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Changes in Antioxidant Enzymes in Metabolic Syndrome Patients after Consumption a Citrus-Based Juice Enriched with *Aronia Melanocarpa*

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Abstract

Increased oxidative stress has been suggested as an early event in the development of the metabolic syndrome and, as such, might contribute to disease progression. The aim of de present study was to evaluate Total Antioxidant Status and endogenous antioxidant enzymes in metabolic syndrome patients after consumption of a citrus-based juice compared with control groups. Before, and at sixth month after treatment the following parameters were determined: Total Antioxidant Status, Superoxide Dismutase, Glutathione Peroxidase and Glutathione Reductases. After six months of citrus-based juice consuming, there was no significant differences at 95% confidence in Total Antioxidant Status between three groups, although it was found significant differences between groups in values of three antioxidant enzymes analysed. As a conclusion, the intake of a citrus-based juice increases the levels of antioxidant enzymes, despite do not increase the Total Antioxidant Status after its intake during 6 months.

Keywords: Oxidative stress; Metabolic syndrome; Glutathione peroxidase; Glutathione reductases; Superoxide dismutase

Introduction

Metabolic syndrome (MetS) is a pool of insulin resistance, hyperinsulinemia, impaired glucose tolerance, dyslipidemia, obesity, and elevated blood pressure. Mets has reached epidemic extents in industrialized countries, exceeding a prevalence above 40% in >40 years old subjects [1]. These risk factors of metabolic origin appear to directly promote the development of atherosclerotic cardiovascular disease, showing prothrombotic and proinflammatory status [2].

Increased oxidative stress has been suggested as an early event in the development of the metabolic syndrome, contributing to disease progression [3]. Not only the increase of pro-oxidant species, but also the drop on antioxidant defenses as endogenous antioxidant enzymes or natural antioxidants, could lead to the development of oxidative stress-related diseases. Under normal physiological conditions, pro-oxidant species can be eliminated or inactivated *in vivo* by different endogenous antioxidant systems. Both intracellular and extracellular antioxidants play important roles in neutralizing these toxic oxygen molecules. The endogenous antioxidant system is formed by some antioxidant enzymes as superoxide dismutase (SOD), catalase, glutathione peroxidase and the tri-peptide (gamma- L-glutamyl-L-cysteintyl-glycine) glutathione or (GSH), and free circulating thiols containing the sulfhydryl group (-SH) [4]. Unless not well established, MetS patients have shown higher predominance of pro-oxidant species and the subsequent increment of oxidative status in the organism [2].

More than 90% of SOD is isolated in the extravascular space bound to heparan sulphate proteoglycans in the glycocalyx of endothelial cell surface and in connective tissue matrix especially in arterial wall [5]. A large amount of GPx is synthesized and secreted from kidney and lungs, maintaining the bioavailability of vascular nitric oxide and scavenges H_2O_2 and peroxidized organic molecules in plasma to reduce systemic oxidative stress [6].

Biological antioxidants can scavenge free radicals, preventing oxidative damage on the cardiovascular system [7]. At physiological concentrations, vitamin C is a potent free radical scavenger in the plasma, protecting cells against oxidative damage caused by ROS [8]. For instance, physiological concentrations of ascorbic acid (50-100 mmol/L) *in vitro* attenuate oxidative modification of LDL induced by transition metals, homocysteine [9], and myeloperoxidase-derived [10]. Procyanidins, anthocyanins and phenolic acids are phenolic compounds claimed to be beneficial in disorders associated with oxidative stress. These compounds are present in *Aronia melanocarpa*, constituting one of the most powerful natural antioxidants food matrices [11].

Owing to the potential dangerous effects of oxidative stress, and the beneficial effects of natural antioxidant against these oxidative molecules, we examined the effect of a citrus-based juice (CJ) on antioxidant status and antioxidant enzymes in patients with metabolic syndrome (MetS) compared with healthy people.

