JOURNAL OF FUNCTIONAL FOODS 9 (2014) 211–224

Available at www.sciencedirect.com

ScienceDirect

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

An ursolic acid-enriched *Cynomorium songarium* extract attenuates high fat diet-induced obesity in mice possibly through mitochondrial uncoupling

Jihang Chen, Hoi Shan Wong, Hoi Yan Leung, Pou Kuan Leong, Wing Man Chan, Kam Ming Ko *

Division of Life Science, The Hong Kong University of Science & Technology, Clear Water Bay, Hong Kong

ARTICLE INFO

Article history:
Received 18 March 2014
Received in revised form 22 April 2014
Accepted 24 April 2014
Available online 14 May 2014

Keywords:
Cynomorium songarium
Mitochondrial uncoupling
Obesity
Glucose intolerance
Fatty liver

ABSTRACT

It has previously been demonstrated that HCY2, an ursolic acid-enriched fraction derived from Cynomorii Herba, induced mitochondrial uncoupling in heart, liver and kidney tissues of rats. In the present study, we investigated whether HCY2 co-treatment can reduce the body weight in high fat diet (HFD)-fed mice by virtue of mitochondrial uncoupling in skeletal muscle. The results showed that HCY2 co-treatment significantly reduced the body weight gain and white fat pads in HFD-fed mice. The weight reduction effect of HCY2 was associated with the induction of mitochondrial uncoupling in skeletal muscle, amelioration of HFD-induced hyperglycaemia and impairment in glucose tolerance as well as dyslipidaemia and fatty liver in HFD-fed mice. It is likely that HCY2 reduces body weight by increasing energy expenditure, presumably via mitochondrial uncoupling in skeletal muscle. HCY2 may therefore offer a promising prospect in preventing obesity and the associated metabolic syndrome and health consequences.

1. Introduction

Obesity is a principal causative factor involved in the development of metabolic syndrome, for which it was characterised by chronic weight gain, non-alcoholic fatty liver, and diabetes associated with insulin resistance and glucose intolerance, leading to reduced life expectancy and/or increased health problems (Isomaa et al., 2001; Prangthip et al., 2013; Reaven, 1988). The increase in the incidence of obesity arising from a combination of socioeconomic factors and lifestyle changes has been an alarming public health trend in the world. It is no doubt that the escalating prevalence of obesity can exert financial pressure on publicly funded healthcare systems in different countries. In this regard, the search for therapeutic interventions aimed at reducing the incidence of obesity and its related body disorders has been an area of active investigation (Baboota et al., 2013).

Although the etiology of obesity is complex, the major cause of obesity is considered to be an imbalance in energy homeostasis – the result of energy intake that is higher than energy expenditure (Lidell & Enerback, 2010; Wu et al., 2013). Hence, any treatments that can help to reduce energy intake, increase energy expenditure, or have an effect on both would be useful for the clinical management of obesity. Fuel molecules are oxidised in mitochondrial matrix, with the resultant formation of reduced electron carriers such as NADH and $FADH_2$. During the electron transport process initiated by NADH

* *Corresponding author.* Tel.: +852 2358 7298; fax: +852 2358 1552.
E-mail address: bcrko@ust.hk (K.M. Ko).
http://dx.doi.org/10.1016/j.jff.2014.04.021
1756-4646/

and $FADH_2$, a proton gradient is generated across the mitochondrial inner membrane, of which the potential energy is utilised for driving ATP synthesis. However, not all potential energy stored in the proton gradient is transformed for ATP synthesis. Mitochondrial uncoupling is such a process by which protons leak across the mitochondrial inner membrane and bypass the ATP synthase, thereby uncoupling the electron transport and ATP synthesis (Gruber et al., 2013). The consequent increase in ADP/ATP ratio triggers the activation of mitochondrial electron transport and the upstream energy metabolic pathways, which allows the unchecked catabolism of dietary fuel molecules, eventually leading to weight loss (Rousset et al., 2004). Skeletal muscle, which constitutes approximately 40% of total body mass, is a major site for glucose and lipid oxidation in the body (O'Neill, Holloway, & Steinberg, 2013). In perfused rat skeletal muscle, proton leak has been estimated to account for around 52% of resting oxygen consumption (Rolfe & Brand, 1996). Therefore, skeletal muscle represents a particularly attractive target for mitochondrial uncoupling. In 1930s, dinitrophenol (DNP), a non-selective uncoupler of mitochondrial oxidative phosphorylation, was used effectively in humans (3–5 mg/kg) as an anti-obesity drug (Lou et al., 2007). Although DNP produces fatal side effects related to overdose that preclude its clinical use, uncoupling of mitochondria is still an attractive drug target for combating obesity if the safety of use can be proven.

