

# A Green Tea Catechin Extract Upregulates the Hepatic Low-Density Lipoprotein Receptor in Rats

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**Abstract** Green tea extracts have hypocholesterolaemic properties in epidemiological and animal intervention studies. Upregulation of the low-density lipoprotein (LDL) receptor may be one mechanism to explain this as it is the main way cholesterol is removed from the circulation. This study aimed to determine if a green tea extract could upregulate the hepatic LDL receptor in vivo in the rat. A green tea extract (GTE) enriched in its anti-oxidant constituents, the catechins, was fed to rats ( $n = 6$ ) at concentrations of either 0, 0.5, 1.0 or 2.0% (w/w) mixed in with their normal chow along with 0.25% (w/w) cholesterol for 12 days. Administration of the GTE had no effect on plasma total or LDL cholesterol concentrations but high-density lipoprotein significantly increased (41%;  $p < 0.05$ ). Interestingly, there was a significant increase in LDL receptor binding activity (2.7-fold) and LDL receptor protein (3.4-fold) in the 2% (w/w) treatment group compared to controls. There were also significant reductions in liver total and unesterified cholesterol (40%). Administration of the GTE significantly reduced cholesterol absorp-

tion (24%) but did not affect cholesterol synthesis. These results show that, despite no effect on plasma cholesterol, the GTE upregulated the LDL receptor in vivo. This appears to be via a reduction in liver cholesterol concentration and suggests that the green tea extract was able to increase the efflux of cholesterol from liver cells.

**Keywords** Green tea · LDL receptor · Cholesterol synthesis · Cholesterol absorption · Lathosterol · Phytosterols

## Introduction

The low-density lipoprotein (LDL) receptor is a cell surface protein that is present on the outer surface of most cells, but in particular liver cells. It is the main mechanism by which cholesterol-carrying LDL can be removed from the circulation [1]. There is, therefore, much interest in agents that increase LDL receptor activity and subsequently lower plasma cholesterol concentrations. Elevated LDL cholesterol levels are associated with increased risk of heart disease, one of the biggest killers in western societies [2].

There is evidence to suggest that green tea and its anti-oxidant constituents, the catechins, can upregulate the LDL receptor. This has evolved from epidemiological studies [3–5] showing that drinking between 5 and 10 cups of green tea per day is associated with lower plasma cholesterol concentrations. Intervention studies in rats, mice and hamsters have also found that green tea and green tea extracts enriched in catechins exhibit hypocholesterolaemic effects [6–10]. An increase in the LDL receptor may be one mechanism by which to explain this observed cholesterol-lowering ability of green tea extracts.

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Studies in vitro [11–13] have provided more direct evidence that green tea extracts and its catechin constituents can upregulate the LDL receptor and modulate cholesterol metabolism in HepG2 cells. Indirect evidence that the LDL receptor may be upregulated by green tea extracts in vivo has also been found [14]. When rats were fed EGCG, the main catechin in green tea, the removal of intravenously injected  $^{14}\text{C}$ -cholesterol from the plasma was enhanced. This increase in the plasma clearance of cholesterol may be due to the upregulation of the LDL receptor [1] but was not assessed.

Currently, the inhibition of cholesterol absorption has been proposed as the mechanism to explain the cholesterol lowering effects of green tea in vivo. This is because the faecal excretion of total lipids and cholesterol were found to be higher in animals consuming green tea extracts [6, 7, 9]. The EGCG has also been observed to inhibit the uptake of  $^{14}\text{C}$ -cholesterol from the intestine [14]. This apparent reduction in intestinal cholesterol absorption has been ascribed to EGCG reducing the solubility of cholesterol into mixed bile salt micelles [15]. It has also been found that hamsters and rats fed green tea extracts had increased faecal excretion of bile acids [9, 10]. A study in rabbits found that a green tea extract also inhibited cholesterol synthesis [16]. Despite these effects on cholesterol absorption and synthesis, it does not rule out the possibility that an upregulation of the LDL receptor could also contribute to the hypocholesterolaemic effects of green tea. Evidence for this has been found in recently published work, where the administration of a green tea extract to rabbits significantly increased the hepatic LDL receptor in vivo [16].

