Journal of Oleo Science
Copyright ©2012 by Japan Oil Chemists' Society
J. Oleo Sci. **61**, (3) 113-120 (2012)

Antioxidant Activities of *Perilla frutescens* against Low-Density Lipoprotein Oxidation *in Vitro* and in Human Subjects

Emi Saita, Yoshimi Kishimoto, Mariko Tani, Maki Iizuka, Miku Toyozaki, Norie Sugihara and Kazuo Kondo*

Institute of Environmental Science for Human Life, Ochanomizu University (2-1-1, Otsuka, Bunkyo-ku, Tokyo 112-8610, JAPAN)

Abstract: Perilla (*Perilla frutescens* (L.) Britt.) is a popular food as well as a traditional medicine in Japan, China, and other Asian countries. The aim of this study was to investigate the inhibitory effects of perilla on low-density lipoprotein (LDL) oxidation *in vitro* and in human subjects. We compared the antioxidant activities of red perilla and green perilla. Both green and red perilla had high 1,1-diphenyl-2-picrylhydrazyl radical scavenging activities and were abundant in polyphenol compounds. In addition, the radical scavenging activity and polyphenol content of red perilla were higher than those of green perilla. Perilla dramatically inhibited azo-radical-induced LDL oxidation and endothelial-cell-mediated LDL oxidation *in vitro*. Moreover, red perilla significantly increased mRNA and protein expression levels of antioxidant enzymes in endothelial cells. We further examined the antioxidant effects against LDL in human subjects after the consumption of perilla extracts. After oral intake of red perilla, the subjects' LDL oxidation lag times were significantly longer than those before the intake. Furthermore, lipid peroxide formation and the electrophoretic mobility of LDL decreased markedly. These results suggested that perilla, especially the red variety, had high antioxidant activity and prevented the oxidation of LDL, which is a process strongly related to the development of atherosclerosis.

Key words: Perilla, LDL oxidation, antioxidant enzyme, atherosclerosis, endotherial cell

1 INTRODUCTION

Recent reports have shown that leaves contain high quantities of polyphenols and could therefore play a positive role in preventing low-density lipoprotein (LDL) oxidation[1, 2]. Perilla (*Perilla frutescens* (L.) Britt.) is a widely cultivated leafy vegetable that is commonly consumed as well as used in traditional medicine in Japan, China, and other Asian countries. Perilla can be categorized into two types based on their morphology and use: *Perilla frutescens* var. *frutescens* is mostly used as an oil crop, whereas *Perilla frutescens* var. *crispa* is eaten raw and used for medicinal and nutritional purposes[3].

Perilla frutescens var. *crispa* can be further categorized into two types: a red variety (red perilla, "Aka-jiso" in Japanese) and a green variety (green perilla, "Ao-jiso"); these types differ in anthocyanin content[4]. Chemical analysis has indicated that only the red variety produces anthocyanins, with malonylshisonin being the main pigment[5]. Many studies have been conducted on the compounds contained in perilla that have anti-inflammatory[6, 7], anti-HIV-1[8], anti-allergic[9, 10], and anti-tumor[11, 12] properties. Nonetheless, the antioxidant activity of perilla remains poorly understood.

The oxidative modification of LDL may play a critical role in the development of atherosclerosis[13–15]. Oxidative stress is one of the main risk factors for LDL oxidation. Oxidized LDL (oxLDL) is internalized by endothelial cells and macrophages, leading to endothelial dysfunction and foam cell formation, respectively. Therefore, reducing LDL oxidation is assumed to be a useful strategy to prevent atherogenic disease.

Reactive oxygen species (ROS) are free radicals produced during metabolism and the aging process. ROS include free radicals such as superoxide ($O_2^{\cdot -}$), hydroxyl ($^{\cdot}OH$), peroxyl ($RO_2^{\cdot}$), and hydroperoxyl ($HRO_2^{\cdot -}$), as well as nonradical species such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). ROS can cause lipid peroxidation, result-

*Correspondence to: Kazuo Kondo, Institute of Environmental Science for Human Life, Ochanomizu University, 2-1-1, Otsuka, Bunkyo-ku, Tokyo 112-8610, JAPAN
E-mail: kondo.kazuo@ocha.ac.jp
Accepted October 4, 2011 (recieved for review September 29, 2011)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manusriptcentral.com/jjocs

ing in oxidative stress. A variety of non-enzymatic antioxidants (e.g., glutathione and uric acid) and enzymatic antioxidants (e.g., catalase (CAT) and Cu-Zn-superoxide dismutase (SOD)) have been known to play an active role against oxidative stress[16, 17]. The mechanism by which perilla protects against oxidative stress still remains largely unknown. In this study, we investigated the effects of perilla on LDL oxidation and antioxidant enzyme expression both *in vitro* and in human subjects.

