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Effect of soy proteins and isoflavones on lipid metabolism and involved gene expression

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1. ABSTRACT

Clinical trials and animal studies showed that ingestion of soy proteins improves blood lipid profiles including lowering triglyceride, total and LDL cholesterol levels and increasing HDL cholesterol content. However, the effective components in the soy and the mechanisms involved in the hypolipidemic actions are not fully understood. Increasing evidence from animal studies have suggested that soy components may regulate lipid metabolism by modulating the activities of key transcription factors and thereby changing the downstream gene expression involved in lipogenesis or lipolysis. It has been shown that intake of soy proteins alters the expression of genes for sterol regulatory element binding protein, peroxisomal proliferator activated receptor, and liver X receptor. Dietary soy proteins suppress the DNA binding activities of hepatic nuclear receptors for thyroid hormones and retinoic acid, and alter the activities of key enzymes including cholesterol 7alpha hydroxylase and ATPase/ATP synthase through post-translational protein modifications. This paper reviews the current understanding of the cellular and molecular events by which soy components affect lipid levels, especially focusing on modulation of transcription factors and regulation of gene expression involved in lipid metabolism by soy proteins and associated isoflavones.

2. INTRODUCTION

Soy foods have been consumed for over 5000 years in Asian countries. The epidemiological evidence suggest that soy consumption is linked to a lower incidence of chronic diseases including coronary heart disease, atherosclerosis, type 2 diabetes, osteoporosis, and certain types of cancers such as prostate cancer and breast cancer. Clinical trials (1) and animal studies (2-4) also showed that dietary soy proteins or isoflavones (ISF) reduce the risk factors for cardiovascular diseases including lowering triglyceride, total and LDL cholesterol levels and increasing the ratio of HDL/LDL cholesterol. Consequently, these studies have led to the approval of a food labeling health claim for soy proteins in the prevention of coronary heart disease by the U.S. Food and Drug Administration in 1999. Since then, soy food consumption in the U.S. has doubled, and over 2500 new foods containing soy have been introduced by U.S. food manufacturers.

Similar health petitions for soy proteins have also been approved thereafter in the United Kingdom, South Africa, Philippines, Brazil, Indonesia, Korea and Malaysia. However, the purported beneficial health effects were quite variable in different clinical studies. The Nutrition

Committee of the American Heart Association has recently assessed 22 randomized trials conducted since 1999, and found that isolated soy proteins with ISF only slightly decreased LDL cholesterol but had no effect on HDL cholesterol, triglyceride, lipoprotein(a) or blood pressure. Soy ISF failed to change HDL cholesterol and other lipid risk factors in 19 studies. The effects of soy proteins and ISF on vasomotor symptoms of menopause, postmenopausal bone loss, and prevention of breast, endometrial, and prostate cancers were not substantiated (5).

More recent results suggest that a variety of factors could cause variations among different studies. These include processing procedures of soy protein isolate (SPI) which have been shown to affect the intactness of the protein subunits (6), different forms of ISF (conjugated or unconjugated to sugar) (7), and the ability of the subjects to metabolize daidzein, one of the major soy ISF, to produce equol (8). In addition, lack of understanding of the bioactive components in the soy and the molecular mechanisms by which lipid metabolism is impacted may contribute a major part to the discrepancies. Existing literature is quite controversial regarding which component (proteins or associated ISF) in soy has the lipid-lowering actions. This paper reviews the current understanding of the molecular events by which soy components affect lipid levels, especially focusing on the modulation of transcription factors and regulation of gene expression involved in lipid metabolism by soy proteins and associated ISF.

3. EFFECT OF SOY PROTEINS AND ISF ON LIPID PROFILES

Soybeans contain about 40% protein, and depending on the processing procedure, the protein content can reach over 90% as in SPI that is usually used in soy-based infant formulas. Most of the attention in soy studies has been focused on the proteins and their associated ISF.

3.1. Human clinical trials

The first human study on the lipid-lowering effect of soy proteins was reported in 1967 (9). It was shown that a replacement of mixed proteins by vegetable proteins, mainly isolated soy proteins, remarkably reduced the average blood cholesterol levels by more than 100 mg/ml, from approximately 295 mg/ml to 172 mg/ml in hypercholesterolemic men. The hypolipidemic action of soy proteins has been further demonstrated in the following clinical studies.

