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Reduced-energy cranberry juice increases folic acid and adiponectin and reduces homocysteine and oxidative stress in patients with the metabolic syndrome

Tathiana Name Colado Simão[1], Marcell Alysson Batisti Lozovoy[2], Andréa Name Colado Simão[3], Sayonara Rangel Oliveira[3], Danielle Venturini[3], Helena Kaminami Morimoto[3], Lúcia Helena Silva Miglioranza[4] and Isaias Dichi[5]*

[1]*Department of Nutrition, Philadelphia University Center (UNIFIL) Londrina, Londrina, Paraná, Brazil*
[2]*Department of Clinical Analysis, University of North Paraná (UNOPAR), Londrina, Paraná, Brazil*
[3]*Department of Pathology, Clinical Analysis and Toxicology, University of Londrina, Londrina, Paraná, Brazil*
[4]*Department of Food Science and Technology, University of Londrina, Londrina, Paraná, Brazil*
[5]*Department of Internal Medicine, University of Londrina, Robert Koch Avenue no 60, Cervejaria, Londrina, CEP 86038-440 Paraná, Brazil*

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Abstract

The metabolic syndrome (MetS) comprises pathological conditions that include insulin resistance, arterial hypertension, visceral adiposity and dyslipidaemia, which favour the development of CVD. Some reports have shown that cranberry ingestion reduces cardiovascular risk factors. However, few studies have evaluated the effect of this fruit in subjects with the MetS. The objective of the present study was to assess the effect of reduced-energy cranberry juice consumption on metabolic and inflammatory biomarkers in patients with the MetS, and to verify the effects of cranberry juice concomitantly on homocysteine and adiponectin levels in patients with the MetS. For this purpose, fifty-six individuals with the MetS were selected and divided into two groups: control group (n 36) and cranberry-treated group (n 20). After consuming reduced-energy cranberry juice (0·7 litres/d) containing 0·4 mg folic acid for 60 d, the cranberry-treated group showed an increase in adiponectin ($P=0·010$) and folic acid ($P=0·033$) and a decrease in homocysteine ($P<0·001$) in relation to baseline values and also in comparison with the controls ($P<0·05$). There was no significant change in the pro-inflammatory cytokines TNF-α, IL-1 and IL-6. In relation to oxidative stress measurements, decreased ($P<0·05$) lipoperoxidation and protein oxidation levels assessed by advanced oxidation protein products were found in the cranberry-treated group when compared with the control group. In conclusion, the consumption of cranberry juice for 60 d was able to improve some cardiovascular risk factors. The present data reinforce the importance of the inverse association between homocysteine and adiponectin and the need for more specifically designed studies on MetS patients.

Key words: **Metabolic syndrome: Cranberry juice: Folic acid: Adiponectin: Homocysteine**

The metabolic syndrome (MetS) comprises pathological conditions that include insulin resistance, arterial hypertension, visceral adiposity and dyslipidaemia, which favour the development of CVD[1]. Existing evidence suggests that the MetS is rising in both developed[2] and developing countries such as Brazil[3]. Abdominal obesity and insulin resistance are the core features of the MetS; however, inflammation is thought to be associated with insulin resistance and the MetS[4]. Central obesity is considered to be one of the most important determinants of low-grade chronic inflammation present in the MetS[5].

Diets rich in fruits and vegetables enhance polyphenolic intake and are protective against CVD. Cranberry (*Vaccinium macrocarpon*) juice is unique among fruit juices because it has a relatively low natural carbohydrate content compared with its high content of vitamins, minerals and polyphenolic compounds including flavonols (myricetin and quercetin), anthocyanins and proanthocyanidins, which confers potent antioxidant activity to any cranberry-derived products such as juice[6]. These phenolic compounds have a wide range of biological effects including the ability to serve as antioxidants, modulate enzyme activity and regulate gene expression[6].

Abbreviations: AOPP, advanced oxidation protein products; MetS, metabolic syndrome; WC, waist circumference.

* **Corresponding author:** I. Dichi, email dichi@sercomtel.com.br

In addition, cranberry juice cocktail contains >100 % of the daily value of vitamin C[6]. Furthermore, cranberry juice has been shown to contain close to the same amount of resveratrol as in grape juice[7]. Resveratrol has several biological effects related to cardiovascular health including quenching reactive oxygen species, inhibiting platelet aggregation and reducing inflammation. Thus, cranberries have been suggested to have a beneficial impact on cardiovascular health[8]. Cranberry juice consumption has been associated with a reduction of surrogate biomarkers of CVD risk, as reported in clinical studies[9]. Intervention trials have reported the beneficial effects of cranberry on oxidative stress, dyslipidaemia and inflammatory biomarkers in healthy volunteers[10,11] and in patients with type 2 diabetes mellitus[12] and the MetS[13].