2 EXPERIMENTAL PROCEDURES

2.1 Antioxidative activities of perilla

2.1.1 Perilla preparation

The leaves of a green- and red-type *P. frutescens* were purchased from a market (Tokyo, Japan). The dried perilla leaves (1 g) were extracted with 10 mL distilled water for 1 h at room temperature under gentle stirring. The extracted solution was then filtered and stored at −20℃ for use in the following *in vitro* studies.

2.1.2 Free radical-scavenging activity

Free radical-scavenging activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Wako Pure Chemical Industries, Osaka, Japan). An aliquot of each extract was mixed with 2 mL of 0.1 mM DPPH in ethanol. Following incubation for 20 min at 37℃, the absorbance of each solution was measured at 516 nm using a Beckman Model DU 640 spectrophotometer. The volume of each perilla extract that was required to cause a 50% decrease in the absorbance at 516 nm relative to the control was then calculated.

2.1.3 Determination of total polyphenol content

The total polyphenol content was determined by the Folin-Ciocalteu assay as described previously[18]. The content was expressed as a (+)-catechin equivalent.

2.1.4 Isolation of LDL from human subjects

Blood samples were collected in sodium EDTA-containing tubes from fasting normolipidemic volunteers after obtaining their informed consent. Plasma samples were immediately prepared by centrifugation at 3,000 rpm for 15 min at 4℃. The LDL was separated by single-spin density gradient ultracentrifugation (100,000 rpm, 40 min, 4℃). LDL protein concentration was determined using a Micro BCA Protein Assay Kit (Pierce Laboratories, Rockford, IL).

2.1.5 Determination of LDL oxidizability (lag time assay)

LDL oxidizability was measured according to the method described in our previous report[19]. The prepared LDL samples (final concentration of protein: 70 μg/mL) were oxidized with or without 5 μL of perilla extract by 200 μM 2,2-azobis-4-methoxy-2,4-dimethylvaleronitrile (V-70; AMVN-CH_3O) (Wako Pure Chemical Industries, Ltd.), which is an oxidative inducer. The kinetics of LDL oxidation were determined by monitoring the absorbance of conjugated dienes at 234 nm using a Beckman Model DU 800 spectrophotometer at 4 min intervals at 37℃.

2.1.6 Endothelial cell-meditated LDL oxidation

Human umbilical vein endothelial cells (HUVECs) (Sanko Junyaku Co., Tokyo, Japan) were cultured in EGM-2 (Lonza Walkersville, Inc., Walkersville, USA). The Cells were grown to confluence at 37℃ in 5% CO_2 and used for experiments at passage 4. HUVECs were pre-incubated for 6 h in the presence or absence of perilla extract in M-199 medium. The medium was subsequently removed, and the cells were washed twice with PBS. LDL (100 μg protein/mL) was incubated with the cells for 18 h in Ham's F10 medium (Lonza Walkersville) containing 3 μM $FeSO_4$ and $CuSO_4$. After this incubation, the medium was analyzed for LDL oxidation as described below.

2.1.7 Thiobarbituric acid reactive substances assay

Malondialdehyde (MDA) generated in a medium containing LDL was measured by using the thiobarbituric acid reactive substances (TBARS) assay as described by Buege and Aust[20]. Sample absorbance was measured at 535 nm. Results were expressed in terms of relative MDA content (nmol/mg-LDL protein) calculated using the extinction coefficient for MDA as previously described.

2.1.8 Lipid peroxide assay

Lipid peroxide (LPO) in LDL was measured by using a Determiner LPO (Kyowa Medex, Tokyo, Japan). Samples were measured spectrophotometrically at 675 nm.

2.1.9 Agarose gel electrophoresis

The media containing LDL were collected and subjected to agarose gel electrophoresis. Electrophoresis was performed at 400 V for 25 min by using a rapid electrophoresis system (Helena Laboratories, Saitama, Japan). After electrophoresis, the gels were stained with CHO/Trig CONBO CH (Helena Laboratories, Saitama, Japan)[21].

2.2 Effect of perilla on antioxidant enzyme

2.2.1 Real-time PCR analysis

Total cellular RNA was extracted using TRIZOL Reagent (Invitrogen, Tokyo, Japan), and first-strand cDNA was synthesized from the total RNA (0.2 μg) by using TaqMan® Reverse Transcription Reagents (Applied Biosystems, CA, USA). Real-time PCR was performed on an ABI 7300 cycler (Applied Biosystems, CA, USA) using SYBR green PCR mix. The results were expressed as the copy number ratio of the target mRNA to GAPDH mRNA. The primer sequences were as follows: for catalase, forward 5′-TGACCAGGGCATCAAAAACC-3′, reverse 5′-CGGATTGCCATAGTCAGGATCTT-3′; for SOD, forward 5′-CATCATCAATTTCGAGCAGA-3′, reverse 5′-GCCACACCATCTTTGTCAGCAG-3′; and for GAPDH, forward 5′-TGCACCACCAACTGCTTAGC-3′, reverse 5′-GGCATGGACTGTGGTCATGAG-3′.