Subjects and Methods

Citrus-based juice

Beverage tested (CJ) is based on a mixture of citrus juice (95%) with berries (*Aronia melanocarpa* concentrate, 5%). The composition and characterization of that drink juice used in the clinical trial has been previously published [12]. The nutritional composition of the beverage was: Energy: 38 Kcal/100 mL; Protein: 0.45 g/100 mL; Carbohydrates: 9 g/100 mL; Fat: 0.03 g/100 mL.

Study design

The study included 53 subjects (24 men and 29 women) 20 healthy subjects and 33 patients who fulfilled the Adult Treatment Panel III

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criteria for the metabolic syndrome [13]. The inclusion criteria were: age 50±10 years old; no vitamin supplements consumption; alcohol consumption ≤ 30 g/day; no renal, hepatic or gastrointestinal diseases, cancer or allergies; no abnormal dietary habits; no Body Mass Index (BMI) higher to 30 kg/m^2; non-smokers. To assess the effects of a citrus-based juice on the markers of oxidative stress in MetS patients, 18 patients daily consumed 300 mL of CJ during 6 month and 15 patients consume 300 mL of a placebo beverage. The control group (20 patients without MetS) consume CJ. The population sample was selected from Primary Care Center Dr. Quesada of Murcia and the analysis were initially performance before consuming the drink, at 4 months (intermediate time) and at 6 months of consuming.

Anthropometric measurements

After recording the clinical history and conducting the physical examination, we obtained the following anthropometric measurements: weight, height, BMI and waist circumference. Weight was measured while the subject was wearing underwear and was in a fasted state (after evacuation). A scale was calibrated before each measurement, was used. Height was obtained with a cursor stadiometer graduated in millimetres. The subject was barefoot with the back and head in contact with the stadiometer in the Frankfurt horizontal plane. BMI was calculated by dividing weight (kg) by height squared (m^2). Waist circumference (cm) was measured to the nearest 0.5 cm with a tape measure at the umbilical scar level. The study was carried out in accordance with the Helsinki Declaration, and the Ethical Committee of the San Antonio Catholic University approved the protocol (6 November 2006, register number: 1424). Participation was voluntary, and each patient gave written informed consent.

Parameters evaluated

Plasma total antioxidant status ORAC-$_{FL}$: The oxygen radical absorbance capacity was determined as described by Dávalos [14] with slight modifications. The antioxidant capacity was expressed as mM Trolox/L.

Red blood cell superoxide dismutase: The method uses xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. Superoxide dismutase (SOD) activity was measured by the degree of inhibition of the reaction. Kinetics was measured at 505 nm. Analysis was carried out with the kit supplied by Randox Laboratories.

Glutathione peroxidase and glutathione reductase: In presence of glutathione reductase (GSR) and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidation of glutathione (GS) by cumene hydroperoxide is catalyzed by glutathione peroxidase (GPx). Oxidized GS is immediately converted into the reduced form with a subsequent oxidation of NADPH to NADP$^+$. The decrease in absorbance was measured at 340 nm, in order to measure the antioxidant capacity of the glutathione system. Analysis was developed with the kit supplied by Randox Laboratories.

Precision

Assays precision was tested by repeatability and reproducibility studies. The inter-assay coefficient of variation (IACV) of assay (TAS, SOD, GPx and GR) were calculated giving values of 4%, 2%, 3% and 2% respectively

Statistical analysis

All data were analyzed by using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL). Descriptive statistic is presented as mean ± standard deviation. Means were compared by the variance test of repeated means. Analysis of variance (ANOVA) was used to examine significant differences in the TAS (intra- and interassay coefficients of variation 6.1% and 7%, respectively), SOD (intra- and interassay coefficients of variation both <3%), Gpx (intra- and interassay coefficients of variation 4.2% and 4.8%, respectively) and GR (intra- and interassay coefficients of variation 4.1% and 5%, respectively) levels of the two groups of study in basal conditions and at the end of the study. A probability of less than 0.05 ($p<0.05$) was considered statistically significant.

