NUTRITION RESEARCH 36 (2016) 220–226

Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Original Research

Elevated plasma and urinary concentrations of green tea catechins associated with improved plasma lipid profile in healthy Japanese women

Ryusuke Takechi[a,b,c], Helman Alfonso[b], Naoko Hiramatsu[d], Akari Ishisaka[d], Akira Tanaka[c], La'Belle Tan[b], Andy H. Lee[b,*]

[a] Curtin Health Innovation Research Institute, Faculty of Health Sciences, Curtin University, Perth, Australia
[b] School of Public Health, Faculty of Health Sciences, Curtin University, Perth, Australia
[c] Nutrition Clinic, Kagawa Nutrition University, Tokyo, Japan
[d] School of Human Science and Environment, University of Hyogo, Himeji, Japan

ARTICLE INFO

Article history:
Received 3 October 2015
Revised 13 November 2015
Accepted 16 November 2015

Keywords:
Biomarker
Cardiovascular disease
Catechin
Diabetes
Green tea

ABSTRACT

This study investigated green tea catechins in plasma and urine and chronic disease biomarkers. We hypothesized that plasma and urinary concentration of green tea catechins are associated with cardiovascular disease and diabetes biomarkers. First void urine and fasting plasma samples were collected from 57 generally healthy females aged 38 to 73 years (mean, 52 ± 8 years) recruited in Himeji, Japan. The concentrations of plasma and urinary green tea catechins were determined by liquid chromatography coupled with mass tandem spectrometer. Low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, glucose, insulin, glycated hemoglobin, and C-reactive protein in plasma/serum samples were analyzed by a commercial diagnostic laboratory. Statistical associations were assessed using Spearman correlation coefficients. The results showed weak associations between plasma total catechin and triglyceride ($r = -0.30$) and LDL cholesterol ($r = -0.28$), whereas plasma (–)-epigallocatechin-3-gallate, (–)-epigallocatechin, (–)-epicatechin-3-gallate, and (–)-epicatechin exhibited weak to moderate associations with triglyceride or LDL cholesterol, but little associations with HDL cholesterol, body fat, and body mass index were evident. Urinary total catechin was weakly associated with triglyceride ($r = -0.19$) and LDL cholesterol ($r = -0.15$), whereas urinary (–)-epigallocatechin-3-gallate ($r = -0.33$), (–)-epigallocatechin ($r = -0.23$), and (–)-epicatechin-3-gallate ($r = -0.33$) had weak to moderate correlations with triglyceride and similarly with body fat and body mass index. Both plasma ($r = -0.24$) and urinary ($r = -0.24$)

Abbreviations: AMPK, AMP-activated protein kinase; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; EC, (–)-epicatechin; ECG, (–)-epicatechin-3-gallate; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin-3-gallate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HPLC-MS/MS, high-performance liquid chromatography mass spectrometry; LDL, low-density lipoprotein; IL-6, interleukin-6; GLUT, glucose transporter.

* *Corresponding author.* School of Public Health, Curtin University, GPO Box U1987, Perth, Western Australia, 6845, Australia. Tel.: +61 8 9266 4180.
E-mail address: Andy.Lee@curtin.edu.au (A.H. Lee).

http://dx.doi.org/10.1016/j.nutres.2015.11.010

total catechin, as well as individual catechins, were weakly associated with glycated hemoglobin. Plasma total and individual catechins were weakly to moderately associated with C-reactive protein, but not the case for urinary catechins. In conclusion, we found weak to moderate associations between plasma and urinary green tea catechin concentrations and plasma biomarkers of cardiovascular disease and diabetes.

1. Introduction

During the past 2 decades, the preventative role of green tea has been reported in certain chronic diseases such as cardiovascular disease (CVD) and type 2 diabetes [1]. A recent large cohort study involving 76 979 individuals aged 40 to 79 years in Japan observed that green tea consumption was associated with lower CVD mortality by up to 38% [2]. Increased tea consumption is also related to an attenuated risk of cardiac death, coronary heart disease, intracerebral hemorrhage, and cerebral infarction according to a systematic review and meta-analysis of prospective observational studies [3]. In a study of participants with myocardial infarction, green tea consumption significantly reduced serum concentrations of low-density lipoprotein (LDL), C-reactive protein (CRP), and interleukin 6 [4]. Similarly, a dose-response meta-analysis of 16 cohort studies found a linear inverse association between tea consumption and risk of type 2 diabetes [5]. However, the mechanisms by which green tea reduces the risk of CVD and diabetes are not fully understood.

