ELSEVIER

Nutrition 22 (2006) 593–599

NUTRITION

www.elsevier.com/locate/nut

Applied nutritional investigation

A role for fruit content in energy-restricted diets in improving antioxidant status in obese women during weight loss

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Manuscript received May 25, 2005; accepted March 19, 2006.

Abstract

Objective: The aim of the present work was to estimate the ability of two hypocaloric diets with different fruit contents to improve antioxidant biomarkers related to lipid peroxidation in obese women.

Methods: Fifteen obese women (age 32 ± 6 y, body mass index 34.9 ± 2.9 kg/m^2) were assigned to two different dietary treatments for 8 wk. The subjects received a hypocaloric diet (600 kcal/d restriction from the measured individual energy expenditure) containing 5% (n = 8) or 15% (n = 7) energy supplied by fructose from fruits. Anthropometric measurements, blood lipid profile, plasma oxidative markers, total antioxidant capacity, and malondialdehyde (MDA) were evaluated before and after the nutritional intervention in addition to some relations among them.

Results: No differences in weight loss were observed between diets (5% energy from fructose in the low fruit diet −6.9 ± 2% versus 15% energy from fructose in the high fruit diet −6.6 ± 2%; P = 0.781). Low-density lipoprotein cholesterol levels significantly decreased (P = 0.048) in obese women who followed the high fruit diet, which was accompanied by a statistical (P = 0.046) diet-related decrease (−30%) in the ratio of MDA to antioxidant capacity. There was a positive association between MDA diet-related change and low-density lipoprotein cholesterol (r = 0.665, P = 0.003), with antioxidant capacity directly proportional to the fiber plus fructose content associated with fruit consumption (r = 0.697, P = 0.025).

Conclusion: A fruit-enriched hypocaloric diet appears to be more effective against oxidative stress. Consumption of antioxidant substances contained in fruit could be a useful strategy in the design of hypocaloric diets that, with the weight reduction, could increase the improvement of cardiovascular risk factors related to obesity.

Keywords: Obesity; Oxidative stress; Fruit; Caloric restriction; Malondialdehyde; Antioxidant capacity in plasma

Introduction

Obesity is associated with a greater risk of developing illnesses such as diabetes, inflammation, atherosclerosis, and coronary heart disease [1]. This relation is likely to be linked to a number of metabolic impairments and accompanied by oxidative stress disturbances [1–3]. Increased reactive oxygen species generation in the obese population may result in oxidative injury on cell lipids and proteins [4,5]. Native low-density lipoprotein (LDL) cholesterol is damaged by oxidative species originated by monocytes/macrophages at the endothelium [6]. Thus, reactive oxygen species promote lipid peroxidation, leading to cardiovascular disease by oxidative stress stimulation [2].

Nutritional intervention by means of a hypocaloric diet could produce protective effects against the redox unbalance [7]. Further, antioxidant-enriched diets could be applied in the nutritional therapy of obesity by increasing the health benefits related to weight loss [7]. Also, it has been proposed that dietary antioxidants could provide protection against a free radical attack, thus decreasing the risk of

This research was supported by the Government of Navarra (Public Health and Education Departments) and Linea Especial about Nutrition, Obesity and Health (University of Navarra LE/97).

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0899-9007/06/$ – see front matter
doi:10.1016/j.nut.2006.03.008

coronary heart disease [8,9]. Fruits are often considered a healthy food because they contain a variety of compounds with antioxidant capacity in plasma (AOP), such as vitamins C and E, carotenoids, flavonoids, and polyphenols, which may produce beneficial actions [10,11]. This protective effect against cardiovascular disease could be related to a decrease in susceptibility of LDL oxidation [12,13] or stimulation of other antioxidative processes [14].

In this context, the aim of this intervention trial was to estimate the ability of two hypocaloric diets with different fruit contents to provide potential AOP and cardiovascular protection in obese women by improving oxidative stress biomarkers related to lipid peroxidation in addition to the effect associated with weight loss.

Materials and methods

Subjects

Fifteen obese women with a mean age of 32 ± 6 y and a mean body mass index of 34.9 ± 2.9 kg/m^2 were recruited though local advertisements. All participants were in apparent good health as determined by medical history, physical examination, and routine biochemical and hematologic laboratory tests.

