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Effect of EGCG on lipid absorption and plasma lipid levels in rats

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Abstract

Catechins, compounds derived from green tea, have been shown to reduce plasma cholesterol levels and the rate of cholesterol absorption. We investigated the dose response and the mechanism of action of epigallocatechin gallate (EGCG) on these parameters in rats. Wistar rats were fed a diet high in cholesterol and fat containing either none, 0.25% (0.2 g/day/kg BW), 0.5% (0.4 g/day/kg/BW) or 1.0% (0.7 g/day/kg BW) of EGCG. After 4 weeks of treatment, total cholesterol and low density lipoprotein plasma levels were significantly reduced in the group fed 1% EGCG when compared to the no treatment group. Plasma triglycerides and high-density lipoprotein levels did not change significantly. Following a single oral application of a liquid test-meal, intestinal cholesterol absorption in Wistar rats was 79.3% in the control group. In the group treated with 0.1 g/kg BW EGCG intestinal cholesterol absorption decreased to 73.7% and in the group treated with 0.5 g/kg BW of EGCG intestinal cholesterol absorption fell significantly to 62.7% ($P = 0.005$). Total fat absorption was very efficient in the control group (99.5% of the applied dose) and decreased significantly but moderately in the group treated with the highest doses of EGCG (0.75, 1 g/kg BW). In an in-vitro biliary micelle model, the addition of 55 μM to 1300 μM EGCG not only decreased cholesterol solubility dose-dependently in these micelles but also altered the size of the mixed lecithin/taurocholate/cholesterol micelles as demonstrated by light scattering. This study provides evidence suggesting that the cholesterol-lowering effect of green tea is mainly elicited by EGCG, one of the most abundant catechins contained in green tea. It is suggested that one of the underlying mechanisms by which EGCG affects lipid metabolism is by interfering with the micellar solubilization of cholesterol in the digestive tract, which then in turn decreased cholesterol absorption.

Keywords: Epigallocatechin gallate (EGCG); Cholesterol; Triglycerides; Lipid; Intestinal absorption; Rats

1. Introduction

In epidemiological studies a significant inverse relationship between tea drinking and plasma cholesterol levels [1,2] has been reported. The health beneficial effects of green tea have been attributed mainly to the catechins, epigallocatechin-3-gallate (EGCG), epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (ECG) [3,4]. The most abundant catechin in green tea is EGCG. Animal studies have shown that catechins inhibited cholesterol absorption and lowered plasma cholesterol [5–11]. Several studies were carried out with green tea extracts containing mainly a mixture of the four major green tea epicatechins [5,9–11]. Muramatsu et al. [5] observed that dietary green tea epicatechins increased fecal excretion of cholesterol and total lipids in cholesterol fed rats. Yang et al. [9] observed that Chinese green tea and Jasmine tea, which contain higher amounts of EGCG than other green teas, more effectively reduced cholesterol levels in rats. Chisaka et al. [7] showed that EGCG did not inhibit cholesterol synthesis but orally administered EGCG decreased cholesterol absorption from rat intestine. EGCG also inhibited the absorption of cholesterol from the rat intestinal lumen in situ [7]. In hamsters, no differences in activities of 3-hydroxy-3-methyl glutaryl coenzyme A reductase and intestinal acyl CoA: cholesterol acyltransferase were observed after supplementation with a green tea epicatechin mixture, but the fecal excretion of total fatty acids, neutral sterols and acidic sterols was increased [10]. Ikeda et al. [12] showed that tea catechins inhibited lymphatic absorption of cholesterol. These studies suggest that green tea or more specifically green tea catechins may decrease cholesterol absorption from the gut. Several animal studies have shown that green tea catechins may also affect fat absorption [5,10,12]. In rats fed green tea catechins, a marked increase in fecal total lipids and cholesterol was observed as compared to the

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control group [5]. Hamsters drinking green tea or green tea polyphenols also had a higher fecal fat excretion than the control group [10]. As there is limited information on the effect of single catechins on lipid absorption and plasma lipid levels we investigated the effect of a purified form of EGCG on lipid metabolism in rats and in an in-vitro biliary micelle model to get an understanding of the underlying mechanism.

