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Modulation of carbohydrate metabolism and peptide hormones by soybean isoflavones and probiotics in obesity and diabetes

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Abstract

Soybean and its isoflavones have been shown to have beneficial effects on carbohydrate and lipid metabolism and on renal function. Probiotics may potentiate the beneficial effects of isoflavones by converting the inactive isoflavone glycoside to aglycones, which are biologically active, thereby producing a synergistic effect. We therefore studied the effects of soybean isoflavones in the presence and absence of probiotics on glucose and triglyceride metabolism and the peptide hormones involved in their metabolism. Lean and obese SHR/N-cp rats were fed AIN-93 diets containing 0.1% soybean isoflavone mixture, 0.1% probiotics mixture or both. Plasma was analyzed for glucose, triglycerides, parameters of renal function and peptide hormones—insulin, leptin, glucagon and ACTH—that are involved in glucose and lipid metabolism. Isoflavones given alone lowered plasma glucose in both phenotypes while triglyceride was decreased only in lean animals. Isoflavones also lowered aspartate amino transferase and alanine amino transferase in both phenotypes. Isoflavones had significant effect on plasma insulin, leptin and glucagon in lean rats but not in obese rats. Thus, our data show that in lean animals, isoflavones have hypoglycemic and hypolipidemic effect, and the effect is mediated by changes in peptide hormones. When lipid levels are very high as in obese rats, isoflavones fail to lower plasma triglyceride levels. Probiotics do not appear to enhance the effect of isoflavones.

Keywords: Metabolism; Soybean; Isoflavone; Probiotics; Peptide hormones; Obesity; Diabetes

1. Introduction

In recent years, there has been a considerable interest in the effects of soybean and soy-based products in human health, especially their potential role in cardiovascular disease. This was further spurred by the approval by the United States Food and Drug Administration in October 1999 of a health claim by food manufacturers that consumption of 20 g of soy protein, as part of a diet low in saturated fat and cholesterol, may help reduce the risk of coronary heart disease [1]. This claim is based on accumulated evidence from numerous studies in humans and animals, showing that soy protein reduces serum cholesterol, which is one of the major risk factors of cardiovascular disease [2–9].

The lipid-lowering effect of soy protein is well documented. Recent studies in normal and hypercholesterolemic human subjects confirm the reduction in plasma total and non-HDL cholesterol without significant effect on HDL cholesterol and triglycerides by soy protein [10–12]. However, it is not clear whether the effect of soy protein is related to isoflavones. Similarly, the data are lacking on the effect of isoflavones on serum triglyceride and uric acid. This is of interest, since both hypertriglyceridemia and hyperuricemia are known to have an independent relationship with other cardiovascular risk factors, such as obesity, type II diabetes mellitus and hypertension, which are major components of the insulin resistance syndrome [13–18]. Moreover, an elevated serum triglyceride level is not only a common lipid abnormality in free-living individuals but also an independent risk factor for coronary artery disease [19–21].

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There have been few studies suggesting that soy foods may have beneficial effect on obesity and diabetes [22]. However, the studies on the effects of soy on glucose metabolism are lacking. Taha and Wasif [23] reported that in alloxan diabetic hypercholesterolemic rats, soy flour added to whole durum meal lowered the elevated plasma glucose concentration. However, in obese Wistar fatty rats, Iritani et al. [24] observed no significant difference between dietary soy protein and casein on plasma glucose concentration. Thus, the effect of soy protein and isoflavones on glucose metabolism is unclear. In addition, studies on the effect of probiotics on carbohydrate metabolism are also lacking. Therefore, in the present study, we sought to determine whether soy isoflavones and/or probiotics from lactobacilli have significant effect on carbohydrate metabolism and triglyceride in a genetic model of obesity and diabetes, and compared it to lean controls. Secondly, since carbohydrate and lipid metabolisms are influenced by peptide hormones, we further studied the effects of soy isoflavones and probiotics on peptide hormones involved in their metabolism.

Commonly used probiotics have been shown to have beneficial effects on cholesterol metabolism in vitro [25,26]. The data on the effects of probiotics on LDL cholesterol in animal studies are equivocal [27]. Similarly, some evidence exist that combined use of probiotics and soygerm powder may enhance beneficial effects of probiotics [28]. The effects of probiotics on triglyceride and glucose metabolism are lacking.

