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Soy protein reduces triglyceride levels and triglyceride fatty acid fractional synthesis rate in hypercholesterolemic subjects

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Abstract

To examine the effects of protein source and isoflavones on triglyceride (TG) fatty acid (TGFA) and cholesterol biosynthesis, subjects (>50 years, LDL cholesterol >130 mg/dl) underwent a four-phase randomized cross-over feeding trial. Diets contained either isolated soy protein or common sources of animal protein (25 g/1000 kcal), without or with isoflavones (49 mg/1000 kcal) and were each fed for 6 weeks. Blood samples from 20 hyperlipidemic subjects (6M, 14F, 62 ± 9 years, BMI 26 ± 3 kg/m^2, LDL cholesterol >160 mg/dl after feeding animal protein without isoflavones) were selected to measure TGFA fractional synthetic rate (TGFA-FSR) and free cholesterol fractional synthetic rate (FC-FSR) over 24 h as deuterium oxide uptake into TGFA and free cholesterol. Soy protein reduced TG by 12.4% ($P < 0.0001$), total cholesterol by 4.4% ($P < 0.001$), and LDL cholesterol by 5.7% ($P = 0.003$) compared to animal protein. The TGFA-FSR was reduced by13.3% ($P = 0.018$) and FC-FSR was increased by 7.6% ($P = 0.017$) after the soy protein relative to the animal protein. Isoflavones had no significant effect on TG and TGFA-FSR. Isoflavones reduced total cholesterol levels by 3.1% ($P = 0.009$) but had no significant effect on LDL, HDL cholesterol levels, or FC-FSR. These data demonstrate that dietary protein type modulates circulating TG and cholesterol levels in hypercholesterolemic individuals by distinct mechanisms.
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Keywords: Soy protein; Animal protein; Isoflavones; Lipid profiles; Cholesterol biosynthesis; Triglyceride fatty acid biosynthesis

1. Introduction

Over the past 30 years numerous studies have investigated the lipid-lowering effects of soy protein. In a meta-analysis [1], soy protein reduced low density lipoprotein (LDL) cholesterol (LDL-C) levels by an average of 13% when compared to animal protein (primarily casein), with a greater reduction achieved in subjects with higher initial total cholesterol (T-C) levels. Decreases in triglyceride (TG) levels were also noted (11%) [1]. Since then a number of studies have re-evaluated the effects of soy protein and/or isoflavones on blood lipid levels and the results, for the most part, have been less dramatic [2–8]. The majority of work has compared soy protein to casein but not to common animal protein containing foods, hence it is difficult to extrapolate results for the purpose of developing public health recommendations.

To date, the mechanism(s) for the potential hypolipidemic effect of soy protein remains elusive and is almost certain to be multifactorial [9,10]. Postulated mechanisms include inhibition of cholesterol absorption or enhanced bile acid excretion [11,12], and increased receptor mediated clearance [13–16], LDL receptor activity [2] or 7α-hydroxylase activity [17].

The association between TG levels and cardiovascular health is the topic of ongoing investigation. With respect to TG levels and soy protein, some work in animals has demonstrated reduced levels when the soy protein contained isoflavones [12,18,19], whereas other work failed to observe a significant effect [10,14,16]. In humans, most work has reported no significant effect of soy protein on TG levels [2–4,7,8], although reductions [6] and increases [5] have been identified. Focusing on isoflavones, with or without soy protein, the data on TG levels are conflicting [4,8,18,20]. The mechanism(s) by which soy protein and/or isoflavones influences TG levels have yet to be postulated.