2.2.2 Western blot analysis

After treatment, cells were washed with ice-cold PBS

and lysed with a protein extraction reagent (Pierce Biotechnology, IL, USA) containing 10 μg/mL leupeptin, 10 μg/mL aprotinin, and 1 mM phenylmethylsulfonyl fluoride. Aliquots of cellular proteins were electrophoresed in a 10% SDS-polyacrylamide gel and transferred to an Immobilon-P membrane (Millipore, MA, USA). The membrane was allowed to react with catalase and SOD (Santa Cruz, CA, USA), and specific proteins were detected by enhanced chemiluminescence. Loading differences were normalized using polyclonal actin antibodies. All signals were detected by LAS-4000 (Fujifilm, Tokyo, Japan). Densitometric analysis was performed by using Multi Gauge version 3.0 (Fujifilm, Tokyo, Japan) to scan the signals.

2.3 Clinical study

The study group consisted of eight healthy female volunteers ranging from 20 to 25 yrs of age; none took any medications or special dietary supplements. All were nonsmokers and in good states of health. This study was approved by the Ethics Committee of Ochanomizu University and conformed to the Declaration of Helsinki (established in 1964 and revised in 2004). All subjects gave their informed consent to participate in the study. After over 12 h of fasting, blood samples were collected between 8:00 and 9:00 a.m. The subjects then ingested 120 mL of perilla extract. The perilla extract contained about 1,000 mg of polyphenols. Plasma samples were taken at baseline, then 0.5, 1, 2, and 4 h after consumption of perilla extract; these samples were then subjected to the isolation and preparation of LDL described above. Next, we measured the lag time. After diluting the samples with PBS to a final concentration of 140 μg/mL LDL protein, the LDL was oxidized by 400 μM AMVN-CH_3O. Lag time, TBARS, LPO products, and LDL mobility were determined as described above.

2.4 Statistical analysis

Results were expressed in the form, mean ± SD. Human study results were expressed in the form, mean ± SEM. Differences between groups were analyzed by ANOVA with Fisher's PLSD using StatView-J5.0 (SAS Institute Inc., NC, USA). The minimum significance level was set at a P value of 0.05 for all analyses.

3 RESULTS

3.1 Antioxidant activities of perilla

The antioxidant activities of green and red perilla are listed in Table 1. The DPPH solutions required 7.9 and 29 μg/mL of red perilla and green perilla, respectively, to scavenge 50% of DPPH radicals. The polyphenol concentration of green perilla was 1.7 mg/mL, and that of red perilla was 4.6 mg/mL.

Table 1 Antioxidant activity of perilla.

	Polyphenol (mg/leaf)	IC_{50} value of perilla extracts for DPPH scavenge
Green perilla	1.7 ± 0.5	29 ± 2.1
Red perilla	4.6 ± 0.5***	7.9 ± 1.1***

Data are mean ± S.D. n = 6. ***$p < 0.001$ vs. green perilla

3.2 Inhibitory effects of perilla on LDL oxidation *in vitro*

We first analyzed the cytotoxic effects of perilla in HUVECs with an MTT assay. Neither green nor red perilla affected cell viability under our experimental conditions (data not shown).

To evaluate the antioxidant effects of perilla extracts on LDL oxidation, we carried out an LDL lag time assay. As shown in Fig. 1, both green and red perilla extracts significantly prolonged LDL oxidation lag time compared with the control (control 24 ± 3 min; green perilla 51 ± 5 min; red perilla 87 ± 7 min ($p < 0.001$)).

We also examined the inhibitory effects of perilla on endothelial-cell-mediated LDL oxidation. The pretreatment of HUVECs with red perilla significantly reduced the TBARS response and LPO formation from oxidation ($p < 0.001$) (Fig 2A, B). Furthermore, we evaluated the change in the surface charge of LDL by agarose gel electrophoresis. The oxidation of LDL could be monitored by comparing the relative electrophoresis mobility of unmodified and modified LDLs. Red perilla significantly suppressed LDL

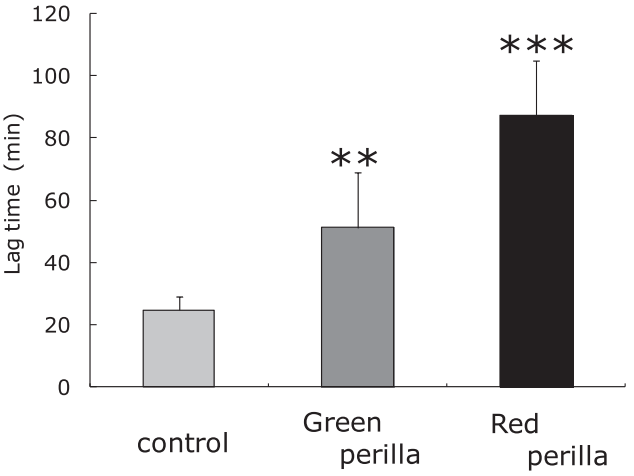

Fig. 1 Effects of perilla extracts on LDL oxidation lag time.
Perilla extract was incubated with 70 μg protein/mL of LDL in PBS (1 mL in total) with V-70 at 37℃ for 400 min, and conjugated diene formation was monitored by the changes in 234 nm wavelength absorbance. Results are expressed in the form, mean ± S.D., n = 3.
***$p < 0.001$, **$p < 0.01$ vs. control

