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ORIGINAL CONTRIBUTION

The effects of high walnut and cashew nut diets on the antioxidant status of subjects with metabolic syndrome

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Abstract *Background* Nut consumption is associated with a protective effect against coronary heart disease, partly due to its high antioxidant content. It is hypothesized that the inclusion of nuts in the diet will improve the antioxidant status of subjects with metabolic syndrome who may be vulnerable to impaired antioxidant status. *Aim* The effects of high cashew nut and high walnut diets on the antioxidant status of subjects with metabolic syndrome are investigated. *Methodology* Sixty-four volunteers (29 male and 35 female, 45 ± 10y) with metabolic syndrome (diagnosed by using the ATP III criteria) received a prudent control diet, prepared in the metabolic kitchen of the North-West University, Potchefstroom campus (NWU-PC) for a period of 3 weeks (run-in). The participants were grouped according to gender and age and randomized into three groups, receiving either the walnut, cashew nut or the control diets for 8 weeks, while maintaining a stable body weight. Nuts provided 20% of daily energy intake. Fasting blood samples were taken after the run-in period (baseline) and at the end of the intervention period and analyzed for various antioxidant capacity markers. *Results* The oxygen radical absorbance capacity (ORAC) of the walnut and cashew nut diets were significantly higher than the control diet. Despite this, the walnut and cashew nut diets had no significant effects on serum ORAC, reduced (GSH), oxidized (GSSG) glutathione, GSH:GSSG or diacron reactive metabolites (dRom) (total oxidant status) levels compared to the control group. However, all three groups showed significant improvements in antioxidant status from baseline to end (GSSG and dRom levels decreased; GSG:GSSG ratio and ORAC levels increased). This may be due to a general increased antioxidant intake from the prudent diet compared to the habitual diets. *Conclusion* Although the inclusion of walnuts and cashew nuts into a prudent diet resulted in an increased antioxidant capacity (ORAC) of the nut diets, compared to the control diet, it did not improve the serum antioxidant profiles of subjects with metabolic syndrome.

Key words cashew nuts – walnuts – antioxidant status – polyphenols – redox status – metabolic syndrome

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Introduction

Metabolic syndrome is characterized by a constellation of cardiovascular risk factors, including atherogenic dyslipidemia, abnormal glucose tolerance, hypertension and abdominal obesity. These factors are closely associated with insulin resistance and elevated insulin concentrations [19]. It has been suggested that hyperglycaemia and obesity may disrupt natural antioxidant defence systems [28] by increasing the generation of free radicals through the reduction of molecular oxygen [30]. There is also some evidence relating oxidative stress to the degree of insulin resistance [37]. It has been suggested that high oxidative stress promotes an impaired insulin action, which in turn may aggravate the degree of oxidation [18].

Little is known about the antioxidant status of subjects with metabolic syndrome. Because a low antioxidant status is associated with obesity [33], which is high among subjects with metabolic syndrome, metabolic syndrome may be associated with a low antioxidant status [13]. Findings from the third National Health And Nutrition Examination Survey (NHANES III) (2003) revealed low concentrations of vitamin C and E among subjects with metabolic syndrome [13]. This may be due to their involvement in antioxidant reactions in these individuals. Furthermore, research is beginning to highlight the potential health benefits of polyphenols in diets, which are known to be powerful scavengers of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in vitro [34, 36].

The nutritional composition of nuts suggest that they may reduce the risk for chronic diseases by various mechanisms [23]. Particularly, nuts are low in saturated fatty acids (SFA), high in unsaturated fatty acids [17], an important source of dietary fibre and plant protein, antioxidants, vitamins, minerals, polyphenols [17], magnesium, potassium and arginine [12]. The rich antioxidant content of nuts (e.g. vitamins and polyphenols) may offer protection against oxidative damage [12]. Compared to other nuts, walnuts are unique due to their high poly-unsaturated fatty acid (PUFA) content, specifically α-linolenic acid [40]. Walnuts are a good source of polyphenols [16] and to a lesser extent, vitamin E [12] that may further contribute to its antioxidant capacity. The main purpose of this paper is, therefore, to explore the effects of a high walnut and unsalted cashew nut diet on the antioxidant status of subjects with metabolic syndrome.

