Original Article

Sweet potato (*Ipomoea batatas L.*) leaves suppressed oxidation of low density lipoprotein (LDL) *in vitro* and in human subjects

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Sweet potato (*Ipomoea batatas L.*) leaves are consumed as vegetables around the world, especially in Southeast Asia. The aim of this study was to investigate the inhibitory effect of sweet potato leaves on low-density lipoprotein oxidation *in vitro* and in human subjects. We compared the antioxidant activity of 8 kinds of sweet potato leaves. Every sweet potato leaf had high radical scavenging activity and prolonged a lag time for starting low-density lipoprotein oxidation *in vitro*. We found that sweet potato leaves contained abundant polyphenol compounds and the radical scavenging activity and prolongation rate of lag time were highly correlated with total polyphenol content. We also confirmed that thiobarbituric acid reactive substances production was increased in endothelial cell-mediated low-density lipoprotein oxidation, which was decreased by treatment with sweet potato leaves. We further measured the low-density lipoprotein oxidizability in 13 healthy volunteers after their intake of 18 g of "Suioh", raw sweet potato leaves. "Suioh" prolonged a lag time for starting low-density lipoprotein oxidation and decreased low-density lipoprotein mobility. These results suggest that sweet potato leaves have antioxidant activity leading to the suppression of low-density lipoprotein oxidation.

***Key Words*: sweet potato leaves, polyphenol, antioxidant activity, low-density lipoprotein, atherosclerosis**

The high consumption of vegetables and fruits has been linked epidemiologically to a reduced risk of cardiovascular disease (CVD).[1–3] Polyphenols are the major photochemical with antioxidant properties in vegetables and fruits, which partly contribute to their beneficial effect on the prevention of CVD. The oxidative modification of low-density lipoprotein (LDL) is related to foam cell formation, and can result in the initiation and progression of atherosclerosis leading to CVD.[4]

Several studies have shown that polyphenolics from various foods such as red wine, green tea, and chocolate could play a positive role in preventing LDL oxidation.[5–7]

Many cultivars of sweet potatoes are consumed around the world. In Japan, the roots of sweet potatoes such as "Naruto Kintoki", "Beni Azuma", "Purple Sweet Road", "Quick Sweet", and "Kogane Sengan" are commonly eaten as root crops and used to make spirits. "Simon No. 1" is used as a folk medicine in Brazil. Sweet potato leaves are consumed as vegetables in tropical areas, especially Southeast Asia.[8] These leaves can be harvested many times per year because they grow well during the rainy season. Recently, the cultivars known as "Elegant Summer" and "Suioh" have been improved to produce more edible leaves and petioles.

Several reports have indicated that sweet potato leaves inhibited HIV replication, mutagenicity, diabetes, and the proliferation of cancer cells,[9,10] although the effect of these leaves in inhibiting LDL oxidation has not been sufficiently demonstrated. The aim of this study was to compare the antioxidant effect on LDL oxidation of several cultivars of sweet potato leaves.

Materials and Methods

Preparation of sweet potato leaves extracts. We used 8 kinds of sweet potato leaves; "Naruto Kintoki", "Suioh", "Elegant Summer", "Beni Azuma", "Purple Sweet Road", "Quick Sweet", "Kogane Sengan", and "Simon No. 1". All sweet potato leaves were achieved as seedlings from Miyazaki in Japan. The leaves freeze-dried and powdered by blender were extracted with 70% methanol with mixing at room temperature for 1 h. The extracted solution was filtered and stored at −20°C for using in the following studies *in vitro*.

Free radical-scavenging activity. The free radical-scavenging activity of sweet potato leaves extracts was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as described previously.[11,12] An aliquot of the sweet potato leaves extracts was mixed with 2 ml of 0.1 mM DPPH in ethanol. Following incubation for 20 min at 37°C, the absorbance was measured at 516 nm with a Beckman Model DU 800 spectrophotometer. We used 1 mM ascorbic acid as a positive control and expressed the DPPH radical scavenging activity of sweet potato leaves as ascorbic acid equivalent.