A meta-analysis of 38 controlled clinical trials published between 1977 and 1994 showed that average intake of 47 g/day of soy proteins (ranged from 17 to 124 g/day) significantly decreased total cholesterol by 9.3%, LDL cholesterol by 12.9%, triglycerides by 10.5%, and increased HDL cholesterol by 2.4% but this increase was not statistically significant. All of these studies used either isolated or textured soy proteins. Among them, 30 studies were conducted with hypercholesterolemic subjects (1). More recent results suggest that intact soy proteins with ISF (3.0~185.0 mg/d) were associated with significant decreases in serum total cholesterol by 3.8%, LDL cholesterol by 5.3%, and triglycerides by 7.3% and significant increases in serum HDL cholesterol by 3.0% in 23 randomized controlled studies published from 1995 to 2002. The effects on total and LDL cholesterol were greater in men than in women (10).

The effects of soy ISF on blood lipid profile appear to be inconsistent among different studies. For instance, a meta-analysis showed that ISF-enriched soy proteins (61.7~317.9 mg/d ISF) markedly decreased serum total cholesterol by 1.8%, and LDL cholesterol by 3.6%, but no changes in HDL cholesterol and triglycerides were significant compared to the ISF-depleted soy proteins (1.2~14.6 mg/d ISF). ISF-depleted soy proteins significantly decreased LDL cholesterol by 2.8%, while ISF-enriched soy proteins significantly decreased LDL cholesterol by 5.0% and increased HDL cholesterol by 3.0% compared to animal proteins (11). With equal amounts of soy protein intake, high ISF intake (averagely 96 mg/d) resulted in significantly greater decreases in serum LDL cholesterol than low ISF intake (6 mg/d), suggesting that the LDL cholesterol-lowering effects of ISF are independent of soy proteins (12).

However, the hypolipidemic benefits of soy ISF in other studies using a range of 40-150 mg/d ISF in either normocholesterolemic or hypercholesterolemic men or women were insignificant. For example, total and LDL cholesterol levels in healthy perimenopausal women receiving 61.8 mg/d of ISF or placebo for 4 weeks had no significant difference (13). The LDL cholesterol levels among the moderately hypercholesterolemic women supplemented with 42 g/d milk protein, soy plus ISF (80 mg/d), or soy without ISF did not differ (14). When hypercholesterolemic postmenopausal women were given a much higher dosage of ISF (150 mg/d) for 6 months, total and LDL cholesterol levels were lower, but not statistically different (15). Similar results were observed in the study with healthy men given 55 mg/d of ISF (16). A recent meta-analysis of 10 randomized controlled trials from 1995 to 2002 indicated that 36 g/d of soy proteins in combination with 52 mg of soy-associated ISF lowered LDL cholesterol by 4%; however, despite large increases in blood ISF concentrations, no significant correlation between soy ISF and change in LDL cholesterol was detected. It has been concluded that ISF did not have an independent effect on lowering LDL cholesterol levels in the presence of soy proteins (17). This conclusion was supported by another meta-analysis showing that supplementation with extracted soy ISF had no significant effect on total cholesterol reduction (10).

Nevertheless, soy proteins, regardless of ISF content, have been shown to lower the ratios of total to HDL cholesterol, and LDL to HDL cholesterol compared to the milk protein in healthy young men (18), and significantly reduced total and LDL cholesterol and triglyceride concentrations in hypercholesterolemic men and women (19). However, the added ISF had no effect on plasma lipid levels. In addition, the hypocholesterolemic

effects of soy proteins were closely associated with the initial blood cholesterol levels. The reductions in total or LDL cholesterol levels are much greater in hypercholesterolemic subjects than in normocholesterolemic subjects (1,10,11).

3.2. Animal studies

Obese Zucker rats fed soy proteins with high ISF (5.8 mg/g protein) for 8 or 11 weeks had lower liver weights, lower liver cholesterol and triglyceride content than those fed either casein or soy proteins with low amounts of ISF (0.1 mg/g protein) (20). Plasma cholesterol concentrations in the rats fed either ethanol-washed SPI or SPI with an ethanol extract of soy were comparable and significantly lower than those fed casein. Addition of ethanol extract to SPI or casein did not influence plasma cholesterol level, suggesting that the cholesterol-lowering effect of SPI in rats was attributed to its protein component but not to the ethanol-extractable minor constituents including ISF (21). Our study in Sprague Dawley rats also showed that intake of 20% alcohol-washed SPI with or without added ISF markedly lowered the plasma triglyceride levels compared to a casein diet, however the added ISF had no additional effects (22).

