Effect of Soybean Hypocotyl Extract on Lipid Peroxidation in GK Rats

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Summary **Vascular complications, as a consequence of atherosclerosis, are main causes of morbidity and mortality in patients with diabetes mellitus. There is increasing evidence that lipid peroxidation and oxidative modification of low density lipoprotein (LDL) is important in atherogenesis. In this study we investigated the effect of soybean hypocotyl extract (SHE), rich in isoflavones and saponins, on lipid peroxide (LPO) levels in liver, plasma and lipoproteins in GK diabetic rats, and its efficacy on the reduction of susceptibility of LDL and high density lipoprotein (HDL) to oxidation. The oxidative modification of LDL and HDL was determined with the lag time of copper ion-induced oxidation curve identified by the conjugated dienes. In SHE group which were fed diet containing 40 g/kg of SHE for 16 weeks, LPO levels in liver, plasma and HDL fraction were significantly decreased compared with the control group. The lag phage of LDL oxidation curve was prolonged noticeably by a mean of 27 min in SHE group as compared to the control group, indicating a reduced susceptibility to oxidation. The results suggest that intake of soybean hypocotyl extract might be useful for the prevention and treatment of diabetes mellitus and diabetes-associated diseases.**

Key Words: **soybean, hypocotyl, oxidation, lipoproteins, diabetic rats**

Introduction

Soybeans contain valuable nutritional attributes. It has been found that the intake of soy foods is closely related to lowering the occurrences of chronic diseases [*1–5*]. There are many functional ingredients contained in soy foods such as soy protein, isoflavones, saponins, phytic acid, phytosterol, and phenolic acid [*2*]. Among them, isoflavones and saponins are most commonly noted, and the both seem to be concentrated in the soybean hypocotyl. Soyasaponins have drawn interest in recent years due to their potential multiple health-promoting properties including plasma cholesterol lowering [*3*], anticarcinogenic [*4*, *5*], hepatoprotective [*6*], and anti-viral activities [*7*]. Recent reports demonstrated that the *in vivo* reduction in hepatic lipid peroxidation in mice intraperitoneally injected with soyasaponins was comparable to that which has been observed for α-tocopherol, and the effects of soyasaponins on lipid peroxidation levels appear to be mediated through the secretion of thyroid hormones [*8*]. Soy primarily contains 3 types of isoflavone glycosides: genistin, daidzin, and glycitin. The isoflavone aglycones, genistein, daidzein, and glycitein, are structurally related to 17-estradiol, hence, they are often referred to as phyto-estrogens [*9*]. Kerry and Abbey reported that genistein inhibits copper- and peroxy radical–mediated low density lipoprotein (LDL) oxidation when added to a cell-free oxidation system but not when incorporated into LDL

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particles before the oxidation reaction [10]. Kapiotis *et al.* also reported that genistein, but not daidzein, inhibits copper-mediated LDL oxidation, as shown by the inhibition of thiobarbituric acid–reactive substances (TBARS) and the formation of hydroperoxides in cell-free systems [11]. In contrast with *in vitro* studies, data from interventional studies have failed to show an antioxidant effect from isoflavone supplements [12–14]. Several studies have suggested that soy intake aids against oxidative stress, as indicated on the basis of measurements of conjugated dienes in the LDL fraction [15, 16], lag time for copper-induced LDL oxidation [17, 18], and plasma concentrations of F_2-isoprostanes [17]. However, results from other studies failed to support the antioxidant effects of soy [19, 20]. The potential decrease in oxidative stress has been ascribed to the isoflavone component of the soybeans. Soybeans and products derived from soybeans represent the major source of dietary isoflavones [21]. Compared with the major anti-oxidants in plasma (ascorbic acid, uric acid, α-tocopherol, β-carotene, and other carotenoids) [22, 23], the concentration of isoflavones in plasma is relatively low and can reach concentrations comparable with those of carotenoids, only after the consumption of meals containing soy products high in isoflavones or isoflavone supplements [17, 24]. One possibility for the discordant observations to date is that there is a potential independent effect of soy isoflavones, or the synergistic effect of isoflavones and other soy-derived phytochemicals. This possibility has not been adequately addressed. Therefore, the aim of this study was to evaluate the anti-oxidative effect of soy hypocotyl, rich in soyasaponins and isoflavones in GK diabetic rats in order to clarify the paradox.

