Published in final edited form as:

Menopause. 2014 October ; 21(10): 1136–1142. doi:10.1097/GME.0000000000000223.

Effect of Soy Isoflavones on Thyroid Hormones in Intact and Ovariectomized Cynomolgus Monkeys (*Macaca fascicularis*)

Marnie G. Silverstein, DVM,

Department of Pathology, Section on Comparative Medicine Integrative Physiology and Pharmacology Graduate Program Wake Forest University School of Medicine, Winston-Salem, NC

Jay R. Kaplan, PhD,

Department of Pathology, Section on Comparative Medicine Wake Forest University School of Medicine, Winston-Salem, NC

Susan E. Appt, DVM,

Department of Pathology, Section on Comparative Medicine Wake Forest University School of Medicine, Winston-Salem, NC

Thomas C. Register, PhD, and

Department of Pathology, Section on Comparative Medicine Wake Forest University School of Medicine, Winston-Salem, NC

Carol A. Shively, PhD

Department of Pathology, Section on Comparative Medicine Wake Forest University School of Medicine, Winston-Salem, NC

Abstract

Objectives—Soy isoflavones are commonly used to alleviate menopause-related symptoms. Menopausal women are at an increased risk for hypothyroidism and there are concerns that isoflavones may be detrimental to thyroid health. The aim of this study was to examine the effects of soy protein and isoflavones on thyroid function and the relationship between thyroid and ovarian function.

Methods—Adult female cynomolgus monkeys (*Macaca fascicularis*) were randomized to consume two diets differing only in protein source: casein-lactalbumin (n = 44) or soy protein with isoflavones (n = 41). After 34 months all animals were ovariectomized via laparotomy. Half of the monkeys from each diet treatment group continued to consume their Pre-Ovariectomy treatment phase diet (either SOY [n = 19] or CL [n = 21]) for an additional 34 months. The remaining animals did not continue their diets, and thus were not considered further. Circulating progesterone, triiodothyronine, thyroxine, and thyroid stimulating hormone were measured at baseline. Thyroid hormones were remeasured during each treatment phase.

Corresponding Author: Carol A. Shively, PhD Department of Pathology-Comparative Medicine Medical Center Blvd. Winston-Salem, NC 27157-1040 Tel: 336-716-1524, Fax: 336-716-1515 cshively@wakehealth.edu.

Disclosures: Drs. Silverstein, Kaplan, Appt, Register and Shively reported no biomedical financial interests or potential conflicts of interest.

Results—Dietary soy increased triiodothyronine in pre-ovariectomized monkeys and prevented a decline in thyroxine following surgical menopause (both p 's < 0.05). Mean progesterone concentrations were positively correlated with triiodothyronine at baseline in pre-ovariectomized monkeys ($p < 0.01$).

Conclusions—Progesterone levels and triiodothyronine are positively correlated in macaques. Dietary soy increases triiodothyronine in pre-ovariectomized monkeys and prevents a decline in thyroxine following surgical menopause. The outcomes observed in this study suggest soy protein and isoflavone consumption does not adversely affect and may even preserve thyroid function in postmenopausal women.

Keywords

Thyroid; monkey; macaque; soy; isoflavone; menopause

INTRODUCTION

The menopausal transition is often accompanied by life-disrupting symptoms such as hot flashes and vaginal dryness, as well as increased risk of osteoporosis, cardiovascular disease, and cognitive decline. While estrogen replacement through hormone therapy (HT) is the most effective method for treating menopausal symptoms,¹ results of the Women's Health Initiative² and Heart and Estrogen/Progestin Replacement Study^{3,4} suggest that the health risks associated with HT may outweigh the benefits. These observations have stimulated the use of alternatives to HT, including soy isoflavones, a plant-derived compound with estrogen-like activity. In humans and animals isoflavones bind to estrogen receptors (ER). They have a greater affinity of ER β than ER α and can exert both estrogen-agonist and estrogen-antagonist properties. Supplements commonly contain soy protein which is a rich source of isoflavones; specifically genistein, daidzein, and glycitein.⁵

