

Soy Isoflavone Intake Lowers Serum LDL Cholesterol: A Meta-Analysis of 8 Randomized Controlled Trials in Humans¹

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ABSTRACT Clinical trials have noted hypocholesterolemic effects of soy protein intake, but the components responsible are not known. This meta-analysis of 8 randomized controlled trials was conducted to more precisely evaluate the effects of isoflavones on blood LDL cholesterol concentration independently of soy protein level. PubMed was searched for English-language "randomized controlled trial" articles published from 1966 to 2003 that described the effects of soy protein isolate (SPI) intake with measured isoflavone levels on blood lipids in humans using the search terms "soy protein," "isoflavones," and "cholesterol." From 31 articles identified by the search, 8 articles (with 10 low vs. high isoflavone comparisons) were selected for the meta-analysis. Subjects in each comparison consumed similar dietary fat, cholesterol, and fiber; the reported body weight of subjects did not change significantly during treatment. Serum LDL cholesterol concentration in subjects who consumed SPI (mean 50 g/d) with high isoflavone content (mean intake 96 mg/d) decreased by 0.15 mmol/L (95% CI: 0.08 to 0.23 mmol/L; $P < 0.0001$) compared with those who consumed the same SPI level with low isoflavone content (mean intake 6 mg/d). Decreases in serum LDL cholesterol concentration in hypercholesterolemic and normocholesterolemic subjects were 0.18 mmol/L (95% CI: 0.01 to 0.35 mmol/L; $P = 0.03$) and 0.14 mmol/L (95% CI: 0.06, 0.23 mmol/L; $P = 0.0008$), respectively. With identical soy protein intake, high isoflavone intake led to significantly greater decreases in serum LDL cholesterol than low isoflavone intake, demonstrating that isoflavones have LDL cholesterol-lowering effects independent of soy protein. *J. Nutr.* 134: 2395–2400, 2004.

KEY WORDS: • isoflavones • LDL • cholesterol • soy protein • meta-analysis

A meta-analysis (1) published in 1995 of 38 controlled clinical studies in 29 scientific articles reported that ingestion of 47 g/d of soy protein was associated with the following net changes in serum lipid concentrations compared with the control diet: decrease in total cholesterol of 0.60 mmol/L (23.2 mg/dL; 95% CI, 0.35 to 0.85 mmol/L [13.5 to 32.9 mg/dL]), or 9.3%; decrease in LDL cholesterol (LDL-C)³ of 0.56 mmol/L (21.7 mg/dL; 95% CI, 0.30 to 0.82 mmol/L [11.2 to 31.7 mg/dL]), or 12.9%; and a decrease in triglycerides of 0.15 mmol/L (13.3 mg/dL; 95% CI, 0.003 to 0.29 mmol/L [0.3 to 25.7 mg/dL]), or 10.5%. The changes in serum total cholesterol and LDL-C concentrations were directly related to the initial serum total cholesterol concentration ($P < 0.001$). The analysis did not address possible mechanisms of the effects of soy protein intake. Whether the changes were attributable to the soy protein per se and/or other soy-derived factors, e.g., constitutive isoflavones (IFs), remained unclear.

Since that time, a number of studies have reexamined the effect of soy protein and/or IFs on blood lipid levels in humans

and observed substantially weaker effects. Changes in LDL- or non-HDL cholesterol (HDL-C) levels attributable to the substitution of 25–50 g of soy protein for animal protein range from 0 to a 5% decline in individuals with moderately elevated total cholesterol levels (2–7). Some studies reported that the potential benefit of soy protein might depend on whether it was ingested with the constituent IFs (3), whereas others did not support this observation (8–12). A similar meta-analysis of 10 clinical trials published in 2003 reported that a daily intake of 36 g soy protein with 52 mg soy-associated IFs decreased LDL-C by 0.17 ± 0.04 mmol/L (mean \pm SE), and increased HDL-C by 0.03 ± 0.01 mmol/L. No dose-response relations between soy-associated IF intake and changes in LDL-C (Pearson correlation coefficient $R = -0.33$, $P = 0.14$) or HDL cholesterol ($R = -0.07$, $P = 0.76$) were reported, suggesting that consumption of soy-associated IFs was not related to changes in LDL- or HDL-C (13).

Clinical trials used various intake levels of soy protein with varying amounts of IFs, and different protocols. To more precisely evaluate the effects on serum LDL-C concentrations of IF while controlling for soy protein intake, we performed a meta-analysis of 8 randomized controlled trials that contained blood baseline cholesterol and endpoint LDL-C concentrations, and had different levels of IF intake for a given amount of soy protein intake. Meta-analysis is a statistical technique in which results of separate studies are combined to increase

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³ Abbreviations used: HDL-C, HDL cholesterol; IF, isoflavone; LDL-C, LDL cholesterol; L-IF, low isoflavone; H-IF, high isoflavone; SPI, soy protein isolate.

statistical power, clarify results, and more accurately estimate the size of treatment effects (14–16).