Both in vivo and in vitro experimental studies have suggested that the beneficial effects of green tea may be attributed to its high content of anti-inflammatory/antioxidative polyphenols. The main phenolic compounds in green tea are catechins, including (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC), with EGCG being the highest accounting for 60% to 65% of the entire catechin content [6]. Among these 4 catechins, most studies focused on the effect of EGCG. For example, EGCG was found to reduce blood pressure and myocardial infarct size and improve endothelial function and insulin sensitivity in a rat model of cardiovascular disorder [7]. On the one hand, rodent models of obesity/diabetes similarly demonstrated significant reductions in body fat and plasma cholesterol, attenuated insulin resistance, decreased blood glucose, and improved insulin sensitivity by EGCG [8-10]. On the other hand, only 1 study showed that EC reduced plasma glucose in streptozotocin-induced insulin resistant rats within 6 days [11]. Moreover, the relationships between green tea catechins in plasma and urine and biomarkers of CVD and diabetes in healthy population have never been reported in the literature.

In this study, we hypothesized that plasma and urinary concentrations of green tea catechins are associated with the CVD and diabetes biomarkers in generally healthy participants. Our objectives were to determine the concentrations of EGCG, EGC, ECG, and EC in plasma and urine of healthy Japanese women and to assess their association with the plasma biomarkers of CVD and diabetes.

2. Methods and materials

2.1. *Participant recruitment*

Participants were recruited from Tsunashimakai Kosei Hospital and University of Hyogo in Himeji, located in Hyogo Prefecture of central Japan, during April to August 2014. After screening for inclusion criteria (female aged ≥35 years), individuals currently on prescription for a chronic condition or had modified their diet within the past year were excluded. The study purpose and procedure were explained to the participants before obtaining their informed written consent. The recruitment process terminated when the target sample of 60 volunteers was met. The study protocol was approved by the Curtin University Human Research Ethics Committee (approval no. 4649) and University of Hyogo Research Ethics Committee (approval no. 068).

2.2. *Urine and plasma sample collection*

Participants were instructed to fast overnight for more than 8 hours before collecting their first morning void in a supplied container. Fasting blood samples were taken by a qualified phlebotomist and collected in a serum separator tube and a heparin tube. Plasma, serum, and urine samples were then stored at −80°C until analysis. Anthropometric and blood pressure measurements were also taken before venous blood sampling, along with basic demographic characteristics. A body composition scale (Tanita, Tokyo, Japan) was used to measure body weight and body fat percentage.

2.3. *Analysis of green tea catechins*

The concentrations of EGCG, ECG, EGC, and EC in plasma and urine were determined with a highly sensitive method using high-performance liquid chromatography (HPLC; Agilent 1100LC, Santa Clara, CA, USA, with binary pump) coupled with tandem mass spectrometer (MS/MS) (Applied Biosystems Sciex API 3000, Waltham, MA, USA), as described previously with some minor modifications [12-15]. Briefly, 500 μL of plasma samples was mixed with 20 μL of 10% ascorbic acid (wt/wt in water) and 100 μL of 1% sulfatase H-1 (wt/wt in sodium acetate buffer, pH 5, Sigma S9626, ≥10 U/mg with β-glucuronidase ≥300 U/mg). After incubation at 37°C for 45 minutes to allow enzymatic hydrolysis, 2.5 μL of 40 μmol/L ethyl gallate was added as an internal standard. Subsequently, deconjugated and free catechins were extracted using 500 μL of 0.1% formic acid in ethyl acetate. The extraction process was repeated 3 times, and the pooled extract was evaporated under vacuum (Tomy CC-105 centrifugal concentrator and Eyel4 Unitrap UT-2000 evaporator). The samples were then reconstituted in a buffer (15% acetonitrile and 0.1% formic