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GREEN TEA EXTRACTS LOWER SERUM FOLATES IN RATS AT VERY HIGH DIETARY CONCENTRATIONS ONLY AND DO NOT AFFECT PLASMA FOLATES IN A HUMAN PILOT STUDY

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Green tea catechins (GTC) have been shown to inhibit the activities of enzymes involved in folate uptake. Hence, regular green tea drinkers may be at risk of impaired folate status. The present experiments aimed at studying the impact of dietary GTC on folate concentrations and metabolism. In a human pilot study (parallel design) healthy men consumed for 3 weeks 6 capsules (~670 mg GTC) per day (2 capsules with each principal meal) containing aqueous extracts of the leaves of *Camellia sinensis* (n=17) or placebo (n=16). No differences in plasma folate concentrations were observed between treatments. We further fed groups of 10 male rats diets fortified with 0, 0.05, 0.5, 1, or 5 g GTC/kg for 6 weeks. Only at the highest intake, GTC significantly decreased serum 5-methyl-tetrahydrofolate concentrations in rats, while mRNA concentrations of reduced folate carrier, proton-coupled folate transporter/heme carrier protein 1, and dihydrofolate reductase (DHFR) remained unchanged in intestinal mucosa. Using an *in vitro* enzyme activity assay, we observed a time- and dose-dependent inhibition of DHFR activity by epigallocatechin gallate and a green tea extract. Our data suggest that regular green tea consumption is unlikely to impair folate status in healthy males, despite the DHFR inhibitory activity of GTC.

Key words: *folates, catechins, bioavailability, human, rat*

INTRODUCTION

In mammals, folate is a water-soluble vitamin required for optimal growth, development, and health throughout all stages of life. Folate deficiency leads to anaemia (1) and an insufficient maternal supply during pregnancy causes neural tube defects (spina bifida) in newborns (2). In addition, low serum folate levels have been associated with a wide range of diseases, including atherosclerotic (3) and psychiatric disorders (4, 5). Folate is a co-substrate for enzymes essential for cell function and plays a key role in one-carbon transfer reactions, which among others is important for protein and DNA methylation and the biosynthesis of nucleotides (6).

Uptake of folate across the brush border membrane of enterocytes in the small intestine is mediated by the reduced folate carrier (RFC) (7) and the proton-coupled folate transporter/heme carrier protein-1 (PCFT/HCP1) (8). Within the enterocytes the fully-oxidised, biologically inactive folic acid is reduced to the bioactive form tetrahydrofolate (H₄folate) through the action of dihydrofolate reductase (DHFR) (9) and methylated *via* one-carbon substitution to 5-methyltetrahydrofolate (5-CH₃-H₄folate). 5-CH₃-H₄folate is taken up by cells and is retained only after conversion to H₄folate, in the presence of vitamin B₁₂, and by polyglutamation. However, there is some evidence from both acute and chronic supplementation studies that the

metabolic capacity of intestinal cells to reduce folic acid may be limited and that ingestion of large doses of folic acid may result in the appearance of unmetabolized folic acid in the circulation (10-12). Folic acid is widely used for supplementation and food fortification, because of its superior chemical stability compared to the naturally occurring reduced folates.

A recent cell culture study (13) showed that certain gallated derivatives of green tea polyphenols, namely epigallocatechin gallate (EGCG), the predominant catechin in green tea, and epicatechin gallate (ECG), inhibited folic acid uptake in Caco-2 cells. Based on *in vitro* findings (9, 14), the authors suggested a competitive inhibition of DHFR to be the most likely explanation. Inhibition of DHFR was previously demonstrated for folate analogues and is the mechanism facilitating the action of cytostatic drugs (so called antifolates) such as methotrexate (15, 16). Based on these findings, we hypothesized that individuals regularly consuming larger amounts of green tea may be at risk of reduced folate availability and in turn for the development of (chronic) folate deficiency.

