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Green tea decoction improves glucose tolerance and reduces weight gain of rats fed normal and high-fat diet☆

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Abstract

Green tea containing polyphenols exerts antidiabetic and antiobesity effects, but the mechanisms involved are not fully understood. In this study, we first analyzed and compared polyphenol compounds [epigallocatechin gallate (EGCG), epigallocatechin (EGC)] in decoction of green tea leaves versus usual green tea extracts. Second, the effects of acute (30 min) or chronic (6 weeks) oral administration of green tea decoction (GTD) on intestinal glucose absorption were studied *in vitro* in Ussing chamber, *ex vivo* using isolated jejunal loops and *in vivo* through glucose tolerance tests. Finally, we explore in rat model fed normal or high-fat diet the effects of GTD on body weight, blood parameters and on the relative expression of glucose transporters SGLT-1, GLUT2 and GLUT4. GTD cooked for 15 min contained the highest amounts of phenolic compounds. In fasted rats, acute administration of GTD inhibited SGLT-1 activity, increased GLUT2 activity and improved glucose tolerance. Similarly to GTD, acute administration of synthetic phenolic compounds (2/3 EGCG+1/3 EGC) inhibited SGLT-1 activity. Chronic administration of GTD in rat fed high-fat diet reduced body weight gain, circulating triglycerides and cholesterol and improved glucose tolerance. GTD-treated rats for 6 weeks display significantly reduced *SGLT-1* and increased *GLUT2* mRNA levels in the jejunum mucosa. Moreover, adipose tissue *GLUT4* mRNA levels were increased. These results indicate that GTD, a traditional beverage rich in EGCG and EGC reduces intestinal SGLT-1/GLUT2 ratio, a hallmark of regulation of glucose absorption in enterocyte, and enhances adipose GLUT4 providing new insights in its possible role in the control of glucose homeostasis.

Keywords: Green tea decoction; EGCG; EGC; SGLT-1; GLUT2; GLUT4; Intestine

1. Introduction

Obesity and type 2 diabetes are major public health issues worldwide contributing to increase morbidity and mortality. Although these diseases draw a large attention, prevention and treatment of obesity and type 2 diabetes have not been resolved yet [1]. However, particular dietary compounds, such as polyphenols, may assist in type 2 diabetes prevention in ways other than weight control. Dietary polyphenols are chemicals of plant origin that are abundant in fruits, vegetables, chocolate and nuts, as well as in beverages such as tea, coffee, wine and soy milk [2]. As such, polyphenols are the most abundant antioxidants in the diet of human beings [3]. Dietary polyphenols consumption is associated with lower rates of diabetes and cardiovascular diseases [3–6]. Studies indicate that green tea (*Camellia sinensis*) has wide effects on diabetes and obesity in animals and humans [7,8]. The most important polyphenolic compounds in green tea are catechins: epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC) and epicatechin [9]. Green tea contains also high levels of other bioactive phenols such as caffeine. Some epidemiological and clinical studies have shown the health benefits of EGCG on obesity and diabetes [10,11] and the underlying mechanisms involve modulations of energy balance, endocrine systems, food intake, lipid and carbohydrate metabolism, and redox status [12]. Green tea flavonoids were also shown to have insulin-like activities [13] as well as insulin enhancing activity [14]. In addition, green tea powder and its polyphenols decrease fasting plasma levels of glucose, insulin, triglycerides and free fatty acid [15] and conversely insulin-stimulated glucose uptake increase in rats fed green tea extract and polyphenols for 12 weeks [15]. In the literature, most investigations on tea beverages explored tea leaves, green tea extracts or their derivatives (catechins) but not green tea decoction (GTD), which nevertheless is a very popular beverage through large areas of North Africa, especially in Tunisia. GTD is characterized by the cooking of the dried tea leaves in boiling water for a variable period of time, overall not exceeding 60 min. The beneficial effects of tea decoction likely depend on the preservation of its polyphenol and caffeine

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contents and activities after the cooking process. No studies were performed to directly examine the effects of GTD on intestinal sugar absorption. The aim of this study was as follows: first, to analyze and compare contents of polyphenol compounds (EGCG, EGC and caffeine) in decoction of green tea leaves versus usual green tea extracts, and second, to explore the effects of acute or chronic oral administration of short-time cooked GTD on intestinal glucose transporter activities and resulting glucose homeostasis in rat fed normal diet (ND) or high-fat diet (HFD).

2. Methods and materials

2.1. Animals

Male Wistar rats weighing 240–280 g (Centre Elevage Janvier, Le Genest-St-Isle, France) were caged under standard laboratory conditions with tap water and regular food provided *ad libitum*, in a 12-h/12-h light/dark cycle at a temperature of 21–23°C. The animals were treated in accordance with the European Community guidelines are based on declaration of Helsinki concerning the care and use of laboratory animals, and all efforts were made to minimize animal suffering and the number of animals used.

2.1.1. Preparation of green tea decoction or infusion

The tea was freshly prepared throughout the experimental period. Fifty grams of green tea leaves (*C. sinensis*) purchased from local market was soaked in 1 liter of hot water and cooked for 15, 30 or 60 min, then cooled to room temperature before analysis and distribution. For comparison, the usual "tea extract" was prepared in the form of green tea infusion (GTI), which consist of 50 g of tea leaves and 1 liter of hot water, brewed for about 3 min. All studies were performed with same batches of tea to avoid possible variations in the properties of the green tea sample.

