JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Intake of Soy Protein Isolate Alters Hepatic Gene Expression in Rats

NOBUHIKO TACHIBANA,*,† ICHIRO MATSUMOTO,§ KENSUKE FUKUI,† SOICHI ARAI,# HISANORI KATO,§ KEIKO ABE,§ AND KIYOHARU TAKAMATSU†

Food Science Research Institute, Fuji Oil Company, Ltd., 1 Sumiyoshi-cho, Izumisano City, Osaka 598-8540, Japan; Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan; and Department of Nutritional Science, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-0054, Japan

Soy protein isolate (SPI) can elicit various physiological effects such as cholesterol lowering and antiobesity effects. To examine whether hepatic gene expression is altered by SPI intake, rats were fed an SPI or casein diet for 8 weeks. After 8 weeks of feeding, liver weight and plasma triglyceride and cholesterol levels were significantly lower in the SPI group than in the casein group. Hepatic gene expression was investigated using DNA microarrays. The expression profiles and statistical analysis showed clear and significant differences between the SPI and casein groups ($p < 0.05$); in the SPI group, 63 genes were up-regulated and 57 genes were down-regulated, most involved in various physiological functions such as lipid metabolism, antioxidant activity, transcriptional regulation, and energy metabolism. Especially in lipid metabolism, the down-regulated genes are related to fatty acid synthesis and the up-regulated genes are related to cholesterol synthesis and steroid catabolism. These results suggest that SPI intake could maintain homeostasis primarily by modulating lipid and energy metabolism.

KEYWORDS: Soy protein; rat; DNA microarray; lipid metabolism; cholesterol

INTRODUCTION

Soybeans contain abundant storage proteins and play an important role as a protein source. The nutritional and physiological effects of a soybean protein isolate (SPI) diet have long been studied, and it has been reported that an SPI diet brings various health benefits such as a cholesterol-lowering effect (*1*−*5*), reduction of plasma triglyceride levels (*6*, *7*), antiobesity effect (*8*), antiatherosclerosis effect (*9*), and anticarcinogenesis effect (*10*, *11*). These physiological effects of an SPI diet can result in alteration of the expression levels of several genes (*6*, *12*). Iqbal et al. (*13*) used differential display of mRNA analysis and reported the influences of soy protein including the difference of isoflavone content in genetically obese rats. However, no detailed and comprehensive information about genes in normal rats is available.

DNA microarray experiments provide data on total gene expression (*14*, *15*). Endo et al. (*16*) reported that various dietary protein sources resulted in the differential of expression about 281 genes in rat liver, suggesting a nutritional function of protein components. Lee et al. (*17*) reported that calorie restriction retards the aging process by causing a metabolic shift revealed by using high-density oligonucleotide arrays. Such nutrigenomics to investigate the effects elicited by the intake of various foods or their components can open new frontiers in nutritional and food sciences because physiological effects can be described in terms of gene expression profiles (*18*−*20*).

In this study, we used DNA microarrays to investigate the effects of feeding rate of an SPI diet for 8 weeks in normal Sprague−Dawley rats and found the differential hepatic expression of many genes, especially those involved in lipid metabolism.

MATERIALS AND METHODS

Animals and Diets. Twelve specific-pathogen-free male Sprague−Dawley rats (SD rats), aged 5 weeks, were purchased from Japan SLC Co. (Hamamatsu, Japan). Vitamin-free casein (casein; Oriental Yeast Co., Tokyo, Japan) and soy protein isolate (SPI; Fujipro, Fuji Oil Co., Osaka, Japan) were provided as dietary proteins. Crude protein content was measured according to the Kjeldahl method with a Kjeltec Auto 1030 analyzer (Nippon General Trading Co., Tokyo, Japan). Experimental diets were based on the AIN-93G formula (*21*) and contained 20% protein supplied by SPI or casein (**Table 1**).

Nutritional Conditions. All animals were treated in accordance with the guidelines established by the Japanese Society of Nutrition and Food Science (Law 105 and Notification 6 of the Japanese government). All rats were housed individually in stainless steel cages under controlled circumstances (temperature = 23 ± 1 °C, humidity = 55 ± 5%, light from 7:00 a.m. to 7:00 p.m.). After acclimation on commercial chow (CRF-1, Oriental Yeast Co., Tokyo, Japan) for 5 days, the rats were divided into two groups with similar average body weights. The experimental diets and water were given ad libitum for 8 weeks. Food intake was recorded daily, and body weight was measured twice a week.

