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Annatto carotenoids attenuate oxidative stress and inflammatory response after high-calorie meal in healthy subjects

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ABSTRACT

The aim of this study was to evaluate the effect of annatto carotenoids intake associated to a single high-calorie meal (high fat and high carbohydrate) in postprandial biochemical, inflammatory and oxidative stress markers. Twelve healthy subjects (6 men, 6 women) were included in this randomised, controlled crossover study. Baseline blood samples were collected from fasting subjects that immediately received high-calorie meal without carotenoid (placebo) or containing 1.2 mg/kg bixin (BIX) or 0.06 mg/kg norbixin (NBIX). Blood samples were taken 60, 120 and 240 min after meal intake. NBIX intake did not affect biochemical blood markers but reduced the postprandial levels of inflammatory cytokines (IL-1, IL-6 and TNF-α) and lipid oxidation 60–120 min after meal. BIX only partially prevented postprandial-induced lipid oxidation. Results indicate that the intake of NBIX may be an alternative to reduce the postprandial inflammatory and oxidative stress responses to high-calorie meals.

1. Introduction

During the last decades there was an increase in sedentary lifestyle and changes in the pattern of food consumption, including gradual increase in fat and carbohydrate consumption due to the fast food-based meals (Shareck, Frohlich, & Kestens, 2014). These factors have increased the prevalence of overweight and obesity in developed and developing countries (Flegal, Kit, & Orpana, 2014). Moreover, humans spend the majority of their time in the postprandial state, which is a pro-inflammatory and pro-oxidative state that may induce insulin resistance in skeletal muscle, also contribute to beta-cell dysfunction and to the development of type 2 diabetes (Chan, Pang, Romic, & Watts, 2013). Inflammation and oxidative stress are also hallmarks of atherosclerosis and cardiovascular disease (Ekström, Björck, & Ostman, 2013; Wang, Wang, Rabinovitch, & Tabas, 2014). Diabetes and atherosclerosis are the leading causes of death for 30–50 years-old people (WHO, 2013).

The postprandial state is a dynamic period of metabolic traffic of macromolecules absorbed from substrates such as carbohydrates, lipids, proteins and other dietary components (Burton-Freeman, 2010). Regular ingestion of high-calorie meals rich in processed carbohydrates and saturated fat can lead to transient exaggerated postprandial spikes of glucose and lipids, which promote oxidative stress. The oxidative stress triggers biochemical inflammatory cascades and endothelial dysfunction (Klop, Proctor, Mamo, Botham, & Castro Cabezas, 2012). When repeated multiple times each day, these postprandial excursions create a milieu that favours the development of atherosclerosis and cardiovascular disease (Ceriello et al., 2008)

Few studies have shown the mechanisms by which high-calorie food intake exerts harmful health effects (Herieka & Erridge, 2014; Magrone et al., 2013). After a meal, serum glucose, triacylglycerols, and neutrophil count increase and lead to the release of pro-inflammatory cytokines and oxidative stress (Van Oostrom, Sijmonsma, Rabelink, Van Asbeck, & Cabezas, 2003). In addition, triacylglycerols and glucose are

Abbreviations: ALT, alanine amino transferase; AST, aspartate amino transferase; BIX, bixin; CAT, catalase; GPx, glutathione peroxidase; HDL, high density lipoprotein; IL-1, interleukin-1; IL-6, interleukin-6; NBIX, norbixin; NFkB, nuclear factor kappa B; PPAR, peroxisome proliferator activated receptor; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive species; TC, total cholesterol; TG, triacylglycerols; TNF-α, tumoral necrosis factor α; TOS, total oxidant status; VLDL, very low density lipoprotein

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able to induce the activation of leucocytes as demonstrated in vitro (Bentley et al., 2011; Brownlee, 2013) Triacylglycerol-rich lipoproteins induce the expression of leucocyte adhesion molecules in the endothelium, which facilitates the recruitment of inflammatory cells and remnant lipoproteins (Wheeler-Jones, 2007). These triacylglycerol-rich lipoproteins also increase inflammatory activation of endothelial cells by increasing the expression of cyclooxygenase-2 and activating intracellular signalling pathways controlled by nuclear factor kappa B and mitogen-activated protein kinases (Wheeler-Jones, 2007).

Some intervention and epidemiological studies have shown that postprandial oxidative stress and inflammation can be modified by dietary intervention or antioxidant supplementation (Kamiyama, Ishimoto, Ani, & Ndoh, 2009) with nutraceuticals or functional foods (Sirtori, Galli, Anderson, & Arnoldi, 2009), which also improved endothelial function (Zhu et al., 2015). Bixin (BIX) and norbixin (NBIX) are carotenoids extracted from annatto (*Bixa orellana*) seeds. These carotenoids are largely used as food colouring additives (Fernandes et al., 2002) and have antioxidant and anti-inflammatory properties (Goto, Takahashi, Hirai, & Kawada, 2010; Goto et al., 2012), which make them potential nutraceutical constituents. BIX (Roehrs et al., 2014) and the annatto extract (Russell, Morrison, & Ragoobirsingh, 2005) have been demonstrated to reduce glycaemia and improve blood lipid profile in animal models. This effect has been ascribed to the activation of PPAR receptors (receptor peroxisome proliferator-activated) that may increase adipocyte sensitivity to insulin increasing glucose uptake (Goto et al., 2010). PPAR receptors regulate the expression of proteins involved in the metabolic pathways of lipids and carbohydrates, and their activation results in anti-inflammatory, immunomodulatory and anti-atherosclerotic effects (Ahmadian et al., 2013). BIX and NBIX acted as PPAR-γ agonists in cultured HepG2 hepatocytes, regulating the expression of proteins responsible for fatty acid oxidation and improving carbohydrate and lipid metabolism (Goto et al., 2012). The treatment of obese KK-Ay mice with BIX attenuated obesity-induced metabolic dysfunctions (dyslipidaemia, lipid accumulation in the liver, hyperglycaemia, hyperinsulinemia, glucose intolerance and insulin resistance) (Goto et al., 2012). Despite these promising results obtained from animal studies and the safety data that supports the use of annatto carotenoids as food colouring additives (acceptable daily intake of 12 mg/kg for BIX and 0.6 mg/kg for NBIX), there is no data on their nutraceutical effects in humans.

