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Soy-Tibolone Combination - Effect on Lipids in Postmenopausal Monkeys and Women

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Abstract

Objectives—To determine whether co-administration of soy during tibolone treatment would prevent tibolone-induced dyslipoproteinemia in postmenopausal monkeys and women.

Methods—Surgically postmenopausal cynomolgus monkeys (n=18) were assigned randomly to one of four dietary regimens in a Latin Square crossover design, such that all animals received all diets for 14 weeks with a 4-week washout period: 1) Casein/lactalbumin (CL); 2) Tibolone (Tib, 1.25mg/day women's equivalent); 3) Soy (138mg isoflavones/day women's equivalent); 4) Soy+Tib. Postmenopausal women on tibolone treatment were randomized to receive soy powder (52 g of soy protein containing 112 mg isoflavones) or placebo (containing 52 g of milk protein) daily in a crossover trial for 8 weeks with a 4-week washout period.

Results—Monkeys given Tib alone had ~14% increase in plasma LDL+VLDL-C; whereas those given soy combined with tibolone had significant (~22%) reductions. Tib treated monkeys had reductions in plasma HDL-C of about 48% vs. no reductions in Soy+Tib. In postmenopausal women using tibolone, soy reduced plasma LDL-C concentrations by ~10% from baseline without a change in HDL-C.

Conclusions—Co-administration of soy during tibolone treatment improved the lipoprotein profile in both monkeys and women; however, the effects were more robust in monkeys.

Keywords

tibolone; soy; menopause; monkeys; lipids; cardiovascular

Introduction

Tibolone, a synthetic steroid, is widely prescribed in many parts of the world to treat menopausal symptoms (hot flashes, vaginal dryness, sexual dysfunction) and to prevent bone

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loss. Tibolone {7- α ,17- α -17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one} is metabolized among human and nonhuman primates into three biologically active metabolites, a 3 α -hydroxy estrogenic metabolite, a 3 β -hydroxy estrogenic metabolite, and a Δ -4 ketoisomer which has both progestogenic and androgenic effects [1,2]. Tibolone's metabolites circulate in inactive forms and their conversion to bioactive forms depends on tissue-specific de-sulfatation [3].

Although tibolone treatment in postmenopausal women has been shown to have some beneficial lipid effects, including reductions in plasma triglyceride (TG) and lipoprotein(a) [Lp (a)] concentrations, there has been concern about its "cardiovascular safety" since it also markedly reduces plasma high density lipoprotein-cholesterol (HDL-C) (up to 30%) [4–7]. Similarly, cynomolgus monkeys experience large decreases in HDL-C following tibolone treatment [8].

Soy supplements have robust effects on the plasma lipid profiles of postmenopausal monkeys. Consistently, postmenopausal monkeys given soy supplements have 20 to 30% increases in HDL-C and reductions in plasma concentrations of low density lipoprotein (LDL) + very low density lipoprotein-cholesterol (VLDL-C) of 30 to 40% [9]. However, in human beings the soy-related beneficial lipid effects are somewhat smaller and thus, the clinical relevance of soy supplementation alone to cardiovascular health remains unclear [10].

The beneficial effects of soy on plasma lipid profiles prompted us to explore whether the co-administration of soy and tibolone would prevent the tibolone-induced dyslipoproteinemia. We report here on the results of a comparative/translational study of postmenopausal monkeys and women.

