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Orange beverage ameliorates high-fat-diet-induced metabolic disorder in mice

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ABSTRACT

Metabolic syndrome (MetS) refers to a group of disorders that includes insulin resistance, central obesity, arterial hypertension and hyperlipidaemia. Regular consumption of bioactive compounds has consistently been associated with a reduced risk of these disorders. The aim of this study was to determine if an orange beverage with high concentrations of bioactive compounds (flavanones, carotenoids, melatonin, and ascorbic acid) and low alcohol content (<1%, v/v) improves metabolic parameters through modulation of oxidative stress, lipid profile and inflammatory response in a rodent model of high fat diet (HFD)-induced obesity. Mice with HFD-induced MetS were fed the orange beverage for 12 weeks (volume equivalent to 250 mL/day in human). Long-term intake of the orange beverage decreased plasma TAG, oxidized LDL and C-reactive protein levels. The present data provide evidence of a beneficial effect of orange beverage intake on some outcome parameters related to HFD-induced MetS.

1. Introduction

Metabolic syndrome (MetS) is characterized by a group of metabolic abnormalities, including obesity, dyslipidaemia, hyperglycaemia and hypertension (Huang, 2009). These conditions refer to the clusters of risk factors that increase the prevalence of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Shin et al., 2013). Chronic inflammation and oxidative stress are recognized as major factors involved in the pathogenesis of MetS (Hotamisligil, 2006; Hutcheson & Rocic, 2012). Lifestyle also plays a pivotal role in the development

Chemical compounds: Melatonin (PubChem CID: 896); Ascorbic acid (PubChem CID: 54670067); Ethanol (PubChem CID: 702); Hesperidin (PubChem CID: 3594); Naringenin-7-O-rutinoside (PubChem CID: 25244529); Beta-cryptoxanthin (PubChem CID: 182237).

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Abbreviations: AI, atherogenic index; AS, aqueous alcohol solution; AUC, area under the curve; CAT, catalase; CTL, control; CRP, C-reactive protein; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; HFD, high fat diet; IPGTT, intraperitoneal glucose tolerance test; MDA, malondialdehyde; OB, orange beverage; OJ, orange juice; oxLDL, oxidized low-density lipoprotein; PAC, plasma antioxidant capacity; SOD, superoxide dismutase; TC, total cholesterol

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of several features commonly recognized as MetS (Brown et al., 2009). In fact, chronic consumption of a HFD is strongly associated with the development of obesity in humans and rodents (Buettner, Scholmerich, & Bollheimer, 2007), promotes hepatic oxidative stress (Milagro, Campion, & Martinez, 2006) and triggers some inflammatory processes (Innis & Jacobson, 2007). Increases in MetS incidence has resulted in an increased need for therapeutic and preventative strategies. Experimental and epidemiological data indicate that consumption of energy diluted foods with naturally occurring phytochemicals, in particular fruits and vegetables, prevents obesity and is associated with a lower risk of coronary artery disease (Liu, 2013). Orange juice is known to be a rich source of bioactive compounds such as flavonoids, carotenoids and melatonin (Fernández-Pachón et al., 2014; Stinco et al., 2012; Tounsi et al., 2011). Accumulating evidence suggests that orange juice consumption could beneficially modulate some of the pathophysiological features associated with MetS, such as obesity (Titta et al., 2010), dyslipidaemia (Aptekmann & Cesar, 2013), hyperglycaemia (Ghanim et al., 2007) and oxidative and inflammatory stress (Coelho, Hermsdorff, & Bressan, 2013; Sánchez-Moreno et al., 2003). Recently, alcoholic fermentation processes have been carried out in fruit juices (Mena et al., 2014; Pérez-Gregorio, Regueiro, Alonso-González, Pastrana-Castro, & Simal-Gándara, 2011), resulting in products with higher concentrations of bioactive compounds than the respective substrates. Moreover, the fermentation process involves a final product with alcohol. Our group has previously described the profile of bioactive compounds in alcoholic fermented orange juice (orange beverage), showing increases in flavanone, carotenoid and melatonin contents in relation to the original juice (Cerrillo, Escudero-López, Hornero-Méndez, Martín, & Fernández-Pachón, 2014; Escudero-López et al., 2013; Fernández-Pachón et al., 2014). To assess the potential functional benefits of the orange beverage, a 12-week intervention study was carried out in healthy mice. The results showed that orange beverage intake could exert greater protection against cardiovascular risk factors than the original orange juice (Escudero-López et al., 2015). Based on these results and the evidence from the literature, the aim of the present study was to investigate whether regular consumption of orange beverage reverses the metabolic parameters by modulating the inflammatory response, lipid profile and oxidative stress in a rodent model of HFD-induced obesity (a physiological model of MetS).