The SHR/N-cp rat is a genetic animal model of obesity and type II diabetes mellitus and exhibits many feature characteristics of the insulin resistance syndrome, namely glucose intolerance, hyperinsulinemia, hypercholesterolemia, hypertriglyceridemia and mild hypertension [29–32]. The purpose of this study is to determine the effects of soy isoflavones on glucose and triglyceride metabolism and the hormones influencing their metabolism. The second aim of the study is to determine whether probiotics have additive or synergistic effect with isoflavones on these parameters.

2. Material and methods

2.1. Animals

Thirty-two male lean and 32 male obese SHR/N-cp rats were obtained from the National Institutes of Health at approximately 5–6 weeks of age. At this age, obesity is already evident in SHR/N-corpulent (cp/cp) rats as indicated by higher body weight (average, 125 g) than their lean littermates (average, 96 g) and increased abdominal girth. The experimental protocol was approved by the Institutional Animal Care and Use Committees of the Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD, and by the George Washington University, Washington, DC. All animals were housed individually in stainless steel wire cages with controlled temperature (21–23°C) and relative humidity (40–50%) and maintained on a reverse 12-h dark (0900–2100 h) and light (2100–0900 h) cycle.

2.2. Diets and experimental protocol

All animals were provided with a Purina rat chow and were maintained on this diet for 2 weeks until 7–8 weeks of age. Food and water were consumed ad libitum. The rats were then randomly divided into four groups of eight lean rats and four groups of eight obese rats and fed AIN-93 diet [33]. Group 1 rats were fed 20% casein; group 2 rats were fed 20% casein with 0.1% soybean isoflavone mixture containing genistein, daidzein and glycetin; group 3 rats were fed 20% casein with 0.1% probiotic mixture (10^{10} colony forming units) containing *Lactobacillus acidophilus* (LA 140), *Lactobacillus casei* subsp. *casei* (LC 107) and *Bifidobacterium bifidum* (BBL 730) and group 4 rats were fed 20% casein with 0.1% isoflavone mixture and 0.1% probiotic mixture. All diets were identical and contain similar amounts of protein, fat, carbohydrates, minerals and vitamins. All diets contained (g/kg) dextrinized cornstarch, 155; sucrose, 100; soybean oil, 40; cellulose, 50; mineral mix (AIN-93M-MX), 35; vitamin mix (AIN-93-VX), 10; L-cystine, 1.8; choline bitartrate, 2.5; and *tert*-butylhydroquinone, 0.008. Casein and L-cystine were purchased from Sigma, St. Louis, MO. Soy isoflavone mixture was obtained gratis from Protein Specialties Division, Archer Daniels Midland, Decatur, IL. *tert*-Butylhydroquinone was purchased from Aldrich, Milwaukee, WI. All other ingredients were purchased from Dyets, Bethlehem, PA.

The diet was supplemented with the probiotic mixture to obtain enhanced intestinal milieu. When probiotics mixture was added to diets, Lactobacilli and Bifidobacterium strains showed growth of 131×10^8 and 35×10^8, respectively. In view of these results, we attempted to supplement the rats fed with mixture of probiotics concentration to meet nutritional requirements.

Table 1
Effects of soy isoflavones and probiotics on plasma glucose and triglyceride in a genetic model of obesity and diabetes

Phenotype	Diet	Glucose (mmol/L)	Triglyceride (mmol/L)
Lean	Casein	19.89±2.90	0.81±0.05
	Isoflavones	14.36±2.71	0.61±0.04
	Probiotics	19.92±3.84	0.76±0.16
	I+P	18.87±2.41	0.63±0.10
Obese	Casein	27.24±3.13	5.55±0.47
	Isoflavones	19.95±2.95	7.10±0.26
	Probiotics	26.06±3.09	5.99±0.14
	I+P	27.51±2.36	8.34±0.27
ANOVA	Phenotype	$P<.001$	$P<.0001$
	Diet	NS	NS
	P×D	NS	$P<.018$

Values are means±S.E.M. of eight rats.
I+P, isoflavones+probiotics; P×D, phenotype×diet.

Table 2
Effects of soy isoflavones and probiotics on plasma enzymes in a genetic model of obesity and diabetes

Phenotype	Diet	AST (μKat/L)	ALT (μKat/L)	LDH(μKat/L)
Lean	Casein	2.515±0.04	1.103±0.02	4.991±0.52
	Isoflavones	1.843±0.13	0.615±0.04	5.775±0.49
	Probiotics	2.302±0.03	1.117±0.05	6.318±0.44
	I+P	1.037±0.08	0.554±0.09	4.222±0.64
Obese	Casein	8.373±0.06	6.570±0.06	42.664±5.2
	Isoflavones	4.222±0.09	3.773±0.15	29.362±4.8
	Probiotics	7.215±0.17	8.735±0.12	41.381±4.9
	I+P	4.402±0.05	4.758±0.10	47.287±8.8
ANOVA	Phenotype	$P<.0001$	$P<.0001$	$P<.0001$
	Diet	$P<.017$	$P<.018$	NS
	P×D	NS	NS	NS

Values are means±S.E.M. of eight rats.
I+P, isoflavones+probiotics; P×D, phenotype × diet.