Methods

The study was part of a larger study with the main aim to investigate the effects of nuts on metabolic syndrome [27, 32].

Subjects

Sixty-eight volunteers with metabolic syndrome were recruited mainly from the Potchefstroom Campus of the North-West University and surrounding areas in Potchefstroom, South Africa. The power calculation for sample size was based on results of Dessein and co-workers [11]. To provide 80% power at 5% significance and by considering a 15% change in the quantitative insulin sensitivity check index (QUICKI) (due to the original research question of the study) as significant, a total number of 22 subjects were calculated per group (i.e. a total of 66 subjects).

Sixty-four subjects completed the study (dropouts discussed later). The National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) criteria for the diagnosis of the metabolic syndrome was used. Subjects with metabolic syndrome were defined as individuals with three or more of the following symptoms: abdominal obesity (waist circumference >88 cm for women or >102 cm for men), TG $\geq$ 1.7 mmol/l, HDL-C $\leq$ 1.3 mmol/l for women and $\leq$1.0 mmol/l for men, blood pressure $\geq$ 130/85 mmHg (the use of antihypertensive medication was also an indication of elevated blood pressure) or fasting glucose $\geq$6.1 mmol/l. Additionally, subjects were included if they were able to comply with the specified feeding conditions, being able to eat walnuts and cashew nuts and being between the ages of 21 and 65 years. Exclusion criteria included the following: pregnancy or lactation, the use of thiazide diuretics (>25 mg/day) or beta-blockers (non-specific, β_1 and β_2), subjects having nut allergies and subjects with diagnosed diabetes.

Study design

A controlled feeding trial with a randomized, controlled, parallel study design was used. The study protocol consisted of a three-week run-in period during which the subjects consumed a control diet (percentage of total energy (%E) from protein: 20%, carbohydrate (CHO): 47%, and fat: 33%) (Table 1). After the run-in period, participants were grouped according to gender and age, after which groups were randomly assigned to one of the three treatment groups by drawing numbers from a hat. Group one received walnuts (n = 21), group 2 received unsalted cashew nuts (n = 21) and group three (n = 22) continued with the control diet without any nuts or nut-based ingredients or food. The intervention periods were followed for 8 weeks. Meals were prepared in the metabolic kitchen of the metabolic unit at the NWU-PC. Fasting blood samples, anthropometric measurements and blood pressure measurements were taken before (after the three-week run-in period) and after

Table 1 Calculated and analyzed diets as well as the habitual diets

Nutrients		Habitual diet prior to dietary intervention (n = 64) FFQ[a]	Walnut diet		Cashew nut diet		Control diet	
			Predicted[a]	Analyzed[b]	Predicted[a]	Analyzed[b]	Predicted[a]	Analyzed[b]
Protein	(%E)	15.1	15.7	17.4 ± 1.54	16.2	19.1 ± 1.69	16.4	19.6 ± 1.74
	(g/100g)		4.97	5.62 ± 0.67	5.18	6.13 ± 0.68	4.92	5.36 ± 0.75
CHO	(%E)	49.2	48.9	42.2 ± 4.31	46.8	44.3 ± 4.85	51.3	47.3 ± 6.55
	(g/100g)		12.6	13.5 ± 1.27	13.1	14.2 ± 2.07	12.4	12.9 ± 2.01
Fat	(%E)	33.2	35.0	40.3 ± 4.95	37.1	36.5 ± 4.06	32.8	33.1 ± 7.14
	(g/100g)		4.93	5.99 ± 1.04	5.13	5.37 ± 0.63	4.16	4.17 ± 0.99
Vitamin E (mg/day)		27.5	15.6	–	15.1	–	23.6	–
Vitamin C (mg/day)		147.3	157.9	–	145.9	–	154.4	–
Polyphenols (mg/g wet mass)		–	–	0.33 ± 0.02[a]	–	0.28 ±0.01[b]	–	0.31 ± 0.02[ab]
ORAC (mmol TE/g wet mass) HF		–	–	1114 ± 8.56[a]	–	819 ± 21.7[b]	–	653 ± 76.4[c]
ORAC (mmol TE/g wet mass) LF		–	–	1162 ± 160[a]	–	1323 ± 90.4[a]	–	804 ± 150[b]
ORAC (mmol TE/g wet mass) TAC		–	–	2277 ± 162[a]	–	2142 ± 72.9[a]	–	1457 ± 226[b]

