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Anti-diabetic and anti-lipidemic effects of chlorogenic acid are mediated by ampk activation☆

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ABSTRACT

Chlorogenic acid (CGA) has been shown to stimulate glucose uptake in skeletal muscle through the activation of AMPK. However, its effect on other metabolic pathways and likewise its effects after long-term consumption have yet to be understood. We investigated the effects of CGA on glucose tolerance, insulin sensitivity, hepatic gluconeogenesis, lipid metabolism and skeletal muscle glucose uptake in *Lepr*$^{db/db}$ mice. Hepatoma HepG2 was used to investigate CGA's effect on hepatic glucose production and fatty acid synthesis. Subsequently, we attempted to evaluate whether these effects of CGA are associated with the activation of AMPK. In *Lepr*$^{db/db}$ mice, acute treatment with CGA lowered $AUC_{glucose}$ in an OGTT. Chronic administration of CGA inhibited hepatic G6Pase expression and activity, attenuated hepatic steatosis, improved lipid profiles and skeletal muscle glucose uptake, which in turn improved fasting glucose level, glucose tolerance, insulin sensitivity and dyslipidemia in *Lepr*$^{db/db}$ mice. CGA activated AMPK, leading to subsequent beneficial metabolic outcomes, such as suppression of hepatic glucose production and fatty acid synthesis. Inhibition and knockdown of AMPK abrogated these metabolic alterations. In conclusion, CGA improved glucose and lipid metabolism, *via* the activation of AMPK.

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1. Introduction

Pharmacological management of diabetes shows promising results but all are associated with unwanted side effects. For instance, sulfonylureas initiated insulin release even when glucose level is low and therefore are more likely to cause hypoglycemia [1]. Thiazolidinediones often caused weight gain which will further deteriorate insulin resistance [2] and increase cardiovascular mortality risk, *e.g.* pioglitazone [3]. While exercise is effective, sustained benefits are difficult to achieve due to difficulties in compliance with consistent life-style changes. Dietary intervention through intake of polyphenol-rich plant products that can modulate glucose metabolism is another modality of management.

Chlorogenic acid (CGA), a type of hydroxycinnamic acids, occurs in many types of fruits and in high concentration in coffee [4]. CGA-rich coffee consumption has been associated with a lower risk of Type 2 diabetes mellitus (T2DM) [5,6]. CGA has been shown to inhibit glucose-6-phosphate translocase 1 and to reduce the sodium gradient-driven glucose transport in the intestine [7]. It suppresses hepatic gluconeogenesis through the inhibition of glucose-6-phosphatase (G6Pase) activity [8]. In a cross-over trial, 1 g CGA caused significant reduction in early fasting glucose and insulin responses to glucose in overweight men during an oral glucose tolerance test [9]. Besides, there are studies demonstrating that CGA stimulates glucose uptake in myotubes [10] and adipocytes [11].

Recently, our lab showed that CGA stimulates glucose uptake in skeletal muscle through the activation of AMP-dependent kinase (AMPK) [12]. However, its effect on other metabolic pathways that regulates blood glucose levels is not yet fully explored. Likewise, the effect of long-term consumption of CGA in T2DM is still to be elucidated as the beneficial metabolic effects of coffee on T2DM result mainly from the long-term consumption of the beverage. Our current study thus investigated the effect of CGA on glucose tolerance before and after 2-week treatment in *Lepr*$^{db/db}$ mice. We also examined the effect of 2-week treatment with CGA on various organs involved in glucose metabolism. Hepatoma HepG2 cell line was used to study CGA's effect on hepatic glucose production. Our study also investigated the effect of CGA on lipid metabolism in both *in vivo* and *in vitro* models as previous study [13] has shown that CGA enhances fat metabolism in the liver. We subsequently also evaluated whether these effects of CGA are associated with the activation of AMPK.

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2. Materials and methods

2.1. Reagents

CGA, DMEM, Krebs-Ringer bicarbonate buffer (KRBB), antibiotic/antimycotic, insulin, wortmannin, cytochalasin B, Fluoroshield with DAPI, Oil Red O, STO-609 and AMP were obtained from Sigma (St. Louis, MO). HepG2 hepatocytes were obtained from ATCC (Manassas, VA, USA). FBS was from Hyclone (Cramlington, UK). DMSO was purchased from MP Biomedicals (Illkirch, France). Glucose oxidase kits, Infinity^{TM} Tryglyceride and Total Cholesterol reagent kits were obtained from Thermo Scientific (Waltham, MA). Compound c and NP 40 were obtained from Merck (Darmstadt, Germany). [γ-^{32}P]-ATP, NaH^{14}CO and ^{14}C-sodium acetate were purchased from PerkinElmer (Waltham, MA). Protease inhibitor cocktail was purchased from Abcam (Cambridge, UK). AMPK α1/2 siRNA and an unrelated siRNA (control siRNA-A) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All antibodies (otherwise stated elsewhere) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-GAPDH and anti-phospho-ACC were from Cell Signaling Technology (Danvers, MA) and Millipore (Billerica, MA) respectively. Oligofectamine, Alexa Fluor 555-conjugated mouse IgG, Fluo-4 direct calcium assay kit and OPTI-MEM were purchased from Invitrogen (Carlsbad, CA). Ultra-sensitive Rat Insulin and Leptin ELISA kits were obtained from Crystal Chem Inc. (Downers Grove, IL) while adiponectin ELISA kit was obtained from Bertin Pharma (Artigues Pres Bordeaux, France). Free fatty acids detection kits were purchased from Wako Diagnostics (Richmond, VA). G-Sepharose beads and ECL detection kit were obtained from GE Healthcare (Piscataway, NJ). SAMS peptide was purchased from Tocris Bioscience (Minneapolis, MN).