All animals were fed the experimental diets for 20 weeks and weighed biweekly throughout the study. Food intake was measured biweekly over 2-day period. At the end of the feeding period, after an overnight fast, animals were anesthetized under carbon dioxide and blood was drawn by cardiac puncture. Blood was collected in tubes containing EDTA (1.4 g/L) and Trasylol (100 kU/L), and plasma was separated for subsequent biochemical analyses and stored at −70°C.

2.3. Analytical measurements

Plasma was analyzed using Alcyon analyzer, ATAC 8000 (Abbott Laboratories) and kits from Elan Diagnostics for glucose (cat. #s. 532-018), triglyceride (cat. # 589-018), creatinine (cat. # 518-480), blood urea nitrogen (BUN) (cat. # 512-028), uric acid (cat. # 590-008), aspartate amino transferase (AST) (cat. # 536-018), alanine amino transferase (ALT) (cat. # 538-018) and lactate dehydrogenase (LDH) (cat. # 550-018). Protein was determined by the method of Lowry et al. [34]. Plasma insulin and leptin were measured by ELISA using kits from ALPCO (cat. # 008-10-1145-01 and 022-LEP-R61, respectively). Plasma glucagon and ACTH were measured by radioimmunoassays using kits from ICN Biomedicals (cat. # 07-152101 and 07-106101).

Lactobacilli from feces of rats fed the mixture of probiotics were enumerated anaerobically as described by Gilliland et al [35]. Bifidobacterium counts were determined anaerobically according to the method of Doleyres et al. [36].

2.4. Statistical analysis

Results are expressed as mean±standard error of the mean (S.E.M.). Comparisons between groups were made using two-way analysis of variance (ANOVA). When an effect was statistically significant ($P<.05$), mean comparisons were done. A Sidak adjusted significance level was used for the pairwise comparisons of the means so that the overall significance level was .05.

3. Results

The effects of isoflavones and probiotics on plasma glucose and triglycerides are summarized in Table 1. Plasma glucose is higher in this rat model compared to Sprague–Dawley rats. There was a significant phenotypic effect on plasma glucose being higher in obese rats than in lean phenotypes. Isoflavones alone appear to lower plasma glucose in both phenotypes, but when combined with probiotics, the effect is not present. Plasma triglyceride was 7- to 10-fold higher in obese rats than lean rats. There was an interaction between isoflavones and probiotics in different phenotypes. Thus, isoflavones appear to lower triglycerides in lean rats but not in obese rats. Probiotics had no significant effect on plasma triglycerides in either lean or obese rats.

Table 2 summarizes the data on the effect of isoflavones and probiotics on plasma enzymes AST, ALT and LDH, which are involved in liver function. All of the parameters were significantly higher in obese rats than in lean littermates. There was also a significant diet effect on AST and ALT. Isoflavones significantly decreased both enzymes in lean as well as obese phenotypes. The decrease was observed in the absence as well as the presence of probiotics. Isoflavones had no significant effect on plasma

Table 3
Effects of soy isoflavones and probiotics on plasma creatinine, BUN, protein and uric acid in a genetic model of obesity and diabetes

Phenotype	Diet	Creatinine (μmol/L)	BUN (mmol/L)	Protein (g/L)	Uric acid (μmol/L)
Lean	Casein	34.48±3.54	7.05±0.49	80.38±8.42	239.11±35.09
	Isoflavones	29.17±2.65	7.34±0.55	60.76±4.50	190.34±32.71
	Probiotics	30.94±2.86	6.47±0.49	60.79±2.81	217.70±33.76
	I+P	28.29±3.25	6.83±0.51	60.69±2.53	195.69±42.74
Obese	Casein	21.22±3.59	8.20±0.57	70.31±3.20	277.18±41.04
	Isoflavones	15.91±2.51	7.41±0.49	80.13±2.71	205.21±35.06
	Probiotics	24.75±3.83	8.06±0.42	60.83±8.44	249.82±38.29
	I+P	15.91±3.24	6.88±0.39	80.68±6.40	267.66±27.19
ANOVA	Phenotype	$P<.0001$	$P<.043$	NS	NS
	Diet	NS	NS	NS	NS
	P×D	NS	NS	$P<.052$	NS

Values are means±S.E.M. of eight rats.
I+P, isoflavones+probiotics; P×D, phenotype × diet.