The enrolled women fulfilled the following inclusion criteria: (1) they were premenopausal; (2) they had no history of diabetes mellitus, high blood pressure, or dyslipidemia; (3) they did not smoke; (4) they had not been alcoholic; and (5) they had not used supplemental vitamin or minerals and regular prescription of medications in the previous 3 mo. The study protocol was approved by the ethical committee at the University Clinic of Navarra. A written informed consent to participate in the experimental intervention to lose weight was obtained before the start of the study.

Study design

The trial was a nutritional intervention controlled by trained dietitians from the Department of Physiology and Nutrition of the University of Navarra. The obese women were randomly assigned to one of two different 8-wk energy-restricted dietary treatments that differed by energy supply from fruits. Thus, one group was instructed to obtain more calories from fruits to receive 15% energy as fructose (n = 7), and the other group was oriented to select fruit to receive 5% energy as this monosaccharide (n = 8). The hypocaloric diets were prescribed according to a food exchange system, in which the menu plans are individually designed. Therefore, volunteers selected the fruits to be consumed according to the dietary instructions established for each intervention group.

The hypocaloric diets were designed to produce an energy restriction of −600 kcal/d with respect to the individual energy expenditure as measured by indirect calorimetry at baseline (Deltratac, Datex Ohmeda, Finland) using conventional protocols [15]. The macronutrient content in the two energy-restricted diets was designed to supply about 15% energy as proteins, 55% as carbohydrates, and 30% as lipids. Compliance to energy and nutrient intakes was assessed by 3-d weighted food records (2 weekdays and 1 weekend day) and calculating nutrient composition with the Medisystem nutritional database (Sanocare Human Systems L.S., Madrid, Spain). Before and after nutritional intervention, anthropometric measurements were taken and fasting blood was drawn.

Blood analysis

Blood samples were obtained during the follicular period, depending on the individual's menstrual cycle, to avoid bias from this potential confounding factor. EDTA plasma, and serum were separated from whole blood by centrifugation and stored at −80°C until assay.

Circulating glucose was assessed by an automatized colorimetric assay (COBAS MIRA, Roche, Switzerland) and insulin concentrations by using a radioimmunoassay method (DPC, Los Angeles, CA, USA). Plasma levels of triacylglycerol, total cholesterol, high-density lipoprotein (HDL) cholesterol, free fatty acids, phosphate, and urate were assayed by specific commercial assays (COBAS MIRA). The reported plasma LDL cholesterol data were calculated by the Friedewald equation as described elsewhere [16]. Conventional units for circulating urate levels (milligrams per deciliter) were converted into International System units (millimolars) through the 0.059 conversion factor (Uric Acid CP Kit, ABX Pentra, Montpellier, France).

Antioxidant status

Total antioxidant capacity in plasma (AOP) was evaluated by means of a colorimetric assay kit (AOP-450, OXIS International, Portland, OR, USA). In brief, serum samples were analyzed by using a six-point standard curve that was previously built. Samples and standards were diluted 1:40 in dilution buffer and 200 μL of plasma dilution was put in each well in duplicate. The microtiter was read the first time at 490 nm (Multiskan Spectrum, Thermo Electron Corporation, Vantaa, Finland), and 50 μL of solution with Cu^{2+} was added to each well and incubated for 3 min at room temperature. The microtiter was later read at 490 nm, and the difference between the two readings was compared with the standard curve, giving the measurement of reducing power, expressed as millimoles per liter.

Lipid peroxidation assay

Plasma malondialdehyde (MDA) was colorimetrically determined with a commercial kit that measured free and