MATERIALS AND METHODS

Study identification and selection. PubMed was searched for English-language articles with publication type “randomized controlled trial” published from 1966 to 2003 that described effects of soy protein intake and IFs on blood lipid levels in humans. Search terms included “soy protein,” “isoflavones,” and “cholesterol” with the constraints noted above. The search strategy resulted in 25 articles (2–5,17–37). We also looked for relevant articles in the reference lists of these articles, resulting in an additional 6 articles (6,7,38–41). Articles were selected for analysis if they met the following criteria: 1) subjects ingested soy protein for 1–3 mo; 2) randomized controlled trials with either a crossover or a parallel design; 3) comparable groups with at least 2 different levels of IFs [high IH (H-IF) and low IF (L-IF)] for the same soy protein intake level; 4) provided blood cholesterol and endpoint LDL-C concentrations. On the basis of the above criteria, 8 articles (3,4,28,29,31–33,38) were selected for the meta-analysis.

Data extraction. In this meta-analysis, we compared the serum LDL-C concentration of subjects who ingested soy protein with H-IF levels with the concentration of those who ingested the same level of soy protein with L-IF levels. Although 7 studies contained 1 (3,4,28,29,33,38) or 2 (32) control (nonsoy protein) diet groups, we analyzed only pairs of comparable data based on different IF content for a given soy protein intake level. Additionally, 1 study (3) contained 4 different levels of IFs, and 2 studies (29,33) contained 3 different levels of IFs; we selected the highest and lowest IF groups for analysis. One study (4) also reported mid-study (4 wk) measurements, but we used the full study period (12 wk) for analysis. One study (33) measured LDL-C concentrations during the 4 phases of the menstrual cycle, but we used mean values for this meta-analysis. Because effects of soy intake on LDL-C appear to depend on baseline LDL-C levels (3,32), we divided our data into 2 subcategories (hypercholesterolemic and normocholesterolemic) using a baseline LDL-C cutoff value of 4.14 (or 4.1) mmol/L (3,4,28,29,31–33,38) or total cholesterol > 6.2 mmol/L (31) for the hypercholesterolemic subcategory. Two studies (3,32) also divided their subject population into 2 groups according to baseline blood LDL-C levels, resulting in a total of 10 comparisons extracted from the 8 selected articles.

Meta-analysis. We performed the meta-analysis using review manager software (RevMan 4.2.2 for Windows, released on 2 June 2003), developed by The Nordic Cochrane Center (42). Many variables of interest in epidemiology, such as blood levels of cholesterol, use continuous scales. When studies use the same continuous scale to measure the outcome variables of interest, RevMan software can be used to calculate a weighted mean difference from studies for which sample sizes, mean scores and SD are available. Weighting factors, based on SD, are assigned to studies and reflect the precision with which each study measures the outcome variable of interest.

Two models are available for the final meta-analysis. The fixed effect model makes the assumption that there is one single mean effect, and that all the studies come from a population of studies measuring this effect (the “fixed effect”). If this is true, all of the observed variation will be due to chance alone. The other model, “random effects,” assumes that there is no one single true effect, but rather that the effect actually varies with a Normal distribution. There is no general agreement on which of these models to use. The random effects model is more conservative (the resulting CI are wider), but the random effects model also gives more weight to studies with smaller sample sizes, which may seem counterintuitive. However, if there is little variation, the 2 models give similar results.

A meta-analysis combines studies to produce an overall effect. Variation among the results of different studies may suggest that the studies are so different that it may not be sensible to combine them. RevMan also performs a statistical test for heterogeneity by calculating the χ^2 and *P*-value, thereby assessing whether there is more variation in the results of studies than would be expected by chance. When the resulting *P*-value is small (in the case of heterogeneous studies, a *P*-value < 0.1 is usually considered significant), there is

more variation than expected by chance, and it may not be advisable to combine those particular studies in a meta-analysis.

Data for this meta-analysis were extracted from 8 articles (3,4,28,29,31–33,38) consisting of 10-pairwise comparisons, and included the following: number of participants in each group, and mean and SD of blood LDL-C concentrations. RevMan software was used to calculate weighted mean differences in LDL-C levels between comparison groups (i.e., weighted mean LDL-C level for those consuming the H-IF diet minus the levels for those consuming the L-IF diet), 95% CI for each comparison, a combined overall effect with *P*-value for the total population and the hypercholesterolemic and normocholesterolemic subcategories, and a χ^2 value with *P*-value used for testing heterogeneity. Both a fixed effect model and random effects model were tested. Pearson correlation coefficients were calculated using SPSS 11.5J for Windows.

RESULTS

Characteristics of the studies. Of the 8 selected articles that met the criteria for analysis, 2 (4,31) used a parallel design, and the others used a crossover design; 4 were carried out in postmenopausal women (4,29,31,38), 2 included men and postmenopausal women (28,32), 1 included men and women (premenopausal and postmenopausal) (3), and 1 included only premenopausal women (33) (Table 1). None of the studies provided data on the ethnicity of participants. In articles with reported data (4,28,29,31,33,38), the mean age of the volunteers ranged from 26.3 to 62.7 y, mean BMI ranged from 22.8 to 26.6 kg/m². Four articles (28,31,32,38) reported serum LDL-C concentrations and 4 articles (3,4,29,33) reported values of plasma. Normally, serum cholesterol is ~3% higher than corresponding plasma cholesterol concentrations (43), but because we were interested in mean differences in each study, we analyzed plasma and serum concentrations without correction for this difference; we report all results as serum concentrations. The reported mean baseline serum LDL-C concentration ranged from 2.32 to 4.81 mmol/L (89.7 to 186.0 mg/dL) (Table 1). Subjects in 3 studies (3,4,32) were mildly or moderately hypercholesterolemic, 2 studies (28,31) contained hypercholesterolemic subjects only, 2 studies (33,38) contained normocholesterolemic subjects only, and 1 study (29) contained normocholesterolemic and mildly hypercholesterolemic subjects.