Several clinical and epidemiological studies have hypothesised that increased concentration of total plasma homocysteine[14] and decreased adiponectin levels[15] could represent an additional independent risk factor for CVD. Studies that have assessed the effects of cranberry juice ingestion on the MetS are scarce[13]. In addition, an inverse association between plasma total homocysteine and adiponectin has only been reported in a MetS-related condition, such as the polycystic ovary syndrome[16], and also in a MetS-unrelated condition, such as prolonged alcohol exposure[17]. To the best of our knowledge, the present study is the first to report the effects of cranberry juice concomitantly on homocysteine and adiponectin levels in patients with the MetS. The present hypothesis is that a juice that contains higher folic acid levels, such as cranberry juice, would lead to a decrease in homocysteine levels followed by an increase in adiponectin levels and a decrease in oxidative stress.

Therefore, the aim of the present study was to evaluate the effects of reduced-energy cranberry juice ingestion on several metabolic and inflammatory biomarkers, and to verify the effects of cranberry juice concomitantly on homocysteine and adiponectin levels in patients with the MetS.

Subjects and methods

A total of eighty patients (*n* 80) with the MetS from ambulatory patients of the University Hospital of Londrina, Paraná, Brazil were contacted by telephone, and twenty-two were considered ineligible. Inclusion criteria included the MetS and age between 18 and 60 years. Exclusion criteria were thyroid, renal, hepatic, gastrointestinal or oncological diseases and utilisation of lipid-lowering drugs, oestrogen replacement therapy, drugs for hyperglycaemia and antioxidant supplements. Patients who were taking anti-hypertensive drugs were not excluded and were allowed to continue taking the same dose of the drugs. None of the subjects followed a specific diet before the study began. After exclusion, fifty-eight (fourteen men and forty-four women) were paired by sex, age, ethnicity and BMI in two groups (control group *n* 37 and cranberry-treated group *n* 21) and began to participate in the study. Non-compliance was verified in two patients, one from the control group and one from the cranberry-treated group (Fig. 1). The distribution of postmenopausal women between the groups was similar (data not shown).

Patients were instructed by a nutritionist to maintain their usual diets, alcohol intake, level of physical activity or other lifestyle factors throughout the intervention period. The

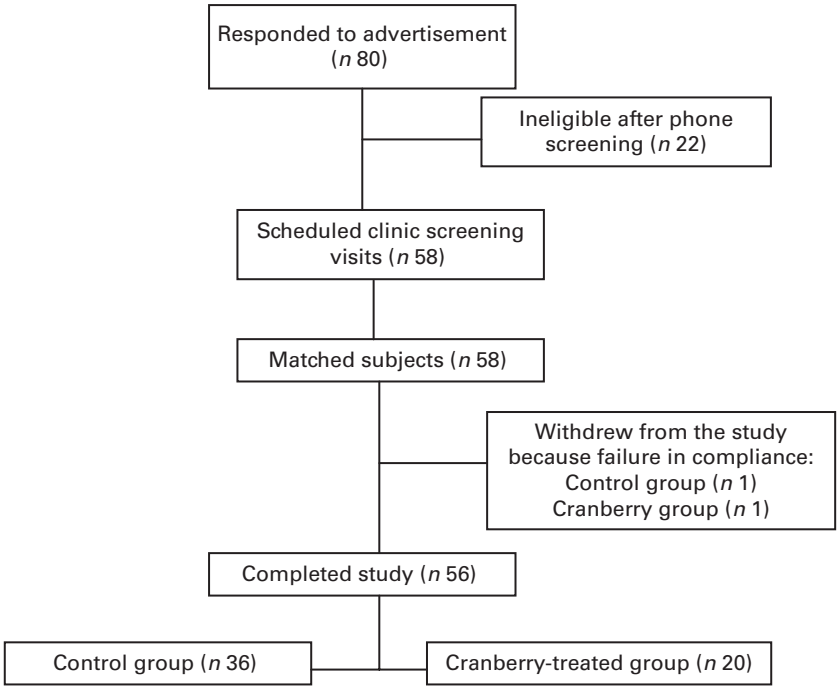

Fig. 1. Schematic of subject flow and reasons for exclusion.

present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Ethical Committee of the University of Londrina, Paraná, Brazil (study protocol CEP 230/2011). Written informed consent was obtained from all subjects/patients.

