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Modulation of Cholesterol Metabolism by the Green Tea Polyphenol (–)-Epigallocatechin Gallate in Cultured Human Liver (HepG2) Cells

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Epidemiological and animal studies have found that green tea is associated with lower plasma cholesterol. This study aimed to further elucidate how green tea modulates cholesterol metabolism. When HepG2 cells were incubated with the main green tea constituents, the catechins, epigallocatechin gallate (EGCG) was the only catechin to increase LDL receptor binding activity (3-fold) and protein (2.5-fold) above controls. EGCG increased the conversion of sterol regulatory element binding protein-1 (SREBP-1) to its active form (+56%) and lowered the cellular cholesterol concentration (−28%). At 50 μM, EGCG significantly lowered cellular cholesterol synthesis, explaining the reduction in cellular cholesterol. At 200 μM EGCG, cholesterol synthesis was significantly increased even though cellular cholesterol was lower, but there was a significant increase seen in medium cholesterol. This indicates that, at 200 μM, EGCG increases cellular cholesterol efflux. This study provides mechanisms by which green tea modulates cholesterol metabolism and indicates that EGCG might be its active constituent.

KEYWORDS: Catechins; cholesterol; cholesterol synthesis; lathosterol; SREBP-1

INTRODUCTION

Hypercholesterolemia is one of the major risk factors for the development of atherosclerosis. Epidemiological studies (*1*, *2*) have identified that drinking green tea is associated with lower plasma cholesterol concentrations. Animal intervention studies have also found that green tea and green tea extracts exhibit hypocholesterolaemic (*3*−*9*) as well as anti-atherogenic effects (*9*, *10*).

Green tea contains an abundance of polyphenolic compounds called catechins, with the four main types being (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epigallocatechin gallate (EGCG). Of these, EGCG is the most abundant, accounting for 58% of the total catechins (*3*).

Catechins account for more than 30% of the solids in a normal infusion of green tea (*11*, *12*) and might therefore be the active constituents in green tea that produce the hypocholesterolaemic effects described. In support of this hypothesis, studies in mice and rats have observed that purified tea catechins, (−)-epicatechin gallate and (−)-epigallocatechin gallate, lower plasma cholesterol and reduce atherosclerosis (*4*, *13*, *14*).

In a study by Chisaka et al. (*13*), it was found that EGCG enhanced the removal of intravenously injected ^{14}C-cholesterol from plasma in rats. This increased clearance rate of cholesterol might have been due to an increase in the expression of the LDL receptor, as this is the main mechanism by which cholesterol is removed from the circulation (*15*). In support of this hypothesis, Bursill et al. (*16*) found that both freshly boiled green tea and a green tea extract enriched in catechins significantly increased LDL receptor binding activity and protein and mRNA levels of cultured human (HepG2) cells. Green tea also increased the conversion of sterol regulatory binding protein-1 (SREBP-1) from its inactive precursor form to its active transcription factor. In support of these findings, Kuhn et al. (*17*) found that EGCG and ECG were able to increase both LDL receptor protein expression and the active form of SREBP-2 in HepG2 cells with subsequent increases in LDL receptor gene transcription. Such an activation of SREBPs might explain the hypocholesterolaemic effects of green tea.

In the present study, we aimed to further elucidate how purified green tea catechins modulate cholesterol metabolism. HepG2 cells were incubated with individual purified catechins to determine their effects on the LDL receptor and SREBP-1, as well as cellular cholesterol synthesis and concentration.

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MATERIAL AND METHODS

Materials. (−)-Epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-gallocatechin gallate (GCG), and epigallocatechin gallate (EGCG) were purchased from Sigma Chemical Company, Castle Hill, NSW, Australia. The green tea catechin extract used in this study was prepared as previously described (*16*) by the method of Huang et al. (*18*) from commercially available "Special Gunpowder" green tea packaged by the China National Native Products and Animal Byproducts Import and Export Corporation, Zhejiang Tea Branch, China. The extract contained at least 58% (w/w) catechins, and the composition of the measured constituents was as follows: 30% EGCG, 21% ECG, 10% caffeine, 6% moisture, 4% EGC, 2% EC, 1% GCG, and 0.5% theanine.

HepG2 Cell Culture. HepG2 cells were grown in monolayer cultures to near confluency in 75-cm^2 flasks with 10 mL of Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal calf serum (DMEM/FCS) at 37 °C with 5% CO_2 (*17*, *19*). Cells were then incubated for 24 h in 75-cm^2 flasks with 10 mL of DMEM/FCS containing different amounts (0−200 μM) of the purified catechins or the green tea catechin extract [100 μM equivalence of (−)-epigallocatechin gallate]. Three flasks of cells were treated with each dose of purified catechin or green tea catechin extract.

