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Isoflavone-poor soy protein alters the lipid metabolism of rats by SREBP-mediated down-regulation of hepatic genes

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Abstract

Soy intake acts hypolipidemically. Besides isoflavones, soy protein itself is suggested to influence plasma lipid concentrations. We investigated the effects of an alcohol-washed isoflavone-poor soy protein isolate on plasma and liver lipids and the hepatic expression of genes encoding proteins involved in cholesterol and fatty acid metabolism. Therefore, rats were fed diets containing 200 g/kg of either ethanol-extracted soy protein isolate or casein over 22 days. Rats fed soy protein isolate had markedly lower concentrations of liver cholesterol and lower concentrations of triglycerides in the liver and in plasma than rats fed casein ($P<.05$). Rats fed soy protein isolate had lower relative mRNA concentrations of sterol-regulatory element-binding protein (SREBP)-2, 3-hydroxy-3-methylglutaryl coenzyme A reductase, low-density lipoprotein receptor, cholesterol 7α-hydroxylase, apolipoprotein B, Δ9-desaturase and glucose-6-phosphate dehydrogenase in the liver than rats fed casein ($P<.05$). Hepatic mRNA concentration of SREBP-1c tended to be lower in rats fed soy protein isolate ($P<.10$). Hepatic mRNA concentrations of insulin-induced gene (Insig) 1 and Insig-2 and of microsomal triglyceride transfer protein, as well as plasma concentrations of free fatty acids, insulin and glucagon, were not different between the two groups. In conclusion, this study suggests that isoflavone-poor soy protein isolate affects cellular lipid homeostasis by the down-regulation of SREBPs and its target genes in the liver, which are involved in the synthesis of cholesterol and triglycerides.

Keywords: Soy protein isolate; Plasma lipoproteins; Liver lipids; Hepatic genes; Rat

1. Introduction

Soy protein, compared with animal protein sources in humans (e.g., Refs. [1,2]) and animals (e.g., Refs. [3,4]), has a hypolipidemic potential. Soybean contains proteins, lipids, fiber and a variety of bioactive phytochemicals such as saponins, phytic acid, trypsin inhibitors, fiber and isoflavones. Many investigations have focused on the isoflavone components of soy protein, which are thought to be active components of soy protein and to be responsible for many of its beneficial effects on plasma lipids of humans and animals [5]. Afterwards, a series of studies suggests that the lipid-lowering effect of soy protein isolate is mainly attributed to protein content [6–12]. However, in other studies, it was found that soy protein washed with alcohol to remove isoflavones had no cholesterol-reducing effect on cynomolgus monkeys and mice [13,14]. Several follow-up studies have been made to elicit possible mechanisms by which isolated protein from soy may act on lipid metabolism. Based on these cell cultures and on animal and human experiments, suggested mechanisms of soy protein include the stimulation of low-density lipoprotein (LDL) receptor expression and activity [15–20] and increase in the synthesis and fecal excretion of bile acids [4], thereby reducing plasma cholesterol concentration. Others have found a soy-protein-mediated down-regulation of sterol-regulatory element-binding protein-1 (SREBP-1) — a transcriptional factor that is primarily responsible for the regulation of genes involved in fatty acid

Abbreviations: apo, apolipoprotein; CYP7A1, cholesterol 7α-hydroxylase; ER, endoplasmic reticulum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; Insig, insulin-induced gene; LDL, low-density lipoprotein; MTP, microsomal triglyceride transfer protein; SCAP, SREBP cleavage-activating protein; SREBP, sterol-regulatory element-binding protein; VLDL, very-low-density lipoprotein.

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biosynthesis [21–23]. Thus, soy protein isolates are suggested to regulate cholesterol and fatty acid metabolism by influencing genes involved in lipid metabolism at the transcription and protein levels.

However, in most of these studies, the soy protein isolate used for the experiment was not further characterized. It is known that many bioactive compounds, such as isoflavones, are found in soy protein isolate, and their abundance has been shown to depend on the processing method used [24]. If soy protein is extracted by a water-isolation process, many bioactive compounds remain in the soy protein isolate, resulting in a high-isoflavone soy protein. In contrast, if soy protein is isolated by an alcohol-wash process, isoflavones, along with many other bioactive compounds, are removed from the protein, resulting in a low-isoflavone soy protein.

This study was designed to assess the role of an alcohol-washed isoflavone-poor soy protein isolate on the expression of genes that appear to play a critical role in lipid pathways such as lipogenesis, cholesterol biosynthesis and cholesterol uptake from plasma to cells and in steroid catabolism. It is suggested that some of the effects observed with soy protein isolates in previous studies may be different from those observed in this study, which used an alcohol-wash-processed isolated soy protein. The experiment was performed with rats to test whether SREBP-mediated effects on triglycerides and cholesterol metabolism were also obvious in animals fed an isoflavone-poor soy protein isolate compared to animals fed casein. In accordance with typical Western diets, the rats were fed diets containing saturated fats and 0.5 g/kg of cholesterol. First, we focused our attention on the possible mechanism of the cholesterol-lowering effect of soy protein isolate. Besides the concentration of cholesterol in plasma, in lipoproteins and in the liver, we determined the relative mRNA concentrations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and LDL receptor in the liver, which play a critical role in cholesterol biosynthesis and uptake into cells. SREBP-2 has been identified as a transcription factor responsible for the transcription activation of HMG-CoA reductase and LDL receptor [25,26]. Since there is currently no information on SREBP-2 expression in response to soy protein isolate, we also measured the relative mRNA concentration of SREBP-2. Nonactivated SREBP is an integral protein of the endoplasmic reticulum (ER). To gain access to nuclear DNA, SREBP must move in vesicles into the Golgi complex, where it is processed proteolytically [27,28]. Transport from the ER to the Golgi complex is mediated by SREBP cleavage-activating protein (SCAP), a polytropic ER membrane protein that forms complexes with newly synthesized SREBP. The retention of the SCAP/SREBP complex in the ER is mediated by sterol-dependent binding of the complex to one of two ER retention proteins designated as insulin-induced gene (Insig) 1 and Insig-2 [29,30]. Insig-1 and Insig-2 effectively block the activation of SREBP by binding SCAP, thereby preventing it from leaving the ER to escort SREBPs into the Golgi complex [29–32]. If consumption of soy protein alters SREBP expression, we can speculate that this could be due to a modified Insig expression. Thus, we measured the relative mRNA concentration of Insig-1 and Insig-2 in the liver of rats in response to the feeding of isoflavone-poor soy protein and casein. The hepatic gene expression of cholesterol 7α-hydroxylase (CYP7A1), the key enzyme in the synthesis of bile acids from cholesterol, should provide additional information about the possible effects of soy protein on cholesterol excretion via bile acids at the transcription level.