Study design

Patients were assigned to one of two groups after stratification by age and BMI. The first group (control group, *n* 36) was only directed to maintain their usual diet; the second group (cranberry-treated group, *n* 20) consumed 0·7 litres/d of reduced-energy cranberry juice. The juice was given at lunch and dinner. Subjects were recommended to avoid resting after meals to prevent unpleasant effects. All of the groups were evaluated at the beginning of the study and after 60 d. Interviews were performed to assure no change in lifestyle factors throughout the study. The nutrient composition of 200 ml cranberry juice was as follows: 84 kJ (20 kcal); 0 g protein; 5 g carbohydrate; 0 g lipids; 0 g fibre; 30 mg Na; 60 mg vitamin C; 66 mg proanthocyanidins; total phenolics of 104 and 0·12 mg folic acid. The total antioxidant power of cranberry juice determined by oxygen radical antioxidant capacity was 183·65 μmol trolox equivalents (TE)/ml.

Anthropometric measurements and laboratory parameters were assessed at the beginning of the study and after 60 d. The MetS was defined following the Adult Treatment Panel III criteria, when three of the following five characteristics were confirmed: (1) abdominal obesity – waist circumference (WC) ≥ 88 cm for women and ≥ 102 cm for men; (2) hypertriacylglycerolaemia ≥ 1500 mg/l; (3) low levels of HDL-cholesterol ≤ 500 mg/l for women and ≤ 400 mg/l for men; (4) high blood pressure ≥ 130/85 mmHg; (5) high fasting glucose ≥ 1100 mg/l[18].

Steps taken to optimise compliance

Various measures were taken to optimise and assess patient compliance. Before each trial began, it was assured that the patients understood that they could be allocated to any group. Boxes of cranberry juice were handed out at the initial interview and at the two later visits. Subjects were asked to bring back any unconsumed juice to assess unmonitored compliance[13]. Treatment adherence, i.e. consumption of cranberry, was 95 %.

Anthropometric measurements

Body weight was measured to the nearest 0·1 kg by using an electronic scale with individuals wearing light clothing and no shoes; all patients were weighed in the morning. Height was measured to the nearest 0·1 cm with a stadiometer. BMI was calculated as weight (kg) divided by height (m) squared. WC was measured with a soft tape on standing subjects midway between the lowest rib and the iliac crest.

Biochemical biomarkers

After fasting for 12 h, patients underwent the following laboratory blood analysis: glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol and TAG, evaluated by a biochemical auto-analyser (Dimension Dade AR; Dade Behring), using Dade Behring® kits; plasma insulin levels determined by microparticle enzyme immunoassay (MEIA) (AxSYM; Abbott Laboratory). Homeostasis model assessment was used as a surrogate measurement of insulin sensitivity.

Folic acid and homocysteine measurements

HPLC (Waters 2695 Separations Module) determined folic acid concentration in accordance with US Pharmacopeia[19] at 290 nm detection (Waters 2996 Photodiode Array Detector), and plasma total homocysteine levels measured by chemiluminescence (ARCHITECT®; Abbott Laboratory).

Inflammatory and immunological biomarker measurements

Serum high-sensitivity C-reactive protein was measured using a nephelometric assay (Behring Nephelometer II; Dade Behring). Serum IL-1, IL-6, TNF-α and adiponectin were measured by a sandwich ELISA using a commercial immunoassay (R&D System).

Oxidative stress measured by tert-butyl hydroperoxide-initiated chemiluminescence

tert-Butyl hydroperoxide-initiated chemiluminescence measures lipid hydroperoxides originating from phospholipids, cholesterol esters, protein and NEFA oxidation and decreased antioxidant levels, created by the free radical action primarily on plasma lipoprotein particles. This method is much more sensitive and less prone to artifact than to others[20]. *tert*-Butyl hydroperoxide-initiated chemiluminescence in plasma was evaluated as described by Flecha *et al.*[21]. For chemiluminescence measurement, reaction mixtures were placed in 20 ml scintillation vials (low-K glass) containing final concentrations of plasma (250 μl), 30 mM-KH_2PO_4/K_2HPO_4 buffer (pH 7·4) and 120 mM-KCl with 3 mM-LOOH in a final volume of 2 ml. *tert*-Butyl hydroperoxide-initiated chemiluminescence was measured in a Beckman LS 6000 liquid scintillation counter (Beckman Coulter) set to the out-of-coincidence mode with a response of 300–620 nm. The vials were kept in the dark up to the moment of assay, and determination was carried out in a dark room at 30°C. Results are expressed as counts per min.