Total polyphenol content. Total polyphenol content of sweet potato leaves extracts was measured by Folin-Ciocalteu assay using 1 mM chlorogenic acid as the standard according to the previous report.[13] In brief, Folin–Ciocalteu phenol reagent (Nakaraitesuku Co., Kyoto, Japan) was added to the sample and incubated in 1.5% $NaCO_3$ solution for 2 h at 20°C, and the absorbance was measured at 750 nm. Results were expressed as chlorogenic acid equivalent.

Isolation of LDL. 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°C. The LDL was separated by single-spin density gradient ultracentrifugation (100,000 rpm, 40 min, 4°C) using a TLA-100.4 fixed angle-rotor (Beckman Instruments, Fullerton, CA).[14] The LDL protein concentration was determined using a Micro

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BCA Protein Assay Kit (Pierce Laboratories, Rockford, IL).

Measurement of LDL oxidizability (lag time assay). The LDL oxidizability was measured according to the method described in the previous report.[15] The prepared LDL samples were oxidized with or without 5 μl of each sweet potato leaves extracts by 200 μM 2,2'-azobis (4-methoxy-2,4-dimethylvaleronitrile) (AMVN-CH_3O; Wako Pure Chemical Industries), which was an oxidative inducer. The kinetics of LDL oxidation was obtained by monitoring the absorbance of conjugated dienes at 234 nm with a Beckman Model DU 800 spectrophotometer at 4 min intervals at 37°C.

Endothelial cell-mediated LDL oxidation. Human umbilical vein endothelial cells; HUVECs (Lonza Walkersville Inc., Walkersville, MD) were cultured in EGM-2 (Lonza Walkersville Inc.). Cells were grown to confluence at 37°C in 5% CO_2 and used for experiments at passage 4. HUVECs were treated with dialyzed LDL (a final concentration 100 μg/ml) in the absence or presence of sweet potato leaves extracts in Ham's F10 medium (Lonza Walkersville Inc.), containing 3 μM $FeSO_4$ and $CuSO_4$. After 24 h, the medium was analyzed for the extent of LDL oxidation as described below.

Measurement of endothelial cell-mediated LDL oxidation TBARS assay. Malondialdehyde (MDA) generated in medium including LDL was measured by using the thiobarbituric acid reactive substances (TBARS) assay according to the previous report.[16] The TBARS reagent (15% (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid- 0.25 N HCl) was added to the sample and heated for 10 min at 90°C. The absorbance of supernatant fraction was measured at 535 nm. Results are expressed as MDA-equivalent content (nanomoles of MDA per milligram of LDL protein) calculated using the extinction coefficient for MDA.

Agarose gel electrophoresis. The change in surface charge of the LDL protein was evaluated by an increase in its electrophoretic mobility. The media including LDL were subjected to agarose gel electrophoresis performed at 400 V for 15 min using the rapid electrophoresis system (Helena Laboratories, Saitama, Japan).[6] After electrophoresis, the plates were stained with Cho/Trig COMBO CH (Helena Laboratories). The increased electrophoretic mobility of LDL was expressed relative electrophoretic mobility (REM) to native LDL.

Clinical study. Our study subjects were 13 healthy volunteers (6 male and 7 female, 22–49 years). This study was approved by the Ethics Committee of Ochanomizu University and carried out in conformity with the Declaration of Helsinki (established in 1964 and revised in 2004). All subjects gave their written consent in this study. After an overnight fast, blood samples were collected between 8:00 and 9:00 a.m. Then subjects consumed 18 g of raw "Suioh" leaves containing about 1,000 mg polyphenol. Plasma samples were taken at 0.5, 1, 2, and 4 h after consumption; these samples then underwent isolation and preparation of LDL. Then we measured lag time. After LDL diluted with PBS to give a final concentration of 140 μg/ml LDL protein, was oxidized by 400 μM AMVN-CH_3O. Lag time, TBARS products and LDL mobility were determined as described above.

Statistical analysis. Results were expressed as mean ± SD, clinical study results were expressed as mean ± SEM. Differences between groups were analyzed by ANOVA with Fisher's PLSD using StatView-J5.0 (SAS Institute Inc., NC). The minimum significance level was set at a p value of 0.05 for all analysis. All experiments were performed at least three times.