Another purpose of the present work was to study whether the effects of soy protein on the triglyceride and fatty acid metabolism observed in previous studies can be confirmed with the use of ethanol-washed soy protein isolate. It is known that key genes of lipid homeostasis are regulated by insulin and SREBP-1c, a transcriptional factor involved in lipogenesis [33] that has emerged as a mediator of insulin effects [34]. Additionally, recent evidence indicates that soy protein modulates serum insulin concentration, which in turn reduces the hepatic synthesis of triglycerides via the down-regulation of SREBP-1c expression [23]. To test the effects of isoflavone-poor soy protein isolate on fatty acid metabolism, we determined the concentrations of triglycerides in plasma and in the liver; the hepatic mRNA concentration of SREBP-1c, including representative SREBP-1c target genes such as *Δ9-desaturase* and glucose-6-phosphate dehydrogenase (*G6PDH*); and the plasma concentrations of insulin and glucagon. The hepatic gene expression of apolipoprotein (apo) B and microsomal triglyceride transfer protein (MTP), the concentrations of apos in plasma lipoproteins, and the plasma concentrations of free fatty acids may help to elaborate possible mechanisms by which isoflavone-poor soy protein acts hypotriglyceridemically.

It is suggested that protein-induced alterations of cholesterol metabolism may be mediated by differences in the amino acid patterns of dietary proteins or by bioactive peptides [20,35–38]. To gain insights into which amino acids could play an important role, we measured the concentrations of amino acid in the diet and in plasma.

2. Materials and methods

2.1. Animals and experimental diets

Twenty-four male Sprague–Dawley rats (Charles River, Sulzfeld, Germany) with an initial body weight of 76.8±6.0 g were randomly assigned to 2 groups of 12 rats each. All rats were kept individually in Macrolon cages in a room maintained at 22±2°C and 50–60% relative humidity, with lighting from 0600 to 1800 h. All experimental procedures followed established guidelines for the care and handling of laboratory animals and were approved by the Council of Saxony-Anhalt, Germany.

All rats were fed a semisynthetic diet containing 200 g/kg of either casein or isolated soy protein. The diets contained the following ingredients (g/kg): soy protein or casein, 200; sucrose, 200; lard, 100; cellulose, 50; corn starch, 389.5; vitamin and mineral mixture, 60; cholesterol, 0.5. Lard (as type of dietary fat) and cholesterol used for the experimental diets were primarily added to the diet not to increase plasma cholesterol concentration but to mimic Western diets, which are commonly rich in saturated fats and cholesterol. The soy protein diet was additionally supplemented with 3 g/kg of DL-methionine at the expense of cellulose to adjust the methionine concentrations of both diets to a similar level. Vitamins and minerals were supplemented according to the recommendations of the American Institute of Nutrition for rat diets [39]. Food intake was controlled, and the amount of food was slightly below that consumed ad libitum in similar diets of rats in preliminary studies. The amount of food offered daily was increased continuously from 6.0 to 14.0 g. Thus, all rats were fed equal feed rations daily within the duration of the experiment. Rats were fed once daily at 0800 h. Water was freely available from nipple drinkers. The rats were fed experimental diets for 22 days.

2.2. Preparation and characterization of dietary proteins

To minimize the possible effects of soy-protein-associated isoflavones that may be partly responsible for cholesterol reduction by soybean protein, the soy protein isolate (Protein Technologies International, Ieper, Belgium) used for this study was, in addition, ethanol-washed — a process that has been shown to be an efficient way to remove remaining isoflavones [40]. The soy protein isolate that was washed twice with 60% ethanol for 2 h was then filtered, freeze-dried and grounded. Quantitative determination of genistein, the major isoflavone in soy protein, was used to prove the efficacy of the ethanol extraction process. Genistein was analyzed by a modification of the method of Bilia et al. [41]. In brief, soy protein isolate was hydrolyzed with HCl (1 mol/L) in 50% ethanol for 1 h at room temperature. After neutralization with NaOH, isoflavones were extracted thrice with 20 ml of ethyl acetate. After the evaporation of ethyl acetate, isoflavones were dissolved in ethanol and used for quantitative high-performance liquid chromatography (HPLC) analysis of genistein using an ELITE LaChrom HPLC system (Hitachi, Mannheim, Germany) with a gradient of phosphoric acid and acetonitrile as mobile phase at a flow rate of 1 ml/min. Genistein was detected at 329 nm. The original soy protein isolate had a genistein concentration of 0.5 mg/g. After the ethanol washing process, the soy protein isolate had a genistein concentration of 0.08 mg/g. Casein was obtained from Meggle (Wasserburg, Germany) and was not processed further. The crude components of the dietary proteins determined by the official methods of Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten [42] were similar in both proteins. The concentrations of crude protein in casein and soy protein were 93.0 and 98.0 g/kg dry matter, the concentrations of crude ash were 4.7 and 3.6 g/kg dry matter and the concentrations of crude fat were 0.9 and 0.6 g/kg of dry matter, respectively. The amino acid concentrations of the diets are shown in Table 1.

2.3. Sample collection

Rats were not fasted before being killed by decapitation under light anesthesia with diethyl ether because food deprivation led to a significant down-regulation of genes encoding enzymes of lipid metabolism [33], which were to be measured in this study. Plasma was separated from heparinized whole blood by centrifugation at 1500×*g* for 10 min at 4°C. The liver was excised, weighed and immediately snap-frozen in liquid nitrogen. Liver aliquots for RNA isolation were stored at −80°C; other samples were stored at −20°C.

2.4. Amino acid analysis

To determine amino acid concentrations in the diet, the samples were oxidized and then hydrolyzed with 6 M HCl [42]. The separation and quantification of amino acids were performed by ion exchange chromatography following postcolumn derivatization in an amino acid analyzer (Biotronic LC 3000; Eppendorf, Hamburg, Germany). Concentrations of free amino acids in the plasma of rats were measured as isoindole derivatives by HPLC (1100 series; Agilent Technologies, Waldbronn, Germany) after precolumn derivatization, according to Schuster [43]. Isoindole derivates were detected at an excitation wavelength of 337 nm and at an emission wavelength of 454 nm.

Table 1
Concentrations of amino acids in the experimental diets

Amino acid	Casein diet	Soy protein diet
Methionine	5.3	5.0
Cysteine	0.8	2.1
Glycine	3.4	7.0
Lysine	14.7	10.7
Arginine	6.3	14.1
Valine	12.1	8.5
Leucine	17.7	14.5
Isoleucine	8.8	8.4
Histidine	6.4	5.8
Tyrosine	8.6	5.9
Phenylalanine	9.7	9.6
Threonine	7.9	6.5
Serine	10.8	9.3
Alanine	5.5	7.2
Proline	20.2	9.8
Glutamic acid	42.2	37.2
Aspartic acid	13.2	20.8

The separation and quantification of amino acids in the diets were performed by ion exchange chromatography with an amino acid analyzer after postcolumn derivatization.

2.5. Plasma and liver lipids

Plasma lipoproteins [chylomicrons+very-low-density lipoproteins (VLDLs); LDLs; high-density lipoproteins (HDLs)] were separated by stepwise ultracentrifugation (900,000×g at 4°C for 1.5 h, Mikro-Ultrazentrifuge; Sorvall Products, Bad Homburg, Germany) by appropriate density cuts commonly used for the measurement of rat lipoproteins [44–46]. Plasma densities were adjusted by sodium chloride and potassium bromide, and the lipoprotein fractions ρ<1.006 kg/L (defined as VLDL+chylomicrons), 1.006 kg/L<ρ<1.063 kg/L (defined as LDL) and ρ>1.063 kg/L (defined as HDL) were removed by suction. Lipids from the liver were extracted with n-hexane/isopropanol mixture (3:2, vol/vol) [47]. To determine the concentrations of lipids in the liver, aliquots of lipid extracts were dried and lipids were dissolved using Triton X-100 [48]. The concentrations of cholesterol and triglycerides in plasma, in lipoproteins and in the liver were determined using enzymatic reagent kits (cat. nos. 1.14830 and 1.14856; VWR International, Darmstadt, Germany). Fractions of free cholesterol and esterified cholesterol in the liver were separated by thin-layer chromatography and were determined densitometrically [49].

2.6. Estimation of apos

Separated plasma lipoproteins (VLDL+chylomicrons, LDL, HDL) were subjected to analytical sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, and apos were estimated after the densitometric scanning of Coomassie-blue-stained bands, according to a modified method of Karpe and Hamsten [50]. Briefly, polyacrylamide gels were cast by a gradient from 4% to 15% acryl amide. The 4% acryl amide solution contained acryl amide (38.96 g/L), bisacryl amide (1.04 g/L), Tris (0.375 M), SDS (0.1%), tetramethylethylene diamine (0.875 μl/ml) and ammonium persulfate (0.05%), whereas the 15% acryl amide solution contained acryl amide (146.1 g/L), bisacryl amide (3.9 g/L), Tris (0.375 M), SDS (0.1%), tetramethylethylene diamine (0.875 μl/ml) and ammonium persulfate (0.05%).

The total protein content of lipoproteins was determined by BCA assay, with bovine serum albumin as standard. Lipoproteins (250 μg/ml) were dissolved (1:1) in buffer containing 0.22 M Tris, 41% glycerol, 0.7% SDS, 5% mercaptoethanol and 0.002% bromophenol blue, and were subsequently denaturated at 95°C for 5 min. After chilling, 20 μl of denaturated probes containing 2.5 μg of protein was applied to the gels. Electrophoresis was run at 210 V for 1 h. Gels were stained in 0.25% Coomassie blue, 40% methanol and 10% acetic acid for 1 h, and destained in 12% methanol and 7% acetic acid overnight. The gels were scanned with a computer-connected gel documentation system (Gel-Pro Analyzer; Intas, Göttingen, Germany). Bands of apoB100, apoB48, apoE and apoAI were identified by comparing the R_f values with those of high-molecular-weight standards (Serva, Heidelberg, Germany).

2.7. Measurement of free fatty acids, insulin and glucagon in plasma

Concentrations of free fatty acids in plasma were assayed using an acyl-CoA-oxidase-based colorimetric kit (Wako NEFA-C; Wako Chemicals, Neuss, Germany). The concentration of plasma glucagon was determined by radioimmunoassay (RIA) using a commercially available rat RIA kit (GL-2K; Linco Research, St. Charles, MO, USA) with a detection limit of 5.8 pmol/L. The concentration of plasma insulin was determined by enzyme-linked immunoassay using a commercially available rat insulin ELISA kit (EIA-2048; DRG Instruments GmbH, Marburg, Germany). The detection limit of insulin was 12.2 pmol/L.

2.8. Relative mRNA concentrations

Isolation of total RNA from the liver and cDNA synthesis were performed as described previously [51]. The relative mRNA quantities of SREBP-1c and SREBP-2, HMG-CoA reductase (EC 1.1.1.34), LDL receptor, apoB, CYP7A1 (EC 1.14.13.17), Insig-1 and Insig-2, MTP, G6PDH (EC 1.1.1.49) and Δ9-desaturase (EC 1.14.99.5), as related to the reference gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA, were determined by reverse transcription polymerase chain reaction (PCR). cDNA was amplified in a 20-μl reaction containing 2 μl of RT mixture, 0.2 μl of Biotherm DNA polymerase, 2 μl of 10× PCR buffer, 0.4 μl of DNA polymerization mix (all from Genecraft, Lüdinghausen, Germany) and gene-specific primers obtained from Carl Roth (Karlsruhe, Germany). The primer sequences used were as follows: 5′-GCA-TGG-CCT-TCC-GTG-TTC-C-3′ (forward) and 5′-GGG-TGG-TCC-AGG-GTT-TCT-TAC-TC-3′ (reverse) for rat GAPDH; 5′-AAG-GGG-CGT-GCA-AAG-ACA-ATC-3′ (forward) and 5′-ATA-CGG-CAC-GGA-AAG-AAC-CAT-AGT-3′ (reverse) for rat HMG-CoA reductase; 5′-ACG-GGC-TGG-CGG-TAG-ACT-GGA-3′ (forward) and 5′-TGA-GGC-GGT-TGG-CAC-TGA-AAA-3′ (reverse) for rat LDL receptor; 5′-GGA-GCC-ATG-GAT-TGC-ACA-TT-3′ (forward) and 5′-AGG-AAG-GCT-TCC-AGA-GAG-GA-3′ (reverse) for rat SREBP-1c; 5′-CCG-GTA-ATG-ATG-GGC-CAA-GAG-AAA-G-3′ (forward) and 5′-AGG-CCG-GGG-GAGACA-TCA-GAA-G-3′ (reverse) for rat SREBP-2; 5′-CAA-GAC-GCA-CCT-CGC-TAT-CC-3′ (forward) and 5′-CCG-GCA-GGT-CAT-TCA-GTT-G-3′ (reverse) for rat CYP7A1; 5′-GGA-AAG-GGG-AGG-GAA-AAG-GTT-3′ (forward) and 5′-TTA-GGT-AGG-GGC-TCA-CAT-TAT-TGG-3′ (reverse) for rat apoB; 5′-CGC-GAG-TCT-AAA-ACC-CGA-GTG-3′ (forward) and 5′-CCC-TGC-CTG-TAG-ATA-GCC-TTT-CAT-3′ (reverse) for rat MTP; 5′-CCA-GCC-TCC-ACA-AGC-ACC-TCA-AC-3′ (forward) and 5′-AAT-TAG-CCC-CCA-CGA-CCC-TCA-GTA-3′ (reverse) for G6PDH; 5′-CCG-TGG-CTT-TTT-CTT-CTC-TCA-3′ (forward) and 5′-CTT-TCC-GCC-CTT-CTC-TTT-G-3′ (reverse) for Δ-9 desaturase; 5′-ATT-TGG-CGT-GGT-CCT-GGC-TCT-GG-3′ (for-