All studies used soy protein isolate (SPI) with intakes ranging from 25 to 100 g/d (mean 50 g/d), and the measured IF intakes ranged from 3 to 132 mg/d (mean of 96 and 6 mg/d for H- and L-IF diets in 10 comparisons, respectively). IF levels were expressed in aglycone units in 7 of the studies (3,4,28,29,32,33,38), but the IF form was not reported in 1 trial (31). In each comparison, subjects ingested the same level of soy protein containing either H- or L-IF. Additionally, in most of the comparisons, subjects consumed similar diets (avoiding foods rich in phytoestrogens such as soy products, legumes, bean sprouts, and flaxseed), similar amounts of fat (total and saturated), cholesterol, and fiber; all studies [except for one (31), which did not provide information] were designed to maintain body weight, and no significant weight changes were reported in the 7 articles with information (data not shown).

Changes in the serum lipid concentrations. The serum LDL-C concentration in subjects who consumed soy protein with H-IF content decreased by 0.15 mmol/L [(5.80 mg/dL); 95% CI, 0.08 to 0.23 mmol/L (3.09 to 8.89 mg/dL); *P* < 0.0001] compared with those who consumed the same level of soy protein with L-IF content (Table 2, Fig. 1). The test for heterogeneity was not significant (*P* = 0.74), suggesting that combining these studies for a meta-analysis was valid. Only

TABLE 1

Characteristics of the 10 comparisons in 8 selected randomized controlled trials of soy isoflavone intake in humans¹

Comparisons	Subjects <i>n</i>	Sample LDL-C ² <i>mmol/L</i>	Study design	Isoflavone intake	
				SPI dose <i>g/d</i>	<i>mg/d</i>
Hypercholesterolemic subjects					
Reference					
(3)	156 (94 Men, 24; PrW; 38 PoW; MC)	Plasma BV: 4.78 ± 0.54 vs. 4.78 ± 0.47	RCT, P, DB, SC, 9 wk	25	AIF, 62 vs. 3
(4)	94 PoW; MC	Plasma BV: 4.0 ± 0.7 vs. 4.2 ± 0.8; SV: 3.9 ± 0.6	RCT, P, 4B; 12 wk	42	AIF, 80 vs. 3
(28)	41 (23 Men, 18 PoW); HC	Serum BV: >4.1; SV: 4.55 ± 0.7 vs. 4.52 ± 0.7	RCT, CO, SB, 3 × 1 mo	50 vs. 52	AIF, 73 vs. 10
(32)	42 (24 PoW; 18 Men); SHC	Serum BV: ≥4.14	RCT, CO, SC, 4 × 6 wk	63	AIF, 124 vs. 8
(31)	49 PoW; HC	Serum SV: 5.07 ± 0.73 vs. 5.11 ± 1.02	RCT, P, DB, 12 wk	28	IF, 65 vs. 4
Normocholesterolemic subjects					
(3)	156 (94 Men, 24 PrW; 38 PoW; MC)	Plasma BV: 3.80 ± 0.23 vs. 3.83 ± 0.23	RCT, P, DB, SC, 9 wk	25	AIF, 62 vs. 3
(32)	42 (24 PoW; 18 Men); SNC	Serum BV: <4.14	RCT, CO, SC, 4 × 6-wk	63	AIF, 124 vs. 8
(33)	13 PrW; NC	Plasma BV: 2.32 ± 0.56	RCT, CO, PLB, 3 × 3-menstrual cycles + 9 d	87.5	AIF, 129 vs. 10
(38)	28 PoW; NC	Serum BV: 2.89 ± 0.1	RCT, CO, DB, 3 × 6-wk	25	AIF, 107 vs. 2
(29)	18 PoW; NMC	Plasma BV: 3.53 ± 0.81	RCT, CO, SB, 3 × 93 d	85	AIF, 132 vs. 7

¹ Abbreviations: PrW, premenopausal women; BV, baseline value; RCT, randomized controlled trial; P, parallel; DB, double blind; SC, subgroup comparison by baseline LDL cholesterol levels; AIF, aglycone isoflavone; PoW, postmenopausal women; MC, mildly or moderately hypercholesterolemic; SV, startpoint value; 4B, participants, investigators, study staff and laboratory technicians blind; HC, hypercholesterolemic; CO, crossover; SB, single blind; SHC, subgroup hypercholesterolemic; SNC, subgroup hypercholesterolemic; NC, normocholesterolemic; PLB, participants and laboratory personnel blind; NMC, normocholesterolemic and mildly hypercholesterolemic.

² Plasma LDL cholesterol concentration, mean ± SD, divide by 0.0259 to convert mmol/L to mg/dL.

results using the “fixed effects” model are reported because the “random effects” model gave identical results.