3.3. Possible effective components

Soybeans contain two major storage globulins, beta-conglycinin (7S) and glycinin (11S). Beta-conglycinin has three subunits (alpha', alpha, and beta), and is believed to be essential for the hypolipidemic actions of soy. Consumption of beta-conglycinin decreased serum triglycerides in both normal and genetic obese mice (3) as well as in humans (23). Addition of beta-conglycinin into cultured HepG2 cells up-regulated LDL receptor activity measured by the uptake and degradation of ^{125}I-labeled LDL (24). However, absence of alpha' subunit in beta-conglycinin derived from an alpha' mutated soy cultivar had no effect (25). This suggests that the alpha' subunit in the beta-conglycinin may be a bioactive component that mediates the hypolipidemic activities of soy.

Different amino acid composition in soy proteins is believed to play a role in mediating the hypolipidemic actions of soy. It has been shown that lysine and methionine have moderate hypercholesterolemic effects (26,27), whereas arginine lowers cholesterol concentrations (28). Soy proteins contain a higher ratio of arginine to lysine and methionine, which may be at least partially responsible for the hypocholesterolemic effects of soy proteins (29,30).

The major soy-derived ISF such as genistein and daidzein are potent regulators of many genes involved in lipid metabolism (20,31-33), however the lipid-lowering actions of soy-derived ISF are quite variable among different studies. The contributing factors causing these inconsistencies are not fully understood, but it has been suggested that the initial lipid levels and the ability of the subjects to metabolize the daidzein or daidzin to equol as well as the forms of ISF taken (glucosides or aglycones) may play a role in determining the bioavailabilities of the ISF and the responsiveness of the subjects to ISF (7,10,12).

4. REGULATION OF TRANSCRIPTION FACTORS RELATED TO LIPID METABOLISM BY SOY PROTEINS AND ISF

Although the molecular mechanism(s) by which soy components affect lipid metabolism is not fully understood, several hypotheses have been proposed to date based on the information obtained from studies in animals, humans, and *in vitro* tissue cultures. These include suppressed cholesterol absorption (34), increased fecal excretion of bile acids (35,36), up-regulated hepatic LDL receptor activity (37), and elevated serum thyroxine (T4) levels (38).

Recent studies suggest that dietary soy components may influence physiological functions via regulation of the expression of genes involved in various biochemical pathways. DNA-microarray analyses show that 33% out of 8000 liver genes were differentially expressed in soy-fed rats as compared to casein-fed rats. Most of those genes were involved in lipid metabolism, transcriptional regulation and energy metabolism. Among those, the ones involved in the fatty acid synthesis were down-regulated, whereas the ones related to cholesterol synthesis or steroid catabolism were up-regulated in soy-fed animals (39,40). More importantly, dietary soy proteins were shown to differentially regulate or modify transcription factors such as sterol regulatory element binding protein (SREBP) (3,41) and several nuclear receptors (42-44).

4.1. Sterol regulatory element binding protein

SREBP is synthesized in the endoplasmic reticulum as a precursor protein, and becomes active after cleavage of the NH_2-terminal domain by two specific proteases, SP1 and SP2 in the Golgi apparatus. The active SREBP translocates to the nucleus, where it activates transcription of multiple target genes by binding to sterol response elements in their promoter regions (45). Transcriptional controls of fatty acid and cholesterol synthesis as well as cholesterol uptake genes are partially mediated by SREBP (46).

There are three SREBP isoforms (SREBP-1a, -1c and SREBP-2) with specific and overlapping transcriptional targets. SREBP-1a and -1c preferentially regulate the enzymes involved in fatty acid and triglyceride biosynthesis such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and microsomal triglyceride transfer protein and are activated in response to insulin. In addition, SREBP-1 is shown to reduce the mRNA expression of stearoyl–CoA desaturase 1 (SCD-1), an important enzyme to catalyze desaturation of long-chain fatty acids and enhance their incorporation into very low density lipoprotein (VLDL) assembly (47). SREBP-2 preferentially binds to promoters of the genes involved in cholesterol biosynthesis and uptake such as hydroxymethylglutaryl-CoA synthase (HMG-CoAs), HMG-CoA reductase (HMG-CoAr) and LDL receptor, and is activated by a low cholesterol content of the cell (45).

Consumption of soy proteins reduces hepatic SREBP-1 mRNA and protein expression and concurrently reduces FAS and malic enzyme (ME) mRNA abundance as well as endogenous fatty acid biosynthesis (4,48-50).