Oxidative stress determined by advanced oxidation protein products

Advanced oxidation protein products (AOPP) were determined in plasma using the semi-automated method described by Witko-Sarsat *et al.*[22]. AOPP results in the oxidation of amino acid residues such as tyrosine, leading to the formation

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Table 1. Demographic and clinical characteristics in the control and cranberry-treated groups (Medians and 25%–75% ranges)

	Control (*n* 36)		Cranberry (*n* 20)		
	Median	25 %–75 % range	Median	25 %–75 % range	*P*
Sex*					0·2917
Male		8		6	
Female		28		14	
Age (years)†	48·5	44·8–56·3	51·0	42·0–53·0	0·8737
White/non-white*		26/10		13/7	0·7679
Anti-hypertensive medication (%)*	19	55·9	9	56·3	0·1307
BMI (kg/m^2)	34·0	31·32–36·9	30·9	26·3–38·4	0·6711
WC (cm)	105·0	97·5–113·5	104·0	94·0–118·4	0·9811

WC, waist circumference.
* χ^2 test.
† Mann–Whitney test.

of dityrosine-containing protein cross-linking products detected by spectrophotometry[20,22]. AOPP concentrations are expressed as μmol chloramine-T equivalents/l.

Statistical analysis

Distributions of sex, ethnicity and medications of hypertension were analysed by a χ^2 test. The Mann–Whitney test was performed to compare differences between the parameters of groups at baseline and differences across the treatment groups (inter-group changes). Wilcoxon matched-pairs test was performed to verify changes from baseline (intra-group changes). Data are presented as medians (25 %–75 % range). Significance was set at $P<0·05$.

Results

There were no differences between the groups in relation to sex, ethnicity, age, anti-hypertensive drugs, BMI and WC (Table 1). With regard to anthropometry, there were no significant differences in BMI and WC between the groups after 60 d (data not shown).

Serum folic acid levels significantly increased ($P=0·033$; Fig. 2) and homocysteine levels decreased ($P<0·001$; Fig. 3) in the cranberry-treated group after 60 d in relation to the baseline values. There was no significant difference between the treatment groups ($P>0·05$).

The metabolic and inflammatory biomarkers C-reactive protein, TNF-α, IL-1 and IL-6 did not differ between the groups (Table 2). On the other hand, serum adiponectin levels showed a significant increase in the cranberry-treated group ($P=0·010$) after 60 d in relation to baseline values. There was a significant difference between the treatment groups ($P<0·05$; Fig. 4).

In relation to oxidative stress measurements, there were no differences in hydroperoxides ($P=0·629$) and AOPP ($P=0·244$) levels in the control group after 60 d when compared with the baseline values. However, decreased lipoperoxidation ($P=0·036$) and protein oxidation ($P=0·008$) levels were found in the cranberry juice group after 60 d in relation to the baseline values. There was a significant difference between the treatment groups (Fig. 5).

Discussion

The present study evaluated reduced-energy cranberry juice ingestion during 60 d in several metabolic and inflammatory cardiovascular risk factors in patients with the MetS. The main findings of the present study were an increase in serum folic acid and adiponectin levels and a decrease in serum homocysteine levels and oxidative stress in patients with the MetS using cranberry juice. Of note, reduced-energy cranberry juice was not supplemented with folic acid.

Effect of cranberry juice on folic acid and homocysteine levels

Elevated homocysteine levels are also thought to be a risk factor for CVD[23]. A meta-analysis of prospective cohort studies has demonstrated that after accounting for known CVD risk factors, a 25 % lower homocysteine level was associated with about an 11 % lower risk of IHD and about a 19 % lower risk of stroke[24]. A causal relationship between

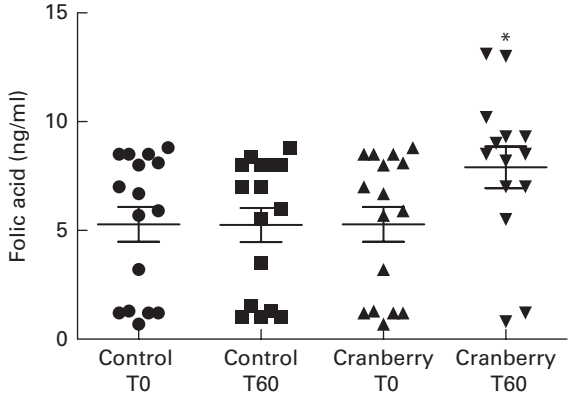

Fig. 2. Serum folic acid levels in patients with the metabolic syndrome at baseline (T0) and after consuming reduced-energy cranberry juice for 60 d (T60). The Wilcoxon matched-pairs test was performed to verify changes from baseline (intra-group changes). The Mann–Whitney test was performed to compare differences between the baseline values and across treatment groups (inter-group changes). Data are the median (25 %–75 % range). There was no difference between the baseline groups. *Cranberry T0 *v.* T60: $P=0·033$;. Differences between treatment groups were not significant ($P>0·05$).

