Effect of tea catechins on postprandial plasma lipid responses in human subjects

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Epidemiological surveys suggest that a higher intake of tea may be associated with a lower risk of CHD. There is accumulating evidence that postprandial lipaemia makes a substantial contribution to the incidence of CHD. Our aim was, therefore, to evaluate the effect of tea catechins (major ingredients in green tea) on postprandial lipid responses in human subjects after the consumption of test meals. In a randomized triple-crossover design, nine male subjects with mild or borderline hypertriacylglycerolaemia consumed 10 (control), 224 (moderate dose) and 674 mg (high dose) of the assigned tea catechins three times each along with a standardized light meal consisting of a piece of bread spread with 20 g butter. Plasma lipids were measured in the fasting state and 1, 2, 3, 4 and 6 h after consuming the light meal. Results showed that, compared with the control, moderate and high doses of tea catechins reduced the incremental area under the plasma triacylglycerol curves by 15·1 and 28·7 %, respectively. Next, the rapid elevation of remnant-like particle cholesterol was significantly inhibited by a high dose of tea catechins 2 h after consuming the light meal ($P < 0.01$). In the range of tea catechin dosages, no significant differences were observed in the postprandial responses for plasma total cholesterol or NEFA at any time point. In conclusion, this trial demonstrated that tea catechins attenuated the postprandial increase in plasma triacylglycerol levels following a fat load. These results may provide evidence for one of the possible mechanisms involved in lowering the incidence of CVD, and may prove useful in further studies on the beneficial health effects of tea drinking.

Tea catechins: Triacylglycerols: Remnant lipoprotein: Postprandial lipaemia

A great deal of scientific interest has been aroused in the beneficial health effects of tea (*Camellia sinensis*). The most extensively investigated benefit is its role in preventing CHD (Yang & Landau, 2000; Peters *et al.* 2001; Kris-Etherton & Keen, 2002; McKay & Blumberg, 2002). Epidemiological surveys provide evidence that tea consumption may be associated with lowering CHD mortality rates (Tijburg *et al.* 1997; Hollman *et al.* 1999; Peters *et al.* 2001).

Several lines of evidence suggest that elevated levels of fasting plasma triacylglycerols may be an independent risk factor for CVD (Jeppesen *et al.* 1998). Over the last decade, a few studies have been conducted on the effects of tea catechins on the fasting plasma triacylglycerols in animal models (Matsumoto *et al.* 1998; Vinson & Dabbagh, 1998). The proposed mechanism underlying the hypolipidaemic activity of tea catechins is most likely mediated by its influence on the intestinal absorption of dietary fat (Chan *et al.* 1999). Moreover, a Japanese cross-sectional study reported that an increased consumption of green tea was associated with a lower fasting serum triacylglycerol level (Imai & Nakachi, 1995).

In recent years, however, there has been a renewal of interest in whether the postprandial elevation of plasma triacylglycerols may be more closely related to atherogenic risk rather than fasting triacylglycerol levels (Sanders, 2003). In this respect, the primary treatment for reducing the risk of CHD is to follow a low-fat diet, while another possible treatment is to ingest something that selectively limits the intestinal absorption of dietary fat. Recent attention has been paid to the latter possibility. Some studies demonstrated that red wine polyphenols attenuate postprandial plasma lipid response in human subjects, possibly by delaying the absorption of dietary fat through the intestine (Kondo *et al.* 1998; Pal *et al.* 2004). As already stated, tea is a rich source of catechin polyphenols. Although an animal study demonstrated that intestinal fat absorption was significantly decreased in rats orally administered a single dose of (−)-epigallocatechin gallate, which is a major component in tea catechins (Raederstorff *et al.* 2003), the clinical contribution of tea catechins to the intestinal absorption of dietary fat is not so far understood.

The aim of the present study was to evaluate the effect of tea catechins on the postprandial response of plasma triacylglycerols after an oral fat load on human subjects.

Abbreviations: IAUC, increment area under the curve; RLP, remnant-like particle.

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Methods

Test beverages

A semi-purified catechin extract from green tea (THEA-FLAN 90S; ITO EN, Ltd, Tokyo, Japan) was used for the preparation of test beverages. This extract was rich in (−)-epigallocatechin gallate and (−)-epicatechin gallate. The present study employed three dose challenges of the following tea catechins in total: (1) 10 mg tea catechins as a control; (2) 224 mg as a moderate dose; (3) 674 mg as a high dose. Table 1 gives the contents of individual tea catechins per bottle serving (250 ml) of each test beverage. In addition to the tea catechins, other ingredients such as cyclodextrin, L-ascorbic acid and a green tea-flavoured catechin-free liquid were added to the respective beverages at the same levels as the control.

