Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 18 (2007) 64–72
Journal of Nutritional Biochemistry

Beneficial impact of crocetin, a carotenoid from saffron, on insulin sensitivity in fructose-fed rats

Liang Xi[a], Zhiyu Qian[a,*], Guanglin Xu[b], Shuguo Zheng[a], Sai Sun[a], Na Wen[a], Liang Sheng[a] Yun Shi[a], Yabing Zhang[a]

[a]*Department of Pharmacology, China Pharmaceutical University, Nanjing 210009, PR China*
[b]*College of Life Science, Nanjing Normal University, Nanjing 210097, PR China*

Received 29 December 2005; received in revised form 6 March 2006; accepted 23 March 2006

Abstract

Crocetin, a unique carotenoid with potent antioxidative and anti-inflammatory activities, is a major ingredient of saffron which is used as an important spice and food colorant in various parts of the world. In the present study, the effect of crocetin on insulin resistance and its related abnormalities induced by high-fructose diet were investigated in male Wistar rats. Compared to the control rats fed on normal laboratory diet, fructose-fed rats developed a series of pathological changes including insulin resistance, hyperinsulinemia, dyslipidemia and hypertension. Although having no evident effect on the body weight, fructose feeding caused a marked increase in the weight of epididymal white adipose tissue. Furthermore, a significant reduction in the expression of both protein and mRNA of adiponectin (an insulin-sensitizing adipocytokine) was observed, whereas those of tumor necrosis factor (TNF)-α and leptin were enhanced in epididymal white adipose tissue in fructose-fed rats. These disorders were effectively normalized in crocetin-treated rats. Crocetin was also demonstrated here to alleviate free fatty acid (FFA)-induced insulin insensitivity and dysregulated mRNA expression of adiponectin, TNF-α and leptin in primary cultured rat adipocytes. These findings suggest the possibility of crocetin treatment as a preventive strategy of insulin resistance and related diseases. The favorable impact on adiponectin, TNF-α and leptin expression in white adipose tissue may be involved in the improvement of insulin sensitivity observed in crocetin-treated rats.

Keywords: Crocetin; Insulin resistance; Adipose tissue; Adiponectin; TNF-α; Leptin

1. Introduction

Functional plant-based foods that contain bioactive components may provide desirable health benefits beyond basic nutrition and are practically useful for the prevention of chronic diseases such as cardiovascular diseases and cancer. Saffron, the world's highest priced spice, is collected from the dried stigmas of *Crocus sativus* L. which originated in the Middle Eastern region of the Eurasian continent [1]. This carotenoid-rich spice is commonly consumed in different parts of the world and also used as an herbal medicine. Among the major ingredients of saffron, crocetin, which is responsible for its coloring property, is a special carotenoid with multi-unsaturated conjugate olefin acid structure. The compound exhibits favorable effects in the prevention or treatment of a variety of diseases such as dyslipidemia, atherosclerosis, myocardial ischemia, hemorrhagic shock, cancer and arthritis [2]. Crocetin also showed obvious inhibitory effects on atherogenic factor-induced disorders in vascular endothelial cells, smooth muscle cells and monocyte-derived macrophages [3] (unpublished data). Recently, we have found that crocetin may prevent low-dose dexmethasone-induced insulin resistance in rats [4].

Insulin resistance is a remarkable and growing health problem tightly associated with obesity, dyslipidemia, hypertension and type 2 diabetes mellitus [5]. The development of insulin resistance is linked to both genetic and environmental factors. A key environmental element is diet

Abbreviations: CON, control; CRO, crocetin; CRO(H), high-dose crocetin; CRO(L), low-dose crocetin; FFA, free fatty acids; FRU, fructose; HDL-C, high-density lipoprotein cholesterol; KRBH, Krebs-Ringer bicarbonate HEPES buffer; LDL-C, low-density lipoprotein cholesterol; MMLV, Moloney murine leukemia virus; RT-PCR, reverse transcription-polymerase chain reaction; TNF-α, tumor necrosis factor-α.

* Corresponding author. Tel.: +86 25 8327 1322; fax: +86 25 832 1355.
E-mail address: zhiyu.qian@yahoo.com (Z. Qian).

0955-2863/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2006.03.010

composition [6]. In this study, a well-established model in which insulin resistance was induced by feeding high-fructose diet was employed, and fructose-fed rats were characterized by hyperinsulinemia and dyslipidemia with normoglycemia.

