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Green tea influences intestinal assimilation of lipids in humans: a pilot study

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Abstract. – OBJECTIVE: Many data show that green tea (GT) consumption has a beneficial effect on human health, including antiinflammatory, antibacterial and anticarcinogenic activities. However, there are no data on the effect of long-term GT intake on lipid assimilation not related to luminal processes. Therefore, in the present study, we aimed to assess the impact of a three-month diet enriched in green tea extract (GTE) on lipid digestion and absorption in obese humans with metabolic syndrome.

PATIENTS AND METHODS: Eight obese subjects aged 56-65 years, for three months, consumed a daily portion of GTE enriched bread. 13C-labelled mixed triglyceride breath test (13C MTG-BT) was performed twice; once before and once after three months of GTE consumption. Cumulative percentage dose recovery (CPDR) was assumed to reflect digestion and absorption of lipids.

RESULTS: Energy and macronutrient intake was stable within the period study. No significant changes in basic anthropological parameters (body weight, BMI, WC, WHR), body fat content (expressed as absolute and relative values), as well as of energy expenditure in the course of the study were observed. Significant decrease in lipid digestion and absorption as assessed using the 13C MTG-BT was observed. CPDR was lower after GTE intake (median <1st-3rd quartile>: 20.8% <14.9-25.6> vs. 15.5 <12.3-20.5>; $p < 0.009$).

CONCLUSIONS: Long-term diet containing GTE decreases lipid assimilation, but probably without involvement of luminal effects. However, further studies are needed to confirm this hypothesis and to clarify underlying mechanism.

Key Words:
Green tea, Metabolic syndrome, Breath test.

Introduction

Many data show that green tea (GT) consumption has a beneficial effect on human health, including antiinflammatory, antibacterial and anticarcinogenic activities[1]. It seems that its health promoting effect is associated with the content of catechins: (-)-epigallocatechin (EGC), (-)-epicatechin (EC) or (-)-epigallocatechin gallate (EGCG). The last one is a GT polyphenol that seems to be primarily responsible for the positive influence on the human body[1-3]. There are numerous studies suggesting that EGCG may be helpful in body weight reduction[4-7] so GT may be useful in the prevention of obesity and its complications, and thus act as a so-called functional food[8].

The effect of GT EGCG is associated with the inhibition of lipid accumulation in adipocytes[9]. GT increases postprandial thermogenesis and fat oxidation[7,10] and it leads to body fat reduction. We have recently documented that body weight loss caused by GT may be also related to decreased lipid digestion and absorption mediated by luminal mechanisms[11]. In humans, dietary triglycerydes are first hydrolyzed in the stomach by gastric lipase, breaking down 10-30% of consumed triglycerydes, and then lipolysis ends up in the duodenum where pancreatic lipase acts[6]. One of the probable mechanisms is associated with EGCG's ability to form complexes with lipids and lipolytic enzymes. It may influence a fat droplet's size making them larger. This likely interferes with the emulsification, hydrolysis and micellar solubilization of lipids[6,9,12-14].

The inhibition of digestive processes by tea catechins seems to explain the above-mentioned phenomenon[6,15]. However, there are no data on the effect of long-term GT intake on lipid assimilation not related to luminal processes. Therefore, in the present study, we aimed to assess the impact of a three-month diet enriched in green tea extract (GTE) on lipid digestion and absorption in obese humans with metabolic syndrome.

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Patients and Methods

Patients Characteristic

Eight obese subjects (7 females and 1 male), aged 56-65 years (median value – 61.5 years), with metabolic syndrome (MS) were enrolled into the study (Table I). Inclusion criteria comprised both of willigness to participate and normal exocrine pancreatic function (fecal elastase-1 concentration > 200 μg/g)[16,17]. Exclusion criteria included: antibiotic therapy within the preceding month, acute or chronic diarrhea, celiac disease, pancreatitis, or severe systemic disease. Other exclusion criteria were: a history or presence of substance abuse, eating disorders, excessive alcohol intake, smoking, significantly abnormal laboratory test results (for example, a systolic blood pressure ≥ 180 mmHg and/or a diastolic blood pressure ≥ 110 mmHg), and previous gastrointestinal surgery for weight reduction. In addition, patients were excluded if they showed a dietary restraint score greater than 14, if they ingested more than 100 mg CAF per day (from coffee, tea, chocolate, cola, or energy drinks), or if they habitually drank GT.

