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Transcriptomics expression analysis to unveil the molecular mechanisms underlying the cocoa polyphenol treatment in diet-induced obesity rats

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ABSTRACT

Cocoa polyphenol (CP), due to their biological actions, may be supplementary treatments for adipose tissue-fat gain. However, the molecular mechanism of CPs is still ambiguous. This study investigated the hypothesis that CP treatment modulates expressing of lipid metabolism genes in mesenteric white adipose tissue (MES-WAT). Sprague–Dawley (SD) rats were fed a low-fat (LF) or high-fat (HF) diet for 12 weeks. Thereafter, HFD rats (n = 10/group) were treated at a dose of 600 mg/kg bw/day CPs (HFD + CPs) for 4 weeks. DNA microarray analysis resulted in 753 genes of the 13,008 genes expressed. Bioinformatics tools showed CP treatment significantly decreased gene expression levels for lipogenic enzymes, while increased the mRNA levels responsible for lipolysis enzymes. CP administration differentially regulates gene expression involved in lipid metabolism in MES-WAT. These data unveil a new insight into the molecular mechanisms underlying the pharmacological effect of CPs on obesity biomarkers in obese rats.

1. Introduction

Adipose tissue is the hub of lipid storage in the form of triglycerides (TGs) or energy release in the form of fatty acid (FA) when demanded by any tissue in the body [12]. A lack of an energy homeostasis due to excess energy storage leads to increase white adipose tissue mass and the development of obesity [17]. However, the regulation mechanisms underlying these associations are much less known. Cocoa, the fruit of *Theobroma cacao* L., was widely used in traditional medicine as a pharmaceutical for blood pressure and cardiovascular prevention. Later, numerous cocoa-derived polyphenolic compounds (i.e. flavonoids and phenolic acids) have shown capacity of obesity preventing via adipose tissue reduction and hypolipidemia effects [18,28,31]. To date, no works have yet studied about the pharmacological effects of CPs on gene expression in diet-induce obese rats. The present study therefore was designed to investigate the hypothesis that CP administration affects expression of key genes related to lipid metabolism in the white adipose tissue of diet-induced obese rats. It has become commonly clear that several pharmaceutical drugs to treat obesity were largely not efficient in normalizing visceral obesity. Moreover, these medications most often coupled with side effects [4]. Therefore, finding new natural products to reduce visceral fat accumulation is highly beneficial for optimal and healthy treatment. A group of the pharmacological drugs to manage of metabolic related disease such as obesity and type 2 diabetes mostly act on the peroxisome prolifertor-activated receptors (PPARs).

The expression patterns of a number of target genes, many of these differentially regulate lipid metabolism and could be modulated by a variety of natural substances, e.g., fatty acids, vitamins, and polyphenols [1,13]. Genome-wide expression data have been clearly elucidated the crosstalk between adipogenesis cascade and various regulatory transcription factors both in vitro and in vivo. PPAR γ, for example, was shown to increase adipocyte hypertrophy or preadipocyte differentiation specific-genes, thereby promoting the obesity development. The hypertrophic adipocytes are frequently associated with a variety of signaling adipokines secretion such as adiponectin, leptin and resistin [26]. In clinical pharmacology, there are currently many classes of lipid-lowering drugs. The mechanisms of actions of these agents depend mainly on drug–transcription factor interaction. Sterol regulatory element-binding protein (SREBP) agonists (statins) are used to treat hypercholesterolemia via suppression HMGCoA reductase, a rate-limiting enzyme for cholesterol synthesis [15]. In the same context, PPARγ agonist thiazolidinediones (TZDs) are given to induce insulin sensitivity in adipose tissue and muscle of obese-diabetic patients, while agonists (fibrates) are given to medicate hyperlipidemia in patients by activate PPARα-target genes [9].