Study Subjects – Monkey Study

Animals and Experimental Design

Female cynomolgus (*Macaca fascicularis*) monkeys (n=18) were imported originally from the Indonesian Primate Center (Pusat Studi Satwa Primata, PSSP) at the Institut Pertanian Bogor (IPB) in West Java, Indonesia. Adult status was confirmed by dentition and radiographic evidence of epiphyseal closure (occurs at about 9 years of age in this species and indicates the end of bone growth). At the time of this study, the average age of the entire cohort was about 16 years of age, approximately equivalent to women of about 45 years of age and they had been surgically postmenopausal for approximately 3 years. The mean body weight (BW) was 3.3 ± 0.03 kg. The monkeys were assigned randomly to one of four dietary regimens (Table 1) in a Latin Square crossover design, such that all animals received all diets (Figure 1). Diets contained either casein/lactalbumin (CL) or soy protein, with or without tibolone (tib). All diets were prepared in our diet laboratory and were formulated to be equivalent for cholesterol and macronutrient content, i.e. protein, fat, carbohydrate. DL-methionine was added to the soy-containing diets to make the content of sulfur-containing amino acids comparable to that in CL diets. The monkeys were fed 120 Cal of diet/kg body weight (BW). To adjust for differences in the metabolic rate between monkeys and women, a women's equivalent dose of soy isoflavones (IF) was calculated based on the average woman consuming approximately 1800 calories per day. Therefore, the monkeys were given 138 mg IF/1800 calories of diet per day which equates to 27.6mg IF/monkey/day (monkeys consume on average ~ 360 calories/day or 1/5th that of the average woman). The soy protein (SUPRO 670-HG, Solae, St. Louis, Missouri, USA) contained, on average, 1.03 mg genistein, 0.72 mg daidzein, and 0.13 mg glycitein per gram of soy protein isolate (expressed in aglycone units).

The dose of tibolone (Org OD 14, Organon, Oss, The Netherlands) that we modeled in this monkey study was a woman's dose of 1.25mg/day. As was done for soy isoflavones, this dose

was adjusted for metabolic differences between monkeys and women and equated to approximately 0.4mg/monkey/day.

Following each 14-week treatment phase, the monkeys were fed a “washout diet” (CL) for 4 weeks before starting the next treatment regimen. All procedures involving animals in this study were conducted in compliance with state and federal laws, standards of the US Department of Health and Human Services, and guidelines established by the Wake Forest University Institutional Animal Care and Use Committee.

Assessments

Serum concentrations of the isoflavonoids (genistein, daidzein, equol) were determined by liquid chromatographic-photodiode array mass spectrometric analysis (LC/PDA/ESI-MS) at the Cancer Research Center of Hawaii, using techniques described previously [11]. Measurements were done on serum samples collected 4 hours after feeding. The assay detection limit for daidzein and genistein was 10nmol/L, and for equol it was 15 nmol/L. Intra-assay coefficients were all less than 14% and varied depending on the analyte, while inter-assay coefficients of variation were 8–22% at levels below 20 nM, 7–14% at 20 nM – 100 nM, and 3–12% at levels over 100 nM.

Blood samples for determination of plasma lipid concentrations were obtained twice during each treatment phase. The samples were collected after food had been withheld for 18 hours. Total plasma cholesterol (TPC), HDL-C, and plasma triglyceride (TG) concentrations were measured in the laboratory of the Wake Forest University Primate Center using enzymatic methods on the COBAS FARA II analyzer (Roche Diagnostics, Inc., Montclair, NJ), with protocols and reagents supplied by Boehringer Mannheim. The Clinical Chemistry Laboratory is fully standardized with this method and is in the continuing surveillance phase of the Centers for Disease Control (Atlanta, GA) Lipid Standardization Program. HDL-C concentrations were measured using the heparin-manganese precipitation procedure and as described in the Manual of Laboratory Operations of the Lipid Research Clinics Program [12,13]. Plasma LDL-C plus VLDL-C was calculated as the difference between TPC and HDL-C. The intra and inter-assay coefficients of variation for plasma lipids were less than 5% for all analytes.

Statistical Analysis

Our primary hypothesis for the monkey study was that soy would inhibit tibolone induced HDLC lowering; and therefore, HDLC was the parameter used to determine sample size. Previous data for these animals indicated that monkeys consuming a soy diet had an increase of 12mg/dl HDLC compared to a casein-lactalbumin diet ($SD \pm 6.5\text{mg/dl}$), and a reduction of 14mg/dl HDLC when treated with tibolone compared to casein. We determined that 18 animals in a crossover design were needed to detect a 5mg/dl HDLC difference between the tibolone and tibolone plus soy groups, with 80% power at the 5% two-sided level of significance. We chose this study design to allow us maximum power to detect differences between treatments with the fewest number of animals.

