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Antioxidative Functions of Natto, A Kind of Fermented Soybeans: Effect on LDL Oxidation and Lipid Metabolism in Cholesterol-Fed Rats

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Natto water-soluble fractions, low-molecular-weight viscous substance, and soybean water extract, which had an inhibitory effect on the oxidation of low-density lipoproteins (LDL) in vitro, were fed to rats for 3 weeks. These fractions had no influence on the growth of rats, which were fed a basal diet containing 1% cholesterol, but lowered plasma triglyceride and total cholesterol. Inhibition of copper-oxidation of plasma and LDL ex vivo, and a reduction in lipid peroxidation in liver and aorta in vivo, were also observed. The antioxidant enzymes were not induced in rats fed natto fraction diets. These results demonstrate that ingestion of the natto fractions led to inhibition of LDL oxidation, and that the fractions perform direct antioxidant action in the body. It is suggested that natto fractions might help to prevent arteriosclerosis, as they appear to reduce lipid peroxidation and improve lipid metabolism.

KEYWORDS: Natto (fermented soybeans); antioxidant; LDL; cholesterol; lipid peroxidation; antioxidant enzyme; superoxide dismutase; catalase; glutathione peroxidase; glutathione

INTRODUCTION

Oxidative injury to the living body by reactive oxygen or free radicals has been shown to play a role in many lifestyle-related diseases (*1*, *2*). Most Japanese meals are considered to be healthy, and it is thought that some of the foodstuffs eaten may help prevent these diseases. Recently, it has been shown that natto, fermented soybeans, a processed traditional food which has been eaten for many years in Japan, has various physiological functions (*3*, *4*).

Water-soluble fractions of natto were shown to have antioxidant activity (*5*) by the XYZ-dish method (*6*). The natto fractions were also shown to have an inhibitory effect on oxidation of plasma low-density lipoproteins (LDL) in vitro (*7*). The oxidation of LDL is known to play an important role in the initiation and progression of arteriosclerosis (*8*, *9*).

In this study, hypercholesterolemia rats were fed a diet containing one of two natto fractions, either low-molecular-weight viscous substance (LMWVS) or soybean water extract (SWE), which were found to have a strong inhibitory effect on LDL oxidation in vitro (*7*). To investigate the inhibitory effect of the natto fractions on LDL oxidation in vivo, lipid peroxidation in plasma and LDL was measured after oxidation of plasma from the natto-fraction-fed rats. Furthermore, the physiological functions of the natto fractions on lipid peroxidation in tissues and lipid metabolism in plasma were examined.

MATERIALS AND METHODS

Preparation of Natto Fractions and Diets. The natto water-soluble fractions (LMWVS and SWE) were isolated from commercial natto (Taishi Foods Co., Ltd., Towada, Japan) as described previously (*7*). LMWVS, which had a molecular weight of under 100 000, was separated from the viscous fraction of natto by an extra filtration, and SWE was extracted from the beans fraction of natto with water. Finally, these fractions were prepared by freeze-drying. The diet compositions are shown in **Table 1**. The basal diet contained 1% cholesterol (CHO), AIN-96VX as a vitamin mixture, and AIN-93G-MX as a mineral mixture (*10*). The LMWVS and SWE diets were prepared by the addition of 9% natto fraction to the basal diet, respectively. LMWVS had 60% protein and 40% carbohydrate contents, and SWE contained 55.5% protein and 44% carbohydrate. Therefore, the amounts of casein and starch in the experimental diets were adjusted on the basis of these contents.

Animals. Male Wistar rats, 4 weeks old, were purchased from Clea Japan Inc., (Tokyo, Japan). After acclimatization for 1 week using commercial diet (MF, Oriental Yeast Co. Ltd., Tokyo, Japan), the animals were divided into three groups of six rats each (control, LMWVS, and SWE groups). The rats were housed individually in stainless wire netting cages for 3 weeks. All rats were given free access to the experimental diet and tap water, and their body weights were monitored daily.