The aim of the current experiments therefore was to study the effects of green tea catechins (GTC) on the activity of DHFR *in vitro*, the gene expression of RFC, PCFT/HCP1 and DHFR in rats and to investigate the impact of dietary green tea catechins on blood folate concentrations in rats and humans.

MATERIAL AND METHODS

Dihydrofolate reductase activity

The inhibition of human dihydrofolate reductase (DHFR) activity by (-) epigallocatechin gallate (EGCG) and a standardized green tea extract (Polyphenon 60 (P60); Sigma Chemical Co., St Louis, MO, USA) was measured using a commercial dihydrofolate reductase assay kit (Sigma-Aldrich) according to the manufacturer's protocol. Methotrexate, a well-known competitive DHFR inhibitor was used as a positive control. EGCG and P60 were dissolved in ultra pure-water (containing 1% ascorbic acid (w/v) (Merck KGaA, Darmstadt, Germany) to stabilize the catechins) on the day of the experiments. DHFR was used at a final activity of 1.5×10^{-3} units per reaction. Final concentrations of EGCG and methotrexate were 1000, 100 and 10 nmol/L per reaction. P60 was used at final concentrations of 1428.57, 142.86, and 14.29 $\mu\text{g/L}$ and, thus, contained 1060, 106, and 10.6 nmol/L EGCG and 1427, 143, and 14.3 nmol/L of the gallated catechins (EGCG, ECG and galocatechin gallate), respectively.

Rat study

Fifty male Wistar rats (Harlan Winkelmann GmbH, Borcheln, Germany) with an initial body weight of 99.8 ± 2.0 g (mean \pm SEM) were randomized into 5 groups of 10 animals each and housed pair-wise with sawdust bedding under controlled environmental conditions ($23 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ relative humidity, 12 h dark-light cycle). The rats were kept for 5 days on a folate-adjusted rat diet for growing animals containing 2 mg of folic acid/kg (C1027; Altromin GmbH, Lage, Germany) and thereafter received their respective experimental diets consisting of the standard diet supplemented with 0, 0.05, 0.5, 1, or 5 g green tea catechins per kg diet using P60 as the source of catechins (see *Table 1* for GTC composition). Feed and tap water were available for ad libitum consumption. Body mass and feed consumption were recorded weekly. After a 42 d feeding period, animals were fasted for 12 h, anesthetized with carbon dioxide and killed by decapitation. Blood samples were collected and serum was allowed to clot for 30 min, separated by centrifugation ($2,000 \times g$, 4°C , 10 min), and stored at -80°C . Samples of liver tissue and duodenal mucosa were collected, snap-frozen in liquid nitrogen, and stored at -80°C .

The animal experiment was conducted in accordance with the German Guidelines and Regulations on Animal Care (Deutsches Tierschutzgesetz, 2006) and was approved by the University of Kiel Ethics Committee on Animal Care.

Human pilot study

Healthy males were recruited by advertisement at the University and local community of Reading (United Kingdom) and amongst volunteers who previously participated in nutritional trials at the Hugh Sinclair Human Nutrition Unit. Inclusion criteria were: male gender, 18-55 y of age, and a BMI in the range of 22-32 kg/m^2 . Subjects were excluded from the trial if they were diagnosed with any illness or on long-term medication, used dietary supplements, participated in >5 h of aerobic exercise activity per week, or were involved in a clinical trial within 3 months prior to the study. The study protocol was approved by the University of Reading ethics committee and all subjects gave written informed consent before participation.

A standardized aqueous green tea extract prepared from the leaves of *Camellia sinensis L.* (a kind gift of Cognis Deutschland GmbH & Co KG, Monheim am Rhein, Germany) was used to make the green tea extract (GTE) capsules. The composition of the

Table 1. Polyphenol and caffeine content of the green tea extracts as specified by the manufacturers.