LDL Receptor Binding Activity. Following incubation, the cells from each flask were harvested and resuspended in phosphate-buffered saline (PBS), and the protein content was determined (*20*). Determination of the specific LDL receptor binding activity was measured by the method of Roach et al. (*21*). The intact HepG2 cells (100 μg of protein) were incubated for 1 h at room temperature with LDL−gold conjugates (20 μg of protein/mL) in the absence and presence of 20 mM EDTA to determine total and nonspecific binding, respectively. After 1 h, the cells were pelleted by centrifugation, washed, and resuspended in 4% (w/v) gum arabic, and a silver enhancement IntenSE BL kit solution (Amersham, Sydney, Australia) was then added. The silver enhancement reaction and absorbance measurements (500 nm) were carried out using a Cobas Bio autoanalyzer (Roche Diagnostica, Nutley, NJ). The amount of LDL bound to the cells is expressed as nanograms of LDL protein bound per milligram of cell protein (ng of LDL/mg of cell protein). Duplicate determinations were made for both total and nonspecific binding, and the specific binding (total binding minus nonspecific binding) was taken to be the measure of LDL receptor binding activity.

LDL Receptor Protein. Determination of the LDL receptor protein was done by Western blotting with a polyclonal anti-LDL receptor antibody (*22*). Cells were frozen at −80 °C for at least 24 h and slowly thawed when required for experimentation. Thawed cells were pelleted by centrifugation at 400*g* for 10 min and resuspended in 200−225 μL of solubilization buffer (250 mM tris-maleate, pH 6.0, 2 mM $CaCl_2$, 1 mM phenylmethylsulfonyl fluoride, and 1 mM *N*-ethylmaleimide) to make a total volume of 300 μL in 1.5-mL plastic eppendorf tubes. To this was added 90 μL of 5% Triton X-100, after which the mixture was vortexed, and the unsolubilized cell matter was removed by pelleting, using centrifugation at 400*g* for 15 min in a swinging-bucket centrifuge. The protein content of the solubilized cell supernatant was measured (*20*). The solubilized cell samples (100 μg) were subjected to electrophoresis on 3−15% SDS−polyacrylamide gradient gels (*23*) and electrotransferred onto nitrocellulose filter membranes (MSI, Westborough, MA) (*24*). The membranes were overlaid with a polyclonal antibody against the LDL receptor followed by an anti-rabbit IgG antibody conjugated to horseradish peroxidase (Sigma, St. Louis, MO). The LDL receptor band was detected on X-ray film (Hyperfilm-ECL, Amersham, North Ryde, NSW, Australia) using an enhanced chemiluminescence (ECL) kit from Amersham (*25*). Quantification of the LDL receptor protein on film was performed using an LKB Ultroscan XL enhanced laser densitometer and Gelscan computer program (Pharmacia LKB Biotechnology, North Ryde, NSW, Australia). Results are expressed as peak areas as determined from the densitometer scan.

Cholesterol, Lathosterol, and Chenodeoxycholic Acid. Cells were frozen at −80 °C for at least 24 h and slowly thawed when required for experimentation. Thawed cells were subjected to centrifugation for 5 min at 400*g*. They were then homogenized by being resuspended in 1 mL of SDS buffer (0.1% SDS, 1 mM EDTA and 0.1 M Tris base, pH 7.4) and taken up in a syringe with an 18-gauge needle 6−8 times. Protein content was then determined (*20*).

Total cholesterol (esterified plus free) and lathosterol concentrations, as an index of cholesterol synthesis (*26*), were measured in cells by gas−liquid chromatography as described by Wolthers et al. (*27*). To measure unesterified cholesterol, the saponification step was omitted. The cholesterol and lathosterol concentrations are expressed relative to the amount of cell protein (mg/mg of cell protein and μg/mg of cell protein, respectively).

In the medium, concentrations of cholesterol and chenodeoxycholic acid, a bile acid present in HepG2 cell medium, were also determined. For cholesterol, 10 mL of the medium was reduced to near dryness using a Savant SpeedVac SC100 apparatus (Selby Anax, Adelaide, Australia), resuspended in 1 mL of water, and analyzed as for cells (*27*). For chenodeoxycholic acid, the cells were grown to near confluency in 75-cm^2 flasks as described above, except DMEM medium free of phenol red was used (*28*). After the 24-h EGCG incubation, media from two flasks were combined, and bile acids were extracted from the 20 mL of medium by passage through a reverse-phase C18 cartridge (Waters Associates, Milford, MA). The cartridge was washed with 10 mL of water and 5 mL of 10% (v/v) methanol, and the bile acids were eluted with 10 mL of 85% (v/v) methanol (*29*). The samples were dried under a stream of nitrogen, and the bile acids were methylated, derivatized with trifluroacetic anhydride, and analyzed by gas chromatography using lithocholic acid as the internal standard (*29*). Because of the low concentration of bile acids in the medium of the Hep G2 cells, only chenodeoxycholic acid was successfully detected. This was quantified using a standard curve and calculated with respect to the internal standard (lithocholic acid).