After an experimental period of 3 weeks, the rats were fasted for 20 h, and blood was collected from the abdominal artery under ether

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Table 1. Compositions of the Diets (%)

composition	control	LMWVS	SWE
casein[a]	20.0	14.6	15.0
α-corn starch[a]	13.0	12.1	12.0
β-corn starch[a]	39.0	36.3	36.0
sucrose	10.0	10.0	10.0
cellulose	5.0	5.0	5.0
corn oil	7.0	7.0	7.0
vitamin mixture[b]	1.0	1.0	1.0
mineral mixture[b]	3.5	3.5	3.5
choline bitatrate	0.25	0.25	0.25
sodium cholate	0.25	0.25	0.25
cholesterol	1.0	1.0	1.0
LMWVS	0	9.0	0
SWE	0	0	9.0

[a] Casein and starch were adjusted by the contents of proteins and carbohydrates in LMWVS and SWE. [b] AIN-96-VX as a vitamin mixture and AIN-93G-MX as a mineral mixture were obtained from Oriental Yeast Co.

anesthesia. The heparinized blood was immediately separated into plasma and red blood cells (RBC) by centrifugation at 3000 rpm for 10 min. The plasma samples for oxidation were stored on ice until oxidation, and the other plasma samples were frozen at −70 °C. After blood collection, physiological saline was perfused, and then the liver and aorta were removed, weighed, and stored at −70 °C.

Oxidation of Plasma. Rat plasma was oxidized as described by Kontush et al. (*11*). Briefly, 30 mM copper(II) sulfate ($CuSO_4$) solution was added to 2.2 mL of plasma and the mixture was oxidized for 4 h in a water bath at 37 °C. The concentration ratio of CHO and $CuSO_4$ in the plasma was adjusted constantly with 0.15 M sodium chloride. At 0, 2, 3, and 4 h after addition of $CuSO_4$, 0.8 mL of mixture was taken, and oxidation was stopped by addition of 24 mM ethylenediaminetetraacetic acid. The oxidized plasma was used for the isolation of LDL and determination of lipid peroxidation.

Isolation of LDL. After dilution of each oxidized plasma sample and adjustment of its specific gravity, LDL was isolated by ultracentrifugation according to the method of Havel et al. (*12*).

Lipid Analysis. Total cholesterol (T-CHO), free cholesterol (F-CHO), high-density lipoprotein-cholesterol (HDL-CHO), LDL-cholesterol (LDL-CHO), triglyceride (TG), phospholipid (PL), and nonesterified fatty acid (NEFA) in plasma were measured.

T-CHO and TG were measured automatically using a Fuji Dri-Chem FDC3000V (Fuji Medical System Co., Tokyo, Japan). F-CHO and LDL-CHO were measured using a Free Cholesterol C-Test (*13*), HDL-CHO was measured using a HDL-Cholesterol Test (*14*), PL was measured using a Phospholipid Test (*15*), and NEFA was measured using a NEFA Test (*16*). These measuring kits were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Measurement of Lipid Peroxidation. Thiobarbituric acid reactive substances (TBARS) in plasma and LDL were determined as a measurement of lipid peroxidation by the fluorescent method (*17*).

TBARS in RBC was measured as described by Ihm et al. (*18*). Briefly, 0.8 mL of 20 mM phosphate buffered saline (pH 7.4) and 25 μL of 8.8 mg/mL butylated hydroxytoluene solution were added to 0.2 mL of RBC, and 0.5 mL of 30% trichloroacetic acid solution was added and mixed. The supernatant was separated from the mixture by centrifugation at 3000 rpm for 15 min after holding the mixture on ice for 2 h. A total of 75 μL of 0.1 M ethylenediaminetetraacetic acid and 0.4 mL of 1% TBA reagent, dissolved in 0.05 N sodium hydroxide solution, were then added to the supernatant, and the mixture was boiled at 95 °C for 15 min. After the solution cooled, the absorbance was measured at 532 nm (A_{532}) and 600 nm (A_{600}). TBARS was calculated from $[A_{532} - A_{600}]$, referred to tetraethoxypropane standard.