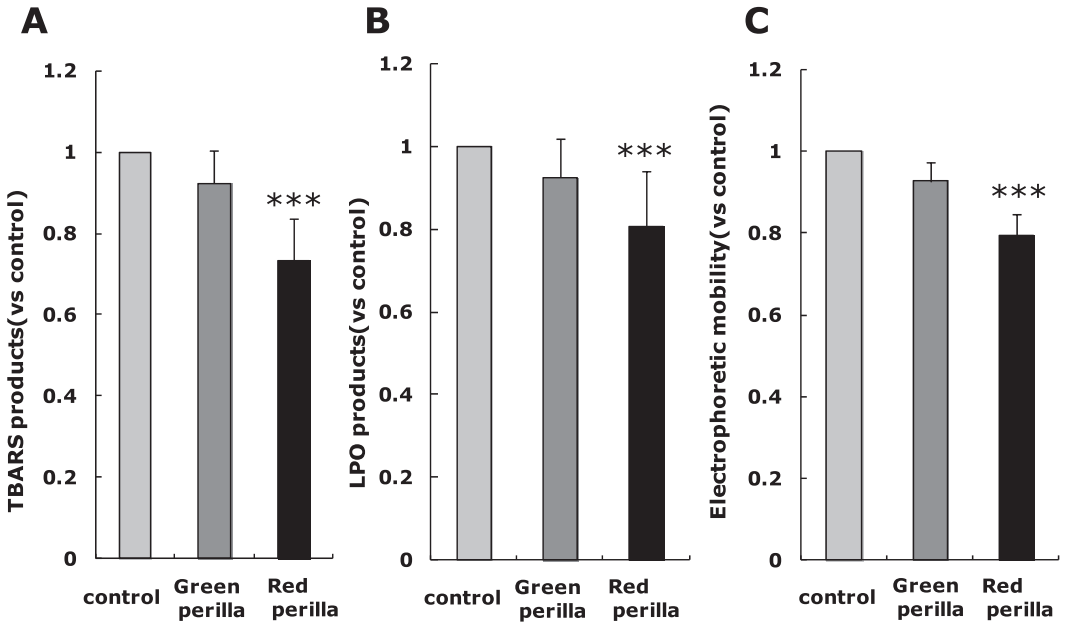

Fig. 2 Effects of perilla extracts on endothelial-cell-mediated LDL oxidation.
HUVECs were pre-incubated with or without perilla extract for 6 h, and were washed twice with PBS; then LDL was added and incubated for 18 h in Ham's F10. The lipid oxidation products were assessed by both the TBARS assay (A) and the LPO assay (B). Electrophoretic mobility was assessed by agarose gel electrophoresis (C). Results are expressed in the form, mean ± S.D., n = 4.
***$p < 0.001$ vs. control

modification in comparison with native LDL in endothelial-cell-mediated LDL oxidation ($p < 0.001$) (Fig. 2C).

3.3 Effect of perilla on antioxidant enzymes

To investigate the mechanism by which endothelial cells mediated LDL oxidation, we tested the effect of perilla on antioxidant enzyme expression in HUVECs. Treatment of HUVECs with red perilla led to a significant increase in SOD and catalase mRNA expression (Table 2). In addition, red perilla increased protein expression of SOD and catalase (Fig. 3), indicating that perilla increased antioxidant enzyme expression by direct modulation of gene transcription.

Table 2 Effects of perilla extracts on mRNA expression of the antioxidant enzymes in HUVECs.

	catalase	SOD
Green perilla	2.3 ± 1.3	1.5 ± 0.6
Red perilla	3.2 ± 1.4***	2.1 ± 0.5***

Values were expressed as the ratio of catalase or SOD to GAPDH mRNA expressions. (mean ± S.D. n = 4) ***$p < 0.001$ vs. control

3.4 Inhibitory effects of perilla on LDL oxidation in human subjects

Eight subjects consumed 120 mL of red perilla extract, which contained about 1,000 mg of polyphenols. As shown in Fig. 4A, the LDL oxidation lag time was prolonged to 2 h and 4 h after the ingestion of red perilla extract compared to that before intake ($p < 0.001$, $p < 0.05$, respectively). The consumption of red perilla extract decreased the amount of LPO products at each time point (Fig. 4B). TBARS products also tended to decrease (Fig. 4C). LDL mobility was markedly decreased at 4 h ($p < 0.05$) (Fig. 4D). These results showed that red perilla could suppress LDL oxidation in human subjects as well as *in vitro*.

4 DISCUSSION

The oxidative modification of LDL is thought to play a central role in the pathogenesis of atherosclerosis. In this study, we demonstrated that perilla reduced LDL oxidizability both *in vitro* and in human subjects.