In October 1999 the US Food and Drug Administration (FDA) authorized the use of a health claim relating intakes

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of at least 25 g of soy protein per day to reduce the risk for developing coronary heart disease [21]. Much of the support for this health claim appears to have been based on a meta-analysis published in 1995 [1]. After comprehensively reviewing the available literature at the time the authors concluded that soy protein, when compared to animal protein, significantly decreased T-C, LDL-C and TG levels and non-significantly increased high density lipoprotein (HDL) cholesterol (HDL-C) levels. Because studies included in the meta-analysis used two different forms of soy protein, one devoid of naturally occurring isoflavones and one in which the isoflavones were intact, it was not clear whether the effects observed were attributable to the soy protein, per se, or the isoflavones. Subsequent work has demonstrated that the potential benefit of soy protein may be dependent on whether it is ingested with the constituent isoflavones [4] whereas other work does not support this observation [22–25]. This study was designed to assess the independent effect and mechanisms of soy protein relative to common sources of animal protein (meat and dairy), distinct from the fatty acid profile and isoflavones, and likewise, the independent effect and mechanisms of soy isoflavones, distinct from protein type and fatty acid profile on TG and cholesterol metabolism, in a controlled trial in individuals at elevated risk of developing cardiovascular disease. We hypothesized that soy protein as compared with animal protein reduce cholesterol and TG levels, and isoflavones work independently in altering lipid metabolism.

2. Materials and methods

2.1. Subjects

The current study is a part of larger cohort [26], which included 42 subjects over the age of 50 years with LDL cholesterol levels greater than 130 mg/l (3.36 mmol/l), recruited from the greater Boston area. These subjects were targeted because dietary intervention is frequently the initial and/or primary approach to normalize their lipid levels. All subjects fulfilled the following criteria: normal kidney, liver, thyroid and cardiac function; normal fasting glucose levels; not taking medications known to affect blood lipid levels; non-smoker, and all females were postmenopausal. Subjects using other medications were required to continue on the same drug throughout the study period. Fifteen subjects dropped out and 27 completed the main study. This study protocol was approved by the Human Investigation Review Committee of New England Medical Center and Tufts University.

2.2. Experimental design

Study subjects were provided with each of the four experimental diets, including (i) soy protein depleted of isoflavones (soy/−), (ii) soy protein enriched in isoflavones (soy/+), (iii) animal protein with no added isoflavones (animal/−), and (iv) animal protein with added isoflavones (animal/+). Diets were each randomly given for a period of 42 days. Investigators and laboratory personnel were blinded as to diet phase. Whereas study subjects were blinded with respect to dietary isoflavones and although the intent was to blind them with respect to type of protein, it is unlikely that this was successful due to the textural difference in the foods. Subjects reported to the metabolic research unit three times per week, had their body weight measured at each visit, and consumed one meal on site. All food and drink were provided to the subjects in containers appropriate for either microwave or conventional ovens obviating the need to transfer food prior to consumption. The caloric intakes (mean ± S.D.) of the female and male subjects selected for the current report were 2147 ± 201 and 2799 ± 519 kcal per day, respectively. After day 35 of each diet phase, blood samples were obtained for lipid determination on three separate days in the fasting state. Twenty-four hours before the end of each phase, 1.2 g D_2O/kg estimated body weight was taken orally by each subject. Blood samples were drawn 24 h after the oral administration of D_2O and plasma was separated and stored at −80 °C. Since a greater effect of soy protein on cholesterol levels was previously reported in individuals with higher baseline cholesterol levels [1], the plasma samples of 20 subjects (6M, 14F) who completed all phases of the study and had LDL-C level equal to or greater than 160 mg/dl after the treatment of animal/− diet (the diet most similar to an average Western diet) were selected post hoc from the large cohort. These samples were sent to McGill University for the measurements of deuterium incorporation into the TG fatty acids (TGFA) and free cholesterols. Characteristics of the subjects in this sub-study at the time of screening are presented in Table 1.

2.3. Diets

The animal/− diet was designed to mimic a non-optimal diet of a hypercholesterolemic subject. Other diets were designed to be similar in total fat, carbohydrate, protein, fatty acid profile, fiber, and cholesterol content, achieved

Table 1
Characteristics of the subjects at the time of screening for the study

Variable	Male (6)	Female (14)	All subjects (20)
Age (years)	58.0 ± 12.8	63.1 ± 6.9	61.6 ± 9.0
BMI (kg/m^2)	25.6 ± 2.0	26.3 ± 3.7	26.1 ± 3.2
Serum concentration (mg/dl)			
TG	101 ± 21	147 ± 72	133 ± 64
T-C	238 ± 30	254 ± 37	249 ± 35
LDL-C	166 ± 33	170 ± 29	169 ± 30
HDL-C	52 ± 9	55 ± 13	54 ± 12