SREBP-1. Five 75-cm^2 flasks per treatment group were harvested, and the cells were pelleted by centrifugation at 500*g* for 10 min. The cells were then fractionated by the method of Wang et al. (*30*, *31*). Aliquots (150 μg) of the resulting nuclear and membrane fractions were diluted with twice the volume of loading buffer (*23*) and then boiled for 3 min. Once cooled, samples were run on 8% SDS PAGE gel (*23*) and then transferred at 20 V overnight onto nitrocellulose membranes (4 °C). The membranes were overlayed with a polyclonal antibody against the SREBP-1 protein (K-10, 1:200 dilution, Santa Cruz Biotechnology, Santa Cruz, CA), followed by a secondary antibody conjugated to horseradish peroxidase (1:5000 dilution, Sigma, St. Louis, MO). The inactive precursor and active transcription factor form of SREBP-1 were detected on X-ray film (Hyperfilm-ECL, Amersham, North Ryde, NSW, Australia) using enhanced chemiluminescence (SuperSignal Substrate, Rockford, IL) and laser densitometry.

Statistical Analyses. Results are expressed as mean ± standard error of the mean (SEM). Statistical evaluation was done using either a linear regression or a two-tailed Student *t*-test, comparing the control with the different treatment groups where appropriate. A value of $p < 0.05$ was the criterion of significance.

RESULTS

Catechins and the LDL Receptor. To confirm that the catechins are the active constituents in green tea that upregulate the LDL receptor, the main catechins (100 μM) in green tea and a green tea extract (positive control) were included separately in the medium of HepG2 cells. Incubation with the green tea extract (positive control) significantly increased LDL receptor binding activity (+86%, $p < 0.05$) above control levels (**Figure 1**). Incubation with EGCG (100 μM) resulted in a significant 2-fold increase in LDL receptor binding activity ($p < 0.05$). The other main catechins tested (i.e., EC, ECG, EGC, 100 μM) had no effect on LDL receptor binding activity.

When cells were incubated with increasing concentrations of EGCG (1−200 μM), LDL receptor binding activity was significantly increased up to 3-fold ($p < 0.05$) in the highest-dose treatment group of 200 μM (**Figure 2A**). There was a positive linear trend between LDL receptor binding activity and

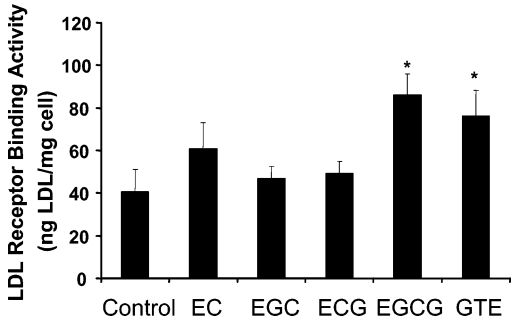

Figure 1. Comparison of purified catechins from green tea and a green tea extract in terms of LDL receptor binding activity. HepG2 cells were incubated for 24 h with 100 μM purified catechins or 100 μM EGCG equivalent of a green tea extract in 10 mL of medium. The LDL receptor binding activity was measured as the calcium-dependent binding of LDL-gold to the intact cells. Each value represents the mean ± SEM of triplicate cell incubations. The asterisks (∗) denote a significant difference compared to control ($p < 0.05$).