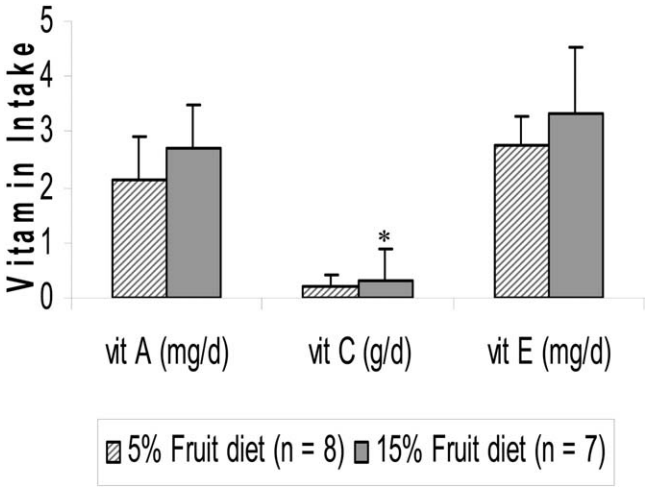

Fig. 1. Vitamin A, E, and C intakes in volunteers who consumed the low (5% content) and high (15% content) fruit diets. *Statistical differences between diets.

total MDA compounds (LPO, OXIS International). Each sample (200 μL of serum) was mixed with 650 μL of N-methyl-2-phenylindole in acetonitrile and 150 μL of 37% (12N) HCl. Tubes were capped, mixed, and incubated at 45°C for 60 min. Samples were centrifuged at 15 000*g* for 10 min and the supernatant was read on a spectrophotometer at 586 nm (Multiskan Spectrum, Thermo Electron Corporation). The assay included a six-point standard curve, the measurement was performed in replicate, and the mean value was computed.

The ability of LDL to form peroxides was assessed by measuring MDA corrected for LDL cholesterol ratio of MDA/LDL cholesterol [17]. The oxidative state was expressed as the MDA/AOP diet-related change ratio.

Statistical analysis

The sample size was calculated by the equation published by Mera et al. [18], taking into account published values of the standard deviation for MDA [19], and was applied for $P < 0.05$. This condition required seven subjects per group as the minimum number. The normal distribution was explored through the Kolmogorov-Smirnov and Shapiro-Wilk tests. Accordingly, the parametric Student's *t* test or nonparametric Wilcoxon's paired and Mann-Whitney U tests were applied to detect differences before and after weight loss and differences between hypocaloric diets. Spearman's correlation coefficient was used to evaluate the association between variables.

When appropriate, percentages of change were calculated as the difference between endpoint and baseline measurements for the 15% and 5% fruit hypocaloric diets. Sequential comparisons of oxidative biomarkers levels were performed with normalized data, with 100% for baseline and endpoint values and expressed as percentage of baseline [5,20].

Results are presented as mean ± standard deviation, and $P < 0.05$ was considered statistically significant. Statistical analysis was performed with SPSS 11.0 (SPSS Inc., Chicago, IL, USA) for Windows XP (Microsoft, Redmond, WA, USA).

Results

As designed, macronutrient distribution was similar in both diets, and no statistical differences were found concerning the energy provided by carbohydrates (P = 0.440), lipids (P = 0.288), and proteins (P = 0.536). With respect to cholesterol content, no statistical differences (P = 0.474) were observed between the low fruit diet (104.5 ± 127.3 mg/d) and the high fruit diet (136.7 ± 81.4 mg/d). As expected, fructose content in the high fruit diet was statistically higher than that in the low fruit diet (180 ± 68 versus 50 ± 19 kcal/d, $P < 0.001$, respectively). Likewise, the high fruit diet showed a higher fiber content (16 ± 4 versus 26 ± 7 g/d, $P < 0.001$) than did the low fruit diet.

Obese women who followed the high fruit diet showed a statistically higher vitamin C intake (P = 0.029) than did volunteers fed the low fruit diet (Fig. 1). However, no differences in vitamin A (P = 0.343) and E (P = 0.556) intakes were found between experimental diets (Fig. 1). Because no differences in the global slimming process were found between hypocaloric diets, data concerning anthropometric and metabolic variables are presented together (Table 1).