The aims of this study were to determine if administration of a crude catechin extract from green tea could upregulate the hepatic LDL receptor in rats and subsequently lower plasma cholesterol in the rat.

## Experimental Procedures

### Catechin Extract

The crude catechin extract was prepared from commercially available “Special Gunpowder” green tea, packaged by the China National Native Products and Animal By-products Import and Export Corporation, Zhejiang Tea Branch, China. The method used was based on the method of Huang et al. [17]. Briefly, 15 kg of green tea was extracted with three volumes (v/v) of methanol at 50 °C for 3 h. Solvent was removed from the extract using a reduced pressure rotary evaporator. The residue was dissolved in two volumes of water (v/v) at 50 °C and extracted twice

with equal volumes of hexane (v/v) and once with an equal volume of chloroform (v/v). The remaining aqueous phase was then extracted once with an equal volume of ethyl acetate (v/v) which extracts the polyphenolic compounds including the catechins. The ethyl acetate was then evaporated, the residue redissolved in the minimum amount of warm water (50 °C) and freeze dried. The extract contained at least 58% (w/w) catechins and the composition of the measured constituents was: 30% EGCG, 21% ECG, 10% caffeine, 6% moisture, 4% EGC, 2% GCG and 0.5% theanine.

### Animal Study

Twenty-four male Sprague Dawley rats (IMVS, Gillies Plains, SA, Australia) were housed at the CSIRO Health Sciences and Nutrition Animal Facility (Kintore Avenue, Adelaide, SA, Australia) in surroundings of controlled temperature ( $20 \pm 1$  °C) and a 12 h light cycle (0600 to 1800). Ethics approval for the study was obtained from the University of Adelaide and CSIRO Health Sciences and Nutrition Animal Ethics Committees.

After an initial plasma collection from the tail vein, the rats were randomised into four different treatment groups. The crude catechin extract was mixed in with their normal rat chow at concentrations of 0, 0.5, 1 or 2% (w/w) along with 0.25% (w/w) cholesterol and fed to the rats for a period of 12 days. The rats were weighed every 2 days. After the 12 days dietary intervention the rats were fasted overnight prior to sacrifice.

Blood was collected into tubes containing EDTA (final concentration 1 g/L) from the abdominal aorta of rats under halothane anaesthesia. Plasma was isolated by centrifugation at  $1,500 \times g$  for 10 min and 1 mL aliquots were frozen at  $-20$  °C for later analysis. Liver tissue was also excised and immediately frozen in liquid nitrogen.

### Plasma Lipid Determinations

The LDL fraction ( $d = 1.019\text{--}1.063$  g/mL) was isolated from 2 mL of plasma by sequential ultracentrifugation. The high-density lipoprotein (HDL) fraction was obtained after precipitating apoB containing lipoproteins with polyethylene glycol 6000 (BDH Chemicals, Kilsyth, Victoria, Australia). Cholesterol in whole plasma and in the LDL and HDL fractions was measured on the Cobas Bio automated centrifugal analyser (Roche, Basel, Switzerland) by enzymatic methods using test kits (Roche Diagnostica, Basel, Switzerland) [18]. The triglyceride concentration of the whole plasma was also determined using enzymatic methods (Roche Diagnostica) on the Cobas Bio.

## Cholesterol Synthesis and Intrinsic Capacity to Absorb Dietary Cholesterol

Plasma lathosterol and phytosterols (campesterol and  $\beta$ -sitosterol) were measured by gas chromatography (GC) [19]. The ratios of serum lathosterol and phytosterol concentrations in the plasma to plasma cholesterol concentration, have been found to correlate with whole body cholesterol synthesis [20] and the intrinsic capacity to absorb dietary cholesterol [21], respectively.

## Hepatic LDL Receptor Binding Assay

To prepare LDL–gold conjugates, normolipidemic human blood (Australian Red Cross, Adelaide, Australia) was used to isolate LDL ( $1.025 < d < 1.050$ ) by sequential ultracentrifugation. Colloidal gold was prepared and the isolated LDL was then conjugated to the colloidal gold as previously described [22].