All variables were first evaluated for their distribution and equality of variances between groups using a univariate analysis and Levene's test for homogeneity of variance. A mixed general linear model with repeated measures was used to determine means and to test for main effects of treatment. All variables were screened in the initial model for phase-by-treatment interactions and none were found. An alpha level 0.05 was used to determine statistical significance. The reported data are means and calculated standard errors (mean $\pm$ SEM).

Postmenopausal Women

We used a dietary challenge to characterize these women for high or low equol production by giving a soy drink (52 g of soy protein containing 112 mg isoflavones) (Solae Co. St. Louis, Missouri) once a day for one week. Serum samples, collected prior and at the end of the soy challenge, were assayed for equol and genistein concentrations. In our study, a 4-fold rise in serum equol concentration was the criterion of equol production capability. Twenty women fulfilled this criterion. Another 20 women without a rise in equol production capability were classified as non-equol producers. There was a minimum of 4 weeks between the soy challenge and the initiation of the actual trial.

The general study design has been presented elsewhere¹⁴. Briefly, the two groups of women (equol producers and non-equol producers), 40 women altogether (mean age 57.7 ± 0.8 and mean BMI 24.6 ± 0.5), were treated in a randomized order, in a double-blind, placebo-controlled, crossover trial for 8 weeks either with a soy powder (52 g of soy protein containing 112 mg isoflavones) or with a placebo powder (containing 52 g of milk protein) (Solae Co., St. Louis, Missouri, USA) that could be mixed with food or beverages (Figure 2). The first treatment period was followed by a washout period of four weeks, and then the treatments were crossed over for another 8 weeks.

Assessments

Serum samples were assayed for equol and genistein concentrations by time-resolved fluoroimmunoassays as published previously^{15,16}. The detection limits were 0.25 nmol/l for equol and 1.5 nmol/l for genistein. Intra-assay and interassay coefficient of variation were 5.5% and 6.0% for equol, and 2.9–4.7% and 5.9–6.7% for genistein, respectively. Plasma lipid profiles including TPC, LDL-C, HDL-C and TG were measured with standard methods¹⁷. Briefly, TPC and TG concentrations were assessed by enzymatic colorimetric methods (ABX Diagnostics). Plasma lipoproteins were isolated by sequential ultracentrifugation using a Beckman optima LE-80K ultracentrifuge and a Ti 50.4 rotor; LDL cholesterol was isolated at density range 1.019–1.063 g/ml and HDL at density range 1.063–1.21 g/ml. Ultracentrifugally isolated LDL and HDL were further purified by gel filtration on Sephadex G25 with phosphate buffered saline (pH 7.4) as eluting solvent. Measuring range for cholesterol assessments was 0.04–18 mmol/l. Intra-assay and interassay coefficient of variation were 0.42–1.26% and 1.48–2.25% for total cholesterol, LDL and HDL, and 1.46–1.91% and 1.85–3.68% for triglycerides, respectively.

Statistical Analysis

The data were analyzed using NCSS software (version 2001, Kaysville, Utah, USA). As with the monkey studies, we used HDLC as the parameter to determine sample size for the study of postmenopausal women. Based on previous soy-only studies with the 40 women in a cross-over design, we determined that the number used for this study should allow the detection of a 10% difference in HDLC with 80% power. The differences between baseline and during treatment values were analyzed by the paired t-test. Comparison of the two treatments (soy versus placebo) and the comparison between equol producers and non-equol producers were done by the two-sample t-test. Since we used a crossover design, the possibility of a period effect was tested by means of the paired t-test, where we compared the differences between the periods in the two groups of subjects (those beginning with soy and those beginning with placebo); no period effect was detected. Because no carryover effect was detected, the women using soy or placebo were analyzed as a single group regardless of the treatment order. The Mann-Whitney U-test or the Wilcoxon Rank Sum Test was used in case of non-normal data distribution. All results are expressed as mean $\pm$ SEM (standard error of mean) and a p-value < 0.05 was considered statistically significant.