In all subjects, body height (BH) was measured using an anthropometer (model WPT 200.0; Rad Wag, Radom, Poland) at the time of entry into the study. Body weight (BW), waist circumference (WC) and fat mass (FM) at the beginning and end of the study were measured. BW was measured using an electronic scale (model WPT 200.0; Rad Wag, Poland), to an accuracy of 0.1 kg, in the morning before breakfast and after defecation, with the subjects dressed only in their underwear. WCs were measured 3 times at the site of the shortest circumference between the rib cage and the ileac crest, using a non-stretch metric tape, with subjects in the standing position. All the circumference measures were recorded to the nearest 0.1 cm. Body composition in terms of adipose tissue (fat mass – FM), was determined immediately after BW measurements with the bioelectric impedance technique using a BIA 101S (Akern-Rjl, Pontassieve, Florence, Italy) bioanalyzer, according to the recommendations of Lukaski et al[18]. To avoid subjective error, all measurements were performed by the same highly qualified person.

Resting metabolic rate (RMR) and respiratory quotient (RQ) were measured using breath-by-breath metabolic measurements with portable metabolic assessment equipment (Cosmed K4b^2, Rome, Italy). Total energy expenditure (TEE) was evaluated through 24 hour heart rate (HR) monitoring with the Polar Sport Tester RS 610 (Polar Electro, Kempele, Finland), according to the procedure proposed by Ekelund et al[19] and Livingstone and Robson[20] HR was recorded continuously over 24 h at one-minute intervals for 3 days (2 weekdays and 1 day of the weekend) in each experimental period. To access physical activity level (PAL), all participants wore an Acti-Graph accelerometer (GT1M in uniaxial mode) for 3 days (2 weekdays and 1 day of the weekend) during each experimental period. Physical activity thresholds were employed according to the method described by Freedson et al[21]. To determine whether the subjects' attitudes towards food intake changed at any point during the experiment, the validated three-factor eating questionnaire (TFEQ) was used[22]. In the present study, only DR factor was analyzed.

Patients completed a 3-day food record for 2 weekdays and 1 day of the weekend at baseline, in the middle, and at the end of the study. The portion sizes of dishes and food products were estimated based on a standard Polish photographic album of food products and dishes[23]. The energy of individual diets was calculated using the *Dietetyk* computer program (Jumar, Pozna , Poland). In addition, the subjects' baseline caffeine (CAF) intake was estimated by a food-history questionnaire.

Study Design

For three months, all subjects consumed a daily portion of bread: 280 g for women, and 360 g for men (as 40 g slices). The bread was consumed in place of the patients' usual breads and other wholegrain products (such as porridge) for four meals (breakfast, two snacks, and lunch). Pasta, potatoes, and rice could be eaten as part of warm dishes (as in dinner) in the amounts described by the dietician. The daily portion of both experimental breads met the requirements for the intake of bread in the daily food rations for women and men aged 25-60 years[24]. The energy and macronutrient content in both kinds of experimental breads was similar. The ingestion of a daily portion of green tea rye bread (GTRB) provided daily totaled 3.3 MJ and 4.3 MJ of energy, 28.0 g and 36.1 g dietary fiber, 123.2 mg and 158.4 mg CAF, 188.3 mg and 242.1 mg epigallocatechin gallate (EGCG), for women and men respectively[25]. The subjects were instructed not to eat any other type of

Table I. Basic anthropological parameters, body fat content and energy expenditure in the course of the study.

	Before mean±SD median (1st-3rd quartile)	After mean±SD median (1st-3rd quartile)
Body weight (kg)	94.4 ± 6.1 91.4 (83.5-109.8)	94.7 ± 4.7 92.2 (86.5-109.2)
BMI (kg/m^2)	34.9 ± 1.9 33.3 (32.3-37.4)	35.1 ± 1.4 34.1 (33.1-36.2)
WC (cm)	111.8 ± 5.3 109.0 (105.8-120.0)	110.1 ± 3.8 109.5 (106.0-116.2)
WHR	0.92 ± 0.03 0.94 (0.87-0.99)	0.94 ± 0.02 0.97 (0.93-0.99)
FM (%)	39.8 ± 1.7 40.9 (36.0-44.0)	39.5 ± 1.8 42.6 (33.2-44.1)
FM (kg)	37.8 ± 3.0 36.1 (34.4-43.6)	37.4 ± 2.2 37.4 (35.6-39.4)
TEE (Kcal)	2323 ± 147 2118 (2012-2584)	2264 ± 98 2100 (2014-2488)
REE (Kcal)	1460 ± 86 1349 (1299-1702)	1491 ± 57 1444 (1324-1644)