Materials and Methods

Reagents and chemicals

Cholesterol (CH), phospholipid (PL) and triglyceride (TG) kits and Glucose B Test kit were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Lipid peroxide (LPO) test kit was obtained from Kyowa Medex Company Ltd. (Tokyo, Japan). Malondialdehyde (MDA) test kit was obtained from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Daidzein, daidzin, malonyldaidzin, glycitein, glycitin, malonylglycitin and malonylgenistin were purchased from Fujicco Company Ltd. (Kobe, Japan). Genistein and genistin were obtained from Extrasynthese (Genay, France).

Preparation and characterization of test extract

Soybean seeds (*Glycine max* (L.) Merrill) were obtained from the National Institute of Agrobiological Resources of Japan. Soybean hypocotyl was shaken with 50% ethanol overnight and the solution was filtered. The filtrate was

spray-dried; the powder was then dissolved in 10% ethanol and applied to ODS column (YMC, ODS-A60-S150, 5 cm × 74 cm). The column was then eluted step-wisely with 10%, 30%, 50%, 80% and 100% ethanol. Each fraction was collected, evaporated to dryness under vacuum, and finally lyophilized. Fractions were analyzed with thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) before further application. The 10% fraction consisted of soybean oligosaccharides; 30%, 50% and 80% fractions consisted of soybean isoflavones and soyasaponins. Therefore, soybean hypocotyl extract (SHE) was the mixture of 30%, 50% and 80% fractions in this experiment. The composition of SHE was as follows (mg/g). Isoflavone: malonyldaidzin 63.2, malonylglycitin 27.5, malonylgenistin 19.1, daidzin 29.3, glycitin 10.4, genistin 6.3, daidzein 12.7, glycitein 3.6, genistein 2.9; saponin: Aa 147.9, Ae 316.4, Ba 20.3, Bb 81.5, Bd 15.7, Be 30.3, αg 21.9, βg 73.8.

To guarantee reproducibility of pharmacological experiments, isoflavone and saponin contents of fractions and SHE were analyzed with TLC and HPLC before further application. For analysis of isoflavones and saponins, each fraction was dissolved in methanol and filtered through a 0.45 μm filter (Millipore). TLC was performed on silica gel (Merck, Kieselgel 60 F-254) with a lower phase of chloroform-methanol-water (65:35:10, v/v). Spots were visualized first by UV-monitoring at 210 nm for saponins and 260 nm for isoflavones, then by heating at 120°C for 10 min after spraying with 10% sulfuric acid. HPLC was carried out as follows. HPLC instrument: Waters 600E system; column: YMC-packed ODS-AM-303 column (5 μm, 4.6 mm × 50 mm); flow rate: 1.0 ml/min; UV-detection: absorption at 210 nm and 260 nm for saponins and isoflavones, respectively; mobile phase: 40% acetonitrile containing 1% trifluoroacetic acid for the detection of saponins, a linear gradient of acetonitrile from 15% to 35% containing 1% trifluoroacetic acid for the detection of isoflavones.