Results

Screening of antioxidant activity of various sweet potato leaves. As shown in Fig. 1A, 8 sweet potato leaf extracts had high free radical scavenging activity ("Naruto Kintoki", 4.5-fold vs 1 mM ascorbic acid; "Suioh", 6.7-fold; "Elegant Summer", 6.6-fold; "Beni Azuma", 7.2-fold; "Purple Sweet Road", 7.3-fold; "Quick Sweet", 7.8-fold; "Kogane Sengan", 8.2-fold; "Simon No. 1", 8.3-fold).

To confirm that sweet potato leaves contribute to free radical scavenging activity, we measured the total polyphenol content in these leaves. As shown in Fig. 1B, all of the sweet potato leaves had a rich total polyphenol content ("Naruto Kintoki", 1.1 mg/ml; "Suioh", 1.5 mg/ml; "Elegant Summer", 1.5 mg/ml; "Beni Azuma", 1.7 mg/ml; "Purple Sweet Road", 1.7 mg/ml; "Quick Sweet", 2.0 mg/ml; "Kogane Sengan", 2.1 mg/ml; "Simon No. 1", 2.3 mg/ml). The free radical scavenging activity of sweet potato leaves was highly correlated with their total polyphenol content (Fig. 1C).

Effect of sweet potato leaves on prolongation of lag time *in vitro*. To evaluate the antioxidant effects of sweet potato leaves on LDL oxidation, we carried out an LDL lag time assay. As shown in Fig. 2A, all types of sweet potato leaves significantly prolonged a lag time for starting LDL oxidation compared with the control ("Naruto Kintoki", 2.9-fold; "Suioh", 3.3-fold; "Elegant Summer", 3.5-fold; "Beni Azuma", 4.0-fold; "Purple Sweet Road", 3.5-fold; "Quick Sweet", 4.3-fold; "Kogane Sengan", 3.9-fold; "Simon No. 1", 4.6-fold). The prolongation of lag time induced by each type of sweet potato leaf was associated with its total polyphenol content (Fig. 2B).

Effect of sweet potato leaves on HUVECs-mediated LDL oxidation. We next examined the inhibitory effect of sweet potato leaves on endothelial cell-mediated LDL oxidation. "Kogane Sengan" and "Simon No. 1" caused significant declines in TBARS products (Table 1). We evaluated the oxidative modification of LDL by monitoring the surface charge of LDL using agarose gel electrophoresis. "Kogane Sengan" and "Simon No. 1" markedly inhibited LDL mobility. As measured by MTT assay, none of the 8 types of sweet potato leaves affected cell viability under our conditions (data not shown).

Effect of sweet potato leaves on LDL oxidation in human subjects. "Suioh" has been developed to eat its leaves more pleasant-tasting. We investigated the effect of "Suioh" intake on LDL oxidation in human subjects. Thirteen subjects consumed 18 g raw "Suioh" leaves, which contained 1,000 mg total polyphenol content. The lag time for starting LDL oxidation was prolonged at 0.5 h and 4 h after the consumption of "Suioh" leaves compared to before intake ($p<0.1$) (Fig. 3A). TBARS production was also prevented at 0.5 h and 4 h (Fig. 3B). LDL mobility was markedly decreased at 2 h and 4 h ($p<0.01$, $p<0.05$, respectively) (Fig. 3C).

Discussion

Oxidation of LDL may play an important role in the generation of foam cells and result in the initiation and development of atherosclerosis.[4] In the present study, we compared the antioxidant activity of various sweet potato leaves in terms of the DPPH radical scavenging activity and LDL oxidation *in vitro* and in human subjects.

There are many cultivars of sweet potatoes around the world, but the antioxidant effects of sweet potato leaves have not yet been sufficiently investigated. First, we compared the antioxidant activity of 8 kinds of sweet potato leaves that are generally consumed in Japan. Each kind of sweet potato leaf had high DPPH radical scavenging activity and contained abundant polyphenol compounds. In addition, the DPPH radical scavenging activity of sweet potato leaves was highly correlated to their total polyphenol content.