TBARS in the liver and aorta were measured as described by Kikugawa et al. (*19*). Briefly, 100 mg of each tissue was weighed and homogenized in 1.0 mL of 1.15% potassium chloride (KCl) solution. A total of 100 μL of 8.1% sodium dodecyl sulfate solution and 0.75 mL acetate buffer (pH 3.5) were added to 50 μL of homogenate and mixed. Then 25 μL of 0.8% butylated hydroxytoluene solution, 0.75 mL of 1% TBA reagent, and 0.35 mL of distilled water were added, and the resulting solution was mixed and stored at 5 °C for 1 h. The mixture was then boiled at 95 °C for 1 h, and 0.5 mL of distilled water and 3.0 mL of *n*-butanol were added to the cooled mixture. After shaking extraction for 10 min, the fluorescent intensity (Ex 515 nm and Em 553 nm) of the *n*-butanol layer, separated from the mixture by centrifugation at 3000 rpm for 10 min, was measured. TBARS was calculated from the fluorescent intensity by standardization against tetraethoxypropane.

Measurement of Protein Concentration. The protein concentration in each sample was measured using a BCA Protein Assay Kit (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard (*20*).

Measurement of Antioxidant Enzymatic Activity. The enzymatic activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), and the concentration of reduced glutathione (GSH) in plasma, RBC, and liver were also measured.

Measurement of SOD Activity. SOD activity was measured with a kit of BIOXYTECH SOD-525 Spectrophotometric Assay for Superoxide Dismutase (OXIS International Inc., Portland, OR) with an iEMF reader (Dainippon Pharmaceutical Co., Tokyo, Japan) and 96-well microplates (*21*). For measurement of Mn-SOD activity, plasma was diluted with 1.15% KCl solution, RBC was hemolyzed with 5 vol of distilled water, and liver sample supernatant was prepared from the hepatic homogenate in 1.15% KCl solution by centrifugation at 10 000 rpm for 10 min. To prepare samples for measurement of Cu/Zn-SOD activity, a water extract layer was obtained from a mixture of each sample and 1.6 vol of ethanol/chloroform (62.5:37.5).

Measurement of Catalase Activity. Catalase activity was measured by a method based on reduction of hydrogen peroxide (*22*). In a quartz cuvette, 0.5 mL of sample was added to 2.0 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 10 mM hydrogen peroxide, and the absorbance change at 240 nm was immediately recorded for 1 min. The samples used in this assay were the same as those used for the measurement of Mn-SOD activity, and were diluted with 50 mM potassium phosphate buffer (pH 7.0). Catalase activity was calculated from the slope of absorbance of the sample (SL) and of the buffer as a blank (SL_b) using the following equation:

$$\text{catalase activity (U/mL)} = \left(\frac{SL - SL_b}{0.0436}\right)\left(\frac{2.5}{0.5}\right)$$

Measurement of GSH-Px Activity. GSH-Px activity was measured with a kit of BIOXYTECH GPx-340 Colorimetric Assay for Cellular Glutathione Peroxidase (OXIS International Inc., Portland, OR) with 96-well microplates and an iEMF reader (*23*). Plasma sample was prepared by dilution with 50 mM Tris−HCl buffer (pH 7.5) containing 5 mM ethylenediaminetetraacetic acid and 1 mM 2-mercaptoethanol, RBC sample was prepared by hemolysis with distilled water, and liver sample supernatant was prepared from the hepatic homogenate in Tris−HCl buffer by centrifugation at 10 000 rpm for 10 min.

Measurement of GSH Concentration. GSH concentration was measured with a kit of BIOXYTECH GSH-400 Colorimetric Assay for Glutathione (OXIS) with 96-well microplates and an iEMF reader. Plasma and RBC samples were prepared by dilution with 5% metaphosphoric acid solution, and liver sample supernatant was prepared from the hepatic homogenates in 5% metaphosphoric acid by centrifugation at 10 000 rpm for 10 min.