* Author to whom correspondence should be addressed (telephone +81-724-63-1830; fax +81-724-61-1356; e-mail 010110@so.fujioil.co.jp).
† Fuji Oil Co., Ltd.
§ The University of Tokyo.
# Tokyo University of Agriculture.

10.1021/jf0481103 CCC: $30.25
Published on Web 04/16/2005

Table 1. Composition of Experimental Diets (g/100g Diet)

	group	
	casein	SPI
casein[a]	22.4	
SPI[b]		23.2
corn starch	37.65	36.85
dextrinized corn starch	13.2	13.2
sucrose	10.0	10.0
soybean oil[c]	7.0	7.0
cellulose powder	5.0	5.0
mineral mixture[d]	3.5	3.5
vitamin mixture[e]	1.0	1.0
choline bitartrate	0.25	0.25
total	100.0	100.0

[a] Crude protein/as is 89.1%. Oriental Yeast Co., Tokyo, Japan. [b] Crude protein/as is 86.2%. Fuji Oil Co., Osaka, Japan. [c] Soybean oil contains 0.02% *tert*-buthylhydroxyquinone. [d] AIN-93G mixtures, Oriental Yeast Co., Tokyo, Japan. [e] AIN-93 mixtures, Oriental Yeast Co., Tokyo, Japan.

Biochemical Profiles in Plasma and Liver. After 8 weeks of feeding, the rats were fasted for 6 h (from 8:00 a.m. to 2:00 p.m.) and anesthetized with sodium pentobarbital [15 μmol/100 g of body weight (BW)]. Blood samples were withdrawn from the abdominal aorta into heparinized syringes. Livers were excised, frozen immediately, and kept at −80 °C until analyzed. Plasma fractions were separated by centrifugation at 1900*g* for 15 min at 5 °C and frozen at −80 °C until analyzed. Plasma glucose, triglycerides, and total cholesterol were measured enzymatically using a Dry Chem 5500 analyzer (Fuji Film Co., Tokyo, Japan). Liver lipids were extracted according to the method of Folch et al. (*22*) and analyzed for cholesterol, triglyceride, and phosphorus according to the methods of Sperry and Webb (*23*), Fletcher (*24*), and Feldman and Feldman (*25*), respectively. Differences in the measurements were analyzed by Student's *t* test using standard software (SPSS 10.0J for Windows, SPSS Japan Inc., Tokyo, Japan).

DNA Microarray Procedure. We selected three average rats from each group as determined by plasma total cholesterol and plasma triglyceride levels. Total hepatic RNA was extracted from each of the selected rats using ISOGEN (Nippon Gene Co. Ltd., Toyama, Japan) and purified with an RNeasy mini kit (Qiagen K.K. Chuo-ku, Tokyo, Japan). The quality and quantity of the total RNA were checked by agarose gel electrophoresis and by spectrophotometry. Microarray analysis was performed according to the manufacturer's instructions (Affymetrix, Santa Clara, CA) as described previously (*26*). Briefly, 5 μg of purified total RNA was used to synthesize double-stranded cDNA. Biotinylated cRNA was transcribed from the cDNA by T7 RNA polymerase, fragmented, and added to an Affymetrix Rat Genome U34A array (contains probes for over 8000 rat genes) as described in the Expression Analysis Technical Manual (Affymetrix). After hybridization at 45 °C for 16 h, the array was washed and labeled with phycoerythrin, and the fluorescent signals were scanned using the Affymetrix GeneChip System. The scanning data were analyzed by Affymetrix software (Microarray Suite 5.0). All data obtained were exported as text files or Microsoft Excel files. After a set of 59 probesets with the AFFX index used for quality control was removed, the remaining data were linearly normalized with the signal value of the β-actin gene (accession no. V01217) to correct for minor differences in the amounts of each cRNA applied to the microarrays.