Experimental animals

Six-week-old male GK/Jcl type 2 diabetic rats with a mean weight of approximately 92 g were purchased from Japan Clea Inc. Twenty GK rats were randomly assigned to two groups: the control and SHE group. The control group was fed on a standard laboratory chow; while SHE group was fed a standard diet containing 40 g/kg of SHE for 16 weeks. The composition of the control diet was as follows (g/kg). Casein 250, corn starch 300, sucrose 250, cellulose powder 50, corn oil 100, mineral mixture 35, vitamin mixture 10, methionine 3, choline bitartrate 2. The composition of the mineral mixture was according to AIN-93G MX and that of the vitamin mixture to AIN-93G VX [25]. SHE was added to the diet at the expense of starch. Experimental animals were housed in an air-conditioned room at 25°C,

under a natural light-dark regimen. Rats were allowed free access to powdered feed and water that was supplied through an automatic watering system *ad libitum*. All experiment was carried out in accordance with the recommendations of the Institutional Animal Care and Use of Committee of Yanbian University.

Determination of MDA in liver homogenate

At the end of the experimental period, rats were sacrificed and the liver was excised, rinsed with chilled 0.2 M phosphate buffer (pH 7.2). The liver (1 g) were homogenized in 0.2 M phosphate buffer saline (pH 7.2) in an ice bath and the homogenate was centrifuged at $145 \times g$ for 10 min at 4°C. The supernatant was used for assay of MDA with the thiobarbituric acid method with the MDA test kit.

Determination of blood biochemical parameters

The blood sample were collected in the test tube containing EDTA after an overnight fast at the beginning and at the end of the 16-week period, and plasma was immediately separated by centrifugation at $1000 \times g$ for 15 min at 4°C. Blood lipids and glucose and other parameters were detected with a biochemical autoanalyser (Hitachi 736-15, HITACHI, Japan) with respective commercial test kits. LPO were determined enzymatically, using a kit according to the manufacturer's instruction. MDA content, a degrading product of lipid peroxidation known as thiobarbituric acid–reactive substances (TBARS), was determined according to the thiobarbituric acid method using a MDA test kit.

Preparation of lipoproteins and determination of lipid

The very low density lipoprotein (VLDL), low density lipoprotein(LDL), high density lipoprotein (HDL) were isolated by sequential ultracentrifugation by a modified method of Havel *et al.* [26] in a HITACHI himac cpα 100 ultracentrifuge. Following ultracentrifugation of HDL, the bottom fraction was taken to represent very high density faction with a density greater than 1.21 (VHDL, also called lipoprotein-deficient fraction). TG, CH and LPO levels in lipoprotein were determined by enzymatic methods using commercial test kits; protein was measured according to the modified Lowry procedure of Markwell *et al.* [27]. All the data were normalized for the original volume of blood plasma.

Copper ion-mediated oxidation of LDL and HDL

The isolated LDL and HDL were incubated with pH 7.4, 10 mM PBS buffer for a total of 20 h at 4°C. Following dialysis, the protein concentration in lipoprotein fractions was determined by the modified Lowry procedure of Markwell *et al.* [27] and adjusted to 0.1 g/L. LDL and HDL oxidation was stimulated by the addition of copper sulfate in PBS to produce a final copper ion concentration of 5 μM. The formation of conjugated dienes (CD) was continuously monitored in a Shimadzu UV-1202 spectrophotometer at 234 nm over a period of 3 h. The indices of lipid peroxidation measured were lag time and oxidation rate. The length of the lag phase was defined as the lag time to the intercept of the tangent of the absorbance curve in the propagation phase with baseline. Propagation rate was expressed as the slope of the tangent (change in absorbance/min) [10, 28].

Statistical analysis

All the results were presented as mean ± SD. Student's *t* test was performed using SPSS 10.0 software. Differences were considered statistically significant when $p < 0.05$.

Results

Table 1 shows the levels of body weight (BW), blood glucose (BG), TG, CH and HDL-C at the beginning (0 week) and at the end of the experimental period (16 week). During the feeding period, BG levels and body weights increased and TG decreased in each group of all the rats. However, no differences in body weights and TG levels were observed in this experiment, when comparing SHE group with the control group. No changes were observed in the levels of total cholesterol or HDL-cholesterol in this experiment, either. At the end of the feeding period, BG levels of rats fed with SHE were significantly lower than those of control animals (Table 1).