Statistical Analysis. Data were obtained as the mean ± standard deviation (SD), and were analyzed by the Scheffe test after a one-way analysis of variance (ANOVA) using the Stat View System (SAS Institute Inc., Cary, NC). A significant difference in the mean values was assumed as $P < 0.05$.

RESULTS AND DISCUSSION

Figure 1 shows the change in body weight of rats fed natto fraction diets for 3 weeks. All three groups (control, LMWVS, and SWE) showed a good change in body weight, and there was no significant difference between the groups. As the body weight curve was usually a straight line, it is suggested that no abnormality in growth had occurred.

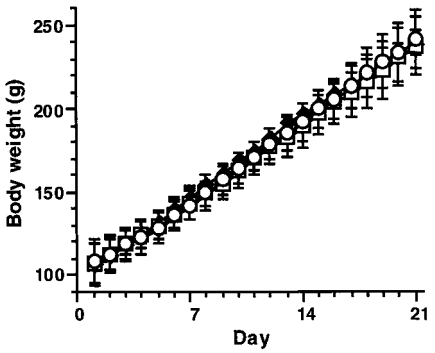

Figure 1. Changes in body weight of male Wistar rats fed natto fraction diets for 3 weeks: ◆, control group; ○, LMWVS group; □, SWE group. Data represent the mean ± SD of six rats.

Table 2. Final Body Weight after Fasting, Body Weight Gain, Liver Weight, and Liver Weight Ratio Per Body Weight of Male Wistar Rats Fed Natto Fraction Diets for 3 Weeks[a]

	units	control	LMWVS	SWE
fasted body weight	(g)	220.0 ± 6.8	215.6 ± 17.4	213.9 ± 15.6
total body weight gain	(g)	132.6 ± 13.4	133.7 ± 21.7	130.6 ± 18.9
daily body weight gain	(g/day)	6.63 ± 0.67	6.68 ± 1.09	6.53 ± 0.94
liver weight	(g)	12.61 ± 0.51	12.68 ± 1.14	12.30 ± 1.10
liver/body weight ratio	(g/100 g)	5.73 ± 0.16	5.88 ± 0.25	5.75 ± 0.30

[a] Rats were fasted for 20 h after experimental period for 3 weeks. Data represent the mean ± SD of six rats.

Table 3. Lipid Concentrations in Plasma of Male Wistar Rats Fed Natto Fraction Diets for 3 Weeks[a]

lipid	units	control	LMWVS	SWE
T-CHO	(mg/mL)	1.563 ± 0.322	1.153 ± 0.270	1.167 ± 0.434
F-CHO	(mg/mL)	0.183 ± 0.058	0.159 ± 0.042	0.159 ± 0.042
HDL-CHO	(mg/mL)	0.105 ± 0.016	0.111 ± 0.036	0.100 ± 0.010
LDL-CHO	(mg/mL)	1.360 ± 0.312	0.977 ± 0.287	0.996 ± 0.416
TG	(mg/mL)	0.493 ± 0.080	0.327 ± 0.074[c]	0.353 ± 0.105[c]
PL	(mg/mL)	0.616 ± 0.125	0.630 ± 0.158	0.556 ± 0.093
NEFA	(mEq/L[b])	0.671 ± 0.051	0.689 ± 0.099	0.683 ± 0.082
F-CHO/T-CHO	(%)	11.86 ± 3.55	13.77 ± 1.23	15.15 ± 9.41

[a] Data represent the mean ± SD of six rats. [b] Palmitic acid equivalent. [c] Significant difference from the control group at $P < 0.05$.

The total body weight gain during the experimental period, daily body weight gain, final body weight after fasting for 20 h, liver weight, and the liver weight ratio per body weight are shown in **Table 2**. No significant differences in these parameters were found between the three groups. Compared with rats fed a normal diet adding no CHO in a preliminary 3 week experiment, the final body weight and total body weight gain of all rats in the three groups increased. These results suggest that the addition of CHO to the diet increased the body weight, while natto fractions had no influence on the growth of rats.