Data Analysis. Genes were classified into 16 groups according to their combination of detection calls, and their data were treated by Affymetrix Statistical Algorithms to find differences between SPI- and casein-fed rats. With the detection calls "P", "A", and "M", meaning "present", "absent", and "marginal", respectively, the data for the detection calls of the genes of three rats were described as three signature combinations, PPP, PPX, PXX, and XXX, in which X represents either "A" or "M" (**Table 4**). The changes in the expression of 2905 genes expressed in all rats fed the SPI and casein diets were statistically analyzed by Student's *t* test at $p < 0.05$.

Table 2. Growth Parameters of Rats Fed Experimental Diets for 8 Weeks

	group	
	casein	SPI
initial body wt[a] (g)	166.8 ± 0.8	166.8 ± 0.8
final body wt[a] (g)	456.2 ± 15.4	421.6 ± 8.7
food intake[a] (g/day)	21.7 ± 0.7	20.3 ± 0.3
relative liver wt[b] (g/100 g of BW)	3.56 ± 0.15	2.87 ± 0.06*[c]

[a] Values are means ± SEM, $n = 6$. [b] Values are means ± SEM, $n = 4$. [c] *, significantly different at $p < 0.05$ from casein group by Student's *t* test.

Table 3. Plasma Chemistries of Rats Fed Experimental Diets for 8 Weeks[a]

	group	
	casein	SPI
glucose (mmol/L)	14.2 ± 1.0	14.1 ± 0.4
triglyceride (mmol/L)	2.12 ± 0.24	1.18 ± 0.16*[b]
cholesterol (mmol/L)	3.15 ± 0.23	1.74 ± 0.07*

[a] Values are means ± SEM; $n = 6$ for casein; $n = 5$ for SPI. [b] *, significantly different at $p < 0.05$ from casein group by Student's *t* test.

Table 4. Classification of DNA Microarray Gene Expression (Total Genes = 8740)[a]

		SPI			
		PPP	**PP**X	**P**XX	XXX
casein	PPP	2905	232	60	9 (**3**[b])
	PPX	92	131	92	43
	PXX	27	81	128	162
	XXX	5 (**2**[a])	38	192	4543 (**4280**[c])

[a] Gene expression levels represented by the signatures "P", "M", and "A", as determined by the Affymetrix Expression Algorithm. "X" represents M or A. P, present expression level; M; marginal expression level; A; absent expression level. [b] Genes classified as AAA in either group. [c] Genes classified as AAA in both groups.

RESULTS

Growth and Blood Lipid Concentration. All rats consumed almost the same amounts of diets throughout the feeding period, but the final body weights of rats fed the SPI diet (SPI-rats) were slightly lower than those fed the casein diet (casein-rats) as shown in **Table 2**. In addition, relative liver weight was significantly lower in the SPI-rats than in the casein-rats. Concentrations of cholesterol and triglycerides in the blood of the SPI-rats were significantly lower than in the casein-rats, indicating that SPI intake has a plasma lipid-lowering effect (**Table 3**). On the other hand, plasma glucose levels were not significantly different between the SPI- and casein-rats.

Three rats from each dietary group, whose plasma lipid concentrations approximated the mean values of the six rats fed the respective diet, were selected for further biochemical and molecular biological analyses including DNA microarray analysis as described below. The levels of cholesterol and triglycerides stored in the livers of the three SPI-rats were moderately lower than those in the three casein-rats, and the level of liver phospholipids in the three SPI-rats was significantly higher than in the casein-rats (data not shown). These results indicate that long-term intake of SPI has an effect, compared the casein diet, on lipid metabolism, especially in the liver.

Differentially Expressed Genes in the Livers of Rats. To investigate the nutritional influence of SPI intake in detail, DNA microarray analysis was carried out, and the data on gene

Table 5. Functional Classification of Genes for Which Expressions Were Significantly Different in the Livers of Rats Fed the SPI Diet for 8 Weeks[a]

function[b]	no. of genes (>1.5-fold) increased	decreased
amino acid metabolism	4 (1)	10 (2)
antioxidant	9 (6)	2 (0)
cell growth and/or maintenance	6 (2)	11 (3)
energy metabolism	4 (1)	7 (2)
fatty acid metabolism	0	9 (7)
immunity	3 (2)	0
signal transduction	7 (0)	5 (1)*[c]
steroid metabolism	12 (10)*	0
structural molecule	0	4(0)
transcriptional regulator	4 (0)	4 (0)
others	12 (1)*	2 (0)**
total	**61 (23)****	**54 (15)*****

