Journal of Functional Foods 17 (2015) 969–978

Available online at www.sciencedirect.com

ScienceDirect

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

Hepatic genome-wide expression of lipid metabolism in diet-induced obesity rats treated with cocoa polyphenols

Faisal Ali [a], *Amin Ismail* [a,*], *Norhaizan Mohd Esa* [a,b], *Chong Pei Pei* [c], *Sander Kersten* [d]

[a] *Department of Nutrition and Dietetics, Metabolism and Genomics Group, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

[b] *Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Selangor 43400, Malaysia*

[c] *Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

[d] *Metabolism and Genomics Group, Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands*

ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form 17 June 2015
Accepted 17 June 2015
Available online

Keywords:
DNA microarray
Cocoa polyphenols treatment
Genes expression profiling
Liver steatosis
Lipid metabolism
Obesity

ABSTRACT

Cocoa polyphenols (CPs) have been shown to exhibit hypolipidaemic actions, suggesting that CPs offer great potential for ameliorating lipid abnormalities. However, the conceivable molecular mechanisms underlying the pharmacological activity of CPs in obesity-induced liver steatosis have yet to be an investigated. This study analysed the hepatic genome-wide expression patterns in high-fat diet (HFD)-induced obese rats using DNA microarray. Rats were fed either a low fat (LFD) or high fat diet (HFD) for 12 weeks. After supplementation, HFD rats (n = 10/group) were treated with 600 mg/kg bw/day CPs (HFD + CPs) for 4 weeks. As a result, compared to the HFD group, CP treatment significantly lowered lipid in the liver and attenuated the increases in body weight as well as visceral fat accumulation in the CP group. DNA microarray analysis resulted in a differential expression of 862 genes of the 12,282 genes expressed in the liver. The differential expression patterns of selected genes were validated with real-time-PCR. Metabolic pathway analysis via bioinformatic tools showed that genes in lipid catabolism, primarily in fatty acid oxidation, were up-regulated in the CP group, whereas genes in lipid synthesis pathways were down-regulated. Together, these findings provide a novel insight into possible molecular mechanisms behind the pharmacological actions of CPs on the management of the obesity-induced steatosis markers in rats with diet-induced obesity.

* *Corresponding author.* Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Metabolism and Genomics Group, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. Tel.: +60 389472435; fax: +60 389426769.
E-mail address: amin@medic.upm.edu.my (A. Ismail).

http://dx.doi.org/10.1016/j.jff.2015.06.047
1756-4646/

1. Introduction

The liver is the master organ of lipid, protein and carbohydrate metabolism. The liver plays a principal role in the maintenance of homeostatic mechanisms and coordinates lipid metabolism in the fed and fasted state. Dysregulation of this balance has been associated with the development of a number of risk factors for metabolic disorders such as hyperlipidaemia and non-alcoholic fatty liver disease (NAFLD) (Gastaldelli et al., 2009; Targher & Arcaro, 2007). A positive imbalance between fat intake and energy expenditure is a requisite for the development of obesity phenotype. Obesity is a global disease and influenced by a number of factors of genetic and environmental origin. Although several attempts have been made to target body weight via anti-obesity drugs, significant problems with side effects remain (Cooke & Bloom, 2006; Halford, 2006). Therefore, dietary supplements that could potentially reduce body weight gain and hyperlipidaemia may represent a more viable strategy. One group of bioactive compounds shown to have various preventive properties, including hypolipidaemic effects (Ikarashi et al., 2010; Vitaglione et al., 2010), antioxidant effects (Vitaglione et al., 2010), anti-diabetic effects (Ikarashi et al., 2010) and anti-inflammatory effects (Rivera, Morón, Sánchez, Zarzuelo, & Galisteo, 2008; Vitaglione et al., 2010), is polyphenols (i.e. flavonoids and phenolic acids) from cocoa. Cocoa-derived polyphenols have been demonstrated as being efficacious in the banning of visceral fat deposits. Furthermore, flavanols, the main group of polyphenols, have been suggested to exert preventive effects in obesity (Kim, Shin, et al., 2004; Kim, Sohn, Lee, & Lee, 2004; Matsui et al., 2005), but the therapeutic benefits of CPs and their underlying molecular mechanism remain ambiguous. A common animal model for obesity is the high fat induced obese model, which better represents the development of human obesity than a number of genetic obesity models (Lin, Thomas, Storlien, & Huang, 2000; Murase et al., 2001). It was also reported that numerous genes encoding enzymes implicated in fat and carbohydrate metabolism were shown to comply with long-term HFD feeding (Han, Kimura, & Okuda, 1999; Kim, Sohn, Ahn et al., 2004; Phillips et al., 1996). On the other hand, two major pharmacological targets for the treatment of metabolic disorders such as dyslipidaemia and insulin resistance are the peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs) (Gervois, Fruchart, & Staels, 2007). Activation of these nuclear receptors leads to the transcriptional upregulation of multiple target genes, many of which play crucial roles in the regulation of the homeostatic process of lipid metabolism by binding to recognition sequences on the DNA called response element (RE). In pharmaceutical drugs, for example, PPARα synthetic agonists (fibrates) are used to treat hyperlipidaemic patients. Flavonoids were obviously shown to be the natural ligands of PPAR-α and LXR-α *in vitro*, suggesting that flavonoids may regulate metabolic pathways through nuclear receptor activation (Goldwasser et al., 2010; Vuppalanchi & Chalasani, 2009). Whether these effects persist *in vivo* remains unclear; thus, a global analysis of gene expression profiling in living organisms using omics technology is needed for a better understanding of the molecular mechanisms. DNA microarray is a powerful technology that has become the standard tool for monitoring multiple gene transcripts. Therefore, in this study DNA microarray was adopted to attempt to gain new shrewdness into the molecular mechanisms involved in the pharmacological actions of CPs on lipid metabolism regulating genes in the liver of diet-induced obesity rats.