First, we checked the antioxidant activity of green perilla and red perilla. Both green and red perilla had DPPH radical-scavenging activities and contained high polyphenol concentrations. Moreover, red perilla showed stronger antioxidant activity than green perilla. Perilla leaves are known to contain several kinds of polyphenols, such as cin-

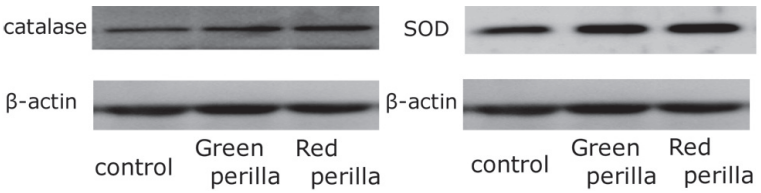

Fig. 3 Effects of perilla extracts on antioxidant enzyme expression in HUVECs.
HUVECs were incubated with or without perilla extract for 6 h. We measured the expression of antioxidant enzyme (catalase, SOD) levels by Western blot analysis. Representative data from four independent experiments are shown.

namic acid derivatives (caffeic acid and rosmarinic acid), flavonoids (apigenin 7-O-caffeoylglucoside, luteolin 7-O-diglucuronide, apigenin 7-O-diglucuronide) and anthocyanins (malonylshisonin)[5, 22–24]. It was already known that anthocyanins are produced only in red perilla and that other phenolic compounds such as rosmarinic acid are more abundant in red perilla than in green perilla. Nakamura *et al*. demonstrated that the scavenging ability of rosmarinic acid from the leaves of perilla was greater than that of ascorbic acid or other phenolcarboxylic acids[25]. They also found that rosmarinic acid effectively exhibited antioxidative activity in biological systems by scavenging $O_2^{\cdot -}$, one of the precursors of ROS. Therefore, the higher antioxidant activity of red perilla to green perilla was easily explained.

Next, we demonstrated the protective effect of perilla against pro-oxidant-initiated oxidative modification or endothelial-cell-mediated oxidation *in vitro*. Perilla significantly prolonged the azo-radical-induced LDL oxidation lag time, indicating that it could prevent free-radical-induced lipid peroxidation of LDL. To examine the effect of perilla on cell-mediated LDL oxidation, we used Ham's F10 medium containing metal ions to induce the oxidation of LDL in HUVECs[21]. As shown in Fig. 2A, B and C, the interaction of LDL and HUVECs significantly increased TBARS and LPO production, but these were dramatically inhibited by incubation with red perilla. Red perilla also reduced the negative charge of LDL particles that resulted from HUVEC incubation. This indicated that perilla could play a role in preventing apolipoprotein B100 modification in LDL. We hypothesized that perilla might exert its antioxidant effects by inducing antioxidant enzyme activity. To test this hypothesis, we examined its effects on antioxidant enzyme expression in HUVECs. SOD catalyzes the dismutation of $O_2^{\cdot -}$ to molecular oxygen and H_2O_2, which in turn is metabolized to harmless water and oxygen by catalase and glutathione peroxidase (GPx). Red perilla upregulates the mRNA and protein expression of SOD and catalase in human endothelial cells. These findings suggested that perilla exerts its antioxidative effects via the induction of SOD and catalase activities in HUVECs.

To verify the ability of perilla to prevent LDL oxidation *in vivo*, we conducted additional experiments in human subjects. We decided to use only red perilla as it showed greater antioxidative activity than green perilla in humans. The consumption of red perilla extract containing 1,000 mg of polyphenols significantly extended the lag time of LDL oxidation and reduced TBARS production, LPO production and LDL mobility.

A past study of the antioxidant abilities of grapes and balsamic vinegar, both containing anthocyanin, reported that the prolongation of the lag time was related to an increase in plasma polyphenol levels[26, 27]. Plasma lipoproteins have been suggested as potential carriers of polyphenols[28]. In the previous *in vivo* study, maximum plasma concentrations of anthocyanins were observed 120-240 min after intake of anthocyanin rich foods[29, 30]. These data suggest that polyphenols may be absorbed into the bloodstream and incorporated into LDL after perilla consumption. Moreover, it must be taken into account that perilla has many different antioxidants, not only polyphenols but also vitamin E and carotenoids. Previous studies have suggested that some hydrophilic antioxidants bind to phospholipids or proteins on the LDL surface, whereas hydrophobic antioxidants bind closer to the LDL core[31]. Based on these past studies, we could postulate that the consumption of red perilla may have inhibited LDL oxidation due to the combination of hydrophilic and hydrophobic antioxidants.

In recent years, it has been theorized that phytochemicals may not only act as "primary antioxidants" by scavenging ROS but also as "secondary antioxidants" by interacting with several signaling pathways to induce production of cytoprotective enzymes[32, 33]. There are reports that antioxidant-rich food may be able to enhance the activity of antioxidant enzymes[34]. Moreover, previous studies have shown that phenolic acids significantly induce antioxidant enzyme production and increase the antioxidizing potential of the liver. These phenolic acids seem to selectively induce transcription of hepatic mRNAs for CuZnSOD, GPx, and catalase, likely through upregulation of gene transcription as well as the NF-E2-related factor 2 (Nrf2) transcription factor[35].