In this experiment, the lipid peroxidation was studied as an index of oxidative damage. An elevated level of lipid peroxides was observed in the plasma of diabetic rats during the experiment. Results show that chronic SHE administration significantly decreased blood LPO and MDA levels of SHE group as compared to the control group (Table 1).

Other biochemical parameters, including total protein (TP), total bilirubin (T-BIL), blood urea nitrogen (BUN),

Table 1. Effect of SHE on body weight, blood glucose, blood lipids, LPO and MDA levels of GK rats

Period (week)	Group	BW (g)	BG (mM)	TG (mg/dl)	CH (mg/dl)	HDL-C (mg/dl)	LPO (μM)	MDA (μM)
0	Control	91 ± 5	5.8 ± 0.9	31.9 ± 3.3	112 ± 21	81.2 ± 11.9	7.0 ± 1.3	5.2 ± 2.1
	SHE	92 ± 5	5.8 ± 1.0	32.2 ± 3.2	112 ± 17	81.1 ± 12.0	7.1 ± 0.7	5.4 ± 1.2
16	Control	357 ± 11	14.3 ± 1.3	17.9 ± 3.1	107 ± 23	78.2 ± 14.3	11.2 ± 2.2	7.9 ± 2.4
	SHE	364 ± 13	11.4 ± 0.9*	18.2 ± 3.5	109 ± 17	82.1 ± 11.9	8.1 ± 0.7*	5.9 ± 1.8*

The results are expressed as means ± SD of ten rats per group. *$p < 0.05$, compared with control group.

Table 2. Effect of SHE on blood biochemical parameters of GK rats

Group	TP (g/dl)	T-BIL (mg/dl)	GOT (U/l)	GPT (U/l)	UA (mg/dl)	BUN (mg/dl)	Cr (mg/dl)
Control	6.2 ± 0.3	0.0 ± 0.0	123 ± 17	120 ± 21	1.4 ± 0.7	14.4 ± 1.1	0.3 ± 0.0
SHE	6.3 ± 0.2	0.0 ± 0.1	127 ± 23	119 ± 25	1.5 ± 0.6	13.8 ± 1.7	0.3 ± 0.0
p value[†]	0.29	0.71	0.67	0.47	0.74	0.32	0.64

The results are expressed as means $\pm$ SD of ten rats per group. [†]Probability (p) was calculated with Student's t test using SPSS 10.0 software.

creatinine (Cr), uric acid (UA), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were not modified by SHE treatment (Table 2).

The hepatic lipid peroxidation in terms of thiobarbituric acid-reactive substances was also measured as an index of oxidative damage to liver. Results show that the hepatic MDA level of SHE group [(3.2 ± 1.1) μmol/g protein] was significantly lower than that of control group [(4.9 ± 1.4) μmol/g protein]. Results confirm that SHE participates in the prevention of oxidative damage to both the plasma and liver.

Lipid peroxidation (LPO) was also studied in lipoprotein fractions at the end of the experiment. Results show that 16 weeks of SHE feeding significantly decreased HDL-LPO (Table 3). The chronic SHE administration slightly decreased the LPO status of LDL, but the differences between the two groups were not significant ($p = 0.12$, Table 3). No differences in TG, CH, PL, and protein levels of lipoprotein fractions were observed in this experiment, when comparing SHE group with the control group (Data were not shown).