Substance	Content (% of dry matter)	
	Cognis GTE ¹	Polyphenon 60
Caffeine	5.2	n.s. ²
Epigallocatechin	12.2	16.7
Epigallocatechin gallate	6.6	34.0
Epicatechin gallate	3.6	8.7
Galocatechin	2.3	n.s.
Galocatechin gallate	2.1	2.8
Epicatechin	1.2	7.3
Catechin gallate	0.7	0.5
Catechin	0.4	n.s.
Total catechins	29.1	70.0

¹The Cognis green tea extract contained 88% native dry extract and 12% of the excipient maltodextrin as well as 1.8% ellagic acid.

²n.s. = not specified.

GTE is given in *Table 1*. Placebo capsules were filled with 95% maltodextrin (a gift of Cognis) and 5% caffeine (by weight; Synopharm GmbH & Co. KG, Barsbuettel, Germany). Both, the GTE and placebo powders were mixed with the flow-regulating excipient silicium dioxide (0.1% by weight) to improve flow properties prior to filling of the gelatine capsules. Each placebo and GTE capsule contained 0 and 119 mg green tea polyphenols, respectively, and 19 mg caffeine and <0.05 μg total folates. Dietary folate intake was estimated at the end of the trial by use of a food frequency questionnaire (FFQ) (using the software Dietist XP version 3.0) polling the frequency of consumption of the major food sources for folates over a period of 4 weeks. Compliance with the food frequency survey was low; only 13 of the 31 subjects returned their completed questionnaires. Analyses of the limited data available, however, suggests that the mean daily folate consumption did not significantly differ between the experimental groups (GTE, 305 ± 39 $\mu\text{g/d}$, $n=7$; placebo, 356 ± 33 $\mu\text{g/d}$, $n=6$; mean \pm SEM) and was similar to reported values for adult men in the UK (mean 311 $\mu\text{g/d}$) and other European countries (17).

The trial was designed as a double-blind placebo-controlled parallel study. Thirty-one volunteers were randomly assigned to one of two treatment groups (GTE, $n=16$ or placebo, $n=15$) with similar BMI and age (data not shown). Subjects took 6 capsules per day, two with each principal meal, for 3 weeks and were instructed to limit their daily tea and coffee consumption to ≤ 3 cups, but to otherwise maintain their normal diet and exercise patterns. Compliance was determined by counting of the returned capsules at the end of the trial and was high ($>98\%$). Blood samples (20 ml) were drawn into tubes containing 0.05 mL 15% K_3 EDTA (Vacutainer; Becton Dickinson UK Ltd., Oxford, UK) after an overnight fast on the first and last day of the intervention period. Plasma was immediately obtained by centrifugation ($1,000 \times g$, 10 min) and 3 ml aliquots were stored at -80°C until analysis.

Folate quantification by HPLC

Procedures for extraction and purification of folates from human plasma and rat serum and liver samples by strong anion exchange solid-phase extraction were described previously by Witthoft *et al.* (18). Dialysed rat serum (500 μg) was used to ensure complete deconjugation of folate polyglutamates in liver samples; modified from Patring *et al.* (19). Analyses were performed using an HPLC system (Agilent 1100) consisting of a

gradient quaternary pump, a cooled autosampler (4°C), a column oven (23°C), a fluorescence detector (excitation/emission, 290/360 nm for reduced folates and 360/460 nm for 10-HCO-H₄folate) and a multiwavelength UV detector. Authentic folates were used as external standards: tetrahydrofolate (H₄folate), 5-methyl-tetrahydrofolate (5-CH₃-H₄folate), 10-formyl-tetrahydrofolate (10-HCO-H₄folate), and 5,10-methenyl-tetrahydrofolate (5,10-CH⁺-H₄folate) (a gift of Merck Eprova AG, Schaffhausen, Switzerland, except 10-HCO-H₄folate, which was purchased from Schircks Laboratories, Jona, Switzerland). Quantification was based on a multilevel (n=7) external calibration curve with a linear range over 1.2-118.0 ng/mL for H₄folate, 0.6-93.1 ng/mL for 5-CH₃-H₄folate, 0.9-184.1 ng/mL for 10-HCO-H₄folate and 9.3-184.5 ng/mL for 5,10-CH⁺-H₄folate.