Results – Monkey Study

a. Plasma Isoflavone Concentration

Total plasma isoflavone concentrations observed in monkeys fed either the soy or soy + tibolone diet were within the range of previously reported studies and were not significantly different from each other ($p=0.99$) [18]. Likewise, the proportion of isoflavone metabolites, equol, daidzein and genistein was as expected, and did not differ between the two diet groups ($p>0.15$ for all metabolites) (Figure 3). Isoflavone values detected in monkeys fed the CL diets were considered below or at the detection level for the assay and not considered significant.

b. Prevention of Tibolone-associated HDL-C Decreases by Soy

A 30% increase in HDL-C (Soy- 1.57 ± 0.1 vs. CL- 1.21 ± 0.1 , $p<0.0001$) was observed in monkeys fed soy, and monkeys treated with tibolone had large reductions in plasma HDL-C (~48%, Tib- 0.63 ± 0.1 vs. CL- 1.21 ± 0.1 , $p<0.0001$) (Figure 4). However, co-administration of soy with tibolone resulted in the conservation of plasma HDL-C concentrations, such that monkeys fed this dietary regimen were not different from control (Soy + Tib- 1.09 ± 0.1 vs. CL- 1.21 ± 0.1 , $p=0.09$).

c. Reductions in Plasma Concentrations of LDL+VLDL-C by Soy + Tib in Contrast to Increases by Tibolone Only (CL + Tib)

A slightly larger increase (~14%) in plasma LDL+VLDL-C was observed in tibolone-treated monkeys compared to those fed the control diet (Tib- 7.67 ± 0.55 vs. CL- 6.76 ± 0.55 , $p=0.004$) (Figure 5). However, soy treatment resulted in an ~18% reduction in plasma LDL+VLDL-C (Soy- 5.55 ± 0.55 vs. CL- 6.76 ± 0.55 , $p=0.0002$), and the addition of tibolone did not negate the LDL+VLDL-C lowering effect of soy (Soy + Tib- 5.26 ± 0.55 vs. Soy- 5.55 ± 0.55 , $p=0.35$).

d. Reductions in Ratio of Total Plasma Cholesterol (TPC) to HDL-C by Soy + Tib in Contrast to Increases by Tibolone Only (CL + Tib)

Although monkeys fed tibolone alone had an increase in TPC:HDL-C ratio of 120% compared to control (Tib- 18 ± 1.5 vs. CL- 8.2 ± 1.5 , $p<0.0001$), those fed Soy or Soy + Tib experienced decreases in the ratio of TPC to HDL-C of 34% (Soy- 5.38 ± 1.5 , $p=0.03$ vs. CL) and 2.4% (Soy + Tib- 8.0 ± 1.5 , $p=0.84$ vs. CL, $p=0.05$ vs. Soy), respectively (Figure 6). This finding is consistent with our earlier report, in which a 53% increase in TPC:HDL-C ratio was found for tibolone-treated monkeys compared to control [8].

Postmenopausal Women

Serum Isoflavones Concentrations

Tibolone-treated postmenopausal women consuming soy had significant increases from baseline in serum concentrations of genistein (1170 ± 183 nmol/l). Women of the daidzein-converting phenotype had equol concentrations of $\sim 144 \pm 36.5$ nmol/l, whereas those who were not considered of the daidzein-converting phenotype had low (24.8 ± 2.9 nmol/l) equol concentrations. Serum levels of the tibolone estrogenic 3α -hydroxy (9.0 ± 5.6 vs. 8.3 ± 4.1 ng/ml) and 3β -hydroxy (2.5 ± 1.7 vs. 2.6 ± 1.3 ng/ml) metabolites showed no difference between the equol producers and the non-equol producers.

Plasma Lipids and Lipoproteins

In the whole study population, soy supplementation for 2 months resulted in significant decreases in plasma LDL-C as compared to placebo treatment (Table 2). A similar trend was observed in TPC concentrations, but soy had no effect on plasma HDL-C or TG. When the equol producers and non-equol producers were evaluated separately (Figure 7), the LDL-C

reduction was significant only in the equol producers. There were no differences in serum lipid or lipoprotein concentrations between equol producers and non-equol producers at baseline.