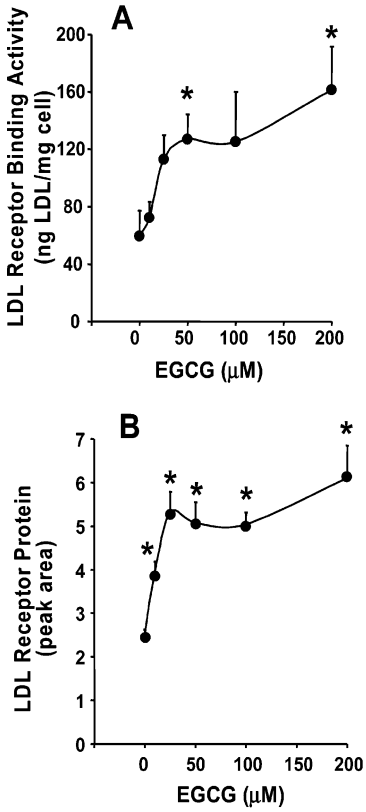

Figure 2. Dose-dependent effect of EGCG on (**A**) LDL receptor binding activity and (**B**) protein. HepG2 cells were incubated for 24 h with increasing amounts of EGCG, 0–200 μM in 10 mL of medium. The LDL receptor binding activity was measured as the calcium-dependent binding of LDL-gold to the intact cells. The LDL receptor protein was measured using a polyclonal antibody against the LDL receptor and the ECL western blot method. Each value represents the mean ± SEM of triplicate cell incubations.

the dose of EGCG incubated with the cells ($p < 0.01$). EGCG also increased the amount of LDL receptor protein, detected by blotting with a polyclonal antibody against the LDL receptor (**Figure 2B**). The scanned peak area was up to 2.5-fold greater in the highest-dose treatment group of 200 μM (6.10 ± 0.75 arbitrary absorbance units) compared to the control (2.5 ± 0.12 units). There was a positive linear trend between the amount of

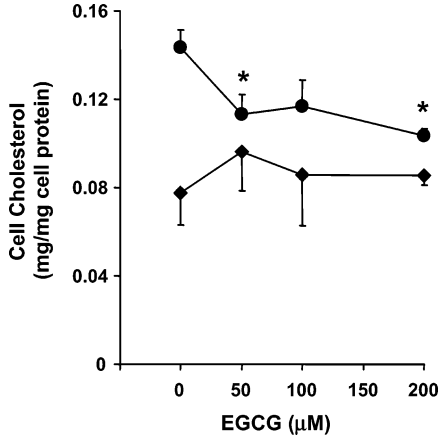

Figure 3. Dose-dependent effects of EGCG on intracellular total and free cholesterol concentrations. The HepG2 cells were incubated for 24 h with increasing amounts of EGCG, 0–200 μM in 10 mL of medium. Homogenized cells were extracted with hexane, and total (●) and unesterified (◆) cholesterol were analyzed using gas chromatography and expressed relative to cell protein. Each value represents the mean ± SEM of triplicate cell incubations.

Figure 4. Effect of EGCG on SREBP-1 protein. HepG2 cells were incubated for 24 h with either 0 (control, lanes 1–4) or 200 μM (lanes 5–8) EGCG in 10 mL of medium. Cell extracts (150 and 200 μg) of nuclear (lanes 1, 2, 5, 6) and microsomal membrane (lanes 3, 4, 7, 8) fractions were subjected to electrophoresis on an 8% SDS PAGE gel and electrotransferred onto nitrocellulose. The SREBP-1 precursor (P, from microsomal membrane fraction) and mature (M, from nuclear fraction) forms were detected using a polyclonal antibody and the ECL western blot method. Each value represents the mean ± SEM of triplicate cell incubations.

LDL receptor protein and the dose of EGCG incubated with the cells ($p < 0.01$).

EGCG and Cellular Cholesterol. Treatment with EGCG decreased intracellular total cholesterol concentrations by up to 28% in the highest-dose treatment group of 200 μM (**Figure 3**). There was a significant inverse linear trend between total intracellular cholesterol and the dose of EGCG incubated with the cells ($p < 0.05$). However, EGCG did not lower intracellular unesterified cholesterol concentrations (**Figure 3**).

EGCG and SREBP-1. Treatment with 200 μM of EGCG resulted in 42% and 56% increases in the active transcription factor form of SREBP-1 (nuclear cell fraction) compared to the control for the 150- and 200-μg cell protein samples, respectively. In addition, EGCG treatment decreased the inactive precursor form of SREBP-1 (membrane fraction) to undetectable levels (**Figure 4**).

EGCG, Cholesterol Synthesis, Medium Cholesterol, and Chenodeoxycholate. A reduction in cholesterol synthesis, a loss of cholesterol from the cell into the medium, and an increase in the conversion of cholesterol into bile acids are three factors that could have decreased the intracellular concentration of cholesterol in the HepG2 cells (*28*, *32*). Indices of these cholesterol homeostatic mechanisms were therefore measured.