mRNA quantification

RNA was isolated from rat duodenal mucosa using the RNeasy Lipid Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. DNA digestion was performed with RNase-Free DNase Set (Qiagen). RNA integrity was checked by electrophoresis on a denaturing agarose gel and ethidium bromide staining. The concentration and purity of isolated RNA was determined by measuring the absorbance (AB) at 260 and 280 nm in a spectrophotometer (DU800, Beckmann Instruments; Munich, Germany). A ratio of >1.8 between AB_{260nm} and AB_{280nm} was considered as acceptable. RNA aliquots were stored at -80°C until analysis. Primer pairs of β-actin, reduced folate carrier (RFC) and proton-coupled folate transporter/heme carrier protein-1 (PCFT/HCP1) were designed to the corresponding sequences of *Rattus norvegicus* mRNA with Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi; 03.05.2007) and purchased from MWG-Biotech AG (Ebersberg, Germany). The sequences of primers used in this study were as follows: Sense primer for β-actin, 5'-GGGGTGTGAAGGTCTCAA-3', antisense primer for β-actin, 5'-TGTCACCAACTGGACGATA-3'; sense primer for RFC, 5'-GGCTCGTGTCTACCTCTGC-3', antisense primer for RFC, 5'-GGTAGTCGGTGAGCAGGAAG-3'; sense primer for PCFT/HCP1, 5'-TGAGCTAAGCACCCCTCT-3', antisense primer for PCFT/HCP1, 5'-TCCGTACCCTGTGAACATGA-3'. The product size was 90 base pair (bp) for β-actin; 183 bp for RFC and 217 bp for PCFT/HCP1. QuantiTect® Primer Assay (Qiagen) was used for DHFR mRNA amplification, with a product size of 88 bp.

For one-step quantitative reverse transcriptase polymerase chain reaction (one-step qRT-PCR) two aliquots of RNA were amplified. External relative standard curves of total RNA were determined with each run. Data was normalized by dividing the concentrations of RFC, PCFT/HCP1 or DHFR by the concentrations of β-actin mRNA. Each PCR reaction (final volume 20 μl) contained 0.5 μmol/L of each primer, 10 μl of 2x QuantiTect® SYBR® Green RT-PCR Master Mix (Qiagen), 0.2 μl QuantiTect RT-Mix (Qiagen), 8 μl of RNA dilution and 1.4 μl water. Real-time cycler conditions were set according to the manufacturers protocol to 40 cycles with annealing temperatures of 56°C for β-actin, 59°C for RFC, 56°C for PCFT/HCP1 and 55°C for DHFR, respectively. Quantification and melting curves of the amplified products were analysed using the RotorGene 6.0 software (Corbett Lifescience; Sydney, Australia). Melting curve analyses and agarose gel electrophoresis with ethidium bromide staining were performed to exclude non-specific products.

Statistical analyses

Statistical calculations were performed with GraphPad Prism 4 software (GraphPad Software Inc., San Diego, CA,

USA). Analyses of the data from the rat study and the *in vitro* assay were performed by means of a one-way ANOVA followed by Dunnett's test for multiple comparisons of group means between animals receiving GTC or control diet. Analyses of the data from the human pilot study were performed by means of a paired Student's t-test for comparison of baseline vs. treatment and by means of an unpaired Student's t-test for comparisons between subjects receiving GTE or placebo. Reported values are means ± SEM and effects were considered significant at P<0.05.

RESULTS

Dihydrofolate reductase activity in vitro

Both pure EGCG and P60, at concentrations of 1000 for EGCG and 1060 nmol/L for EGCG from P60, respectively, time-dependently inhibited DHFR activity (*Fig. 1A*). As shown in *Fig. 1B*, DHFR activity decreased with increasing concentrations of pure EGCG (10-1000 nmol/L) and P60 (EGCG concentrations, 10.6-1060 nmol/L). As expected, methotrexate, which was used as a positive control, inhibited the enzyme almost completely at any concentration used (10-1000 nmol/L).