In the present study, we examined the effect of annatto carotenoids added to a high-calorie meal on postprandial biochemical parameters and markers of oxidative stress and inflammatory response in healthy subjects.

2. Material and methods

2.1. Bixin and norbixin extracts

Food grade extracts of annatto carotenoids that are industrially used as colouring additives were provided by Christian Hansen Co. Ltd. (Valinhos, SP, Brazil). The commercial BIX extract is provided as an oily solution containing sunflower oil, whereas the NBIX extract is an aqueous solution (alkali-aqueous). The content of annatto carotenoids in the extracts was preliminary assessed by spectrophotometric analysis at 470 nm and amounted to 10% in both extracts. Thereafter, the concentration of BIX and NBIX was assessed by high performance liquid chromatography (HPLC) coupled to a photodiode arrangement (PDA) detector. The oily BIX extract was diluted in chloroform whereas the aqueous NBIX extract was diluted in methanol. Solutions were then filtered on a 0.22 μm filter and analysed in a liquid chromatograph (CBM-20A Prominence, Shimadzu LC) coupled to pre-column (Shim-pack GODS, 10 × 4 mm, 5 μm), C18 reverse phase column (Shim-pack CLC ODS, 250 × 4.6 mm, 5 μm) and PDA detector. The injection volume was 20 μL. Mobile phase was acetonitrile:2% acetic acid (65:35, v/v) at a flow rate of 1 mL/min (Levy, Regalado, Navarrete, & Watkins, 1997). BIX and NBIX were identified by comparing their retention times and PDA spectra with pure standards and literature data (Levy et al., 1997). BIX amounted to 89.3% of all carotenoids found the oily BIX extract, whereas NBIX amounted to 87.2% of all carotenoids found in the aqueous NBIX extract (Supplementary material).

2.2. Study subjects

Twelve healthy volunteers (six women and six men; 73.0 ± 3.9 kg body weight; 24.8 ± 0.9 kg/m^2 body mass index; 25.7 ± 1.3 years; range: 20–33 years) were included in this randomised, controlled crossover study. The study protocol was approved by the local ethics committee of Federal University of Santa Maria (UFSM) (CAAE number: 27364114.1.0000.5346). Subjects that were taking vitamins, mineral supplements or medications and those with case history of smoking, alcoholism, chronic diseases as diabetes, hypertension, inflammatory diseases (asthma, lupus, arthritis rheumatoid disease), cancer, infectious diseases or any viral or bacterial infection in the last month were excluded from the study.

2.3. Test meals

The high-calorie meal consisted of one hamburger and 350 mL of soft drink, which amounted to approximately half the daily intake recommended for an adult concerning to caloric value and cholesterol content (Table 1). The protein and saturated fat content amounted to the whole recommended daily intake, whereas the sodium content amounted to 80% of the recommended daily intake (Table 1). Food grade liquid extracts of annatto carotenoids were added to the burgers to yield the three meal treatments: burger with no added carotenoid (placebo), burger containing 1.2 mg BIX/kg body weight (BIX) or burger containing 0.06 mg NBIX/kg body weight (NBIX). The doses of BIX and NBIX chosen amounted to 10% of the acceptable daily intake established by Food and Agriculture Organization of the United Nations (JECFA, 2007) for these carotenoids.

2.4. Study protocol

One day before the beginning of the experiment, blood biochemical markers [urea, creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT) and glycosylated haemoglobin] were evaluated to confirm that all subjects were healthy. All subjects were refrained from consuming fatty foods, foods with red or orange dyes, spicy foods and alcoholic beverages in the 24 h before the experiment. If the subjects reported symptoms of the gastrointestinal tract or any other discomfort on the experimental day, the examination was postponed. All blood collections (10 mL each one) were made by puncturing the median cubital vein or basilic vein with three vacuum system tubes containing no anticoagulant (to obtain serum), containing ethylenediaminetetraacetic acid (EDTA) or heparin (for obtaining whole blood).

Table 1
Nutritional composition and caloric value of test meal.

	Hamburger	Soft drink (350 mL)	Total	% of the recommended daily intake[a]
Caloric value (kcal)	861	127.5	988.5	49.4
Carbohydrate (g)	57	31.5	88.5	32.8
Protein (g)	54	–	54	108
Total fat (g)	46	–	46	65.7
Saturated fat (g)	21	–	21	105
Trans fat (g)	1.7	–	1.7	–
Cholesterol (mg)	145	–	145	48.3
Sodium (mg)	1917	15	1932	80.5

[a] Calculated based on the recommended daily amounts from FAO/OMS - 2003.