Values represent mean ± S.D. BMI, body-mass index; T-C, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride. To convert values from mg/dl to mmol/l, divide by 38.67 for cholesterol and by 88.54 for triglyceride.

by using as many of the same foods as possible, while substituting isolated soy protein for animal protein (2/3 of total protein, 10% of energy), and adjusting the fatty acid profile to be similar across diets using commonly available fats and oils. For the animal/− and animal/+ diets, the protein component was provided by milk and milk products, and meat. Isoflavones, in the form of a powered concentrate (Archer Daniels Midland Company, Decatur, IL), were mixed into different food items of the animal/+ diet consumed throughout the day. For the soy/− and soy/+ diets, the protein component was contributed by specially prepared batches of isolated soy protein, one depleted (0.12 mg aglycone/g protein) and one enriched (1.96 mg aglycone/g protein) in isoflavones (Protein Technologies, St. Louis, MO). The mean soy intakes of the female and male subjects were 107 ± 10 and 140 ± 26 g per day, respectively. The isolated soy protein was incorporated into sauces, cereals, casseroles, baked goods and deserts. The soy/+ and animal/+ diets were formulated to have similar levels of isoflavones as confirmed by chemical analysis (soy/+ diet, 46 mg aglycone isoflavones/1000 kcal; animal/+ diet, 52 mg aglycone isoflavones/1000 kcal). A relatively high isoflavone dose was chosen because it was previously reported to be efficacious [4]. The distribution of genistein, daidzein and glycitein was 59, 30 and 11%, respectively, in the soy/+ and 52, 40 and 8% in the animal/+ diets, respectively. Food items low and high in isoflavones were indistinguishable with respect to appearance and taste. The isoflavone intakes of female and male subjects were 105 ± 10 and 137 ± 25 kcal per day, respectively. Chemical analysis of food homogenates was carried out by Covance Laboratories Inc. (Madison, WI). Dietary isoflavones analysis was carried out in the laboratory of Dr. Patricia A. Murphy (Iowa State University, Ames, IA) (Table 2).

Table 2
Nutrient composition of experimental diets as determined by chemical analysis of food

Constituent	Soy/−	Soy/+	Animal/−	Animal/+
Percentage of energy				
Carbohydrate	45	46	47	48
Protein	17	16	17	15
Fat	38	37	36	36
C16:0	5.7	5.1	5.9	5.7
C18:0	2.4	2.2	2.4	2.4
C18:1 *n*-9	14.0	13.8	13.5	13.6
C18:2 *n*-6	5.1	5.1	4.7	4.8
C18:3 *n*-3	0.7	0.7	0.6	0.6
mg/1000 kcal				
Fiber	1.0	1.1	1.2	1.1
Cholesterol	151	150	154	168
Isoflavones	1.25	46.21	ND	51.71

Soy/−, soy protein depleted in isoflavones; soy/+, soy protein enriched in aglycone isoflavones; animal/−, animal protein without isoflavones; animal/+, animal protein with aglycone isoflavones; ND, not detectable.

2.4. Biochemical analysis

Blood samples were collected after a 14 h fast. Serum was separated by centrifugation at 1100 × *g* at 4 °C and assayed for T-C and TG with a Spectrum CCX bichromatic analyzer using enzymatic reagents [27]. LDL-C was measured directly (Sigma Diagnostics, St. Louis, MO). High density lipoprotein cholesterol was measured after precipitation of apolipoprotein (apo) B-containing lipoproteins using dextran-magnesium sulfate [28]. Very low density lipoprotein (VLDL) cholesterol (VLDL-C) was calculated as the difference between T-C and the sum of LDL-C and HDL-C. The assays were standardized through the Lipid Standardization Program of the Centers for Disease Control, Atlanta, GA.

