Journal of Functional Foods 41 (2018) 62–71

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Cyanidin-3-glucoside attenuates high-fat and high-fructose diet-induced obesity by promoting the thermogenic capacity of brown adipose tissue

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ARTICLE INFO

Keywords:
Cyanidin-3-glucoside
Thermogenesis
Brown adipose tissue
Mitochondrial function
High-fat and high-fructose diet-induced obesity

ABSTRACT

Obesity results from a sustained positive energy balance, occurring when energy intake exceeds energy expenditure. One promising therapeutic strategy to mitigate obesity is the promotion of thermogenesis of brown adipose tissue (BAT) to increase energy expenditure. Cyanidin-3-glucoside (C3G) is an anthocyanin compound widely distributed in human diets. In this study, researchers studied the effect and mechanism of C3G on the prevention and treatment of high-fat and high-fructose diet (HFFD) induced obesity. The study shows that C3G enhances energy expenditure and thermogenic capacity of BAT, protecting mice against HFFD obesity. Further, C3G increases expression of UCP1 and other thermogenic genes in inguinal white adipose tissue (iWAT), as well as in BAT. Results demonstrate that C3G promotes the heat production of BAT and iWAT by increasing mitochondrial biogenesis and function. These findings reveal that C3G activates BAT, which may provide a safe, effective approach to prevent and treat diet-induced obesity.

1. Introduction

Obesity occurs when there is an energy imbalance within the body, specifically when energy intake exceeds energy expenditure. Obesity is a global epidemic, contributing to a dramatic increase in the prevalence of type 2 diabetes, hypertension, cardiovascular disease, and certain cancers (Kivimäki et al., 2017; Ng et al., 2014). Existing methods for treating obesity focus on limiting energy intake and/or absorption. However, these treatments are far from perfect (Johansson, Sundström, Neovius, Rössner, & Neovius, 2010; Siebenhofer et al., 2016). As such, it is critical to develop alternative strategies to improve both metabolic efficiency and energy consumption in key metabolic organs, such as adipose tissue (Ravussin & Galgani, 2011).

Adipocytes in humans are divided into three categories, defined by morphological and functional differences: white, beige, and classical brown adipocytes. Unlike white adipocytes that deposit excess energy into triglycerides (TG), beige and classical brown adipocytes are distinguished by their unique ability to dissipate mitochondrial energy into heat via uncoupling protein 1 (UCP1) (Rosenwald & Wolfrum, 2014). Classical brown human adipocytes possess molecular attributes similar to the interscapular brown adipose tissue (iBAT) of rodents, sharing traits such as constitutive UCP1 expression, homogeneous multilocular morphology, and a myogenic origin ($Myf5^+$) (Seale et al., 2007, 2008). Conversely, beige adipocytes originate from nonmyogenic ($Myf5^-$) progenitors prompted by environmental stimuli (such as cold and exercise) (van der Lans et al., 2013), and show low expression of UCP1 under unstimulated conditions. Although there has been some uncertainty surrounding the cellular identity, recruitment, and bidirectional transdifferentiation of beige fat (Rosenwald, Perdikari, Rulicke, & Wolfrum, 2013; Rosenwald & Wolfrum, 2014), the metabolic significance of both classical brown and beige adipocytes in regulating systemic energy balance is well established (Jeremic, Chaturvedi, & Tyagi, 2017; Mulya & Kirwan, 2016; Schrauwen & van Marken Lichtenbelt, 2016). Brown adipose tissue (BAT) is a natural energy-consuming fat as opposed to the white adipose tissue (WAT) that stores energy. BAT converts fat into energy through non-shivering thermogenesis in small mammals as they try to keep body temperature constant during cold temperatures, dormancy, and under other similar

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https://doi.org/10.1016/j.jff.2017.12.025
Received 23 October 2017; Received in revised form 5 December 2017; Accepted 12 December 2017

stimuli (Casteilla, Champigny, Bouillaud, Robelin, & Ricquier, 1989).

Energy burning is a metabolic activity critical to rodents' and human infants' ability to maintain a constant body temperature (Lowell & Spiegelman, 2000). It is estimated that BAT mass ranges from ~30 to 300 g in humans, which could contribute 20% of total resting energy expenditure when maximally stimulated (Carey & Kingwell, 2013; Cypess et al., 2009). Studies demonstrate that reduction in BAT thermogenesis function may lead to obesity and related metabolic disorders (Lowell & Spiegelman, 2000; Sidossis & Kajimura, 2015). Therefore, BAT is a promising therapeutic target for combating human obesity and its associated metabolic diseases. However, the positive rate of functional BAT in adults is low at room temperature (Cypess et al., 2009). Thus, approaches that can safely and effectively activate BAT are urgently needed in both medical and nutritional fields.