[a]Determined by using the FoodFinder 2 Programme (Medical Research Council of South Africa, Tygerberg); [b]Laboratory analysis (means ± SD). Means with different symbols differed statistically significantly. FFQ: Food Frequency questionnaire; CHO: Carbohydrates; %E: % of total energy intake; HF: Hydrophilic; LF: Lipophilic; TAC: Total antioxidant capacity

the intervention period (8-week controlled feeding). The study participants were weighed twice a week throughout both the run-in and the intervention periods and the energy intake adjusted in order to maintain body weight. Body mass index (BMI) (kg/m^2) was calculated. The participants were informed of all the aspects of the study before commencement and gave consent. The Ethics Committee of the NWU-PC, approved the study. Participants were required to maintain their activity levels and subjects using chronic medication other than thiazide diuretics or β-blockers were to continue with the same dosage for the duration of the study. Due to practical reasons (the metabolic kitchen facility only allows the preparation of meals for 30 people per day), the study was divided into three cohorts distributed over a one-year period.

Diet

The walnuts and cashew nuts provided 20% E of the diet (63–108 g/day). In order to ensure that all groups received equal energy intakes from the meals, proportional reductions to all food substances in the walnut and cashew nut diet menus were made to accommodate the additional energy supplied by the respective nuts. The participants followed a 14-day menu that consisted of commonly consumed food items and was predicted using the computer programme FoodFinder 2 (Medical Research Council of South Africa, Tygerberg, South Africa), based on the South African Food Composition Tables [25]. The participants were allowed a specific number of free points (10%E)—food they could choose themselves from a list, in order to help subjects comply with the study protocol. Validated food frequency questionnaires (FFQs) [26] and physical activity questionnaires (PAQ) [24] were analyzed in order to determine the correct energy intake requirements for the maintenance of body weight for each of the participants during the intervention. Underreporting was established when the ratio of energy intake:basal metabolic rate was less than 1.2 [4, 5]. The registered dietitian then interviewed the subjects that underreported again to obtain a more accurate report of their habitual energy intake. Five different energy intake levels for each diet were developed ranging from 8,000 kJ to 14,000 kJ with 1,500 kJ increments. The participants were assigned to the energy intake level closest to their habitual energy intake to ensure maintenance of body weight. Participants were allowed to consume tea and coffee ad libitum during the intervention period.

The macronutrient profiles and antioxidant capacity of the three diets were analyzed chemically to determine the actual composition of the diet. Duplicate portions of breakfast, lunch and dinner for the 14-day menu cycle were collected daily, homogenized in a container and frozen at −84°C until the analysis was done.

Compliance

Quality control with regard to the test meals was ensured by weighing food to the nearest gram before

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consumption and of the leftovers after consumption. Subjects ate lunch at the Metabolic ward of the NWU-PC, whereas dinner and the following day's breakfast were supplied in take-away format. A registered dietician supervised mealtimes and ensured the complete intake of the respective meals. Leftover foodstuffs were collected and weighed by researchers in order to determine the compliance. Any deviations from the study protocol was recorded in diaries and reviewed by the investigators during the study.

Blood sampling

After an overnight (12 h) fast, a registered nurse collected venous blood samples from the antebrachial vein using a sterile winged infusion set and syringes. Samples were drawn with minimal stasis between 07:00 and 10:00 to avoid diurnal variation. For the preparation of serum 20 ml of blood was drawn and left to clot. For the determination of plasma glucose concentrations, 5 ml blood was collected in tubes containing 10 mg potassium oxalate and 12.5 mg sodium fluoride. EDTA-treated blood was used for glutathione analyses and heparin-treated blood was used to prepare plasma for ORAC analyses. Blood was centrifuged at 2,000*g* to yield serum and plasma, respectively. Aliquots of serum, plasma and deproteinated samples were stored at −82°C until analysis.