A 2–3 g piece of liver was homogenised and microsomal membranes (800–100,000 $\times$ g centrifuge fraction) were prepared and solubilised with 1% (w/v) Triton X-100, 5 mM phenylmethylsulfonyl fluoride and 5 mM *N*-ethylmaleimide, to prevent degradation and dimerisation of the rat LDL receptor protein. Once solubilised, Triton X-100 was removed using Amberlite XAD-2 [23] and the protein content of the microsomal membranes was determined.

To measure LDL receptor binding activity, 8  $\mu$ g of the solubilised liver membranes were applied to nitrocellulose paper (Schleicher and Schuell, Westborough, MA, USA) which was then blocked with 4% (w/v) bovine serum albumin solution [22, 24]. The nitrocellulose membranes were then incubated in buffer containing either 20  $\mu$ g/mL LDL–gold in the absence and presence of 20 mM EDTA to determine total and non-specific binding, respectively. The nitrocellulose paper was soaked in water for 30 min and then incubated with intense BL silver enhancement kit (Amersham, UK) for further 30 min. This was washed with water, dried and scanned using an LKB Ultrascan XL enhanced laser densitometer (Pharmacia LKB Biotechnology, North Ryde, NSW, Australia). The specific binding (total minus the non-specific binding) was taken to be the LDL receptor binding activity which is expressed as peak height, determined from the laser densitometer scan.

## Quantification of LDL Receptor Protein

To determine relative amounts of LDL receptor protein, solubilised rat liver membranes (150  $\mu$ g) were subjected to electrophoresis on 3–15% SDS polyacrylamide gradient gels and electrotransferred onto nitrocellulose paper. The membranes were then overlaid with a polyclonal antibody [24] against the LDL receptor (1:2,000) followed by an

anti-rabbit IgG antibody conjugated to horseradish peroxidase (Sigma, St Louis, MO, USA). The LDL receptor band was then detected on X-ray film (Hyperfilm-ECL, Amersham, North Ryde, NSW, Australia) using enhanced chemiluminescence (Amersham, North Ryde, NSW, Australia). Quantification of LDL receptor protein was performed by laser densitometry. Results are expressed as peak area, determined from the densitometer scan.

## Liver Lipid Determinations

Total cholesterol, unesterified cholesterol and triglycerides were measured on the liver homogenates. Liver preparations were initially sonicated then diluted 1:1 with a 2% (w/w) Triton X-100 and 2 mM  $\text{CaCl}_2$  solution. This was agitated on a rotating wheel for 30 min at 4 °C and protein content determined. Lipid measurements were performed using enzymatic methods on the Cobas Bio and expressed relative to the protein concentrations.

## Statistical Analyses

Results are expressed as mean  $\pm$  SEM. Percentage changes were calculated by determining the percentage change in the highest dose treatment group (2% w/w) relative to the control group. Similarly, fold changes were calculated by dividing the mean of the 2% (w/w) treatment group by the control group.

Statistical evaluation was done using a one way analysis of variance (ANOVA) and a Tukey's posthoc test. A value of  $p < 0.05$  was the criterion of significance. The statistics were performed using the SPSS statistics package.

## Results

### Crude Catechin Extract from Green Tea has No Effect on Plasma Lipids in Rats

There were no significant differences in plasma total cholesterol concentration between groups before intervention with the crude catechin extract (data not shown). There was also no significant changes in body weight between groups throughout the treatment period. This would indicate that there was no variability in food intake between groups, although this was not systematically measured. The inclusion of a green tea catechin extract to regular rat chow did not therefore affect the appetite of the rats.

Administration of the crude catechin extract along with 0.25% cholesterol for 12 days did not significantly change plasma total cholesterol, LDL cholesterol or triglyceride concentrations compared to the control group (Table 1). The HDL cholesterol concentrations were however,

**Table 1** Plasma lipid concentrations in rats after the administration of a crude catechin extract

	Crude catechin extract (%) (w/w)			
	0	0.5	1	2
Total cholesterol (mmol/L)	1.01 ± 0.10	1.39 ± 0.21	1.20 ± 0.12	1.49 ± 0.23
Triglycerides (mmol/L)	0.55 ± 0.04	0.68 ± 0.08	0.40 ± 0.03	0.52 ± 0.04
LDL cholesterol (mmol/L)	0.32 ± 0.06	0.39 ± 0.10	0.35 ± 0.07	0.44 ± 0.08
HDL cholesterol (mmol/L)	0.66 ± 0.05	0.87 ± 0.10	0.77 ± 0.05	0.93* ± 0.10