Table 2
Concentrations of amino acids in the plasma of rats fed the experimental diets

Amino acid	Casein diet (μmol/L)	Soy protein isolate diet (μmol/L)
Methionine	56±10	61±8
Taurine	60±20	122±38*
Glycine	158±27	288±47*
Lysine	474±61	258±54*
Arginine	89±17	134±23*
Valine	186±56	103±18*
Leucine	119±32	86±21*
Isoleucine	83±20	72±14
Histidine	43±12	36±9
Tyrosine	89±23	63±11*
Phenylalanine	42±7	45±7
Tryptophan	67±11	69±8
Threonine	533±140	189±32*
Serine	267±37	236±29*
Alanine	533±61	540±78
Glutamic acid	93±12	98±16
Glutamine	665±88	603±50
Asparagine	450±107	568±76*

Values are expressed as mean±SD ($n=12$).
The concentrations of free amino acids in the plasma were measured by HPLC as isoindole derivatives after precolumn derivatization.
* Different from rats fed the casein-based diet ($P<.05$).

ward) and 5′-GCG-TGG-CTA-GGA-AGG-CGA-TGG-TG-3′ (reverse) for Insig-1; 5′-AAG-CGT-GGC-CCC-TAC-ATT-TCC-TC-3′ (forward) and 5′-GGC-CAC-GCA-GCG-CAT-AAC-AC-3′ (reverse) for Insig-2. The DNA of GAPDH and the genes involved in lipid metabolism, which were to be measured in this study, were amplified in cycles of 20 s of denaturation at 95°C, 30 s of annealing at primer-specific temperatures and 40 s of elongation at 72°C. Fluorescence was measured at 72°C. A final melting curve guaranteed the authenticity of the target product.

2.9. Statistical analysis

The means of the two groups were compared by Student's t test for each experiment. Values are presented as mean±S.D. The means were considered significantly different at $P<.05$.

3. Results

3.1. Body weight gain and concentrations of plasma amino acids

Food intake throughout the feeding period was the same for each rat, averaging 10.5 g/day. Body weight gains did not differ between the two groups of rats (casein group: 5.45±0.22 g/day; soy protein isolate group: 5.54±0.27 g/day; $n=12$). Rats fed isoflavone-poor soy protein isolate had lower concentrations of plasma lysine, valine, leucine, tyrosine, threonine and serine, and higher concentrations of plasma taurine, glycine, arginine and asparagine than rats fed casein (Table 2). The plasma concentrations of methionine, isoleucine, histidine, phenylalanine, tryptophan, alanine, glutamic acid and glutamine were not different between the two groups of rats (Table 2).

3.2. Lipid concentrations in plasma, lipoproteins and liver, and apo concentrations in lipoproteins

Rats fed soy protein isolate had lower concentrations of cholesterol in the triglyceride-rich lipoprotein fraction (chylomicrons+VLDL; Table 3). Concentrations of cholesterol in plasma, LDLs and HDLs were not different between the two groups of rats (Table 3). Concentrations of triglycerides in plasma, triglyceride-rich lipoprotein fraction (chylomicrons+VLDL), LDL and HDL were lower in rats fed soy protein isolate than in rats fed casein (Table 3). The concentrations of total protein, apoB100 and apoB48 in the triglyceride-rich lipoprotein fraction (chylomicrons+VLDL) were lower in rats fed soy protein isolate than in rats fed casein (Table 3). Concentrations of apoE in the triglyceride-rich lipoprotein fraction ($\rho<1.006$ kg/L; chylomicrons+VLDL), of apoB100 in LDL and of apoAI in HDL were not different between the two groups of rats (Table 3). Liver weight per 100 g of body weight was lower in rats fed soy protein isolate than in rats fed casein (casein group: 4.51±0.37 g/100 g body weight; soy protein isolate group: 4.14±0.28 g/100 g of body weight; $n=12$, $P<.05$). Concentrations of total cholesterol, cholesteryl esters and triglycerides in the liver were lower in rats fed soy protein isolate than in rats fed casein (Fig. 1).

Table 3
Composition of plasma lipoproteins in rats fed the experimental diets

Dietary protein	Casein diet	Soy protein isolate diet
Plasma		
Total cholesterol (mmol/L)	2.30±0.30	2.13±0.30
Triglycerides (mmol/L)	2.35±0.86	1.20±0.79*
VLDL+chylomicrons		
Total cholesterol (mmol/L)	0.47±0.12	0.23±0.09*
Triglycerides (mmol/L)	1.67±0.58	1.02±0.34*
Total protein (mg/L)	414±125	269±147*
ApoB100 (relative AU)	1.00±0.41	0.34±0.36*
ApoB48 (relative AU)	1.00±0.25	0.46±0.22*
ApoE (relative AU)	1.00±0.29	0.76±0.26
LDL		
Total cholesterol (mmol/L)	0.44±0.09	0.49±0.09
Triglycerides (mmol/L)	0.32±0.08	0.22±0.07*
Total protein (mg/L)	235±46	260±43
ApoB100 (relative AU)	1.00±0.09	1.00±0.15
HDL		
Total cholesterol (mmol/L)	1.46±0.31	1.48±0.24
Triglycerides (mmol/L)	0.12±0.05	0.08±0.03*
Total protein (mg/L)	1671±420	1706±425
ApoAI (relative AU)	1.00±0.06	0.81±0.34

Values are expressed as mean±S.D. ($n=12$).
AU, absorbance units.
The concentrations of triglycerides and cholesterol in plasma and lipoproteins were determined using commercially available enzymatic reagent kits. Apos of different lipoprotein fractions were separated by SDS-PAGE and estimated after the densitometric scanning of Coomassie-blue-stained bands.
* Different from rats fed the casein-based diet ($P<.05$).