2. Materials and methods

2.1. *Isolation of polyphenols from cocoa powder*

CPs were prepared as described in our previous study (Ali, Ranneh, Ismail, & Esa, 2013). Briefly, five polyphenolic molecules (gallic acid, protocatechuic acid, chlorogenic acid, epicatechin, catechin) were extracted from defatted cocoa powder (KL-Kepong Cocoa Products Sdn. Bhd., Port Klang, and Selangor, Malaysia). The polyphenolic compounds in cocoa powder were isolated and characterized using 80% (v/v) ethanol, column chromatography followed by high performance liquid chromatography methods. Afterwards, the polyphenolic substances in isolated fractions were further identified by high-performance liquid chromatography with ultraviolet detector and electrospray ionization–tandem mass spectrometry with mass detector (HPLC-UV-/ESI-MS–MS) (Agilent 1100, Palo Alto, CA,USA) performing in a negative mode. The total phenolic content (i.e. phenolic acids and flavonoids) in the cocoa polyphenolic samples was determined based on the previous methods utilized by Liu, Lin, Wang, Chen, and Yang (2009).

2.2. *Animals and treatment*

A total of thirty male Sprague-Dawley (SD) rats weighing 100–150 g (6 weeks old) were obtained from the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia. The animal study and protocol were revised and approved by the Animal Care and use Committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (Approval NO: UPM/ FPSK/ PADS/BR-UUH/00469). The SD rats were kept separately in plastic rodent cages made of spotless steel cover. The experimental rats were then acclimated for a week in laboratory conditions (26–28 °C, 50–60% humidity and under a 12:12 h light–dark cycle) to stabilize metabolic conditions prior to each study. Each rat was permitted to a normal rat chow (Gold Coin, Selangor, Malaysia) and tap water *ad libitum* during this adaptation period. After one week of acclimation with the normal rat chow, the body weight was measured (average weight 203.7 ± 4.65 g) and 30 acclimated rats were divided randomly into 3 dietary groups (n = 10 per group). A rat model of diet-induced obesity model was evolved by a feeding system with purified high-fat diet (HFD) feeding, consisting of 49% fat of total energy (kcal) from corn oil and pure ghee (milk fat) for 12 weeks. While the SD rats in the control healthy group (n = 10) were fed an LFD (14% fat of total energy, kcal) for 16 weeks. A high-fat diet was provided to the rats that were adapted for one week in groups 2 and 3 for 12 weeks. The LFD and HFD were used according to research diet formulas: D12450B and D12451, with some modifications in terms of fat content (additional data file). In this study, the energy intensity (calories) of all nutrients was given identical in each diet daily, excluding fat and carbohydrate.