Values given as mean ± SEM. Twenty-four Sprague Dawley rats were divided into four different treatment groups of six rats each. The different treatment groups were fed a crude catechin extract for 12 days at concentrations of either 0, 0.5, 1.0, 2.0% (w/w) mixed in with normal rat chow and 0.25% cholesterol. Plasma lipids were determined using sequential ultracentrifugation and enzymatic methods on the Cobas Bio

\*  $p < 0.05$  (significant difference to control)

significantly increased (41%;  $p < 0.05$ ) in rats supplemented with the highest dose of crude catechin extract (2%) when compared with the control rats (Table 1).

#### Crude Catechin Extract from Green Tea Decreases Cholesterol Absorption

The ratio of plasma phytosterol to cholesterol was used as an index of the intrinsic capacity to absorb dietary cholesterol. Administration of the crude catechin extract was found to significantly decrease cholesterol absorption (24%) in the highest dose treatment group compared to controls (Table 2). No significant changes were seen in cholesterol synthesis between treatment groups.

#### Crude Catechin Extract from Green Tea Upregulates the Hepatic LDL Receptor

The calcium-dependant, LDL–gold binding capacity of solubilised liver membranes was used to determine hepatic LDL receptor binding activity. Administration of the highest dose of crude catechin extract (2% w/w) significantly increased (2.7-fold;  $p < 0.05$ ) the hepatic LDL receptor binding activity compared to the control (Fig. 1). Using a polyclonal antibody against the LDL receptor of liver homogenates, the relative amounts of LDL receptor protein was found to be significantly higher ( $p < 0.05$ ) in all the treatment groups compared to the control. It was at

its highest in the in the 2% (w/w) treatment group (3.4-fold higher than control) (Fig. 1).

#### Crude Catechin Extract from Green Tea Lowers Liver Cholesterol

Administration of the crude catechin extract significantly reduced ( $p < 0.05$ ) total and unesterified cholesterol in liver homogenates (40% for both) in the 2% (w/w) treatment group compared to controls (Fig. 2). Unesterified cholesterol constituted approximately 70% of the total cholesterol content and consumption of the crude catechin extract did not alter this percentage significantly. Administration of the crude catechin extract also significantly lowered triglyceride concentrations in liver homogenates by 40% (control:  $80.4 \pm 6.8$ , 0.5%: $74.7 \pm 10.0$ , 1.0%: $65.4 \pm 8.0$ , 2.0%: $49.0 \pm 4.0$  mmol/L;  $p < 0.05$ ).

## Discussion

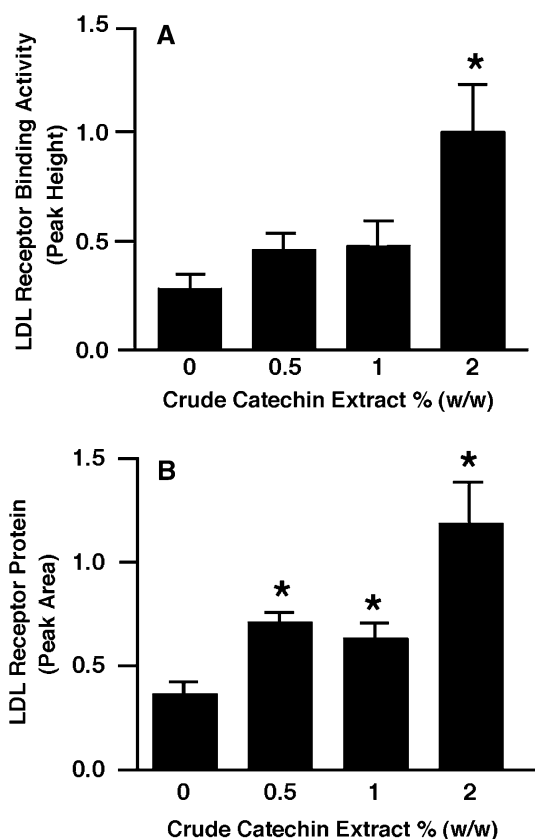
The aim of this study was to investigate if administration of a crude catechin extract, from green tea, could increase the hepatic LDL receptor in vivo in the rat. An upregulation of the LDL receptor would provide a mechanism to explain the hypocholesterolaemic effects of green tea that have been observed in epidemiological and animal intervention studies. In contrast with previous studies, administration of

