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β-Cryptoxanthin ameliorates metabolic risk factors by regulating NF-κB and Nrf2 pathways in insulin resistance induced by high-fat diet in rodents

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ABSTRACT

The aim of this experiment was to determine the effects of β-cryptoxanthin (BCX) on the cardiometabolic health risk factors and NF-κB and Nrf2 pathway in insulin resistance induced by high-fat diet (HFD) in rodents. Twenty-eight Sprague-Dawley rats were allocated into four groups: (1) Control, rats fed a standard diet for 12 weeks; (2) BCX, rats fed a standard diet and supplemented with BCX (2.5 mg/kg BW) for 12 weeks; (3) HFD, rats fed a HFD for 12 weeks, (4) HFD + BCX, rats fed a HFD and supplemented with BCX for 12 weeks. BCX reduced cardio-metabolic health markers and decreased inflammatory markers ($P < 0.001$). Rats fed a HFD had the lower total antioxidant capacity and antioxidant enzymes activities and higher MDA concentration than control rats ($P < 0.001$ for all). Comparing with the HFD group, BCX in combination with HFD inhibited liver NF-κB and TNF-α expression by 22% and 14% and enhanced liver Nrf2, HO-1, PPAR-α, and p-IRS-1 by 1.43, 1.41, 3.53, and 1.33 fold, respectively ($P < 0.001$). Furthermore, in adipose tissue, BCX up-regulated Nrf2, HO-1, PPAR-α, and p-IRS-1 expression, whereas, down-regulated NF-κB and TNF-α expression. In conclusion, BCX decreased visceral fat and cardiometabolic health risk factors through modulating expressions of nuclear transcription factors.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) contains a spectrum of liver damage that progresses to cirrhosis with various mechanisms including simple steatosis, alcohol-free steatohepatitis (NASH), advanced fibrosis and rarely insulin resistance, inflammation and oxidative stress (Wilkins et al., 2013; Higuera-de la Tijera and Servin-Caamano, 2015). High-fat diets (HFD) intake that can lead to NAFLD is the most significant risk factor for obesity, diabetes, non-alcoholic fatty liver, and cancer (Kennedy et al., 2004; Paschos and Paletas, 2009). High-fat diet consumption negatively affects metabolism, thereby increasing body fat, triacylglycerol, and plasma glucose levels, and impairing insulin sensitivity (Buettner et al., 2007; Banin et al., 2014; Hirata et al., 2015). Increased abdominal fat accumulation due to HFD intake or other factors promotes cytokine production and stimulation of inflammatory cytokine signaling pathways (Hotamisligil and Erbay, 2008). In previous studies, it has been reported that high fat consumption also causes oxidative stress, low serum carotenoid levels and increases tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) secretion (Kennedy et al., 2004; Seo et al., 2012; de Meneses Fujii et al., 2014). In addition, transcription factors including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) are also adversely affected by

Abbreviations: BCX, β-cryptoxanthin; CAT, catalase; GSH-Px, glutathione peroxidase; HFD, high fat diet; HO-1, heme-oxygenase 1; IL-6, interleukin-6; Keap1, kelch like-ECH-associated protein 1; MDA, malondialdehyde; NAFLD, nonalcoholic fatty liver disease; NASH, alcohol-free steatohepatitis; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclear factor (erythroid-derived 2)-like 2; p-IRS-1, insulin receptor substrate 1; PPAR-α, peroxisome proliferator-activated receptor-alpha; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidant capacity; TNF-α, tumor necrosis factor-alpha.

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HFD (Kennedy et al., 2004; Tuzcu et al., 2011; Seo et al., 2012).

Obesity-induced oxidative stress can adversely affect transcription factors of cellular components, RNA processing, translation, metabolism, and membrane structure and function (Iwagami, 1996; Vaquerizas et al., 2009). NF-κB, the transcription factor, is responsible for controlling DNA transcription and in cellular responses and promotes free radicals (Gilmore, 2006). It is normally present in the cytosol in an inactive form and enters the nucleus to activate the expression of specific genes (Nelson et al., 2004). Another transcription factor, Nrf2, which is the main regulator of the antioxidant response, binds to another protein called Kelch like-ECH-associated protein 1 (Keap1) in the cytosol (Itoh et al., 1997, 1999). Deterioration of cysteine residues in Keapl due to oxidative stress causes Nrf2 accumulation in the cytosol (Yamamoto et al., 2008). Additionally, Nrf2 is a potent positive regulator of the heme oxygenase (HO-1) gene in many cell types (Alam et al., 1999; Calabrese et al., 2008).