bread, and to not replace the experimental bread with any other wholegrain product, such as bran flakes. Additionally, during both phases, subjects were instructed to avoid drinking GT and all other products containing CAF (including bars, other kinds of teas, energy drinks, and cola drinks). Compliance was evaluated through feedback from each participant during their scheduled visits to the Department of Human Nutrition and Hygiene for dietary assessment and nutritional status evaluation and bread collection. All subjects were encouraged to inform the staff of any problems or discomfort relating to the consumption of the rye bread (RB). To avoid bias, a dietitian formulated menu plans with home-prepared recipes and meal instructions. Supporting materials were provided to facilitate adherence, including frozen-meal options, food-brand options, meal-preparing tools, and shopping lists. The diet contained 25% of energy as fat, 55% as carbohydrate, and 20% as protein.

The RB used for the study was a noncommercial product provided by Fawor bakery, Poznan, Poland. The RB was prepared using a two-phase method (with sourdough), containing either 0% (control RB) or 1.1% GTE per 100 g flour (GTRB), according to the procedure described by Bajerska et al[25]. Energy value (MJ), protein (g), fat (g), total dietary fiber (g), insoluble dietary fiber (g), and soluble dietary fiber (g) content per 100 g experimental breads were 1.19 (0.02), 5.6 (0.1), 0.1 (0.01), 10.0 (0.3), 6.8 (0.2) and 3.2 (0.06) respectively.

GTE was prepared according to the method described by Bajerska et al[5] using Japanese Sencha Fukuju Green Tea, which was bought at a specialty store (The House of Tea). The tea leaves (100 g) were ground and then boiled in double-distilled water (1000 mL), followed by stirring for 15 minutes at 70°C (the procedure was repeated 3 times). Collected extracts were filtered through filter paper, centrifuged (at 2700 × g, for 15 min), frozen and lyophilized under a vacuum (Multi Branch Trade & Manufacturing Company "Elena," Zelazkow, Poland).

HPLC analyses of green tea catechin content were performed on a Waters Alliance HPLC System 2695 (Milford, MA, USA) equipped with an X-Terra RP18 5 μm column (Milford).

In all subjects, a ^{13}C-labelled mixed triglyceride breath test (^{13}C MTG-BT) was performed twice; once before and once after three months of GTE consumption. The ^{13}C MTG-BT was performed as described earlier[26]. ^{13}C-labeled MTG was delivered by Wagner Analysen, Technik GmbH in Bremen (Germany). The samples were analyzed with an IRIS ^{13}C-Analyser System (Wagner, Bremen, Germany). Cumulative percentage dose recovery (CPDR) was assumed to reflect digestion and absorption of lipids; values lower than 13% were considered to be abnormal[27].

Table II. Energy and selected macronutrient intake in the studied group.

	mean±sd median (1st-3rd quartile)
Energy (Kcal)	2293 ± 119 2075 (2045-2589)
Protein (g)	91.8 ± 7.8 84.1 (77.8-97.0)
Carbohydrates (g)	318.8 ± 8.6 312.7 (300.0-343.1)
Fat (g)	80.0 ± 8.6 69.7 (61.7-95.2)
SFA (g)	26.4 ± 2.9 21.9 (20.9-30.2)
MUFA (g)	31.1 ± 3.0 29.0 (24.3-36.0)
PUFA (g)	15.9 ± 2.7 12.0 (10.3-17.0)
Fibre (g)	29.8 ± 3.0 27.1 (25.0-28.3)

Statistical Analysis

Results are expressed as medians and as a 1st-3rd quartile range. The statistical significance of differences in CPDR before and after GTE consumption was determined with the use of the Wilcoxon-rank test. The level of significance was set at $p < 0.05$. Statistical analysis was performed using STATISTICA 8.0. (StatSoft Inc., 2008).

The protocol of the investigation was approved by the Bioethical Committee of Poznan University of Medical Sciences, Poland. All subjects gave their written informed consent. The study protocol was approved by the local Bioethics Committee of the Institutional Review Board at Poznan University of Medical Sciences, Poland (approval number 321/08).

Results

Body height of the studied subjects ranged from 156 to 182 (median value – 159.5 cm). A description of basic anthropological parameters (body weight, BMI, WC, WHR), of body fat content (expressed as absolute and relative values), as well as of energy expenditure (BMR and TEE) have been given in Table I. No significant changes in the course of the study were observed.

Table III. The changes in lipid digestion and absorption (as assessed using 13C MTG-BT) in the course of the study.