Subjects

The clinical aspects of this study were proposed in accordance with the Helsinki Declaration of 1964 as revised in 1989, and were approved by the Ethical Committee of the Nihonbashi Egawa Clinic (Tokyo, Japan). Subjects were recruited from the Nihonbashi Egawa Clinic. The procedures were explained in detail to all subjects in advance, and all gave their informed written consent for participation in the study. Exclusion criteria included chronic ailments such as hepatic, renal or cardiac dysfunction, and malabsorption. Individuals who were on medication or consuming health foods that may affect the lipid metabolism were not enrolled in this study. Based on screening blood tests carried out within 2 weeks, nine male adults with borderline and mild hypertriacylglycerolaemia (range 1·38–2·49 mmol/l) were sampled. Physical characteristics and fasting metabolic profiles of the participants are shown in Table 2. Subjects were required to abstain from any alcoholic beverages for 2 d before each visit.

Experimental protocol

Upon entry into the study, subjects were randomly divided into three groups. Each group was assigned to one of three treatment sequences to ensure a study conducted in a double-blind-controlled manner. The three testing sessions were separated by 1-week

Table 1. Tea catechin composition of the test beverages (mg/250 ml)*

	Catechin dose		
	Control	Moderate	High
Tea catechins (total)	10	224	674
(−)-Epigallocatechin gallate	1	68	243
(−)-Gallocatechin gallate	0	88	233
(−)-Epicatechin gallate	0	33	118
(−)-Catechin gallate	1	30	70
(−)-Epigallocatechin	1	0	0
(−)-Gallocatechin	5	5	5
(−)-Epicatechin	1	0	5
(−)-Catechin	1	0	0

* A tea catechin extract (THEA-FLAN 90S; ITO EN, Ltd, Tokyo, Japan) was used for preparing test beverages (moderate and high). THEA-FLAN 90S mainly contains 47 % of (−)-epigallocatechin gallate and 15 % of (−)-epicatechin gallate of the dry weight. In the manufacturing process of green tea beverages, a part of such catechin is epimerized into (−)-gallocatechin gallate and (−)-catechin gallate.

Table 2. Subject characteristics and fasting plasma lipid levels at screening (Mean values with their standard errors for nine subjects)

	Mean	SE	Range
Age (years)	46	3	(33−63)
Body weight (kg)	78	8	(65−88)
BMI (kg/m^2)	26·8	0·8	(23·6−30·6)
Triacylglycerols (mmol/l)	1·98	0·14	(1·38−2·49)
Total cholesterol (mmol/l)	5·77	0·38	(4·11−7·44)
HDL-cholesterol (mmol/l)	1·17	0·07	(0·91−1·51)
NEFA (mEq/l)	0·44	0·04	(0·32−0·72)

intervals. In between sessions, the subjects were free to continue their usual lifestyle, eating and exercise habits. On the evening before an experiment, the subjects ate a standardized light dinner before 21.00 hours to avoid the effects of previous food intake, and then refrained from all food and beverages (except water) until 09.00 hours on the following morning. After blood was taken from the antecubital vein under overnight fasting conditions, each subject consumed the fat-enriched bread and test beverage within 10 min. The test meal consisted of one piece of sliced white bread (70 g) (Yamazaki Baking Co. Ltd, Tokyo, Japan) and butter (20 g) (Snow Brand Milk Products, Co. Ltd, Sapporo, Japan), the total nutrient composition of which was calculated as 7·0 g protein, 18·8 g fat and 31·8 g carbohydrate (total energy 1364 kJ). Blood samples were collected 1, 2, 3, 4 and 6 h after the completion of the light meal. No food (except 300 ml water) was allowed until the end of the study.

Biochemical analyses

Plasma was separated from whole blood by centrifuging at 1200 **g** for 20 min, and subjected to biochemical analyses of triacylglycerols, total cholesterol and NEFA using automatically enzyme-based methods. Remnant-like particle (RLP) cholesterol was determined with a JIMRO-II assay kit (Japan Immunoresearch Laboratories, Takasaki, Japan) using an immunoseparation method.

Statistics

Results are expressed as means with their SEM. The increment areas under the time *v.* concentration curves (increment area under the curves, IAUC) were evaluated after subtracting the initial individual values (0 h) from all respective postprandial measurements, yielding the net postprandial change as calculated by the trapezoidal rule (Matthews *et al.* 1990). Comparisons between results from the test beverages were made by repeated-measures ANOVA followed by Dunnett's test. A value of $P < 0.05$ was considered statistically significant. These statistical calculations were performed with GraphPad Prism for Windows version 4.0 (GraphPad Software, San Diego, CA, USA).