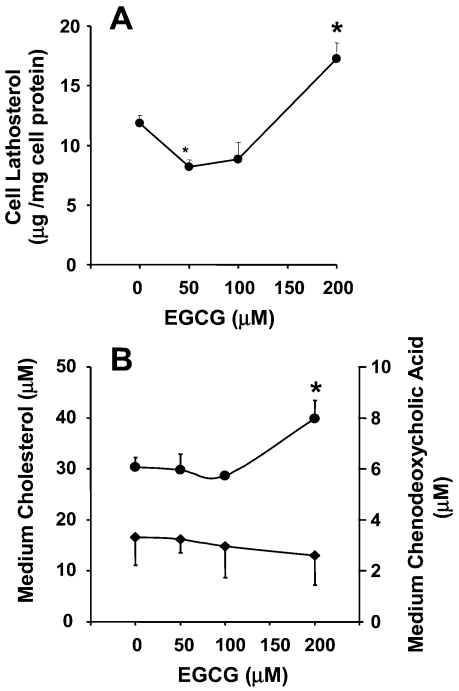

Figure 5. (**A**) Dose-dependent effect of EGCG on cholesterol synthesis. HepG2 cells were incubated for 24 h with increasing amounts of EGCG, 0–200 μM in 10 mL medium. Lathosterol was extracted from homogenized cells and measured using gas chromatography. (**B**) Medium cholesterol (●) and chenodeoxycholic acid (◆) were determined using gas chromatography. Each value represents the mean ± SEM of triplicate cell incubations. The asterisks (∗) denote a significant difference compared to control ($p < 0.05$).

Cellular lathosterol concentration, measured as an index of cholesterol synthesis, was significantly reduced at the lowest dose of 50 μM EGCG (**Figure 5A**). However, at the highest dose of 200 μM, there was a significant increase (50%) in the cellular lathosterol concentration (**Figure 5A**).

At the lower doses of 50 and 100 μM EGCG, there was no effect on the cholesterol concentration in the medium compared to the control (**Figure 5B**). At the highest dose of 200 μM, however, EGCG caused a significant increase (30%) in the medium cholesterol concentration (**Figure 5B**). This increased excretion of cholesterol into the medium at this high dose of 200 μM might explain why the intracellular cholesterol concentrations remained significantly lower despite the 50% increase in cholesterol synthesis at this dose. There was a strong correlation ($r = 0.773$, $p < 0.01$) between the cell lathosterol concentration (**Figure 5A**) and the medium cholesterol concentration (**Figure 5B**).

EGCG did not produce any significant changes in the concentration of chenodeoxycholic acid in the medium (**Figure 5B**). The lowered intracellular cholesterol concentration (**Figure 3**) was therefore not likely to be due to an increase in the conversion of cholesterol to bile acids.

DISCUSSION

The aim of this study was to elucidate how purified green tea catechins modulate cholesterol metabolism in HepG2 cells. It was found that EGCG was the only catechin, of the four main catechins in green tea, to significantly increase the LDL receptor above the control. It increased both the LDL receptor binding activity and the relative amounts of LDL receptor protein. Evidence that this effect can occur at the level of gene transcription was also found, as EGCG treatment increased the active transcription factor form of SREBP-1. These results are consistent with the study by Kuhn et al. (*17*), which showed that EGCG could also increase the active form of SREBP-2 and the LDL receptor protein in treated HepG2 cells. If EGCG can exhibit the same effects on the liver in vivo and increase the hepatic LDL receptor, it would explain the enhanced clearance of ^{14}C-cholesterol from the plasma seen in rats fed EGCG (*13*) and the hypocholesterolaemic effects of green tea in epidemiological and animal intervention studies (*1*−*9*).

When cells were incubated with EGCG, cellular cholesterol was 30% lower than in the control cells. Although a lowering of cellular cholesterol can trigger an increase in the LDL receptor through a sterol negative feedback system (*15*), it is the unesterified form of cholesterol that is thought to be regulatory (*30*, *31*). Unesterified cholesterol can be converted to oxysterols (*33*), which regulate the activation of SREBPs by inhibiting their proteolytic cleavage from the endoplasmic reticulum (*34*). Normally, when cleaved, the active transcription factor form of the SREBP is released from the endoplasmic reticulum and travels to the nucleus, where it can bind upstream of the LDL receptor and activate transcription (*35*).

In this study, however, EGCG did not significantly change unesterified cholesterol concentrations in the cells. This indicates that the regulatory pool of unesterified cholesterol might not be measurable in HepG2 cells, as suggested by Havekes et al. (*32*). However, in cultured cells, SREBP-1 has been shown to be sensitive to changes in intracellular sterol levels (*36*) and might be a better marker of sterol levels than unesterified cholesterol itself. Alternatively, as suggested by Kuhn et al. (*17*), the mature form of SREBP-1 might have accumulated as a result of a reduced turnover because its proteolytic degradation by the chymotrypsin-like activity of the ubiquitin−proteasome was inhibited by EGCG. Although the findings by Kuhn et al. (*17*) were related to SREBP-2, the same is likely to apply to the degradation of SREBP-1 in the HepG2 cells. However, in contrast to cells in culture, regulation of the LDL receptor in vivo appears to be more under the control of SREBP-2 than SREBP-1 (*37*).

