16.2	19.1	16.4	19.6
Carbohydrate (% of energy)	49.2	48.9	42.1	46.8	44.4	51.3	47.2
Fat (% of energy)	33.2	35.0	40.3	37.1	36.5	32.8	33.2
SFA (% of energy)	11.0	7.49	8.2	9.25	9.4	7.7	12.2
MUFA (% of energy)	11.3	10.6	10.7	15.9	16.3	9.4	11.3
PUFA (% of energy)	7.5	15.1	21.4	7.83	10.8	8.1	9.5
Cholesterol (mg/d)	316	226	–	224	–	246	–
Fibre (g/d)	30.7	34.1	–	33.4	–	27.8	–
Glycaemic index§	–	54	–	53	–	56	–
Glycaemic load§	–	14	–	14	–	15	–

† Determined by using the FoodFinder 2 Program (Medical Research Council of South Africa, Tygerberg; mean of 14 d menu).

‡ Laboratory analysis.

§ Calculated with the aid of the glycaemic index and glycaemic load tables of Foster-Powell *et al.* (2002).

of the trial. Fasting blood samples, oral glucose tolerance tests, anthropometric measurements and BP measurements were taken before (after the 3-week run-in period) and after the intervention period (8-week controlled feeding). BMI (kg/m^2) was calculated. In addition, the weighing of subjects was done twice weekly throughout the run-in period and the experimental phase. The subjects were informed of all aspects of the study before commencement.

Diet

The proportion of total energy (ranging from 63 to 108 g/d) from nuts was 20%. Except for the nuts, the diets were identical. This was achieved by making proportional reductions to all food portions in the walnut and the unsalted cashew nut diet menus to accommodate the energy supplied by the respective nuts (Tables 1 and 2). The study featured a highly controlled feeding protocol. In this regard, all subjects were required to have their lunch at the metabolic ward of the Department of Nutrition at the Potchefstroom Campus of the North-West University. Breakfast and dinner were provided in take-away format. Using pre-packed food parcels and a variety of set menu options ensured compliance. On a daily basis 10% of the total energy intake was calculated in the form of 'additional points'. In this regard, a list of foods with their associated number of points was provided to the participants. In order to ensure total energy intake and some freedom of choice, participants were advised to choose any foods from the list, provided they added up to the allotted number of points for their respective energy intakes for that day. A validated FFQ and physical activity questionnaire, measuring activity index, were analysed in order to determine the correct energy intake requirements for the maintenance of body weight for each participant. The validated physical activity questionnaire is based on the Baecke physical activity questionnaire (Kruger *et al.* 2004). Evidence of underreporting was found when the ratio of energy intake to BMR was less

than 1.2 (Bingham, 1991; Briefel *et al.* 1997). In the light of this fact, the subjects that underreported were interviewed again by the registered dietitian to obtain a more accurate report on their habitual energy intake.

A 14 d menu cycle was designed for five amounts of energy intake, ranging from 8000 to 14 000 kJ/d (1905–3333 kcal/d). It was planned by using the FoodFinder 2 program (Medical Research Council of South Africa, Tygerberg), which is based on the South African food composition tables (Langenhoven *et al.* 1991). The macronutrient profiles and fatty acid distribution of the three diets were analysed chemically to validate the diet composition. Duplicate portions of breakfast, lunch and dinner for the 14 d menu cycle were collected daily, homogenized and pooled in a container and frozen at -84°C until the analysis was done.

Compliance to dietary intervention

Quality control and compliance with the protocol were ensured among study participants by the following means: (1) foods were weighed to the nearest gram before being served to the participants; (2) the principal investigator, a registered dietitian, supervised mealtimes and ensured the complete intake of all study foods; (3) participants kept food diaries of the additional points used and possible left-overs were collected and weighed (by researchers). In addition, any deviation from the study protocol were recorded in these diaries (these diaries were reviewed by the investigators during the study); (4) participants were weighed twice weekly and the energy intake was adjusted (especially during the first 3-week run-in period) in order to maintain body weight; and (5) participants were urged to maintain the same activity level throughout the study. Lastly, those individuals who used chronic medication (e.g. lipid-lowering medication) at baseline were instructed to continue use and to maintain the same dosage for the duration of the trial.