Although the underlying molecular mechanism(s) are not fully understood, regulation of SREBP-1 gene expression by dietary soy proteins may be mediated through either insulin-dependent or -independent pathways. It has been shown that insulin stimulates SREBP-1 expression in liver (4) and rat primary hepatocytes (51), and that soy proteins decrease insulin secretion and increase hepatic insulin removal (52). However, recent evidence indicates that despite high insulin concentrations in hyperinsulinemic obese fa/fa rats, rats fed soy proteins have lower SREBP-1 expression than rats fed casein (47). Further investigation demonstrated that reduction in SREBP-1 by soy proteins might be through the negative regulation of liver X receptor (LXR) mRNA expression (47). Interaction of SREBP with nuclear factors is a common mechanism through which SREBP activates gene transcription. For example, SREBP-mediated activation of the genes for HMG-CoAs (53) and FAS (54) is dependent on the interaction between SREBP and nuclear factor-Y. Other studies have shown that SREBP activation of LDL receptor gene is dependent on the interaction between SREBP and sterol regulatory element-1 (55).

Daidzein, genistein, or soy ISF have no effects on SREBP-1 content in cultured hepatocytes (56) nor in rats (47). However, a study in HepG2 cells demonstrated that genistein reduced the expression of site-1 protease which is responsible for the processing and activation of SREBP-1, and the processing of SREBP-1 but failed to change the expression of SREBP-1 mRNA. Meanwhile, the expression of SREBP-1-regulated lipogenic genes including SCD1, ACCalpha and ACCbeta were suppressed (31).

SREBP-2 is a transcription factor regulating the transcriptional activation of HMG-CoAr and LDL receptor (45). Consumption of ISF-poor SPI decreased the mRNA levels of SREBP-2, HMG-CoAr, CYP7A1, and LDL receptor in the liver of rats and markedly reduced liver cholesterol and triglyceride content and plasma triglyceride concentrations (50). In contrast, ISF-containing soy extract and the individual ISF increased the mature form of SREBP-2 and HMG-CoAr protein as well as HMGCoAs mRNA levels in cultured HepG2 cells (56) and in rats (4,57), and elevated serum cholesterol clearance (4,57). The discrepancy between these studies may attribute to the variable content of ISF in SPI.

4.2. Peroxisomal proliferator activated receptor

Peroxisomal proliferator activated receptor (PPAR) is a ligand-activated transcription factor (58) and has three isoforms (alpha, gamma and delta). These isoforms are approximately 60-80% homologous in their ligand- and DNA-binding domains and have overlapping tissue distribution. PPARs are important in the regulation of lipid metabolism. For example, PPARalpha controls fatty acid oxidative metabolism through transcriptional induction of carnitine–palmitoyl transferase 1 (CPT-1). CPT-1 is a key enzyme involved in the transfer of fatty acids into the mitochondria to promote β-oxidation in the liver (59). PPARalpha knockout in mice remarkably elevates blood and hepatic cholesterol and triglyceride levels (60,61).

Intake of soy proteins significantly decreased hepatic triglyceride levels and epididymal adipose tissue weight, and increased skeletal muscle CPT1 activity as well as CPT1 and PPARalpha mRNA levels in male Sprague-Dawley rats (62). Up-regulation of CPT-1 mRNA expression by soy proteins was shown to be mediated through activation of PPAR in rat liver and thereby increased fatty acid oxidation (47).

The effect of ISF on lipid metabolism might be mediated through both PPARalpha-dependent and independent pathways. The wild-type and PPARalpha knockout mice were fed soy proteins with or without ISF for 6 weeks. Soy proteins with ISF activated hepatic PPARalpha in wild-type mice and decreased serum triglyceride levels in both wild-type and PPARalpha knockout mice (61). Additionally, an ISF-containing soy extract doubled PPAR-directed gene expression in RAW 264.7 cells containing either a PPARalpha or PPARgamma expression plasmid. A similar induction was observed when the soy ISF genistein or daidzein were used to treat cells (20).

PPARgamma is a key regulator of glucose homeostasis and adipogenesis and is required for normal adipocyte differentiation. Activation of PPARgamma or its obligate heterodimer PPARgamma:RXR inhibited cholesterol accumulation by enhancing cholesterol efflux (63). Dietary soy proteins increased PPARgamma mRNA steady-state levels (47) and protein content (64) in adipose tissue of Zucker diabetic fatty fa/fa rats compared to a casein diet, and reduced the total and liver adiposity and maintained a lower number of dysfunctional adipocytes.