The decrease in the total cellular cholesterol by EGCG could have occurred via three mechanisms: an inhibition of cholesterol synthesis, an increase in the efflux of cholesterol from the cell to the medium, and an increase in the conversion of cholesterol to bile acids. At the lowest concentration of EGCG (50 μM), there appeared to be a decrease in cholesterol synthesis as measured using cell lathosterol, an index of cholesterol synthesis (*26*). At this concentration, EGCG might have inhibited cholesterol synthesis, which, in turn, might have contributed to the reduction in cell cholesterol. In support of this hypothesis, a study by Abe et al. (*38*) reported that green tea gallocatechins, including ECGC, were potent inhibitors of squalene epoxidase, an enzyme in the cholesterol biosynthesis pathway.

In contrast to the lower-dose concentration (50 μM), cholesterol synthesis appeared to be significantly higher in the group treated with 200 μM EGCG. Nevertheless, cholesterol did not accumulate within the cells, as the cell cholesterol decreased by 28%. Rather than having accumulated in the cells, the extra cholesterol seemed to be in the medium, where its concentration was increased by +30% over the control. It therefore appears that, at the highest concentration, EGCG increased the efflux of cholesterol from the cells into the medium to such an extent that it caused an increase in cholesterol synthesis but this did not fully compensate for the loss of cell sterol. There was a high correlation ($r = 0.773$) between cell lathosterol and the

concentration of cholesterol in the medium, suggesting that the concentration of cholesterol in the medium was directly linked to the amount of cholesterol synthesized by the cells. The increase seen in the SREBP-1 mature form with 200 μM EGCG is also consistent with the observed increase in cholesterol synthesis, as SREBP-1 also upregulates the HMG-CoA reductase gene, the rate-limiting enzyme in cholesterol synthesis (*31*).

Treatment with EGCG did not appear to increase the conversion of cell cholesterol into bile acids (*28*), as the chenodeoxycholic acid content of the medium did not change significantly. This therefore would not have contributed to a decrease in cellular cholesterol.

A study by Ikeda et al. (*39*) found that EGCG formed insoluble complexes with cholesterol, thereby reducing the solubility of cholesterol into bile acid micelles. This ability of EGCG to form complexes with cholesterol might explain the effects seen in vitro with the highest dose of EGCG (200 μM) in the present study. At this concentration, EGCG might complex with enough cholesterol in the medium to render it essentially cholesterol-deficient. Cholesterol could then move from the cells into the medium by normal diffusion down the concentration gradient. This could explain why the concentration of cholesterol is seen to increase in the medium while the cells are not able to regain their normal intracellular cholesterol levels, despite an increase in cholesterol synthesis. This increased EGCG-mediated excretion of cholesterol from the HepG2 cells was also unlikely to be due to an increased secretion of apoB-100 and cholesterol-containing lipoproteins given that Yee et al. (*40*) previously found EGCG to decrease, not increase, the excretion of apoB-100 from HepG2 cells.

Overall, the effects seen with pure EGCG on cholesterol metabolism mirror the effects previously observed for green tea extracts (*16*). This provides evidence that EGCG is likely to be the active constituent and is consistent with EGCG being the most abundant catechin in green tea (*3*).

In conclusion, EGCG was found to modulate cholesterol metabolism in HepG2 cells. The LDL receptor was upregulated, providing a mechanism to explain the hypocholesterolaemic effects of green tea. EGCG appears to increase the LDL receptor by increasing the active transcription factor form of SREBP-1. Cellular total cholesterol was also decreased by treatment with EGCG. The mechanism for this appears to be inhibition of cholesterol synthesis at the lower dose (50 μM) and an increase of the efflux of cholesterol from the cells into the medium at the highest dose (200 μM). The EGCG polyphenol constituent of green tea might be a potential therapeutic agent to reduce plasma cholesterol and atherosclerosis.

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LITERATURE CITED

(1) Kono, S.; Shinchi, K.; Wakabayashi, K.; Honjo, S.; Todoroki, I.; Sakurai, Y.; Imanishi, K.; Nishikawa, H.; Ogawa, S.; Katsurada, M. Relation of green tea consumption to serum lipids and lipoproteins in Japanese men. *J. Epidemiol.* **1996**, *6*, 128−133.

(2) Imai, K.; Nakachi, K. Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. *Br. Med. J.* **1995**, *310*, 693−696.