Discussion

Co-administration of soy during tibolone treatment had beneficial plasma lipid effects in both monkeys and women. Monkeys fed a diet rich in soy, while being treated with tibolone, did not experience the large reductions (~48%) in plasma HDL-C observed in the tibolone only-treated monkeys in this study, and in previous studies of monkeys and women [4–8]. In addition, monkeys given soy combined with tibolone had significant (~22%) reductions in plasma LDL+VLDL-C. Similarly, in postmenopausal women treated with tibolone, soy supplementation reduced plasma LDL-C concentrations ~10% from baseline, and women who converted daidzein to equol appeared to have an even greater beneficial effect on plasma LDL-C than those who did not produce equol (12 % vs. 8 % reductions in LDL-C, respectively). However, unlike the monkeys, soy supplementation did not increase HDL-C in the postmenopausal women taking tibolone.

There are several explanations that may account for the difference in magnitude of effect on plasma lipoproteins when comparing the monkey study and the study of postmenopausal women. First, the monkeys were given 1.25mg/day (women's equivalent dose of tibolone) whereas the women were taking 2.5mg/day. The lower dose was used in the monkey study because this was the tibolone dose that was pending approval by the USDA for use in the United States. It is not known whether the postmenopausal women may have had greater plasma lipid benefits from co-administration of soy if they had been treated with the lower dose of tibolone. Likewise, we cannot determine from this study whether monkeys treated with the higher dose of tibolone (2.5mg/day) would have experienced as much of a beneficial effect on plasma lipids as was seen by combining soy with the 1.25mg/day dose. Second, the length of time on study varied; the monkeys consumed soy along with tibolone treatment for a total of 14 weeks, whereas the women were given soy for only 8 weeks while being treated with tibolone. Third, differences between cholesterol metabolism in monkeys and postmenopausal women may account, in part, for the differential plasma lipid responses between monkeys and postmenopausal women. Macaques readily absorb dietary cholesterol and when exposed to a high cholesterol diet, the majority of the increase in plasma cholesterol is of dietary origin [19,20]. In the monkey study presented here, the diet contained 0.15 mg of cholesterol/Calorie of diet. This is a dietary level of cholesterol that is reasonable for women in Finland; however, at that dietary level, macaques will reduce hepatic synthesis of cholesterol by about 85% and thus, the majority of the plasma cholesterol was most likely of dietary origin [21]. In contrast, human primates do not absorb dietary cholesterol as readily as macaques, and therefore do not reduce hepatic cholesterol synthesis at that dietary level, and thus most of their plasma pool of cholesterol is due to hepatic synthesis [22].

Finally, a probable explanation for the difference in magnitude of effect on plasma lipoproteins when comparing postmenopausal monkeys and women concerns the differences between macaques and human primates in their ability to metabolize daidzein to equol (so called daidzein-converting phenotype). *Cynomolgus* monkeys, and other nonhuman primates and rodents as well, are robust converters of daidzein to equol (see Figure 3) resulting in equol being the predominate isoflavone metabolite in the plasma [23]. In contrast, only 25 to 30% of non-Asian women are the daidzein-converting phenotype, and among those, the amounts in the plasma are markedly less than monkeys, on average about 20% of the total isoflavones [23,24]. The results of the study presented here are consistent with previous studies; mean equol concentrations in the monkeys were ~3.5-fold higher than in postmenopausal women consuming similar amount of soy isoflavones per day (500nmol/L vs. 144 nmol/L). The clinical importance of being an equol producer is poorly understood currently. Setchell et al. have

suggested that equol, being a nonsteroidal estrogen that binds to both ER α and ER β , has physiologic effects that may account for the beneficial effects of soy among women with the daidzein-metabolizing phenotype [25]. We tested that hypothesis in an experiment with cynomolgus monkeys in which we administered synthetic equol in amounts to achieve plasma concentrations equivalent to those of monkeys fed soy [26]. Equol alone had no effect on the monkey's plasma lipids nor lipoproteins, and it therefore appears that the ability to convert daidzein to equol may be a marker for a gut flora phenotype that is capable of metabolizing steroid hormones in some manner that is associated with better health outcomes. As a part of the same experiment on postmenopausal women reported here, we found that women taking tibolone and being of the daidzein-converting phenotype, had lower blood pressure and better vascular function than their non-equol producing counterparts before being administered the soy supplement [14,27].