Table 2. Macronutrient composition of 100 g walnuts and unsalted cashew nuts

Selected nutrients	Walnuts			Cashew nuts		
	Laboratory analysis		Food composition tables†(g)	Laboratory analysis		Food composition tables†(g)
	g	%‡		g	%‡	
Energy (kJ)			2686			2402
Energy (kcal)			642			574
Protein	13.0		14.3	20.5		15.3
Carbohydrate	3.9		13.5	19.4		26.6
Fibre	5.75		6.10	5.62		4.80
Total fat	73.2		61.9	48.4		46.4
MUFA	13.4	18.3	14.2	25.6	52.9	27.3
Oleic acid (C18:1)	13.2	18.0	21.5	25.5	52.6	57.8
PUFA	53.8	73.5	39.1	15.3	31.6	7.84
Linoleic acid (C18:2)	44.7	61.0	51.3	15.0	31.0	16.5
α -Linolenic acid (C18:3)	9.15	12.50	11.00	0.25	0.51	0.34
SFA	6.00	8.20	5.59	7.51	15.5	9.16
Palmitic acid (C16:0)	4.41	6.02	6.85	4.08	8.43	9.38
Stearic acid (C18:0)	1.47	2.01	1.74	3.09	6.38	6.40

† Langenhoven *et al.* (1991); Kruger *et al.* (1992).

‡ Percentage of total fatty acids.

Blood sampling and oral glucose tolerance test

The subjects were required to fast overnight (12 h). A qualified nursing sister collected venous blood samples. For the preparation of serum, 20 ml blood were drawn and left to clot. For determination of plasma glucose concentrations 5 ml blood were collected in tubes containing potassium oxalate (10 mg) and sodium fluoride (12.5 mg).

After the fasting blood samples were collected, the oral glucose tolerance test was continued: 75 g glucose were dissolved in 300 ml water; blood samples for the measurement of glucose were drawn again after 2 h; blood was centrifuged for 15 min at 2000 *g* to yield serum; aliquots of serum were stored at -82°C until the analysis was performed.

Analytical methods

The fatty acid composition of the nuts and diets was measured by GC as described by van Jaarsveld *et al.* (2000). The percentage protein was analysed by a general combustion method (AOAC Method 992.23; AOAC International, 2002) by using a LECO FP 528 (LECO Corporation, St Joseph, MI, USA), the percentage fat by a GAVIEZEL[®] method using the Büchi B 820 fat determination system with the Büchi B 815 extraction unit (Büchi Labortechnik AG, Flawil, Switzerland), the percentage fibre by the filter bag technique using the ANKOM 220 fibre analyser with F57 filter bags (ANKOM Technologies, Fairport, NY, USA), the percentage moisture with the air-oven (aluminium plate) method (AACC Method 44-161; American Association of Clinical Chemistry, 2003) and percentage ash with the AOAC Method 942.05 (AOAC International, 2002). The carbohydrate content was then calculated as the sum of the protein, fat, fibre, moisture and ash subtracted from 100.

CV for all the laboratory analyses of blood samples were less than 5%. Serum TC, HDL-C, TAG, uric acid and plasma glucose were measured on a Vitros DT60 II Chemistry System (Ortho-Clinical Diagnostics, Rochester, NY, USA) with Vitros reagents (catalogue numbers 153 2175, 133 5504, 153 2159, 153 2134 and 1532316, respectively) and controls (catalogue numbers 842-0317 and 144-8042). Serum LDL-C was calculated using the Friedewald formula: $\text{LDL-C (mmol/l)} = \text{TC} - \text{TAG}/2.2 - \text{HDL}$. S-hs CRP was measured using the Synchron LX20[®] clinical system (Beckman Coulter, Inc., Fullerton, CA, USA). Serum fructosamine was measured with a calorimetric method (catalogue number 1930010; Roche, Basel, Switzerland). BP measurements were obtained by taking a 7 min continuous measurement of cardiovascular parameters using the Finometer[™] device (FMS; Finapres Measurement Systems, Arnhem, Netherlands).

Statistical analyses

The computer software package Statistica[®] (Statsoft Inc., Tulsa, OK, USA) was used for the analyses of the data. The statistical analysis was done in five steps. Initially, the variables were tested for normality using the Shapiro–Wilk's *W* test. Non-normally distributed data were transformed into an approximately normal distribution by logarithmic and square-root transformations and again tested for normality.