Plasma concentrations of T-CHO, F-CHO, HDL-CHO, LDL-CHO, TG, NEFA, and PL, and the concentration ratio of F-CHO/T-CHO in rats fed natto fraction diets are shown in **Table 3**. The LMWVS and SWE groups showed a tendency toward lower T-CHO, F-CHO, and LDL-CHO concentrations than the control group. There was no significant difference in HDL-CHO, NEFA, and PL among the three groups. TG of both natto fraction diet groups was significantly lower than that of the control group. It was expected that most of the reduction of CHO was of the esterified type in the natto fraction groups, as

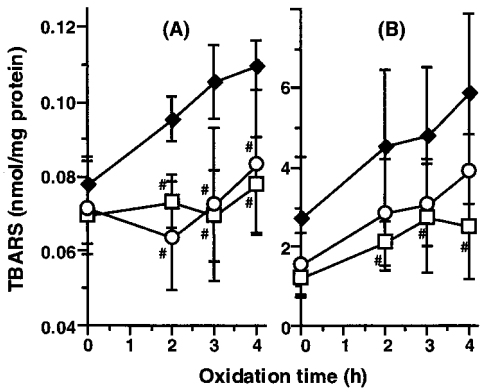

Figure 2. Time-course changes of TBARS in plasma (A) and LDL (B) from $CuSO_4$-oxidized plasma of male Wistar rats fed natto fraction diets for 3 weeks ex vivo: ◆, control group; ○, LMWVS group; □, SWE group. Plasma was oxidized with $CuSO_4$, and then LDL was separated from the oxidized plasma. Data represent the mean ± SD of six rats. #Significant difference from the control group at $P < 0.05$.

the LMWVS and SWE groups showed a higher ratio of F-CHO/T-CHO than the control group. Moreover, T-CHO and TG were higher in the control group than in rats fed the normal diet adding no CHO in our preliminary experiment (T-CHO, 0.665 ± 0.132 mg/mL; TG, 0.329 ± 0.078 mg/mL; $n = 6$). These results suggest that although the addition of CHO to the diet increased lipids, ingestion of the natto fractions appeared to decrease CHO and TG. In particular, the reduction of CHO in the natto fraction groups was reflected by lower LDL-CHO levels. It cannot be denied that the natto fractions suppress the intestinal absorption of lipids. However, there was no difference of body weights and liver weights between control and natto fraction groups. Moreover, in the natto fraction groups plasma T-CHO, LDL-CHO, and TG levels were lower than those in the control group, but HDL-CHO levels were similar to that of control group. This phenomenon suggests that the natto fractions not only suppressed absorption of lipids, but also influenced the distribution of cholesterol. As these lipids are not only dangerous factors in arteriosclerosis (*7*), but also cause overweight, natto fractions may have a useful effect on lipid metabolism.

Figure 2 shows the changes of TBARS in plasma and LDL, separated from oxidized plasma in rats fed natto fraction diets for 3 weeks, at 0, 2, 3, and 4 h during oxidation with $CuSO_4$. TBARS in plasma from LMWVS and SWE groups was significantly lower than that in the control group at 2 and 4 h oxidation, and showed gently increasing curves. Although the LMWVS group showed a tendency toward lower TBARS in LDL, it showed the increasing slope of TBARS similar to that of the control group. On the other hand, the SWE group showed significantly lower TBARS and gentler slope in LDL than the control group. SWE showed a stronger effect than LMWVS, and the order of effects does not agree with their inhibitory effect on oxidation of LDL by $CuSO_4$ and azo pigment in vitro (*7*). However, these results demonstrate that the ingestion of natto fractions could inhibit oxidation of plasma and LDL by $CuSO_4$ ex vivo, confirming the in vitro findings. Moreover, the results ex vivo suggest that the natto fractions were absorbed in the body.