The popularity of soy isoflavones in menopausal women results from an apparent absence of adverse effects. However soy isoflavones may negatively affect thyroid function.⁶ Thyroid function relies on the release of thyroid stimulating hormone (TSH) from anterior pituitary in response to circulating triiodothyronine (T3) and thyroxine (T4) levels. TSH regulates thyroid peroxidase (TPO) activity and iodine uptake by the thyroid. TPO couples iodine to thyroglobulin in order to yield T3 or T4. *In vitro*, soy isoflavones inhibit TPO, but this inhibition can be prevented by iodine supplementation.⁷ The thyroid predominantly releases T4 which is then converted to the more active T3 by peripheral deiodinases (type 1 and 2).

Soy was originally thought to be goitrogenic and cause hypothyroidism.⁷ In humans, rats, and monkeys hypothyroidism has been associated with menstrual irregularities and infertility.⁸⁻¹¹ In post-menopausal women hypothyroidism prevalence reaches 10%¹² and may exacerbate disorders associated with menopause such as osteoporosis, cardiovascular disease, and neuropsychiatric disease.¹³

While mechanisms have been identified through which soy or soy isoflavones could interfere with thyroid function, reported effects are inconsistent. The majority of human studies, including both men and women's studies, have found no significant effect of soy

isoflavones on adult thyroid function.⁶ These studies were all short-term, cross-sectional, and used variable doses of isolated soy protein (ISP) supplementation. Persky et al¹⁴ found no significant effect of ISP on thyroid hormone concentrations after 3 and 6 months in women aged 49-83 years. This did not control for individual differences in age, ethnicity,¹⁵ BMI,¹⁶ smoking status,¹⁷ diet,¹⁸ and underlying disease¹⁹ which may have confounded the results. However, Duncan et al²⁰ studied a far more select and homogeneous sample and also found no thyroidal effects in post-menopausal women after 3 months of ISP treatment. Neither study controlled for the effects of caloric intake, circadian rhythm, and seasonality all of which may affect thyroid function.^{21,22}

Studies using animal models can control for many of the environmental variables thought to influence thyroid function. Controlled experiments with rodents demonstrate that isoflavones induce increases in serum T4 concentration^{23,24} However, rodent thyroid function and regulation differs from that of primates.²⁵ The main thyroid hormone carrier protein in primates, thyroid binding globulin (TBG), is not present in rodents. Isoflavone metabolism also differs markedly between rodents and humans.²⁶ Accordingly, we used non-human primates (NHPs) in a long-term, controlled randomized study to determine the effects of dietary soy on thyroid function, and the relationship between thyroid and ovarian function.

METHODS

Animals

Eighty-two adult (based on radiographic evidence of complete epiphyseal closure at the distal radius, ulna, and proximal tibia) female cynomolgus monkeys (*Macaca fascicularis*) were imported from Indonesia, and housed in social groups (5-6 animals each), indoors, on a 12h:12h light:dark cycle. All animal manipulations were performed according to the guidelines of state and federal laws, the US Department of Health and Human Services, and the Animal Care and Use Committee, Wake Forest University School of Medicine. Wake Forest University is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Study Design (Figure 1)

Baseline Phase—During an 8 month Baseline phase, monkeys were fed a casein-lactalbumin (CL) diet (19% of calories from protein; 35% of calories from fat; 46% of calories from carbohydrate; 0.28 mg cholesterol/kcal which was reduced to 0.20 mg/kcal later in the study). This diet was designed to have the composition of a typical American diet, which is thought to increase the risk of chronic disease.^{27,28}

Pre-Ovariectomy (Pre-OVX) Treatment Phase—Social groups of monkeys were assigned to one of two treatment conditions for 34 months. Since individual differences in plasma cholesterol responses to dietary fat and cholesterol could influence outcomes, the two treatment groups were matched on total serum cholesterol and high-density lipoprotein cholesterol responses to the baseline diet. The two treatment groups differed only in the main protein source: SOY (n=41), derived from isolated soy protein (SUPRO ©, Solae

Corporation, St. Louis, MO, USA); or CL (n=41), derived from casein-lactalbumin (the same diet consumed by all animals during the Pre-Treatment Phase). In addition, approximately 20% of protein fed in each diet was derived from wheat flour.