Minutes	CPDR (%) Before mean ± sd median (1st-3rd quartile)	After mean±sd median (1st-3rd quartile)	Statistical significance
30	0.1±0.1 0 (0-0.1)	0.2 ± 0.1 0 (0-0.2)	NS
60	0.5 ± 0.2 0.2 (0-0.6)	0.6 ± 0.3 0 (0-0.8)	NS
90	1.5 ± 0.5 1.2 (0.2-1.8)	1.8 ± 0.7 0.5 (0.1-3,3)	NS
120	3.3 ± 1.0 2.9 (1.3-4.3)	3.5 ± 1.1 2.5 (0.6-5.7)	NS
150	6.3 ± 1.5 4.2 (3.7-8.1)	6.0 ± 1.5 5.3 (2.3-8.3)	NS
180	10.0 ± 2.1 7.2 (6.6-11.9)	8.7 ± 2.0 7.9 (4.4-10.8)	NS
210	13.3 ± 2.5 11.1 (9.5-14.2)	11.3 ± 2.4 10.5 (6.7-14.1)	NS
240	16.3±2.9 14.4 (12.0-16.5)	13.2 ± 2.6 12.6 (9.0-15.5)	0.021825
270	19.0 ± 3.3 17.2 (13.0-19.4)	14.7 ± 2.9 13.8 (10.1-16.5)	0.005062
300	21.1 ± 3.8 19.5 (13.5-22.4)	16.0 ± 3.2 14.0 (11.2-18.4)	0.006911
330	23.0 ± 4.6 20.5 (14.2-24.7)	17.1 ± 3.4 14.8 (12.1-20.2)	0.006911
360	24.2 ± 5.1 20.8 (14.9-25.6)	17.9 ± 3.6 15.5 (12.3-20.5)	0.009345

Energy and macronutrient intake was stable within the period study. Observed differences for all parameters did not exceed 10% in any of the subjects studied. The obtained values have been summarized in Table II.

The consumption of bread containing GTE resulted in significant changes in lipid digestion and absorption as assessed using the ^{13}C MTG-BT. CPDR was lower from the 240 minute mark of the test until its end (Table III).

Discussion

The consumption of bread containing GTE resulted in significant changes in lipid digestion and absorption based on the results of ^{13}C MTG-BT. CPDR was lower from the 240 minute mark of the test until its end (Table III). Observed CPDR values suggest decreased lipid assimilation. In an earlier study[11], we have documented the impact of a single GTE dose on lipid disestion and absorption. Juhel et al[6] proved that GT catechins affect pancreatic lipase activity *in vitro*. There are few data in humans and in animal models confirming the inhibition of lipase activity[6,28] which is reflected in its hypocholesterolemic effects[5,29] or the inhibition of the postprandial rise of serum triglycerides[28]. This mechanism may be associated with a change in the size and surface area of lipid emulsion droplets in combination with the active substances contained in green tea, EGCG particularly. Such an interaction increases their size, reduces their surface area, and it decreases pancreatic lipase efficiency[30]. However, in the present study, GTE was given for three months, but was not added to test meals. This excludes the direct luminal effect of GTE. There was no factor to interfere with lipid emulsion in the gastric or duodenal lumen. It is possible that active ingredients contained in GTE affect the secretion of lipase into the duodenal tract which is the effect of long-term supplementation.

Another possible mechanism may be related to the uptake of lipids by jejunal enterocytes. Such a proces requires specific transporters on the brush border membrane, and it is probable that green tea catechins may interact with them and, thus, inhibit the lipid transport[12,31]. It has been documented that postprandial triacylglycerol and cholesterol activity may be lowered, and this could potentially be caused by green tea ingestion[28,32,33].

There are numerous studies suggesting that EGCG in green tea may be helpful in body weight reduction[4,5,6,7]. However, in the meta-analysis by Hursel et al[34], it was concluded that active substances contained in green tea, either catechins or a EGCG-caffeine mixture, have a small positive effect on weight loss and weight maintenance after a period of weight loss. No significant changes in BW, BMI, WC, WHR or body fat content were observed in the course of the present study. Energy and macronutrient intake was stable within the period of the study. Such a situation could be related to a minor (although statistically significant) effect of GTE on lipid digestion and absorption. Moreover, patients were on weight maintenance treatment after a period of weight loss.

Conclusions

Long-term GTE intake decreases lipid assimilation, but probably without involvement of luminal effects. However, further studies are needed to confirm this hypothesis and to clarify underlying mechanism.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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