Thereafter, descriptive statistics were done. Data that were normally distributed are expressed as mean and 95% CI. Data that are not normally distributed or logarithmic and square-root transformed are expressed as median (25, 75 percentiles). Furthermore, changes within groups, from baseline to end, were tested for significance by using the *t* test for dependent samples in the case of parametric data and the Wilcoxon matched-pairs test in the case of non-parametric data. Also, differences in baseline and Δ (change from baseline to end) between the three groups were determined by using the ANOVA for parametric data and the Kruskal–Wallis ANOVA for non-parametric data. When significance between the changes in the three groups was indicated with the ANOVA, the Tukey honest significant difference test for unequal *N* for parametric data was used to determine between which groups the differences occurred. Lastly, weight-adjusted differences in baseline and Δ between the three groups were determined by using the analysis of covariance. Significance was set at $P \leq 0.05$.

Results

Subjects

Four subjects discontinued the study for the following reasons: two had work obligations outside Potchefstroom, one had an unrelated medical condition and one went on a holiday during the study period. The remaining sixty-four subjects (twenty-nine men and thirty-five women, aged 45 (SD 10) years) all completed one of the three intervention diets. Compliance with the experimental diets was calculated as 90%, taking into account the left-overs, food diaries and any deviation from the prescribed dietary protocols. Also, activity level was maintained throughout the study. In this regard, most of the participants had a sedentary lifestyle. The baseline characteristics of the subjects did not differ between groups (ANOVA; Table 3). Most of the subjects were obese (91%) with high waist circumference values exceeding those indicated by the ATP III criteria. Of the subjects, 53% had high TAG concentrations, 42% had high systolic BP, 13% had high diastolic BP, 91% had low HDL-C and only 5% had high fasting glucose concentrations as indicated by the ATP III criteria. At baseline 13% of subjects had high hs-CRP concentrations, greater than 7.5 mg/l (as indicated by the producers of the diagnostic kit). Evidently, the subject characteristics at baseline were indicative of the metabolic syndrome. Also, of the entire study population only four were smokers (Table 3). Weight, BMI and waist circumference remained unchanged during the intervention period.

Diet composition

The participants' habitual energy intakes ranged from 5500 to 13 000 kJ/d (1310–3095 kcal/d), however, 52% of the subjects underreported their energy intake. The chemical analysis of the composition of the diets was comparable to the calculated diets, except for the total fat and carbohydrate content of the walnut diet (Table 1). The analysed fat content was higher because the actual fat content of the walnuts was higher than indicated in the food composition tables (Table 2). All three experimental diets had a low to moderate glycaemic

Table 3. Characteristics at baseline for all subjects

Variables	Walnut diet (n 21)		Cashew nut diet (n 21)		Control diet (n 22)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Men/women	10/11	–	8/13	–	11/11	–
Age (years)	45	40.4, 50.2	46	40.7, 50.7	45	40.8, 49.3
Weight (kg)	107	99.4, 115	99	92.5, 106	106	99.2, 113
BMI (kg/m ²)	36.0	33.3, 38.7	34.4	32.2, 36.6	35.1	32.8, 37.4
WC (cm)	109	103, 115	105	98.5, 111	108	102, 113
Cigarette smokers	1	–	0	–	3	–

WC, waist circumference.

index and glycaemic load (Wolever & Jenkins, 1985; Foster-Powell *et al.* 2002; Table 1). The composition of walnuts (high in α -linolenic acid and linoleic acid) and cashew nuts (high in oleic acid; Table 2) was reflected in the diets and resulted in the anticipated increase in PUFA and MUFA concentrations, in the diets respectively. In particular, the fatty acid composition of the walnut, cashew nut and control diets (expressed as % of total fatty acids) was 6.42% α -linolenic acid, 46.31% linoleic acid, 25.65% oleic acid; 1.05% α -linolenic acid, 27.82% linoleic acid, 43.65% oleic acid and 0.86% α -linolenic acid, 27.13% linoleic acid, 32.88% oleic acid, respectively.

Lipid profiles

In Table 4, the serum lipid concentrations displayed no significant changes between walnut, cashew nut and control groups at baseline and in response to the intervention. The subjects in the control diet group showed a small significant increase in HDL-C compared to baseline (Table 4). Results from both nut diets displayed no significant change in HDL-C, TAG, TC or LDL-C concentrations when compared to the baseline.