Sweet potato leaves are known to contain several polyphenols, mainly caffeoylquinic acid (CQA) derivatives.[17] These polyphenol amounts are higher than those of sweet potato tubers including the peel, whole root and flesh tissues.[18] The total polyphenol content varies in cultivars of sweet potato leaves, which are categorized as high (>9 g/100 g dry weight) or medium (5–9 g/100 g dry weight)

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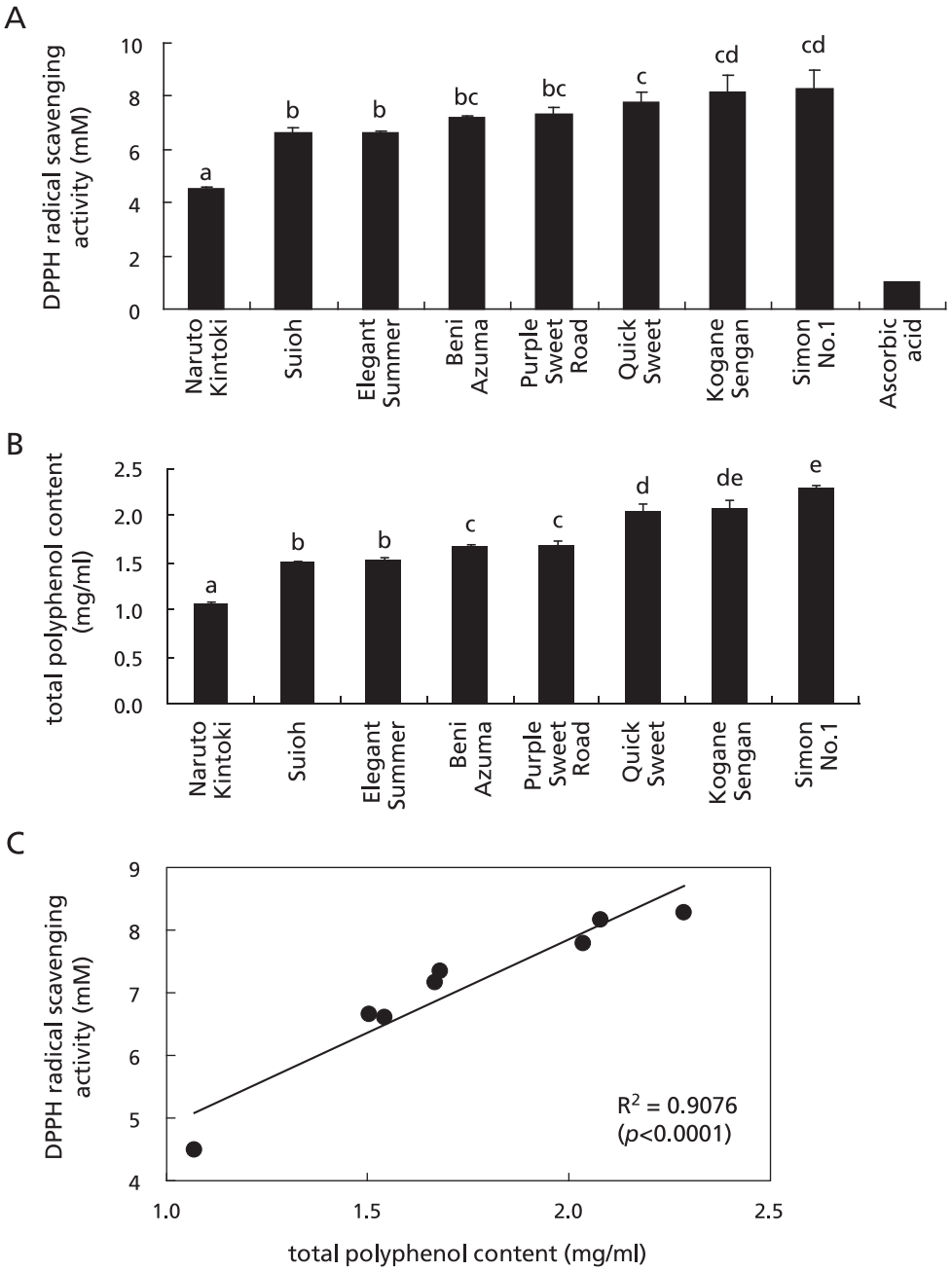

Fig. 1. Relationship between DPPH radical scavenging activity and total polyphenol content of 8 kinds of sweet potato leaves. DPPH radical scavenging activity was expressed as ascorbic acid equivalent (A). Total polyphenol content was determined by Folin-Ciocalteu assay and expressed as chlorogenic acid equivalent (B). Values are means ± SD (n = 3). Different letters indicate statistically significance (p<0.05) among different groups by Fisher's PLSD test after ANOVA. There was a significant positive correlation between total polyphenol content and DPPH radical scavenging activity (C).