4.3 Thyroid hormone receptor

It has been established that thyroid hormones play important roles in the regulation of lipid metabolism. A deficiency in thyroid hormones results in elevated cholesterol levels in blood, however, it can be normalized by thyroid hormone substitution (65,66). Thyroid hormones influence lipid metabolism at several critical steps in the liver, including (a) regulation of fatty acid synthesis via ACC (67), FAS (68) and ME (69); (b) control of cholesterol biosynthesis via HMG-CoAr (70); (c) regulation of bile acid synthesis via CYP7A1 (71,72); and (d) mediation of cholesterol uptake from the circulation via LDL receptor (70,73).

Increased T4 levels were proposed as a putative mechanism responsible for the hypocholesterolemic actions of soy proteins (38). T4 levels were negatively correlated with plasma cholesterol and triglyceride concentrations in gerbils (74), rats (75), and hamsters (76) fed soy protein-based diets. However, this notion appears to be inconsistent with the results obtained from human clinical studies. Daily intake of SPI with either 1.64 or 61.7 mg ISF for 57 d had no effect on total triiodothyronine (T3), free T3, total T4, free T4, thyroid stimulating hormone (TSH) and thyroid binding globulin in healthy young men (77). The blood lipid levels including total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apolipoprotein (apo) B, and

apo A-I were not different among three dietary groups. However, the ratios of total to HDL cholesterol, LDL to HDL cholesterol, and apo B to apo A-I were significantly lower with both SPI treatments than with milk protein isolate treatment (18).

Of the seven trials (77-83) assessing the effects of soy proteins on serum TSH, total T3, and T4, only one study (82) showed elevated serum TSH, T3 and T4 by soy proteins compared with casein group. However, when compared to the normal baseline level, these increases were minimal. Another study also found that soy intake significantly decreased the plasma cholesterol concentration, but had no effect on T4 level in postmenopausal women with type 2 diabetes (79). In addition, although SPI and soy protein concentrates are both hypocholesterolemic in hamsters, only SPI increases T4 levels (84). These results indicate that modulation of thyroid hormone status may not be the only mechanism responsible for the cholesterol-lowering action of soy proteins.

Thyroid hormone receptor (TR) mediates the thyroid hormone-regulated physiological functions. Mammalian TR is encoded by two genes, TRalpha and TRbeta. The primary transcript from each gene can be alternatively spliced to form different isoforms. Four TR isoforms have been reported to date and they are TRalpha1, TRalpha2, TRbeta1, and TRbeta2 (85). TRalpha and TRbeta differ slightly in structure and substantially in tissue distribution. TRalpha regulates heart rate and the speed and force of systolic contraction (86). TRbeta has diverse effects on lipid metabolism and hypothyroidism (87) and is highly expressed in liver (88). They are key regulators of many genes involved in lipid metabolism.

We have shown that consumption of alcohol-washed SPI markedly increased hepatic TRbeta1 protein content in rats compared with a casein-based diet, but had no effect on TRbeta1 mRNA abundances. Supplemental ISF had no additional effect compared with SPI alone. We further demonstrated that effects of SPI on TR were isoform- and tissue-specific (43). Interestingly, the binding ability of nuclear TR to the target genes measured by electrophoretic mobility shift assay was remarkably suppressed by dietary SPI (42).

TRbeta isoform is the major functional TR in liver, accounting for 80% of hepatic T3-binding activity (89), and plays a unique role in the regulation of cholesterol metabolism. For instance, the normal stimulation of T3 on CYP7A1, a rate limiting enzyme in bile acid biosynthesis, was lost in TRbeta-/- but not in TRalpha-/- mice (90), and overexpression of TRalpha1 could not substitute for absence of TRbeta (91).

Inhibition of TRbeta1 binding to target genes by SPI could block the stimulatory effects of thyroid hormones and alter downstream gene expression. In addition, suppression of TRbeta DNA binding may also affect lipid metabolism through altering PPARgamma-mediated gene expression involved in lipid homeostasis. It has been recently demonstrated that TRbeta can compete with PPARgamma and bind to the peroxisome proliferator response element (PPRE) as homodimers and heterodimers with PPARgamma or the retinoid X receptor (RXR) (92), thereby inhibiting PPAR-mediated gene expression at the level of PPAR binding to PPRE (93). Decreased DNA binding ability of TRbeta by SPI may result in increased binding of PPARgamma to PPRE and up-regulation of PPARgamma-mediated gene expression.

4.4. Retinoic acid receptor

RAR family is activated by both all-trans and 9-cis retinoic acid (94). Three RAR subtypes (alpha, beta, and gamma) have been characterized (94,95) and are encoded by distinct genes. Several isoforms are produced from each gene. For example, RARbeta has four isoforms (beta1, beta2, beta3 and beta4) which are generated via alternative gene splicing of primary transcripts initiated from 2 promoters (96,97). Retinoic acid (RA), a metabolite of vitamin A, is important in the prevention and treatment of various cancers (98,99) and also plays important roles in controlling lipid metabolism (100,101).