Serum fructosamine and plasma glucose

In Table 5, serum fructosamine and plasma glucose ($t = 0$ min and $t = 120$ min) showed no significant difference at baseline between groups. Also, there was no significant difference in serum fructosamine between groups after the intervention diets. Plasma glucose concentrations ($t = 0$) increased significantly by 0.70 mmol/l ($P = 0.04$) in the cashew nut group compared to the control group. Fructosamine significantly increased in the control diet group at the end of the study period, when compared to the baseline concentrations. The 2 h oral glucose tolerance test showed that there was no significant difference between the groups and when comparing baseline to end values.

Blood pressure, uric acid and serum high-sensitivity C-reactive protein

Of all three intervention diets, systolic and diastolic BP as well as uric acid concentrations displayed no significant change between groups at baseline and from baseline to end. The changes in S-hs CRP did not differ significantly between groups (Table 6). There was a significant increase in S-hs

CRP concentrations in the walnut intervention group from baseline to end and no significant change in the cashew nut and control groups when comparing baseline to end values. Although not statistically significant, S-hs CRP was also increased, approximately to the same extent, in the control and cashew nut groups. The increase in the walnut group is, therefore, probably not an independent effect of walnuts.

Discussion

As far as we know this well-designed parallel, randomized, controlled feeding trial investigating the effects of nuts on participants having the metabolic syndrome is the first study of its kind. Regarding our main objective, we found that both the walnut and the unsalted cashew nut intervention diets had no significant effect on the HDL-C, TAG, TC, LDL-C, serum fructosamine, S-hs CRP, BP and serum uric acid concentrations when compared to the control diet. Plasma glucose concentrations increased significantly in the cashew nut group compared to the control group.

In a recent systematic review (Mukuddem-Petersen *et al.* 2005), it was concluded from randomized controlled intervention trials that the consumption of 50–100 g (approximately 1.5–3.5 servings) of nuts five or more times/week as part of a heart healthy diet with a total fat content (high in MUFA and/or PUFA) of approximately 35%E may significantly decrease TC and LDL-C. In lieu of the dynamic make-up of nuts, it deserves a mention that this decrease is not solely due to the changes in the fatty acid composition that results when nuts are included in the diet, but also as a result of the other components found in nuts.

Contrary to the outcomes of the present controlled feeding trial, four out of seven well-designed walnut studies (40–84 g/d) displayed a significant decrease in TC and LDL-C when compared to Step I (healthy; Sabate *et al.* 1993), Mediterranean (hypercholesterolaemic; Zamboni *et al.* 2000; Ros *et al.* 2004) and Japanese (healthy; Iwamoto *et al.* 2002) diets. The average %E from fat in the aforementioned nut diets and the control diets were 31 and 29%, respectively. Two of the studies that showed no significant change in the lipid profile of hyperlipidaemic subjects who followed a walnut intervention diet (64–78 g/d) when compared to a low-fat (30%E from fat) and Step I diet (33%E from fat) provided ≥ 38 %E from fat (Chisholm *et al.* 1998; Morgan *et al.* 2002), suggesting that the beneficial effects of nuts disappear with high fat intakes. Similarly, in the current study, the walnut diet (60–100 g/d) was high in fat (41%E from fat)