Results

Both MetS and control groups showed Total Antioxidant Status (TAS) baseline levels between the limits of normality for healthy populations (1.16-1.94 mMol/L) with no significant differences ($p>0.05$) between groups (Figure 1). The intake of CJ or placebo did not cause a significant increase ($p>0.05$) on the TAS, neither during 4 or 6 months of treatment. In addition, the control group evidenced a higher increase on the TAS after 4 months of treatment. However, the increase of the treatment until the sixth month slightly reduced the TAS compared with the fourth month –fact that could be explained by a phenomenon of accommodation, regarding to the intake of the drink. Consumption of CJ or placebo did not produce significant differences in the values of the antioxidant activity in MetS. However, a slight increase was observed with respect to initial values (Figure 1).

Values of SOD at baseline were significantly ($p<0.05$) higher in

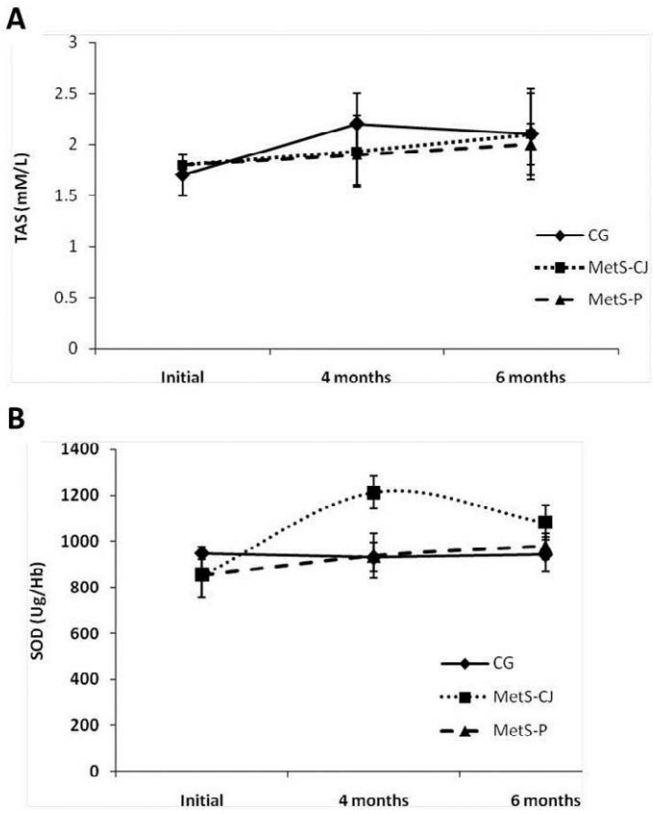

Figure 1: TAS, Total Antioxidant Status; SOD, Superoxide Dismutase CG, Control Group; MetS-CJ, patients with Metabolic Syndrome who intake the citrus-based juice; MetS-P, patients with Metabolic Syndrome who intake the Placebo drink.

control group than in MetS group (947.8 ± 27.2 Ug/Hb and 853.8 ± 25.4 Ug/Hb respectively). In both analyzed groups (MetS and CG) the values were top in women that in men, nevertheless we did not observe significant differences. After four months of CJ intake, it was observed an increase of SOD values in MetS group (1212.15 ± 97.7 Ug/Hb) with significant differences ($p<0.05$) compared to baseline (853.80 ± 25.4 Ug/Hb). After 6 months of CJ consuming, these values decreased (1080.82 ± 75.7 Ug/Hb) with respect to those obtained at fourth month; however, it remains significantly higher than at baseline (Figure 1). Finally, the difference of the intake of CJ and placebo was not significant regarding the control group ($p>0.05$). However, significant differences between the intakes of both beverages were found in MetS patients at fourth month, being homogenised after six months of intake (Figure 1).