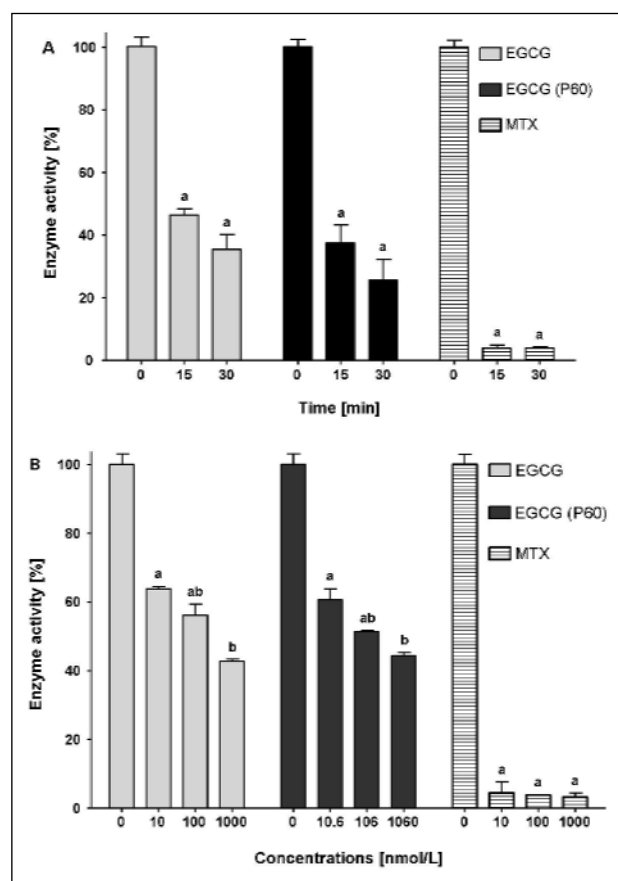


Fig. 1. (A) Time-dependent inhibition of dihydrofolate reductase (DHFR) activity (in percent of control) by pure epigallocatechin gallate (EGCG; 1000 nmol/L), methotrexate (MTX; 1000 nmol/L), and P60 (EGCG concentration, 1060 nmol/L). (B) Concentration-dependent inhibition of DHFR activity by EGCG (10-1000 nmol/L), MTX (10-1000 nmol/L), and P60 (EGCG concentrations, 10.6-1060 nmol/L). Values are means ± SEM, n=4; a = significantly different from control, P<0.05.

Serum and liver folate concentrations in rats

Feed consumption and final body mass (318.7 ± 4.8 g) of the Wistar rats were similar in all groups. Intake of diets containing 0.5% GTC over a period of 42 days significantly decreased the serum concentration of 5-CH₃-H₄folate compared to control rats, whereas the concentrations of H₄folate remained unchanged (Fig. 2A). Serum 10-HCO-, and 5,10-CH⁺-H₄folate values were below detection limit. No differences in liver folate concentrations were observed between dietary groups (Fig. 2B).

Relative mRNA levels of reduced folate carrier and dihydrofolate reductase in rat duodenal mucosa

The housekeeping gene β -actin was expressed at similar levels in all animals and no significant differences in the relative mRNA levels of RFC, PCFT/HCP1 or DHFR in the duodenal mucosa were observed (Fig. 3).

Plasma folate concentrations in humans

Consumption of 670 mg of GTC per day or placebo did not affect plasma folate concentrations in healthy male volunteers. No significant differences in plasma concentrations of 5-CH₃-H₄folate were observed between the treatment groups at baseline (placebo, 16.3 ± 2.6 nmol/L; GTE, 19.1 ± 2.4 nmol/L) or after intervention (placebo, 15.5 ± 2.1 nmol/L; GTE, 17.6 ± 2.4 nmol/L).