Table 4. Weight and serum lipids during interventions*

		Walnut diet (n 21)		P value (B v. E)	Cashew nut diet (n 21)		P value (B v. E)	Control diet (n 22)		P value (B v. E)	P value between groups (ANOVA)	
		Mean	95 % CI		Mean	95 % CI		Mean	95 % CI			
Weight (kg)	B	106	99.4, 115	0.50	99.2	92.5, 106	0.72	106	99.2, 113	0.14	0.22	
	E	106	99.4, 114		99.1	91.9, 106		105	98.5, 112			
	Δ	-0.22	-0.87, 0.44		-0.17	-1.13, 0.79		-0.51	-1.19, 0.17			0.78
Serum lipids (mmol/l)												
	HDL-C	B	0.94	0.83, 1.05	0.34	1.02	0.88, 1.16	0.71	0.85	0.77, 0.93	0.04	0.08
		E	0.91	0.80, 1.01		1.01	0.90, 1.12		0.91	0.82, 1.0		
Δ		-0.03	-0.10, 0.04	-0.01		-0.09, 0.06	0.06		0, 0.11	0.11		
TAG	B	1.90	1.48, 2.32	0.79	1.81	1.33, 2.30	0.36	1.86	1.55, 2.16	0.41	0.96	
	E	1.86	1.44, 2.28		1.64	1.23, 2.05		1.96	1.62, 2.31			
	Δ	-0.04	-0.35, 0.27		-0.16	-0.52, 0.20		0.11	-0.16, 0.38			0.45
		Median	25, 75 Percentiles	P value (B v. E)	Median	25, 75 Percentiles	P value (B v. E)	Median	25, 75 Percentiles	P value (B v. E)	P value between groups (ANOVA)	
TC	B	4.80	4.54, 5.18	0.83	4.49	3.98, 5.34	0.62	4.90	4.39, 5.52	0.09	0.55	
	E	4.72	4.28, 5.76		4.51	3.87, 5.64		5.39	4.44, 5.83			
	Δ	-0.03	-0.30, 0.37		0.07	-0.22, 0.49		0.07	-0.06, 0.60			0.46
LDL-C	B	2.99	2.63, 3.29	0.36	2.64	2.33, 3.16	0.25	3.21	2.79, 3.79	0.22	0.11	
	E	3.19	2.38, 3.94		2.84	2.26, 3.60		3.36	2.86, 3.84			
	Δ	0.18	-0.21, 0.50		0.12	-0.25, 0.55		0.19	-0.11, 0.46			1.00

B, baseline (after 3-week run-in period); B v. E, P values for change from baseline to end of intervention period; E, end; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TC, total cholesterol; Δ, change from baseline to end.

*Significance was set at $P \leq 0.05$.

Table 5. Serum fructosamine and plasma glucose during interventions

Markers		Walnut diet		<i>P</i> value (B v. E)	Cashew nut diet		<i>P</i> value (B v. E)	Control diet		<i>P</i> value (B v. E)	<i>P</i> value between groups (ANOVA)
		Mean	95% CI		Mean	95% CI		Mean	95% CI		
Serum fructosamine (μ mol/l)	B	210	202, 218	0.79	212	206, 218	0.32	204	198, 210	0.04	0.23
	E	209	201, 217		214	206, 222		210	203, 218		
	Δ	-0.95	-8.16, 6.26		2.29	-2.38, 6.96		6.00	0.20, 11.8		
		Median	25, 75 Percentiles	<i>P</i> value (B v. E)	Median	25, 75 Percentiles	<i>P</i> value (B v. E)	Median	25, 75 Percentiles	<i>P</i> value (B v. E)	<i>P</i> value between groups (ANOVA)
Plasma glucose <i>t</i> =0 (mmol/l)	B	4.50	4.30, 5.20	0.64	4.70	4.30, 5.10	0.03	4.55	4.30, 5.40	0.18	0.73
	E	4.70	4.00, 5.40		5.30	4.70, 6.20		4.55	3.80, 5.40		
	Δ	0.40	-0.60, 0.70		0.70*	-0.30, 1.60		-0.75	-1.40, 0.80		
Plasma glucose. <i>t</i> = 120 (mmol/l)	B	4.70	4.50, 5.40	0.09	4.60	4.10, 5.30	0.25	4.60	4.30, 5.10	0.87	0.94
	E	5.50	4.40, 6.30		5.00	4.20, 5.90		4.80	3.90, 6.10		
	Δ	0.50	-0.20, 1.30		0.40	0.70, 1.40		-0.20	-0.90, 1.10		

B, baseline (after 3-week run-in period); B v. E, *P* values for change from baseline to end of intervention period; E, end; Δ , change from baseline to end. Median value was significantly different from that of the control group: **P*≤0.05.

Table 6. Blood pressure, uric acid and C-reactive protein changes within and between groups during intervention*