These transcription factors bind to the promoter region of specific genes, which in turn, increase their transcription and thereby the protein synthesis encoded by these genes [30]. A group of researchers found that a long-term high fat diet (HFD) supplementation decreased mRNA levels for lipogenic enzymes, AMP-activated protein kinase

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(AMPK), but increased PPARγ expression [11,29]. The AMPK plays a central role in regulating lipid and glucose metabolism homeostasis by sensing to the cellular level of ATP concentration. Therefore, the interesting interest toward AMPK as a possible curative target for metabolic disease maintenance has become increasingly apparent. Although the determination of molecular mechanism in living organisms is largely complicated, a global gene expression analysis under the effect of nutritional status appears to be substantial for greater understanding of transcriptional regulation. Microarray system has been increasingly proven to be a very robust tool to study expression alterations of a large number of genes in a single experiment [1,19]. Consequently, in this study, DNA microarray comprising ~13,000 transcripts coupled with validation analysis were carried out. A comparison of HFD-fed rats with a HFD + CP-treated rats partially revealed a new insight in the molecular mechanism for the pharmacological effect of CP treatment on attenuating adiposity in the MES-WAT of diet-induced obesity rats.

2. Material and methods

2.1. Isolation and characterization of polyphenols from cocoa powder

CPs were prepared as described in our previous study [2]. Briefly, five polyphenolic molecules (gallic acid, protocatechuic acid, chlorogenic acid, epicatechin, catechin) were extracted from defatted cocoa powder (40 g) (KL-Kepong Cocoa Products Sdn. Bhd., Port Klang, and Selangor, Malaysia). 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 et al. [16]. The polyphenol profiling in CP was isolated and characterized using 80% (v/v) ethanol, column chromatography followed by high performance liquid chromatography method. Polyphenolic substances 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, USA).

2.2. Animals and treatments

To mimic the development of human obesity, diet-induced obese rats were used rather than genetic obese model. 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). 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 each study. Each rat was permitted to normal rat chow (Gold Coin, Selangor, Malaysia) and tap water ad libitum during this adaptation period. After one week of acclimation with normal rat chow, the body weights were 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 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. The SD rats in normal group (n = 10) were fed low fat diet (14% fat of total energy, kcal) throughout the study. A high-fat diet was provided to the rats that were adapted for one week in groups 2 and 3 (n = 10 per group) for 12 weeks.

LFD and HFD were used according to research diet formulas: D12450B and D12451, with some modifications in terms of fat content, mimicking the fatty acid component of a human. The composition of LFD (g/kg diet) was: cornstarch, 180; sucrose, 172.8; casein, 200; corn oil, 20; cellulose, 50; S10026 mineral mix, 10; v10001 vitamin mix, 10; methionine, 3; maltodextrin, 100; and pure ghee, 20. While, the composition of HFD (g/kg diet) was: cornstarch, 160; sucrose, 172.8; casein, 200; corn oil, 90; cellulose, 50; S10026 mineral mix, 10; V10001 vitamin mix, 10; methionine, 3; maltodextrin, 100; and pure ghee, 100.

In this study, the energy intensity (calories) of all nutrients was given identical in each diet daily, excluding fat and carbohydrate. HFD intake of rats was daily recorded on a cage basis and body weight (g) was individually weighed every week to ensure the development of obesity phenotype. After 12 weeks of HFD administration, the two groups of HFD (n = 10 per each group) were distributed by sort of body weight to reduce the differences between groups and the experimental study was accordingly conducted. CP extract was primarily resolved in 3 mL of carboxymethyl cellulose (CMC) (0.03% w/v) (Sigma-Aldrich (M) Sdn Bhd, Malaysia), as an excellent solvent and was administrated every day through gastric gavage by using a needle of force feeding throughout the treatment period. HFD rats in the test group were treated with a dose of 600 mg/kg bw/day of CPs (CP group) and those in the control group were supplemented with a dose of 600 mg/kg bw/day of carboxymethyl cellulose (CMC) for 4 weeks (control group).

The dosage in this study was provided based on our previous study, in which the above concentration has demonstrated biological activities in vivo [20] and it was adequately equivalent to the dose of fenofibrate in the treatment of dyslipidemia in adult [5]. The diet that rats were fed while being treated with the CPs was normal rat show. During the experimental study, the rats' body weight was weekly recorded, while the diet and energy (kcal) were daily measured per rat. The rate in body weight changes upon CP treatment was determined via this equation: Weight decrease (g) = initial body weight (g) − final body weight (g), and weight decrease (%) = weight decrease (g)/initial body weight (g) × 100%. This equation was only used for HFD and CP groups at the end of this study (Table 1).