DISCUSSION

Green tea is a widely consumed beverage in many countries and contains appreciable amounts of polyphenols. Catechins (flavanols) are the major subclass of bioactive compounds within the polyphenol fraction of green tea. Epidemiological studies associated a high dietary intake of catechins with a reduced risk to suffer from a variety of diseases (reviewed in 20), including

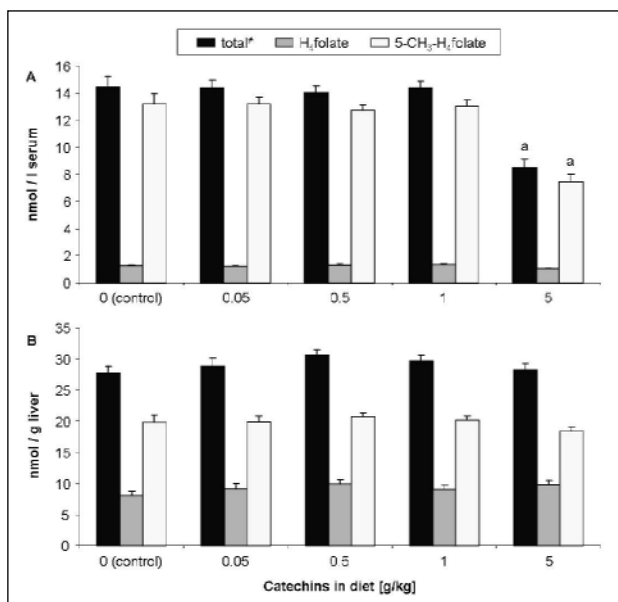


Fig 2. Concentrations of 5-CH₃-H₄folate and H₄folate in serum [A] and liver [B] samples from rats fed 0, 0.05, 0.5, 1, or 5 g green tea catechins/kg diet for 42 d. Values are means \pm SEM, n = 10, a = significantly different from control, P<0.05.

Sum of H₄folate and 5-CH₃-H₄folate calculated as folic acid.

certain forms of cancer (21). The underlying molecular and cellular mechanisms by which green tea catechins may mediate anticarcinogenic activity seem to be diverse: Cell culture experiments as well as studies in rodents indicate that green tea catechin may inhibit angiogenesis *via* a down-regulation of vascular endothelial growth factor (reviewed in 22). Furthermore it has been suggested that the anticancer activity of green tea catechins against different kind of cancers may find an explanation in direct targeting of lipid rafts (23). Recent *in vitro* studies have shown that epigallocatechin gallate (EGCG), the predominant catechin in green tea, competitively inhibits the enzyme dihydrofolate reductase (DHFR) (9, 13). DHFR inhibition is the mechanism by which so-called antifolates, such as the cytostatic drug methotrexate, inhibit cell division and reduce tumor growth (15, 24). Co-administration of folic acid and

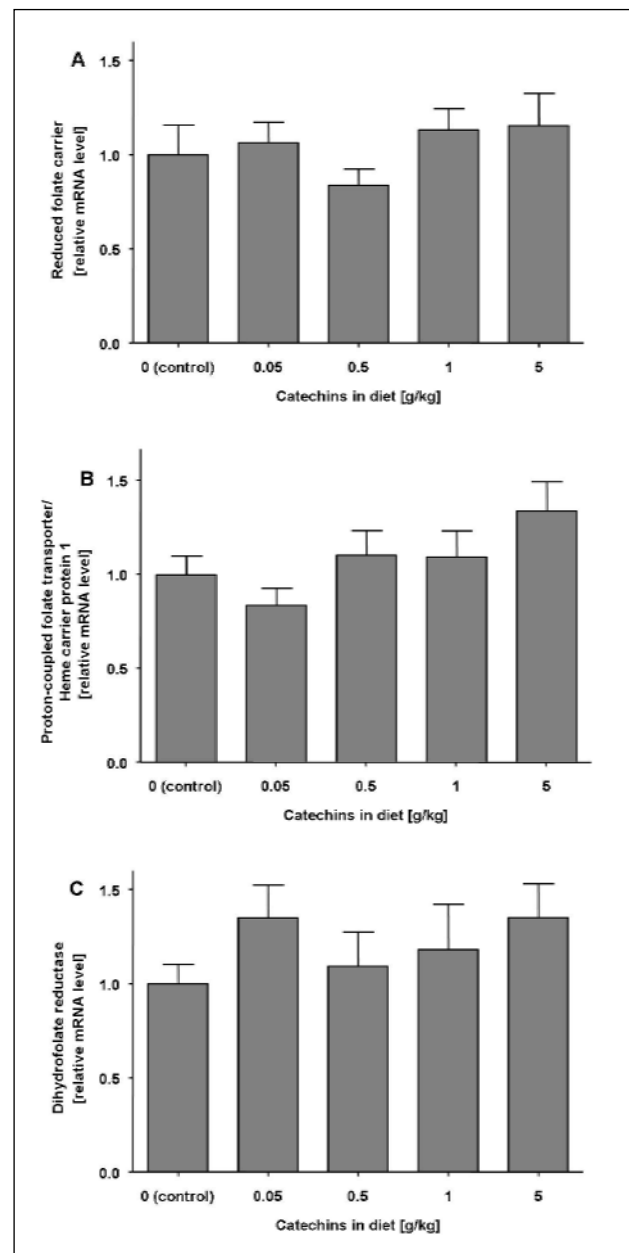


Fig. 3. Relative mRNA levels of reduced folate carrier (A), proton-coupled folate transporter/heme carrier protein-1 (B) and dihydrofolate reductase (C) in duodenal mucosa samples from rats fed 0, 0.05, 0.5, 1, or 5 g green tea catechins/kg diet for 42 d. Values are means \pm SEM, n = 6.

the DHFR inhibitors methotrexate and pyrimethamine, respectively, reduced plasma folate concentrations in rats (25, 26). Thus, the beneficial properties of GTC with respect to the development of certain forms of cancer may potentially induce negative effects with regard to folate status. To date, however, the inhibition of DHFR by gallated catechins has only been demonstrated *in vitro*. We therefore designed the present studies in order to investigate the impact of GTC on folate metabolism *in vivo* using rats and performing a human pilot study. In addition, we carried out *in vitro* studies to show for the first time, that a mixture of GTC, in their naturally occurring proportions, does inhibit DHFR activity more potently than isolated EGCG.

The commercial green tea extract Polyphenon 60 (P60) used in the rat study and its principle bioactive ingredient EGCG inhibited DHFR activity time- and concentration-dependently *in vitro* (Fig. 1). This is in agreement with the data of Navarro-Peran *et al.* (9, 27), who first reported a competitive inhibition of DHFR by EGCG and other gallated catechins. The P60 extract and the isolated EGCG were used at nearly equimolar concentrations of EGCG (EGCG concentrations in the P60 mixture used were 10.6, 106, and 1060 nmol/L; pure EGCG was used at 10.0, 100, and 1000 nmol/L) in the *in vitro* assay. Interestingly, we found that both inhibited DHFR activity to a similar degree, although the total concentrations of gallated catechins in the P60 extract used were ~1.4 times those of pure EGCG. These results suggest that only EGCG significantly contributed to the DHFR-inhibitory effects observed, while the remaining (gallated) catechins in the mixture did not (significantly) inhibit the activity of the enzyme.