Adipose tissue mass (especially visceral adipose) enlargement has been identified as a crucial factor responsible for insulin resistance [7]. As a primary energy-storing organ, adipose tissue accumulates triglycerides during nutrition excess and provides energy in the form of free fatty acids (FFAs), which may induce insulin resistance if produced excessively [8]. Besides, adipose tissue releases numerous bioactive molecules named adipocytokines that participate in a variety of physiological functions. For example, adiponectin is an important adipocyte-specific circulating protein that possesses insulin-sensitizing, anti-atherosclerotic and anti-inflammatory activities [9,10]. TNF-α is a central modulator of adipocyte metabolism which interferes in insulin-mediated biological processes. In addition to a direct inhibitory effect on insulin signaling cascade, TNF-α also raises FFA concentration by decreasing lipogenesis and increasing lipolysis [7,11]. Leptin, which is also chiefly secreted by adipocytes, has also been shown to affect insulin sensitivity [7,12]. Dysregulation of adipocytokines due to fat accumulation is implicated in the progress of insulin resistance, but the involved mechanisms are seldom known.

Oxidative stress caused by enhanced concentrations of FFA, glucose and inflammatory cytokines plays a major role in the development of insulin resistance, and increased oxidative stress in accumulated fat has been recognized as an early instigator of insulin-resistance syndrome [13,14]. In cultured adipocytes, elevated FFA increases oxidative stress, which leads to a dysregulated production of adipocytokines [13], and long-term oxidative stress impairs insulin signaling [15]. The use of antioxidants is proposed as a potential new approach for the treatment of insulin resistance and related diseases [13,14,16,17]. Besides, owing to the close relationship between insulin resistance and dyslipidemia [18], dietary fish oils and other lipid-regulating agents have been shown to be able to prevent adiposity and insulin resistance [19].

Based on these facts, it can be expected that crocetin has more favorable health-promoting effects. The current study adopted a fructose-fed rat model to investigate the effect of crocetin on insulin action. The results showed that fructose-induced insulin resistance and other accompanied abnormalities were significantly attenuated by crocetin. Since adipocytokine secretion and adipose-specific gene are proposed as potential important targets for management of insulin resistance by controlling their expression or actions [16], regulation of adipocytokine expression represents a candidate mechanism underlying the beneficial effect of crocetin on insulin sensitivity. Therefore, the influence of crocetin on the expression of protein and mRNA of adiponectin, TNF-α and leptin in epididymal white adipose tissue was investigated. Furthermore, the effect of crocetin on FFA-induced impairment of insulin sensitivity and disordered mRNA expression of adiponectin, TNF-α and leptin was observed in primary cultured adipocytes.

2. Methods and materials

2.1. Chemicals

Crocetin (>96%, HPLC) was purified from saffron in our laboratory. 2-Deoxy-D-[1-^{3}H]-glucose (5.4 Ci/mmol) was purchased from Atom High-Tech (Beijing, China). Other chemicals were obtained from Sigma or local manufacturers unless otherwise stated.

2.2. General protocol

Male Wistar rats (Slac Laboratory Animal, Shanghai, China) with a body weight of 120–150 g were housed at 24±2°C with a 12/12 h light–dark cycle. All rats were supplied with normal laboratory chow and water for 1 week. The experimental protocols were performed in accordance with the institutional guidelines for animal care of China Pharmaceutical University and approved by the local animal research committee.

After acclimation, the animals were randomly divided into the following groups consisting of 10 rats each: a control group (CON), a crocetin (40 mg/kg)-treated group (CRO), a fructose-fed group (FRU), two fructose-fed groups plus crocetin at a dose of 40 mg/kg [FRU+CRO(H)] and 20 mg/kg [FRU+CRO(L)], respectively. Fructose was supplied in drinking water at a concentration of 10% for 8 weeks, while the CON group received no supplemented fructose. Crocetin powder was mixed thoroughly into the powdered chow at a concentration of 0.02–0.08%, and crocetin-containing diet was given along with fructose supplement through the experiment. To ensure accurate dosing, food intake was measured for 5 days before the study and the dietary crocetin concentration was reset according to the changes in body weight and food consumption every 2 weeks during the study. Additional crocetin was given by oral gavage if the consumed diet did not match enough level of crocetin. Body weights were monitored twice weekly. The intake of food and fluid was recorded daily, using metabolic cages. Systolic blood pressure, heart rate, serum glucose and insulin were measured every 2 weeks from the beginning of the experiment (Week 0). Systolic blood pressure and heart rate were measured with the tail-cuff method, using a programmed sphygmomanometer (DeSci Biotech, Nanjing, China).

2.3. Biochemical analyses

Serum glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using a Beckman LX-20 automatic analyzer. Serum insulin was measured with a rat insulin radioimmunoassay kit (Linco Research, St. Charles, USA). Serum FFA was measured by a validated colorimetric method using a commercial kit (Jiancheng Bioengineering Institute, Nanjing, China).