All volunteers lost body weight, as induced by the energy restriction, which was accompanied by marked decreases (*P*

Table 1
Effects of a calorie-restricted nutritional treatment on anthopometric and metabolic variables*

Biological parameters (n = 15)	Day 0	Day 56	P
Body weight (kg)	91.3 ± 10.0	85.1 ± 9.1	<0.001
Body mass index (kg/m^2)	34.9 ± 2.9	32.6 ± 0.75	<0.001
Fat mass (kg)	38.7 ± 5.9	33.9 ± 5.6	<0.001
Resting energy expenditure (MJ/d)	6.6 ± 0.8	6.0 ± 0.6	<0.001
Resting energy expenditure (kJ · kg^{-1} · d^{-1})	72.2 ± 5.3	70.8 ± 4.1	0.302
Insulin (μU/mL)	6.7 ± 6.4	5.7 ± 5.3	0.113
Glucose (mM)	5.1 ± 0.5	4.9 ± 0.5	0.076
Total cholesterol (mM)	4.2 ± 0.4	3.9 ± 0.5	0.044
Triacylglycerol (mM)	0.76 ± 0.2	0.7 ± 0.2	0.127
Free fatty acids (mM)	0.012 ± 0.005	0.010 ± 0.003	0.264
Total cholesterol/HDL-c index	4.06 ± 1.20	3.94 ± 0.94	0.512

HDL-c, high-density lipoprotein cholesterol

* Data concerning women who followed the high or low fruit-based diets are presented together because no statistical differences ($P > 0.05$) were found in these biological parameters between hypocaloric diets.

Table 2
Oxidative state and LDL cholesterol response to calorie-restricted intervention by hypocaloric diets with different fruit contents

Oxidative biomarkers	Low fruit diet (5% energy from fructose) ($n = 8$)			High fruit diet (15% energy from fructose) ($n = 7$)		
	Day 0	Day 56	Normalized endpoint value versus baseline (%)	Day 0	Day 56	Normalized endpoint value versus baseline (%)
MDA (μM)	1.86 ± 0.23	1.70 ± 0.74	94 ± 45	1.89 ± 0.52	1.48 ± 0.75	79 ± 38
AOP (mM)	1.50 ± 0.34	1.40 ± 0.62	92 ± 38	1.57 ± 0.25	1.73 ± 0.46	110 ± 21
Urate (mM)	0.25 ± 0.05	0.23 ± 0.06	92 ± 15	0.26 ± 0.057	0.26 ± 0.060	102 ± 15
LDL cholesterol (mM)	2.55 ± 0.30	2.52 ± 0.54	99 ± 16	3.04 ± 0.54	2.70 ± 0.39*	90 ± 14
HDL cholesterol (mM)	1.15 ± 0.26	1.08 ± 0.17	96 ± 15	1.03 ± 0.18	0.99 ± 0.17	96 ± 11
MDA/LDL cholesterol ratio (μM/mM)	0.75 ± 0.11	0.69 ± 0.26	93 ± 35	0.65 ± 0.18	0.57 ± 0.31	86 ± 37
MDA/AOP ratio (μM/mM)	1.31 ± 0.43	1.86 ± 1.79	147 ± 160	1.22 ± 0.32	0.87 ± 0.49†	70 ± 30*
MDA/urate ratio (μM/mM)	7.38 ± 1.47	7.81 ± 3.40	98 ± 55	7.79 ± 1.71	6.18 ± 3.45	89 ± 4

AOP, antioxidant capacity of plasma; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDA, malondialdehyde
* $P < 0.05$, intervention-related change.
† $P < 0.09$, intervention-related change.

< 0.001) in body mass index and fat mass (Table 1). Further, no differences were observed between diets concerning weight loss (low fruit diet −6.9 ± 2% versus high fruit diet −6.6 ± 2%, $P = 0.779$) and body fat reduction (low fruit diet −11.7 ± 4.8% versus high fruit diet −13.3 ± 6.4%; p = 0.755). As expected, resting energy expenditure was significantly decreased ($P < 0.001$) after the nutritional intervention. However, when resting energy expenditure was corrected for body weight, statistically significant differences were not found (Table 1).

Circulating levels of insulin, glucose, triacylglycerol, free fatty acids, and cholesterol/HDL cholesterol ratio did not change under either dietary treatment. However, fasting total cholesterol significantly ($P = 0.044$) decreased at the endpoint (4.2 ± 0.4 versus 3.9 ± 0.5 mM) in relation to baseline. This decrease was statistically significant in volunteers who received the high fruit diet ($P = 0.020$).