Blood sample analyses

Markers for the metabolic syndrome

Serum TG, TC, HDL-C and plasma glucose were measured using a Vitros DT60 II Chemistry Analyser (Ortho-Clinical Diagnostics, Rochester, New York, USA), with Vitros reagents (catalogue numbers 153 2159, 153 2175, 133 5504, and 153 2316) and controls (catalogue numbers 842 0317 and 144 8042). Serum LDL-C was calculated using the Friedewald equation (LDL-C (mmol/l) = TC−TG/2.2−HDL-C) [15]. Fasting serum insulin was measured with an ELISA method on the Immulite 2000 Analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). The QUICKI formula was used to calculate insulin sensitivity as follows: QUICKI = 1/[log(fasting venous insulin(μIU/ml) + log(fasting venous glucose(mg/dl)] [22]. Furthermore, insulin resistance was calculated using the homeostasis model assessment (HOMA) formula: HOMA = (fasting insulin (μIU/ml) × fasting venous glucose (mmol/l))/22.5 [22]. During the recruitment of subjects, fasting capillary glucose was measured by fingerprick with a SureStep™ blood glucose meter (Lifespan Inc., Milpitas, CA, USA), using Fine Point Lancets and SureStep™ test strips (code 11). Blood pressure was measured by a 7-minute continuous measurement of cardiovascular parameters using the Finometer™ device (Finapres Measurement Systems, Amsterdam, Netherlands).

Plasma antioxidant activity

Plasma antioxidant capacity of deproteinated plasma was determined by the ORAC assay [7] on a BioTEK fluorescence plate reader at an excitation wavelength at 530 nm and emission wavelength at 590 nm [6]. AAPH (240mM, 2,2′-azobis(2-amidinopropane) dihydrochloride) was used as a peroxyl radical generator, which oxidizes both lipopohylic and hydrophylic antioxidants. Trolox was used as a reference standard and the total plasma antioxidant capacity was expressed as μM trolox equivalents (TE).

Total oxidant status of serum

The Diacron reactive metabolites (dROMs) test (DIACRON International, Grosseto, Italy) was used to measure the oxidant status of serum. The colorimetric assay uses a chromogen, *N*,*N*-diethylparaphenilendiamine, which is a substrate for alkoxyl and hydroperoxyl radicals, and was performed kinetically in a plate reader measuring change in 560 nm over a period of 15 min at 25°C. Samples were quantified using a standard and expressed as Carratelli units (CARR U) where 1 CARR U corresponds to 0.08 mg/100 ml H_2O_2.

Glutahione redox state

The redox state of glutathione (GSH:GSSG) was measured using a spectrophotometric GSH:GSSG ratio assay kit as instructed by the supplier (GSH/GSSG-412™, OXIS Research Inc. Portland, USA). EDTA-treated blood was used and for the determination of GSSG the GSH-scavenger, 1-methyl-2-vinyl-pyridinium trifluoromethane sulfonate (M2VP), was used.

Diet and nut sample analyses

Oxygen radical absorbance capacity (ORAC)

The hydrophilic and lipophilic components of the food samples were analyzed separately. ORAC analyses of these two components were done as previously described [7, 20].

Macronutrient content

The percentage protein was analyzed by a general combustion method (AOAC Method 992.23) [29] by using a LECO FP 528 (LECO Corporation, Michigan,

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 fiber by the filter bag technique using the ANKOM 220 fiber analyzer with F57 filter bags (ANKOM Technologies, Fairport, New York, USA), the percentage moisture with the air-oven (aluminium plate) method (AACC method 44-16) [1] and percentage ash with the AOAC Official Method 942.05 [29]. The CHO content was then calculated as the sum of the protein, fat, fiber, moisture and ash subtracted from 100. Even though the values for vitamin C and E were not chemically analyzed, they were predicted using the South African Food Composition Tables (FoodFinder 2 Medical Research Council of South Africa, Tygerberg, South Africa) [25].