In order to study whether or not the effects observed *in vitro* bear a meaning for the more complex physiological processes *in vivo*, Wistar rats were fed for 42 days with diets fortified with increasing concentrations of green tea catechins (GTC) using a standardized green tea extract (P60). The diets contained 2 mg folic acid per kg, which is equivalent to twice the dietary recommendations for laboratory rats as given by the National Research Council (28). It is noteworthy that folates synthesized by the microflora of the large intestine are absorbed and may significantly contribute to blood folate concentrations (reviewed in 29). The diet used in this study was therefore formulated to provide a minimum of substrate to the intestinal microflora to limit bacterial folate synthesis. Only in those animals fed the highest concentrations of the green tea extract (0.5% GTC), did we observe a significant decrease in serum 5-CH₃-H₄folate concentrations as compared to the control group (Fig. 2A). GTC-feeding did not affect the hepatic concentrations of any form of folates measured (Fig. 2B). Serum folate concentrations, however, are earlier responsive to depletion than tissue concentrations and may begin to drop after approximately 2-3 weeks of insufficient folate supply (30, 31). Furthermore, the liver is the main storage site for folates both in humans (32) and in rats (33). This may explain why serum folate levels in rats fed the highest dose of GTC were reduced while liver folate concentrations remained unchanged. The inhibition of DHFR activity combined with a decreased cellular uptake of folate, resulting in a reduced transepithelial transfer of folates (13), may partially explain the decrease in serum folate concentrations in our rats fed 0.5% GTC. In support of this notion, Lemos *et al.* showed that EGCG, upon co-incubation with folic acid, dose-dependently inhibited uptake of the vitamin into Caco-2 cells with an IC₅₀ value of 7.7 μmol/L. Similarly, green and black teas potently inhibited folic acid uptake in Caco-2 cells (34).

At a given substrate affinity and substrate concentration, the capacity of enzymatic turnover of folates as well as the amount of their carrier-mediated transport across cellular membranes is mainly affected by the amount of enzymes/carriers present at the tissue level. Because catechins are known to alter the gene

expression for a variety of proteins (35), we quantified relative mRNA concentrations of the RFC, PCFT/HCP1, and DHFR in the duodenal mucosa of rats fed GTC. No significant differences in mRNA concentrations of RFC, PCFT/HCP1, and of DHFR were found between the experimental groups (Fig. 3). These data indicate that GTC do not reduce the expression of RFC, PCFT/HCP1 and DHFR *in vivo* and further support a direct interaction with the enzyme DHFR. Assuming a competitive inhibition of DHFR by GTC (9), however, measuring the activity of DHFR in the duodenal mucosa of our rats seemed futile, because samples were collected from rats that had been deprived of feed for at least 12 h. By this time, any GTC consumed with the diet would already have been absorbed, metabolized and excreted, and thus would not have been available as a substrate for the enzyme.

The current findings suggested that GTC might decrease serum folate concentrations only if supplied at supra-nutritional doses. A 70 kg human would have to drink almost 100 cups of green tea infusion per day to match the highest dose fed to rats in the present study. Because such a human study would be unfeasible as well as unrealistic, we designed a pilot study with a standardized green tea extract to assess whether or not regular consumption of high doses of GTC might affect plasma folate concentrations in humans. The intake of 670 mg of GTC per day, which corresponds to about 20 cups of green tea, caused no significant differences in plasma concentrations of 5-CH₃-H₄folate between the treatment and placebo groups, both of which consuming a normal diet containing on average ~328 ± 26 μg folate/d. Insufficient dietary intake of folates for as short as 2-3 weeks has been reported to result in reduced blood concentrations of the vitamin (30). Our findings therefore suggest that green tea drinking is unlikely to affect plasma folate concentrations in healthy, free-living subjects and that a longer treatment period and/or even higher doses of dietary GTC may be necessary to induce changes in folate concentrations, if possible at all. Further human studies with GTC and a standardized supply of folic acid (in the absence of naturally occurring reduced folates) are warranted to investigate the influence of GTC on DHFR activity *in vivo*. In addition, the measurement of (oxidized) serum folic acid should be considered because folic acid has been found in serum of subjects consuming folic acid-fortified foods for 5 d (11).

Based on the experiments presented here, it appears unlikely that daily green tea consumption, even at high levels, may affect folate concentrations in healthy humans.

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