Our finding that soy supplementation had modest effects on plasma lipid profiles in postmenopausal women using tibolone is consistent with a report by Dewell and coworkers [28]. In that study, the effect of soy protein and soy isoflavones on plasma lipid profiles of mainly postmenopausal women was investigated. Most of the women were not currently being treated with tibolone or other hormone therapy. The authors concluded that both soy protein and soy isoflavone extracts caused only very small reductions in TPC concentrations—primarily LDL-C concentrations—and no changes in HDL-C levels. The findings reported by Dewell et al. are also consistent with the 2006 American Heart Association advisory on soy and cardiovascular health [10]. They reported that soy reduced plasma concentrations of LDL-C about 3% on average, with no significant effects on HDL-C, TG, Lp(a), or blood pressure.

Previous studies indicate that tibolone appears to neither increase nor decrease atherosclerosis of postmenopausal cynomolgus monkeys [8]. Despite the tibolone-treated monkeys having markedly reduced plasma concentrations of HDL-C, atherosclerosis was not exacerbated in the coronary nor common and internal carotid arteries compared to the control group. Bots et al. reported on the effect of tibolone on the progression of carotid artery atherosclerosis in women, using carotid artery intima-media thickness (CIMT) as the indicator of progression or regression [29]. Unfortunately, because of unexplained inconsistencies in the trial outcomes, there remains uncertainty about whether and to what extent tibolone affects atherosclerosis extent in postmenopausal women [30].

It is possible that soy supplementation could have plasma lipid-independent atherosclerosis inhibitory effects. For example, isoflavone treatment has been reported to have beneficial effects on arterial compliance and aortic stiffness, despite a lack of effect on plasma lipid profiles [31,32]. Furthermore, studies have found reductions in markers of LDL oxidation in humans consuming soy protein with isoflavones or isoflavone pills [33–37]. Consequently, while we found that soy supplementation had only modest effects on plasma lipid profiles of postmenopausal women taking tibolone, the possibility of plasma lipid-independent benefits on cardiovascular risk cannot be ruled out.

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Figure 1.
Design of Monkey Study.

Figure 2.
Design of the Postmenopausal Women Study.

Figure 3. Plasma isoflavone concentrations 4 hours post feeding in surgically postmenopausal cynomolgus monkeys fed soy (138 mg women's equivalent/day) and soy + tibolone (Tib) (1.25mg women's equivalent/day) diets. Data are expressed as means per diet group (n=18/diet).

Figure 4. Plasma high density lipoprotein cholesterol (HDL-C) concentrations of surgically postmenopausal cynomolgus monkeys fed either casein + lactalbumin (CL), CL + tibolone (Tib) (1.25 mg/day women's equivalent), soy (138 mg women's equivalent total isoflavones) or soy + tib. Data are expressed as means $\pm$ standard error.

Figure 5. Plasma low density plus very low density lipoprotein cholesterol (LDL + VLDL-C) concentrations of surgically postmenopausal cynomolgus monkeys fed casein + lactalbumin (CL), CL + tibolone (Tib) (1.25 mg/day women's equivalent), soy (138 mg women's equivalent total isoflavones) or soy + tib. Data are expressed as means $\pm$ standard error.

Figure 6. Effect of casein + lactalbumin (CL), CL + tibolone (Tib), soy (138 mg women's equivalent total isoflavones), tibolone (1.25 mg/day women's equivalent) and soy + tib on the ratio of total plasma cholesterol (TPC) to high density lipoprotein cholesterol (HDL-C) concentrations of surgically postmenopausal cynomolgus monkeys. Data are expressed as means $\pm$ standard error.

Figure 7. Plasma LDL-C concentrations in postmenopausal women being treated with Tibolone (2.5mg/day) after consumption of soy for 8 weeks. Equol producers were defined by a 4-fold increase in equol concentration following a soy challenge. Data are expressed as percent change from baseline.