Total polyphenols

The total polyphenol content of the three diets and the walnuts and cashew nuts was determined according to the Folin-Ciocalteu method [39]. Lyophilized food samples were extracted with 80% methanol by solification for 5 min and centrifuged for 10 min. Combined extracts were taken to dryness under nitrogen. Samples were resuspended in 200 ml of water followed by 1 ml Folin Ciocalteu's reagent (Sigma—F9252). This was allowed to stand for 8 min at room temperature. Next, 0.8 ml sodium carbonate (7.5%) was added, mixed and allowed to stand for 30 min. Absorption was measured at 765 nm (Shimadzu UV—1601 UV–vis). Total phenolic content was expressed as gallic acid (Aldrich) equivalents (GAE) in grams per liter (g/l).

Statistical analysis

The computer software package Statistica® (Statsoft Inc, Tulsa, OK, USA) was used for data analyses. Initially, variables were tested for normality using the Shapiro-Wilk's *W*-test. Non-normally distributed data were transformed into an approximately normal distribution using logarithmic transformations. Normally distributed data is expressed as mean (95% confidence interval (CI)) and data not normally distributed or logarithmic transformed as median (25, 75 percentiles). The *t*-test for dependent samples was used to test for changes within groups, from baseline to end (parametric data). The Wilcoxon matched pairs test was used for non-parametric data. Differences between changes from baseline to end in the three groups were determined using the ANOVA for parametric data and the Kruskall Wallis ANOVA for non-parametric data. The differences between groups were also determined while controlling for baseline values using the analysis of covariance (ANCOVA) test. The two-way analysis of variance test was used to test for any interaction effects between dietary group and cohort in which the subjects received their dietary treatment for all the variables. Differences between predicted and chemical analyzed diets were determined using the ANOVA and Tukey HSD test for post-hoc analysis. Significance was set at $P \leq 0.05$. Multiple regression analysis was performed with insulin resistance, insulin sensitivity and insulin concentrations at baseline as dependent variables and GSH, GSSG, GSH/GSSG, ORAC and serum oxidant status as independent variables to determine the association between antioxidant and insulin markers.

Results

Subjects

Four subjects failed to complete the study: 2 subjects had out of town job responsibilities, 1 subject had an unrelated illness and 1 subject went on holiday during the study period. Therefore 64 subjects (29 men and 35 women) completed the study. Most of the participants were sedentary prior to the intervention period and remained so for the duration of the study. A dietary compliance of ±90% was calculated based on weighing of leftover food and food dairies. The baseline characteristics (Table 2) of the subjects did not differ between groups (ANOVA). Most of the subjects (91%) had waist circumference values greater than indicated by the ATP III criteria. Fifty three percent of the subjects had high TG, 42% had high systolic blood pressure, 13% had high diastolic blood pressure, 91% had low HDL-C and only 5% had high glucose concentrations as indicated by the ATP III criteria. Evidently, the subjects' characteristics at baseline were clear indications of the metabolic syndrome. Only four participants were smokers. Weight, BMI and waist circumference remained unchanged during the intervention period. The responses in variables for men and women, as well as smokers and non-smokers did not differ significantly. The data was, therefore, analyzed and reported for the combined study group, irrespective of gender and smoking habits.

Diet composition

The habitual diets of the participants consisted of between 5 500 and 13 000 kJ/day (1 310–3 095 kcal/d). The chemical analysis of the diets compared well to the calculated compositions, with the exception of the total fat and CHO content of the walnut diet (Table 1). The analyzed fat content was higher because the ac-

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Table 2 Baseline characteristics

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 (yr)	45.0	40.4, 50.2	46.0	40.7, 50.7	45.0	40.8, 49.3
Weight (kg)	107	99.4, 115	99.0	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	–
Systolic BP (mmHg)	128	126, 131	131	126, 135	131	126, 137
Diastolic BP (mmHg)	78.7	76.3, 81.2	77.0	74.0, 79.9	79.2	76.1, 82.2
Serum TG (mmol/L)	1.90	1.48, 2.32	1.81	1.33, 2.30	1.86	1.55, 2.16
Serum HDL-C (mmol/L)	0.94	0.83, 1.05	1.02	0.88, 1.16	0.85	0.77, 0.93
	Median	25, 75 Percentiles	Median	25, 75 Percentiles	Median	25, 75 Percentiles
Serum TC (mmol/L)	4.80	4.54, 5.18	4.49	3.98, 5.34	4.90	4.39, 5.52
Serum LDL-C (mmol/L)	2.99	2.63, 3,29	2.64	2.33, 3.16	3.21	2.79, 3.79
Serum insulin (μIU/ml)	14.0	10.0, 18.0	12.0	9.00, 16.0	12.5	11.0, 17.0
Plasma glucose (mmol/L)	4.50	4.30, 5.20	4.70	4.30, 5.10	4.55	4.30, 5.40
Insulin sensitivity (QUICKI)	0.57	0.50, 0.59	0.58	0.52, 0.62	0.56	0.52, 0.58
Insulin resistance (HOMA)	2.61	2.24, 4.48	2.35	1.84, 3.82	2.81	2.35, 3.76