2.5. Measurements of triglyceride fatty acid and cholesterol biosynthesis

Plasma samples obtained before and after 24 h of oral administration of D_2O were used to measure deuterium enrichment in TG and cholesterol fractions as previously described [29,30]. Deuterium enrichment of the hydrogen was analyzed by isotope ratio mass spectrometry (VG Isomass, 903D, Cheshire, UK) with an internal analytical error of 0.17/mil (0/00). The H^+ contribution was checked daily and appropriate correction factors were applied [31]. The mass spectrometer was calibrated daily using three standards. The TGFA fractional synthetic rate (TGFA-FSR) and free cholesterol fractional synthetic rate (FC-FSR) were calculated using the methods reported by Jones et al. [29].

2.6. Statistical analysis

A two-way analysis of variance with main effects of dietary protein type and isoflavone content (minimal or supplemented) and the interactions between protein and isoflavones, with subject as a repeated measure, was carried out for each outcome measure. Differences between the means of dietary protein type or isoflavone levels were performed using the Tukey's Studentized range test at a significance level of 0.05. Values are presented as mean ± S.D. The method of Pearson Correlation Coefficients was used to determine associations between changes in FC-FSR and changes in plasma T-C as well as the associations between changes in TGFA-FSR and changes in plasma TG levels within each pair of treatments. The relationships between FC-FSR and T-C levels and between TGFA-FSR and TG levels were also tested within each treatment. All statistical computations were made using the general linear model procedure of the SAS software [32].

3. Results

Body weights of the subjects remained relatively constant and no significant changes were observed during the course

Table 3
Fasting serum lipid profiles at the end of each diet phase

Variable (mg/dl)	Soy/−	Soy/+	Animal/−	Animal/+	*P*-value	
					Protein	Isoflavone
TG	116 ± 52	109 ± 45	127 ± 47	131 ± 47	<0.0001	0.858
T-C	267 ± 43	258 ± 48	278 ± 40	271 ± 42	<0.001	0.009
LDL-C	186 ± 34	181 ± 43	196 ± 35	192 ± 39	0.003	0.062
VLDL-C	26 ± 13	21 ± 11	26 ± 11	26 ± 11	0.102	0.173
HDL-C	55 ± 13	55 ± 14	56 ± 13	53 ± 15	0.447	0.094
T-C:HDL-C	4.98 ± 1.06	4.84 ± 1.18	5.14 ± 0.97	5.32 ± 1.19	0.037	0.863
LDL-C:HDL-C	3.48 ± 0.90	3.42 ± 1.03	3.62 ± 0.76	3.77 ± 0.97	0.018	0.951

Values represent mean ± S.D. ($n = 20$). HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; T-C, total cholesterol; TG, triglyceride; VLDL-C, very low density lipoprotein cholesterol; soy/−, soy protein depleted in isoflavones; soy/+, soy protein enriched in aglycone isoflavones; animal/−, animal protein without isoflavones; animal/+, animal protein with aglycone isoflavones.

of the study (data not shown). A significant reduction in plasma TG levels (12.4%, $P < 0.0001$) was observed after the hypercholesterolemic subjects (LDL-C > 160 mg/dl) consumed the soy protein containing diets compared with the animal protein diet (Table 3, Fig. 1). This result is similar to the large cohort [26] which showed 11% ($P < 0.0001$) reduction of plasma TG levels by soy protein relative to animal protein. Consistent with a reduction in plasma TG levels, TGFA-FSR was suppressed by 13.3% ($P = 0.018$) after subjects consumed the soy protein relative to animal protein containing diets (Table 4, Fig. 2).

Significant reductions in plasma T-C and LDL-C levels (4.4%, $P < 0.001$ and 5.7%, $P = 0.003$, respectively) were observed after the hypercholesterolemic subjects consumed the soy protein containing diets compared with the animal protein diets (Table 3, Fig. 1). The type of dietary protein had no significant effect on HDL-C levels. Significant reduc-

Table 4
Free cholesterol and triglyceride fatty acid fractional synthetic rate at the end of each diet phase