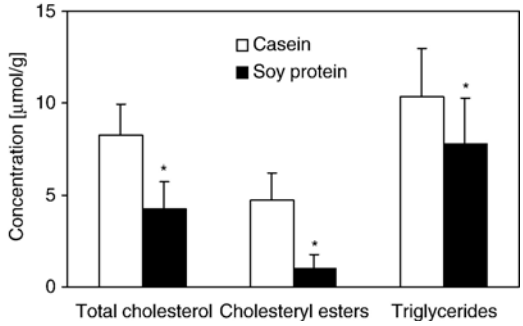

Fig. 1. Concentrations of total cholesterol, cholesteryl esters and triglycerides in the liver of male rats fed diets containing 200 g/kg of casein or soy protein isolate for 22 days. Each bar represents mean±S.D. ($n=12$). *Different from rats fed the casein-based diet (expressed as 100; Student's t test, $P<.05$).

3.3. Plasma concentrations of free fatty acids, insulin and glucagon

The plasma concentrations of free fatty acids (casein group: 0.46±0.13 mmol/L; soy protein isolate group: 0.42±0.12 mmol/L; $n=12$), insulin (casein group: 63.3±16.9 pmol/L; soy protein isolate group: 54.1±10.1 pmol/L; $n=12$) and glucagon (casein group: 22.6±7.9 pmol/L; soy protein isolate group: 23.2±7.0 pmol/L; $n=12$) were not different between rats fed casein and those fed isoflavone-poor soy protein isolate. Additionally, no significant difference in insulin/glucagon ratio was observed between rats fed casein and those fed soy protein isolate (casein group: 2.80±1.41 pmol/pmol; soy protein isolate group: 2.33±0.77 pmol/pmol; $n=12$).

3.4. mRNA concentrations of hepatic genes

Rats fed soy protein isolate had lower relative mRNA concentrations of SREBP-2, HMG-CoA reductase, LDL receptor and CYP7A1 (Fig. 2). The relative gene expressions of apoB, G6PDH and Δ9-desaturase were also lower in rats fed soy protein isolate than in rats fed casein (Fig. 3).

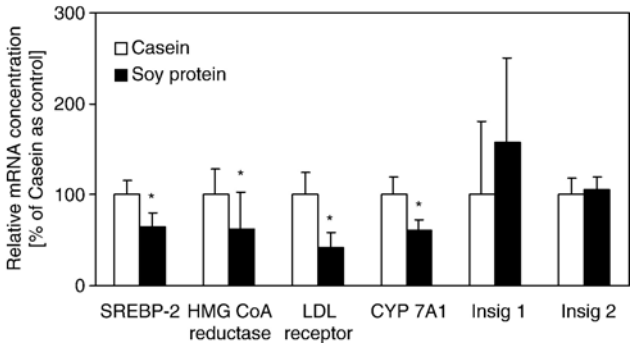

Fig. 2. Relative mRNA concentrations of SREBP-2, HMG-CoA reductase, LDL receptor, CYP7A1, Insig-1 and Insig-2 in the liver of male rats fed diets containing 200 g/kg of casein or soy protein isolate for 22 days. Values were related to the reference gene *GAPDH*. Each bar represents mean±S.D. ($n=12$). *Different from rats fed the casein-based diet (expressed as 100; Student's t test, $P<.05$).

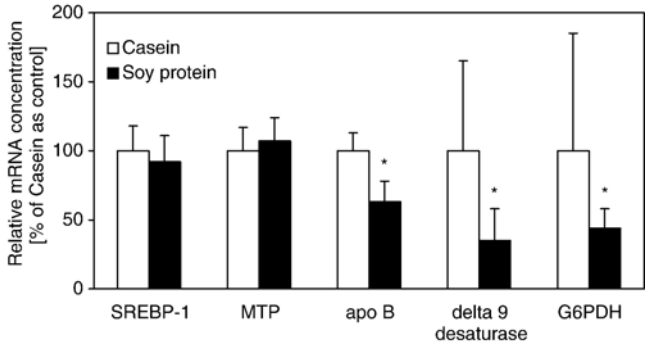

Fig. 3. Relative mRNA concentrations of SREBP-1c, MTP, apoB, Δ9-desaturase and G6PDH. Values were related to the reference gene *GAPDH*. Each bar represents mean±S.D. ($n=12$). *Different from rats fed the casein-based diet (expressed as 100; Student's t test, $P<.05$).

Gene expression of SREBP-1c tended to be lower in the group fed soy protein than in the group fed casein ($P<.10$). The relative mRNA concentrations of Insig-1 and Insig-2 and MTP were not different between the two groups of rats (Fig. 3).

4. Discussion

The results of this study demonstrate that ingestion of an ethanol-extracted isoflavone-poor soy protein isolate compared to casein led to a marked decrease of the concentrations of liver cholesterol, liver triglycerides and plasma triglycerides. We found that isoflavone-poor soy protein isolate down-regulates genes involved in cholesterol synthesis (such as *HMG-CoA reductase*) and cholesterol uptake (such as *LDL receptor*) via a reduced level of mRNA coding for SREBP-2. This finding is in contrast to those of previous experiments, in which soy protein increased mRNA concentrations of genes involved in cholesterol synthesis [4] and LDL uptake [15–20]. However, the SREBP-2-mediated up-regulation of these genes has been also observed after the incubation of HepG2 cells with an isoflavone mixture [52]. Thus, we can speculate that the increased mRNA concentrations of HMG-CoA reductase and LDL receptor observed in previous studies with nonpurified soy protein are possibly caused by soy-protein-associated isoflavones, while soy-protein-purified isoflavones probably exert the opposite effect.