The plasma, RBC, hepatic, and aortic concentrations of TBARS in rats fed the natto fraction diets for 3 weeks are shown in **Table 4**. The TBARS in these tissues was lower in both natto fraction groups than in the control group. In particular, plasma TBARS in the SWE group was significantly lower than that in

Table 4. TBARS in Plasma, RBC, Liver, and Aorta of Male Wistar Rats Fed Natto Fraction Diets for 3 Weeks[a]

	nmol/mg protein		
tissue	control	LMWVS	SWE
plasma	0.069 ± 0.005	0.056 ± 0.013	0.049 ± 0.005[b]
RBC	19.00 ± 12.80	12.62 ± 3.88	12.71 ± 3.93
liver	0.207 ± 0.076	0.158 ± 0.019	0.138 ± 0.024
aorta	0.230 ± 0.078	0.204 ± 0.062	0.151 ± 0.058

[a] Data represent the mean ± SD of six rats. [b] Significant difference from the control group at $P < 0.05$.

Table 5. Antioxidant Enzymatic Activities in Plasma, RBC, and Liver of Male Wistar Rats Fed Natto Fraction Diets for 3 Weeks[a]

tissue	enzyme	control	LMWVS	SWE
plasma	Mn-SOD (U/mg protein)	1.295 ± 0.421	1.797 ± 0.219[b]	1.563 ± 0.146
	Cu/Zn-SOD (U/mg protein)	1.931 ± 0.290	2.266 ± 0.457	2.609 ± 0.742
	catalase (U/mg protein)	0.534 ± 0.209	0.576 ± 0.195	0.647 ± 0.224
	GSH-Px (mU/mg protein)	36.49 ± 5.74	43.29 ± 5.11	45.41 ± 4.22[b]
	GSH (nmol/mg protein)	28.16 ± 4.09	31.14 ± 6.66	27.55 ± 2.74
RBC	Mn-SOD (U/mg protein)	4.948 ± 0.777	5.547 ± 0.814	5.033 ± 0.814
	Cu/Zn-SOD (U/mg protein)	9.491 ± 2.657	10.65 ± 2.94	12.58 ± 3.45
	catalase (U/mg protein)	12.38 ± 2.12	11.34 ± 2.92	8.783 ± 4.163
	GSH-Px (U/mg protein)	0.156 ± 0.027	0.193 ± 0.040	0.188 ± 0.044
	GSH (nmol/mg protein)	166.0 ± 20.1	169.8 ± 23.6	167.3 ± 20.2
liver	Mn-SOD (U/mg protein)	12.83 ± 2.47	17.05 ± 1.67[b]	15.52 ± 0.71
	Cu/Zn-SOD (U/mg protein)	110.5 ± 20.8	122.7 ± 31.6	112.9 ± 36.1
	catalase (U/mg protein)	54.46 ± 11.35	62.96 ± 13.94	59.43 ± 10.98
	GSH-Px (U/mg protein)	0.465 ± 0.086	0.516 ± 0.138	0.498 ± 0.04
	GSH (nmol/mg protein)	206.6 ± 49.9	249.5 ± 64.3	290.1 ± 71.9

[a] Data represent the mean ± SD of six rats. [b] Significant difference from the control group at $P < 0.05$.

the control group. Moreover, hepatic TBARS per tissue weight in the SWE group, and aortic TBARS per tissue weight in both natto fraction diet groups was significantly lower than in the control group (data not shown). These results demonstrate the inhibition of the natto fractions on lipid peroxidation in plasma, RBC, liver, and aorta, and show that the effect of SWE was stronger than that of LMWVS. The antioxidant effect in the body was of similar order to the inhibitory effect on the oxidation of plasma and LDL by $CuSO_4$ ex vivo. In this study, although the rats were given CHO as a substrate for oxidation, they were not subjected to oxidative stress. Therefore, natto fractions were suggested to have depressed oxidation and prevented absorption and accumulation of lipid peroxides in the body. Furthermore, it is suggested that the difference in the order of inhibitory effect of natto fractions on oxidation between in vitro (*7*) and ex vivo was caused by a difference in their active ingredient, absorption, and metabolism.

