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Original Research

# Green tea catechins prevent low-density lipoprotein oxidation via their accumulation in low-density lipoprotein particles in humans

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ABSTRACT

Green tea is rich in polyphenols, including catechins which have antioxidant activities and are considered to have beneficial effects on cardiovascular health. In the present study, we investigated the effects of green tea catechins on low-density lipoprotein (LDL) oxidation in vitro and in human studies to test the hypothesis that catechins are incorporated into LDL particles and exert antioxidant properties. In a randomized, placebo-controlled, double-blind, crossover trial, 19 healthy men ingested green tea extract (GTE) in the form of capsules at a dose of 1 g total catechin, of which most (>99%) was the gallated type. At 1 hour after ingestion, marked increases of the plasma concentrations of (–)-epigallocatechin gallate and (–)-epicatechin gallate were observed. Accordingly, the plasma total antioxidant capacity was increased, and the LDL oxidizability was significantly reduced by the ingestion of GTE. We found that gallated catechins were incorporated into LDL particles in nonconjugated forms after the incubation of GTE with plasma in vitro. Moreover, the catechin-incorporated LDL was highly resistant to radical-induced oxidation in vitro. An additional human study with 5 healthy women confirmed that GTE intake sufficiently increased the concentration of gallated catechins, mainly in nonconjugated forms in LDL particles, and reduced the oxidizability of LDL. In conclusion, green tea catechins are rapidly incorporated into LDL particles and play a role in reducing LDL oxidation in humans, which suggests that taking green tea catechins is effective in reducing atherosclerosis risk associated with oxidative stress.



*Abbreviations:* ANOVA, analysis of variance; EC, (–)-epicatechin; ECG, (–)-epicatechin gallate; EDTA, ethylenediamine-tetraacetic acid; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin gallate; EGCG3″Me, (–)-epigallocatechin 3-*O*-(3-*O*-methyl) gallate; GTE, green tea extract; LDL, low-density lipoprotein; TAC, total antioxidant capacity.

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## 1. Introduction

Various lines of research indicate that oxidized low-density lipoprotein (LDL) within the arterial wall promotes the development of atherosclerosis [1]. Protection against LDL oxidation is an effective strategy to prevent atherosclerosis [2], and growing evidence from epidemiologic studies has shown that dietary antioxidants contribute to the prevention of coronary heart disease [3,4].

Polyphenols are found in most foods and beverages of plant origin and are known to have antioxidant properties. Green tea is a large source of polyphenols among the Japanese population [5–7], and epidemiologic studies by the Japan Public Health Center–based Prospective Study group have revealed that the consumption of green tea can reduce the risk of all-cause mortality and the risk of mortality due to the 3 leading causes of death including heart disease, cerebrovascular disease, and respiratory disease [8]. Green tea extract (GTE) contains a number of catechins, including (–)-epigallocatechin gallate (EGCG), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC), and (–)-epicatechin (EC) and has been known to have many beneficial properties that can help prevent atherosclerotic diseases via the regulation of obesity [9], hypertension [10], diabetes [11], and oxidative stress [12,13].

The molecular mechanism of the antiatherogenic activity of green tea catechins, particularly EGCG, which is the most abundant catechin, has also been determined in several studies [14,15]. Among the various antiatherogenic activities, the antioxidant effects are presumed to play a major role in mediating the cardioprotective role of green tea catechins. Our previous study reported that green tea catechins inhibited LDL oxidation when they were directly added to isolated LDL [16]. Moreover, we reported that the acute intake of green tea (5 g of green tea powder) successfully increased the resistance against LDL oxidation as well as the plasma catechin levels in healthy volunteers [17]. Nakagawa et al [18] reported that EGCG was incorporated into human plasma and could decrease the levels of plasma phosphatidylcholine hydroperoxide, a marker of oxidized lipoproteins, after a single oral intake of green tea catechins (254 mg of catechins containing 82 mg of EGCG). These findings prompted us to hypothesize that catechins, especially gallated catechins, may play a preventive role in LDL oxidation by being incorporated into LDL particles in plasma. It was recognized that catechins undergo glucuronidation and sulfation in the intestinal mucosa, liver, and kidney, and only 0.2% to 5.0% of the ingested doses were present in the circulating plasma after the ingestion of tea catechins [19–22]. Compared with other flavonoids like quercetin, catechins can be present in a free (nonconjugated) “active” form in human plasma [19]; however, no information regarding the incorporation of catechins into LDL is available.

Thus, the aim of this study was to test our hypothesis that green tea catechins are incorporated into LDL particles and exert antioxidant properties. The protective mechanism of green tea catechins against LDL oxidation was investigated in vitro and in human studies by measuring the LDL oxidizability and catechin concentration both in plasma and in LDL.

## 2. Methods and materials

### 2.1. Reagents

Decaffeinated GTE powder (THEA-FLAN 90S), mainly composed of gallated catechins (as shown in Table 1), was obtained from ITO EN, LTD (Shizuoka, Japan). Purified catechins (EGCG, ECG, EGC, and EC), β-glucuronidase from *Escherichia coli*, and sulfatase type VIII from abalone entrails were purchased from Sigma-Aldrich (St Louis, MO, USA). The (–)-epigallocatechin-3-*O*-(3-*O*-methyl) gallate (EGCG3″Me) was purchased from Nagara Science (Gifu, Japan). A Micro BCA Protein Assay Kit was obtained from Thermo Fisher Scientific (Rockford, IL, USA). Ethyl gallate was purchased from Tokyo Chemical Industry (Tokyo, Japan). Ethyl acetate, acetonitrile, and formic acid were purchased from Wako Pure Chemicals (Osaka, Japan). Green tea extract capsules containing 1 g of catechins and matching placebo capsules containing starch were formulated by the Sunsho Pharmaceutical (Shizuoka, Japan).

### 2.2. Experimental design

To evaluate the effect of green tea catechins on LDL oxidizability and their ability to bind to LDL both in vitro and in vivo, we conducted human studies with healthy volunteers. These studies were approved by the Ethics Committee of Ochanomizu University in accordance with the principles of the Declaration of Helsinki. In addition, all participants were fully informed regarding the content and methods of this trial. Written informed consent was obtained prior to enrollment from each participant. Blood was drawn from an antecubital vein after fasting and was collected in tubes containing ethylenediamine-tetraacetic acid (EDTA) or clot accelerant for the separation of plasma and serum. Plasma samples were immediately prepared by centrifugation, and LDL was separated by single-spin density gradient ultracentrifugation at 417 000*g* for 40 minutes at 4°C [23]. The LDL protein concentration was determined using a Micro BCA Protein Assay Kit.

#### 2.2.1. Plasma loading with catechins (in vitro study)

Human plasma from healthy volunteers was incubated with purified catechins (EGCG, ECG, EGC, or EC, at 50 μmol/L) or GTE (3 μg/mL) for 20 minutes at 37°C, and LDL was immediately isolated [24,25]. The LDL oxidizability and catechins concentration in plasma or LDL were measured.

**Table 1 – Catechin composition of GTE**

| | (g/100 g) |
|---|---|
| EGCG | 53.6 |
| ECG | 12.5 |
| Gallocatechin gallate (GCG) | 2.8 |
| EGC | 0.4 |
| Catechin gallate (CG) | 0.4 |
| Gallocatechin (GC) | 0.1 |
| EC | ND |
| Catechin (C) | ND |

Abbreviation: ND, not detected.