Post-Ovariectomy (Post-OVX) Treatment Phase—After 34 months surgical menopause was induced in all animals by ovariectomy via laparotomy. Half of the monkeys from each diet treatment group continued to consume their Pre-treatment phase diet (either SOY [n = 19] or CL [n = 21]) for an additional 34 months. The remainder of the animals did not continue their diets, and thus were not considered further.

Diet

The SOY diet contained 1.88 mg total isoflavone/g protein and was fed at a concentration designed to provide the equivalent of a women's daily consumption of approximately 140 mg aglycone units of isoflavone, assuming a daily intake of about 1800 kcal (i.e. 0.0775 mg isoflavone/kcal). Monkeys received 120 kcal of diet/kg body weight or approximately 9.3 mg isoflavone/kg body weight. Caloric adjustment of dose reflects the higher metabolic rate in monkeys as compared to humans. The diets were matched in macronutrients (19% of calories from protein; 35% of calories from fat; 46% of calories from carbohydrate), cholesterol (0.20 mg/kcal), and vitamin/mineral content.²⁹

Ovarian Function

The methods used to collect data describing ovarian function has been previously described.³⁰ Briefly, monkeys were trained to present for daily blood sampling without anesthesia during the last 6 months of the Baseline phase. Due to the extensive training required for awake blood sampling, ovarian function was assessed in a subset of n = 55 animals (29 of which were later assigned to the SOY treatment group, and 26 of which were later assigned to the CL treatment group). Blood samples were assessed for progesterone concentrations and the two highest progesterone concentrations from the luteal phase of each cycle were averaged (mean peak progesterone for each cycle). These values were then averaged over all cycles to provide a mean progesterone value for each animal.

Isoflavone Concentration

Seven and 19 months after the Pre-OVX treatment phase began blood samples were collected from all animals after ketamine sedation (10-15 mg/kg) to determine circulating isoflavone levels.³⁰ Animals were fed in the morning and sedated for blood collection 4 h post-feeding. Blood was immediately processed, frozen, and protected from light until analysis. Serum isoflavones from soy protein (genistein, daidzein, and glycitein) and a daidzein metabolite (equol) were analyzed by liquid chromatography photo-diode array tandem mass spectrometry. Isoflavone assessments were repeated at 12 and 24 months following initiation of the Post-OVX treatment phase.

Thyroid Hormones

Blood samples were taken between 08:00 and 10:30 h, following ketamine sedation (10-15 mg/kg), 3 weeks after the onset of the study (Baseline phase), 30 months after onset of

treatment (Pre-OVX treatment phase), and 9 months after ovariectomy (Post-OVX treatment phase). After clotting all samples were centrifuged, serum was drawn off, aliquoted, and frozen at -70°C until samples from all study phases were ready for analysis.

Serum levels of total T3, free T4, and TSH were determined by radioimmunoassay (T3, T4) and immunoradiometric assay (TSH) kits (Diagnostic Product Corp; Los Angeles, CA, USA) at the Yerkes Biomarker Laboratory (Atlanta, GA, USA). The inter-assay variations for total T3 were 8.96% at 78.41 ng/dl, 11.17% at 150.91 ng/dl, and 4.57% at 291.51 ng/dl. Intra-assay variation for total T3 was 2.06%. Inter-assay variations for free T4 were 2.0% at 0.40 ng/dl, 5.6% at 1.21 ng/dl, and 6.9% at 2.08 ng/dl. Intra-assay variation for free T4 was 12.78% at 1.82 ng/dl. Inter-assay variations were 5.9% at 0.33uIU/ml, 3.4% at 3.50uIU/ml, and 5.5% at 27.0 uIU/ml. Intra-assay variations for TSH were 5.8% at 0.36uIU/ml, 2.5% at 3.83uIU/ml, and 2.3% at 29.20uIU/ml.