However, induction of hypertriglyceridemia is one of the major adverse effects in the treatment of cancers with retinoids in both rats (102) and humans (98,103-105). Replacement of dietary casein with SPI markedly reduced the severity of RA-induced hypertriglyceridemia in rats (100,106). Although the involved molecular events are unclear, the RA-induced hypertriglyceridemia was shown to be mediated via retinoid receptors (100,107) and the serum retinoid level in RA-treated rats was not affected by dietary SPI (106).

We have recently reported that ingestion of 20% alcohol-washed SPI significantly elevated hepatic RARbeta2 protein content, but had no effect on its mRNA abundance in rats, compared with a casein diet. However, the DNA binding abilities of hepatic RARbeta2 to its target genes were significantly suppressed. Increasing amounts of added soy ISF had no effect on RARbeta. We further demonstrated that dietary SPI induces modification of the receptor protein, which is believed to be responsible for the functional inhibition of RARbeta (44). RARbeta2 is the most abundant RARbeta isoform in the body (108). Suppression of RARbeta DNA binding activity may be an important cellular event by which dietary soy proteins alleviate the retinoid-induced hypertriglyceridemia.

4.5. Liver X receptor

LXR is predominantly expressed in the liver and can be activated by oxysterols. LXR regulates a variety of genes involved in the catabolism, transport, and uptake of cholesterol and its metabolites. CYP7A1 is one of those genes and contains a LXR response element in its promoter region (109). LXR acts as a cholesterol sensor and up-regulates the expression of CYP7A1, which results in increased bile acid synthesis and subsequently excretion of cholesterol (110). Through regulation of ATP-binding cassette-A1 transporter system in extrahepatic cells, LXR controls the efflux of cholesterol from these cells which will then be taken up by HDL and transported back to the liver for further catabolism and elimination in bile (111). In

addition, LDL receptor and SREBP-1c genes possess the LXR response element through which LXR mediates endocytic uptake of LDL cholesterol in the liver (112) and insulin-induced SREBP-1 gene expression (113). Ingestion of non-extracted soy proteins (containing normal level of ISF) reduced hepatic cholesterol concentrations and down-regulated the expression of liver LXR, CYP7A1, ATP-binding cassette-A1 transporter and SREBP-1 mRNA in obese rats (47).

5. REGULATION OF GENES RELATED TO LIPID METABOLISM BY SOY PROTEINS AND ISF

5.1. Fatty acid biosynthesis

Three key enzymes are involved in fatty acid synthesis. ACC catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, which is the first step of the long-chain fatty acid biosynthesis (114). FAS catalyzes malonyl-CoA to generate long-chain fatty acids, a complex NADPH-dependent seven-step reaction (115). ME catalyzes oxidative decarboxylation of malate to pyruvate and CO_2, with the concomitant reduction of the cofactor NAD(P)(+) to NAD(P)H (116), a process providing the source of NADPH for FAS. Alteration in the gene expression or enzymatic activity in any of these enzymes may affect fatty acid synthesis.

5.1.1. ACC

Two ACC isoforms have been identified and are encoded by distinct genes. ACCalpha is a cytosolic protein, highly expressed in liver and adipose tissue that exhibit high rates of fatty acid synthesis, whereas ACCbeta is located in mitochondria and is predominantly expressed in heart and muscle, and to a lesser extent in liver. ACCbeta plays a pivotal role in the regulation of mitochondrial fatty acid beta-oxidation (117) through feedback inhibition of CPT-1, an enzyme that controls the entry of long-chain fatty acid CoA esters into the mitochondria for degradation (118). Suppression of ACCbeta increases CPT-1 activity and enhances fatty acid beta-oxidation.

It has been found that feeding soy proteins reduces hepatic ACCalpha mRNA content in fatty rats (119). This is further supported by our study in normal rats showing that intake of alcohol-washed SPI significantly reduced ACCalpha and ACCbeta mRNA and protein expression in the liver (22). Moreover, the ratio of phospho-ACCalpha/ACCalpha and phospho-ACCbeta/ ACCbeta were not different in liver, kidney and heart between rats fed SPI- or casein-based diet in our study (22), suggesting that dietary SPI may affect ACC mainly through transcriptional regulation rather than phosphorylation or dephosphorylation.