2. Material and methods

2.1. Materials

Epigallocatechin gallate (EGCG) was extracted and purified from green tea leaves. The total epicatechin content of the green tea extract was 85% of which 90% was EGCG. [carboxyl-^{14}C]-Triolein (104 mCi/mmol) and [1,2-^{3}H]-Polyethylene glycol (1.51 mCi/g, MW ≅ 4000) were purchased from NEN Life Science Product (Boston, MA, USA). [4-^{14}C]-cholesterol was obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK). All other chemicals and solvents were of analytical grade from Fluka (Buchs, Switzerland), Sigma (St. Louis, MO, USA) and E. Merck (Darmstadt, Germany).

2.2. Dietary treatment experiment

Female Wistar rats (SPF) from Research and Consulting Company (RCC, Füllinsdorf, Switzerland), weighing 125-135 g were randomly divided into four experimental groups consisting of 8 animals each. They were housed in wired cages, in pairs with free access to water and feed, with an alternating 12-hr light-dark cycle. The rat model with high fat and cholesterol diet was chosen to study the nutritional cholesterol overload. Therefore, the animals were fed a semisynthetic diet high in cholesterol and fat for 4 weeks. In previous studies hypercholesterolemic animal model responded positively to inhibitor of cholesterol absorption while chow fed animals failed to respond to the drug [13]. The macro-nutrient compositions of the diet was as follows (g/100g anhydrous mix): protein 21; fiber 8; fat 18; carbohydrate 41. The diet also contained 0.5 wt% cholesterol, 1 wt% sodium cholate and a standard vitamin and mineral mix, prepared according to rat nutritional requirements. The main fats consisted of coconut kernel (18 wt%; 12% fat), coconut oil (2.5 wt%) and corn oil (2.5 wt%). The basic ingredients were selected to be free of flavonoids, and EGCG was added in quantities of 0.25% (0.2 g/day/kg BW), 0.5% (0.4 g/day/kg BW) and 1.0% (0.7 g/day/kg BW) to the diet. The doses of EGCG expressed in g/day/kg body weight (BW) were evaluated from the daily food intake and body weight of the animals. The controls received the same basic diet but without added EGCG. Individual body weight was recorded twice a week, and feed consumption was registered once a week for each group. After 4 weeks the animals were sacrificed by withdrawing blood from the vena cava under Isoflurane anesthesia. Blood was collected into tubes containing heparin as an anticoagulant. Plasma was prepared from the heparinized blood by immediate centrifugation at 900 X g for 10 min at 4°C. Assays of plasma cholesterol, triglycerides and HDL-cholesterol (precipitation method) were determined enzymatically on a COBAS FARA analyzer (Roche Diagnostics, Switzerland). Non-HDL cholesterol was calculated by taking the difference between total plasma cholesterol and HDL-cholesterol. The livers were excised, washed in ice cold isotonic NaCl and stored at -20°C until further analysis. The frozen livers were lyophilized for dry weight determinations. Cholesterol and fat extraction were performed in dried liver powders. Aliquots of about 100 mg were saponified in methanolic KOH for 4 hr at 60°C. The hydrolysates were then extracted in cyclohexane and evaporated to dryness. Liver fat was estimated by weighing out the lipid extracts. The extracts were dissolved in ethanol and assayed for cholesterol with the method used for plasma. Feces were collected quantitatively and lyophilized in pools per group for the last experimental week. The method for the cholesterol determination in lyophilized, powdered feces was the same as for the liver. Fat was extracted directly from the dried and milled feces with hexane.