Markers		Walnut diet		<i>P</i> value (B v. E)	Cashew nut diet		<i>P</i> value (B v. E)	Control diet		<i>P</i> value (B v. E)	<i>P</i> value between groups (ANOVA)	
		Mean	95% CI		Mean	95% CI		Mean	95% CI			
BP (mmHg)	Systolic	B	128	126, 131	0.26	131	126, 135	0.28	131	125, 137	0.40	0.66
		E	130	126, 133		128	123, 134		133	128, 137		
		Δ	2.21	-1.74, 6.16		-2.45	-7.09, 2.19		1.74	-2.45, 5.92		
Diastolic	B	78.7	76.3, 81.0	0.49	77.0	74.0, 80.0	0.72	79.2	76.1, 82.2	0.79	0.50	
	E	79.1	75.9, 82.0		76.0	73.1, 80.0		79.6	76.9, 82.4			
	Δ	1.21	-2.39, 4.81		-0.59	-4.01, 2.83		0.48	-3.11, 4.07			0.76
Serum uric acid (μ mol/l)	B	388	347, 429	0.30	373	329, 418	0.65	412	368, 456	0.57	0.42	
	E	401	361, 440		377	328, 426		416	376, 457			
	Δ	12.6	-12.2, 37.3		3.86	-13.5, 21.2		4.64	-12.1, 21.4			0.78
		Median	25, 75 Percentiles	<i>P</i> value (B v. E)	Median	25, 75 Percentiles	<i>P</i> value (B v. E)	Median	25, 75 Percentiles	<i>P</i> value (B v. E)	<i>P</i> value between groups (ANOVA)	
S-hs CRP (mg/l)	B	2.40	0.60, 4.80	0.01	4.30	1.80, 5.50	0.07	3.05	1.30, 6.10	0.21	0.17	
	E	3.00	1.10, 5.50		4.20	2.10, 6.40		3.65	2.20, 7.30			
	Δ	0.50	0.10, 1.50		0.20	-0.50, 3.20		0.65	-0.30, 2.20			0.38

B, baseline; B v. E, *P* values for change from baseline to end of intervention period; BP, blood pressure; E, end; S-hs CRP, serum high-sensitivity C-reactive protein; Δ , change from baseline to end.

*Significance was set at *P*≤0.05.

compared to the control diet (33 %E from fat). However, high-PUFA diets, similar to the walnut diet in the current study, are predicted to reduce TC and LDL-C concentrations (Mensink & Katan, 1992). Based on the predictive equations of Mensink & Katan (1992), which predict changes in TC and LDL-C concentrations according to changes in the fatty acid content of the diet, the walnut diet should have reduced the TC and LDL-C concentrations with 0.34 and 0.30 mmol/l, respectively, compared to the control diet. The lack of effect can possibly be ascribed to the low TC and LDL-C concentrations of the subjects at baseline, as these low concentrations make it difficult to show a further benefit.

Most of the walnut studies to date did not show an effect on HDL-C and TAG concentrations (Mukuddem-Petersen *et al.* 2005). Sabate *et al.* (1993) showed a decrease in HDL-C that could possibly have been due to the high PUFA content on the walnut diet (17 %E) compared to the control diet (10 %E). High intakes of PUFA (> 10 %E) may decrease HDL-C (Riccardi *et al.* 2003). Although the PUFA content of the walnut diet in the current study was also much higher compared to the control diet (21.4 v. 9.5 %E), the HDL-C did not differ between the diets.

As no clinical trials have been done on cashew nuts before, we considered almond studies as they are similar in composition to cashew nuts. We expected the cashew nut study to have a beneficial effect on TC and LDL-C concentrations as seen in previous almond nut studies. In this context three out of four well-designed almond studies (54–100 g/d) ranging from 32 to 39 %E from fat, significantly decreased TC and LDL-C in hypercholesterolaemic (Spiller *et al.* 1998; Jenkins *et al.* 2002) and normocholesterolaemic (Sabate *et al.* 2003) subjects compared to subjects on a control diet (35 %E from fat), low-fat (26.3 %E from fat) and Step I diet (30 %E from fat) (Sabate *et al.* 2003). In the present study, the cashew nut (66–115 g/d) diet (37 %E from fat) had no significant beneficial effect on the lipid profile (Table 4) when compared to the control diet that is lower in fat (33 %E from fat). This outcome was very similar to results found by Lovejoy *et al.* (2002) who showed that 57–113 g almonds/d (39 %E from fat) had no significant beneficial effect on the lipid profile of diabetic subjects when compared to a high-fat (37 %E from fat) control group. The same outcome was achieved when the same amount of almonds (27 %E) as part of a low-fat diet was compared to a low-fat (26 %E from fat) control diet (Lovejoy *et al.* 2002). Based on differences in fatty acid content of the cashew nut and control diets in the current study the predicted reductions in TC and LDL-C concentrations with the cashew nut diet were very small (–0.14 mmol/l for both TC and LDL-C). However, it has been shown that nuts may reduce TC and LDL-C concentrations beyond the effects predicted based solely on fatty acid profiles (Mukuddem-Petersen *et al.* 2005). As mentioned earlier, the low TC and LDL-C concentrations at baseline might explain the lack of effects. Other possible reasons for the non-significance in lipid concentrations need to be explored.