To investigate the mechanism of action of the natto fractions in the body, the antioxidant enzymatic activities of Mn-SOD, Cu/Zn-SOD, catalase, and GSH-Px, and concentration of GSH in plasma, RBC, and liver were determined (**Table 5**). Plasma GSH-Px activity in the SWE group was significantly higher than that in the control group. RBC and hepatic GSH-Px activities were also higher in both natto fraction groups than in the control. No significant differences in other enzymatic activities were observed between the three groups. In addition, no significant difference in GSH concentration, a substrate of GSH-Px, was observed among the three groups. SOD and GSH-Px activities are reported to be decreased by hypolipidemic drugs such as clofibrate (*24*). Although a decrease in plasma TG and T-CHO was observed in rats fed the natto fractions, no decrease in antioxidant enzymatic activity was observed in our study. Because these antioxidant enzymes have an important role of protecting organelles from oxidative injury, the activities of the enzymes were compared with the concentrations of TBARS in tissues. No statistical correlation was found between enzymatic activity and TBARS concentration. In this study, no fluctuation in activity of specific antioxidant enzyme was observed as the rats had not received an oxidative load. Therefore, the lack of a relationship between reduced TBARS and antioxidant enzymatic activity in tissues demonstrates that the absorbed natto fractions, which have radical scavenging activity (*7*), directly affects oxidation in the body; ingestion of natto fractions for 3 weeks did not induce antioxidant enzymes.

It is well-known that isoflavones, which are contained in soybeans, have an inhibitory effect on oxidation of LDL (*25*, *26*). The antioxidant activity of fermented natto was stronger than that of steamed soybeans, although there was no difference in the tocopherol and isoflavone content of natto and steamed soybeans (*27*, *28*). Moreover, Yokota et al. (*29*) reported that an antioxidant-containing crude fraction from natto lowered serum TBARS, T-CHO, LDL-CHO, and TG, and depressed the incidence of atherosclerotic lesions in CHO-fed rabbits. The antioxidant activity of the crude fractions, extracted from natto with aqueous methanol (*27*, *28*) or ethanol (*29*), was investigated, but no antioxidant ingredients were identified in either fraction. On the other hand, although Toda et al. (*30*) has reported that 6''-*O*-succinyl-daidzin and 6''-*O*-succinyl-genistin increase by fermentation, they used aqueous methanol for extracting isoflavones. In our study, natto water-soluble fractions were found to have an inhibitory effect on oxidation of LDL, not only in vitro (*7*) but also ex vivo. Moreover, the observation that natto fractions decreased TBARS in tissues, plasma T-CHO, and TG in CHO-fed rats agrees with the findings of Yokota et al. (*29*). It was considered that the natto water-soluble fractions contained very little isoflavone aglycon, which had strong antioxidant activity, because our crude fractions were prepared with water only as the solvent.

It is clear that soybean proteins inhibit lipid peroxidation in plasma and LDL (*31*). The active ingredients in this process are likely to be proteins, peptides, and amino-carbonyl reactive substances in LMWVS and SWE (*7*), as these fractions had stronger antioxidant activity than other fractions with the time after production of natto (*5*). Isolation and identification of these antioxidant ingredients is now in progress. In this study, natto water-soluble fractions appeared to inhibit oxidation in plasma, LDL, and tissues, and it is suggested that the absorbed ingredients had direct antioxidative action, because induction of antioxidant enzymes was not observed in rats fed natto fractions for 3 weeks. Furthermore, as the natto fractions had no adverse effect on the growth of rats and also appeared to improve lipid metabolism by decreasing CHO and TG, it is suggested that these natto fractions might help to prevent atherosclerosis.

ABBREVIATIONS USED

LMWVS, low-molecular-weight viscous substance; SWE, soybean water extract; LDL, low-density lipoprotein; TBARS, thiobarbituric acid reactive substance; RBC, red blood cell; CHO, cholesterol; TG, triglyceride; HDL-CHO, high-density lipoprotein cholesterol; T-CHO, total cholesterol; F-CHO, free cholesterol; LDL-CHO, low-density lipoprotein cholesterol; NEFA, nonesterified fatty acid; PL, phospholipid; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GSH, reduced glutathione.

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