2.3. Measurement of the intestinal cholesterol absorption after a single oral test dose

The inhibition of intestinal cholesterol absorption of dietary cholesterol was measured by the fecal isotope ratio method after a test meal containing radioactive cholesterol and polyethylene glycol (PEG) as nonabsorbable marker [14,15]. The absorption was calculated as the loss of cholesterol relative to PEG during intestinal transit. Female wistar rats (SPF) from Research and Consulting Company (RCC, Füllinsdorf, Switzerland), weighing 160-180 g were housed individually in wired cages with an alternating 12-hr light-dark cycle. Animals had free access to standard rodent chow (Klibamühle n° 3430 in pellet form, Kaiseraugst, Switzerland) and water for one week. After a 18-hr fasting period, three groups of eight rats received by oral intubation either 0.5 or 0.1 g/kg body weight of EGCG or vehicle (5ml/kg BW). This was immediately followed by a liquid test meal containing [4-^{14}C]-cholesterol and [^{3}H]- polyethylene-glycol (5ml/kg BW). EGCG was dissolved in phosphate buffered saline (PBS) (10 mM) at the required concentrations. The liquid test-meal was prepared by mixing starch (2.5%), glucose (24%), olive oil (9%), [4-^{14}C]-cholesterol (0.5mg/ml, 0.94 μCi/ml of test meal), defatted milk powder (12%) and [^{3}H] polyethylene-glycol (PEG; 0.37 μCi/ml of test-meal) in 0.9% NaCl. The homogeneity of the test meal was controlled by measuring the radioactivity of the aliquots in a liquid scintillation counter. Thereafter, the standard rodent chow (Klibamühle n° 3430 in pellet form, Kaiseraugst, Switzerland) was given ad libitum. The feces

Table 1
Plasma lipid levels after 4 weeks of dietary treatment[a]

	Control	0.25% EGCG	0.5% EGCG	1% EGCG
Total cholesterol	3.81 ± 0.65	4.13 ± 0.83	3.17 ± 1.05	2.40 ± 0.58[b]
HDL cholesterol	1.69 ± 0.20	1.73 ± 0.27	1.59 ± 0.40	1.44 ± 0.16
non-HDL cholesterol	2.12 ± 0.65	2.41 ± 1.01	1.58 ± 0.83	0.96 ± 0.48[b]
non-HDL/HDL cholesterol ratio	1.28 ± 0.42	1.50 ± 0.90	0.99 ± 0.48	0.66 ± 0.29
Triglyceride	0.47 ± 0.05	0.53 ± 0.08	0.46 ± 0.09	0.44 ± 0.09
Free fatty acids	1.44 ± 0.24	1.46 ± 0.15	1.30 ± 0.33	1.28 ± 0.18

[a] Values are expressed as mean ± SD given in mmol/L (n = 8 per group).
[b] Significantly different from control ($p < 0.05$).

were collected at the following intervals: 0-24, 24-32, 32-48 and 48-55 hr. The weight of each fresh feces portion was determined and homogenized with water using a polytron homogenizer and the weight of the mixture was determined. The samples were stored at -20°C. To measure the radioactivity in the feces, aliquots were placed in a "Combusto cone" (PACKARD Biosciences). The samples were air dried overnight at room temperature before combustion in a Packard sample oxidizer. The CO_2 was collected in 8 ml "Carbosorb E". The radioactivity was determined by liquid scintillation counting after the addition of 14 ml "Permafluor". Inclusion of the [^{3}H] PEG allowed correction for incomplete fecal recovery. Absorption of [^{14}C]-cholesterol after 55 hr was calculated from the difference between the orally administered dose (100%) and the fecally recovered and corrected radioactivities. Cholesterol absorption was calculated using the following formula: = {1-[(fecal cholesterol radioactivity/fecal PEG radioactivity)*(administered PEG radioactivity/administered cholesterol radioactivity)]}*100.