The isolated LDLs and HDLs were subjected to copper ion-induced oxidation. The results indicated a significant

Table 3. Effect of SHE on LPO levels in lipoprotein fractions

Group	LPO concentration in lipoprotein fractions (μM)			
	VLDL	LDL	HDL	VHDL
Control	1.4 ± 0.4	2.7 ± 0.9	6.6 ± 1.6	0.3 ± 0.1
SHE	1.2 ± 0.3	2.2 ± 0.4	$4.8 \pm 1.2^*$	0.2 ± 0.1

The results are expressed as means $\pm$ SD of ten rats per group. Data have been normalized for the original volume of blood plasma. *$p<0.05$, compared with control group.

mean prolongation of the LDL lag phase by more than 27 min in SHE group as compared to control group after 16 weeks of SHE feeding (Table 4). The lag phase of HDL oxidation curve also increased, but insignificantly. There was also a significant decrease in the propagation rate of LDL but the accumulated LDL and HDL conjugated dienes were not significantly different between SHE and control animals during the incubation period (Table 4). When the lipid peroxide content in lipoprotein fractions was expressed on a per cholesterol basis, then LDL and HDL of SHE group were found to contain less LPO molecules at the beginning of copper ion-induced oxidation than the control group ($p = 0.09$ and 0.01 for LDL and HDL, respectively, Table 4).

Discussion

Over the last few years, research has focused on soybean constituents such as isoflavones, phytosterols, saponins, water- and fat-soluble vitamins, and minerals [29, 30]. Tanizawa et al. [31] reported that soyasaponins inhibit the in vivo Adriamycin-induced increase of lipid peroxide levels in the myocardium of mice and saponin constituents are also responsible for a reduction of NADPH-induced lipid peroxidation (in vitro) in mice hepatic microsomes. In a small study, Tikkanen et al. [18] also documented that the consumption of soy bars for 2 week resulted in a longer lag phase of copper-induced LDL oxidation than that at baseline. However, there is a contradiction about the in vivo effect of isoflavones and saponins on lipid oxidation [12–14]. It encourages us to investigate the in vivo antioxidant properties of soy hypocotyl, rich in isoflavones and saponins, in whole plasma and isolated lipoprotein fractions.

In this study isoflavones and saponins in soybean hypo-

Table 4. Change of susceptibility of LDL and HDL to oxidation

Group	Initial LPO[†] (nmol/mg cholesterol)		Lag time (min)		CD[‡] (nmol/mg protein)		Propagation rate (ΔA_{234}/min $\times 10^{-2}$)	
	LDL	HDL	LDL	HDL	LDL	HDL	LDL	HDL
Control	9.5 ± 2.4	8.4 ± 1.7	38 ± 21	19 ± 10	95 ± 37	20 ± 9	3.5 ± 1.2	0.52 ± 0.22
SHE	8.1 ± 1.9	$6.4 \pm 2.0^*$	$65 \pm 29^*$	26 ± 12	89 ± 33	18 ± 10	$3.1 \pm 1.1^*$	0.49 ± 0.20

The results are expressed as means $\pm$ SD of six rats per group. [†]The initial hydroperoxide contents in lipoprotein fractions at the beginning of oxidation were expressed on a per mg cholesterol basis. [‡] Absorbance units were converted to molar units of conjugated dienes (CD) using the molar extinction coefficient 29500 $M^{-1} \cdot cm^{-1}$. *$p<0.05$, compared with control group.

cotyl occur as glucose-conjugated forms. Once ingested, these glucosides are hydrolyzed to absorbable aglycones [*32*]. It is commonly assumed that isoflavone aglycones are absorbed quickly compared with glucoside forms. However, the bioavailability of aglycones and glucosides of soy isoflavones is controversial [*33*, *34*]. Soyasaponins may have very low absorbability in the human gut. According to the study of Hu *et al.* [*35*], soyasaponins are taken up by gut epithelial cells in a saturable manner in small amounts; in contrast, the uptake of soyasapogenol may depend upon its concentration in the lumen. The absorbed soyasaponin or sapogenol may also contribute to anticancer activity [*35*].