Regulation of ACC gene expression by dietary SPI might be mediated through at least two different pathways which could be TR-dependent or SREBP-dependent. ACC gene contains thyroid hormone response element in its promoter region and its expression is regulated by thyroid hormones (120). In addition, SREBP-1 is a transcription factor of the ACCalpha gene and is down-regulated by dietary SPI. However, a recent study showed that dietary SPI significantly decreased hepatic ACCalpha mRNA, but did not affect hepatic SREBP-1 mRNA content (121). Further study revealed that ACCalpha expression was controlled by two promoters, PI and PII (121). Dietary SPI significantly decreased PI-generated ACCalpha mRNA expression through inhibiting the binding ability of SREBP-1 to ACCalpha promoter, but did not affect PII and PII-generated ACCalpha mRNA (122), suggesting that SPI regulates ACCalpha mRNA mainly by regulating SREBP-1 binding to PI via nuclear factors other than SREBP-1 itself.

5.1.2. FAS and ME

Mammalian FAS is the product of a single non-duplicated gene that generates a 250 kDa polypeptide chain. FAS is active as a homodimer (115) and its activity is regulated by diet, glucose, T4 and SREBP-1c through transcriptional control (123). Consumption of soy proteins reduced the mRNA expression of FAS and ME, and lowered hepatic triglyceride depots in rats (4,48,49). This regulation is suggested to be mediated through down-regulation of SREBP-1 (4,48,49). However, FAS (124) and ME (125) also contain a thyroid hormone response element in their promoters. Inhibition of the DNA binding activity of TR by SPI (43) may be an alternative pathway for controlling gene expression and lipid synthesis.

5.1.3. ATPase/ATP synthase

ATPase/ATP synthase is an enzymatic complex responsible for ATP synthesis and hydrolysis in mitochondria. It consists of a membrane-bound F0 portion and a soluble F1 portion. F1 has five subunits (alpha, beta, gamma, delta, epsilon), and the beta subunit contains the catalytic sites of the ATP synthesis. F1 catalyzes the synthesis of ATP from adenosine diphosphate and inorganic phosphate. ATPase/ATP synthase plays important roles in the regulation of carbohydrate, protein and lipid metabolism via modulating energy homeostasis. Its beta subunit on the hepatocyte plasma membrane was recently identified as a high-affinity HDL receptor (126). HDL mediates the efflux and transport of cholesterol from peripheral cells to the liver for further metabolism, suggesting that ATPase/ATP synthase may play a role in the regulation of cholesterol metabolism.

Dietary supplementation with soy isolated proteins containing ISF prevented reduction of Na^+, K^+-ATPase activity, another member of the ATPase family, in diabetic rats (127). Genistein, one of the main soy ISF, suppressed brain and hepatic mitochondrial ATP synthase activity *in vitro* (128). We have recently shown that hepatic mitochondrial ATPase activity was significantly higher in the rats fed alcohol-washed SPI than in those fed casein. Addition of ISF to SPI eliminated the action of SPI. ATPase/ATP synthase beta protein content in the liver were unchanged; however its patterns (isoelectric points) measured by 2 dimensional Western blot were different among dietary groups. We further demonstrated that dietary SPI increases the dephosphorylation of the mitochondrial ATPase/ATP synthase beta subunit, which is responsible for the increase in the enzymatic activity (129). However, the physiological importance of this cellular response to the dietary SPI remains to be investigated.

5.2. Cholesterol metabolism

5.2.1. Synthesis

In most cells, cholesterol is derived either from endogenous synthesis via the mevalonate pathway or from the uptake of circulating cholesterol-rich LDL. HMG-CoAr is the rate-limiting enzyme in cholesterol biosynthesis and catalyzes the formation of mevalonate from HMG-CoA. Genistein has been shown to decrease cholesterol synthesis in cultured HepG2 (32) and inhibit HMG-CoAr activity in MCF-7 human breast cancer cells (33). Consumption of SPI significantly decreased HMG-CoAr mRNA abundance and cholesterol synthesis in the liver of nephrotic rats (49). We also found that hepatic HMG-CoAr protein content was significantly lower in the rats fed SPI with ISF supplement compared to casein control (unpublished data).

5.2.2. Catabolism

One of the mechanisms proposed to explain the hypocholesterolemic effect of soy proteins is through an increase in bile acid secretion. Rabbits fed casein diet excreted less neutral steroids and produced less bile acid than animals fed a soy protein diet. In addition, rabbits fed casein excreted mainly cholesterol whereas those fed soy proteins excreted unabsorbable coprostanol (35). Peptides prepared by the *in vitro* digestion of soy proteins were found to stimulate fecal steroid excretion and consequently lower serum cholesterol in rats (130) as well as in human volunteers (131).