Table 4
Effects of soy isoflavones and probiotics on plasma peptide hormones in a genetic model of obesity and diabetes

Phenotype	Diet	Insulin (pmol/L)	Leptin (ng/L)	Glucagon (pmol/L)	ACTH (nmol/L)
Lean	Casein	192.2±26.1	11.33±2.60	228.2± 76.5	1.70±0.47
	Isoflavones	76.8±10.2	2.79±0.32	137.5±37	2.02±0.44
	Probiotics	181.5±21.4	8.83±0.96	223.6±70.8	0.93±0.24
	I+P	103.7±14.5	3.25±0.41	114.0±38	0.66±0.20
Obese	Casein	2886±240	78.40±10.2	372.3±43.8	1.12±0.24
	Isoflavones	3151±233	89.08±11.6	281.0±37	0.86±0.25
	Probiotics	4647±355	88.89±9.8	344.4±76.5	0.98±0.22
	I+P	4602±358	68.25±8.4	340.3±40	0.89±0.24
ANOVA	Phenotype	$P<.0001$	$P<.001$	$P<.0003$	NS
	Diet	NS	NS	NS	NS
	P×D	$P<.05$	$P<.05$	NS	NS

Values are means±S.E.M. of eight rats.
I+P, isoflavones+probiotics; P×D, phenotype × diet.

LDH. Probiotics alone had no significant effect on any of these enzymes.

Since isoflavones significantly decreased kidney weight in lean and obese rats [37], we studied the effects of isoflavones and probiotics on the parameters of kidney function. Plasma creatinine levels were lower in obese rats than lean rats while BUN levels were higher in obese rats than lean rats (Table 3). The effects of isoflavone appeared to be different in lean obese rats. In lean rats, isoflavones decreased plasma protein and uric acid levels but not in obese rats. Thus, there was no overall significant effect of isoflavones or probiotics on any of these parameters.

Table 4 summarizes the effects of isoflavones and probiotics on plasma levels of insulin, leptin, glucagon and ACTH in lean and obese SHR/N-cp rats. Note that plasma insulin, leptin and glucagon were significantly higher in obese rats than in lean littermates. Isoflavones lowered plasma insulin, leptin and glucagon in lean rats but not in obese phenotypes. Probiotics increased the levels of insulin and leptin in obese rats but not in lean rats. There was a significant interaction between phenotype and diet for insulin and leptin, thereby negating the diet effects of isoflavones on plasma insulin leptin in these rats. Neither phenotype nor diet had any significant effect on plasma ACTH levels.

4. Discussion

We have previously described the effects of isoflavones and probiotics on body weight, fat distribution, cholesterol metabolism and steroid and thyroid hormones in this animal model [37]. In the present study, we did not observe overall significant effect of isoflavones on plasma glucose or triglycerides though both were lowered in lean animals. Our results are thus similar to previous results observed by us using soy protein [21,38,39]. Thus, soy protein or soy isoflavones [2–9] affect cholesterol but not plasma glucose or triglyceride levels in obesity or diabetes. It is interesting to note that previous studies in humans have shown that soy polysaccharide [40,41] but not soy protein [42,43] lowers postprandial glucose and triglyceride concentrations. However, in male Wistar rats, Lavigne et al. [44] observed lower fasting plasma glucose and lower incremental area under the curve glucose after an intravenous glucose load than casein.

Hypertriglyceridemia is a characteristic lipid abnormality in obesity and type II diabetes mellitus and has been linked to the development of fatty liver in obesity [10,45]. Numerous studies in experimental animals and humans have documented the hypolipidemic effects of soy protein [2–12]. In the present study, soy isoflavones reduced triglycerides in lean rats by 25%, but in obese rats, there was no significant effect. The levels of triglyceride in obese rats were 7- to 10-fold higher than in lean rats, and that soy isoflavones may not be effective in lowering triglycerides when levels are extremely high.