2.4. Measurement of triglyceride absorption after a single oral dose

The ability of orally administered EGCG to inhibit intestinal fat absorption was quantified by measuring the fecal excretion of labeled triglyceride after a liquid test-meal containing 0.45 g fat/kg body weight and using the method described above for cholesterol. The rats were randomized in six groups. After an 18-hr fasting period, the following treatment was administered by oral intubation (intragastric): vehicle (n = 12); 10 mg/kg BW tetrahydrolipstatin (THL, n = 8); 0.1 or 0.5 g/kg BW of EGCG (n = 12); 0.75 or 1 g/kg BW of EGCG (n = 8). The EGCG was dissolved in PBS (10 mM) at the required concentrations (5ml/kg BW). THL was suspended in 5% gum arabic and 5% defatted milk powder at the required concentration. This was immediately followed by a liquid test meal containing [^{14}C]-triolein and [^{3}H]-polyethylene-glycol (5ml/kg BW). Thereafter, the standard rodent chow (Klibamühle n° 3430 in pellet form, Kaiseraugst, Switzerland) was given ad libitum. The liquid test-meal was prepared as described above for cholesterol but labeled cholesterol was replaced by [carboxyl-^{14}C]-triolein (0.8 μCi/ml of test meal). The feces were collected at the following intervals: 0-24, 24-48, 48-55 and 55 hr. The samples were processed as described above for cholesterol.

2.5. Solubility of cholesterol in mixed micelles

The mixed micelles were prepared by sonication of a 15 mM phosphate buffer saline (PBS) solution containing 6.6mM taurocholate, 2.4 mM lecithin, 0.4 or 0.5 mM cholesterol at pH 7.4. The lipids were dissolved in methanol and dried before adding the PBS buffer. The micellar solution was kept at 37°C for 24 hr. The EGCG solution (50 μl) in PBS was added to 2.5 ml of mixed micellar solution. The final concentration of EGCG was 0, 25, 50, 100, 200, 400, 600 μg/ml micelle [0,55,109,218,436,873,1309 μM]. The micellar solution was incubated again for 1 h at 37°C and centrifuged at 1000 x g for 10 min. In order to separate the precipitated cholesterol from the intermicellar cholesterol the solution was filtered through a 0.22 μm MILLEX-GP filter (Millipore, Bedford, MA, USA). The intermicellar concentration of cholesterol was then determined enzymatically on a COBAS FARA analyzer (Roche diagnostica, Switzerland). The particle size of the mixed micelles was measured by dynamic light scattering at 90° using a Coulter N4 PLUS (Beckman coulter, Fullerton, CA, USA).

2.6. Statistics

All data are expressed as means ± standard deviation (SD) for animals in each diet group, with n being the number of rats. Statistical significance of the mean differences between dietary groups was tested by one-way analysis of variance (ANOVA). If significant differences were found, the Dunnett's test for multiple comparison was used to compare each group to the control group. The group treated with THL was not included in the statistical evaluation of the effects of EGCG. P values less than 0.05 were considered significant. All analyses were performed with Statistica (ver. 5.5A, StatSoft, Inc).

Table 2
Liver plasma lipid levels after 4 weeks of dietary treatment[a]

	Control	0.25% EGCG	0.5% EGCG	1% EGCG
Liver fresh weight (g)	9.51 ± 1.50	10.03 ± 1.36	10.31 ± 1.00	9.67 ± 0.99
Total lipid (mg/g)	566 ± 46	595 ± 39	541 ± 77	541 ± 54
Cholesterol (mmol/g)	0.47 ± 0.05	0.49 ± 0.03	0.45 ± 0.07	0.38 ± 0.07[b]

[a] Values are expressed as mean ± SD (n = 8 per group).
[b] Significantly different from control ($p < 0.05$).

3. Results

3.1. Dietary treatment experiment

Weight gain was similar in the control group and in the groups treated with EGCG during the 4-week feeding period. The average food intake for the 4 week period of the four dietary regimes was 15 g/day/rat. Dietary treatment with EGCG had no significant effect on body weight, food consumption and liver weight.