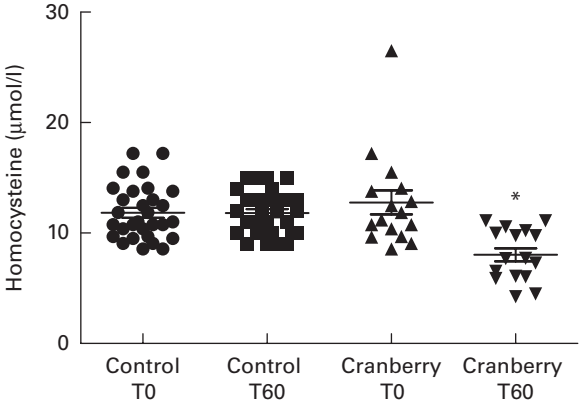

Fig. 3. Serum homocysteine levels in patients with the metabolic syndrome at baseline (T0) and after consuming reduced-energy cranberry juice for 60 d (T60). The Wilcoxon matched-pairs test was performed to verify changes from baseline (intra-group changes). The Mann–Whitney test was performed to compare differences between the baseline values and across treatment groups (inter-group changes). Data are the median (25 %–75 % range). * Cranberry T0 *v.* T60: $P<0{\cdot}001$. Differences between treatment groups were not significant ($P>0{\cdot}05$).

homocysteine and CVD has been proposed based on genetic and prospective studies, and lowering concentrations by 3 μmol/l from current levels would reduce the risk of IHD by 16 %, deep vein thrombosis by 25 % and stroke by 24 %[25]. Elevated homocysteine levels can occur due to a lack of vitamins, in particular folate and/or cobalamin, that are cofactors required for homocysteine metabolism through the remethylation pathway[14,23]. Kawashima *et al.*[26] evaluated the effect of fruit and vegetable juice concentrates, including cranberry, given as capsules on serum homocysteine levels in healthy subjects. In that study, serum folate levels rose significantly in the group supplemented with fruit and vegetable juice concentrates compared with the placebo group. There was a corresponding significant reduction in plasma homocysteine levels in the active compared with the placebo group. As expected, these changes in plasma homocysteine were negatively correlated with the increase in serum folate concentrations. Significant increases in folate and decreases in homocysteine levels have been observed in other studies in which fruit and vegetable juice concentrates were supplemented to healthy subjects[27–29] and also to MetS patients after folate and vitamin B_{12} therapy[30]. Berries are significant dietary sources of folic acid, a water-soluble B vitamin which is essential in preventing neural tube defects in newborn babies, and may also play a role in reducing the risk of heart disease and cancer through a range of mechanisms including lowering homocysteine levels, catalysing NO formation and maintaining DNA stability[31]. In the present study, the decrease in homocysteine levels can be explained by the increase in serum folic acid levels due to the daily ingestion of approximately 0·4 mg folic acid (present in 700 ml), the median amount ingested, which is the US RDA for adults and the dose precisely needed for maximal homocysteine lowering (400 μg)[32].

However, several meta-analyses have failed to show a decrease in all-cause mortality and cardiovascular events when folic acid supplements were given to high-risk or vascular disease patients, despite the decrease verified in homocysteine levels[33–37]. These long-term prospective studies have reduced the expectations in relation to the importance of decreasing homocysteine levels and reinforced the concept that increased homocysteine levels would be a marker, but not a cause, for vascular disease risk. However, many arguments can be raised to justify the importance of decreasing homocysteine levels, mainly through food fortification. First, mandatory folic acid fortification of foods implemented in the USA and Canada (140 μg folic acid in 100 g wheat flour) to prevent neural tube defects decreased plasma total homocysteine concentrations and neural tube defects, and also reduced stroke mortality in the aforementioned countries in comparison with stoke mortality in England and Wales where folic acid fortification is not mandatory[38]. Second, B vitamins in pharmacological doses may exert some harmful effects through mechanisms involving direct effects on smooth muscle proliferation and matrix formation in coronary arteries[37], and the dose of folic acid used in that study (0·8–2·5 mg) was far higher than the aforementioned dose for maximal homocysteine lowering[32]. Third, methylenetetrahydrofolate reductase C677T T-allele homozygotes, who have greater mean homocysteine concentrations for the C (wild-type) allele, have a significantly increased risk for stroke[39], and

Table 2. Anthropometric, blood pressure, laboratory and inflammatory markers in the control and cranberry-treated groups at baseline and after consuming reduced-energy cranberry juice for 60 d*

(Medians and 25%–75% ranges)

	Controls (*n* 36)					Cranberry (*n* 20)				
	T0		T60			T0		T60		
Parameters	Median	25 %–75 % range	Median	25 %–75 % range	*P*	Median	25 %–75 % range	Median	25 %–75 % range	*P*
IL-1 (pg/ml)	2·00	2·00–2·60	2·00	2·00–2·10	0·750	2·0	2·0–6·65	2·0	2·00–4·30	1·000
IL-6 (pg/ml)	1·6	1·00–4·35	1·20	1·00–4·13	0·944	1·3	1·0–3·4	1·1	1·00–4·13	0·812
TNF-α (pg/ml)	2·00	2·00–2·95	2·00	2·00–4·35	0·250	2·0	2·00–2·89	2·0	2·00–3·34	0·437
CRP (mg/l)	44	22–96	42	14·7–95	0·131	56	10·6–104·0	42·8	14·4–135·1	0·391

CRP, C-reactive protein.