polyphenol accumulators.[17] In this study, we observed that the total polyphenol content ranged from 6.3–13.5 g/100 g dry weight in the 8 kinds of sweet potato leaves, indicating that sweet potato leaves could be a strongly antioxidative leafy vegetable with high amount of polyphenol. On the other hand, sweet potato leaves have been known to contain various antioxidants such as vitamin E, β-carotene and lutein which might be contribute to their radical scavenging effects as well as polyphenols.[19]

We next demonstrated the protective effect of sweet potato leaves on LDL oxidation against pro-oxidant-initiated oxidative modification or endothelial cell-mediated oxidation *in vitro*. Sweet potato leaves significantly prolonged a lag time for starting LDL oxidation, indicating that they could prevent free radical-induced lipid peroxidation in LDL. The prolongation rate of lag time showed a significant positive correlation with their total polyphenol content. We also used Ham's F10 medium containing metal ions to induce the oxidation of LDL in HUVECs. Interaction between LDL and HUVECs remarkably increased TBARS production, which was decreased by treatment with sweet potato leaves. Sweet potato leaves also inhibited the increase of negative charge in LDL particles, indicating that the leaves may play a role in the prevention of apolipoprotein B100 modification in LDL.

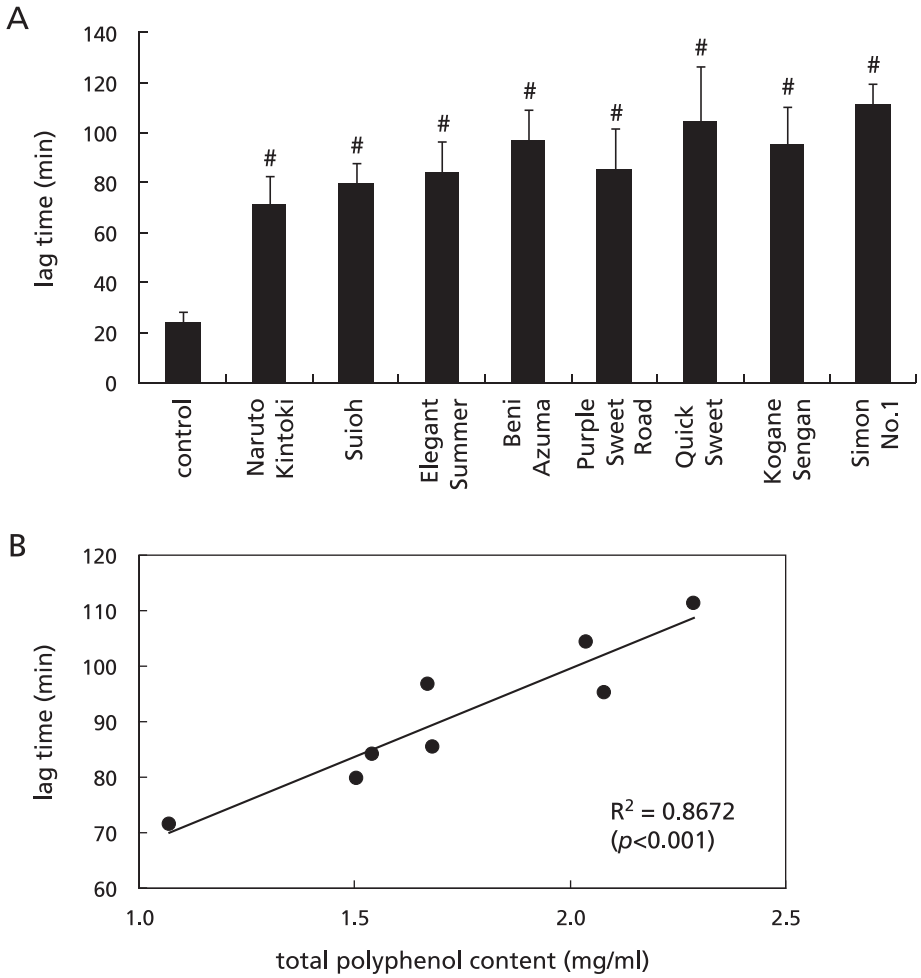

Fig. 2. Relationship between a lag time for starting LDL oxidation and total polyphenol content of 8 kinds of sweet potato leaves. LDL (70 μg/ml protein) was incubated with AMVN-CH_3O (final concentration 400 μM) at 37°C in the absence or presence of sweet potato leaves extracts. The lag time for starting LDL oxidation was defined as the time interval between the initiation and intercept of the two tangents drawn to the lag and propagation phase of the absorbance curve at 234 nm (A). Values are means ± SD (n = 4). #p<0.001 compared with untreated LDL, by Fisher's PLSD test after ANOVA. There was a significant positive correlation between a lag time for starting LDL oxidation and total polyphenol content (B).