There were 5 comparisons in the hypercholesterolemic subcategory and 5 in the normocholesterolemic subcategory. Significant reductions in serum LDL-C of 0.18 mmol/L [(6.96 mg/dL); 95% CI, 0.01 to 0.35 mmol/L (0.39 to 13.53 mg/dL); $P = 0.03$] and 0.14 mmol/L [(3.87 mg/dL); 95% CI, 0.06 to 0.23 mmol/L (2.32 to 8.89 mg/dL); $P = 0.0008$], were found for the hypercholesterolemic and normocholesterolemic subcategories, respectively; tests for heterogeneity in both subcategories were not significant ($P = 0.71$ and $P = 0.45$, respectively), indicating that it was valid to combine the comparisons in each subcategory. No linear correlations were observed between SPI or IF intake differences and weighted mean differences in serum LDL-C. Pearson correlation coefficients were -0.062 ($P = 0.738$) and 0.0706 ($P = 0.847$) for the relations between the weighted mean difference of serum LDL-C concentration and SPI and IF intakes, respectively.

Effects of other variables. Because the age, sex, and BMI data for subjects in 2 studies (3,32) were not reported, the effects of different IF intakes according to age, sex, and BMI could not be analyzed. Reported mean differences of serum LDL-C concentration between H-IF and L-IF treatment groups were independent of changes in body weight and dietary intakes of total fat, saturated fat, cholesterol, and fiber.

DISCUSSION

This meta-analysis of 10 comparisons reported in 8 randomized controlled trials showed that serum LDL-C significantly decreased in hypercholesterolemic and/or normocholesterolemic subjects after ingestion of SPI with H-IF content

compared with those who ingested the same level of SPI with L-IF content. The results of this meta-analysis suggest that ingesting 90 mg/d soy-derived IFs (mean difference between H-IF and L-IF groups), independent of the intake level SPI, over 1–3 mo would lower serum LDL-C concentration by 0.15 mmol/L (5.80 mg/dL) on average, or 0.18 mmol/L (6.96 mg/dL) for hypercholesterolemic individuals and 0.14 mmol/L (3.87 mg/dL) for normocholesterolemic individuals. An intake of 90 mg/d soy IFs (daidzein and genistein) can be attained by daily consumption of ~2 glasses (383 g) of soymilk, 2 packs (92 g) of natto (fermented soybeans), one block (207 g) of tofu (bean curd), or 249 g miso (fermented soybean paste) (44), amounts that are consumed habitually in countries such as Japan. Therefore, these results suggest that increasing the consumption of soy by inclusion of foods such as soy milk or tofu in the daily diet could significantly lower blood cholesterol. A recent meta-analysis reported a decrease of 0.17 mmol/L in LDL-C with a daily mean intake of 36 g SPI and 52 mg IFs (13). However, because we observed similar changes after controlling for SPI intake (by examining the mean difference between H-IF and L-IF for a given SPI intake), this suggests that IFs play an important role in lowering LDL-C. The mechanism of the cholesterol-lowering effect of IFs is not well understood but may be a result of the chemical and biological similarity to mammalian estrogens, which were shown to have cholesterol-lowering effects in humans (45). IFs measured in the studies (4,32,38) used in this meta-analysis included genistein and daidzein, the main isoflavones found in soybeans (46), as well as some glycitein.

Although most individual studies in this meta-analysis showed a slight decrease in LDL-C, the 95% CI often included

TABLE 2

Mean LDL-C concentrations in the H-IF and L-IF groups and weighted mean difference (WMD) for 10 comparisons in 8 selected randomized controlled trials of soy isoflavone intake in humans

Comparisons	High-IF group		Low-IF group		Weight ²	WMD (fixed) ³ 95% CI
	n	LDL-C ¹ mmol/L	n	LDL-C ¹ mmol/L		
Hypercholesterolemic subjects						
Reference						
(3)	15	4.22 ± 0.47	12	4.53 ± 0.44	4.81	-0.31 [-0.65, 0.03]
(4)	31	3.50 ± 0.50	33	3.80 ± 0.80	5.41	-0.30 [-0.62, 0.02]
(28)	41	4.14 ± 0.70	41	4.18 ± 0.70	6.22	-0.04 [-0.34, 0.26]
(32)	22	4.70 ± 1.12	22	4.82 ± 0.91	1.57	-0.12 [-0.72, 0.48]
(31)	25	4.71 ± 0.73	24	4.78 ± 0.96	2.49	-0.07 [-0.55, 0.41]
Subtotal (95% CI)	134		132		20.50	-0.18 [-0.35, -0.01]
Test for heterogeneity: $\chi^2 = 2.13$, df = 4 ($P = 0.71$), $I^2 = 0\%$						
Test for overall effect: $Z = 2.14$ ($P = 0.03$)						
Normocholesterolemic subjects						
(3)	15	3.83 ± 0.31	16	3.85 ± 0.52	6.37	-0.02 [-0.32, 0.28]
(32)	20	3.83 ± 0.43	20	3.80 ± 0.59	5.57	0.03 [-0.29, 0.35]
(33)	13	2.14 ± 0.18	13	2.31 ± 0.18	29.81	-0.17 [-0.31, -0.03]
(38)	24	2.86 ± 0.49	24	2.87 ± 0.49	7.43	-0.01 [-0.29, 0.27]
(29)	18	3.01 ± 0.21	18	3.22 ± 0.21	30.32	-0.21 [-0.35, -0.07]
Subtotal (95% CI)	90		91		79.50	-0.14 [-0.23, -0.06]
Test for heterogeneity: $\chi^2 = 3.72$, df = 4 ($P = 0.45$), $I^2 = 0\%$						
Test for overall effect: $Z = 3.34$ ($P = 0.0008$)						
Total (95% CI)	224		223		100.00	-0.15 [-0.23, -0.08]
Test for heterogeneity: $\chi^2 = 6.00$, df = 9 ($P = 0.74$), $I^2 = 0\%$						
Test for overall effect: $Z = 3.94$ ($P < 0.0001$)						

¹ Endpoint value, mean ± SD.