Statistical Analysis

Baseline characteristics of the population (body weight, T3, T4, TSH, and progesterone) were compared between treatment groups using the Student's *t*-test. The effect of treatment on thyroid hormones was analyzed by 2 (treatment phase) -by- 2 (SOY, CL) mixed model analysis of variance (ANOVA) and by analysis of covariance (ANCOVA) using Baseline or Pre-OVX treatment phase values as covariates. Baseline thyroid function measurements were not obtained for three animals later assigned to the CL condition. Correlations between T3, T4, and TSH and progesterone were assessed by Pearson's *r*. Statistical significance was set at $p < 0.05$ (two-tailed), and all results were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed using STATISTICA 10.0 for Windows, StatSoft Inc. (Tulsa, OK).

RESULTS

Baseline Characteristics of the Study Population

During the Baseline phase there were no significant differences in body weight, T3, T4, and progesterone among animals later assigned to either CL or SOY; however TSH was higher in animals later assigned to CL as compared to the SOY condition (Table 1). Baseline phase measures obtained from all animals demonstrated that mean progesterone concentrations correlated with T3 ($r = +0.41$, $p < 0.01$; Figure 2) but not T4 ($r = +0.11$, $p = 0.41$) or TSH ($r = +0.01$, $p = 0.94$).

Isoflavones (Table 2)

Total serum isoflavones were present in only trace amounts during the Baseline phase and did not differ significantly between treatment groups.³⁰ Total serum isoflavones, genistein, daidzein, and equol were significantly elevated in the SOY group during both Treatment Phases (Table 2).

Diet Effects on Thyroid Function in Reproductively Intact Monkeys

The means ($\pm$ SEMs) of thyroid hormones and body weight during the Treatment Phases are given in Table 3. Repeated measures ANOVA comparing Baseline and Pre-OVX treatment

phase indicated no significant differences in BW. Repeated measures ANOVA comparing Baseline and Pre-OVX treatment phase values indicated a significant time-by-diet interaction for T3 ($F_{1,80} = 7.01$, $p = 0.01$; Figure 3), but not T4 ($F_{1,80} = 2.73$, $p = 0.10$; data not shown). Although nonsignificant, differences in Baseline means for animals later assigned to CL and SOY suggested the use of ANCOVA to adjust for data collected during Baseline. Covariance of the Baseline values revealed a significant increase in T3 but not T4 following SOY treatment (T3: $F_{1,79} = 6.23$, $p = 0.01$; Figure 3; T4: $F_{1,79} = 1.71$, $p = 0.19$).

A repeated measures ANOVA comparing Baseline and Pre-OVX treatment phase values indicated that TSH increased significantly over time ($F_{1,80} = 44.58$, $p < 0.01$), but was not affected by diet ($F_{1,80} = 1.80$, $p = 0.18$; data not shown). Moreover, the time-by-diet interaction was not significant ($F_{1,80} = 2.76$, $p = 0.20$). Because TSH assessed during Baseline differed between animals later assigned to the CL and SOY conditions, an ANCOVA using Baseline phase values as the covariate was again performed; here there were no significant differences between dietary conditions ($F_{1,79} = 1.69$, $p = 0.20$).

Diet Effects on Thyroid Function in Ovariectomized Monkeys

Repeated measures ANOVA comparing Pre-OVX and Post-OVX treatment phase values revealed a significant ovariectomy-by-diet interaction for T4 ($F_{1,38} = 4.64$, $p = 0.04$; Figure 4), but not T3 ($F_{1,38} = 0.11$, $p = 0.74$; data not shown). A subsequent ANCOVA adjusting for Pre-OVX treatment phase values demonstrated the presence of a significant effect of diet on T4 ($F_{1,38} = 7.25$, $p < 0.01$; Figure 4) but not T3 ($F_{1,38} = 1.70$, $p = 0.20$). There were no significant effects of ovariectomy, diet, or their interaction on TSH (Ovariectomy: $F_{1,37} = 1.80$, $p = 0.19$; Diet: $F_{1,37} = 0.76$, $p = 0.39$; Ovariectomy-x-Diet: $F_{1,37} = 1.38$, $p = 0.25$; data not shown).