Weight reduction induced by both nutritional interventions was associated with a decrease in MDA circulating levels, which did not reach statistical significance (Table 2). In this context, MDA diet-related change (percentage) showed a marginal positive association with body weight loss ($r = 0.500$, $P = 0.082$).

When comparing the ability of LDL to form peroxides (MDA/LDL cholesterol index), no differences were observed between diets. However, LDL cholesterol levels significantly decreased ($P = 0.048$) in obese women who followed the high fruit diet (Table 2). Remarkably, total cholesterol ($r = 0.665$, $P = 0.013$) and LDL cholesterol (Fig. 2) diet-related changes were highly correlated with MDA diet-related change.

Concerning AOP, obese women following the high fruit diet tended to have increased levels, although no statistical significance was achieved, and this trend was not observed for the low fruit diet group (Table 2). Likewise, plasma urate levels (Table 2) were similar in obese women who followed the high fruit diet and those who followed the low fruit diet ($P = 0.152$), as occurred with phosphate values ($P = 0.694$). Interestingly, obese women who followed the high fruit diet showed a decrease ($P = 0.046$) in the oxidative stress state, as expressed by the MDA/AOP diet-related change ratio (Table 2). This statistical observation was not evident in the obese women who followed the low fruit diet (Table 2).

Further, change in AOP was directly proportional to fiber plus fructose intake in relation to consumption of fruit ($r = 0.697$, $P = 0.025$). Also, AOP change was highly correlated with plasma urate (Fig. 3).

Discussion

Obesity and associated morbidities such as cardiovascular diseases have been related to low-grade inflammation, which could benefit from weight reduction and antioxidative control [1]. Lipid peroxidation appears to be involved in oxidative modifications of LDL that yield

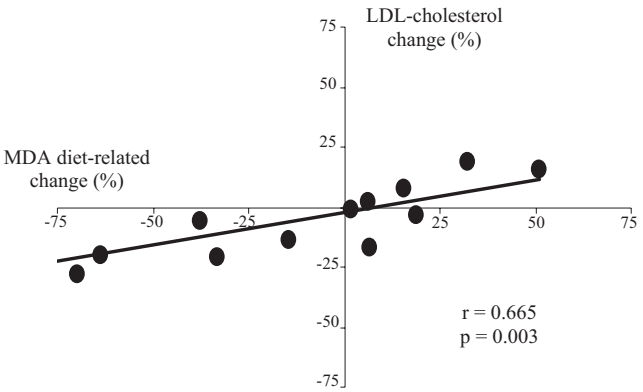

Fig. 2. Relations between MDA diet-related change and changes in LDL cholesterol fraction under the experimental conditions. LDL, low-density lipoprotein; MDA, malondialdehyde.

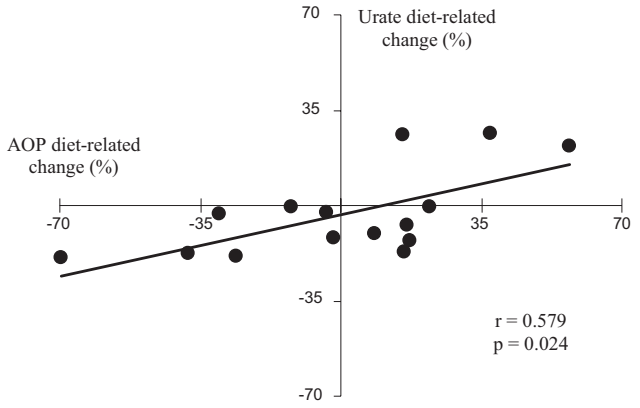

Fig. 3. Relations between AOP and circulating urate diet-related change under the experimental conditions. AOP, antioxidant capacity of plasma.

the formation of atherosclerotic injury [21,22]. Malondialdehyde (MDA) has been proposed as an indicator of lipid peroxidation because this molecule is one of the end products of this oxidative process [23]. Previous studies have shown that circulating MDA levels are higher in obese subjects than in non-obese healthy controls [24]. Moreover, a decrease in this lipid peroxidation marker has been related to weight loss [25].

On the other hand, daily intake of fruit that provides fructose, fiber, and different bioactive compounds (antioxidant vitamins, polyphenols, carotenoids, etc.) could produce specific effects on weight management and oxidant status [26]. Thus, several investigators have reported that daily fructose intake may contribute to increased energy intake and weight gain [27,28], whereas other researchers have proposed that intake of fruit, which contains a large amount of fructose, may contribute to weight loss [29]. In this study, which was designed to evaluate whether a high fruit diet could specifically contribute to the MDA diet-related decrease as an indicator of oxidative changes and changes in AOP as a marker of antioxidant status, a similar weight reduction was induced with two energy-restricted diets with different fruit contents.

In agreement with other investigators who showed a relation between MDA and LDL cholesterol and total cholesterol [25,30], we found a statistical association between MDA diet-related change and cholesterol and LDL cholesterol diet-related change.

LDL oxidation is associated with atherogenesis [22] and the ability of LDL cholesterol to form peroxides (MDA/LDL cholesterol) was reported to be responsible specifically for atherogenesis [17,31]. In this context, we found a greater decrease in LDL cholesterol levels in obese women who followed the high fruit diet, although no differences were observed in the MDA/LDL cholesterol index. The high fruit diet was richer in fiber and this food component is known to influence fat metabolism. Gel-forming fibers are particularly effective in decreasing high LDL cholesterol without changing HDL cholesterol mediators [32]. Several mechanisms by which fiber lowers blood cholesterol have been reported. Thus, evidence suggests that soluble fibers bind bile acids or cholesterol during intraluminal formation of micelles. The resulting decrease in cholesterol content of liver cells leads to upregulation of LDL receptors and thus increased clearance of LDL cholesterol [33]. Other suggested mechanisms include inhibition of hepatic fatty acid synthesis byproducts of fermentation such as acetate, butyrate, and propionate [33]. Therefore, these results could indicate that atherosclerotic risk could be decreased through a nutritional intervention by means of fiber content in fruit-enriched hypocaloric diets [8,34].

In addition, it has been reported that the antioxidant mixture provided from fruits, which includes vitamin C and other antioxidant compounds, is more helpful than single vitamins in preventing oxidative damage in vivo [35–37]. Moreover, consumption of a dietary supplement representing the antioxidant content from five to seven servings of fruit has been shown to increase plasma antioxidant capacity similar to levels obtained through inclusion of fruits in the diet [38]. The present results demonstrated that the oxidative stress status of obese women, measured as a MDA/AOP ratio, improved more after a nutritional intervention with a high fruit diet compared with a low fruit diet.

Reinforcing this concept, the significant correlation between AOP and dietary fiber plus fructose substantiated the beneficial effect of fruit intake on antioxidant capacity. In agreement with this outcome, an increase in vitamin C intake was observed in obese volunteers fed the high fruit diet. Thus, vitamin C intake could also contribute to the increased plasma antioxidant status observed in this study [39]. Fruits contain a variety of compounds with antioxidant capacity, such as vitamins C and E, carotenoids, and polyphenols, that could be involved in these findings [40]. Further, feeding animals on a diet with a high content in fructose has been reported to lead to a significant increase in total antioxidant capacity, which may prevent lipid peroxidation [41]. In addition to this, a previous study suggests that urate is responsible for the increase in AOP upon consuming an apple [42]. Fruits increase plasma urate due to rapid metabolism of fructose by fructokinase [43, 44], so it is conceivable that some reported antioxidant health effects of flavonoids from fruits are confounded by the metabolic effect of fructose on urate [42]. In agreement with this hypothesis, we found that obese women who followed the high fruit diet increased plasma urate without significant changes in circulating phosphate, thus demonstrating the safety of the intervention [45]. Therefore, urate changes could contribute to improve the antioxidant state in women fed a high fruit diet [46].

Summary

The outcome of this nutritional trial shows that a fruit-enriched hypocaloric diet appears to be effective in decreasing oxidative stress. The supply of fiber and antioxidant substances naturally occurring in fruits could be a useful strategy in the design of hypocaloric diets that, with weight reduction, could produce an improvement of cardiovascular risk factors related to obesity.

Acknowledgments

The authors thank Salomé Pérez, nurse, María Hernandez, dietitian, and Ana Lorente and Verónica Ciaurriz, technicians, for excellent technical assistance.

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