² Weighting factors assigned by Revman software using SD.

³ WMD, weighted mean difference; fixed effect model.

zero. However, the overall effect observed in this meta-analysis was significantly different from 0 (95% CI), suggesting that limited sample sizes often prevent detection of significant effects in individual studies.

The weighted mean differences of serum LDL-C concentration showed no linear correlation with the soy protein intakes or IF differences (high IF intake level minus low IF intake level) for a given soy protein intake, in agreement with 2 other meta-analyses (1,13). One possible explanation for the lack of a linear correlation with soy protein intakes may be the different levels of constituent IFs or other components in the various soy proteins tested. Another explanation is that one IF may be more effective than the others in lowering serum LDL-C concentration; thus, measurement of total IF might obscure any linear correlation. If the relative amounts of genistein, daidzein, and glycitein differed among the articles analyzed here, this would weaken the estimated effect. Of 8 articles, only 3 (4,32,38) reported individual IF values. Thus, we were unable to analyze the specific effects of the individual IFs due to the small number of studies.

Alternatively, the lack of a linear correlation may be due to individual differences in the capacity of intestinal flora to convert the IF daidzein into its metabolite, equol. Approximately one third of Western individuals can metabolize daidzein into equol (47,48). Watanabe et al. (49,50) reported that equol was excreted by 50% of all Japanese middle-aged women studied and that equol was observed in 2 of 7 healthy men. Equol is easily absorbed and possesses substantial estrogenic activity due to its affinity for both the estrogen α and β

receptors (51). A crossover trial found that only in the 8 equol producers were LDL-C concentrations significantly decreased by replacing dairy products with soy-based milk or yogurt, whereas in the 15 poor-equol producers, there were no significant changes (52). Thus, significant LDL-C lowering effects of IFs may be limited to individuals who can produce equol; therefore, the LDL-C lowering effects observed in this meta-analysis may underestimate the effects for equol producers, but overestimate the effects for equol nonproducers. Because few studies analyze equol production, it is currently impossible to include equol-producing ability in a meta-analysis, but future studies should separate these groups.

SPI with L-IF was obtained by alcohol extraction in 6 of the 8 studies (3,4,28,29,33,38), and extraction methods were not reported in the other 2 articles (31,32). Alcohol washing may denature soy protein or modify soy-associated IFs or some other component of isolated soy protein that is important for the effect on blood concentration (53). This might also explain the lack of a linear correlation between different IF intakes and the observed cholesterol-lowering effect.

Many studies have not found an effect of IF on serum lipids. Nestel et al. (8) reported that 80 mg/d isoflavones (45 mg genistein) did not affect the plasma lipids concentrations in 21 primarily postmenopausal women aged 54.0 ± 6.0 y (mean ± SD) over 5- to 10-wk periods in a placebo-controlled, crossover trial. Hodgson et al. (9) also found that administration of a tablet containing 55 mg isoflavones (predominantly genistein) did not alter serum lipid concentrations in 46 men and 13 postmenopausal women in a randomized, double-blind,

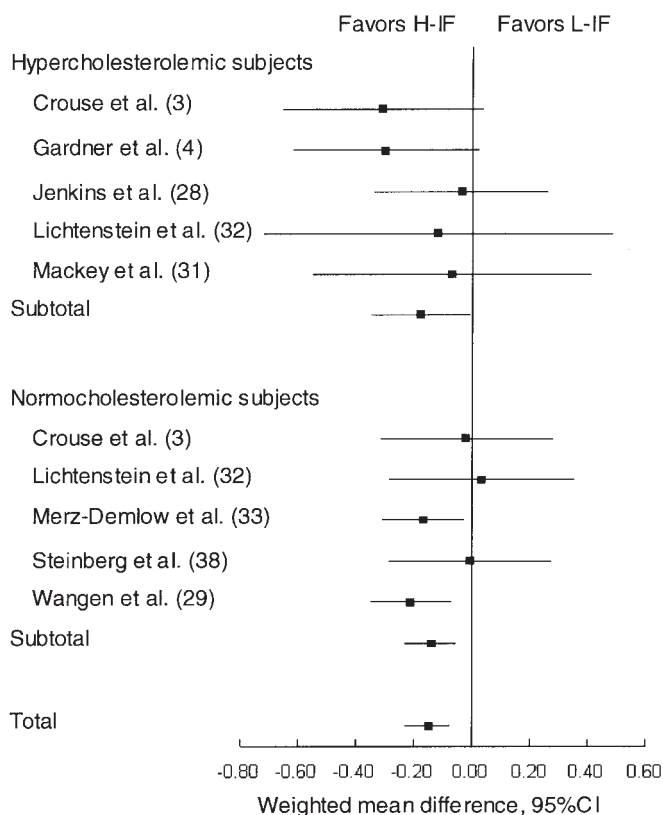


FIGURE 1 Weighted mean difference (WMD) between LDL-C concentration endpoints in the H-IF and L-IF intake groups for 10 comparisons in 8 selected randomized controlled trials. The WMD was calculated by subtracting the endpoint plasma LDL-C concentration for the L-IF group from that of the H-IF group. Lines correspond to 95% CI.