Plasma lipid levels after 4 weeks of dietary treatment are shown in Table 1. The 1% EGCG dose significantly lowered plasma lipids. Total plasma cholesterol and non-HDL cholesterol (VLDL-cholesterol + LDL-cholesterol) levels were significantly lower by 37% ($p = 0.004$) and 55% ($P = 0.013$), respectively in the 1% EGCG-treated group when compared to the control group. Plasma HDL-cholesterol, triglyceride and free fatty acid levels tended to decrease in the 1% EGCG-treated group although the levels did not change significantly when compared to the control group. Lipid concentrations of the liver are depicted in Table 2. Liver weight and total lipid concentration were unaltered by EGCG treatment. Hepatic total cholesterol concentration was decreased by 17% ($p = 0.009$) in the 1% EGCG-treated animals when compared to the control group. The group-pooled fecal excretion of cholesterol and fat is shown in Table 3. Fecal cholesterol excretion in week 4 was 10.5% of the dietary cholesterol intake in the control group and increased to 14.7% of the cholesterol intake in the 1% EGCG-treated group. The fecal fat excretion was 3.5% of the intake in the controls and increased with rising dietary EGCG to 5.8% of intake in the 1% EGCG-treated group (Table 3).

3.2. Measurement of the intestinal cholesterol and fat absorption

The cholesterol and fat absorption, calculated as the loss of cholesterol or triglygeride relative to PEG during intestinal transit is shown in Table 4. After 48 hr more than 90% of the administered [^{3}H]-PEG was excreted in the feces of the control and EGCG treated groups. This indicates that a 55h collection period is sufficient for the measurement of cholesterol and fat absorption in the rat. The control rat absorbed 79.3% of ^{14}C-cholesterol when corrected for ^{3}H-PEG recovery, showing that the absorption of cholesterol is very efficient when given in the form of an emulsion in a liquid test meal. The cholesterol absorption was decreased by 7% in the group receiving 0.1 g/kg BW of EGCG and significantly reduced by 21% in the group getting 0.5 g/kg BW of EGCG as compared to the control group. THL was used as the positive control in the experiment measuring fat absorption, as it is an established inhibitor of fat absorption in humans. Triglyceride absorption was 99.4% in the control group. After 55 hr, the active control, THL, decreased fat absorption from 99.5% to 36.7% of the administered dose confirming previous studies in rats [16]. The animals receiving THL were excluded from the statistical evaluation of the effect of EGCG on fat absorption since THL was considerably more potent than EGCG. EGCG dose-dependently increased fat excretion. Thus, the highest doses of EGCG used in this study (0.75 and 1 g/kg BW) decreased significantly but moderately the intestinal fat absorption by 3%, from 99.5% in the control to 96.5%.

Table 3
Effect of EGCG on fecal excretion of cholesterol and fat after 4 weeks of dietary treatment[a]

Treatment	Cholesterol			Fat		
	Intake (g/week)	Excretion (g/week)	Excretion as % of Intake	Intake g/week	Excretion (g/week)	Excretion as % of Intake
Control	4.2	0.44	10.5	151.2	5.3	3.5
0.25% EGCG	4.45	0.46	10.2	160.2	5.9	3.7
0.5% EGCG	4.3	0.52	12.1	154.8	7.1	4.6
1.0% EGCG	4.25	0.63	14.7	153	8.9	5.8

[a] The cholesterol and fat excretion were determined in the pooled feces per group of week 4. Cholesterol and fat intake were calculated from the dietary content and the feed consumption (week 4). Values are given in g/week or as % of intake.