The most widely used methods for monitoring lipid oxidation are the measurement of lipid peroxides, thiobarbituric acid reactive substance and conjugated diene levels. In our study the addition of SHE to daily diet clearly reduced the plasma and hepatic LPO levels. Moreover, LPO levels in the isolated HDL samples from SHE group were significantly lower than those from the control group. The result indicates reduced lipid peroxidation of the liver, plasma and high density lipoprotein fraction. The chronic SHE administration also slightly decreased the LPO status of LDL, but the differences between the two groups were not significant. The result may be explained with the fact that HDL is the principal vehicle for circulating plasma lipid hydroperoxides and may be more rapidly oxidized than those in LDL *in vivo* [*36*]. Lipid peroxides may accumulate more rapidly in HDL than in LDL *in vivo* because of the antioxidant action of CoQH$_2$ in the LDL [*31*].

According to the study of Bowry *et al.* [*36*], even though HDL particles carry most (≈85%) of the detectable oxidized core lipoprotein lipids, LDL core lipids were oxidized more rapidly after most ubiquinol-10 present in LDL was consumed. Therefore, we have also examined the effect of SHE on the susceptibility of LDL and HDL to oxidation. All samples of SHE group showed similar shifts of the propagation curves to the right as compared to the control group. Our finding that 16 weeks of SHE feeding caused a significant prolongation of lag time of LDL is potentially important, as oxidative modification of LDL is regarded as atherogenic. The elevated concentrations of circulating antioxidant phytochemicals such as isoflavones and saponins during SHE feeding could have protected LDL from oxidation and resulted in a reduction of the initiation rate. Even given a significant reduction of HDL-LPO after SHE administration, there was no significant difference in the formation of conjugated dienes and lag time of HDL oxidation between the two groups. The much lower initiation rate and shorter lag time of HDL oxidation curve may explain the "lack" of a protective effect of SHE against HDL oxidizability in some way.

In this experiment, antioxidant activity of SHE was confirmed. In diabetic rats, the consumption of soybean hypocotyl rich in isoflavones and saponins reduced the liver, plasma and HDL lipid peroxidation and the oxidative modification of LDL. These findings suggest that soybean hypocotyl, the natural combination of isoflavones and saponins, might have a therapeutic value for the prevention and treatment of diabetes-associated atherosclerosis.

Acknowledgement

References

[1] Adlercreutz, C.H., Goldin, B.R., Gorbach, S.L., Hockerstedt, K.A., Watanabe, S., Hamalainen, E.K., Markkanen, M.H., Makela, T.H., Wahala, K.T., and Adlercreutz, T.: Soybean phytoestrogen intake and cancer risk. *J. Nutr.*, **125**, 757S–770S, 1995.

[2] Messina, M.: Modern applications for an ancient bean: soybeans and the prevention and treatment of chronic disease. *J. Nutr.*, **125**, 567S–569S, 1995.

[3] Potter, S.M.: Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J. Nutr.*, **125**, 606S–611S, 1995.

[4] Konoshima, T.: Anti-tumor-promoting activities or triterpenoid glycosides; cancer chemoprevention by saponins. *Adv. Exp. Med. Biol.*, **404**, 87–100, 1996.

[5] Rodrigues, H.G., Diniz, Y.S., Faine, L.A., Galhardi, C.M., Burneiko, R.C., Almeida, J.A., Ribas, B.O., and Novelli, E.L.: Antioxidant effect of saponin: potential action of a soybean flavonoid on glucose tolerance and risk factors for atherosclerosis. *Int. J. Food Sci. Nutr.*, **56**, 79–85, 2005.

[6] Kinjo, J., Imagire, M., Udayama, M., Arao, T., and Nohara, T.: Structure-hepatoprotective relationships study of soyasaponins I-IV having soyasapogenol B as aglycone. *Planta Med.*, **64**, 233–236, 1998.

[7] Hayashi, K., Hayashi, H., Hiraoka, N., and Ikeshiro, Y.: Inhibitory activity of soyasaponin II on virus replication *in vitro*. *Planta Med.*, **63**, 102–105, 1997.