BMI: Body mass index; BP: Blood pressure; CI: Confidence interval; HDL-C: High density lipoprotein cholesterol; hs-CRP: High sensitivity C-reactive protein; LDL-C: Low density lipoprotein cholesterol; TC: Total cholesterol; TG; Triacylglycerol; WC: Waist circumference; QUICKI: Quantitative insulin sensitivity check index; HOMA: Homeostasis model assessment. No significant P-values were obtained ($P \leq 0.05$)

tual fat content of the walnuts was higher than indicated in the food composition computer programme (73g/100 g vs. 62g/100 g) (Medical Research Council of South Africa, Tygerberg) [25]. The vitamin E content was higher in the control diet than both the walnut and cashew nut diets. The vitamin C content was the same in all three diets. The polyphenol content of the walnuts used in this study was significantly higher than the cashew nuts (0.83 ± 0.02 vs. 0.76 ± 0.01 mg/g wet mass, P = 0.01). This was also reflected in the diets where the polyphenol content of the walnut diet was significantly higher than the cashew nut diet (P = 0.02). The polyphenol content of the control diet did not, however, differ significantly from the nut diets (Table 1). Walnuts had higher hydrophilic (125 ± 4.72 vs. 69.8 ± 1.91 mmol TE/g wet mass, P = 0.001), similar lipophilic (76.7 ± 10.9 vs. 75.7 ± 13.9 mmol TE/g wet mass, P = 0.93) and higher total ORAC (202 ± 6.20 vs. 146 ± 15.8 mmol TE/g wet mass, P = 0.01) values compared to cashew nuts. The hydrophilic, lipophilic and total ORAC values of the control diet was significantly lower compared to the walnut and cashew nut diets (Table 1). The walnut diet had a significantly higher hidrophilic ORAC compared to the cashew nut diet whereas the lipophilic and total ORAC values were similar in the two nut diets.

Antioxidant profiles

No interaction effects could be seen between dietary group and cohorts in which the subjects received their dietary treatment for all the variables. The results of the different cohorts are therefore combined. In Table 3, significant improvements from baseline to end were reported for GSSG, GSH/GSSG, dRom and ORAC levels in the blood of all three diet groups. GSH levels showed no significant changes when comparing baseline and end values of all three groups (Table 3). Similarly, no significant differences in the changes from baseline to end between the three groups were seen (ANOVA) for any of the markers. The results did not change when the markers were controlled for baseline concentrations (ANCOVA).

Associations between insulin and markers of antioxidant status

Multiple regression analysis showed that only 7% of the variance in insulin sensitivity (R^2 = 0.07, P = 0.54) and 10% of the variance for both insulin resistance (R^2 = 0.101, P = 0.30) and insulin concentrations at baseline (R^2 = 0.104, P = 0.276) could be explained by the effect of GSH, GSSG, GSH/GSSG, ORAC and dRom.