**Table 2** Effect of a crude catechin extract on cholesterol absorption and cholesterol synthesis

	Crude catechin extract (%) (w/w)			
	0	0.5	1	2
Phytosterols/cholesterol ( $\mu\text{M}/\text{mM}$ )	21.92 ± 2.12	23.93 ± 2.85	19.75 ± 3.34	16.74* ± 1.70
Lathosterol/cholesterol ( $\mu\text{M}/\text{mM}$ )	0.11 ± 0.01	0.07 ± 0.005	0.10 ± 0.020	0.10 ± 0.032

Values given as mean ± SEM. Twenty-four Sprague Dawley rats were divided into four treatment groups of six rats each. The different treatment groups were fed a crude catechin extract for 12 days at concentrations of either 0, 0.5, 1.0, 2.0% (w/w) mixed in with normal rat chow and 0.25% cholesterol. Phytosterols, lathosterol and cholesterol were measured using gas chromatography

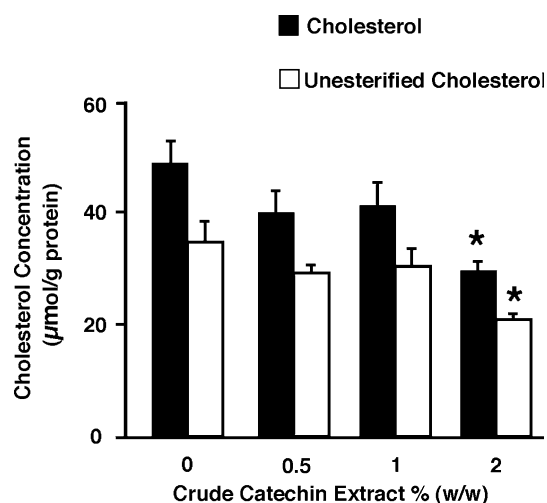
\*  $p < 0.05$  (significant difference compared to the control)



**Fig. 1** Green tea extract upregulates the LDL receptor. Twenty-four Sprague Dawley rats were divided into four treatment groups of six rats each. The different treatment groups were fed a crude catechin extract for 12 days at concentrations of either 0, 0.5, 1.0, 2.0% (w/w) mixed in with normal rat chow and 0.25% cholesterol. **a** Calcium-dependant LDL receptor binding activity was measured using solubilised livers dot-blotted onto nitrocellulose membranes and colloidal-gold LDL. **b** Relative amounts of LDL receptor protein were measured using a polyclonal antibody and western blotting. \* $p < 0.05$  (significant difference compared to the control)

the crude catechin extract did not alter plasma total or LDL cholesterol concentrations. There was, however, a significant increase in HDL cholesterol. Interestingly, administration of the highest dose (2% w/w) of extract was able to significantly increase both LDL receptor binding activity and the relative amounts of LDL receptor protein. There were also significant reductions in liver total and unesterified cholesterol concentrations. In accordance with previous studies, there was a significant reduction in the intrinsic capacity to absorb cholesterol. Despite no effect on plasma cholesterol concentrations, the crude catechin extract used in this study upregulated the LDL receptor. This appears to be via a reduction in liver cholesterol concentrations, suggesting that the crude catechin extract increases the efflux of cholesterol from liver cells.