* The Wilcoxon matched-pairs test was performed to verify changes from baseline (intra-group changes). The Mann–Whitney test was performed to compare differences between the baseline values and across treatment groups (inter-group changes).

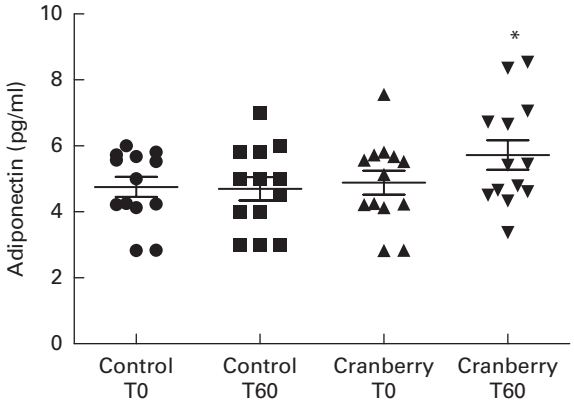

Fig. 4. Serum adiponectin levels in patients with the metabolic syndrome at baseline (T0) and after consuming reduced-energy cranberry juice for 60 d (T60). The Wilcoxon matched-pairs test was performed to verify changes from baseline (intra-group changes). The Mann–Whitney test was performed to compare differences between the baseline values and across treatment groups (inter-group changes). Data are the median (25 %–75 % range). * Cranberry T0 *v.* T60: $P=0{\cdot}010$. Differences between treatment groups were significant ($P<0{\cdot}05$).

the relationship between methylenetetrahydrofolate reductase C677T polymorphism and folate status is consistent with the possibility that population-wide folic acid fortification helps to prevent stroke[40]. Fourth, even folic acid supplements have shown beneficial results in stroke prevention after exclusion of the subset of patients with B_{12} deficiency and renal impairment that were unlikely to respond to B-multivitamin therapy[41]. Therefore, it seems that emphasis should be laid on food fortification with folic acid rather than on supplements, as previously stated by Lichtenstein & Russell[42].

Effect of cranberry juice on inflammatory and anti-inflammatory cytokines

Abdominal obesity and insulin resistance are core features of the MetS. Abdominal subcutaneous tissue produces a variety of adipokines, such as TNF-α and IL-6, which has an important role in inflammation and insulin resistance via endocrine, paracrine or autocrine signals[43,44]. IL-6 is considered to be the major mediator of the hepatic acute-phase reaction, and is thought to play a central role in the pathogenesis of CVD in patients with insulin resistance[45]. Inflammation, demonstrated primarily by the elevated levels of serum C-reactive protein, is thought to be associated with insulin resistance and the MetS[4,46]. However, adipose tissue also secretes adiponectin, a protein showing anti-inflammatory activity, which inhibits TNF-α production[47], adhesion molecule expression and nuclear transcriptional factor κB signalling, a pivotal pathway in inflammatory reactions in endothelial cells[48,49]. In addition, adiponectin is anti-atherogenic and an insulin-sensitising agent[50]. Adipose-derived TNF-α may have negative effects on the expression of adiponectin and vice versa, and these two proteins also have opposite effects on insulin sensitivity[51,52]. Given this antagonistic relationship, obesity, especially visceral obesity, may lead to a decreased secretion of adiponectin through feedback inhibition, thereby suppressing the beneficial effects of adiponectin on insulin sensitivity. Levels of adiponectin are lower in patients with obesity[53], type 2 diabetes mellitus[54], arterial hypertension[55] and the MetS[56]. Similarly to the present findings, a previous study also showed that adiponectinaemia was not associated with pro-inflammatory status in women with the MetS[56].