Table 4
Inhibition of cholesterol and fat absorption after a single dose of EGCG and a liquid test meal[a]

	% Absorption after 55 hours	
	Cholesterol	Fat
Control (n = 12)	79.3 ± 8.3	99.5 ± 0.1
EGCG 0.1 g/kg BW (n = 12)	73.7 ± 6.4	99.1 ± 0.5
EGCG 0.5 g/kg BW (n = 12)	62.7 ± 5.8[b]	98.6 ± 0.8
EGCG 0.75 g/kg BW (n = 8)		96.5 ± 2.4[b]
EGCG 1 g/kg BW (n = 8)		96.6 ± 3.0[b]
Tetrahydrolipstatin (THL) 10 mg/kg (n = 8)		36.7 ± 16.8

[a] Values are given as mean ± SD expressed as percent absorption after 55 hours (n = 8–12). Rats received an intragastric dose of EGCG immediately followed by a liquid test meal containing ^{14}C-cholesterol and ^{3}H-PEG or ^{14}C-triolein and ^{3}H-PEG. The regular diet was given after the liquid test meal. The feces were collected 24, 32, 48, 55 hours after the administration of radioactive cholesterol or triolein. The radioactivity was quantified as described in materials and methods. The absorption is calculated as the loss of cholesterol or triglyceride relative to [^{3}H] polyethyleneglycol (PEG) during intestinal transit. [^{3}H]-PEG was used as a non-absorbable marker.

[b] Significantly different from control ($p < 0.05$). The animals receiving THL were excluded from the statistical evaluation of the effect of EGCG on fat excretion since THL was considerably more potent than EGCG.

3.3. Cholesterol solubility in micelles

The effect of EGCG on the micellar solubility of cholesterol is shown in Fig. 1. The intermicellar concentration of cholesterol decreased in a dose-dependent manner with increasing concentrations of EGCG (55-1300 μM). The micellar cholesterol concentration was decreased by 50% after the addition of 353 μM of EGCG. The effect of EGCG on the mixed micelle particle size is shown in Fig. 2. The diameter of the micelles gradually increased as the concentration of EGCG in the solution increased from 109 μM to 870 μM, subsequently leveling off at higher EGCG concentrations. The change in the micellar solubility of cholesterol paralleled the change in the mixed micelle particle size.

4. Discussion

To our knowledge, this is the first study to show that pure EGCG lowers dose-dependently plasma cholesterol levels in rats. Our data provide evidence that the cholesterol-lowering effect of green tea [9–11] may be brought about primarily by EGCG, the most abundant catechin in green tea. The addition of 1% EGCG to the diet significantly lowered total plasma cholesterol and non-HDL cholesterol levels. The ratio of non-HDL: HDL-cholesterol an indicator of atherosclerosis risk, was also positively affected in the animals who were on the diet containing 1% of EGCG. However, we should bear in mind that animals received a diet high in fat and cholesterol along with a rather high dose of EGCG. Thus, it remains to be demonstrated to which extend a reasonable intake of pure EGCG may affect cholesterol plasma levels in humans.

EGCG also dose-dependently increased fecal excretion of total lipids and cholesterol. These findings are consistent with those of other investigators, who show that green tea catechin (1-2%) significantly lowers the levels of plasma total cholesterol in diet-induced hypercholesterolemic rats [5,17]. These studies showed that green tea does not affect the incorporation of ^{14}C acetate into cholesterol or the activity 3-hydroxy-3methylglutaryl-CoA reductase or cholesterol 7α-hydroxylase [7,11], suggesting that the hypolipidemic activity of epicatechins may be attributable to a luminal effect in the gut rather than an inhibitory effect on cholesterol synthesis.