[a] Number of genes for which mRNA levels were significantly different ($p < 0.05$) by Student's *t* test between the casein and SPI group. [b] Genes tentatively categorized according to their physiological functions. [c] *, **, and ***, one, two, and three genes, respectively, represent specifically expressed in one experimental group.

expression in livers of both SPI- and casein-rats ($n = 3$) were individually obtained. The correlation coefficients showing the overall similarity of gene expression between any two individuals in the same dietary group were very high (0.973−0.986 and 0.986−0.992 among SPI- and casein-rats, respectively). Next, differences in gene expression between the two dietary groups were identified. Data on gene expression were classified into 16 groups according to the detection call by Affymetrix software Microarray Suite (**Table 4**). Genes showing reproducible results in both dietary groups, a total of 4280, were further analyzed. Statistical analysis by Student's *t* test of 2905 genes expressed in all rats in both dietary groups articulated 115 genes showing significant differences in expression levels (**Figure 1**). Among these 115 genes, 61 were significantly up-regulated and 54 were significantly down-regulated in SPI-rats as compared with casein-rats. The expressions of one-third of the 115 genes changed at least 1.5-fold. The presence of 2 genes in SPI-rats and 3 genes in casein-rats was considered to demonstrate biologically significant differences (**Table 4**). Consequently, 120 genes were identified as showing significant alteration of

Table 6. Genes for Which Expressions Showed Differences >1.5-Fold

fold ± SEM	gene name	functions	accession no.	UniGene-ID
3.08 ± 0.20	cysteine sulfinic acid decarboxylase	amino acid metabolism	M64755	Rn.43232
3.52 ± 0.49	cytochrome P-450e	antioxidant	M13234	Rn.91353
3.20 ± 0.38	metallothionein-II	antioxidant	AI176456	Rn.64596
2.53 ± 0.22	metallothionein-2 and metallothionein-1	antioxidant	M11794	Rn.54397
1.82 ± 0.15	glutathione *S*-transferase Ya subunit	antioxidant	K00136	Rn.40574
1.61 ± 0.13	epoxide hydrolase 1	antioxidant	M26125	Rn.3603
1.60 ± 0.09	glutathione *S*-transferase Yb subunit	antioxidant	J02592	Rn.625
1.69 ± 0.13	PRG1	cell growth and/or maintenance	X96437	
1.52 ± 0.08	deoxyribonuclease KIAA0218	cell growth and/or maintenance	AA799581	Rn.6207
2.10 ± 0.13	carbonic anhydrase III (CA3)	energy metabolism	AF037072	Rn.1647
1.86 ± 0.08	macrophage migration inhibitory factor	immunity	S73424	
1.56 ± 0.07	interferon-inducible protein variant 10	immunity	X61381	Rn.6945
2.32 ± 0.20	sterol-C4-methyl oxidase-like	steroid metabolism	AI172293	Rn.7167
4.12 ± 0.60	isopentenyl diphosphate-dimethylallyldiphosphate isomerase	steroid metabolism	AF003835	Rn.10780
2.23 ± 0.13	lanosterol 14-demethylase	steroid metabolism	AB004096	Rn.6150
2.09 ± 0.08	carboxylesterase	steroid metabolism	M20629	Rn.2549
2.03 ± 0.03	carboxylesterase	steroid metabolism	D00362	
1.89 ± 0.10	carboxylesterase (Es-HVEL)	steroid metabolism	X65296	
1.81 ± 0.08	3-hydroxy-3-methylglutaryl-coenzyme A synthase 1	steroid metabolism	AI177004	Rn.5106
1.63 ± 0.07	3-hydroxy-3-methylglutaryl-coenzyme A synthase 1	steroid metabolism	X52625	Rn.5106
1.81 ± 0.16	farnesyl diphosphate farnesyl transferase 1	steroid metabolism	M95591	Rn.3252
1.74 ± 0.16	farnesyl diphosphate farnesyl transferase 1	steroid metabolism	M95591	Rn.3252
activated[a]	squalene epoxidase	steroid metabolism	D37920	Rn.33239
2.96 ± 0.09	GADII	others	E13557	
activated[a]	T-complex 1	others	AA859980	Rn.7102
−1.60 ± 0.13	kynurenine/α-aminoadipate aminotransferase	amino acid metabolism	Z50144	Rn.11133
−1.90 ± 0.17	serine dehydratase (SDH2)	amino acid metabolism	J03863	Rn.9918
−1.62 ± 0.12	trans-golgi network protein 1	cell growth and/or maintenance	AA926292	Rn.11349
−2.23 ± 0.23	Arg/Abl-interacting protein ArgBP2	cell growth and/or maintenance	AF026505	Rn.24612
−3.05 ± 0.38	fatty acid-binding protein	cell growth and/or maintenance	S69874	
−1.53 ± 0.11	UDP-galactose:*N*-acetylglucosamine β-1,4-galactosyltransferase	energy metabolism	S81025	
−1.67 ± 0.12	L-type pyruvate kinase	energy metabolism	X05684	Rn.48821
−2.25 ± 0.17	cytochrome P450 4A1	fatty acid metabolism	AA924267	Rn.5721
−1.91 ± 0.17	carnitine octanoyltransferase	fatty acid metabolism	J02844	Rn.4896
−1.56 ± 0.08	malic enzyme 1	fatty acid metabolism	AI171506	Rn.3519
−1.67 ± 0.12	malic enzyme 1	fatty acid metabolism	AI171506	Rn.3519
−1.54 ± 0.08	peroxisomal 3-ketoacyl-CoA thiolase	fatty acid metabolism	J02749	Rn.8913
−1.99 ± 0.13	enoyl-coenzyme A, hydratase/3-hydroxy-acyl-coenzyme A dehydrogenase	fatty acid metabolism	K03249	Rn.3671
−4.95 ± 1.49	stearoyl-coenzyme A desaturase 1	fatty acid metabolism	AI175764	Rn.1023
−1.53 ± 0.07	protein kinase C δ-binding protein	signal transduction	D85435	Rn.12281
suppressed[b]	serine-threonine kinase receptor type I	signal transduction	L36088	Rn.10631
suppressed[b]	EST	others	AI639525	Rn.74079
suppressed[b]	EST	others	AA875288	Rn.2791