[8] Ishii, Y. and Tanizawa, H.: Effects of Soyasaponins on Lipid Peroxidation through the Secretion of Thyroid Hormones. *Biol. Pharm. Bull.*, **29**, 1759–1763, 2006.

[9] Branham, W.S., Dial, S.L., Moland, C.L., Hass, B.S., Blair, R.M., Fang, H., Shi, L., Tong, W., Perkins, R.G., and Sheehan, D.M.: Phytoestrogens and mycoestrogens bind to the rat uterine estrogen receptor. *J. Nutr.*, **132**, 658–664, 2002.

[10] Kerry, N. and Abbey, M.: The isoflavone genistein inhibits copper and peroxyl radical mediated low density lipoprotein oxidation *in vitro*. *Atherosclerosis*, **140**, 341–347, 1998.

[11] Kapiotis, S., Hermann, M., Held, I., Seelos, C., Ehringer, H., and Gmeiner, B.M.K.: Genistein, the dietary-derived angio-

genesis inhibitor, prevents LDL oxidation and protects endothelial cells from damage by atherogenic LDL. *Arterioscler. Thromb. Vasc. Biol.*, **17**, 2868–2874, 1997.

[12] Nestel, P.J., Yamashita, T., Sasahara, T., Pomeroy, S., Dart, A., Komesaroff, P., Owen, A., and Abbey, M.: Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. *Arterioscler. Thromb. Vasc. Biol.*, **17**, 3392–3398, 1997.

[13] Samman, S., Lyons, W.P.M., Chan, G.S.M., Smith, S.J., and Petocz, P.: The effect of supplementation with isoflavones on plasma lipids and oxidisability of low density lipoprotein in premenopausal women. *Atherosclerosis*, **147**, 277–283, 1999.

[14] Hodgson, J.M., Puddey, I.B., Croft, K.D., Mori, T.A., Rivera, J., and Beilin, L.J.: Isoflavonoids do not inhibit *in vivo* lipid peroxidation in subjects with high-normal blood pressure. *Atherosclerosis*, **145**, 167–172, 1999.

[15] Jenkins, D.J., Kendall, C.W., Jackson, C-J.C., Connelly, P.W., Parker, T., Faulkner, D., Vidgen, E., Cunnane, S.C., Leiter, L.A., and Josse, R.G.: Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *Am. J. Clin. Nutr.*, **76**, 365–372, 2002.

[16] Jenkins, D.J.A., Kendall, C.W.C., Vidgen, E., Vuksan, V., Jackson, C.J., Augustin, L.S., Lee, B., Garsetti, M., Agarwal, S., Rao, A.V., Cagampang, G.B., and Fulgoni, V.: Effect of soy-based breakfast cereal on blood lipids and oxidized low-density lipoprotein. *Metabolism*, **49**, 1496–1500, 2000.

[17] Wiseman, H., O'Reilly, J.D., Adlercreutz, H., Mallet, A.I., Bowey, E.A., Rowland, I.R., and Sanders, T.A.: Isoflavone phytoestrogens consumed in soy decrease F2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. *Am. J. Clin. Nutr.*, **72**, 395–400, 2000.

[18] Tikkanen, M.J., Wähälä, K., Ojala, S., Vihma, V., and Adlercreutz, H.: Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 3106–3110, 1998.

[19] Steinberg, F.M., Guthrie, N.L., Villablanca, A.C., Kumar, K., and Murray, M.J.: Soy protein with isoflavones has favorable effects on endothelial function that are independent of lipid and antioxidant effects in healthy postmenopausal women. *Am. J. Clin. Nutr.*, **78**, 123–130, 2003.

[20] Swain, J.H., Alekel, D.L., Dent, S.B., Peterson, C.T., and Reddy, M.B.: Iron indexes and total antioxidant status in response to soy protein intake in perimenopausal women. *Am. J. Clin. Nutr.*, **76**, 165–171, 2002.