Discussion

This study was unique in investigating the effects of diet on various antioxidant status markers in subjects with diagnosed metabolic syndrome, a condition associated with disrupted antioxidant defence systems [37]. However, no significant differences were

Table 3 Oxidative stress related parameters in blood

Markers		Walnut diet (n = 21)			Cashew nut diet (n = 21)			Control diet (n = 22)			
		Mean	95% CI	P-value (B versus E)	Mean	95% CI	P-value (B versus E)	Mean	95% CI	P-value (B versus E)	P-value between groups (ANOVA)
GSH (mmol/L)	B	655	610, 700	0.54	626	588, 664	0.30	668	613, 724	0.68	0.44
	E	636	602, 671		647	614, 681		673	639, 706		
	Δ	−18.6	−75.3, 38.1		21.0	−22.5, 64.4		4.40	−49.0, 57.8		0.34
GSSG (mmol/L)	B	2.87	2.37, 3.35	0.01	2.89	2.50, 3.29	0.02	2.89	2.43, 3.34	0.03	0.99
	E	2.09	1.81, 2.36		2.27	1.93, 2.62		2.30	1.95, 2.64		
	Δ	−0.78	−1.38, −0.19		−0.62	−1.14, −0.10		−0.59	−1.12, −0.06		0.72
GSH/GSSG	B	250	214, 286	<0.001	241	201, 281	0.01	256	215, 297	0.01	0.82
	E	328	285, 371		319	265, 374		324	276, 372		
	Δ	78.0	29.0, 128		78.0	22.0, 134		68.0	14.0, 122		0.86
dRom (CARR U)	B	502	434, 569	<0.001	524	476, 572	<0.001	531	454, 608	<0.001	0.79
	E	388	347, 429		397	355, 440		416	353, 479		
	Δ	−114	−179, −49		−126	−168, −85		−115	−153, −77		0.92
ORAC (mg TE/g)	B	1073	951, 1195	<0.001	1105	939, 1271	<0.001	1067	921, 1213	<0.001	0.87
	E	1489	1312, 1667		1315	1139, 1491		1340	1179, 1501		
	Δ	417	216, 617		209	-18, 437		273	97, 449		0.10

B: Baseline (after 3 week run-in period); E: End; CI: Confidence interval; dRom: Diacron reactive metabolites; GSH: Reduced glutathione; GSSG: Oxidized glutathione; ORAC: Oxygen radical absorbance capacity; Δ: Change from baseline to end

observed between the walnut, cashew nut and control diets. Furthermore, markers of antioxidant capacity improved significantly from baseline to end in both the intervention groups and the control group. These results are in accordance with results from a randomized controlled cross-over study by Ros et al. who could also not show any differences in biomarkers of oxidative stress (lag time of LDL conjugated dienes production, oxidized LDL or malondialdehyde) between a walnut (contributing to ≈18% E of the diet) and control diet that was followed for 4 weeks [35]. The walnut diet in the study of Ros et al. did, however, improve endothelium dependent vasodilation and reduced vascular cell adhesion molecule-1 levels compared to the Mediterranean control diet [35].

It was expected that the antioxidant profiles of the participants consuming the walnut and cashew nut diets would improve compared to those not consuming nuts. Walnuts have favorable phenolic concentrations compared to apple juice [31], or a glass of red wine [14], all of which are known for their high phenolic and antioxidant concentrations [2]. The number of servings of these foods that would have to be consumed to equal the phenolics in a serving of walnuts is as follows: 2.2 servings of red wine and 4.6 servings of apple juice [2]. Recent findings showed that cashew nut kernel oil had an ability to increase the antioxidant status of animals [38].

A walnut extract containing polyphenols, ellagic acid, and gallic acid was reported to inhibit the oxidation of human plasma low density lipoproteins (LDL) in vitro [14]. Recently, researches indicated that antioxidants (vitamin A, vitamin E, β-carotene, selenium, zinc, and $_L$-cysteine, carotene, citrus flavonoids, vitamin C, coenzyme Q_{10}, vitamin D and pyridoxine hydrochloride) given at combinations close to one recommended daily allowance (RDA), reduced oxidative stress [10]. However, a fluid form (containing distilled water) was shown to be more active and bioavailable than a dry form (capsule). Considering this, the antioxidants in the nuts may have been more bioavailable had they been consumed in an alternative form as to their natural state [10]. To date, little is known about the bioavailability, absorption and metabolism of polyphenols in humans.