We have previously shown that isoflavones significantly lowered liver weight in both lean and obese SHR/N-cp rats [37]. However, the data on the effects of isoflavones and probiotics on plasma levels of enzymes that are involved in liver function are lacking. We observed significant decrease in AST and ALT in rats fed isoflavones while feeding probiotic mixture had no significant effect. Li et al. [46] fed a probiotic mixture to obese mice along with high-fat diet and observed decrease in serum ALT levels. Lower levels of ALT were also observed in hinds fed a probiotic mixture [47]. Thus, our results in rats differ from those in obese mice and hinds. In the present study, neither isoflavones nor probiotics had any significant effect on LDH. Choi and Lee [48] have reported a dose-dependent effect of genistein on LDH in rat brain, the level being higher at high dose (20 mg/day) and lower with lower dose of genistein (2 mg/day). In the present study, the activity of all three enzymes was higher in obese phenotypes compared to lean.

Isoflavones alone appeared to reduce plasma creatinine, BUN and uric acid in obese rats and plasma creatinine, protein and uric acid in lean rats. However, the effects were not statistically significant. In earlier study, soy protein also failed to show any significant effect on these parameters in two different rat models [21]. In humans, Garrel et al. [49] have reported increased plasma uric acid after ingestion of soy protein compared to casein or lactalbumin. It thus appears that neither soy protein nor its isoflavones have

significant beneficial effect on renal function. However, soy protein has been shown to retard the development and progression of chronic renal disease in humans and animals [50–59]. However, this effect may not occur when there is persistence of diabetes and insulin resistance as in SHR/N-cp rat [38] or in patients with type II diabetes mellitus, obesity and hypertension [12].

The effects of isoflavones and probiotics on peptide hormones are not well defined. In the present study, neither isoflavones nor probiotics had significant effect on plasma insulin levels in obese rats, but isoflavones did decrease plasma insulin, leptin and glucagon in lean rats. Persky et al. [60] also observed no significant effect of isoflavones on plasma insulin in hypercholesterolemic postmenopausal women. In postmenopausal women, Goodman-Gruen and Kritz-Silverstein [61] reported that the consumption of isoflavones was associated with lowered plasma insulin response to glucose load. Akahoshi et al. [62] reported that consumption of soy protein compared to casein lowered plasma insulin levels in rats. Several other studies [63–66] have also reported lower insulin concentration and increased insulin sensitivity in rats fed soy protein compared to those fed casein. Thus, soy protein may act differently than the isoflavones present in soybean or that isoflavones are less effective than soy protein in affecting peptide hormones. In the present study, we observed low insulin/glucagon ratio in obese rats but not in lean rats fed isoflavones. Sanchez and Hubbard [67] also observed low postprandial insulin/glucagon ratio in humans after a single meal containing soy protein compared to casein.

The data on the effect of probiotics on plasma insulin and glucagon levels are lacking. In Holstein cows, direct-fed microbial supplementation increased plasma insulin and glucose levels during postprandial period [68]. In our study, probiotics had no significant effect on plasma insulin or glucagon levels in rats.

In the present study, we observed a differential effect of isoflavones on plasma leptin levels. Isoflavones significantly lowered plasma leptin in lean rats but not in obese phenotypes. Soy protein compared to casein has been reported to lower plasma leptin, though not significantly, in male rats [62]. In humans also, high dose of isoflavones had no significant effect on plasma leptin levels [69]. Lack of effect of isoflavones on plasma insulin, leptin and glucagon in obese rats may, in part, explain the lack of effect of isoflavones on plasma triglyceride in obese rats. Probiotics had no significant effect on plasma leptin in either lean or obese rats. In healthy male and female subjects, administration of *Lactobacillus plantarum* 299 v was shown to reduce plasma leptin levels [70].

In our study, plasma ACTH levels were not significantly altered by either isoflavones or probiotics. Lephart et al. [71] observed significantly increased plasma ACTH levels in male rats fed phytoestrogens. Thus, the data on the effects of isoflavones and probiotics on plasma peptide hormones are equivocal and need further study.

In summary, we have shown that soy isoflavones lower plasma glucose and triglyceride levels in animals. However, when levels of triglycerides are extremely high as observed in obese rats, isoflavones fail to lower the triglyceride levels. Further, changes in glucose and triglycerides are brought about by the changes in the peptide hormones that control their metabolism. Our data also show that probiotics that were used in the present study do not potentiate the beneficial effects of isoflavones on glucose or triglyceride metabolism or the peptide hormones involved in their metabolism.