Recent studies have shown that serum and liver carotenoids levels decrease in chronic diseases (Leo et al., 1993; Yadav et al., 2002), and carotenoids have been shown to be useful in preventing obesity, a rapidly growing chronic disease in recent years (Bahcecioglu et al., 2010; Karmakar et al., 2011; Yilmaz et al., 2015). Beta-cryptoxanthin (BCX) is considered a provitamin A carotenoid found in high concentrations in tangerines, oranges, papayas and red sweet peppers and belongs to a family of carotenoids called xanthophylls (Burri, 2015; Gammone et al., 2015). Administration of BCX has variable beneficial effects such as anticancer, anti-inflammatory, anti-obesity, antidiabetic, anti-atherosclerosis, bone, brain and dental health effects (Katsuura et al., 2009; Fu et al., 2010; Donaldson, 2011; Yamaguchi, 2012; Ciccone et al., 2013; Ni et al., 2015). Previous experiments have shown that BCX supplements, an antioxidant carotenoid, are inversely proportional to the risk of insulin resistance and liver dysfunction in obese mice induced by a HFD (Kobori et al., 2014; Ni et al., 2015). BCX also helps protect cellular damage from free radical damage (Ciccone et al., 2013) and suppresses the expression of inflammatory cytokines including IL-1, IL-6, and others (Katsuura et al., 2009). In addition, serum BCX is inversely proportional to oxidative DNA damage indices and lipid peroxidation (Haegele et al., 2000). However, the effect of BCX supplementation on the liver and abdominal adipose tissue NF-κB and Nfr2/HO-1 pathway is unknown. Therefore, this study was conducted with an animal model to examine the effects of BCX on visceral fat levels, NF-κB and Nrf2 pathways. In addition, we also evaluated the metabolic effects of BCX mechanism and changes in transcription factors and other biological markers such as peroxisome proliferator-activated receptor-alpha (PPAR-α) and insulin receptor substrate 1 (p-IRS-1) proteins, antioxidant properties and oxidative stress effects in rats fed a HFD.

2. Material and methods

2.1. Animals

This study was carried out using 28 male Sprague-Dawley rats (8 weeks old, 180 ± 20 g) obtained from Inonu University Laboratory Animal Research Center (Malatya, Turkey). Rats were housed in cages in a temperature and humidity controlled environment, on a 12-hr light and 12-hr dark cycle, designed for the purpose of the study. The temperature inside the rat cages was 21 ± 2 °C, relative humidity was 55± 5%. The protocol of the study was approved by Inonu University Animal Experiment Ethics Committee (Malatya, Turkey). All procedures for rats have been carried out in strict accordance with the relevant legislation, the Animal Welfare Act, the Public Health Service Policy, and the guidelines established.

2.2. Experimental protocol

After one week adaptation period, the animals were randomly assigned to one of the four experimental groups: (i) Control (n = 7); rats fed a standard diet for 12 weeks (12% of calories from fat); (ii) BCX (n = 7); rats fed a standard diet and supplemented with BCX (2.5 mg/kg BW/day) for 12 weeks; (iii) HFD (n = 7); rats fed a high-fat diet (42% of calories from fat); (iv) HFD + BCX (n = 7); rats fed a high-fat diet (42% of calories from fat) and supplemented with BCX (2.5 mg/kg BW/day) for 12 weeks. BCX (BCXcel™) was supplied by Omni Active Health Technologies Pvt. Ltd., Mumbai, India (B.No: 141025). BCX concentrate, which contains about 10–80% by weight total xanthophylls, of which about 75–98% by weight is trans-beta-cryptoxanthin, the remaining including zeaxanthin, trans-capsanthin, beta-carotene and trace amounts of other carotenoids, derived from oleoresin. BCX was dissolved in corn oil. The composition of the experimental diets is shown in Table 1.

At the end of the study, all rats were sacrificed by cervical dislocation. Blood samples were taken from rats in the morning upon overnight fasting for biochemical analyses and their visceral fat and liver samples were removed and weighed after sacrificing the animals. Liver and abdominal adipose tissues were washed with phosphate-buffered saline (PBS). Tissues were snap-frozen immediately in liquid nitrogen and stored at −80 °C for further analysis.

2.3. Laboratory measurements

Blood was collected by cardiac puncture using a non-anticoagulated vacutainer tube, then centrifuged at 3000×*g* for 10 min to obtain serum and frozen at −80 °C until assayed for biochemical parameters and malondialdehyde (MDA). Serum biochemical parameters were estimated using an automated analyzer (Samsung LABGEO PT10, Samsung Electronics Co., Suwon, Korea). Repeatability and device/method precision of LABGEO^{PT10} was established according to the IVR-PT06 guideline. Serum insulin, leptin, and adiponectin levels were measured by ELISA (Elx-800, Bio-Tek Instruments Inc., Vermont, USA) and Rat Insulin Kit (Linco Research Inc., St. Charles, Mo., USA).

Total antioxidant capacity (TAC) was measured using kits from Abcam (Cambridge, UK) according to the manufacturer's instructions. Lipid peroxidation was measured in terms of MDA formation, which is the main product of membrane lipid peroxidation

Table 1
Composition of diets (g/kg diet) fed to rats.

	Regular diet	HFD[a]
Casein	200.0	200.0
Starch	579.5	150.0
Sucrose	50.0	149.5
Soybean oil	70.0	–
Beef tallow	–	400.0
Cellulose	50.0	50.0
Vitamin-Mineral Premix[b]	45.0	45.0
l-cysteine	3.0	3.0
Choline bitartrate	2.5	2.5

[a] HFD: High fat diet.
[b] The vitamin-mineral premix provides the following (per kg): all-*trans*-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-*rac*-a-tocopherol acetate, 12,5 mg; menadione (menadione sodium bisulfate), 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; vitamin B-6, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B-12, 0.02 mg; folic acid, 0.55 mg; *d*-biotin, 0.1 mg manganese (from manganese oxide), 40 mg; iron (from iron sulfate), 12.5 mg; zinc (from zinc oxide), 25 mg; copper (from copper sulfate), 3.5 mg; iodine (from potassium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg.