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A Comparative Analysis of Genistein and Daidzein in Affecting Lipid Metabolism in Rat Liver

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Summary **Effects of soy isoflavones, genistein and daidzein, on the hepatic gene expression profile and indices for lipid metabolism were compared in rats. In the first experiment (Expt. 1), animals were fed diets containing 2 g/kg of either genistein or daidzein, or a control diet free of isoflavone for 14 days. In the second experiment (Expt. 2), rats were fed diets containing 1 or 2 g/kg of genistein, or an isoflavone-free diet for 16 days. Genistein at a dietary level of 2 g/kg reduced serum triacylglycerol concentrations in both experiments, and serum concentrations of cholesterol in Expt. 2. However, daidzein at 2 g/kg did not decrease serum lipid concentrations in Expt. 1. A DNA microarray analysis in Expt. 1 showed that genistein was stronger than daidzein in affecting gene expression in liver, targeting many genes involved in lipid and carbohydrate metabolism. Detailed analyses indicated that alterations in the expression of genes related to lipogenesis are primarily responsible for the serum lipid-lowering effect of genistein. This notion was supported by analyses of the activity of enzymes involved in lipogenesis in Expt. 2.**

Key Words**: DNA microarray, fatty acid, gene expression, isoflavone, liver**

Introduction

Dietary soy protein lowers blood lipid concentrations and reduces the incidence of cardiovascular diseases in animals and humans [*1*]. The soy protein preparations employed in previous studies usually contained considerable amounts of isoflavones, saponins, fiber, and phytic acid [*2*]. These extra compound(s) may be partly responsible for the lipid-lowering effect. Studies [*3–10*] indicate that soy isoflavones have the physiological effect of lowering blood lipid levels. We previously showed that serum triacylglycerol concentrations were comparable between rats fed casein and rats fed a methanol-washed soy protein preparation low in isoflavones; however, supplementation of the soy protein preparation with isoflavones dose-dependently decreased this parameter [*10*]. Also, some studies [*6–9*] showed that soy isoflavones added to an experimental diet containing casein as a protein source reduced cholesterol and triacylglycerol concentrations in the serum and/or liver of rodents. With regard to the mechanism underlying the hypolipidemic effect of isoflavones, studies have indicated that isoflavones affected the activity of enzymes involved in lipid metabolism [*6*, *9*] and mRNA levels of proteins related to β-oxidation and energy metabolism in the liver of animals [*5*, *7*]. These observations suggest that dietary isoflavone plays a crucial role in regulating lipid metabolism.

Genistin and daidzin are isoflavones abundant in soybean, and their aglycones, genistein and daidzein, are released by the action of intestinal glucosidase and absorbed from the gastrointestinal tract [*11*]. It has been reported that genistein is stronger than daidzein in its agonistic activity for the

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estrogen receptors [*12*]. Meanwhile, daidzein is metabolized by enterobacteria to produce equol which has stronger estrogenic and antioxidative activities than daidzein or the other isoflavone metabolites [*11*, *12*]. Therefore, it is plausible that dietary daidzein and genistein influence lipid metabolism differently. However, information on this topic is lacking. Here we compared the physiological activity of dietary genistein and daidzein in affecting the mRNA expression of proteins involved in lipid metabolism in rat liver using a DNA microarray technique.

Materials and Methods

Animals

This study was approved by the review board of animal ethics in our institute, and we followed the institute's guidelines for the care and use of laboratory animals. Male Sprague-Dawley rats purchased from Charles River Japan (Kanagawa, Japan) at 4 weeks of age were housed individually in a room with controlled temperature (20–22°C), humidity (55–65%), and lighting (0700–1900 h), and fed a commercial diet (Type NMF; Oriental Yeast, Tokyo, Japan). Animals had free access to the diets and water during the acclimatization and experimental periods. After 5 days of acclimatization to the conditions, rats were randomly divided into experimental groups with similar mean body weights consisting of 7 animals each. In the first experiment (Expt. 1), 3 groups of rats were fed an experimental diet containing 2 g/kg of either genistein (LC Laboratories, Woburn, MA) or daidzein (LC Laboratories), or a control diet free of isoflavone for 14 days. In the second trial (Expt. 2), 3 groups of rats were fed diets containing 0, 1.0, or 2.0 g/kg of genistein for 16 days. The basal composition of the experimental diet was (in g/kg): casein, 200; palm oil, 100; corn starch, 150; cellulose, 20; mineral mixture (AIN-93G, Oriental Yeast), 35; vitamin mixture (AIN-93, Oriental Yeast), 10; L-cystine, 3.0; choline bitartrate, 2.0 and sucrose to 1 kg. Genistein or daidzein was added to the experimental diets instead of sucrose. At the end of the experimental period, the animals were killed by bleeding from the abdominal aorta under diethyl ether anesthesia after 3 h of fasting.

Analyses of serum lipid and isoflavone concentrations

Serum triacylglycerol, cholesterol, phospholipid, and free fatty acid concentrations were analyzed using commercial enzyme kits (Wako Pure Chemical Industries, Osaka, Japan). Serum isoflavone concentrations were analyzed by HPLC as detailed previously [*10*].

Microarray analysis

RNA extracted from the livers of 5 rats from each group was subjected to microarray analyses (Expt. 1). The selection was based on serum triacylglycerol levels. Those rats with the highest and lowest values for this parameter were eliminated from the microarray analyses. Hepatic RNA was extracted and purified using the RNeasy Mini Kit (Qiagen, Valencia, CA). Total RNA (10 μg) purified from each sample was processed to prepare biotin-labeled cRNA using One-Cycle Target Labeling and Control Reagents (Affymetrix, Santa Clara, CA). cRNA was fragmented and used as a probe for hybridization to the Affymetrix Rat Genome 230_2.0 GeneChip microarray. After the washing and staining processes, the arrays were scanned with a GCS 3000 scanner (Affymetrix) and analyzed by Affymetrix GeneChip Operating Software 1.2 (GCOS). Subsequent analyses of microarray data were carried out using GeneSpring 7.3.1 software (Agilent Technologies, Santa Clara, CA). Per chip and per gene normalizations were carried out using a 50 percentile value of signal intensity in each chip, and the mean values in the group of rats fed a control diet free of isoflavone, respectively. After the normalizations, unreliable data with low signal intensity were eliminated: data had to fulfill the following criteria. First, the average raw signal intensity should be greater than 50 in at least 2 of 3 dietary treatments. Second, the expression data should call present- or marginal-flags according to the GCOS algorithm in more than 5 of 15 arrays. After the filtering process, the expression data for 9,307 of 31,099 transcripts survived, and the lists were further analyzed to clarify the genes whose expression was significantly ($p<0.05$) affected more than 1.5-fold by dietary isoflavones. The functions of genes were clarified using several data-bases including the Rat Genome Database (http://rgd.mcw.edu/), KEGG (http://www.kegg.jp/kegg/pathway.html) and GenMAPP (http://www.genmapp.org/).

Analysis of hepatic enzyme activities involved in fatty acid synthesis and β-oxidation

Approximately 1.5 g of each liver was homogenized with 10 ml of 0.25 M sucrose containing 1 mM EDTA and 3 mM Tris-HCl (pH 7.2) (Expt. 2). The homogenate was centrifuged at 200,000 × g for 30 min. The activities of enzymes involved in lipogenesis were measured spectrophotometrically using the supernatant as an enzyme source as detailed previously [*10*]. The activities of enzymes related to β-oxidation were analyzed using the whole liver homogenate as an enzyme source [*10*].

Statistical analysis

The values were expressed as means ± standard errors (SE). The data were analyzed with the Kruskal-Wallis test to detect significant effects of dietary compounds, and the differences between means at the level of $p<0.05$ were evaluated with Dunn's multiple comparison procedure.

Table 1. Effect of dietary isoflavones on growth parameters, and levels of serum isoflavones and lipids in rats[1]

	Dietary isolfavone (Expt. 1)			Dietary genistein (g/kg diet) (Expt. 2)		
	none	genistein	daidzein	0	1.0	2.0
Body weight gain (g/day)	9.61 ± 0.33	8.28 ± 0.37	9.60 ± 0.19	9.85 ± 0.35	9.30 ± 0.36	8.84 ± 0.20
Food intake (g/day)	20.8 ± 0.8	19.3 ± 0.8	21.4 ± 0.4	23.4 ± 0.8	22.9 ± 0.7	21.6 ± 0.5
Liver (g/100 g body weight)	5.51 ± 0.27	5.44 ± 0.13	6.04 ± 0.15	5.48 ± 0.10	5.32 ± 0.09	5.19 ± 0.09
Serum isoflavones (μmol/l)[2]						
Genistein	ND	8.17 ± 0.48	ND	ND	6.82 ± 0.41	10.1 ± 1.0
Daidzein	ND	ND	6.41 ± 0.68	ND	ND	ND
Equol	ND	ND	5.48 ± 1.15	ND	ND	ND
Serum lipids (mmol/l)						
Triacylglycerol	3.84 ± 0.61^{b}	2.18 ± 0.29^{a}	2.61 ± 0.32^{ab}	4.57 ± 0.58^{b}	4.17 ± 0.39^{ab}	3.22 ± 0.31^{a}
Cholesterol	2.55 ± 0.04^{ab}	2.11 ± 0.10^{a}	3.09 ± 0.11^{b}	3.15 ± 0.25^{b}	2.58 ± 0.21^{ab}	2.43 ± 0.11^{a}
Phospholipid	2.82 ± 0.21^{ab}	2.23 ± 0.11^{a}	2.99 ± 0.07^{b}	3.04 ± 0.15	2.90 ± 0.18	2.80 ± 0.11
Free fatty acid	1.25 ± 0.25	0.934 ± 0.095	1.21 ± 0.20	1.16 ± 0.110	1.13 ± 0.13	1.15 ± 0.06

[1] Values represent the mean ± SE for 7 rats. Values in the same experiment with different superscripts differ significantly at $p<0.05$.
[2] ND, not detectable.

Results

Growth parameters, and serum isoflavone and lipid concentrations

No significant differences were seen in growth, food intake and liver weight among the groups in either experiment (Table 1). Genistein was detected in the serum of rats given genistein (Expts. 1 and 2), but not in the animals fed an isoflavone-free diet or a diet containing daidzein (Table 1). The concentration of genistein increased dose-dependently in response to dietary genistein in Expt. 2. Not only daidzein but also equol was detected in rats fed daidzein (Expt. 1). In Expt. 1, a diet containing 2 g/kg of genistein compared to an isoflavone-free diet significantly lowered serum triacylglycerol levels (Table 1). Although the differences were not significant, genistein tended to lower cholesterol, phospholipid, and free fatty acid concentrations (17.3, 20.9 and 25.3% reductions, respectively) in serum. Daidzein caused a 32.0% reduction in the serum triacylglycerol concentration, but this was not significant. Daidzein was ineffective in altering serum concentrations of cholesterol, phospholipid, and free fatty acid. In Expt. 2, a significant decrease in serum triacylglycerol concentrations in rats fed a diet containing 2 g/kg of genistein compared to those fed an isoflavone-free diet was confirmed. In addition, we observed that dietary genistein at 2 g/kg significantly lowered serum concentrations of cholesterol in Expt. 2. Although the differences were not significant, the levels of serum triacylglycerol and cholesterol tended to be lower in rats given a diet containing 1 g/kg of genistein than in those fed an isoflavone-free diet.

Gene expression profile in the liver of rats fed genistein or daidzein (Expt. 1)

Compared to a diet free of isoflavone, genistein significantly caused >1.5-fold changes in the expression of 154 genes and daidzein altered the expression of 38 genes in liver (Table 2). The genes whose expression was modulated by genistein and/or daidzein were classified into categories according to function. Many of the genes affected by genistein were categorized as a playing role in the metabolism of lipid, carbohydrate, amino acid, and protein, and other metabolic processes. However, no gene was found in the category of metabolism in rats given daidzein. Among the genes classified into the metabolism group, we found that genistein affected the mRNA expression of many genes related to lipid and carbohydrate metabolism. Because analyses of serum lipid indicated that genistein affected lipid metabolism (Table 1), we picked out the genes associated with lipid metabolism whose expression was modified more than 1.5-fold by dietary isoflavones and listed them in Table 3. Dietary genistein significantly lowered the gene expression levels of lipogenic enzymes (Me1 and Acyl), and of a transcription factor (Srebf1) that regulates the expression of many enzymes involved in fatty acid and triacylglycerol synthesis [*13*]. Daidzein-dependent changes in the expression of these transcripts were not significant. Acsl4 catalyzes the ligation of a fatty acid and CoA to produce acyl-CoA with preferential activity for arachidonic and eicosapentaenoic acids, and plays a role in the regulation of fatty acid oxidation and triacylglycerol synthesis [*14*]. Genistein caused a significant increase in the mRNA expression of Acsl4. The daidzein-evoked change in this parameter was not significant. The microarray analysis showed that

Table 2. Distribution into functional categories of gene transcripts whose expression was altered more than 1.5-fold by dietary isoflavones[1]

Functional category	Genistein versus Isoflavone-free		Daidzein versus Isoflavone-free	
	Up	Down	Up	Down
Metabolism				
Lipid and carbohydrate metabolism	8	10	0	0
Amino acid and protein metabolism	2	9	0	0
Other metabolism	1	4	0	0
Signal transduction	7	10	0	3
Transcription	3	4	0	2
Stress responsive, xenobiotic metabolism and electron transport	5	10	6	3
Other transport	2	8	1	0
Cell cycle, apoptosis, and DNA/RNA modification	3	0	3	3
Cell adhesion	2	1	0	4
Other biological function	0	4	0	2
Biological function unknown	4	14	4	2
Expressed sequence tags and unknown genes	21	22	1	4
Total	58	96	15	23

[1] Numbers of genes for which mRNA expression was significantly different in genistein vs isoflavone-free and daidzein vs isoflavone-free, respectively ($p<0.05$).

genistein also affected the expression of genes related to lipid transport. Genistein significantly increased the mRNA level of Cd36, also known as fatty acid translocase, but decreased that of Sorl1, a member of the LDL receptor gene family. Daidzein caused similar changes in these lipid transporters, but was less effective than genistein, and the changes were not significant in all cases. Dietary genistein, but not daidzein, significantly reduced the gene expression of two enzymes participating in cholesterol synthesis (Hmgcs1 and Sc4mol). We found that genistein significantly increased the hepatic mRNA level of Akr1d1 related to bile acid biosynthesis and the metabolism of steroid hormones [*15*]. However, the change in this mRNA level obtained with daidzein was not significant. In addition to Akr1d1, enzymes (Smp2a, Sult2a2, Hsd3b, Hsd17b2, and Cyp2a1) involved in the metabolism of steroid hormones were targeted by isoflavones. Dietary genistein down-regulated the mRNA expression of Hsd3b, while it up-regulated the expression of other genes. The changes obtained with daidzein resembled those observed with genistein, but were considerably attenuated and insignificant. Genistein but not daidzein significantly lowered the mRNA level of Pld1 which catalyzes the conversion of phosphatidylcholine to diacylglycerol [*16*].

Enzyme activities involved in lipogenesis and β-oxidation in rats given genistein (Expt. 2)

The observations made in Expt. 1 indicated that genistein and daidzein affected lipid metabolism differently, with the former being more competent. We therefore analyzed the activities of enzymes involved in lipogenesis and β-oxidation in rats fed diets containing 1 and 2 g/kg of genistein in the next trial (Expt. 2). Dietary genistein at 2 g/kg significantly reduced the activities of enzymes participating in lipogenesis including ATP-citrate lyase, glucose 6-phospate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme and pyruvate kinase (Table 4). These activities were also considerably lower in rats fed a diet containing 1 g/kg of genistein than in those fed an isoflavone-free diet. However, a significant reduction was observed in ATP-citrate lyase but not in the other enzymes. Dietary genistein at 1 and 2 g/kg caused a 15.2 and 21.5% decrease in the activity of fatty acid synthase, respectively. However, the reductions were not significant. Diets containing 1 and 2 g/kg of genistein compared to an isoflavone-free diet increased the activity of carnitine palmitoyltransferase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase involved in β-oxidation. The increases were all significant except on one occasion (carnitine palmitoyltransferase activity in rats fed 1 g/kg of this isoflavone). The activity levels of these β-oxidation enzymes were comparable between rats fed 1 and 2 g/kg of genistein. However, dietary genistein failed to affect the rate of peroxisomal palmitoyl-CoA oxidation and the activity of acyl-CoA oxidase and 2,4-dienoyl-CoA reductase.