Results

All participants were able to follow the study protocol without difficulty. No adverse effects from the experimental beverages were reported by subjects during or after the testing sessions. There were no significant differences in the baseline levels

of plasma triacylglycerols, total cholesterol and NEFA among the three different sessions (data not shown).

The incremental postprandial responses of plasma triacylglycerols are illustrated in Fig. 1(A). When the subjects consumed the test meal with a control beverage, plasma triacylglycerol concentrations quickly rose from the baseline (0 h), and reached their highest levels 3 h after the test-meal intake. Concentrations did not return to baseline for 6 h. Clearly, the peak plasma triacylglycerols were reduced by co-administrations of tea catechins in a dose-dependent manner. Repeated-measures one-way ANOVA with *post hoc* comparisons using Dunnett's test applied to the data showed that, compared with control, the plasma triacylglycerol response to the oral fat load was significantly lower 2 and 3 h after a high dosage of tea catechins ($P < 0.05$, both ways). This was reflected in the significant effect of tea catechins from the high-dosage treatment on the mean IAUC as shown in Table 3 ($P < 0.05$). Expressed as a percentage of control, the average reductions in IAUC after a moderate and a high-catechin dosage were 15·1 and 28·7 %, respectively. However, no significant effect on IAUC was observed between the control and the moderate dosage of tea catechins.

The same was true of the plasma level of RLP-cholesterol. Fig. 1(B) shows that the maximum level of plasma RLP-cholesterol was reached 2 h after consuming the test meal with a control beverage. With moderate and high dosages, however, the rise in plasma RLP-cholesterol continued for 4 h. The rapid elevations in plasma RLP-cholesterol were suppressed by the tea catechin supplementations, with a statistically significant difference between the control and the high dosage at 2 h ($P < 0.01$), whereas the repeated-measures ANOVA for detecting differences across treatments showed no statistically significant difference in IAUC among the three test beverages ($P = 0.10$) (Table 3).

There was a mild increase in plasma total cholesterol levels during the study period (Fig. 2(A)). Meanwhile, plasma-NEFA showed a temporary downturn from the baseline, and then turned upward (Fig. 2(B)). Postprandial levels of total cholesterol and NEFA were found to be unrelated to the different dosage levels of tea catechins at any time point.

Discussion

To date, a number of clinical studies have been conducted to evaluate the effect of tea consumption on the potential risk factors for CVD. Investigation of the cardiovascular-protective effects of tea can be focused on the prevention of oxidative damage of LDL, interference with the atherosclerotic inflammatory process, reduction of thrombosis, and the promotion of normal endothelial function (Kris-Etherton & Keen, 2002). In keeping with the current attention being paid to the association of postprandial lipaemia with the increased risk factors for CHD, the present study was carried out to investigate whether tea catechins could modulate postprandial lipaemia using human subjects in a triple-crossover design.