[a] Gene arbitrated as PPP in the SPI group and AAA in the casein group. [b] Gene arbitrated as AAA in the SPI group and PPP in the casein group.

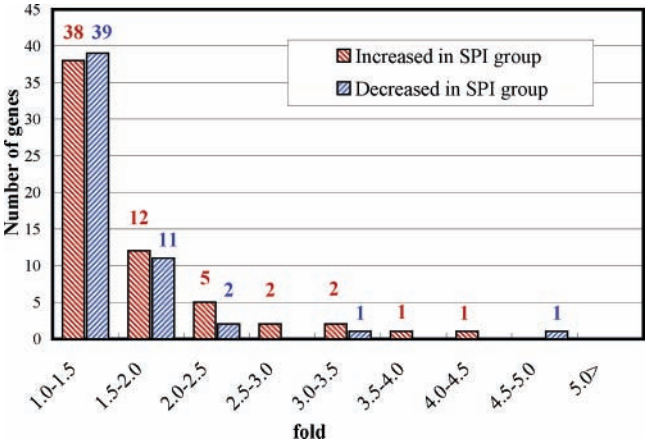

Figure 1. Results of Student's *t* test ($p < 0.05$). The expressions of 115 of 2905 genes were significantly different ($p < 0.05$) between the SPI and casein groups.

expression in rat liver between SPI- and casein-rats. Their products may be related to various physiological functions such as immunity, transcription, antioxidation, and amino acid metabolism (**Table 5**). Dietary SPI up-regulates the expression of genes related to antioxidation and steroid metabolism and down-regulates the expression of genes related to fatty acid metabolism, cell growth and/or maintenance, cytoarchitecture, and amino acid metabolism. Especially, genes showing significant differences of >1.5-fold or expressed in only one of the diet groups were mainly categorized as playing roles in cholesterol metabolism, fatty acid metabolism, and antioxidation, indicating that the effects on metabolism by SPI intake are predominantly in lipid metabolism and antioxidation (**Tables 5** and **6**). These results suggest that global modifications with respect to the progression of steroid metabolism and the suppression of fatty acid synthesis in the liver can reduce plasma lipid concentrations by dietary SPI and that the suppression of the expression of genes concerned with cell growth and/or maintenance, cytoarchitecture, and amino acid metabolism can be related to the lower liver and body weights of SPI-rats.