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References

[1] Food and Drug Administration. Food labeling: health claims; soy protein and coronary heart disease. Food and Drug Administration, HHS. Final rule. Fed Regist 1999;64:57700–33.
[2] Carroll KK. Review of clinical studies on cholesterol-lowering response to soy protein. J Am Diet Assoc 1991;91:820–7.
[3] Wagner JD, Cefalu WT, Anthony MS, Litwak KN, Zhang L, Clarkson TB. Dietary soy protein and estrogen replacement improve cardiovascular risk factors and decrease aortic cholesteryl ester content in ovariectomized cynomolgous monkeys. Metabolism 1997; 46:698–705.
[4] Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of effects of soy protein intake on serum lipids in humans. N Engl J Med 1959;333:276–82.
[5] Crouse JR, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones in plasma concentrations of lipids and lipoproteins. Arch Intern Med 1999; 159:2070–6.
[6] Sugano M, Yamada Y, Yoshida K, Hashimoto Y, Matsuo T, Kimoto M. The hypocholesterolemic action of the undigested fraction of soy bean protein in rats. Atherosclerosis 1988;72:115–22.
[7] Anthony MS, Clarkson TB, Waddle DL, Wolfe MS. Effects of soy protein phytoestrogens on cardiovascular risk factors in rhesus monkeys. J Nutr 1995;l25(Suppl):803S–4S.
[8] Morita T, Oh-hashi A, Takei K, Ikai M, Kasaoka S, Kiriyama S. Cholesterol-lowering effects of soybean, potato and rice proteins depend on their low methionine contents in rats fed a cholesterol free purified diet. J Nutr 1997;127:470–7.
[9] Beynen AC, West CE, Spaaij CJ, et al. Cholesterol metabolism, digestion rates and postprandial changes in serum of swine fed purified diets containing either casein or soybean protein. J Nutr 1990;120:422–30.
[10] Bosello O, Cominacini L, Zocca L, et al. Short- and long-term effects of hypocaloric diets containing proteins of different sources on plasma lipids and apoproteins of obese subjects. Ann Nutr Metab 1998;32: 206–14.
[11] Hermansen K, Sondergaard M, Hoie L, et al. Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. Diabetes Care 2001;24:228–33.