[21] Bingham, M., Gibson, G., Gottstein, N., de Pascual-Teresa, S., Minihane, A-M., and Rimbach, G.: Gut metabolism and cardioprotective effects of dietary isoflavones. *Curr. Top. Nutraceutical Res.*, **1**, 31–48, 2003.

[22] Nyyssönen, K., Porkkala-Sarataho, E., Kaikkonen, J., and Salonen, J.T.: Ascorbate and urate are the strongest determinants of plasma antioxidative capacity and serum lipid resistance to oxidation in Finnish men. *Atherosclerosis*, **130**, 223–233, 1997.

[23] Aldini, G., Yeum, K-J., Russell, R.M., and Krinsky, N.I.: A method to measure the oxidizability of both the aqueous and lipid compartments of plasma. *Free Radic. Biol. Med.*, **31**, 1043–1050, 2001.

[24] Yeum, K-J., Booth, S.L., Sadowski, J.A., Liu, C., Tang, G., Krinsky, N.I., and Russell, R.M.: Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. *Am. J. Clin. Nutr.*, **64**, 594–602, 1996.

[25] Reeves, P.G., Nielsen, F.H., and Fahey, G.C. Jr.: AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.*, **123**, 1939–1951, 1993.

[26] Havel, R.J., Eder, H.A., and Bragdon, J.H.: The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.*, **34**, 1345–1353, 1955.

[27] Markwell, M.A., Haas, S.M., Bieber, L.L., and Tolbert, N.E.: A modification of the lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.*, **87**, 206–210, 1978.

[28] Esterbauer, H., Gebicki, J., Puhl, H., and Jurgens, G.: The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic. Biol. Med.*, **13**, 341–390, 1992.

[29] Watanabe, S., Haba, R., Terashima, K., Arai, Y., Miura, T., Chiba, D., and Takamatsu, M.: Antioxidant activity of soya hypocotyl tea in humans. *Biofactors*, **12**, 227–232, 2000.

[30] Watanabe, S., Terashima, K., Sato, Y., Arai, S., and Eboshida, A.: Effects of isoflavone supplement on healthy women. *Biofactors*, **12**, 233–241, 2000.

[31] Tanizawa, H., Sazuka, Y., Takino, Y., Kitagawa, I., Hayashi, T., and Arich, S.: Inhibitory effect of soyasaponins on the increase of lipid peroxide by adriamycin (ADR) in mice. *Proc. Symp. Wakan-Yaku*, **15**, 119–123, 1982.

[32] Setchell, K.D.R., Brown, N.M., Zimmer-Nechemias, L., Brashear, W.T., Wolfe, B.E., Kirschner, A.S., and Heubi, J.E.: Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *Am. J. Clin. Nutr.*, **76**, 447–453, 2002.

[33] Izumi, T., Piskula, M.K., Osawa, S., Obata, A., Tobe, K., Saito, M., Kataoka, S., Kubota, Y., and Kikuchi, M.: Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr.*, **130**, 1695–1699, 2000.

[34] Setchell, K.D.R., Brown, N.M., Desai, P., Zimmer-Nechemias, L., Wolfe, B.E., Brashear, W.T., Kirschner, A.S., Cassidy, A., and Heubi, J.E.: Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *J. Nutr.*, **131**, 1362S–1375S, 2001.

[35] Hu, J., Reddy, M.B., Hendrich, S., and Murphy, P.A.: Soyasaponin I and sapongenol B have limited absorption by Caco-2 intestinal cells and limited bioavailability in women. *J. Nutr.*, **134**, 1867–1873, 2004.

[36] Bowry, V.W., Stanley, K.K., and Stocker, R.: High density lipoprotein is the major carrier of lipid hydroperoxides in human blood plasma from fasting donors. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 10316–10320, 1992.