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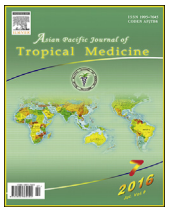

Original research http://dx.doi.org/10.1016/j.apjtm.2016.05.017

Green coffee bean extract improves obesity by decreasing body fat in high-fat diet-induced obese mice

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ARTICLE INFO

Article history:
Received 15 Apr 2016
Received in revised form 16 May 2016
Accepted 23 May 2016
Available online 30 May 2016

Keywords:
Green coffee bean extract
High-fat diet induced obese mice
Adipogenesis
Body fat
Dual energy X-ray absorptiometry

ABSTRACT

Objectives: To evaluate possible lipid catabolism and body fat regulation effects of 3-caffeoylquinic acid in Green coffee bean extract (GCBE) in high-fat diet (HFD)-induced obese mice.

Methods: Obesity was induced in mice using a HFD for four weeks. Then, mice were fed only HFD or HFD with GCBE at 50, 100, and 200 mg/kg. Fatty acid synthesis mechanism regulation of body fat was investigated through real-time PCR and Western blot assay. Body fat reduction was measured through dual-energy X-ray absorptiometry.

Results: In HFD-induced obese mice, GCBE treatment significantly decreased body weight gain, liver weight and white adipose tissue weights with regulation of adipose tissue lipolysis hormones, like adiponectin and leptin. GCBE treatment decreased mRNA expression levels of adipogenesis and adipocyte metabolism related genes in adipose tissues and the liver, and decreased the corresponding protein expression. Dual energy X-ray absorptiometry measurements were used to compare body fat between mice on high-fat and those treated with GCBE. GCBE treated mice had a lower fat mass compared to HFD alone fed mice and relative body weight and fat mass were markedly decreased.

Conclusions: GCBE has a potential anti-obesity effect with lowering body fat accumulation by regulating adipogenesis and lipid metabolism-related genes and proteins in WAT and liver.

1. Introduction

In recent decades, obesity has become a serious clinical disease that is contributed by a high-fat diet. The World Health Organization defines obesity was abnormal or excessive fat accumulation is present in many diseases. Another definition of obesity is the accumulation of body fat from the imbalance between calorie input and energy expenditure. Incremental evidence suggests obesity is related to epidemiological diseases including diabetes, heart disease, stroke, arthritis, inflammation, and cancers [1]. Therefore, the role of fat in obesity development is important to study to prevent and treat obesity. Adipocytes store energy in triglyceride form and break down lipids into free fatty acids when energy is required [2]. Furthermore, adipocytes play a major role in obesity and related disease through the secretion of wide range of regulatory factors. Remarkably, adipocytes hormonally control metabolism through the secretion of autocrine, paracrine, and endocrine hormones and effect insulin sensitivity, immune function, eating behavior, and most importantly regulate differentiation of preadipocytes into adipocytes [3].

Polyphenols are abundant secondary metabolites in plants and are known to prevent diseases associated with oxidative stress and its related complications. The glycosylated derivate forms of polyphenol, chlorogenic acids (CGA) (ester of caffeic

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Peer review under responsibility of Hainan Medical College.
Foundation project: This work was supported by the Cooperative Research Program for Agriculture Science & Technology Development (No. PJ01134802).

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acid and quinic acid) are the main polyphenol in coffee [4]. There is an increase in scientific evidence that coffee affects metabolic syndromes such as obesity, type 2 diabetes, atherosclerosis, and insulin-resistance [5–9].

Green coffee is raw coffee beans that have not been roasted. Many different pharmacological studies about green coffee bean extract (GCBE) demonstrates that the CGA in green coffee regulates hypertensive, vasoreactivity, and glucose metabolism [10–12]. There are several prospective studies regarding how 5-caffeoylquinic acid (5-CQA), the major chlorogenic acid in coffee, decreases diabetes risk by decreasing glucose uptake in the small intestine. However, only the short-term effects were analyzed and more research is needed [7,13]. In particular, recent studies propose that attenuation of obesity and lipid accumulation by green coffee bean extract is derived from 5-CQA in diet-induced obesity and insulin resistance [14,15].

In the present study, we investigate a quantitative analysis of the 3-caffeoylquinic acid (3-CQA) in GCBE and examined whether it has an ameliorative effect against high-fat diet (HFD, 60% calories from fat) induced obesity in mice. Furthermore, decreases in lipid accumulation and metabolism related genes, proteins, and body fat composition provide scientific evidence to support GCBE as a supplement to prevent obesity.