DISCUSSION

DNA microarray technology that reveals an all-inclusive gene expression profile makes it possible to analyze the overall effects of nutritional components; thus, nutrigenomics has arisen as a new analytical field of food functions and nutrition (*16*, *17*). In this study, we succeeded in investigating the physiological influences of dietary soy protein isolate in terms of biochemical analysis and nutrigenomics.

Especially, alterations in the expressions of genes related to lipid metabolism by SPI intake were characteristic. Potter (*3*) reviewed various effects of SPI and reported that its intake increased low-density lipoprotein (LDL) receptor activity. These results indicated that SPI intake significantly up-regulated the expression of genes related to cholesterol de novo synthesis and steroid catabolism, but significantly decreased serum cholesterol levels. These results suggest that SPI intake brings about the progression of steroid excretion. In fact, the levels of the excretion of acidic steroids, for example, bile acid, and neutral steroids, for example, cholesterol, were significantly higher in SPI-rats than in casein-rats after 2 weeks of feeding (Tachibana et al., unpublished data). Taken together, the data suggest that the progression of steroid excretion due to SPI intake may result in a reduction of the serum cholesterol level and that its intake for 8 weeks can maintain serum cholesterol at a lower level. On the other hand, the expression of genes related to fatty acid synthesis was significantly down-regulated in SPI-rats as compared with casein-rats. The stearoyl-CoA desaturase-1 gene, which was the most down-regulated gene, is also concerned with fatty acid metabolism (*27*). Iritani et al. (*6*) reported that SPI intake suppresses the expression of genes encoding key enzymes involved in fatty acid synthesis, such as fatty acid synthase, ATP-citrate lyase, and malic enzyme. In addition, the expressions of genes concerned with series of enzymes involved in fatty acid synthetic pathways were also decreased by SPI intake. This indicates that the collective suppression of the expression of genes involved in fatty acid synthesis contributes to a reduction in the concentration of lipids in the serum and liver. On the metabolic map, de novo steroid synthesis links to fatty acid synthesis via acetyl-CoA. Therefore, the effects of SPI intake, such as the suppression of fatty acid synthesis and the progression of de novo steroid synthesis, indicate an overall alteration in the expression of genes involved in lipid catabolism and anabolism, indicating that SPI intake comprehensively modulates lipid levels in vivo.

One of the physiological effects of SPI intake is the repression of renal damage. SPI intake brings about a significant progression of urinary urea excretion, suppression of urinary protein excretion, and reduction of blood urea-N (*28*, *29*). In our present study, the concentration of urea-N in the SPI-rats (13.1 ± 0.6, $n = 5$) was significantly lower than in the casein-rats (18.3 ± 0.7, $n = 6$), suggesting an association of SPI with the suppression of renal damage. The enzymes involved in the urea cycle are all present in the liver, and the results of DNA microarray analysis also showed that genes concerned with amino acid metabolism were altered by the difference in dietary proteins. Serine dehydratase 2, which mediates nonoxidative deamination and cleaved $-NH_2$ from the amino acid directly into the urea cycle, was significantly up-regulated in SPI-rats as compared with casein-rats. In energy metabolism, carbonic anhydrase III, which is concerned with ammonia catabolism, was also significantly up-regulated in the SPI-rats. These results suggest that these genes play important roles in reducing the stress of renal function and promoting amino acid and energy metabolism.

Genes involved in antioxidative effects showed higher expressions in the livers of SPI-rats than in casein-rats. Dietary SPI contains such antioxidants as partially digested SPI peptides (*30*) as well as isoflavones and saponins (*31*−*33*), and, therefore, antioxidation is one of the major physiological roles of SPI. Takenaka et al. (*34*) reported that SPI intake suppressed hyperoxidation confirmed by TBARs level in the liver. Therefore, SPI intake led to some sort of preventive effects. Among the genes showing higher expressions in the livers of SPI-rats than in casein-rats are some genes related to xenobiotics, such as genes encoding glutathione *S*-transferase, cytochrome P-450, and epoxide hydrolase. It is suggested that ingested SPI induces the expression of antioxidants to promote xenobiotic metabolism and reduce oxidative stress.