Upregulation of the LDL receptor is triggered when there is a reduction in intracellular cholesterol concentrations [1]. Administration of the crude catechin extract



**Fig. 2** Green tea extract lowers liver cholesterol. Twenty-four Sprague Dawley rats were divided into four treatment groups of six rats each. The different treatment groups were fed a crude catechin extract for 12 days at concentrations of either 0, 0.5, 1.0, 2.0% (w/w) mixed in with normal rat chow and 0.25% cholesterol. Total and unesterified cholesterol concentrations were measured on homogenised livers using enzymatic methods on the Cobas Bio. \* $p < 0.05$  (significant difference compared to the control)

lowered liver cholesterol and increased the hepatic LDL receptor. In particular there was a reduction in liver unesterified cholesterol which is thought to be the regulatory form of cholesterol [25]. These results are consistent with in vitro studies which found that incubation with green tea or ECGC increased the LDL receptor and decreased intracellular cholesterol concentrations in HepG2 cells [11, 12]. This work is also consistent with a recent study, which found that administration of a green tea extract to rabbits increased the hepatic LDL receptor and lowered intracellular liver cholesterol [16].

Liver cellular cholesterol concentration can be lowered by three main mechanisms: (1) a decrease in cholesterol entry to the cell, (2) an increase in the packaging of cholesterol into lipoproteins and secretion or, (3) an increase in bile acid production. The first mechanism is unlikely as there should have been an increase in cholesterol entry into the cell due to the increase in the LDL receptor. The third mechanism is possible. However, if bile acid production and excretion had increased, it would have been expected that the plasma cholesterol concentration would have subsequently decreased. As there was no change in plasma cholesterol concentrations it would seem that an increase in packaging of cholesterol into lipoproteins and secretion from the liver cells is most likely. So despite the increased entry of cholesterol into the cell via the upregulation of the LDL receptor, plasma cholesterol levels may not have been lowered due to the increased secretion of cholesterol back into the circulation. This apparent increase in LDL recycling



may be a beneficial effect of the crude catechin extract as the LDL particle would be in the circulation for less time, thus reducing its chances of being oxidised. Oxidised LDL has been found to be pro-atherogenic [26, 27].

An increase in cholesterol efflux from the liver cells is also consistent with *in vitro* HepG2 cell studies [11, 12]. Incubation with green tea and EGCG appeared to increase cholesterol efflux from the cells as cholesterol concentration in the cell media was significantly increased.

Administration of the crude catechin extract increased HDL cholesterol concentration. This increase in HDL concentration may have offset any potential reduction in plasma total cholesterol concentration. However, an increase in HDL cholesterol could potentially be an athero-protective effect of the crude catechin extract. The HDL is involved in a process called reverse cholesterol transport where it transfers cholesterol from the tissues and arteries and transports it back to the liver for processing [28]. Large clinical trials have also found that there is a strong inverse correlation between heart disease and plasma HDL concentrations [29, 30]. The mechanism for the increase in HDL cholesterol by the green tea extract is not known. It is possible that the crude catechin extract is able to upregulate the factors involved in the transfer of cholesterol from cells to HDL. The results from this study suggest that there is an increased efflux of cholesterol from liver cells. It is therefore likely that the crude catechin extract may have this effect in other cells, for example, in the tissues and arteries. This is supported in a recently published study [16] that found administration of a green tea extract to rabbits decreased cholesterol concentrations in the thoracic aorta. In this study, however, plasma cholesterol concentrations were significantly reduced which would also contribute to a lowering of cholesterol in the thoracic aorta.

Consistent with previous studies the crude catechin extract significantly reduced the intrinsic capacity of cholesterol to be absorbed. This was measured by the plasma ratio of phytosterols to cholesterol. Other studies have found that faecal excretion of total lipids and cholesterol were higher in animals consuming green tea extracts [6, 7, 9] and EGCG has been observed to inhibit the uptake of  $^{14}\text{C}$ -cholesterol from the intestine [14]. This apparent reduction in intestinal cholesterol absorption has been ascribed to EGCG reducing the solubility of cholesterol into mixed bile salt micelles [15]. It has also been found that hamsters and rats fed green tea extracts had increased faecal excretion of bile acids [9, 10]. Despite the reduction in cholesterol absorption, there was no reduction in plasma cholesterol concentrations. A reduction in plasma cholesterol concentration may have been expected as less cholesterol was entering the circulation. The reduction in cholesterol absorption is consistent, however, with the reduction in liver cholesterol concentrations and reinforces

that the crude catechin extract was increasing the efflux of cholesterol from the liver cells.