Since the *in vitro* study found that perilla upregulated both SOD and catalase, we may conclude that the consumption of red perilla inhibited LDL oxidation by increasing the level of the antioxidant enzymes. The reason for the increase might be related to up-regulation of hepatic

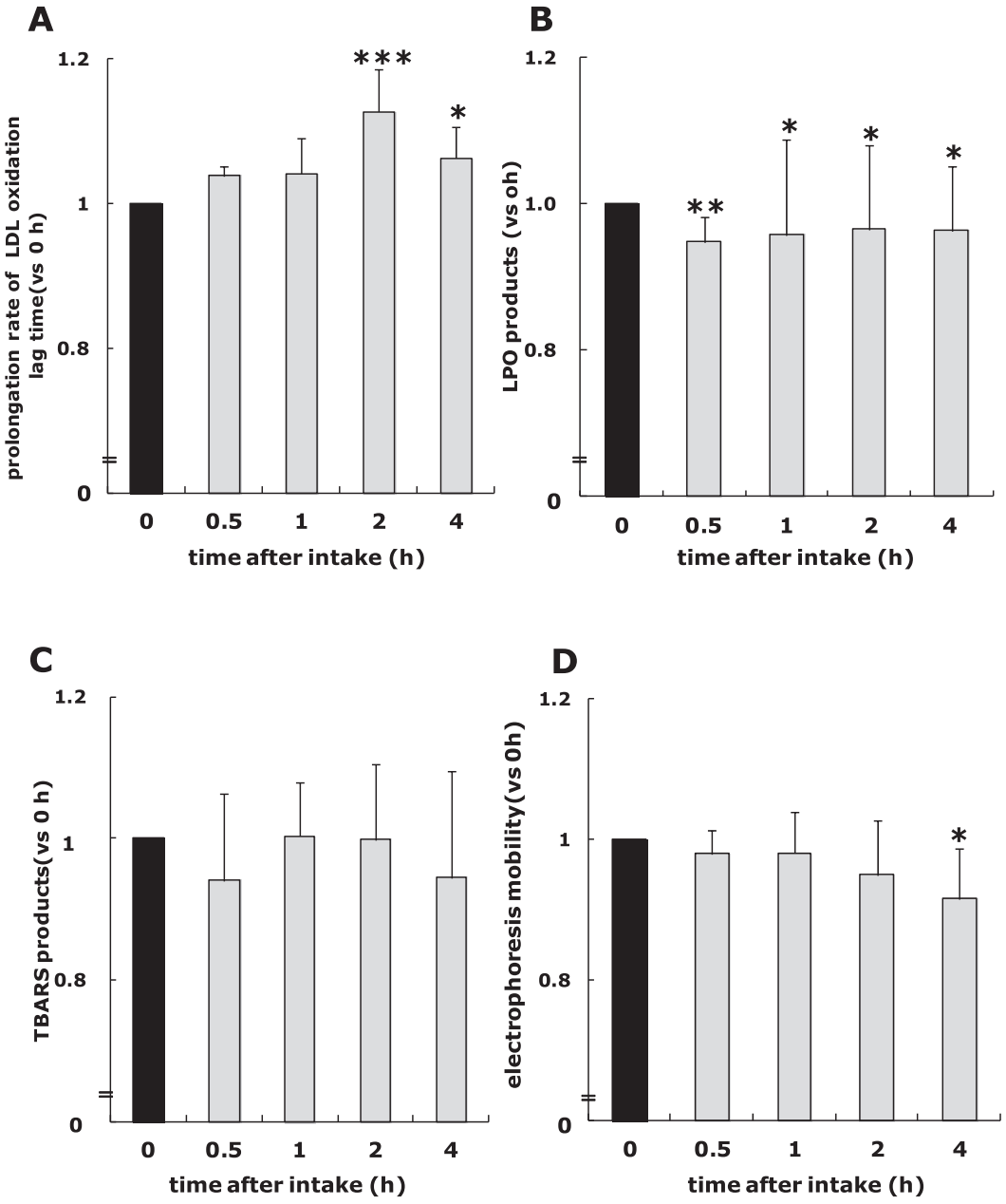

Fig. 4 Effects of perilla extract consumption on oxidation against LDL in healthy subjects.
After overnight fasting, eight healthy volunteers consumed red perilla extract. Blood was sampled before intake, then 0.5, 1, 2, and 4 h after intake. We measured LDL oxidizability in the presence of V-70 (final concentration: 200 μM) by lag time assay (A), LPO assay (B), TBARS assay (C), and agarose gel electrophoresis (D). Results are expressed in the form, mean ± SEM, n = 8.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs. before consumption of perilla

multidrug resistance-associated protein and transcription factor Nrf2, although we do not have data to support this claim.