Table 1
Effects of CPs on physical and biochemical parameters in the HFD-induced obesity SD rats as compared with both LFD and HFD groups.[a]

Group determination	LFD	HFD	HFD + CPs
Initial body weight (g) before treatment	203.7 ± 4.65^{b}	573.93 ± 7.44^{a}	575.49 ± 4.38^{a}
Final body weight (g) after treatment	327.1 ± 11.82^{c}	529.56 ± 9.18^{a}	395.19 ± 3.32^{b}
Body weight loss (g)	ND	44.37 ± 6.29^{a}	180.3 ± 3.93^{b}
Weight decrease (%)	ND	7.73 ± 1.76^{c}	31.32 ± 1.75^{a}
Food intake (g/rat/day)	29 ± 0.44^{a}	24.58 ± 0.58^{a}	27 ± 0.69^{a}
Energy intake (kcal/rat/day)	83 ± 2.17^{a}	86 ± 1.6^{a}	85 ± 1.9^{a}
Water intake (mL/rat/day)	39.36 ± 0.75^{a}	43.57 ± 1.42^{a}	37.74 ± 0.61^{a}
Heart (g)	1.12 ± 0.05^{b}	1.8 ± 0.08^{b}	1.32 ± 0.11^{b}
Kidneys (g)	1.14 ± 0.09^{a}	1.9 ± 0.18^{a}	1.3 ± 0.04^{a}
Spleen (g)	0.68 ± 0.06^{a}	1.2 ± 0.2^{a}	0.89 ± 0.09^{a}
Liver (g)	11.7 ± 1.5^{c}	25.7 ± 1.2^{b}	14.5 ± 1.3^{a}
MES-WAT (g)	8.2 ± 0.7^{a}	21 ± 1.6^{b}	13.3 ± 0.097^{c}
Perirenal WAT	1.7 ± 0.03^{c}	3.9 ± 0.4^{b}	2.6 ± 0.1^{a}
Epididymal WAT	3.52 ± 3.24^{a}	7.7.58 ± 15.54^{c}	5.17 ± 7.04^{b}
Serum			
Triglyceride (mmol/L)	4.12 ± 1.42^{a}	6.42 ± 0.57^{b}	5.03 ± 0.30^{a}
Total cholesterol (mmol/L)	3.41 ± 0.16^{a}	4.83 ± 0.15^{b}	3.58 ± 0.17^{a}
LDL-cholesterol (mg/dL)	11.72 ± 0.53^{a}	12.77 ± 0.84^{a}	12.19 ± .61^{a}
HDL-cholesterol (mg/dL)	50.28 ± 2.55^{a}	44.73 ± 4.2^{a}	52.68 ± 2.49^{a}
Free fatty acid (mmol/L)	1.26 ± 0.16^{b}	1.63 ± 0.4^{b}	0.97 ± 0.12^{a}
Insulin (ng/mL)	1.46 ± 0.77^{b}	1.93 ± 1.17^{b}	0.90 ± 0.42^{a}
Leptin (ng/mL)	43.4 ± 11.8^{a}	48.3 ± 12.5^{a}	19.8 ± 5.03^{b}
Adiponectin (μg/ml)	66.5 ± 13.6^{b}	70 ± 14.0^{b}	55.9 ± 12.3^{a}

LFD, low fat diet; HF, high fat diet; HFD + CPs, high fat + cocoa polyphenols; ND, not detected; LDL, low density lipoprotein; HDL, high density lipoprotein; MES-WAT, mesenteric white adipose tissue. Details of the nutrient contents, feeding and treatment period are given in the "Material and methods" section. Data in a row with superscripts without a common letter were significantly different, $p < 0.05$.

[a] Each value is represented as mean ± SDs (n = 10 per group).