2.1.2. Determination of total polyphenol compounds

Briefly, 1 ml of GTD or GTI was mixed with 3 ml of water, vortexed vigorously and sonicated for 20 min. Then, 7 ml of cold acetone (−20°C) was added to the mixture. Following a centrifugation at 10,000×*g* for 15 min, the residue was reextracted twice with 5 ml acetone (−20°C). The supernatants were collected, pooled and concentrated using a rotary evaporator (60°C) to a final volume of 3 ml [16]. To prevent oxidation of the polyphenols, extraction was rapidly achieved and extracts were immediately used or stored in darkness at −20°C until further use. The total polyphenol compound (TPC) in GTD or GTI was estimated spectrophotometrically by the Folin–Ciocalteu assay using gallic acid as standard. The profile of polyphenols from GTD and GTI was further analyzed from extract by RP-HPLC-MS technique [17].

2.1.3. Acute administration of GTD or solution of EGCG+EGC

Animals were fasted overnight with free access to water. The day of the experiment, animals were given an oral load (0.5 ml/100 g BW) of water (control), GTD or a solution containing 4 mg EGCG+2mg EGC (Sigma Aldrich, France). After 30 min, the animals were sacrificed and the jejunum (5 cm distal to the ligament of Treitz) was dissected out and rinsed in cold Krebs–Ringer bicarbonate (KRB) solution of the following composition: NaCl 115.4 mM, KCl 5 mM, $MgCl_2$ 1.2 mM, NaH_2PO_4 0.6 mM, $NaHCO_2$ 25 mM and $CaCl_2$ 1.2 mM to remove luminal contents. In some experiments, one segment of the jejunum was also used for galactose transport using the isolated jejunal loops *in vitro*.

2.1.4. Ussing chamber

Intestine was opened along the mesenteric border and placed between the two halves of an Ussing chamber (Easy Mount P2312; Physiologic Instrument, San Diego, CA, USA) (exposed area: 0.50 cm^2) and bathed with 4 ml of KRB solution and glucose 10 mM or mannitol 10 mM in serosal and mucosal compartment, respectively. Solutions were gassed with 95% O_2–5% CO_2 and kept at a constant temperature of 37°C (pH at 7.4). Electrogenic ion transport was monitored continuously as the short-circuit current (Isc) by using an automated voltage clamp apparatus (DVC 1000; WPI, Aston, England, UK) linked through a MacLab 8 to a MacIntosh computer. Tissue ionic conductance was calculated according to Ohm's law. Sodium-dependent glucose transporter SGLT-1 was challenged by 10 mM glucose apically as described [18]. After a plateau was reached, tissues were challenged by serosal addition of 100 μM cholinergic agonist carbachol to stimulate electrogenic chloride secretion. Results were expressed as the intensity of the Isc (μA/cm^2) or as the difference (ΔIsc) between the peak Isc (measured within 10 min) and the basal Isc (measured just before the addition of the compound).

2.1.5. Transport of ^{14}C-galactose in rat jejunum loops in vitro

The jejunal segments from water or GTD-treated rats were used. Briefly, a 5-cm segment of jejunum was filled with KRB solution pH 6 containing 30 mM D-galactose added with 0.1 μCi/ml [D-^{14}C]-galactose (specific activity 57 mCi/mmoL) and mucosal-to-serosal transport of ^{14}C-galactose was monitored. Jejunal segments were ligated at both ends and were incubated at 37°C in a thermostated bath of KRB solution at pH 7.4. Samples were withdrawn from the bath at t=0, 5, 15 and 30 min. ^{14}C-galactose concentration was calculated after radioactivity was measured using a beta counter (LS 6000 TA liquid scintillation counter). The effects of GTD on transport of [^{14}C]-mannitol as nontransported substrate were also determined. The incubation medium was sampled and radioactivity was counted. Apparent permeability (P_{app}) was used to assess mucosal-to-serosal transport according to the following equation $P_{app}=(dQ/dt)\cdot(V/Q_0\cdot A)$, where V is the volume of the incubation medium, A is the area of the loop, Q_0 is the total radiolabeled galactose introduced into the loop and dQ/dt is the flux across the jejunum loop.

2.1.6. Glucose tolerance tests

Rats were fasted for 16 h before being subjected to an oral glucose tolerance test or an intraperitoneal glucose tolerance test. The animals were gavaged with water or GTD (0.5 ml/100 g BW) 30 min before the tests. Glucose (2 g/kg) was given as an oral load by gavage or through an intraperitoneal injection. Blood was sampled from the tail vein before (t=0) and 15, 30, 60 and 120 min after the administration of glucose. Glycemia was measured with the ACCU-CHEK System (Roche Diagnostics, Meylan, France), and the areas under the curves were calculated.

2.1.7. Chronic administration of GTD in ND or HFD-fed rats

Rats were fed *ad libitum* ND or HFD (1320 and C1090-45 Genestil, Altromin) without (control) or with GTD for 6 weeks. The ND provides 2820 kcal/kg of food and contains 3% fat accounting for 9.6% of calories, 48% complex carbohydrates accounting for 67.7% of calories (primarily starch) and 16% protein providing 22.7%

Table 1
Sequences of primers used for real-time quantitative polymerase chain reaction

Gene name	Accession number	Primers (5′–>3′)
β-Actin	NM_031144.3	Forward CCCGCGAGTACAACCTTCT
		Reverse CGTCATCCATGGCGAACT
SGLT-1	NM_012879.2	Forward GAAGGGTGCATCGGAGAAG
		Reverse CAATCAGCACGAGGATGAAC
GLUT2	NM_013033.2	Forward AAAGCCCCAGATACCTTTACCT
		Reverse TGCCCCTTAGTCTTTTCAAGC
GLUT4	NM_012751.1	Forward TTGCAGTGCCTGAGTCTTCTT
		Reverse CCAGTCACTCGCTGCTGA