The down-regulation of HMG-CoA reductase observed in the soy-protein-fed rats in this study is suggested to be, at least in part, responsible for the diminished cholesterol concentration in the liver. However, an increased excretion of cholesterol from the liver into the intestine via bile acids is unlikely to be the reason for the diminished cholesterol concentration in the liver because the concentration of mRNA coding for CYP7A1, the initial and rate-limiting enzyme in the conversion of cholesterol to 7α-hydroxylated bile acids, was decreased by 40% in rats fed soy protein isolate compared to rats fed casein. This confirms the findings from a recent study in which the soy-protein-induced

reduction of hepatic cholesterol was associated with a low expression of liver X receptor-α and the target gene *CYP7A1* [53], and supports the assumption that cholesterol excretion via bile acids may probably not account for low cholesterol concentrations in the liver.

The concentration of cholesterol in plasma and LDL, which was not different from that in the groups fed soy protein or casein, is in contrast to previously published results obtained from human and animal studies showing a distinct reduction of plasma and/or LDL cholesterol after the administration of soy protein [1,54]. The LDL receptor is a major regulator of circulating LDL cholesterol [55]. A decreased removal of LDL from the circulation due to a diminished LDL receptor expression, which was observed in soy-protein-fed rats in this study, may explain the finding that the concentration of cholesterol in plasma and LDL increased although the key gene involved in cholesterol synthesis was regulated. We suggest that the lower mRNA concentrations of LDL receptor in rats fed soy protein isolate may explain the finding that plasma LDL concentration remained unchanged although the liver cholesterol concentration was markedly decreased.

The diminished triglyceride concentrations observed in the liver and in the plasma of rats fed isoflavone-poor soy protein isolate could result from the moderate down-regulation of SREBP-1c ($P<.10$) and the significant down-regulation of SREBP-1c target genes ($P<.05$) involved in fatty acid synthesis, such as *G6PDH* and *Δ9-desaturase*. However, these findings are consistent with previous studies in which rats were fed soy protein diets [22,23,53]. Thus, the triglyceride-lowering effect observed with soy intake seems to be, at least in part, caused by the protein itself. Recent evidence indicates that liver key genes involved in triglyceride homeostasis are regulated by insulin [34], and the findings of Ascencio et al. [23] indicate that soy protein regulates SREBP-1c expression by modulating insulin concentration and insulin/glucagon ratio in the serum. However, significant differences in the plasma concentrations of insulin and glucagon were not observed in our study, although a slight reduction of the insulin/glucagon ratio was obvious in rats fed soy protein isolate compared to rats fed casein. However, the reason for the failure of soy protein to affect plasma insulin concentration in this study could be due to the fact that we focused on a single measurement of hormone concentration at one point in time. The measurement of plasma insulin concentration in defined time intervals after the serving of food according to a fixed time schedule, combined with the determination of the area under the curve of insulin, should be elucidated in further studies.

In addition to the fact that triglyceride synthesis in the liver seemed to be lower in animals fed soy protein than in animals fed casein via the down-regulation of lipogenic genes, another interesting finding was that the level of mRNA coding for apoB and the concentrations of apoB100 and apoB48 were reduced in response to soy protein administration. This is consistent with a previous work showing that incubation of HepG2 cells with soy protein peptides devoid of isoflavone components inhibits apoB production in the liver and the secretion of apoB from the liver into the plasma [20]. Recent studies using HepG2 cells and primary rat hepatocytes have shown that the rate of cholesterol synthesis and the availability of cellular cholesteryl esters may regulate apoB production and secretion and the gene expression of MTP [56–58]. However, although our data are not indicative of an altered MTP expression in the liver of rats fed soy protein isolate compared to rats fed casein, the significant differences of apoB expression and concentrations between the two groups of rats could result from the diminished availability of cellular cholesteryl esters.

Together, this study suggests that the diminished deposition of cholesterol and triglycerides in the livers of rats fed alcohol-washed soy protein isolate compared to rats fed casein is mediated by a down-regulation of SREBPs and its target genes. SREBPs are synthesized as inactive precursors bound to ER membranes. Insig-1 and Insig-2 have been recently identified as modulators of SREBP activity [29,30]. Insig-1 and Insig-2 are proteins that prevent the movement of the SREBP/SCAP complex from the ER to the Golgi complex, thus blocking proteolytic cleavage and the transcriptional activation of SREBP [29,30]. Our study is therefore the first to show that Insig expression, which has been shown to be critical for SREBP processing, was not altered by soy protein isolate. Therefore, we suggest that SREBP-mediated effects on lipid metabolism were regulated mainly at the transcription level and not via an Insig-mediated alteration of SREBP processing.

Identifying which of the components of soy protein isolate is the major contributor to effects on lipid metabolism is still a subject of controversy. Alterations of plasma amino acids, mediated by differences in amino acid patterns of dietary proteins, could contribute to the observed effects on lipid metabolism. Some studies showed that the effect of soy protein on lipid metabolism is at least partially mediated by its low methionine content [35–37]. We suggest that methionine might not be involved in the effects of soy protein on the lipid metabolism observed in this study because we adjusted methionine concentrations of both diets to a similar level. A recent study has shown that dietary proteins with low lysine/arginine ratios act hypocholesterolemically [38], and we found that this ratio was distinctly lower in the soy protein diet than in the casein diet used in our rat study and in the plasma of corresponding rats (the lysine/arginine ratio was 2.33 for casein and 0.76 for soy protein). Therefore, it is possible that the effects of soy protein, in comparison to those of casein, were mediated by the concentrations of arginine and lysine in the respective diets. It is also possible that the effects of soy protein were caused by specific peptides. Alpha and alpha′ subunits from 7S soy globulin have been identified as peptides from soy protein that may regulate cholesterol homeostasis in HepG2

cells [20]. Whether amino acids or peptides are actually responsible for the observed effects of soy protein on hepatic genes remain to be clarified in further studies. However, it seems not very likely that the remaining isoflavones in the ethanol-washed soy protein isolate may contribute to the observations made because their concentration is extremely low compared to the concentrations normally used for the induction of hypolipidemia (e.g., Refs. [52,59]).

In conclusion, this study shows that a dietary protein with a low isoflavone content acts hypotriglyceridemically and lowers the concentrations of cholesterol and triglycerides in the rat liver. This effect may be due to the significant down-regulation of SREBP-2, a trend toward lower SREBP-1c mRNA concentrations, and the diminished mRNA concentrations of its target genes in the liver, which are involved in the synthesis of cholesterol and triglycerides, respectively.