2. Materials and methods

2.1. Chemicals and reagents

Chemicals were purchased from Sigma-Aldrich Quimica (Alcobendas, Spain).

2.2. Characteristics of the orange beverage

The company Grupo Hespérides Biotech S.L. carried out the controlled alcoholic fermentation and subsequent pasteurization of commercial orange juice made from *Citrus sinensis* L. var. *Navel late* (Huelva, Spain). The beverage obtained was rich in bioactive compounds with high antioxidant activity. The quality parameters, bioactive compound contents and antioxidant activities of the commercial orange juice and the final orange beverage were previously evaluated in Escudero-López et al. (2013) and Fernández-Pachón et al. (2014) (Table 1). Briefly, the orange beverage has: (i) 806 ± 5 mg/L flavanones, among which the most abundant are naringenin-7-*O*-rutinoside (412 ± 0. 8 mg/L) and hesperidin (hesperetin 7-*O*-rutinoside) (310 ± 1.7 mg/L); (ii) 6.4 ± 0.26 mg/L carotenoids, among which the most abundant is cryptoxanthin (0.85 ± 0.04 mg/L); (iii) 394 ± 5.4 mg/L ascorbic acid; and (iv) 16.8 ± 1.5 ng/mL melatonin (Table 1). In addition, the orange beverage has moderate alcohol content (0.85 ± 0.01%, v/v).

Table 1 – Quality parameters, bioactive compounds content and antioxidant activity of orange juice (OJ) and orange beverage (OB).

Composition	OJ	OB
pH	3.48 ± 0.2	3.43 ± 0.2
Total carbohydrates (g/L)	78.2 ± 5.64	53.7 ± 4.65
Alcohol (% v/v)	0	0.85 ± 0.01
Total flavanones (mg/L)	698.9 ± 20.5	806.2 ± 5.1
Naringenin-7-O-glucoside (mg/L)	0.6 ± 0.0	0.7 ± 0.0
Naringenin-7-O-rutinoside (mg/L)	363.7 ± 8.4	412 ± 0.8
Hesperetin-7-O-rutinoside (mg/L)	274.9 ± 10.2	310.5 ± 1.7
Hesperetin-7-O-glucoside (mg/L)	11.5 ± 0.7	22.0 ± 3.0
Isosakuranetin-7-O-rutinoside (mg/L)	47.9 ± 1.3	60.7 ± 3.1
Total carotenoids	5.37 ± 0.21	6.41 ± 0.23
Neochrome (mg/L)	0.37 ± 0.01	0.44 ± 0.01
Auroxanthin (mg/L)	0.65 ± 0.03	0.78 ± 0.04
Mutatoxanthin (mg/L)	0.32 ± 0.01	0.38 ± 0.02
All-trans-zeaxanthin (mg/L)	0.40 ± 0.02	0.47 ± 0.02
All-trans-lutein (mg/L)	0.30 ± 0.01	0.35 ± 0.02
β-Crytoxanthin (mg/L)	0.71 ± 0.03	0.85 ± 0.04
Carotene (mg/L)	0.37 ± 0.01	0.46 ± 0.02
Provitamin A (RAEs/L)	75.3 ± 3.58	90.7 ± 3.97
Ascorbic acid (mg/L)	409 ± 1.8	394 ± 5.4
Melatonin (ng/mL)	3.15 ± 0.03	16.88 ± 1.42
ORAC (μmol/L)	6044 ± 247	8169 ± 652
FRAP (μmol/L)	10.3 ± 0.4	9.9 ± 0.1
TEAC (μmol/L)	5.4 ± 0.1	5.4 ± 0.0
DPPH (% inhibition)	58.1 ± 26	77.4 ± 1

Values are expressed as the mean ± SD. RAEs, retinol activity equivalents; ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing antioxidant power assay; TEAC, Trolox equivalent antioxidant capacity; DPPH radical scavenging assay. Modified from Escudero-López et al. (2013) and Fernández-Pachón et al. (2014) with permission from the authors.

2.3. Experimental design and HFD protocol

Forty-five male OF1 mice (eight weeks old at the beginning of the experiment, Charles River Laboratories, Barcelona, Spain) were housed individually in cages in a controlled environment (12 h daylight cycle, 22 °C) with free access to food and water. After one week of acclimatization, the mice were randomly divided into two groups: control (CTL) group (n = 10), fed with a control diet (standard diet; 4% of total calorie intake from fat; Scientific Animal Food and Engineering, Spain), and HFD group (n = 35), fed with a HFD (45% of total calorie intake from fat), for 12 weeks, respectively (Fig. 1).