The test meals used in the present study were low in fat (20 g butter) relative to other alimentary studies that used more than 50 g butter to stimulate responses (Thomsen *et al.* 1999, 2003; Mattes, 2001). A recent study by Igarashi *et al.* (2003) recommended a newly designed oral fat-loading test with the clinical use of 20 g butter along with an enteral nutrition drink having a combined energy level of 553 kcal. Our test meals induced an average 0·70 mmol/l increase in plasma triacylglycerols over the baseline level at 3 h in the control beverage sessions. Under such feeding conditions, tea catechin supplements could have exerted suppressing effects on the postprandial elevations in plasma triacylglycerols in a dose-dependent manner, and on the high dosage of tea catechins accompanied by a statistically significant difference in IAUC of plasma triacylglycerols as compared with the control. The assumption that exaggerated lipaemia in the postprandial phase may be implicated in the early development of atherosclerosis is now widely accepted, but a further hypothesis has gained attention recently, i.e. that impaired remnant lipoproteins specifically promote atherosclerosis and CVD. Plasma triacylglycerols are clearly a measure of triacylglycerol-rich lipoproteins (chylomicrons and VLDL) of intestinal and hepatic origin. Remnant lipoproteins are formed in the bloodstream from these triacylglycerol-rich lipoproteins by lipoprotein lipases and to a lesser extent by hepatic lipases (Syvänne & Taskinen, 1997; Jialal & Devaraj, 2002; Schaefer, 2002). The issue of the remnant characteristics of certain lipoprotein fractions has recently provoked new interest due to the development of a technique for the determination of RLP-cholesterol in plasma. The RLP-cholesterol assay provides a valid measure of atherogenic triacylglycerol-rich lipoprotein remnants, especially in patients with metabolic abnormalities such as hyperlipidaemia, type II
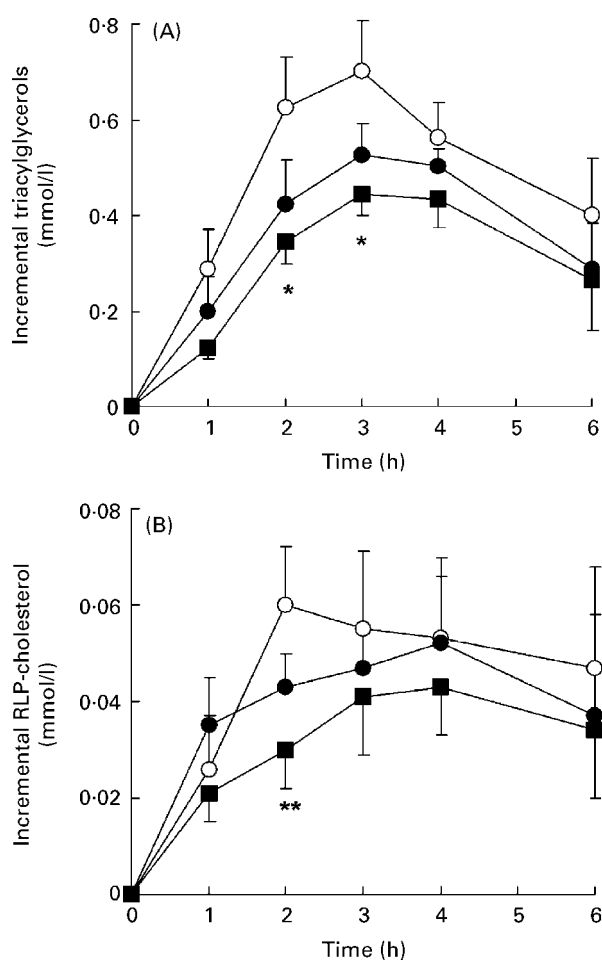

Fig. 1. Changes in plasma triacylglycerols (A) and remnant-like particle (RLP)-cholesterol (B) after an oral administration of a butter-enriched light meal along with each of a control (○), moderate (●) and high (■) tea catechin-containing beverage. Values are means for nine subjects with their standard errors represented by vertical bars. Mean values were significantly different from those of the control group at each time point: *$P < 0.05$, **$P < 0.01$.

Table 3. Effects of tea catechins on incremental area under the curve following an oral fat load

(Mean values with their standard errors)

	Control		Moderate		High		
	Mean	SE	Mean	SE	Mean	SE	P value†
Triacylglycerols (mmol h/l)	2·87	0·32	2·21	0·17	1·86*	0·20	0·036
Remnant-like particle-cholesterol (mmol h/l)	0·28	0·05	0·26	0·03	0·20	0·03	0·100

Mean value was significantly different from control: *$P<0.05$ (*post hoc* comparison with Dunnett's test).
† For differences across treatments (ANOVA).

diabetes mellitus and renal disorders (Twickler *et al.* 2004). Recently, the effect of diet intervention on postprandial plasma RLP-cholesterol levels has been evaluated extensively, with a conceptual design to promote a reduction of postprandial RLP-cholesterol concentrations by soya protein (Shige *et al.* 1998) and dietary fibre (Kondo *et al.* 2004). In the present study, treatment with tea catechin supplements also resulted in the suppression of RLP-cholesterol at an early postprandial stage, but it failed to achieve

statistical significance in IAUC of RLP-cholesterol. This seems likely to be due to the sample size of our subjects.