	Variable (pools per day)	
	TGFA-FSR	FC-FSR
Soy/−	0.107 ± 0.043	0.073 ± 0.030
Soy/+	0.087 ± 0.028	0.070 ± 0.016
Treatment		
Animal/−	0.109 ± 0.045	0.063 ± 0.017
Animal/+	0.116 ± 0.040	0.068 ± 0.027
P-value		
Protein	0.018	0.017
Isoflavone	0.781	0.431

Values represent mean±S.D. ($n = 20$). FC-FSR, free cholesterol fractional synthetic rate; TGFA-FSR, triglyceride fatty acids fractional synthetic rate; soy/−, soy protein depleted in isoflavones; soy/+, soy protein enriched in aglycone isoflavones; animal/−, animal protein without isoflavones; animal/+, animal protein with aglycone isoflavones.

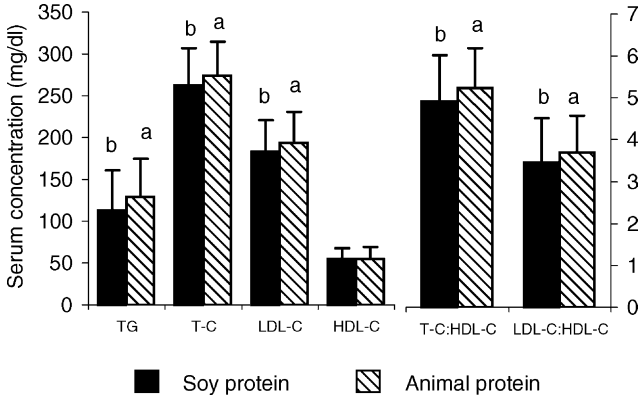

Fig. 1. Effect of soy protein relative to animal protein on lipid profiles in moderately hypercholesterolemic individuals. A two-way analysis of variance with main effects of dietary protein type and aglycone isoflavone content (minimal or supplemented) with subject as a repeated measure was carried out for each outcome measure in all 20 subjects. Differences between the means of dietary protein types or isoflavone levels were performed using the Tukey's Studentized range test at a significance level of 0.05. Values represent mean ± S.D. and those with different superscripts are significantly different ($P < 0.05$). T-C, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride.

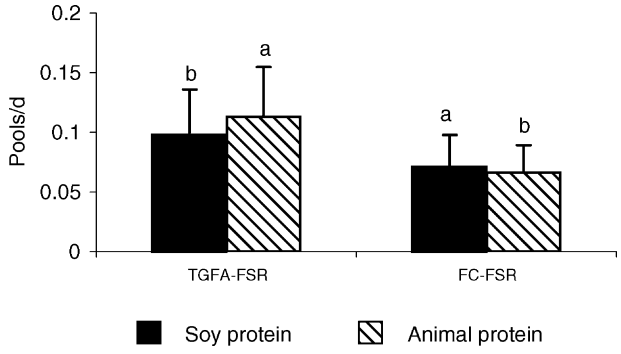

Fig. 2. Effect of protein source on triglyceride fatty acid and free cholesterol fractional synthesis rate. A two-way analysis of variance with main effects of dietary protein type and isoflavone content (minimal or supplemented) with subject as a repeated measure was carried out for each outcome measure utilizing all 20 subjects. Differences between the means of dietary protein types or isoflavone levels were performed using the Tukey's Studentized range test at a significance level of 0.05. Values are presented as mean ± S.D. FC-FSR, free cholesterol fractional synthetic rate; TGFA-FSR, triglyceride fatty acid fractional synthetic rate. For FC-FSR and TGFA-FSR, values with different superscripts are significantly different ($P < 0.05$).

tions in T-C/HDL-C (6.1%, $P = 0.037$) and LDL-C/HDL-C (6.6%, $P = 0.018$) ratios were observed after the subjects consumed the soy protein relative to animal protein diets. Free cholesterol fractional synthetic rate was reciprocally influenced by soy protein and increased by 7.6% ($P = 0.017$) after subjects consumed the soy protein diets compared with the animal protein diets (Table 4, Fig. 2).