[12] Anderson JW, Blake JE, Turner J, Smith BM. Effects of soy protein on renal function and proteinuria in patients with type 2 diabetes. Am J Clin Nutr 1988;68(Suppl):1347S–53S.
[13] Bhathena SJ. Dietary fatty acids and fatty acid metabolism in diabetes. In: Chow CK, editor. Fatty acids in foods and their health implications. 2nd ed. New York: Marcel Dekker; 2000. p. 915–61.
[14] Andersen T, Gluud C. Liver morphology in morbid obesity: a literature study. Int J Obes 1984;8:97–106.
[15] Vuorinen-Markkola H, Yki-Jarvinen H. Hyperuricemia and insulin resistance. J Clin Endocrinol Metab 1994;78:25–9.
[16] Schmidt Ml, Watson RL, Duncan BB, et al. Clustering of dyslipidemia, hyperuricemia, diabetes, and hypertension and its association with fasting insulin and central and overall obesity in general population, Atherosclerosis Risk in Community Study Investigators. Metabolism 1966;45:699–706.
[17] Bonora E, Targher G, Zenere MB, et al. Relationship of uric acid concentration to cardiovascular risk factors in young men. Role of obesity and central fat distribution. The Verona Young Men Atherosclerosis Risk Factors Study. Int J Obes Relat Metab Disord 1996;20:975–80.
[18] Rathman W, Funkhouser E, Dyer AR, Roseman JM. Relations of hyperuricemia with various components of the insulin resistance syndrome in young black and white adults: the CARDIA study. Coronary artery risk development in young adults. Ann Epidemiol 1998;8:250–61.
[19] Castelli WP. Epidemiology of triglycerides: a view from Framingham. Am J Cardiol 1993;70:3H–9H.
[20] Gotto AM. Triglyceride as a risk factor for coronary artery disease. Am J Cardiol 1998;82:22Q–5Q.
[21] Bhathena SJ, Ali AA, Mohamed AI, et al. Differential effects of dietary flaxseed protein and soy protein on plasma triglyceride and uric acid levels in animal models. J Nutr Biochem 2002;13:684–9.
[22] Bhathena SJ, Velasquez MT. Beneficial role of dietary phytoestrogens in obesity and diabetes. Am J Clin Nutr 2002;76:1191–201.
[23] Taha SA, Wasif MM. Hypoglycemic effect and protein nutritive quality of soy and methionine supplemented whole durum pasta products. Nahrung 1996;40:281–7.
[24] Iritani N, Sugimoto T, Fukuda H. Dietary soybean protein increases insulin receptor gene expression in male Wistar fatty rats when dietary polyunsaturated fatty acid level is low. J Nutr 1977;127: 1077–83.
[25] Gilliland SE, Nelson CR, Maxwell C. Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl Environ Microbiol 1985;49:377–81.
[26] Gilliland SE, Walker DK. Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. J Dairy Sci 1990;73:905–11.
[27] Taylor GRJ, Williams CM. Effects of probiotics and prebiotics on blood lipids. Br J Nutr 1998;80(Suppl 1):S225–30.
[28] De Doever P, Wouters R, Verstraete W. Combined use of *Lactobacillus reuteri* and soygerm powder as food supplement. Lett Appl Microbiol 2001;33:420–4.
[29] Michaelis IV OE, Ellwood KC, Judge JM, et al. Effect of dietary sucrose on the SHR/N-corpulent rat: a new model for insulin-independent diabetes. Am J Clin Nutr 1984;39:612–8.
[30] Michaelis IV OE, Patrick DH, Hansen CT, et al. Spontaneous hypertensive/NIH-corpulent rat. Animal model for insulin-independent diabetes mellitus (Type II). Am J Pathol 1986;23:398–400.
[31] Voyles NR, Powell AM, Timmers KI, et al. Reversible impairment of glucose-induced insulin secretion in SHR/N-cp rats. Genetic model of type II diabetes. Diabetes 1988;37:398–404.
[32] Michaelis IV OE, Carswell N, Velasquez MT, Hansen CT. The role of obesity, hypertension, and diet in diabetes and its complication in the spontaneous hypertensive/NIH-corpulent rat. Nutrition 1989;5:56–9.
[33] 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 diets. J Nutr 1993;123:1939–51.
[34] Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol chem 1951;193:265–75.
[35] Gilliland SE, Speck ML, Morgan CG. Detection of *Lactobacillus acidophilus* in feces of humans, pigs, and chickens. Appl Microbiol 1975;30:541–5.
[36] Doleyres Y, Paquin C, Leroy M, Lacroix C. *Bifidobacterium longum* ATCC 15707 cell production during free- and immobilized-cell cultures in MRS-whey permeate medium. Appl Microbiol Biotechnol 2002;60:168–73.
[37] Ali AA, Velasquez MT, Hansen CT, et al. Effects of soy bean 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.
[38] Velasquez MT, Bhathena SJ, Ranich T, et al. Dietary flaxseed meal reduces proteinuria and ameliorates nephropathy in an animal model of type II diabetes mellitus. Kidney Int 2003;64:2100–7.
[39] Bhathena SJ, Ali AA, Haudenschild C, et al. Dietary flaxseed meal is more protective than soy protein concentrate against hypertriglyceridemia and steatosis of the liver in an animal model of obesity. J Am Coll Nutr 2003;22:157–64.
[40] Tsai AC, Mott EL, Owen GM, et al. Effects of soy polysaccharide on gastrointestinal functions, nutrient balance, steroid excretions, glucose tolerance, serum lipids, and other parameters in humans. Am J Clin Nutr 1983;38:504–11.
[41] Ahmed M, Gannon MC, Nuttall FQ. Postprandial plasma glucose, insulin, glucagon and triglyceride response to a standard diet in normal subjects. Diabetologia 1976;12:61–7.
[42] Lang V, Bellisle F, Opper J-M, et al. Satiating effect of proteins in healthy subjects: a comparison of egg albumin, casein, gelatin, pea protein, and wheat gluten. Am J Clin Nutr 1998;67:1197–204.
[43] Lang V, Bellisle F, Alamowitch C, et al. Varying the protein source in mixed meal modifies glucose, insulin and glucagon kinetics in healthy men, has weak effects on subjective satiety and fails to affect food intake. Eur J Clin Nutr 1999;53:959–65.
[44] Lavigne C, Marette A, Jacques H. Cod and soy proteins compared with casein improve glucose tolerance and insulin sensitivity in rats. Am J Physiol Endocrinol Metab 2000;278:E491–E500.
[45] Yamashita T, Sasahara T, Pomeroy SE, et al. Arterial compliance, blood pressure, plasma leptin, and plasma lipids in women are improved with weight reduction equally with a meat-based diet and plant-based diet. Metabolism 1998;47:1308–14.
[46] Li Z, Yang S, Lin H, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology 2003;37:343–50.
[47] Zomborszky Z, Feher T, Horn E, et al. Comparison of some blood parameters of captured and farmed red deer (*Cervus elaphus*) hinds. Acta Vet Hung 1996;44:433–41.
[48] Choi EJ, Lee BH. Evidence for genistein mediated cytotoxicity and apoptosis in rat brain. Life Sci 2004;75:499–509.
[49] Garrel DR, Verdy M, PetitClerc C, et al. Milk- and soy-protein ingestion: acute effect on serum uric acid concentration. Am J Clin Nutr 1991;53:665–9.
[50] Iwasaki K, Gleiser CA, Masoro EJ, et al. The influence of dietary protein source on longevity and age-related disease processes of Fischer rats. J Gerontol 1988;43:B5–B12.
[51] Tomobe K, Philbrick DJ, Ogborn MR, et al. Effect of dietary Soy protein and genistein on disease progression in mice with polycystic kidney disease. Am J Kidney Dis 1998;31:55–61.
[52] Aukema HM, Housini I, Rawling JM. Dietary soy protein effects on inherited polycystic kidney disease are influenced by gender and protein level. J Am Soc Nephrol 1999;10:300–8.
[53] Ogborn MR, Bankovic-Calic N, Shoesmith C, et al. Soy protein modification of rat polycystic kidney disease. Am J Physiol 1998;274: F541–9.
[54] Ogborn MR, Nitschmann E, Weiler HA, et al. Modification of polycystic kidney disease and fatty acid status by soy protein diet. Kidney Int 2000;57:159–66.