2. Materials and methods

2.1. Chemicals and reagents

Isopropanol, TRI reagent and protease inhibitor cocktail were purchased from Sigma–Aldrich (MO, USA). HPLC grade acetonitrile and phosphoric acid were purchased from Merck (Darmstadt, Germany). Polyclonal antibodies against FAS, SREBP-1c, PPARγ, C/EBPα, AMPK, phospho-AMPK, PPARα and β-actin were purchased from Santa Cruz Biotechnology (CA, USA). Horseradish peroxidase-linked anti rabbit IgG and HRP-linked anti mouse IgG were purchased from Bio-Rad (CA, USA). SYBR Green reaction buffer was purchased from Takara (Shiga, Japan).

2.2. Compound analysis of GCBE

GCBE was provided from KPLC group (Montagne, France). Chromatographic analysis of 3-CQA in GCBE was performed using the Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA) with a UV detector. The C18 column (5 μm, 4.6 × 250 mm; Supelco, MO, USA) was maintained at 40 °C for chromatographic separation. The mobile phase was mixture of 0.5% phosphoric acid in distilled water (A) and 0.5% phosphoric acid in acetonitrile (B) and delivered at 1.2 mL/min in a gradient flow as follows: 0 min 8.0%, followed by 20 min 25.0%, 35 min 100.0%, and 45 min 8%. The injected volume was 5.0 μL and optimal detection was achieved at 325 nm. The standard samples for 3-CQA was purchased from Sigma Aldrich (MO, USA) and freshly prepared for analysis.

2.3. Animal studies

Male C57BL/6J mice were obtained from DaeHan BioLink (Chungbuk, South Korea) at four weeks of age. The mice were individually housed in stainless steel cages and were maintained under temperature of (23 ± 3) °C in a humidity-controlled room with a 12–12 h light–dark cycle. All mice were given free access to water and food. After acclimatization for one week, they were fed either the normal-fat diet (NFD, n = 8, certified irradiated global 18% protein diet, 2918C, Harlan Laboratories, Indiana, USA) or High-fat diet (HFD, n = 40, Rodent diet with 60% Kcal from Fat, #101556; Research Diets, USA) for four weeks to induce obesity. After obesity induction, the mice were divided into five experimental groups (n = 8/group) and were matched by body weight.

The following five groups were studied for six weeks: normal-fat diet, HFD, and HFD with oral administration of GCBE at 50 mg/kg of body weight (HFD + GCBE 50); HFD with GCBE at 100 mg/kg of body weight (HFD + GCBE 100); HFD with GCBE at 200 mg/kg of body weight (HFD + GCBE 200). Food intake of the mice was recorded daily and their body weights were measured twice per week. At the end of the experiment, the mice were anesthetized and the liver, kidney, and white adipose tissue (WAT) were excised immediately. Each tissue was then rinsed with phosphate-buffered saline, and stored at −80 °C until analysis. The experimental procedures were approved by Ethics Committee of the Wonkwang University (Iksan, Korea) and the mice were maintained in accordance with their guidelines.

2.4. Plasma biochemical analysis

After six weeks of feeding, 12 h fasted mice were anesthetized using ether and blood was collected. Collected samples were centrifuged at 2500×g for 15 min at 4 °C for biochemical analyses of plasma parameters. The separated plasma was stored at −80 °C until analysis. The levels of serum glucose, total cholesterol (T-CHO), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), Alanine transaminase (ALT), Aspartate transaminase (AST), free fatty acid (FFA), leptin, and adiponectin were measured using commercial kits (Sigma–Aldrich, MO, USA) according to the manufacturer's instructions.

2.5. Histological analysis

Liver and adipose tissue were dissected, fixed in 10% neutral buffered formalin, and embedded in paraffin for histological examination. The formalin-fixed and paraffin-embedded tissue blocks were cut to a thickness of 4 μm and stained with hematoxylin and eosin (H&E). The sections were photographed under 200× magnification.

2.6. Protein extraction and Western blot analysis

Cold phosphate-buffered saline washed adipose and liver tissues were homogenized in RIPA buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM PMSE) and 1% protease inhibitor cocktail. The homogenates were centrifuged 8000×g for 15 min at 4 °C and the supernatants were collected. Total protein concentration was calculated by BCA protein assay (Pierce, IL, USA). Proteins were separated on 10% sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gels and transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were blocked with 5% non-fat skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and were incubated overnight at 4 °C with