Table 3. Microarray analyses of genes involved in lipid metabolism whose expression was affected (>1.5-fold) by either genistein or daidzein[1]

GenBank accession no.	Gene name	Gene symbol	Fold change (microarray)		
			Isoflavone-free	Genistein	Daidzein
Fatty acid metabolism					
M30596	Malic enzyme 1	Me1	1.00 ± 0.07[b]	0.559 ± 0.128[a]	1.03 ± 0.10[b]
NM_016987	ATP citrate lyase	Acly	1.00 ± 0.07[b]	0.532 ± 0.155[a]	0.758 ± 0.13[ab]
AF286470	Sterol regulatory element-binding factor 1	Srebf1	1.00 ± 0.07[b]	0.568 ± 0.101[a]	0.666 ± 0.138[ab]
NM_053623	Acyl-CoA synthetase long-chain family member 4	Acsl4	1.00 ± 0.03[a]	1.68 ± 0.23[b]	1.36 ± 0.14[ab]
Fatty acid transport					
NM_031561	Cd36 antigen	Cd36	1.00 ± 0.11[a]	2.89 ± 0.86[b]	1.29 ± 0.22[ab]
AF072411	Cd36 antigen	Cd36	1.00 ± 0.08[a]	2.78 ± 0.76[b]	1.14 ± 0.24[ab]
AA850618	Sortilin-related receptor, LDLR class A repeats-containing	Sorl1	1.00 ± 0.03[b]	0.359 ± 0.069[a]	0.757 ± 0.046[ab]
Cholesterol synthesis					
NM_017268	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	Hmgcs1	1.00 ± 0.04[b]	0.654 ± 0.051[a]	0.920 ± 0.047[ab]
NM_080886	Sterol-C4-methyl oxidase-like	Sc4mol	1.00 ± 0.13[b]	0.546 ± 0.031[a]	0.864 ± 0.063[ab]
Bile acid and steroid hormone metabolism					
D17309	Aldo-keto reductase family 1, member D1	Akr1d1	1.00 ± 0.10[a]	1.86 ± 0.22[b]	1.44 ± 0.11[ab]
NM_012695	Rat senescence marker protein 2A gene, exons 1 and 2	Smp2a	1.00 ± 1.19[a]	25.9 ± 8.0[b]	3.84 ± 1.04[ab]
D14989	Sulfotransferase family 2A, dehydroepiandrosterone (DHEA)-preferring, member 2	Sult2a2	1.00 ± 0.07[a]	4.97 ± 3.81[b]	1.08 ± 0.22[a]
NM_012584	7-Dehydrocholesterol reductase	Hsd3b	1.00 ± 0.12[b]	0.0837 ± 0.0828[a]	0.73 ± 0.10[ab]
NM_024391	Hydroxysteroid (17-beta) dehydrogenase 2	Hsd17b2	1.00 ± 0.36[a]	3.98 ± 1.93[b]	2.27 ± 0.50[ab]
NM_012692	Cytochrome P450 IIA1 (hepatic steroid hydroxylase IIA1) gene	Cyp2a1	1.00 ± 0.20[a]	3.10 ± 0.29[b]	1.30 ± 0.20[ab]
Glycerolipid metabolism					
AB000779	Phospholipase D1	Pld1	1.00 ± 0.04[b]	0.634 ± 0.063[a]	0.781 ± 0.048[ab]

[1] Fold change represents the mean ratio ± SE from each of 5 dependent microarray experiments, comparing genistein and daidzein groups with an isoflavone-free diet group. Values in a row with different superscripts are significantly different at $p<0.05$.

Discussion

It has been reported that dietary soy isoflavones exert hypolipidemic actions in experimental animals [*5–10*]. However, studies to compare physiological activities of genistein and daidzein, major isoflavone species in soy, are scarce. The present study showed that genistein is more effective than daidzein in lowering serum triacylglycerol levels and in changing the expression of genes associated with lipid metabolism in liver.