placebo-controlled trial of two-way parallel and 8 wk duration. Nestel et al. (11) reported that 40 and 80 mg isoflavones derived from red clover containing genistein, daidzein, biochanin A, and formononetin did not affect plasma lipids in 17 postmenopausal women in a 5-wk placebo-controlled, cross-over trial. Samman et al. (12) also reported that 86 mg isoflavones/d (biochanin A, 51.4 mg; formononetin, 18.6 mg; genistein, 8.6 mg; and daidzein, 7.4 mg) extracted from red clover did not significantly affect plasma lipids in 14 premenopausal women for 4 menstrual cycles (~4 mo). Further studies are warranted to confirm which components of soy-related isoflavones play a role in lowering blood cholesterol.

In conclusion, consumption of soy protein with a high IF content significantly decreased serum LDL-C concentration compared with the same soy protein intake with a low IF content in hypercholesterolemic and normocholesterolemic individuals in this meta-analysis of 8 randomized controlled trials.

LITERATURE CITED

- Anderson, J. W., Johnstone, B. M. & Cook-Newell, M. E. (1995) Meta-analysis of the effects of soy protein intake on serum lipids. *N. Engl. J. Med.* 333: 276-282.
- Baum, J. A., Teng, H., Erdman, J. W., Jr., Weigel, R. M., Klein, B. P., Persky, V. W., Freels, S., Surya, P., Bakhit, R. M., Ramos, E., Shay, N. F. & Potter, S. M. (1998) Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. *Am. J. Clin. Nutr.* 68: 545-551.
- Crouse, J. R., 3rd, Morgan, T., Terry, J. G., Ellis, J., Vitols, M. & Burke, G. L. (1999) A randomized trial comparing the effect of casein with that of soy

protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. *Arch. Intern. Med.* 159: 2070-2076.