No direct studies have been done to investigate the effects of nuts on BP. Despite this, we anticipated an improvement in the BP readings with the walnut and cashew nut diets based on their fatty acid composition. In particular, emerging research has suggested possible health benefits associated with

modest increases in dietary α -linolenic acid (walnuts), including reduced BP (Ferrara *et al.* 2000; Hermansen, 2000; Hunter, 1990). Also, numerous studies conducted in healthy and hypertensive individuals have shown a beneficial effect of MUFA (cashew nuts) on a number of outcomes related to cardiovascular risk, including BP (Roche *et al.* 1998). However, no improvement in BP readings was seen after the nut intervention diets in the current controlled feeding trial.

Zhao *et al.* (2004) concluded that a diet high in α -linolenic acid, obtained from walnuts, walnut oil and flaxseed oil, elicited cardioprotective effects and vascular anti-inflammatory effects. Regarding the latter, it has been reported that walnuts are amongst the dietary plants that contain the most antioxidants (Halvorsen *et al.* 2002) and several health effects have been ascribed to flavonoids (antioxidants) including reduced inflammation (Nijveldt *et al.* 2001). In the present study, the walnut diet (high in α -linolenic acid) resulted in an increase in S-hs CRP concentrations, although this was probably not an independent effect of walnuts. Recently, some evidence has been presented for a beneficial effect of MUFA on a number of outcomes related to cardiovascular risk, including reduced inflammation (Ferrara *et al.* 2000). Consequently, a cashew nut diet (high in MUFA) could be expected to improve the inflammatory parameter CRP. However, this was not evident in the present study.

Numerous studies compared a low-fat diet (21 %; 23 %; 29 %E from fat; Luscombe *et al.* 1999; Thomsen *et al.* 1999; Rodriguez-Villar *et al.* 2000; Perez-Jimenez *et al.* 2001) to a high-MUFA diet (35 %; 40 %E from fat; Luscombe *et al.* 1999). The results of these studies provided similar glycaemic control. Those authors concluded that provided the intake of SFA is low, a MUFA diet with a total fat content of up to 40 %E has effects on glycaemic control that are similar to those of the traditional high-carbohydrate diet with fat limited to 25–30 %E. An almond nut study conducted by Lovejoy *et al.* (2002) showed no effect on plasma glucose concentrations compared to a low-fat (26 %E from fat) control group (olive and rapeseed oil) in diabetics. In contrast to these previous findings, the significant increase of the plasma blood glucose ($t = 0$) seen in the cashew nut diet (high MUFA; 36.5 %E from fat) group was unexpected. Even though serum fructosamine is a short-term marker of glycaemic control, its concentrations remained unchanged in the cashew nut group compared to the control group that increased. It could be speculated that the aforementioned increase in serum fructosamine concentrations in the control group may be due to this group having lower baseline values compared to the other two groups.

ATP III recommends that obesity should be the primary target of intervention for the metabolic syndrome. In turn, the first line of therapy should be weight reduction reinforced with increased physical activity. A notion related to this is evident in the study by Esposito *et al.* (2004) where they displayed how a Mediterranean diet (high in MUFA and PUFA) that included nuts, weight loss and increased physical activity resulted in a significant reduction in S-hs CRP amongst other beneficial anti-inflammatory responses.

In conclusion, individuals having the metabolic syndrome showed no improvement in the markers of this syndrome after following a walnut diet or a cashew nut diet (8 weeks)

compared to a control diet while maintaining body weight. Most of the study population was obese (average BMI 35) and sedentary therefore it could be speculated that with such a high degree of obesity that even a 'good diet including nuts' would not suffice in inducing beneficial effects without weight loss. Firstly, it may be suggested that maintenance of body weight may have masked the positive metabolic effects of the nut diets. Especially, if these diets mediate its beneficial effects mainly through central appetite suppression and consequent body weight reduction. Wien *et al.* (2003) showed how a low-energy nut diet resulted in sustained weight reduction and improved the preponderance of abnormalities associated with the metabolic syndrome. Future nut research on these aspects are worthy of exploration.

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