Isoflavones and Thyroid Function

Correlational analyses between individual and total serum isoflavones and T3, T4, and TSH concentrations in the SOY condition revealed a significant positive association between T3 and genistein ($r = 0.33$, $p = 0.03$), and between T3 and total serum isoflavones ($r = 0.31$, $p < 0.05$; Figure 5) during the Pre-OVX treatment phase. No significant associations were observed in the data collected during the Post-OVX treatment phase. There were no significant associations between thyroid hormones and equol.

DISCUSSION

Reported here are the results of the first pre-clinical experiment evaluating the effects of dietary soy on thyroid function in a single NHP cohort treated both before and after surgical menopause. Notably, all animals consumed a typical North American diet that differed only in the source of protein (high isoflavone soy vs. casein-lactalbumin). The major findings were that dietary soy increased T3 in intact adult female monkeys and prevented a decline in T4 following surgical menopause. Additionally, ovarian function (progesterone concentration) was correlated with thyroid function (T3), demonstrating for the first time a relationship between these physiologic pathways in female NHPs.

This study is unique and these observations are compelling due to the use of NHPs, a human-like diet, and the multi-year experimental design that studied the same animals before and after surgical menopause. The translation to human beings of data from rodent studies is limited due to species differences in thyroid function and isoflavone metabolism. The available data from human studies derive largely from cross-sectional studies and are thus correlational, and the interpretation of the data are further limited by lack of control over environmental variables known to influence thyroid function, e.g. diet, caloric intake, health status, social environment, or circadian and seasonal rhythms. In addition to the use of an appropriate model and experimental design, a number of variables known to influence thyroid function were controlled including diet, physical and social characteristics of housing, light:dark cycle, and sampling timing.

In intact animals, SOY increased T3 but not T4 or TSH. These results differ from rodent studies that showed an increase in T4^{23,24} and one small, short-term premenopausal women's study which showed a decrease in T3³¹. Changes in only T3 suggest that soy affects T4 conversion to T3 by peripheral deiodinases. Isoflavones have estrogenic activity and estradiol has been shown to increase type 1 deiodinase activity in rodent liver.³² The caveat is that T3 is produced at the tissue level and plasma levels do not represent total metabolic T3 production.³³ Additionally a modest amount of T3 is released directly from the thyroid gland. TSH has been shown to trigger preferential release of T3 from the thyroid gland in man.³⁴ Although TSH was not significantly different between the diet treatment conditions, preferential T3 secretion represents a possible mechanism by which isoflavones increase plasma T3 concentration.

SOY prevented the dramatic decrease in T4 observed in the CL condition following ovariectomy. A post-ovariectomy drop in T4 has also been described in rodents, a response that is reversed by exogenous estradiol administration.^{35,36} Although there are no studies in humans directly addressing the effects of surgical menopause on thyroid function, a dietary isoflavone supplementation study in oophorectomized women found alterations in free T3 concentrations across time (increased at 6 weeks, decreased at 12 weeks).³⁷ Isoflavone studies done in naturally menopausal women have not shown significant changes in thyroid hormones.^{14,20,38-40} In general, these were short term studies that were not fully controlled and individual variation may have obscured the results. Alterations in thyroid function are dependent on a subject's normal set point.⁴¹ In the current study baseline measurements allowed the demonstration of a change in function in thyroid function in response to dietary isoflavones.

The observed alterations in T4, but not T3, in the SOY condition may have occurred at the thyroid or peripheral deiodinase level. In ovariectomized rats, T3 and T4 decreases are accompanied by reduced thyroid size and histologic evidence of thyroid hypoactivity. All are reversed by physiologic doses of estradiol.^{35,36} Isoflavones may act similarly, possibly via thyroidal ER binding. In ovariectomized sheep an isoflavone rich diet results in greater ER α immunoactivity, higher concentrations of T3 and T4, and morphologic changes to the thyroid follicles.⁴² In the periphery isoflavones may boost free T4 levels by decreasing TBG. Greater than 99% of plasma T4 is protein bound, TBG being the predominant binding protein. Reduction of TBG following isoflavone administration³¹ may have increased free

T4 in SOY fed animals. Unfortunately, while TBG has been isolated in NHP species, assays have not been validated in macaques.