In conclusion, our results showed that perilla exhibits antioxidant abilities and inhibits LDL oxidation. Furthermore, the consumption of perilla may reduce the risk factors associated with atherosclerosis.

ACKNOWLEDGMENTS

This study was supported in part by Grant-in-Aid (20300244 to K. K.) from the Japan Society for the Promotion of Science. E.S is supported by research fellowships of the Japan Society for the Promotion of Science for young scientists.

We thank all members of the Kondo Laboratory for criti-

cal discussion.

Reference

1) Katsube T.; Imawaka, N.; Kawano, Y.; Yamazaki, Y.; Shiwaku, K.; Yamane, Y. Antioxidant flavonol glycosides in mulberry (*Morus alba* L.) leaves isolated based on LDL antioxidant activity. *Food Chem.* **97**, 25-31 (2006).
2) Hseu, Y. C.; Chang, W. H.; Chen, C. S.; Liao, J. W.; Huang, C. J.; Lu, F. J.; Chia, Y. C.; Hsu, H. K.; Wu, J. J.; Yang, H. L. Antioxidant activities of *Toona sinensis* leaves extracts using different antioxidant models. *Food Chem Toxicol.* **46**, 105-114 (2008).
3) Lee J. K.; Nitta M; Park C. H.; Yoon K. M.; Shin Y. B.; O, O. Genetic diversity of perilla and related weedy types in Korea determined by AFLP analyses. *Crop Sci.* **42**, 2161-2166 (2002).
4) Saito, K.; Yamazaki, M. Biochemistry and molecular biology of the late-stage of biosynthesis of anthocyanin: lessons from *Perilla frutescens* as a model plant. *New Phytologist* **155**, 9-23 (2002).
5) Yamazaki, M.; Nakajima, J.; Yamanashi, M.; Sugiyama, M.; Makita, Y.; Springob, K.; Awazuhara, M.; Saito, K. Metabolomics and differential gene expression in anthocyanin chemo-varietal forms of Perilla frutescens. *Phytochem.* **62**, 987-995 (2003).
6) Ueda, H.; Yamazaki, C.; Yamazaki, M. Luteolin as an anti-inflammatory and anti-allergic constituent of *Perilla frutescens*. *Biol. Pharm. Bull.* **25**, 1197-1202 (2002).
7) Banno, N.; Akihisa, T.; Tokuda, H.; Yasukawa, K.; Higashihara, H.; Ukiya, M.; Watanabe, K.; Kimura, Y.; Hasegawa, J.; Nishino, H. Triterpene acids from the leaves of *Perilla frutescens* and their anti-inflammatory and antitumor-promoting effects. *Biosci. Biotechnol. Biochem.* **68**, 85-90 (2004).
8) Yamasaki, K.; Nakano, M.; Kawahata, T.; Mori, H.; Otake, T.; Ueba, N.; Oishi, I.; Inami, R.; Yamane, M.; Nakamura, M.; Murata, H.; Nakanishi, T. Anti-HIV-1 activity of herbs in Labiatae. *Biol. Pharm. Bull.* **21**, 829-833 (1998).
9) Makino, T.; Furuta, Y.; Wakushima, H.; Fujii, H.; Saito, K.; Kano, Y. Anti-allergic effect of *Perilla frutescens* and its active constituents. *Phytother. Res.* **17**, 240-243 (2003).
10) Takano, H.; Osakabe, N.; Sanbongi, C.; Yanagisawa, R.; Inoue, K.; Yasuda, A.; Natsume, M.; Baba, S.; Ichiishi, E.; Yoshikawa, T. Extract of Perilla frutescens enriched for rosmarinic acid, a polyphenolic phytochemical, inhibits seasonal allergic rhinoconjunctivitis in humans. *Exp. Biol. Med.* (*Maywood*). **229**, 247-254 (2004).
11) Lin, C. S.; Kuo, C. L.; Wang, J. P.; Cheng, J. S.; Huang, Z. W.; Chen, C. F. Growth inhibitory and apoptosis inducing effect of *Perilla frutescens* extract on human hepatoma HepG2 cells. *J. Ethnopharmacol.* **112**, 557-567 (2007).
12) Kolettas, E.; Thomas, C.; Leneti, E.; Skoufos, I.; Mbatsi, C.; Sisoula, C.; Manos, G.; Evangelou, A. Rosmarinic acid failed to suppress hydrogen peroxide-mediated apoptosis but induced apoptosis of Jurkat cells which was suppressed by Bcl-2. *Mol. Cell Biochem.* **285**, 111-120 (2006).
13) Witztum, J. L.; Steinberg, D. Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.* **88**, 1785-1792 (1991).
14) Harrison, D.; Griendling, K. K.; Landmesser, U.; Hornig, B.; Drexler, H. Role of oxidative stress in atherosclerosis. *Am. J. Cardiol.* **91**, 7A-11A (2003).
15) Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **320**, 915-924 (1989).
16) Schultz, J.; Johansen; Harris, A. K.; Rychly, D. J.; Ergul, A. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovasc. Diabetol.* **4**, 1-11 (2005).
17) SIES, H. Oxidative Stress: Oxidants and antioxidants. *Exper. Physiol.* **82**, 291-295 (1997).
18) Ainsworth, E. A.; Gillespie, K. M. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.* **2**, 875-877 (2007).
19) Hirano, R.; Kondo, K.; Iwamoto, T.; Igarashi, O.; Itakura, H. Effects of antioxidants on the oxidative susceptibility of low-density lipoprotein. *J. Nutr. Sci. Vitaminol.* (*Tokyo*). **43**, 435-444 (1997).
20) Buege, J. A.; Aust, S. D. Microsomal lipid peroxidation. *Methods Enzymol.* **52**, 302-310 (1978).
21) Yoshida, H.; Ishikawa, T.; Hosoai, H.; Suzukawa, M.; Ayaori, M.; Hisada, T.; Sawada, S.; Yonemura, A.; Higashi, K.; Ito, T.; Nakajima, K.; Yamashita, T.; Tomiyasu, K.; Nishiwaki, M.; Ohsuzu, F.; Nakamura, H. Inhibitory effect of tea flavonoids on the ability of cells to oxidize low density lipoprotein. *Biochem. Pharmacol.* **58**, 1695-1703 (1999).
22) Meng, L.; Lozano, Y.; Bombarda, I.; Gaydou, E. M.; Li, B. Polyphenol extraction from eight *Perilla frutescens cultivars Comptes Rendus Chimie* **12**, 602-611 (2009).
23) Meng, L.; Lozano, Y. F.; Gaydou, E. M.; Li, B. Antioxidant activities of polyphenols extracted from *Perilla frutescens* varieties. *Molecules* **14**, 133-140 (2009).
24) Sawabe, A.; Satake, T.; Aizawa, R.; Sakatani, K.; Nishimoto, K.; Ozeki, C.; Hamada, Y.; Komemushi, S. Toward use of the leaves of *Perilla frutescens* (L.) Britton var. Acuta Kudo (red perilla) with Japanese dietary pickled plum (Umeboshi). *J. Oleo Sci.* **55**, 413-422