Table 1. Effect of sweet potato leaves extracts on TBARS production and electrophoretic LDL mobility in HUVECs-mediated LDL oxidation

	% of control	
	TBARS products	LDL mobility
control	100.0	100.0
Naruto Kintoki	84.3	91.4
Elegant Summer	81.1†	88.2
Suioh	80.7†	85.0†
Beni Azuma	82.5†	91.4
Purple Sweet Road	82.8†	86.2
Quick Sweet	82.2†	88.4
Kogane Sengan	80.5*	82.7*
Simon No.1	67.3**	74.4**

Values are means ± SD (n = 3). †p<0.1, *p<0.05, **p<0.01 compared with control, by Fisher's PLSD test after ANOVA.

Although TBARS production and LDL mobility in HUVECs-mediated LDL oxidation did not show the significant correlation with total polyphenol content (data not shown), "Kogane Sengan" and "Simon No. 1", which had the highest polyphenol contents showed strong inhibitory effects.

In view of the numerous studies concerning their biofunctional activities, polyphenols have been demonstrated as effective free radical scavengers and metal ion chelators. Thus we speculated that sweet potato leaves might inhibit endothelial cell-mediated LDL oxidation, mostly due to their direct chelating action for transition metal ions and/or their interaction with oxidants in the reaction medium. In addition, 12/15-lipoxygenase from endothelial cells have been proposed to be another endogenous pro-oxidant factor involved in LDL oxidation.[20] Some polyphenols such as quercetin and epicatechin have been reported to inhibit 15-lipoxigenase activity,[21] suggesting that sweet potato leaves might have the possibility to inhibit lipoxigenase-induced LDL oxidation in endothelial cells.

To identify the contribution made by sweet potato leaves to protection against LDL oxidation, we conducted additional experiments in human subjects. Recently, several sweet potatoes such as "Elegant summer" and "Suioh" were developed in Japan for eating those leaves mainly. In the present study, the consumption of raw "Suioh" leaves containing 1,000 mg total polyphenol significantly extended the lag phase of LDL oxidation and reduced TBARS production and LDL mobility. We previously reported that the consumption of grapes containing 1,000 mg total poly-