The microarray analysis showed that the genes involved in hepatic fatty acid synthesis (Acly, Me1, and Srebf1) were the targets to be down-regulated by isoflavones (Table 3). The effect was much stronger with genistein than with daidzein. Consistent with this observation, dietary genistein decreased the activities of lipogenic enzymes (Table 4). Moreover, we observed that genistein increased the activities of several enzymes related to β-oxidation (Table 4). Therefore, it is suggested that dietary genistein decreased serum lipid levels through a reduction in fatty acid synthesis and an increase in β-oxidation in the liver. However, our microarray analysis barely detected isoflavone-dependent changes in mRNA levels of enzymes related to β-oxidation. None of the gene for enzymes involved in β-oxidation was up-regulated more than 1.5-fold either by genistein or by daidzein (Table 3). Detailed examination of the DNA micro-

Table 4. Effect of dietary genistein on the activities of enzymes involved in hepatic fatty acid synthesis and β-oxidation in rats[1]

	Dietary genistein (g/kg diet)		
	0	1.0	2.0
Enzymes involved in fatty acid synthesis (nmol/min per mg protein)			
Fatty acid synthase	53.9 ± 6.3	45.7 ± 4.6	42.3 ± 5.8
ATP-citrate lyase	154 ± 11[b]	109 ± 8[a]	106 ± 10[a]
Glucose 6-phospate dehydrogenase	210 ± 22[b]	149 ± 13[ab]	137 ± 13[a]
6-Phosphogluconate dehydrogenase	141 ± 9[b]	119 ± 9[ab]	110 ± 8[a]
Malic enzyme	155 ± 16[b]	132 ± 5[ab]	107 ± 10[a]
Piruvate kinase	656 ± 28[b]	573 ± 29[ab]	553 ± 29[a]
Enzymes involved in β-oxidation (nmol/min per mg protein)			
Peroxisomal palmitoyl-CoA oxidation	1.77 ± 0.10	2.05 ± 0.04	2.09 ± 0.17
Acyl-CoA oxidase	1.24 ± 0.05	1.33 ± 0.07	1.37 ± 0.05
Carnitine parmitoyltransferase	2.92 ± 0.21[a]	3.79 ± 0.19[ab]	4.12 ± 0.33[b]
Enoyl-CoA hydratase	6815 ± 207[a]	7732 ± 208[b]	7702 ± 210[b]
3-Hydroxyacyl-CoA dehydrogenase	425 ± 11[a]	508 ± 22[b]	503 ± 16[b]
3-Ketoacyl-CoA thiolase	170 ± 16[a]	233 ± 15[b]	238 ± 13[b]
2,4-Dienoyl-CoA reductase	2.55 ± 0.18	2.98 ± 0.20	3.01 ± 0.10

[1] Values represent the mean ± SE for 7 rats. Mean values within a row with different superscripts differ significantly at $p<0.05$.

array results revealed that daidzein caused a significant 1.44-fold increase in the mRNA expression of enoyl-CoA hydratase/3-hydroxyacyl CoA dehydrogenase (Ehhadh), but the genistein-dependent change in this parameter (1.33-fold) was not significant. No other significant isoflavone-dependent changes were observed in the mRNA expression of enzymes related to β-oxidation. Genistein-dependent changes in the activity of enzymes involved in hepatic β-oxidation were rather small (10.4–41.1%) (Table 4). Given that the enzyme activity levels parallel the mRNA levels, the DNA microarray analysis may not be sensitive and accurate enough to detect these small changes.

The liver is a central organ of cholesterol homeostasis [*17*]. It has been reported that dietary isoflavone decreased cholesterol levels in the liver and serum of rats fed a high-cholesterol diet, accompanying a marginal increase in the activity of hepatic cholesterol 7-hydroxylase, the key enzyme regulating the degradation of cholesterol to bile acids [*9*]. In the present study, we observed that serum cholesterol levels were significantly lower or tended to be lower in rats fed diets containing genistein than in the animals fed an isoflavone-free diet (Table 1). As genistein significantly increased the mRNA level of Akr1d1, but decreased those of Hmgcs1 and Sc4mol observed in the microarray analysis (Table 3), enhanced degradation of cholesterol as well as the decrease in cholesterogenesis may account for the cholesterol-lowering effect of genistein. We also found that several genes related to steroid hormone metabolism were affected by isoflavones. For example, dietary genistein considerably reduced the mRNA level of Hsd3b (Table 3), which plays an important role in the biosynthesis of all classes of hormonal steroids including androgens and estrogens [*18*]. Also, Akd1d1 is an enzyme that affects the first step of the degradation of steroid hormones [*15*]. Therefore, it is possible that dietary isoflavones also affect hepatic steroid hormone metabolism.