- Gardner, C. D., Newell, K. A., Cherin, R. & Haskell, W. L. (2001) The effect of soy protein with or without isoflavones relative to milk protein on plasma lipids in hypercholesterolemic postmenopausal women. *Am. J. Clin. Nutr.* 73: 728-735.
- Teede, H. J., Dalais, F. S., Kotsopoulos, D., Liang, Y. L., Davis, S. & McGrath, B. P. (2001) Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. *J. Clin. Endocrinol. Metab.* 86: 3053-3060.
- Nilausen, K. & Meinertz, H. (1998) Variable lipemic response to dietary soy protein in healthy, normolipemic men. *Am. J. Clin. Nutr.* 68: 1380S-1384S.
- Duane, W. C. (1999) Effects of soybean protein and very low dietary cholesterol on serum lipids, biliary lipids, and fecal sterols in humans. *Metabolism* 48: 489-494.
- Nestel, P. J., Yamashita, T., Sasahara, T., Pomeroy, S., Dart, A., Komesaroff, P., Owen, A. & Abbey, M. (1997) Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. *Arterioscler Thromb. Vasc. Biol.* 17: 3392-3398.
- Hodgson, J. M., Puddey, I. B., Beilin, L. J., Mori, T. A. & Croft, K. D. (1998) Supplementation with isoflavonoid phytoestrogens does not alter serum lipid concentrations: a randomized controlled trial in humans. *J. Nutr.* 128: 728-732.
- Simons, L. A., von Konigsmark, M., Simons, J. & Celermajer, D. S. (2000) Phytoestrogens do not influence lipoprotein levels or endothelial function in healthy, postmenopausal women. *Am. J. Cardiol.* 85: 1297-1301.
- Nestel, P. J., Pomeroy, S., Kay, S., Komesaroff, P., Behrsing, J., Cameron, J. D. & West, L. (1999) Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. *J. Clin. Endocrinol. Metab.* 84: 895-898.
- Samman, S., Lyons Wall, P. M., Chan, G. S., Smith, S. J. & Petocz, P. (1999) The effect of supplementation with isoflavones on plasma lipids and oxidisability of low density lipoprotein in premenopausal women. *Atherosclerosis* 147: 277-283.
- Weggemans, R. M. & Trautwein, E. A. (2003) Relation between soy-associated isoflavones and LDL and HDL cholesterol concentrations in humans: a meta-analysis. *Eur. J. Clin. Nutr.* 57: 940-946.
- Irwig, L., Tosteson, A. N., Gatsonis, C., Lau, J., Colditz, G., Chalmers, T. C. & Mosteller, F. (1994) Guidelines for meta-analyses evaluating diagnostic tests. *Ann. Intern. Med.* 120: 667-676.
- Gibaldi, M. (1993) Meta-analysis. A review of its place in therapeutic decision making. *Drugs* 46: 805-818.
- Sacks, H. S., Berrier, J., Reitman, D., Ancona-Berk, V. A. & Chalmers, T. C. (1987) Meta-analyses of randomized controlled trials. *N. Engl. J. Med.* 316: 450-455.
- Teixeira, S. R., Potter, S. M., Weigel, R., Hannum, S., Erdman, J. W., Jr. & Hasler, C. M. (2000) Effects of feeding 4 levels of soy protein for 3 and 6 wk on blood lipids and apolipoproteins in moderately hypercholesterolemic men. *Am. J. Clin. Nutr.* 71: 1077-1084.
- Cruz, M. L., Wong, W. W., Mimouni, F., Hachey, D. L., Setchell, K. D., Klein, P. D. & Tsang, R. C. (1994) Effects of infant nutrition on cholesterol synthesis rates. *Pediatr. Res.* 35: 135-140.
- Takatsuka, N., Nagata, C., Kurisu, Y., Inaba, S., Kawakami, N. & Shimizu, H. (2000) Hypocholesterolemic effect of soymilk supplementation with usual diet in premenopausal normolipidemic Japanese women. *Prev. Med.* 31: 308-314.
- Dewell, A., Hollenbeck, C. B. & Bruce, B. (2002) The effects of soy-derived phytoestrogens on serum lipids and lipoproteins in moderately hypercholesterolemic postmenopausal women. *J. Clin. Endocrinol. Metab.* 87: 118-121.
- Gooderham, M. H., Adlercreutz, H., Ojala, S. T., Wahala, K. & Holub, B. J. (1996) A soy protein isolate rich in genistein and daidzein and its effects on plasma isoflavone concentrations, platelet aggregation, blood lipids and fatty acid composition of plasma phospholipid in normal men. *J. Nutr.* 126: 2000-2006.
- Hermansen, K., Sondergaard, M., Hoie, L., Carstensen, M. & Brock, B. (2001) Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. *Diabetes Care* 24: 228-233.
- Jayagopal, V., Albertazzi, P., Kilpatrick, E. S., Howarth, E. M., Jennings, P. E., Hepburn, D. A. & Atkin, S. L. (2002) Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. *Diabetes Care* 25: 1709-1714.
- Jenkins, D. J., Kendall, C. W., Connelly, P. W., Jackson, C. J., Parker, T., Faulkner, D. & Vidgen, E. (2002) Effects of high- and low-isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. *Metabolism* 51: 919-924.
- Jenkins, D. J., Kendall, C. W., Vidgen, E., Vuksan, V., Jackson, C. J., Augustin, L. S., Lee, B., Garsetti, M., Agarwal, S., Rao, A. V., Cagampang, G. B. & Fulgoni, V., 3rd (2000) Effect of soy-based breakfast cereal on blood lipids and oxidized low-density lipoprotein. *Metabolism* 49: 1496-1500.
- Jenkins, D. J., Kendall, C. W., Garsetti, M., Rosenberg-Zand, R. S., Jackson, C. J., Agarwal, S., Rao, A. V., Diamandis, E. P., Parker, T., Faulkner, D., Vuksan, V. & Vidgen, E. (2000) Effect of soy protein foods on low-density lipoprotein oxidation and ex vivo sex hormone receptor activity—a controlled crossover trial. *Metabolism* 49: 537-543.
- Puska, P., Korpelainen, V., Hoie, L. H., Skovlund, E., Lahti, T. & Smerud,