Regarding to GPx, control group exhibited higher GPx values than MetS group, although no significant differences ($p>0.05$) were found. In the same way, the values obtained of GR were statistically higher in control group than in MetS group (Table 1). In both MetS and control groups the mean values of GR were higher in women (62.3 ± 2.4 U/L in control group and 52.2 ± 1.6 U/L in patients with MetS) than in men (56.3 ± 2.5 U/L in control group and 47.8 ± 2.5 U/L U/L in patients with MetS). The intake of CJ produced an increase in GPx values at fourth and sixth months, both in control and MetS patients compared to baseline ($p<0.05$). However, such an increase was not observed in MetS group when ingested placebo drink (Table 1). Finally, GR values in MetS group were found to be influenced by CJ intake after 4 and 6 months of treatment, reaching those values obtained at baseline. The intake of CJ by control group during four months did not present differences compared to baseline, reaching statistically significant differences after six months of treatment. The intake of placebo beverage did not lead to changes in GR values in any MetS or control patients (Table 1).

Discussion

Increased oxidative stress has been suggested as an early event in the development of the Metabolic Syndrome, subsidizing to disease progression [3]. Esposito [15] found that patients with MetS had higher levels of oxidative stress associated with an increase in insulin resistance and endothelial dysfunction. These alterations may interact and amplify the set of metabolic and vascular disorders as diabetes, obesity, hypertension, hyperlipidaemia and the subsequent endothelial dysfunction. Endothelial dysfunction could be produced due to the reduced availability of nitric oxide (NO) required for vascular relaxation. Guilder [16] reported higher quantity of oxidative stress systemic markers in obese adults with MetS compared with obese adults without MetS. These findings are consistent with previous studies demonstrating that obesity and MetS are independently associated with increased oxidative stress and inflammatory disease [17]. Demircan [18] found lower TAS values for MetS patients. However, many authors found similar TAS plasmatic values in MetS patients compared to healthy volunteers, besides significantly lower TAS levels in men compared to women [19-21].

		Glutathione-Peroxidase (U/L)	Glutathione Reductase (U/L)
Baseline	**Control**	7913,6 ± 265	59,3 ± 2,3*
	MetS	7257,5 ± 432	50 ± 1,9
4 Month	**Control-CJ**	10964 ± 495*	58,8 ± 1,2
	MetS-P	7203,5 ± 285*	54,2 ± 2
	MetS- CJ	9961,7 ± 711*	56,8 2,3*
6 Month	**Control-CJ**	9934 ± 492*	60,3 ± 1,4
	MetS-P	7932 ± 536*	54,5 ± 1,9
	MetS-CJ	9453,1 ± 566*	57,7 ± 1,5*

Control, Control Group; MetS-CJ, patients with Metabolic Syndrome who intake the citrus-based juice; MetS-P, patients with Metabolic Syndrome who intake the Placebo drink. (*) represents statistical differences.

Table 1: Glutathione peroxidase and Glutathione reductases values over six months of intake of citrus-based juice or placebo drink.

Many studies reported similar results than those obtained in the present study. Common to MetS patients as insulin resistance, hypertension or obesity produce a lower resistance to oxidative stress, due to decreased antioxidant defenses [22,23]. However, other authors disagree with those results, reporting similar SOD values in MetS patients and in healthy subjects [19,24].

Some authors noticed similar results regarding GPx values in MetS patients, reporting lower values of GPx in MetS patients, with respect to a control group [19,25-27]. Our results are in agreement with other authors who found an increase in SOD values with supplements of vitamin C [28]. Bronzel [27] reported a significant increase in GPx and SOD with supplements of *Aronia melanocarpa* in patients with MetS. However, that relationship has also been inversely described by others authors, who found higher values of GPx in patients with MetS with respect to healthy population [24,26].

A broad range of polyphenolic compounds from *Aronia melanocarpa* including phenolic acids, flavonoids, anthocyanins, proanthocyanidins and stilbenes have all demonstrated potent antioxidant capacities *in vivo* or *in vitro* [29]. Pilaczynska-Szczesniak [30] described an increase in antioxidant capacity after administration of black chokeberry-red pigment on *in vitro* and *in vivo* systems; nevertheless after consumption of CJ we observed a slight increase respect initial values, although not significant differences were observed.

Conclusions

Our study demonstrated that intake of citrus-based juice increases the levels of antioxidant enzymes; however, were not found significant increases in total antioxidant status after 6 months of intake. Additional studies are needed to establish the mechanisms of protection of the bioactive compounds in the novel beverage against the range of condition of MetS.

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