Cynomorii Herba (the stems of *Cynomorium songaricum* Rupr., Cynomoriaceae), also known as Suo-Yang in Chinese, is an obligate root parasitic plant, which is mainly distributed over the deserts in Western China as well as Central Asia, Iran and Mongolia (Meng, Wang, Li, Kuang, & Ma, 2013). This plant is a source of both medicine and healthy food used for treating lumbar weakness and enhancing sexual ability for both men and women for hundreds of years in China. It is also frequently added into teas, coffees or beverages and consumed by local people as invigorating food (Meng & Ma, 2013). Previous experimental findings in our laboratory have demonstrated that long term treatment with HCY2, an ursolic acid-enriched active fraction isolated from Cynomorii Herba ethanol extract, invariably protected against oxidative tissue damage in the rat models of myocardial ischaemia/reperfusion injury, carbon tetrachloride hepatotoxicity and gentamicin nephrotoxicity, with the possible involvement of mitochondrial uncoupling (Chen & Ko, 2013; Chen, Wong, & Ko, 2014a; Chen et al., 2014b). In the present study, we sought to examine the effect of HCY2 co-treatment on high fat diet (HFD)-induced obesity and the associated metabolic changes in both male and female ICR mice. To elucidate the biochemical mechanism underlying the weight loss effect of HCY2, activities of enzymes related to glucose and fatty acid metabolism as well as mitochondrial respiration in skeletal muscle of mice were measured.

2. Materials and methods

2.1. Chemicals

PicoLab Rodent diet 20 (normal diet) was purchased from LabDiet (St. Louis, MO, USA). HFD (Diet-induced Obesity Formula D12492, 60% energy from fat) was purchased from Research Diets, Inc. (New Brunswick, NJ, USA) (Table S1 in the online version at doi:10.1016/j.jff.2014.04.021). Triglyceride (TG) and cholesterol assay kits were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were purchased from Sigma Chemical (St. Louis, MO, USA).

2.2. Herbal material and extraction

Cynomorii Herba was purchased from a local herbal dealer (Lee Hoong Kee, Ltd., Hong Kong SAR, China). The herb was authenticated by the supplier and a voucher specimen (HKUSTY01001) was deposited in the Division of Life Science, the Hong Kong University of Science and Technology (HKUST). An UA-enriched active fraction, HCY2, was isolated from Cynomorii Herba ethanol extract as previously described, and characterised with a HPLC/MS/UV method to ensure the consistency between batches. Briefly, for the MS/MS analysis, an Agilent QQQ-MS/MS (6410A) equipped with an ESI ion source was operated in positive ion mode. Two suitable transition pairs (457,411) were chosen for acquisition in MRM mode for UA. For quantitative analysis, a photodiode array UV-vis detector was used. The detection wavelength was set at 210 nm. The quantitation of UA in the HCY2 fraction was determined from a calibration curve. The calibration curve of UA was linear and the regression equation of the peak area (y) as a function of concentration (x) was $y = 473.2x + 532.6$ ($r = 0.999$). The quantitative analysis indicated that HCY2 majorly contained UA at 74.8% (w/w) (Chen & Ko, 2013; Chen, Wong, & Ko, 2014a) (Fig. S1 in the online version at doi:10.1016/j.jff.2014.04.021).

2.3. Animal care

Both male and female ICR mice (8 weeks old) were obtained from the Animal and Plant Care Facility at HKUST. The animals were maintained under a 12-h dark/light cycle at about 22 °C, and allowed food and water *ad libitum*. The experimental protocol was approved by the Animal Ethics Committee at HKUST (approval number: 2013049; approval date: 25 September 2013; experiment duration: 3 years).

2.4. Animal treatment

Eight-week-old ICR mice, including both females and males, were randomly divided into control and HCY2 co-treatment groups in each gender, with 5–15 mice per group. The control groups fed a normal diet (ND) or HFD *ad libitum*. In the HCY2 co-treatment groups, mice were intragastrically administered with HCY2 (112 or 336 mg/kg for male, 180 or 360 mg/kg for female) simultaneously with either ND or HFD feeding for 8 weeks. Control mice received vehicle only. The dosages of HCY for male and female mice were determined with reference to previous findings which showed the mitochondrial uncoupling effect of HCY in heart, liver and kidney tissues of rats (Chen & Ko, 2013; Chen et al., 2014b). During the 8-week experiment period, the amount of food intake as well as body weight was monitored once every week. At the end of experiment, blood samples were collected from phenobarbital-anaesthetised mice by cardiac excision using syringes rinsed with 0.5% heparin in saline (w/v) after 24-h fasting, and the mice were then sacrificed by cardiac excision. Various fat pads,