There is accumulating evidence that quercetin, a flavonol found in large quantities in cranberries, is a potent down-regulator of the NF-κB pathway[57]. In addition, resveratrol, a polyphenol also present in cranberry juice, has been shown to suppress the expression of inflammatory genes relevant to CVD through the activation of NF-κB and Janus kinase/signal transducer and activator of transcription (JAK/STAT)[3] pathways in cultured cells[6]. Numerous genes of inflammatory proteins are under the regulation of NF-κB, including adhesion molecules, IL-6 and TNF-α. Other components such as proanthocyanidin, anthocyanidins, hydroxycinnamic acid and acetylsalicylic acid that can be found in cranberries have all been shown to prevent the expression of adhesion molecules induced by TNF-α through their inhibitory action on NF-κB

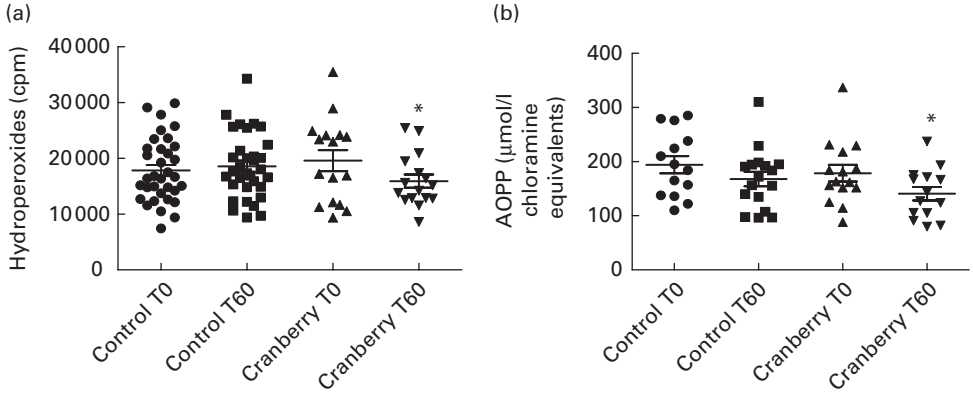

Fig. 5. Oxidative stress in patients with the metabolic syndrome at baseline (T0) and after consuming reduced-energy cranberry juice for 60 d (T60). The Wilcoxon matched pairs test was performed to verify changes from baseline (intra-group changes). The Mann–Whitney test was performed to compare differences between the baseline values and across treatment groups (inter-group changes). Data are the median (25 %–75 % range). (a) Hydroperoxide levels given in counts per min (cpm). * Cranberry T0 *v.* T60: $P=0{\cdot}036$. Differences between treatment groups were significant ($P<0{\cdot}05$). (b) Advanced oxidation protein products (AOPP) levels. * Cranberry T0 *v.* T60: $P=0{\cdot}008$. Differences between treatment groups were significant ($P<0{\cdot}05$).

activation[8,11,58]. In addition, flavonols, such as quercetin, found in some fruit including berries, have been shown to inhibit cyclo-oxygenase and lipoxygenase activities[59], enzymes which are released after arachidonic acid stimulus, the initiator of a general inflammatory response. Although consuming reduced-energy cranberry juice for 60 d was unable to provoke any change in pro-inflammatory cytokines or in C-reactive protein, there was a significant increase in adiponectin levels. It is likely that a decrease in pro-inflammatory biomarkers has not been detected because it is clear that detected plasma cytokines do not represent the concentration of cytokines locally produced in the tissue. TNF-α does not seem to be released into the circulation and is thus unable to signal systemically, therefore functioning as a paracrine pathway[4,51,60].

The present study showed that reduced-energy cranberry juice ingestion had no effects on BMI and WC similarly to a recent study that examined the effects of cranberry juice in women with the MetS[13]; thus, serum adiponectin increase cannot be attributed to changes in body composition.

This is the first study, to our knowledge, to evaluate the effect of cranberry juice on adiponectin, an important anti-inflammatory cytokine, which has been considered the link between obesity and the MetS[61]. Some hypotheses can be raised to suggest adiponectin increase with cranberry. First, it has been demonstrated that daily ingestion of blueberry for 6 weeks increased IL-10[62], an anti-inflammatory cytokine which inhibits TNF-α, and thus can be indirectly related to increased adiponectin levels. Second, despite not having an important fat content, the high proportion of long-chain *n*-3 fatty acids in berries (0·25 g α-linolenic acid/100 g) makes them an important proportion of daily intakes in some populations[63], and fish oil *n*-3 fatty acids have demonstrated an increase in adiponectin levels[64,65]. Third, another rich source of flavonoids (isoflavone), found in soya and soya-derived products, have also shown an increase in adiponectin levels[66,67]. Recently, our group also showed an increase in serum adiponectin after the ingestion of fish oil or a soya-derived product (kinako) during 90 d in patients with the MetS[68]. Fourth, some studies have shown an inverse association between homocysteine and adiponectin levels[17,69].