Although we did not elucidate in this study the mechanisms by which tea catechins attenuated postprandial plasma triacylglycerols, the work of others helps to account for our results. According to the *in vitro* study by Juhel *et al.* (2000), green tea extracts exhibited a marked ability to inhibit gastric and pancreatic lipases. It is well recognized that digestive lipases play key roles in lipid absorption through the intestinal tract to hydrolyse triacylglycerols to 2-monoacylglycerol and NEFA, both of which are incorporated into bile salt–phospholipid micelles, which in turn are absorbed into the brush border of the small intestine, eventually entering the bloodstream. In view of this, the assumption that tea catechins attenuate lipid absorption via digestive lipase inhibition at the *in vivo* stage may make sense. It should also be noted that factors such as differing lipid emulsification and droplet size affect intestinal lipid absorption. There is evidence to show that a smaller fat droplet size facilitates fat digestion by digestive lipases (Armand *et al.* 1999). A recent *in vitro* study showed that (−)-epigallocatechin gallate increased gradually in the scale of mixed micelles (Raederstorff *et al.* 2003). In this connection, it would be possible to argue that, although the evidence is not compelling, limited intestinal lipid absorption by tea catechins may contribute to postprandial plasma triacylglycerol responses at the *in vivo* stage (but the matter may be not quite as simple as this suggests).

One serving of the black tea consumed in the UK typically contains approximately 137–141 mg total flavonoids per 235 ml for 40–60 s of brewing time (Lakenbrink *et al.* 2000). Briefly, the amounts of tea catechins at moderate (224 mg) and high (674 mg) doses account for the equivalent of 1·6 and 4·8 cups, respectively. The point to keep in mind is that individual tea catechins such as (−)-epicatechin gallate, (−)-epigallocatechin gallate, (−)-catechin gallate and (−)-gallocatechin gallate, accounting for a large percentage of total tea catechins in the test beverages, exert strong inhibitory effects on the digestive lipases *in vitro*, whereas tea catechins such as (−)-epicatechin, (−)-epigallocatechin, (−)-catechin and (−)-gallocatechin have little effect (unpublished results). Provided that direct interaction between tea catechins and digestive lipases could occur in the gut, the choice of effective forms of tea catechins is of major importance in achieving a significant impact on postprandial lipaemia.

To our knowledge, this is the first controlled clinical trial to provide evidence confirming the suppressive effects of tea catechins on the postprandial levels of triacylglycerols and RLP-cholesterol. Given that tea is often consumed both during and after meals, such effects of tea catechins may, in fact, link to the epidemiological findings on the inverse association between tea drinking and CHD. However, an overall assessment of the beneficial effects of tea catechins ingested over long periods of
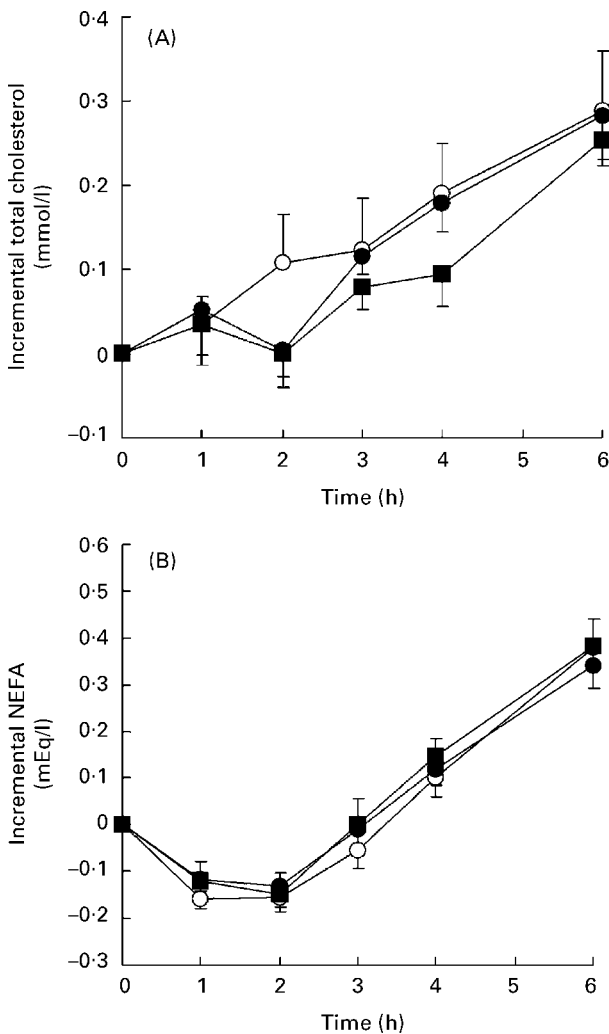

Fig. 2. Changes in plasma total cholesterol (A) and NEFA (B) after an oral administration of a butter-enriched meal along with each of a control (○), moderate (●) and high (■) tea catechin-containing beverage. Values are means for nine subjects with their standard errors represented by vertical bars.

time remains to be completed. Further studies are required to elucidate the long-term effects of tea catechins on lipid metabolism in human subjects as well as their detailed mechanisms of action.

Acknowledgements

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