Table 1**Diet Composition.**

Ingredient (g/100grams)	Control	Tibolone	Soy	Tibolone + Soy
Soy Protein Isolate			17.09	17.09
DL-methionine			0.3	0.3
Tibolone	None	0.00027	None	0.00027
Casein	8.5	8.5		
Lactalbumin	8.5	8.5		
Wheat Flour, self-rising	35.76	35.76	35.76	35.76
Dextrin	9.0	9.0	8.9	8.9
Sucrose	7.0	7.0	7.0	7.0
Alphacel (non-nutrient bulk)	8.05	8.05	8.25	8.25
Lard	5.0	5.0	5.0	5.0
Beef Tallow	4.0	4.0	4.0	4.0
Butter, lightly salted	3.1	3.1	3.1	3.1
Safflower Oil	3.0	3.0	2.6	2.6
Crystalline Cholesterol	0.04	0.04	0.04	0.04
Complete Vitamin Mix	2.5	2.5	2.5	2.5
Modified Ausman-Haves Mineral Mix #2	5.0	5.0	5.0	5.0
Calcium Carbonate	0.4	0.4	0.36	0.36
Calcium Phosphate, Monobasic	0.15	0.15	0.15	0.15
Composition				
Protein (% of calories)	19.0	19.0	19.0	19.0
Carbohydrate (% of calories)	35.3	35.3	35.3	35.3
Lipid (% of calories)	45.8	45.8	45.8	45.8
Saturated (% of calories)	41.8	41.8	41.4	41.4
Monounsaturated (% of calories)	35.6	35.6	35.5	35.5
Polyunsaturated (% of calories)	22.6	22.6	23.1	23.1
Cholesterol (mg/Calorie)	0.15	0.15	0.15	0.15
Calcium (mg/1800 Calories)	1330.0	1330.0	1330.0	1330.0
Phosphorus (mg/1800 Calories)	1297.7	1297.7	1298.0	1298.0
Total isoflavones (genistein, daidzein, glycitein, mg/1800 Calories)			137.7	137.7

Table 2

Changes in total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) from baseline after soy or placebo treatment. P-value denotes the difference between baseline and after treatment (or placebo vs. soy). Data are expressed at means $\pm$ SE.

All (n=32)						
Variable	Baseline	Placebo	p	Soy	p	Placebo vs. Soy
TC	5.25 $\pm$ 0.1	5.1 $\pm$ 0.1	0.09	4.89 $\pm$ 0.2	0.001	p=0.06
LDL-C	3.75 $\pm$ 0.1	3.61 $\pm$ 0.1	0.07	3.37 $\pm$ 0.2	0.0003	p=0.006
HDL-C	1.38 $\pm$ 0.1	1.33 $\pm$ 0.1	0.3	1.36 $\pm$ 0.1	0.7	p=0.3
TG	0.97 $\pm$ 0.1	0.92 $\pm$ 0.1	0.3	0.93 $\pm$ 0.1	0.4	p=0.8
Equal producers (n=14)						
Variable	Baseline	Placebo	p	Soy	p	Placebo vs. Soy
TC	5.35 $\pm$ 0.2	5.09 $\pm$ 0.3	0.02	4.86 $\pm$ 0.2	0.0009	p=0.2
LDL-C	3.88 $\pm$ 0.3	3.63 $\pm$ 0.2	0.05	3.39 $\pm$ 0.2	0.0002	p=0.04
HDL-C	1.33 $\pm$ 0.1	1.27 $\pm$ 0.1	0.5	1.34 $\pm$ 0.1	0.9	p=0.3
TG	0.95 $\pm$ 0.1	1 $\pm$ 0.1	0.5	0.94 $\pm$ 0.1	0.5	p=0.5
Non-equal producers (n=18)						
Variable	Baseline	Placebo	p	Soy	p	Placebo vs. Soy
TC	5.18 $\pm$ 0.1	5.13 $\pm$ 0.2	0.7	4.92 $\pm$ 0.3	0.1	p=0.2
LDL-C	3.65 $\pm$ 0.1	3.59 $\pm$ 0.2	0.6	3.35 $\pm$ 0.2	0.04	p=0.07
HDL-C	1.42 $\pm$ 0.1	1.38 $\pm$ 0.1	0.5	1.38 $\pm$ 0.1	0.5	p=0.9
TG	0.99 $\pm$ 0.1	0.86 $\pm$ 0.1	0.09	0.92 $\pm$ 0.1	0.4	p=0.3