Anthocyanins are natural compounds possessing pharmacological properties. Cyanidin-3-glucoside (C3G) is the most abundant anthocyanin in the plant kingdom and a quite popular research subject in this field as a result; it exhibits significant pharmacological activities, including anti-oxidative, anti-inflammatory, and anti-obesity effects (Nasri, Roghani, Baluchnejadmojarad, Rabani, & Balvardi, 2011; Scazzocchio et al., 2011; Wang et al., 2016). However, the molecular mechanism underlying these effects remains elusive. Current studies on C3G's resistance to obesity focus largely on WAT (Matsukawa, Inaguma, Han, Villareal, & Isoda, 2015; Scazzocchio et al., 2011). Furthermore, most research on obesity has been conducted on genetically obese mice (e.g. db/db mice), or high-fat diet induced obese mice (Yuan et al., 2017; Zhang et al., 2014). Few studies have been conducted specifically on high-fat and high-fructose diet (HFFD) induced obese mice model. The high morbidity of obesity can be attributed partly to modern dietary patterns. Diets rich in fats and sugars are the main culprits (Johnson et al., 2007; Yamauchi et al., 2001). Fructose is widely used in the production of beverages and sweets due to its low cost and preferred taste, and is also widely consumed (Dills, 1993). However, dietary fructose is almost totally absorbed and metabolized rapidly by the liver, which is vastly different from glucose metabolism. This results in deleterious effects, such as insulin resistance (IR), obesity, and hyperuricemia (Balakumar et al., 2016; Tappy & Lê, 2010). Consequently, there is a heightened interest in identifying highly effective compounds that lessen the problematic effects of fructose and fats while maintaining food palatability. However, research into the effect of C3G on the BAT activation in HFFD obese mice model is needed. Given the adverse effects of fructose on metabolic balance, such as hepatic damage, dyslipidemia and IR (Alwahsh et al., 2014; Sloboda et al., 2014), as well as the prevalence of both high fat and fructose in the typical western diet, it is important to examine the effects of C3G on combined dietary fat- and fructose-induced obesity.

It is critically important to determine a suitable animal model as a preliminary step in studying obesity. However, there are many factors that contribute to obesity, including genetic inheritance, metabolic disorders, chemical stimulation, and lifestyle (diet and exercise). Since different obesity causes have different pathogenesis, they also require different approaches to treatment and prevention. Food composition and energy intake excess are the most prominent culprits of the current obesity epidemic, which makes the diet induced obesity animal model the preferred choice to study human obesity. One previous study showed that C3G mediated weight loss in genetically obese db/db mice by activating BAT thermogenesis (You et al., 2017b). Therefore, in order to further study the effect and mechanism of C3G resistance to obesity, it is still necessary to further confirm whether C3G has an activation effect on BAT in the diet induced obesity model. Moreover, the effect of C3G on the activation of BAT in the HFFD obese mice model must be studied further. In this study, we investigated the effects of dietarily supplied C3G on high-fat and high-fructose diet (HFFD)-induced obese mice and explored their potential mechanisms. Results demonstrate that C3G could regulate a thermogenesis program in both BAT and inguinal white adipose tissue (iWAT) to reduce diet-induced obesity. Our data establishes C3G's previously unrecognized role in increasing energy expenditure, which could have a therapeutic role in the treatment of diet-induced obesity.

2. Materials and methods

2.1. Chemicals and antibodies

C3G (purity 98%) was purchased from Chengdu Must Biological Technology Co. Ltd. (Chengdu, China). The antibodies used for immunoblotting included anti-UCP1 and anti-OXPHOS (Abcam plc.), anti-β-actin (Cell Signaling Technology, Inc.). All other chemicals were obtained from Sigma Chemical Co. unless otherwise specified.

2.2. Animal model

Thirty-six male C57BL/6J mice (21 days of age) were purchased from Vital River Laboratory Animal Technology. Co. Ltd., China. Mice were housed three per cage in a facility certified by the Office of Laboratory Animal Welfare under a 12-h light/12-h dark cycle and were fed with standard laboratory chow for one week. After adaptation period, mice were randomly divided into three groups (n = 12/group) with equal body weight and assigned to one of three dietary treatments for 15 weeks: (1) normal chow diet group (CHOW, 3.2 kcal/g, 4.5% fat, w/w); (2) diet induced obesity group (DIO, 4.7 kcal/g, 25% fructose and 25% lard); (3) C3G group (DIO + C3G, 4.7 kcal/g, 25% fructose and 25% lard). C3G group mice received C3G dissolved in drinking water (1 mg/mL); Drinking water served as the vehicle for the CHOW and DIO treatments. Stability tests of C3G in water were determined before the experiment was begun, and it was determined that no significant degradation occurred during a 48 h period at room temperature. Thus, fresh drinking water containing C3G was replaced every other day. Mice had ad libitum access to food and drinking water. Body weight was measured weekly. Drinking water and food consumption was monitored daily. At the end of the experiment, animals were sacrificed after overnight fasting (16 h). Principles of laboratory animal care were followed and all procedures were conducted according to the guidelines established by the National Institutes of Health, and every effort was made to minimize suffering. This study was approved by the Animal Experiment Committee of College of Food Science and Nutritional Engineering, China Agricultural University. The specific authorization reference number is CFSNE20160023.

At the end of the experimental process, blood was collected into tubes containing EDTA (5 mM final concentration) and protease inhibitors. Plasma was prepared from blood by centrifugation at 2000*g* for 10 min at 4 °C. The total body weight and weight of individual organs (liver, BAT, inguinal WAT, and epididymal WAT) were measured after careful dissection. Tissues isolated for gene expression and western blotting analyses were rapidly collected, frozen in liquid nitrogen, and stored at −80 °C; tissues isolated for histology, immunohistochemistry, and transmission electron microscope experiments were immediately treated with fixative solution.

2.3. Glucose tolerance testing (GTT) and insulin tolerance testing (ITT)

Mice were fasted for 16 h with free access to water. Blood glucose was measured with an Accu-Chek glucometer (Roche Diagnostics Corp) at 0, 15, 30, 45, 60, 90, and 120 min after an intraperitoneally (i.p.) administered injection of glucose at 1.5 g/kg. An insulin tolerance test was performed on 6 h-fasted mice. The glucose concentrations were measured by venous bleeding at 0, 15, 30, 45, 60 and 90 min after an i.p. injection of human insulin at 1.0 U/kg.

2.4. Metabolic rate and physical activity

Oxygen consumption and physical activity were determined for