Effect of cranberry juice on oxidative stress

Oxidative stress is believed to be a pathway through which atherosclerosis develops in insulin-resistant and dyslipidaemic subjects[8]. There is considerable evidence *in vitro*[70] and *in vivo*[71] that cranberry phenolics are potent antioxidants. In screening for antioxidant activity of different plant phenolics, berries scored as the most promising among ninety-two plant materials examined[72]. Also, in a review of ninety-three intervention studies concerning the relevance of polyphenols to human subjects, cranberry has also been shown to decrease the formation of lipid oxidation products[73]. They appear to have free-radical-scavenging properties against superoxide radical, H_2O_2, hydroxyl radicals and singlet oxygen, and can also inhibit lipid and protein oxidation[70]. In addition, cranberry flavonoids reduce the vulnerability of endothelial cells to increased oxidative stress[58]. Human studies have also demonstrated an increase in antioxidant capacity in healthy[10] and MetS subjects[13]. Similarly to Basu *et al.*[13] who evaluated the effects of reduced-energy cranberry juice on MetS patients for 8 weeks, we also found a decrease in lipid oxidation. In addition, in the present study, we also verified a decrease in protein oxidation. Certainly, total phenolics in cranberry juice ingested by our patients (362·5 mg/d) have a prominent role in these findings.

Link between serum homocysteine, adiponectin and oxidative stress

An inverse association between homocysteine and adiponectin[17,69,74–76] and a direct association between homocysteine and oxidative stress[55,56] have been reported in different studies.

Sieminska *et al.*[69] verified lower adiponectin levels, whereas Badawy *et al.*[74] showed increased homocysteine levels in patients with the polycystic ovary syndrome, a MetS-related condition. They attributed their finding to a higher prevalence of insulin resistance in the polycystic ovary syndrome. In addition, this inverse association was verified in patients with essential hypertension[71] and type 1 diabetes with the progression of coronary artery disease[76]. However, the potential mechanism to explain this association comes from a study on prolonged alcohol exposure. Song *et al.*[17] demonstrated that chronic alcohol consumption altered methionine metabolism with consequent hyperhomocysteinaemia not only in the liver but also in adipose tissue. Homocysteine due to chronic alcohol consumption induced the inhibition of adiponectin gene expression in primary adipocytes and reduced circulatory adiponectin levels in an animal model of mild hyperhomocysteinaemia, suggesting that elevated homocysteine in adipose tissue may play a causal role in suppressing adiponectin production in alcoholic liver disease. The results of the present study reinforce the importance of this association also in patients with the MetS.

The association between homocysteine and oxidative stress has been shown in type 2 diabetes mellitus, hypertension, atherosclerosis and the MetS[77,78]. Tyagi *et al.*[79] showed in cardiac microvascular endothelial cells that homocysteine induces oxidative stress by increasing inducible NO synthase and decreasing endothelial NO synthase. The interaction of ROS, such as superoxide, with NO generates peroxynitrite, which then reacts with tyrosine residues to produce nitrotyrosine. In addition, homocysteine inhibits dimethylarginine dimethylaminohydrolase, which is responsible for degrading asymmetric dimethylarginine; the later inhibits endothelial NO synthase and competes with L-arginine for NO production[77]. Thus, endothelial dysfunction is an important mechanism that mediates increased oxidative stress caused by increased homocysteine levels.

The following limitations need to be considered in the present study: first, the small number of participants; second, the incomplete characterisation of cranberry juice in terms of individual polyphenol content; third, the absence of a placebo control group, although a similar design has been previously

used in several studies[11,80–82]. Nevertheless, the present study also has several strengths. First, to our knowledge, this is the first study to evaluate folic acid, homocysteine, adiponectin and oxidative stress concentrations in patients with the MetS using cranberry juice. Second, we rigorously tried to assure that patients did not take any drug or presented any disease that could interfere with the results. Therefore, patients with renal impairment, vitamin B_{12} insufficiency, hypothyroidism and haemolysis or using drugs, such as phenytoin, isoniazid, methrotexate and L-DOPA[28], were excluded from the study to avoid interference with homocysteine results. Also, statistical analyses between the groups were not significantly different in patients using anti-hypertensive drugs, such as angiotensin-converting enzyme inhibitors which may elevate plasma adiponectin levels[83]. Third, both groups were similar in relation to all parameters evaluated at the beginning of the study.

In conclusion, the present study show that reduced-energy cranberry juice ingestion for 60 d increased serum adiponectin and folic acid levels and decreased serum homocysteine levels and oxidative stress. The present data in addition to showing the beneficial effects of reduced-energy cranberry juice ingestion reinforce the importance of decreasing homocysteine levels, which contributes in turn to decreased oxidative stress and increased adiponectin levels. More studies are needed to specifically evaluate the aforementioned data in patients with the MetS.

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