Recent in vitro studies have revealed that polyphenols in almond skins work synergistically with vitamin E and C to decrease the susceptibility of LDL to oxidation [8]. The lack of effect reported in this study may be attributed to the fact that the incorporation of walnuts and cashew nuts into the diets resulted in a lower vitamin E content compared to the habitual and control diets. The lower vitamin E content is probably due to the fact that predetermined amounts of spreads and fats (high in vitamin E) were omitted, in order to compensate for the inclusion of the nuts in these diets. Although chemical analysis of the Vitamin C and E concentrations in these foods would have been more accurate, for the purpose of this discussion, the estimated values for vitamin C and E from food composition tables, adapted for South African foods, were used. Despite the lower vitamin E and similar total polyphenol concentrations in the nut diets compared to the control and habitual diets, the antioxidant capacity (as measured by

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ORAC) in the walnut and cashew nut diets tended to be higher when compared to the control diet. This may be explained by the fact that the total antioxidant concentration of a mixture is not the only factor which influences its antioxidant capacity. The structural arrangements (number and position of hydroxyl groups, double bonds and aromatic rings) of the various individual antioxidants and their individual concentrations may also play a role [34] resulting in different magnitudes of effectiveness, i.e. some polyphenols may have better radical scavenging effects and others may have better ferric reducing effects [16], hence using a different method, such as the ferric reducing antioxidant power (FRAP) assay to determine antioxidant capacity, may have lead to different results. Despite the ORAC concentrations of the nut diets being significantly higher than that of the control diet, it did not change the fasting serum ORAC concentrations of the subjects receiving nuts compared to the control diet group, over the period in which the study was conducted. It could be speculated that the high fat content of the walnut diet induced oxidative stress in vivo [21] that could have reduced the walnut diets' antioxidant capacity.

Longer durations of exposure of individuals to the nuts may have had a different outcome. Bioavailability studies [3, 41] have shown that the maximum polyphenol concentrations are reached 1–2 h after ingestion, followed by a rapid reduction in these concentrations. It may, therefore, be worthwhile to investigate the effects of nuts on the antioxidant capacity in the postprandial state. Antioxidant capacity analyses done on fasting blood samples, as measured in this study, is however not expected to measure the acute antioxidant capacity changes induced by the polyphenols in the blood stream (typically seen a couple of hours after ingestion), but would rather measure the body's inherent antioxidant mechanisms and the influence of a chronic dietary polyphenol intake on these.

It was difficult to determine whether the study had sufficient power to investigate the effects of nuts on the antioxidant status since it is not known to what extent antioxidant status needs to improve to be clinically significant. It is, however, clear that there were no conspicuous differences between the groups for ORAC, GSH, GSSG, GSH/GSSG and hydroperoxides. With regard to ORAC, the increase in the walnut group was noticeably greater than in the cashew nut and control groups ($P = 0.10$) and a larger study group might have resulted in a statistically significant change. However, in order to make conclusions regarding the effect on antioxidant status, one would expect to see the change in more than one antioxidant variable, since different antioxidant systems work synergistically in order to improve the total antioxidant capacity [9].

There are indications from the literature that oxidative stress may be related to the degree of insulin resistance through prolonged exposure to high glucose levels. This may result in further deterioration of glucose intolerance and insulin resistance [18, 37]. However, in this study only a small percentage of the variance in insulin resistance and insulin sensitivity could be explained by antioxidant markers as most of the participants presented with normal plasma glucose levels at baseline. Only 5% ($n = 3$) of the subjects had fasting blood glucose concentrations >6.1 mmol/l.

In conclusion, compared to the control diet, the intake of walnuts and cashew nuts did not improve the fasting antioxidant profiles of subjects with metabolic syndrome. Therefore, in practice, when nuts are incorporated into a prudent diet, it increases the antioxidant capacity (ORAC) of the diet, but this increase does not result in an improved antioxidant capacity of subjects with metabolic syndrome over a relatively short period. Additionally, the antioxidant capacity in food may not correlate well with the antioxidant capacity in biological samples and may be influenced by many factors that we still know little about, such as the bioavailability, absorption, metabolism and anti-oxidant capacity of different polyphenols in vivo. The effects of polyphenols in different food structures and the nutrient changes that occur in the diet when food products high in antioxidants are included. This study emphasizes the importance that the identification of foods with high antioxidant capacity is followed up with intervention trials to determine cause and effect.

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