(3) Muramatsu, K.; Fukuyo, M.; Hara, Y. Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *J. Nutr. Sci. Vitaminol.* **1986**, *32*, 613−622.

(4) Matsuda, H.; Chisaka, T.; Kambomura, Y.; Yamahara, J.; Sawada, T.; Fujimura, H.; Kimura, H. Effects of crude drugs on experimental hypercholesterolemia. I. Tea and its active principles. *J. Ethnopharmacol.* **1986**, *17*, 213−224.

(5) Yang, T. T.; Koo, M. W. Hypocholesterolemic effects of Chinese tea. *Pharm. Res.* **1997**, *35*, 505−512.

(6) Fukuyo, M.; Hara, Y.; Muramatsu, K. Effect of tea leaf catechin, (−)-epigallocatechin gallate, on plasma cholesterol level in rats. *J. Jpn. Soc. Nutr. Food Sci.* **1986**, *39*, 495−500.

(7) Chan, P. T.; Fong, W. P.; Cheung, Y. L.; Huang, Y.; Ho, W. K.; Chen, Z. Y. Jasmine green tea epicatechins are hypolipidemic in hamsters (*Mesocricetus auratus*) fed a high fat diet. *J. Nutr.* **1999**, *129*, 1094−1101.

(8) Yang, T. T.; Koo, M. W. Chinese green tea lowers cholesterol level through an increase in fecal lipid excretion. *Life Sci.* **2000**, *66*, 411−423.

(9) Vinson, J. A.; Teufel, K.; Wu, N. Green and black teas inhibit atherosclerosis by lipid, antioxidant and fibrinolytic mechanisms. *J. Agric. Food Chem.* **2004**, *52*, 3661−3665.

(10) Miua, Y.; Chiba, T.; Tomita, I.; Kiozuma, H.; Miura, S.; Umegaki, K.; Hara, Y.; Ikeda, M.; Tomita, T. Tea catechins prevent the development of atherosclerosis in apoprotein E-deficient mice. *J. Nutr.* **2001**, *131*, 27−32.

(11) Graham, H. N. Green tea composition, consumption, and polyphenol chemistry. *Prev. Med.* **1992**, *21*, 334−350.

(12) Harbowy, M. E.; Balentine, D. A. Tea chemistry. *Crit. Rev. Plant Sci.* **1997**, *16*, 415−480.

(13) Chisaka, T.; Matsuda, H.; Kubomura, Y.; Mochizuki, M.; Yamahara, J.; Fujimura, H. The effect of crude drugs on experimental hypercholesterolaemia: mode of action of (−)-epigallocatechin gallate in tea leaves. *Chem. Pharm. Bull.* **1988**, *36*, 227−233.

(14) Chyu, K. Y.; Babbidge, S. M.; Zhao, X.; Dandillaya, R.; Rierveld, A. G.; Yano, J.; Dimayuga, P.; Cercek, B.; Shah, P. K. Differential effects of green tea-derived catechin on developing versus established atherosclerosis in apolipoprotein E-null mice. *Circulation* **2004**, *109*, 2448−2453.

(15) Brown, M. S.; Goldstein, J. A. A receptor-mediated pathway for cholesterol homeostasis. *Science* **1986**, *232*, 34−47.

(16) Bursill, C.; Roach, P. D.; Bottema, C. D.; Pal, S. Green tea upregulates the low-density lipoprotein receptor through the sterol-regulated element binding protein in HepG2 liver cells. *J. Agric. Food Chem.* **2001**, *49*, 5639−5645.

(17) Kuhn, D. J.; Burns, A. C.; Kazi, A.; Dou, Q. P. Direct inhibition of the ubiquitin−proteasome pathway by ester bond-containing green tea polyphenols is associated with the increased expression of sterol regulatory-binding protein 2 and LDL receptor. *Biochim. Biophys. Acta* **2004**, *1682*, 1−10.

(18) Huang, M. T.; Ho, C. T.; Wang, Z. Y.; Ferraro, T.; Finnegan-Olive, T.; Lou, Y. R.; Mitchell, J. M.; Laskin, J. D.; Newmark, H.; Yang, C. S.; Conney, A. Inhibitory effect of topical application of a polyphenol fraction on tumour initiation and promotion in mouse skin. *Carcinogenesis* **1992**, *13*, 947−954.

(19) Kambouris, A. M.; Roach, P. D.; Calvert, G. D.; Nestel, P. J. Retroendocytosis of high-density lipoproteins by the human hepatoma cell line, HepG2. *Arteriosclerosis* **1990**, *10*, 582−590.