(2006).
25) Nakamura, Y.; Ohti, Y.; Murakami, A.; Ohigashi, H. Superoxide scavenging activity of rosmarinic acid from *Perilla frutescens* Britton var. Acuta f. Viridis. *J. Agric. Food Chem.* **46**, 4545-4550(1998).
26) Kamiyama, M.; Kishimoto, Y.; Tani, M.; Andoh, K.; Utsunomiya, K.; Kondo, K. Inhibition of low-density lipoprotein oxidation by Nagano purple grape(*Vitis vinifera x, Vitis labrusca*). *J. Nutr. Sci. Vitaminol.* (*Tokyo*). **55**, 471-478(2009).
27) Iizuka, M.; Tani, M.; Kishimoto, Y.; Saita, E.; Toyozaki, M.; Kondo, K. Inhibitory effects of balsamic vinegar on LDL oxidation and lipid accumulation in THP-1 macrophages. *J. Nutr. Sci. Vitaminol.*(*Tokyo*). **56**, 421-427(2010).
28) Carbonneau, M. A.; Leger, C. L.; Monnier, L.; Bonnet, C.; Michel, F.; Fouret, G.; Dedieu, F.; Descomps, B. Supplementation with wine phenolic compounds increases the antioxidant capacity of plasma and vitamin E of low-density lipoprotein without changing the lipoprotein Cu(2+)-oxidizability: possible explanation by phenolic location. *Eur. J. Clin. Nutr.* **51**, 682-690 (1997).
29) Mazza, G.; Kay, C. D.; Cottrell, T.; Holub, B. J. Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *J. Agric. Food Chem.* **50**, 7731-7737(2002).
30) Cao, G.; Russell, R. M.; Lischner, N.; Prior, R. L. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *J. Nutr.* **128**, 2383-2390(1998).
31) Milde, J.; Elstner, E. F.; Grassmann, J. Synergistic effects of phenolics and carotenoids on human low-density lipoprotein oxidation. *Mol. Nutr. Food Res.* **51**, 956-961(2007).
32) Surh, Y. J.; Kundu, J. K.; Na, H. K. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta Med.* **74**, 1526-1539(2008).
33) Stevenson, D. E.; Hurst, R. D. Polyphenolic phytochemicals-just antioxidants or much more? *Cell Mol. Life Sci.* **64**, 2900-2916(2007).
34) Kim, H. Y.; Kim, O. H.; Sung, M. K. Effects of phenol-depleted and phenol-rich diets on blood markers of oxidative stress, and urinary excretion of quercetin and kaempferol in healthy volunteers. *J. Am. Coll. Nutr.* **22**, 217-223(2003).
35) Yeh, C. T.; Yen, G. C. Induction of hepatic antioxidant enzymes by phenolic acids in rats is accompanied by increased levels of multidrug resistance-associated protein 3 mRNA expression. *J. Nutr.* **136**, 11-15 (2006).