Soy derived isoflavones, as a constituent part of soy protein or added to the animal protein diet, reduced T-C levels by 3.1% ($P = 0.009$) (Table 3). However, the effect was not significant when the changes in T-C levels were further assessed on the basis of lipoprotein subfractions (VLDL, LDL, and HDL) cholesterol. The T-C/HDL-C and LDL-C/HDL-C ratios were not significantly affected by isoflavones. Similarly, isoflavones did not affect FC-FSR (Table 4). Isoflavones had no significant effects on TG levels (Table 3) and TGFA-FSR (Table 4).

There was no significant interaction between protein source and isoflavones on plasma TG levels. A significant interaction ($P = 0.019$) was observed between protein source and isoflavones on plasma TGFA-FSR, i.e. isoflavones decreased TGFA-FSR when added to soy protein but increased TGFA-FSR when added to animal protein. TGFA-FSR was significantly associated with plasma TG levels within each treatment period. The correlation coefficient was 0.459 ($P = 0.042$) for soy/−, 0.716 ($P < 0.001$) for soy/+, 0.653 ($P < 0.003$) for animal/−, and 0.558 ($P = 0.016$) for animal/+. However, there was no relationship between the difference in plasma TG levels and the difference in TGFA-FSR after consumption of the soy versus animal protein diets or after consumption of diets with isoflavones versus diets without isoflavones.

There were no significant interactions between protein source and isoflavones with respect to plasma T-C, LDL-C, and HDL-C levels as well as FC-FSR. Free cholesterol fractional synthetic rate was not associated with plasma T-C levels within each treatment period. No correlations were observed between changes in FC-FSR and the changes in T-C levels after soy versus animal protein diets or after diets with isoflavones versus diets without isoflavones.

4. Discussion

The design of the present study allowed an independent assessment of protein source and isoflavone levels alone and in combination on plasma lipid profiles, TGFA-FSR and FC-FSR in hypercholesterolemic subjects. The pronounced effect of soy protein, relative to animal protein, on plasma TG levels was somewhat unexpected and persisted in the non-fasting state (data not shown). The current study demonstrated that soy protein decreased TGFA-FSR compared to animal protein. The degree of reductions in TGFA-FSR (13.3%) is close to that of the reductions in TG levels (12.9%), suggesting that the reduction of TGFA-FSR was a contributing factor to plasma TG reductions by soy protein. Although soy protein consumption had originally been reported to reduce TG levels [1], the observation has not been corroborated by the more recent studies [2–4,7,8] with the exception of one [6]. The more controlled nature of the current study, the minimum intake of soy protein, 50 g per day, and the use of common sources of animal protein, in contrast to casein, may have contributed to the discrepancies among reports. Interestingly, the change in TG levels was not accompanied by a reciprocal response in HDL-C levels as is frequently observed. Significant associations between TG levels and TGFA-FSR within each treatment period suggest an important relationship between de novo biosynthesis of TGFA and plasma TG levels. However, there was no significant correlation between the changes in TGFA-FSR and the changes in plasma TG levels after consumption of the soy versus animal protein diets. These data suggest that soy protein reduces plasma TG levels through multiple mechanisms, one of which is TGFA-FSR.

The data that emerged from the current study are in agreement with some previous work showing decreases in T-C and LDL-C or non-HDL-C levels in hypercholesterolemic subjects consuming soy protein compared with casein [7,33–36], but not other work reporting no effect [8,37]. In ovariectomized adult female cynomolgus monkeys soy protein has been reported to reduce T-C and non-HDL-C levels [10]. Endogenous de novo cholesterol biosynthesis is an important input to the cholesterol pool and impacts on cholesterol kinetics and plasma levels. Animal studies have shown that soy protein increases the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase [17,38], suggesting an increase in the cholesterol biosynthesis. There are few studies, if any, which have examined rates of cholesterol biosynthesis using a direct method in humans, possibly due to limitations in methodology. The current study demonstrated that cholesterol synthesis rate, measured using the deuterium incorporation method, was increased in hypercholesterolemic subjects when fed diets enriched in soy compared to animal protein. No correlations between T-C and FC-FSR were observed. These results, together with the previous work, suggest that the reductions in T-C and LDL-C levels observed by soy protein are not attributable to alterations in rates of cholesterol biosynthesis.