Hyperlipidemia and hypercholesterolemia invite various disorders such as obesity and atherosclerosis (*1*). Thus, it is important that blood lipids are reduced in daily life. Especially, differences of the diet may bring about the possibility of lowering blood lipids in connection with physiological changes. Although the number of genes with altered expression profiles on microarrays represents only 1.4% of in the total genes in the rat genome, it is worth noting that these may trigger desirable physiological effects of SPI intake, such as reductions in the levels of liver and blood lipids, steroid excretion, and the

mitigation of oxidative stress. Further investigations on rats of different ages and feeding period will provide more precise information about the multifunctionalities of this important food protein, SPI.

LITERATURE CITED

(1) Carroll, K. K. Hypercholesterolemia and atherosclerosis: effects of dietary protein. *Fed. Proc.* **1982**, *41*, 2792−2796.

(2) Anderson, J. W.; Johnstone, B. M.; Cook-Newell, M. E. Meta-analysis of the effects of soy protein intake on serum lipids. *N. Engl. J. Med.* **1995**, *333*, 276−282.

(3) Potter, S. M. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J. Nutr.* **1995**, *125*, 606S−611S.

(4) Fukui, K.; Tachibana, N.; Wanezaki, S.; Tsuzaki, S.; Takamatsu, K.; Yamamoto, T.; Hashimoto, Y.; Shimoda, T. Isoflavone-free soy protein prepared by column chromatography reduces plasma cholesterol in rats. *J. Agric. Food Chem.* **2002**, *50*, 5717−21.

(5) Bakhit, R. M.; Klein, B. P.; Essex-Sorlie, D.; Ham, J. O.; Erdman, J. W.; Potter, S. M. Intake of 25g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations. *J. Nutr.* **1994**, *124*, 213−222.

(6) Iritani, N.; Hosomi, H.; Fukuda, H.; Tada, K.; Ikeda, H. Soybean protein suppresses hepatic lipogenic enzyme gene expression in wistar fatty rats. *J. Nutr.* **1996**, *126*, 380−388.

(7) Sugano, M.; Tanaka, K.; Ide, Y. Secretion of cholesterol, triglyceride and apolipoprotein A-I by isolated perfused liver from rats fed soybean protein and casein or their amino acid mixtures. *J. Nutr.* **1982**, *112*, 855−862.

(8) Aoyama, T.; Fukui, K.; Takamatsu, K.; Hashimoto, Y.; Yamamoto, T. Soy protein isolate and its hydrolysate reduce body fat of dietary obese rats and genetically obese mice (Yellow KK). *Nutrition* **2000**, *16*, 349−354.

(9) Huff, M. W.; Roberts, C. K. D.; Carroll, K. K. Long-term effects of semipurified diets containing casein or soy protein isolate on atherosclerosis and plasma lipoprotein in rabbits. *Atherosclerosis* **1982**, *41*, 327−336.

(10) Messina, M. J.; Persky, V.; Setchell, K. D. R.; Barnes, S. Soy intake and cancer risk. *Nutr. Cancer* **1994**, *21*, 113−131.

(11) Adlercreutz, C. H. T.; Goldin, B. R.; Gorbach, S. L.; Hockerstedt, K. A. V.; Watanabe, S.; Hamalainen, E. K.; Markkanen, M. H.; Makela, T. H.; Wahala, K. T.; Hase, T. A.; Fotsis, T. Soybean phytoestrogen intake and cancer risk. *J. Nutr.* **1995**, *125*, 757S−770S.

(12) Tovar, A. R.; Fermanda, M.; Cristino, C.; Hernandez-Pando, R.; Aguilar-Salinas, C. A.; Pedraza-Chaverri, J.; Correa-Rotter, R.; Torres, N. A soy protein diet alters hepatic lipid metabolism gene expression and reduces serum lipids and renal fibrogenic cytokines in rats with chronic nephritic syndrome. *J. Nutr.* **2002**, *132*, 2562−2569.

(13) Iqbal, M. J.; Yaegashi, S.; Ahsan, R.; Lightfoot, D. A.; Banz, W. J. Differentially abundant mRNAs in rat liver in response to diets containing soy protein isolate. *Physiol. Genomics* **2002**, *11*, 219−226.