2.2. Experimental animals

Lepr$^{db/db}$ mice were obtained from The Jackson Laboratory (Sacramento, CA). Ten C57BL/6 mice were purchased from Centre for Animal Resources (CARE), National University of Singapore (NUS). They were allowed to acclimatize to conditions in the Animal Holding Unit (AHU), NUS. They were housed throughout the experiment on a 12 h light/dark cycle. Water and feeds were available to the animals *ad libitum.*

2.3. Ethics statement

The Principles of Laboratory Animal Care (NIH, 1985) were followed throughout the duration of the experiment. The experimental protocol for animal study was approved by NUS Institutional Animal Care and Use Committee [Protocol No: 085/07(A3)10].

2.4. Oral glucose tolerance test

Lepr$^{db/db}$ mice were randomly assigned to five groups ($n = 4$) and four C57BL/6 mice were assigned as lean control group. They were fasted for six hours before the test. For inhibitor study with compound c, mice were pre-treated with i.p. 50 mg/kg compound c. Blood samples were collected from the tail vein

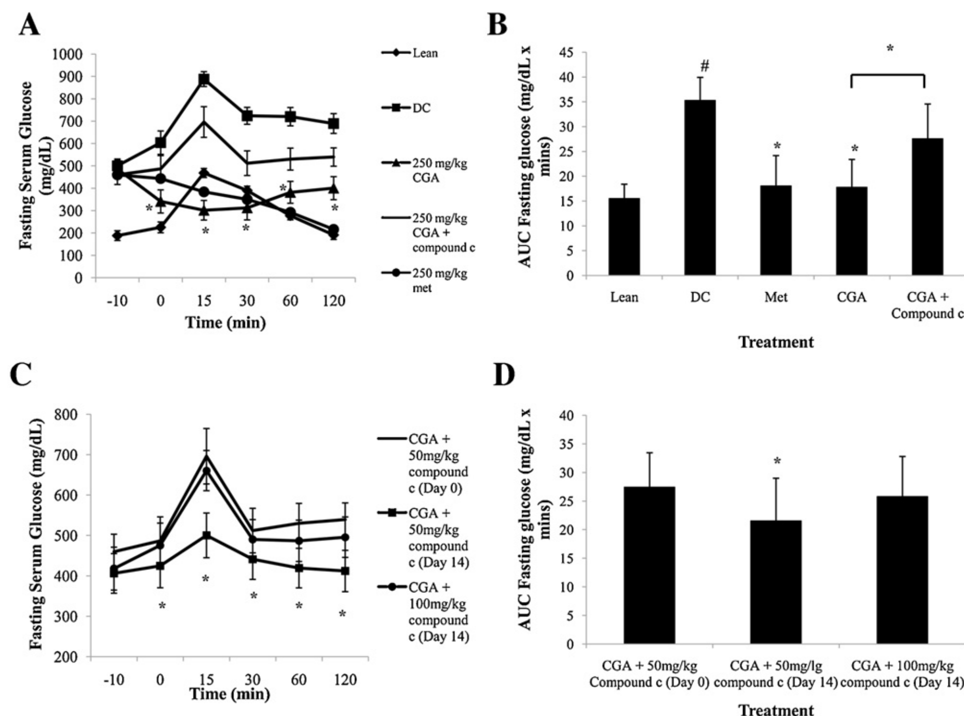

Fig. 1. Acute effects of CGA on glucose tolerance in *Lepr*$^{db/db}$ mice and decreased inhibitory effect of compound c in suppressing CGA-mediated glucose lowering after 2-week treatment with CGA. (A) Oral glucose tolerance test was performed in 6 h fasted *Lepr*$^{db/db}$ mice. For inhibitor study, mice were pre-treated with compound c for 10 min. Blood samples were collected from the tail vein for fasting glucose measurement before treatments (vehicle, ip 250 mg/kg CGA, oral 250 mg/kg metformin). Ten minutes after the treatments, blood samples were collected again followed by oral gavaging of 2 g/kg glucose. Blood samples were collected 15, 30, 60 and 120 min after the glucose challenge. (B) AUC of fasting glucose levels in OGTT (A). (C) Efficiency of different concentrations of compound c in inhibiting CGA-mediated glucose lowering was measured before and after 2-week treatment with CGA. Pre-treatment of compound c was performed intreaperitoneally 10 min before administration of CGA. (D) AUC of fasting glucose levels in OGTT (C). Data shown represent the means ± SE of three independent experiments. (A–B) $^{*}P < 0.05$ compared to diabetic control, $^{*}P < 0.05$ compared to CGA only-treated mice, #$P < 0.05$ compared to lean control. (C–D) $^{*}P < 0.05$ compared to mice treated with compound c at Day 0. DC = Diabetic Control, Met = Metformin.

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