Since the FC-FSR was increased after soy protein relative to animal protein intake, the reductions in T-C and LDL-C levels by soy protein must be a result of other mechanisms. Some studies have suggested that when soy protein is fed, cholesterol absorption or bile acid reabsorption, or both, are impaired. This has been observed in animal species such as rabbits [11], rats [12,39], monkeys [10], and hamsters [16], but not in humans. Increases in LDL receptor activity in both animals and humans have been reported after ingestion of soy protein or various extracts of soy, or both [17]. Khosla et al. [13] reported that removal of LDL from the circulation is enhanced in rabbits fed soy protein. Increases in LDL receptor activity resulting from soy protein feeding have been observed in rats [14] and hypercholesterolemic

individuals [15]. Elevated LDL receptor mRNA levels in mononuclear cells have been reported in subjects consuming soy protein relative to casein [2], suggesting an effect of soy protein on gene expression and LDL receptor production. The increased cholesterol biosynthetic rate is likely a compensatory response to the reductions in cholesterol levels resulting from inhibition of cholesterol absorption and enhancement of cholesterol clearance. The increased cholesterol biosynthesis did not completely compensate for the reductions in cholesterol input from changes in absorption or clearance in contrast to what has been observed with phytosterols, which reciprocally affect cholesterol absorption and synthesis [40,41].

It has been suggested that the isoflavones present in soy protein are responsible for or contributing to lower cholesterol levels in populations consuming high soy diets [42]. Accordingly, work has focused on the plasma cholesterol modulating effect of isoflavones [2,4,9,43,44]. Some studies have shown reductions in total [43] or LDL [44] or non-HDL cholesterol [2,9] levels when subjects (postmenopausal women and elderly men) consume soy protein plus high levels of isoflavones. Only one study, to our knowledge, has shown a dose response of isoflavones in reducing T-C and LDL-C levels when consumed with soy protein diet [4]. Isoflavones, at the dose provided in the current study (49 mg/1000 kcal), showed a moderate effect in reducing T-C with no influence on LDL-C and HDL-C as well as TG levels in hypercholesterolemic subjects. These results are in contrast to those reported by Crouse et al. [4] while being in agreement with those reported by Urban et al. [43]. Other studies administering isoflavones at levels similar to that used in the current study, in the absence of soy protein, have reported no significant effect on lipid and lipoprotein levels [22,24,25,29,45,46]. The discrepancy in the effect of isoflavones on lipid metabolism may be a result of differences in the components of isoflavones and structural features (aglycone or glycone), diet composition, duration of supplementation, and baseline lipid profiles of the subjects. The results of the current study demonstrated that isoflavones did not have a significant effect on plasma TG levels and TGFA-FSR. There was a trend ($P = 0.182$) for an interaction between protein and isoflavones on TG levels, i.e. the addition of isoflavones reduced TG levels when fed with soy protein, but raised TG levels when fed with animal protein. Isoflavones affected TGFA-FSR in a way that is associated with protein source. As compared with animal protein without isoflavones, animal protein with isoflavones increased TGFA-FSR. In contrast, isoflavones acted synergistically with soy protein to decrease TGFA-FSR.

In summary, these data demonstrate that consumption of relatively high levels of soy protein decreased TG levels in hypercholesterolemic subjects, an effect which appears to be in part attributable to the suppression of TGFA biosynthesis. In contrast to the effect of soy protein on TG metabolism, soy protein moderately reduced T-C and LDL-C levels in hypercholesterolemic subjects in a manner that is not due to a reduction in cholesterol biosynthesis. Other mechanisms such as suppressed cholesterol absorption, increased cholesterol removal from the tissues, or increased cholesterol and bile acid excretion may be involved. The increased biosynthesis rate observed likely reflects a compensatory response. Correspondingly, biosyntheses of cholesterol and TGFA were not influenced by isoflavone intake. In conclusion, results of the present study indicate that relatively high intakes of soy protein in hypercholesterolemic individuals (LDL-C $>$ 160 mg/dl) may have a moderately beneficial effect on plasma lipid profiles.

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