(14) Weindruch, R.; Kayo, T.; Lee, C.-K.; Prolla, T. A. Microarray profiling of gene expression in aging and its alteration by caloric restriction in mice. *J. Nutr.* **2001**, *131*, 918S−923S.

(15) Moraes, R. C.; Blondet, A.; Birkenkamp-Demtroeder, K.; Tirard, J.; Orntoft, T. F.; Gertler, A.; Durand, P.; Naville, D.; Begeot, M. Study of the alteration of gene expression in adipose tissue of diet-induced obese mice by microarray and reverse transcription-Polymerase Chain Reaction analyses. *Endocrinology* **2003**, *144*, 4773−4782.

(16) Endo, Y.; Zhengwei, Fu; Abe, K.; Arai, S.; Kato, H. Dietary protein quantity and quality affect rat hepatic gene expression. *J. Nutr.* **2002**, *132*, 3632−3637.

(17) Lee, C. K.; Klopp, R. G.; Weindruch, R.; Prolla, T. A. Gene expression profile of aging and its retardation by caloric restriction. *Science* **1999**, *285*, 1390−1393.

(18) Muller, M.; Kersten, S. Nutrigenomics: goals and strategies. *Nat. Rev. Genet.* **2003**, *4*, 315−22.

(19) Trayhurn, P. Nutritional genomics—"Nutrigenomics". *Br. J. Nutr.* **2003**, *89*, 1−2.

(20) Kato, H.; Kimura, T. Evaluation of the effects of the dietary intake of proteins and amino acids by DNA microarray technology. *J. Nutr.* **2003**, *133*, 2073S−2077S.

(21) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C., Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc eriting committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939−1951.

(22) Folch, J.; Lees, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.

(23) Sperry, W. M.; Webb, M. Arevision of the Schoenheimer−Sperry method for cholesterol determination. *J. Biol. Chem.* **1950**, *187*, 97−106.

(24) Fletcher, M. J. A colorimetric method for estimating serum triglycerides. *Clin. Chem. Acta* **1968**, *22*, 393−397.

(25) Feldman, G. L.; Feldman, L. S. Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots. *Lipid* **1965**, *1*, 85−87.

(26) Matsumoto, I.; Emori, Y.; Nakamura, S.; Shimizu, K.; Arai, S.; Abe, K. DNA microarray cluster analysis reveals tissue similarity and potential neuron-specific genes expressed in cranial sensory ganglia. *J. Neurosci. Res.* **2003**, *74*, 818−828.

(27) Paul, C.; Miyazaki, M.; Socci, N. D.; Aaron, H. G.; Wolfgang, L.; Alexander, A. S.; Sharma, R.; Lisa, C. H.; Ntambi, J. M.; Friedman, J. M. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science* **2002**, *297*, 240−243.

(28) Henry, K. M.; Kon, S. K. Effect of level of protein intake and of age of rat on the biological value of proteins. *Br. J. Nutr.* **1957**, *11*, 305−313.

(29) Sakemi, T.; Ikeda, Y.; Shimazu, K. Effect of soy protein added to casein diet on the development of glomerular injury in spontaneous hypercholesterolemic male Imai rats. *Am. J. Nephrol.* **2002**, *22*, 548−554.

(30) Chen, H. M.; Muramoto, K.; Yamauchi, F.; Fujimoto, K.; Nokihara, K. Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. *J. Agric. Food Chem.* **1998**, *46*, 49−53.

(31) Wei, H.; Wei, L.; Frenkel, K.; Bowen, R.; Barnes, S. Inhibition of tumor promoter-induced hydrogen peroxide formation *in vitro* and *in vivo* by genistein. *Nutr. Cancer* **1993**, *20*, 1−12.

(32) Disilvestro, R. A. Antioxidant actions of soya. *Food Ind. J.* **2001**, *4*, 210−220.

(33) Cai, Q.; Wei, H. Effect of dietary genistein on antioxidant enzyme activities in SENCAR mice. *Nutr. Cancer* **1996**, *25*, 1−7.

(34) Takenaka, A.; Annaka, H.; Kimura, Y.; Aoki, H.; Igarashi, K. Reduction of paraquat-induced oxidative stress in rats by dietary soy peptide. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 278−283.

Received for review November 12, 2004. Revised manuscript received March 12, 2005. Accepted March 15, 2005.

JF0481103