Regarding fat absorption, we found that the control rat

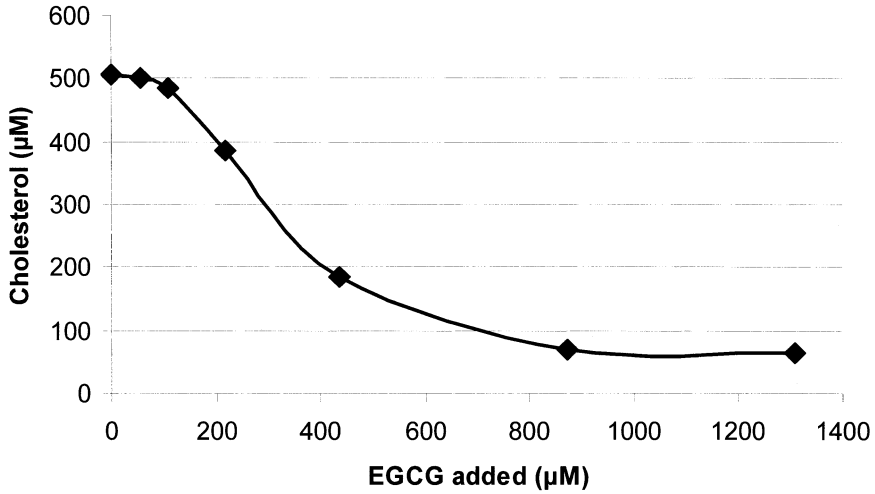

Fig. 1. Effect of EGCG on the micellar solubility of cholesterol in vitro. EGCG was added to taurocholate/lecithin/cholesterol mixed micelles containing: 6.6mM sodium taurocholate, 2.4mM phosphatidylcholine, 0.5mM cholesterol and 132mM sodium chloride in 15mM sodium phosphate buffer (pH 7.4) at 37°C. The micellar solution was incubated for 1h at 37°C. After centrifugation and filtration the cholesterol content of micelle was measured.

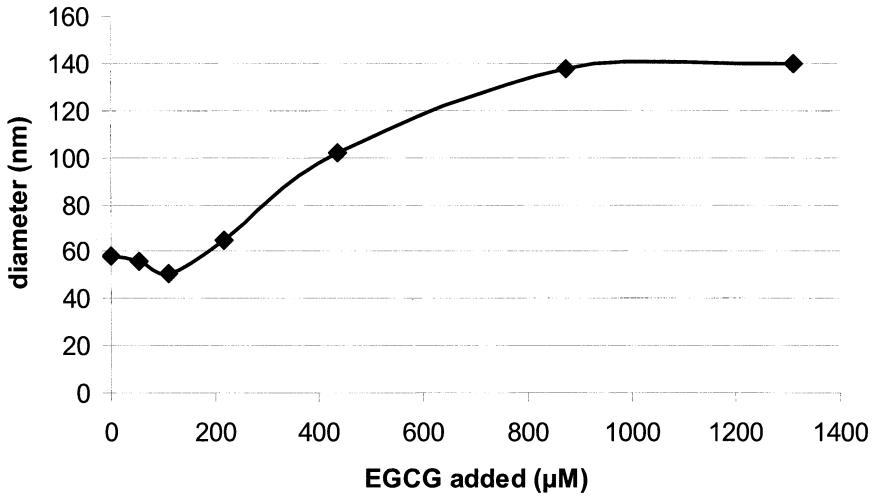

Fig. 2. Effect of EGCG on the micellar size. EGCG was added to the micellar solution and the particle size was measured by dynamic light scattering at 90° after 1 hr of incubation at 37°C, centrifugation and filtration as described in materials and methods.