The present study showed that dietary genistein compared to daidzein was more competent in affecting serum lipid concentrations and mRNA levels of proteins associated with lipid metabolism in liver. It has been reported that genistein, compared with daidzein, has greater affinity for the estrogen receptors [*12*] and a stronger inhibitory effect on the activity of tyrosine kinase [*19*]. Therefore, it is also possible that genistein has more influence than daidzein on lipid metabolism. In fact, genistein has been previously demonstrated to be stronger than daidzein in increasing the gene expression of apolipoprotein A-I, a major component of high density lipoprotein, in HepG2 cells [*20*]. As the gene expression of apolipoprotein A-I is under the control of estrogen receptor-alpha [*21*], this effect is ascribable to the difference between these isoflavones in affinity for this receptor. Also, it has been reported that genistein interferes with the activation of CCAAT/enhancer-binding protein (C/EBP)β by inhibiting tyrosine-phosphorylation [*22*]. This results in impairments of the mRNA expression of peroxisome proliferator-activated receptor (PPAR)γ and C/EBPα, which promote adipogenesis. Daidzein with no tyrosine kinase inhibitory activity had minimal effect on this process.

It has been reported that equol, a metabolite of daidzein, is stronger than daidzein and similar to genistein in its affinity

for the estrogen receptors, and is stronger than daidzein and genistein in its antioxidative activity [*11*]. So it is possible that equol also exerts profound effects on lipid metabolism. In the present study, a considerable amount of equol was detected in the serum of rats fed daidzein (Table 1). In spite of this finding, dietary daidzein was weaker than genistein in influencing lipid metabolism. Therefore, it is unlikely that equol has strong activity in improving lipid metabolism in rats. In relation to this, it has been reported that equol is weaker than genistein or daidzein in activating PPARα and PPARγ [*12*].

In the present study, genistein at a dietary level of 2 g/kg effectively reduced hepatic lipogenesis and serum lipid levels. Because rats consumed approximately 20 g of the experimental diets in a day (Table 1), daily consumption of genistein is estimated to be 40 mg in the animals fed an experimental diet containing this compound. As one gram of soybean contains about 1 mg of genistein [*23*], this value corresponds to the consumption of approximately 40 g of soybean in a day. When extrapolated from rats of 300 g body weight to humans of 60 kg body weight, the amount becomes 8 kg of soybean per day per person, which is not achievable. However, it has been reported that considerable differences exist in the metabolism of isoflavones between rats and humans. Gu *et al.* [*24*] showed that the plasma genistein concentration was 11-fold higher in women than in female rats at 4 h after a single administration of this isoflavone at a dose of 1 mg/kg body weight in the form of a soy protein isolate. This difference may represent a consequence of diversity in the absorption, metabolism or elimination of the isoflavone between humans and rats. Also, this observation raised the possibility that genistein exerts its physiological activity in humans at a dose much lower than that required in rats. This needs to be clarified.

In conclusion, genistein was more effective than daidzein in lowering serum triacylglycerol levels. The DNA microarray analysis revealed that genistein affected mRNA levels of several genes involved in fatty acid metabolism, and cholesterol and steroid metabolism in rat liver. The changes were much weaker with daidzein than genistein for all these genes. We also showed that genistein decreased the activities of many lipogenic enzymes, but increased those of enzymes related to β-oxidation in the liver. These changes may account for the different effects of genistein and daidzein on the serum triacylglycerol level.

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Abbreviations

C/EBP, CCAAT/enhancer-binding protein; GCOS, Affymetrix GeneChip Operating Software; PPAR, peroxisome proliferator-activated receptor.

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