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- K. T. (2002) Soy in hypercholesterolaemia: a double-blind, placebo-controlled trial. *Eur. J. Clin. Nutr.* 56: 352–357.
28. Jenkins, D. J., Kendall, C. W., Jackson, C. J., Connelly, P. W., Parker, T., Faulkner, D., Vidgen, E., Cunnane, S. C., Leiter, L. A. & Josse, R. G. (2002) Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *Am. J. Clin. Nutr.* 76: 365–372.
29. Wangen, K. E., Duncan, A. M., Xu, X. & Kurzer, M. S. (2001) Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. *Am. J. Clin. Nutr.* 73: 225–231.
30. Urban, D., Irwin, W., Kirk, M., Markiewicz, M. A., Myers, R., Smith, M., Weiss, H., Grizzle, W. E. & Barnes, S. (2001) The effect of isolated soy protein on plasma biomarkers in elderly men with elevated serum prostate specific antigen. *J. Urol.* 165: 294–300.
31. Mackey, R., Ekangaki, A. & Eden, J. A. (2000) The effects of soy protein in women and men with elevated plasma lipids. *Biofactors* 12: 251–257.
32. Lichtenstein, A. H., Jalbert, S. M., Adlercreutz, H., Goldin, B. R., Rasmussen, H., Schaefer, E. J. & Ausman, L. M. (2002) Lipoprotein response to diets high in soy or animal protein with and without isoflavones in moderately hypercholesterolemic subjects. *Arterioscler. Thromb. Vasc. Biol.* 22: 1852–1858.
33. Merz-Demlow, B. E., Duncan, A. M., Wangen, K. E., Xu, X., Carr, T. P., Phipps, W. R. & Kurzer, M. S. (2000) Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. *Am. J. Clin. Nutr.* 71: 1462–1469.
34. Potter, S. M., Baum, J. A., Teng, H., Stillman, R. J., Shay, N. F. & Erdman, J. W., Jr. (1998) Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am. J. Clin. Nutr.* 68: 1375S–1379S.
35. Sanders, T. A., Dean, T. S., Grainger, D., Miller, G. J. & Wiseman, H. (2002) Moderate intakes of intact soy protein rich in isoflavones compared with ethanol-extracted soy protein increase HDL but do not influence transforming growth factor beta(1) concentrations and hemostatic risk factors for coronary heart disease in healthy subjects. *Am. J. Clin. Nutr.* 76: 373–377.
36. Dalais, F. S., Ebeling, P. R., Kotsopoulos, D., McGrath, B. P. & Teede, H. J. (2003) The effects of soy protein containing isoflavones on lipids and indices of bone resorption in postmenopausal women. *Clin. Endocrinol.* 58: 704–709.
37. Washburn, S., Burke, G. L., Morgan, T. & Anthony, M. (1999) Effect of soy protein supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in perimenopausal women. *Menopause* 6: 7–13.
38. Steinberg, F. M., Guthrie, N. L., Villablanca, A. C., Kumar, K. & Murray, M. J. (2003) Soy protein with isoflavones has favorable effects on endothelial function that are independent of lipid and antioxidant effects in healthy postmenopausal women. *Am. J. Clin. Nutr.* 78: 123–130.
39. Vigna, G. B., Pansini, F., Bonaccorsi, G., Albertazzi, P., Donega, P., Zanotti, L., De Aloysio, D., Mollica, G. & Fellin, R. (2000) Plasma lipoproteins in soy-treated postmenopausal women: a double-blind, placebo-controlled trial. *Nutr. Metab. Cardiovasc. Dis.* 10: 315–322.
40. Dent, S. B., Peterson, C. T., Brace, L. D., Swain, J. H., Reddy, M. B., Hanson, K. B., Robinson, J. G. & Alekel, D. L. (2001) Soy protein intake by perimenopausal women does not affect circulating lipids and lipoproteins or coagulation and fibrinolytic factors. *J. Nutr.* 131: 2280–2287.
41. Meinertz, H., Nilausen, K. & Hilden, J. (2002) Alcohol-extracted, but not intact, dietary soy protein lowers lipoprotein(a) markedly. *Arterioscler. Thromb. Vasc. Biol.* 22: 312–316.
42. The Cochrane Collaboration (2003) RevMan 4.2.2 for Windows. <http://www.cochrane.org/software/revman.htm>. [Last accessed 15 Jan 2004].
43. Laboratory Methods Committee of the Lipids Research Clinics Program (1977) Cholesterol and triglyceride concentrations in serum/plasma pairs. *Clin. Chem.* 23: 60–63.
44. Arai, Y., Watanabe, S., Kimira, M., Shimoi, K., Mochizuki, R. & Kinae, N. (2000) Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *J. Nutr.* 130: 2243–2250.
45. Rossouw, J. E. (1999) Hormone replacement therapy and cardiovascular disease. *Curr. Opin. Lipidol.* 10: 429–434.
46. Dwyer, J. T., Goldin, B. R., Saul, N., Gualtieri, L., Barakat, S. & Adlercreutz, H. (1994) Tofu and soy drinks contain phytoestrogens. *J. Am. Diet. Assoc.* 94: 739–743.
47. Axelson, M., Sjøvall, J., Gustafsson, B. E. & Setchell, K. D. (1984) Soya—a dietary source of the non-steroidal oestrogen equol in man and animals. *J. Endocrinol.* 102: 49–56.
48. Setchell, K. D., Borriello, S. P., Hulme, P., Kirk, D. N. & Axelson, M. (1984) Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. *Am. J. Clin. Nutr.* 40: 569–578.
49. Arai, Y., Uehara, M., Sato, Y., Kimira, M., Eboshida, A., Adlercreutz, H. & Watanabe, S. (2000) Comparison of isoflavones among dietary intake, plasma concentration and urinary excretion for accurate estimation of phytoestrogen intake. *J. Epidemiol.* 10: 127–135.
50. Watanabe, S., Yamaguchi, M., Sobue, T., Takahashi, T., Miura, T., Arai, Y., Mazur, W., Wahala, K. & Adlercreutz, H. (1998) Pharmacokinetics of soybean isoflavones in plasma, urine and feces of men after ingestion of 60 g baked soybean powder (kinako). *J. Nutr.* 128: 1710–1715.
51. Morito, K., Hirose, T., Kinjo, J., Hirakawa, T., Okawa, M., Nohara, T., Ogawa, S., Inoue, S., Muramatsu, M. & Masamune, Y. (2001) Interaction of phytoestrogens with estrogen receptors alpha and beta. *Biol. Pharm. Bull.* 24: 351–356.
52. Meyer, B. J., Larkin, T. A., Owen, A. J., Astheimer, L. B., Tapsell, L. C. & Howe, P. R. (2004) Limited lipid-lowering effects of regular consumption of whole soybean foods. *Ann. Nutr. Metab.* 48: 67–78.
53. Anthony, M. S., Blair, R. M. & Clarkson, T. B. (2000) Neither isoflavones nor the alcohol-extracted fraction added to alcohol-washed soy protein isolate restores the lipoprotein effects of soy protein isolate. *J. Nutr.* 132: 583S (abs.).