In contrast to previous studies, we did not find a hypo-cholesterolaemic effect with green tea. This indicates that the crude catechin extract used in this study, for this time period, must affect lipid metabolism differently in rats than observed in other studies. Previous studies have found that green tea extracts fed to rats, mice, hamsters and rabbits have significantly lowered plasma cholesterol concentrations [6–10]. It is possible that the 12 days dietary intervention was not sufficient as other studies tended to be longer (4–8 weeks). It may also be due to the particular strain of rats used in the present study having a different response [31].

The green tea extract used in this study contained 10% caffeine. There is evidence in the literature that unfiltered coffee has cholesterol-raising effects. This is thought to be due to components called diterpenes [32]. However, the effects of caffeine alone, as present in the green tea extract, are inconclusive [32]. It has been found, however, that caffeine can inhibit cholesterol absorption [33]. It is possible, therefore that caffeine had a minor effect on cholesterol absorption in this study. Catechins, particularly EGCG are also known to inhibit cholesterol absorption [6, 7, 9, 14], consistent the current study.

There is certainly no evidence that caffeine has an effect on the LDL receptor. In addition to this, previous studies by our group have found that purified, commercially purchased, catechins upregulate the LDL receptor [12]. This would suggest that it is the catechin constituents in the green tea extract and not the caffeine that are upregulating the LDL receptor in rats.

In conclusion, administration of a crude catechin extract from green tea upregulated the hepatic LDL receptor in the rats. This appeared to be due to an increase in the efflux of cholesterol from the liver cells into the circulation, as there was a decrease in liver intracellular cholesterol and no change in plasma cholesterol.

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## References

1. Brown MS, Goldstein JL (1986) A receptor-mediated pathway for cholesterol homeostasis. *Science* 232:34–47
2. Glass CK, Witztum JL (2001) Atherosclerosis: the road ahead. *Cell* 104:503–516
3. Kono S, Shinchi K, Ikeda N, Yanai F, Imanishi K (1992) Green tea consumption and serum lipid profiles: a cross-sectional study in northern Kyushu, Japan. *Prev Med* 21:526–531
4. Stensvold I, Tverdal A, Solvoll K, Foss OP (1992) Tea consumption: relationship to cholesterol, blood pressure, and coronary and total mortality. *Prev Med* 21:546–553