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References

[1] Bakhit RM, Klein BP, Essex-Sorlie D, Ham JO, Erdman Jr JW, Potter SM. Intake of 25 g soybean protein reduces plasma cholesterol in men with elevated cholesterol concentrations. J Nutr 1994;124:213–22.
[2] Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soybean protein intake on serum lipids. N Engl J Med 1995; 333:276–82.
[3] Sirtori CR, Lovati MR, Manzoni C, Gianazza E, Bondioli A, Staels B, et al. Reduction of serum cholesterol by soybean proteins: clinical experience and potential molecular mechanisms. Nutr Metab Cardiovasc Dis 1998;8:334–40.
[4] Tachibana N, Matsumoto I, Fukui K, Arai S, Kato H, Abe K, et al. Intake of soy protein isolate alters hepatic gene expression in rats. J Agric Food Chem 2005;53:4253–7.
[5] Potter SM. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J Nutr 1995;125:606S–11S.
[6] Forsythe WA. Comparison of dietary casein or soy protein effects on plasma lipids and hormone concentrations in the gerbil (*Meriones unguiculatus*). J Nutr 1986;116:1165–71.
[7] Greaves KA, Parks JS, Willians JK, Wagner JD. Intact dietary soy protein, but not adding an isoflavone-rich soy extract to casein, improves plasma lipids in ovariectomized cynomolgus monkeys. J Nutr 1999;129:1585–92.
[8] Gardner CD, Newell KA, Cherin R, Haskell WL. The effect of soy protein with or without isoflavones relative to milk protein on plasma lipids in hypercholesterolemic postmenopausal women. Am J Clin Nutr 2001;73:728–35.
[9] Adams MR, Golden DL, Anthony MS, Register TC, Williams JK. The inhibitory effect of soy protein isolate on atherosclerosis in mice does not require the presence of LDL receptors or alteration of plasma lipoproteins. J Nutr 2002;132:43–9.
[10] Fukui K, Tachibana N, Wanezaki S, Tsuzaki S, Takamatsu K, Yamamoto T, et al. Isoflavone-free soy protein prepared by column chromatography reduces plasma cholesterol in rats. J Agric Food Chem 2002;50:5717–21.
[11] Jenkins DJ, Kendall CW, Jackson JC, Connelly PW, Parker T, Faulkner D, et al. Effects of high- and low-isoflavones soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. Am J Clin Nutr 2002;76:365–72.
[12] Lichtenstein AH, Jalbert SM, Adlercreutz HZ, Goldin BR, Rasmussen EJ, Schaefer EJ, et al. Lipoprotein response to diets high in soy or animal protein with and without isoflavones in moderately hypercholesterolemic subjects. Arterioscler Thromb Vasc Biol 2002; 22:1852–8.
[13] Anthony MS, Clarkson TB, Bullock BC, Wagner JD. Soy protein versus soy phytooestrogens in the prevention on diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. Arterioscler Thromb Vasc Biol 1997;17:2524–31.
[14] Kirk EA, Sutherland P, Wang SA, Chait A, LeBoeuf RC. Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. J Nutr 1998; 128:954–9.
[15] Sirtori CR, Galli G, Lovati MR, Carrara P, Bosisio E, Kienle MG. Effects of dietary proteins on the regulation of liver lipoprotein receptors in rats. J Nutr 1984;114:1493–502.
[16] Lovati MR, Manzoni C, Canavesi A, Sirtori M, Vaccarino V, Marchi M, et al. Soybean protein diet increases low density lipoprotein receptor activity in mononuclear cells from hypercholesterolemic patients. J Clin Invest 1987;80:1498–502.
[17] Lovati MR, Manzoni C, Agostinelli P, Ciappellano S, Mannucci L, Sirtori CR. Studies on the mechanism of the cholesterol lowering activity of soy proteins. Nutr Metab Cardiovasc Dis 1991;1:18–24.
[18] Lovati MR, Manzoni C, Gianazza E, Sirtori CR. Soybean protein products as regulators of liver low-density lipoprotein receptors: I. Identification of active β-conglycinin subunits. J Agric Food Chem 1998;46:2474–80.
[19] Manzoni C, Lovati MR, Gianazza E, Sirtori CR. Soybean protein products as regulators of liver low-density lipoprotein receptors: II. α-α′ Rich commercial soy concentrate and α′ deficient mutant differently affect low-density lipoprotein receptor activation. J Agric Food Chem 1998;46:2481–4.
[20] Lovati MR, Manzoni C, Gianazza E, Arnoldi A, Kuroswka E, Carroll KK, et al. Soy protein peptides regulate cholesterol homeostasis in Hep G2 cells. J Nutr 2000;130:2543–9.
[21] Tovar AR, Murguía F, Cruz C, Hernández-Pando R, Aguilar-Salinas CA, Pedraza-Chaverri J, et al. 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;132:2562–9.
[22] Nagasawa A, Fukui K, Kojima M, Kishida K, Maeda N, Nagaretani H, et al. Divergent effects of soy protein diet on the expression of adipocytokines. Biochem Biophys Res Commun 2003;311:909–14.
[23] Ascencio C, Torres N, Isoard-Acosta F, Gómez-Pérez FJ, Hernández-Pando R, Tovar AR. Soy protein affects serum insulin and hepatic SREBP-1 mRNA and reduces fatty liver in rats. J Nutr 2004; 134:522–9.
[24] Liu K. Soybeans: chemistry, technology and utilization. Gaithersburg: Aspen; 1999.
[25] Vallett SM, Sanchez HB, Rosenfeld JM, Osborne TF. A direct role of sterol regulatory element binding protein in activation of 3-hydroxy-3-methylglutaryl coenzyme A reductase gene. J Biol Chem 1996;271: 12247–53.
[26] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002;109:1125–31.
[27] Nohturfft A, DeBose-Boyd RA, Scheek S, Goldstein JL, Brown MS. Sterols regulate cycling of SREBP cleavage-activating protein (SCAP) between endoplasmic reticulum and Golgi. Proc Natl Acad Sci U S A 1999;96:11235–40.
[28] Nohturfft A, Yabe D, Goldstein JL, Brown MS, Espenshade PJ. Regulated step in cholesterol feedback localized to budding of SCAP from ER membranes. Cell 2000;102:315–23.
[29] Yabe D, Brown MS, Goldstein JL. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol

regulatory element-binding proteins. Proc Natl Acad Sci U S A 2002;99:12753–8.
[30] Yang T, Espenshade PJ, Wright ME, Yabe D, Gong Y, Aebersold R, et al. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. Cell 2002;110:489–500.
[31] Sever N, Song BL, Yabe D, Goldstein JL, Brown MS, DeBose-Boyd RA. Insig-dependent ubiquitination and degradation of mammalian 3-hydroxy-3-methylglutaryl-CoA reductase stimulated by sterols and geranylgeraniol. J Biol Chem 2003;278:52479–90.
[32] Sever N, Yang T, Brown MS, Goldstein JL, DeBose-Boyd RA. Accelerated degradation of HMG-CoA reductase mediated by binding of insig-1 to its sterol-sensing domain. Mol Cell 2003;11:25–33.
[33] Shimano H, Yahagik N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, et al. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 1999;274:35832–9.
[34] Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Mol Cell Biol 1999;19:3760–8.
[35] Sugiyama K, Kanamori H, Akachi T, Yamakawa A. Amino acid composition of dietary proteins affects plasma cholesterol concentration through alteration of hepatic phospholipid metabolism in rats fed a cholesterol-free diet. J Nutr Biochem 1996;7:40–8.
[36] Morita T, Oh-Hashi A, Takei K, Ikai M, Ksaoka S, Kiriyama S. Cholesterol-lowering effects of soybean, potato and rice proteins depend on their low methionine contents in rat fed a cholesterol-free purified diet. J Nutr 1997;127:470–7.
[37] Sugiyama K, Yamakawa AA, Kumazawa A, Saeki S. Methionine content of dietary proteins affects the molecular species composition of plasma phosphatidylcholine in rats fed a cholesterol-free diet. J Nutr 1997;127:600–7.
[38] Gudbrandsen OA, Wergedahl H, Liaset B, Espe M, Berge RK. Dietary proteins with high isoflavone content or low methionine–glycine and lysine–arginine ratios are hypocholesterolaemic and lower the plasma homocysteine level in male Zucker fa/fa rats. Br J Nutr 2005; 94:321–30.
[39] Reeves PG, Nielsen FH, Fahey Jr GC. 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 1993;123:1939–51.
[40] Fukui K, Tachibana N, Fukuda Y, Takamatsu K, Sugano M. Ethanol washing does not attenuate the hypocholesterolemic potential of soy protein. Nutrition 2004;29:984–90.
[41] Bilia AR, Salvini D, Mazzi G, Vincieri FF. Characterization of calendula flower, milk-thistle fruit, and passion flower tinctures by HPLC-DAD and HPLC-MS. Chromatographia 2001;53:3–4.
[42] Naumann C, Bassler R. Methodenbuch band: III. Die chemische Untersuchung von Futtermitteln. Darmstadt (Germany): VDLUFA-Verlag; 1993.
[43] Schuster R. Determination of amino acids in biological, pharmaceutical, plant and food samples by automated precolumn derivatization and high-performance liquid chromatography. J Chromatogr 1988; 431:271–84.
[44] Sparks JD, Phung TL, Bolognino M, Cianci J, Khurana R, Peterson RG, et al. Lipoprotein alterations in 10- and 20-week-old Zucker diabetic fatty rats: hyperinsulinemic versus insulinopenic hyperglycemia. Metabolism 1998;47:1315–24.
[45] Giudetti AM, Beynen AC, Lemmens AG, Gnoni GV, Geelen MJH. Hepatic fatty acid metabolism in rats fed diets with different contents of $C_{18:0}$, $C_{18:1}$ *cis* and $C_{18:1}$ *trans* isomers. Br J Nutr 2003;90:887–93.
[46] Sirtori CR, Lovati MR, Manzoni C, Castiglioni S, Duranti M, Magni C, et al. Proteins of white lupin seed, a naturally isoflavone-poor legume, reduced cholesterolemia in rats and increase LDL receptor activity in HepG2 cells. J Nutr 2004;134:18–23.
[47] Hara A, Radin NS. Lipid extraction of tissues with a low toxicity solvent. Anal Biochem 1978;90:420–6.
[48] De Hoff JL, Davidson JH, Kritchevsky D. An enzymatic assay for determining free and total cholesterol in tissues. Clin Chem 1978; 24:433–5.
[49] Hojnacki JL, Nicolosi RJ, Hayes KC. Densitometric quantitation of neutral lipids on ammonium sulfate impregnated thin-layer chromatograms. J Chromatogr 1976;128:133–9.
[50] Karpe F, Hamsten A. Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS PAGE. J Lipid Res 1994;35:1311–7.
[51] Brandsch C, Nass N, Eder K. A thermally oxidized dietary oil does not lower the activities of lipogenic enzymes in mammary glands of lactating rats but reduces the milk triglyceride concentration. J Nutr 2004;134:631–6.
[52] Mullen E, Brown RM, Osborne TF, Shay NF. Soy isoflavones affect sterol regulatory element binding proteins (SREBPs) and SREBP-regulated genes in HepG2 cells. J Nutr 2004;134:2942–7.
[53] Tovar A, Manzano N, Torres N. Metabolism of cholesterol and fatty acids in nephrotic syndrome and its regulation by sterol regulatory element binding protein (SREBPs). Effect of soy protein consumption. Gac Med Mex 2005;141:407–15.
[54] Carroll KK, Kurowska EM. Soy consumption and cholesterol reduction: review of animal and human studies. J Nutr 1995;125: 587S–94S.
[55] Meddings J, Spady D, Dietschy J. Kinetic constants for receptor-dependent and receptor-independent low density lipoprotein transport in the tissues of the rat and hamster. J Clin Invest 1986;77:1474–81.
[56] Cianflone KM, Yasruel Z, Rodriguez MA, Vas D, Sniderman AD. Regulation of apoB secretion form HepG2 cells: evidence for a critical role for cholesteryl ester synthesis in the response to a fatty acid challenge. J Lipid Res 1990;31:2045–55.
[57] Tanaka M, Jingami H, Otani H, Cho M, Ueda Y, Arai H, et al. Regulation of apolipoprotein B production and secretion in response to the change of intracellular cholesteryl ester contents in rabbit hepatocytes. J Biol Chem 1993;268:12713–8.
[58] Ooyen C, Zecca A, Bersino AM, Catapano AL. NK-104, a potent 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, decreases apolipoprotein B-100 secretion from Hep G2 cells. Atherosclerosis 1999;145:87–95.
[59] Ali AA, Velasquez MT, Hansen CT, Mohamed AI, Bhathena SJ. Effects of soybean isoflavones, probiotics, and their interactions on lipid metabolism and endocrine system in an animal model of obesity and diabetes. J Nutr Biochem 2004;15:583–90.