To our knowledge this is the first study to demonstrate a relationship between thyroid and ovarian function in macaques. Records from a rhesus monkey breeding colony suggest an association between hypothyroxinemia (decreased plasma T4) and diminished reproductive performance.¹⁰ In this study T3, but not T4, concentrations were associated with progesterone. This may reflect the fact that T3 possesses greater biological activity compared to T4. Thyroid hormones can influence reproductive function through their effects on gonadotropin-releasing hormone pulse generation, prolactin release, sex hormone-binding globulin turnover, and by binding to thyroid hormone receptors (THRs) in the reproductive tract.⁷ The human ovary⁴³ and the uterus of cynomolgus macaques⁴⁴ express THRs. It is possible that THRs are also present in the macaque ovary and function as they do in humans. In humans, T3 and T4 are present in follicular fluid and addition of these hormones to granulosa cells has been shown to enhance progesterone production *in vitro*.⁴⁵ However, the relationship between thyroid and ovarian function is complex and bidirectional, and the effect of isoflavones on thyroid function depends on ovarian status (intact versus surgical menopause).

There were some limitations to this present study. First, this study did not account for menstrual cycle related fluctuations in thyroid function. Kaplan et al³⁰ previously published no differences in reproductive measures (ovarian hormone profile or menstrual cyclicity) between the two diet treatment conditions in this cohort; however, thyroid samples were not collected in relation to menstrual cycle phase. This may have affected outcomes as both estradiol and progesterone affect thyroid hormone concentrations.⁴⁶ A related study limitation was that we were unable to measure TBG which may have been affected by isoflavone administration and reflected in the diet treatment group differences in thyroid hormone concentration. The main translational limitation of this study was that women consume a variety of protein sources; our treatment diets consisted of only wheat protein and either soy or casein-lactalbumin derived protein. This and other dietary components may have affected dietary soy-thyroid relationships. However, the effects of dietary soy in this NHP model do reflect many of those observed in premenopausal women including reduced atherogenic plaque formation,²⁹ reduced ovarian aging,⁴⁷ and the absence of effects on bone mineral content.⁴⁸

Future studies might benefit from assessment of thyroid gland histological and morphometric changes. Additionally, it is essential to analyze deiodinase activity, including type 3 deiodinase which is involved in T3 and T4 degradation, to better understand the effects of soy isoflavones on thyroid hormone production and metabolism.

CONCLUSIONS

These observations suggest that dietary soy increases T3 in intact female monkeys, and prevents a decline in T4 following surgical menopause in nonhuman primates. Further there was a positive correlation between ovarian-derived progesterone levels and T3 secretion.

The outcomes observed in this study suggest soy protein and isoflavone consumption does not adversely affect and may even preserve thyroid function in postmenopausal women.

Acknowledgments

This work was supported in part by the National Center for Research Resources S10 RR020890, the National Heart, Lung, and Blood Institute P01 HL45666 (JRK), R01HL79421 (JRK), 2 R01 HL 087103 (CAS), and the National Institute of Health T32 OD 010957 (MS).

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Figure 1.

Experimental Protocol. Morning blood samples were obtained for thyroid hormone (thyroxine, triiodothyronine, thyroid stimulating hormone; TH) determination 3 weeks after the onset of the study (Baseline phase), 30 months after onset of treatment (Pre-OVX treatment phase), and 9 months after ovariectomy (Post-OVX treatment phase). During the last 6 months of the Baseline phase awake blood samples were obtained for peak luteal phase progesterone (P4) concentration determination in a subset of $n = 55$ animals. Circulating isoflavone (I) concentrations were sampled at 7 and 19 months after the Pre-OVX treatment phase began and were repeated at 12 and 24 months following initiation of the Post-OVX treatment phase. CL, casein-lactalbumin diet; SOY, isolated soy protein diet; OVX, ovariectomy

Figure 2.