5. Imai K, Nakachi K (1995) Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. *BMJ* 310:693–696
6. Muramatsu K, Fukuyo M, Hara Y (1986) Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *J Nutr Sci Vitaminol (Tokyo)* 32:613–622
7. Matsuda H, Chisaka T, Kubomura Y, Yamahara J, Sawada T, Fujimura H, Kimura H (1986) Effects of crude drugs on experimental hypercholesterolemia. I. Tea and its active principles. *J Ethnopharmacol* 17:213–224
8. Yang TT, Koo MW (1997) Hypocholesterolemic effects of Chinese tea. *Pharmacol Res* 35:505–512
9. Anonymous (2000) Chinese green tea lowers cholesterol level through an increase in fecal lipid excretion. *Life Sci* 66:411–423
10. Chan PT, Fong WP, Cheung YL, Huang Y, Ho WK, Chen ZY (1999) Jasmine green tea epicatechins are hypolipidemic in hamsters (*Mesocricetus auratus*) fed a high fat diet. *J Nutr* 129:1094–1101
11. Bursill C, Roach PD, Bottema CD, Pal S (2001) Green tea upregulates the low-density lipoprotein receptor through the sterol-regulated element binding protein in HepG2 liver cells. *J Agric Food Chem* 49:5639–5645
12. Bursill CA, Roach PD (2006) Modulation of cholesterol metabolism by the green tea polyphenol (–)-epigallocatechin gallate in cultured human liver (HepG2) cells. *J Agric Food Chem* 54:1621–1626
13. Kuhn DJ, Burns AC, Kazi A, Dou QP (2004) Direct inhibition of the ubiquitin–proteasome pathway by ester bond-containing green tea polyphenols is associated with increased expression of sterol regulatory element-binding protein 2 and LDL receptor. *Biochim Biophys Acta* 1682:1–10
14. Chisaka T, Matsuda H, Kubomura Y, Mochizuki M, Yamahara J, Fujimura H (1988) The effect of crude drugs on experimental hypercholesterolemia: mode of action of (–)-epigallocatechin gallate in tea leaves. *Chem Pharm Bull (Tokyo)* 36:227–233
15. Ikeda I, Imasato Y, Sasaki E, Nakayama M, Nagao H, Takeo T, Yayabe F, Sugano M (1992) Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. *Biochim Biophys Acta* 1127:141–146
16. Bursill CA, Abbey M, Roach PD (2007) A green tea extract lowers plasma cholesterol by inhibiting cholesterol synthesis and upregulating the LDL receptor in the cholesterol-fed rabbit. *Atherosclerosis* 193:86–93
17. Huang MT, Ho CT, Wang ZY, Ferraro T, Finnegan-Olive T, Lou YR, Mitchell JM, Laskin JD, Newmark H et al (1992) Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin. *Carcinogenesis* 13:947–954
18. Clifton PM, Chang L, Mackinnon AM (1988) Development of an automated Lowry protein assay for the Cobas-Bio centrifugal analyser. *Anal Biochem* 172:165–168
19. Wolthers BG, Walrecht HT, van der Molen JC, Nagel GT, Van Doormaal JJ, Wijnandts PN (1991) Use of determinations of 7-lathosterol (5 alpha-cholest-7-en-3 beta-ol) and other cholesterol precursors in serum in the study and treatment of disturbances of sterol metabolism, particularly cerebrotendinous xanthomatosis. *J Lipid Res* 32:603–612
20. Kempen HJ, Glatz JF, Gevers Leuven JA, van der Voort HA, Katan MB, (1988) Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. *J Lipid Res* 29:1149–1155
21. Tilvis RS, Miettinen TA (1986) Serum plant sterols and their relation to cholesterol absorption. *Am J Clin Nutr* 43:92–97
22. Roach PD, Zollinger M, Noel SP (1987) Detection of the low density lipoprotein (LDL) receptor on nitrocellulose paper with colloidal gold–LDL conjugates. *J Lipid Res* 28:1515–1521
23. Roach PD, Noel SP (1985) Solubilization of the 17 alpha-ethinyl estradiol-stimulated low density lipoprotein receptor of male rat liver. *J Lipid Res* 26:713–720
24. Roach PD, Kerry NL, Whiting MJ, Nestel PJ (1993) Coordinate changes in the low density lipoprotein receptor activity of liver and mononuclear cells in the rabbit. *Atherosclerosis* 101:157–164
25. Grundy SM (1991) George Lyman Duff memorial lecture. Multifactorial etiology of hypercholesterolemia. Implications for prevention of coronary heart disease. *Arterioscler Thromb* 11:1619–1635
26. Albertini R, Moratti R, De Luca G (2002) Oxidation of low-density lipoprotein in atherosclerosis from basic biochemistry to clinical studies. *Curr Mol Med* 2:579–592
27. Steinberg D (1997) Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 272:20963–20966
28. Ohashi R, Mu H, Wang X, Yao Q, Chen C (2005) Reverse cholesterol transport and cholesterol efflux in atherosclerosis. *QJM* 98:845–856
29. Randomised trial of cholesterol lowering in 4,444 patients with coronary heart disease: the Scandinavian simvastatin survival study (4S) (1994) *Lancet* 344:1383–1389
30. MRC/BHF heart protection study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial (2002) *Lancet* 360:7–22
31. Roach PD BS, Hirata F, Abbey M, Szanto A, Simons LA, Nestel PJ (1993) The low-density lipoprotein receptor and cholesterol synthesis are affected differently by dietary cholesterol in the rat. *Biochim Biophys Acta* 1170(2):165–172
32. Wang S, Noh SK, Koo SI (2006) Epigallocatechin gallate and caffeine differentially inhibit the intestinal absorption of cholesterol and fat in ovariectomized rats. *J Nutr* 136(11):2791–2796
33. Cornelis MC, El-Sohehy A (2007) Coffee, caffeine, and coronary heart disease. *Curr Opin Lipidol* 18(1):13–9