[55] D'Amico G, Gentile MG, Manna G, et al. Effect of vegetarian soy diet on hyperlipidaemia in nephrotic syndrome. Lancet 1992;339:1131–4.
[56] Gentile MG, Fellin G, Cofano F, et al. Treatment of proteinuric patients with a vegetarian soy diet and fish oil. Clin Nephrol 1993;40: 315–20.
[57] Soroka N, Silverberg DS, Greemland M, et al. Comparison of a vegetable-based (soya) and an animal-based low-protein diet in predialysis chronic renal failure patients. Nephron 1998;79:173–80.
[58] Wardle EN. Soy protein diet therapy in renal disease. Nephron 1998; 8:328–31.
[59] Maddox DA, Alavi FK, Silbernick EM, Zawada ET. Protective effects of a soy diet in preventing obesity-linked renal disease. Kidney Int 2002;61:96–104.
[60] Persky VW, Turyk ME, Wang L, et al. Effect of soy protein on endogenous hormones in postmenopausal women. Am J Clin Nutr 2002;75:145–53.
[61] Goodman-Gruen D, Kritz-Silverstein D. Usual dietary isoflavone intake is associated with cardiovascular risk factors in post-menopausal women. J Nutr 2001;131:1202–6.
[62] Akahoshi A, Koba K, Ichinose F, et al. Dietary protein modulates the effect of CLA on lipid metabolism in rats. Lipids 2004;39:25–30.
[63] Hurley C, Galibois I, Jacqes H. Fasting and post-prandial lipid and glucose metabolisms are modulated by dietary proteins and carbohydrates: role of plasma insulin concentrations. J Nutr Biochem 1995; 6:540–6.
[64] Sugano M, Lshiwaki N, Nagata Y, Imaizumi K. Effects of arginine and lysine addition to casein and soya-bean protein on serum lipids, apolipoproteins, insulin and glucagon in rats. Br J Nutr 1982;48: 211–21.
[65] Baba N, Radwan H, Van Itallie T. Effects of casein versus soyprotein diets on body composition and serum lipid levels in adult rats. Nutr Res 1992;12:279–88.
[66] Hurley C, Richard D, Deshaies Y, Jacques H. Soy protein isolate in the presence of cornstarch reduces body fat gain in rats. Can J Physiol Pharmacol 1998;76:1000–7.
[67] Sanchez A, Hubbard RW. Plasma amino acids and the insulin/glucagon ratio as an explanation for the dietary protein modulation of atherosclerosis. Med Hypotheses 1991;36:27–32.
[68] Nocek JE, Kautz WP, Leedle JA, Block E. Direct-fed microbial supplementation on the performance of dairy cattle during the transition period. J Dairy Sci 2003;86:331–5.
[69] Phipps WR, Wangen KE, Duncan AM, et al. Lack of effect of isoflavonic phytoestrogen intake on leptin concentrations in premenopausal and postmenopausal women. Fertil Steril 2001;75:1059–64.
[70] Naruszewicz M, Johansson ML, Zapolska-Downar D, Bukowska H. Effect of *Lactobacillus plantarum* 299v on cardiovascular disease risk factors in smokers. Am J Clin Nutr 2002;76:1249–55.
[71] Lephart ED, Galindo E, Bu LH. Stress (hypothalamic–pituitary–adrenal axis) and pain response in male rats exposed lifelong to high vs. low phytoestrogen diets. Neurosci Lett 2003;342:65–8.