absorbed 99.5% of ^{14}C-triolein when corrected for ^{3}H-PEG recovery. Thus, in contrast to cholesterol, fat is absorbed to almost 100% in the intestine making it difficult to study the dietary effect of EGCG on fat absorption. High doses of EGCG (0.75 and 1 g/kg BW) significantly decreased fat absorption in rats. In relative terms, fat excretion was increased 2 to 6 fold from 0.5% to 3% for a dose range of 0.1 to 1 g/kg BW of EGCG. However, in absolute terms, the effect of EGCG is very small and of little physiological relevance. Thus, a very small decrease in fat absorption was observed from 99.5% in the control group to 97% after treatment with 0.75 and 1 g/kg BW of EGCG. Previous, studies have shown that most ingested EGCG is detected in the intestine and at the intestinal mucosa [18,19]. The very small effect observed in vivo is most likely due to the effect of EGCG on micellar solubilization or interference with the diffusion of fat across the unstirred water layer and the mucosa cell membrane since the local concentration of EGCG in the intestine is thought to be very high. High concentration of EGCG in the intestine may also affect the activity of the digestive enzymes [20]. Thus, whether the effect of EGCG on fat absorption is due to altered intraluminal digestion , to effects on micellar solubilization, to modification in surface transport barriers, to altered intestinal production of lipoproteins, or to more than one of the factors remains to be determined.

In order to investigate the effect of EGCG on cholesterol absorption and to better understand the underlying mechanism of action, we quantified in rats the inhibition of intestinal cholesterol and fat absorption after a single oral dose of EGCG, given with a liquid test meal. EGCG (0.5g/kg BW) significantly reduced intestinal cholesterol absorption by 21% in rats. The principal steps in the absorption of cholesterol are emulsification in the stomach, hydrolysis of the ester bond by a specific pancreatic esterase, micellar solubilization, absorption in the proximal jejunum, re-esterification within the intestinal cells, and transport to the lymph by chylomicrons [21]. Due to the insolubility of cholesterol in water, solubilization of cholesterol in mixed micelles is a requirement for its efficient absorption. Micelles are polymolecular aggregates that act as a carrier and a solubilizer of cholesterol and other lipids. The solubilized cholesterol is then transferred from the micelles to the cell membrane of the intestinal brush border. Thus, the action of molecules that influence cholesterol uptake could interfere either with the affinity of micelles for membranes or with the affinity of cholesterol for micelles. These alterations in the intestinal lumen affect hepatic cholesterol metabolism and may affect synthesis and catabolism of lipoproteins. To further address the mechanism of action of EGCG on cholesterol absorption, we studied the effect of pure EGCG on the micelle size and cholesterol solubility. The addition of pure EGCG (from 109 μM to 436 μM) to mixed micelles decreased the intermicellar cholesterol concentration by 65%. The cholesterol concentration leveled off at higher EGCG concentrations (Fig. 1). Our findings are supported by the data from Ikeda et al. [12] showing that green tea catechins reduce the cholesterol absorption from the intestine by reducing solubility of cholesterol in mixed micelles. In addition, we showed that pure EGCG induced an increase in the mean particle size of the micelles. The change in the micelle size may affect the solubility of cholesterol in micelles and the affinity of micelles for membrane [22]. Previous, in-vitro studies showed that the incorporation of catechins into lipid bilayers reduced the membrane fluidity by affecting membrane structure [23,24]. In those in-vitro models the gallate esters of catechins were more effective than the free catechins. Our data suggest that similar changes might be induced by the presence of pure EGCG in the intestine, which in turn will result in a decrease in cholesterol uptake by enterocytes and will also explain the observed in vivo inhibition of cholesterol absorption. Thus, the hypocholesterolemic activity of EGCG, the main catechin of green tea, appears to be due to the inhibition of intestinal cholesterol

absorption by reducing micellar solubilization of cholesterol.

In conclusion, dietary EGCG dose-dependently decreased plasma total cholesterol and non-HDL cholesterol concentrations by inhibiting cholesterol absorption in association with mild fat malabsorption. Moreover, EGCG was shown by light scattering to rearrange the size of mixed lecithin/taurocholate/cholesterol micelles. Therefore, EGCG reduced cholesterol absorption, most likely by altering the micelle structure, consequently displacing cholesterol from the mixed micelle phase.

This results of EGCG on cholesterol absorption in animals and in an in-vitro system are encouraging it remains to be shown to what extent this finding will translate into an effect in the human body.

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