(20) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

(21) Roach, P. D.; Hosking, J.; Clifton, P. M.; Bais, R.; Kusenic, B.; Coyle, P.; Wight, M. B.; Thomas, D. W.; Nestel, P. J. The effects of hypercholesterolaemia, simvastatin and dietary fat on the low-density lipoprotein receptor of unstimulated mononuclear cells. *Atherosclerosis* **1993**, *103*, 245−254.

(22) Roach, P. D.; Balasubramaniam, S.; Hirata, F.; Abbey, M.; Szanto, A.; Simons, L. A.; Nestel, P. J. The low-density lipoprotein receptor and cholesterol synthesis are affected differently by dietary cholesterol in the rat. *Biochim. Biophys. Acta* **1993**, *1170*, 165−172.
(23) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−684.
(24) Burnette, W. N. "Western Blotting": Electrophoretic transfer of proteins from sodium dodecyl sulphate−polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* **1981**, *112*, 195−203.
(25) Krika, L. J. Chemiluminescent and bioluminescent techniques. *Clin. Chem.* **1991**, *37*, 1472−1481.
(26) Kempen, H. J.; Glatz, J. F.; Gevers Leuven, J. A.; van der Voort, H. A.; Katan, M. B. Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. *J. Lipid Res.* **1988**, *29*, 1149−1155.
(27) Wolthers, B. J.; Walrecht, H. T.; van der Molen, J. C.; Nagel, G. T.; Van Doormaal, J. J.; Wijnandts, P. N. Use of determinants of 7-lathosterol (5α-cholest-7-en-3β-ol) and other cholesterol precursors in serum in the study and treatment of disturbances of sterol metabolism, particularly cerebrotendinous xanthomatosis. *J. Lipid Res.* **1991**, *32*, 603−612.
(28) Axelson, M.; Mörk, B.; Everson, G. T. Bile acid synthesis in cultured human hepatoblastoma cells. *J. Biol. Chem.* **1991**, *266*, 17770−17777.
(29) Ross, P. E.; Pennington, C. R.; Bouchier, I. A. Gas−liquid chromatographic assay of serum bile acids. *Anal. Biochem.* **1977**, *80*, 458−465.
(30) Wang, X.; Sato, R.; Brown, M. S.; Goldstein, J. L. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell* **1994**, *77*, 53−62.
(31) Brown, M. S.; Goldstein, J. L. Sterol regulatory element binding proteins (SREBPs): Controllers of lipid synthesis and cellular uptake. *Nutr. Rev.* **1998**, *56*, S1−S3.
(32) Havekes, L. M.; De Wit, E. C.; Princen, H. M. Cellular free cholesterol in Hep G2 cells is only partially available for down-regulation of low-density-lipoprotein receptor activity. *Biochem. J.* **1987**, *247*, 739−746.
(33) Grundy, S. M. Multifactorial etiology of hypercholesterolaemia. *Arterioscler. Thromb.* **1991**, *11*, 1619−1635.
(34) Winegar, D. A.; Salisbury, J. A.; Sundseth, S. S.; Hawke, R. L. Effects of cyclosporin on cholesterol 27-hydroxylation and LDL receptor activity in HepG2 cells. *J. Lipid Res.* **1996**, *37*, 179−191.
(35) Brown, M. S.; Goldstein, J. L. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane bound transcription factor. *Cell* **1997**, *89*, 331−340.
(36) Shimomura, I.; Shimano, H.; Horton, J. D.; Goldstein, J. L.; Brown, M. S. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. *J. Clin. Invest.* **1997**, *99*, 838−845.
(37) Horton, J. D.; Goldstein, J. L.; Brown, M. S. SREBPs: Activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **2002**, *109*, 1125−1131.
(38) Abe, I.; Seki, T.; Umehara, K.; Miyase, T.; Noguchi, H.; Sakakibara, J.; Ono, T. Green tea polyphenols: Novel and potent inhibitors of squalene epoxidase. *Biochem. Biophys. Res. Commun.* **2000**, *268*, 767−771.
(39) Ikeda, I.; Imasato, Y.; Sasaki, E.; Nakayama, M.; Nagao, H.; Takeo, T.; Yayabe, F.; Sugano, M. Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. *Biochim. Biophys. Acta* **1992**, *1127*, 141−146.
(40) Yee, W. L.; Wang, Q.; Agdinaoay, T.; Dang, K.; Chang, H.; Grandinetti, A.; Franke, A. A.; Theriault, A. Green tea catechins decrease apolipoprotein B-100 secretion from HepG2 cells. *Mol. Cell Biochem.* **2002**, *229*, 85−92.

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