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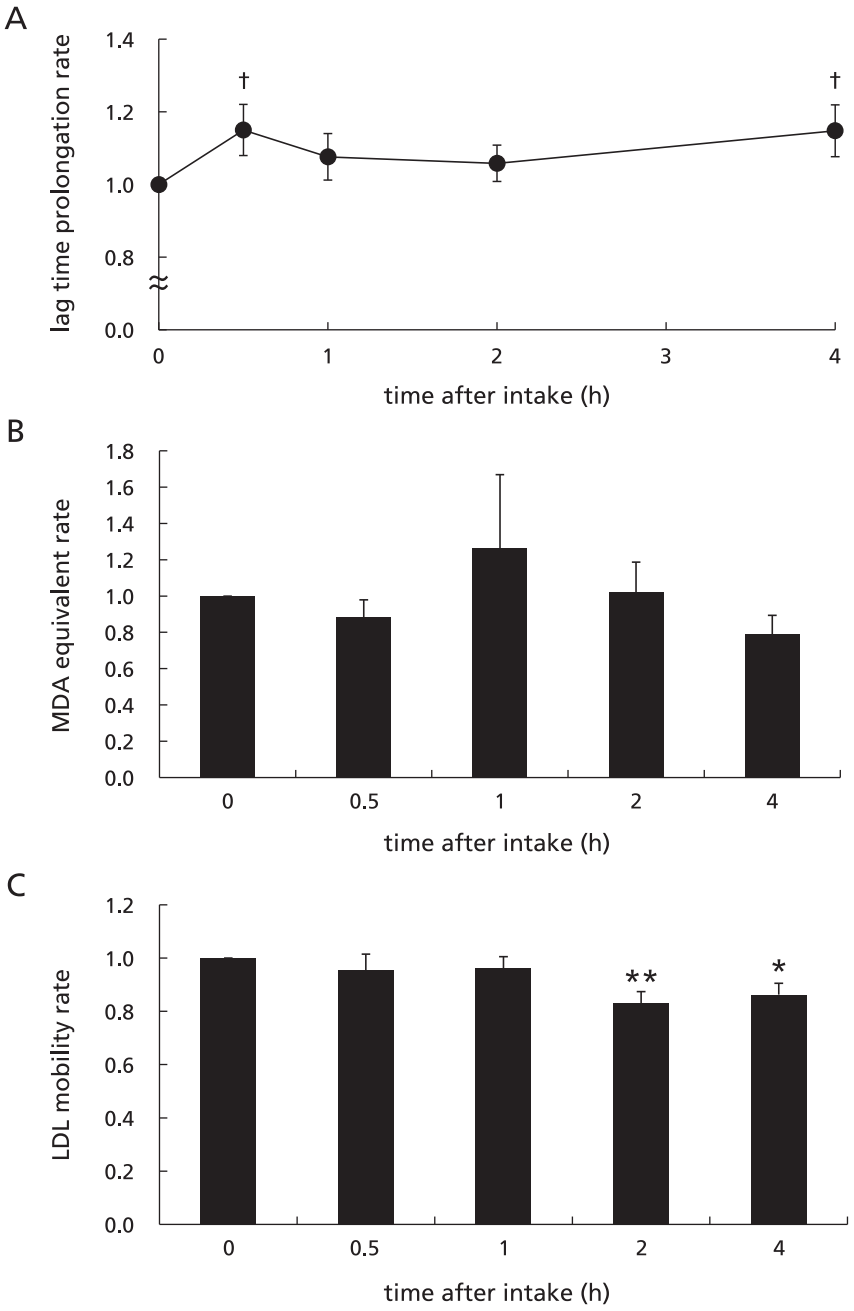

Fig. 3. Effect of "Suioh" intake on LDL oxidation in healthy subjects. After overnight fasting, 13 healthy volunteers consumed 18 g of raw "Suioh" leaves. Blood was sampled before and 0.5, 1, 2, and 4 h after intake. We measured LDL oxidizability in the presence of AMVN-CH_3O (final concentration 200 μM) by lag time assay (A), TBARS assay (B) and agarose gel electrophoresis (C). Data are mean ± SEM (n = 13). $^{\dagger}p<0.1$, $*p<0.05$, $**p<0.01$ compared with before consumption of "Suioh" by Fisher's PLSD test after ANOVA.

phenol prolonged the lag time of LDL oxidation, and that this prolongation was related to an increase in the plasma polyphenol levels.[22] A similar result has been obtained for green tea.[23]

Plasma lipoproteins have been suggested to be potential carriers of polyphenols.[24] In the previous study, plasma concentration of polyphenols was reached a peak at 30–60 min after intake of polyphenol-rich beverages such as red wine[25] and coffee.[26] Although our study could not examine, we speculated that plasma concentration of polyphenols might increase around 30 min after intake and affect on LDL oxidazability. A recent *in vivo* study also showed that CQA and diCQA were identified in plasma around 1 μmol/l at 1.75–2.33 h.[27] These data suggest that polyphenols may be absorbed into the bloodstream and incorporated into LDL after "Suioh" consumption. Moreover, it must be taken into account that "Suioh" has various antioxidants, not only polyphenols but also vitamin E and carotenoids. Previous study suggested that some hydrophilic antioxidants, such as polyphenols, bind to phospholipids or proteins on the LDL surface, whereas hydrophobic antioxidants, such as β-carotene, bind closer to the LDL core.[28] "Suioh" would be suitable for use as a vegetable which can help prevent LDL oxidation due to the combination of hydrophilic and hydrophobic antioxidants.

In conclusion, this study provided the novel findings that sweet potato leaves had antioxidant ability and inhibited the oxidation of LDL.

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