Bile acids are amphipathic and polar derivatives of cholesterol. The liver excretes bile acids to the intestine where ~ 95% of bile acids are then reabsorbed in the terminal ileum and returned to the liver. If bile acids are no longer available for transport back to the liver, they are lost in the faeces. As a result, there is an up-regulation of hepatic enzymes involved in bile acid biosynthesis such as CYP7A1.

The activity of CYP7A1 is negatively regulated by bile acid, diet, drug and hormones. The effects of consumption of soy proteins on hepatic CYP7A1 gene expression are inconsistent in different studies. One study showed that dietary soy proteins increased CYP7A1 gene expression in the liver (30), whereas other studies reported opposite effects (47,50,132). We have recently demonstrated that ingestion of 20% alcohol-washed SPI (containing minimal amount of ISF) remarkably reduced the total cholesterol levels in the blood and liver of the rats compared with a casein diet. However, the hepatic CYP7A1 protein contents measured by Western blot were not different between dietary groups. Our further study showed that SPI appears to increase the phosphorylation of hepatic CYP7A1, which may lead to a higher enzymatic activity (unpublished data). Although this remains to be confirmed, other studies have already demonstrated that increased protein phosphorylation is correlated with enhanced activity of CYP7A1 (133).

5.2.3. Uptake

LDL cholesterol is mainly from three sources: peripheral cholesterol synthesis, hepatic cholesterol synthesis, and intestinal cholesterol absorption. LDL receptor is a major regulator of circulating LDL cholesterol. It has been suggested that the hypocholesterolemic effects of soy proteins may be mediated through up-regulation of LDL receptors (37,134-137). Soy proteins enhance the expression of the LDL receptor in cultured human hepatoma cells (37,134), animals (136,137), and hypercholesterolemic type 2 diabetic patients (135). A similar effect has been observed with a soy protein polypeptide in cultured HepG2 cells (25). However, results obtained from a study with LDL receptor null mice did not support this hypothetical mechanism, in which SPI lowered the plasma LDL and VLDL cholesterol concentrations and inhibited atherosclerosis despite the absence of the LDL receptor in the mice (138). This suggests that LDL receptor–independent pathway(s) by which dietary SPI lowers cholesterol levels may exist.

6. SUMMARY

The hypolipidemic actions of soy components (mainly proteins and associated ISF) appear to be consistent in both animal and human studies. However, the size of the effect is variable, which might be attributed to the variation in the processing procedures of SPI, the content of ISF, the amount of unknown bioactive component(s) or the physiology of the subjects. It has been shown that different processing procedures affect the intactness of soy protein subunits that might be crucial for their physiological functions (6). Although the *in vitro* and animal studies showed that beta-conglycinin, particularly the alpha' subunit, has most of the functions of the soy, all the human trials and animal studies were based on the amount of total soy proteins rather than the amount of the bioactive components which might be very different among different SPI preparations. In addition, the ability of the subjects to metabolize daidzein or daidzin to equol is suggested to be associated with their responsiveness to dietary soy. However, only 30-50% of the adult population can excrete equol in urine when challenged daily with soy foods (8). Soy proteins and ISF regulate lipid metabolism via multiple cellular pathways (fatty acid biosynthesis, cholesterol synthesis, catabolism and uptake) and through modulation of the key transcription factors including SREBP, PPAR, TR, RAR, and LXR. In addition to regulation at transcriptional levels, soy proteins have also been shown to posttranslationally modify the receptor (such as RAR) and enzymatic (ATPase/ATP synthase and CYP7A1) proteins via a phosphorylation and dephosphorylation mechanism, and thereby altering the DNA binding ability of the receptor or activity of the enzymes. Since most of the mechanism studies on soy actions have been conducted in either cultured cells or animal models, whether the same mechanisms are shared in humans remains to be verified using appropriate biomarkers.

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Abbreviations: ACC: acetyl-CoA carboxylase; CYP7A1: cholesterol-7alpha hydroxylase; FAS: fatty acid synthase; HMG-CoAr: hydroxymethylglutaryl-CoA reductase; HMG-CoAs: hydroxymethylglutaryl-CoA synthase; ISF: isoflavones; LDL: low density lipoprotein; LXR: liver X receptor; ME: malic enzyme; PPAR: peroxisomal proliferators activated receptor; RAR: retinoic acid receptor; SPI: soy protein isolate; SREBP: sterol regulatory element binding protein; T3: triiodothyronine; T4: thyroxine; TR: thyroid hormone receptor.

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