Association between T3 and mean luteal phase progesterone from across the Pre-Treatment Phase. T3, triiodothyronine. $n = 55$ (29 of which were later assigned to the SOY treatment group, and 26 of which were later assigned to the CL treatment group)

Figure 3.

T3 concentration during the Pre-Treatment Phase and the Pre-OVX Treatment Phase (30 months after dietary intervention). Values presented as mean $\pm$ SEM; n = 41 per group. *p < 0.05 versus CL. T3, triiodothyronine; OVX, ovariectomy; CL, casein- lactalbumin diet; SOY isolated soy protein diet.

Figure 4.

T4 concentrations during the Pre-OVX Treatment Phase and the Post-OVX Treatment Phase (9 – 12 months post ovariectomy). Values are presented as mean $\pm$ SEM; CL group, n=21; SOY group, n=19. *p < 0.05 versus CL. T4, thyroxine; OVX, ovariectomy; CL, casein-lactalbumin diet; SOY, isolated soy protein diet.

Figure 5.

Association between mean total serum isoflavones and T3 in SOY fed monkeys during the Pre-OVX Treatment Phase. $n = 41$. OVX, ovariectomy; T3, triiodothyronine; SOY, isolated soy protein diet.

Table 1

Baseline Characteristics

	CL ^a	SOY ^a	T	P
n	41	41		
Body Weight (kg)	2.9 ± 0.09	2.9 ± 0.06	-0.56	0.57
T3 (ng/dL)	123.3 ± 3.1	119.2 ± 3.2	-0.92	0.36
T4 (ng/dL)	2.3 ± 0.05	1.2 ± 0.06	-1.0	0.32
TSH (uIU/mL)	1.0 ± 0.1	0.7 ± 0.06	-2.26	0.03
P4 (ng/mL) ^b	4.5 ± 0.04			

Values expressed as mean ± SEM.

CL, casein and lactalbumin diet; SOY, isolated soy protein diet; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone; P4, progesterone; T, T-value; P, P-value

^a Refers to Pre-Ovariectomy/ Post-Ovariectomy treatment conditions; all animals were on the CL diet during baseline measures

^b n = 55

Table 2

Isoflavone concentrations

	Baseline ^a				Treatment					
					Pre-Ovariectomy			Post-Ovariectomy		
	CL ^b	SOY ^b	T	P	CL	SOY	T	CL	SOY	P
n	44	41			44	41		21	19	
Total Isoflavones (nmol/L) ^c	15.0 ± 2.5	16.6 ± 3.7	0.37	0.71	6.2 ± 1.5	405 ± 39	17	3.7 ± 2.8	354 ± 46	<0.01
Daidzein (nmol/L)	12.5 ± 2.1	12.1 ± 2.6	-0.10	0.92	4.6 ± 1.1	212 ± 21	10	2.1 ± 1.5	196 ± 21	<0.01
Genistein (nmol/L)	2.5 ± 0.5	4.5 ± 1.2	1.6	0.12	1.4 ± 0.6	164 ± 17	10	1.6 ± 1.3	159 ± 22	<0.01
Equol (nmol/L)	259 ± 29	237 ± 21	-0.60	0.55	3.3 ± 1.5	624 ± 39	17	6.7 ± 5.6	498 ± 104	<0.01

Values expressed as mean ± SEM.

CL, casein and lactalbumin diet; SOY, isolated soy protein diet; T, T-value; P, P-value

^a All animals were on the CL diet at this timepoint^b Pre-Ovariectomy/ Post-Ovariectomy dietary treatment condition^c Total serum isoflavones include daidzein, genistein, and glycitein

Table 3

Treatment Phase Characteristics

	Pre-Ovariectomy		Post-Ovariectomy	
	CL	SOY	CL	SOY
n	44	41	21	19
Body Weight	3.5 ± 0.09	3.5 ± 0.12	3.4 ± 0.19	3.4 ± 0.16
T3 (ng/dL)	123 ± 3.5	132 ± 3.5	117 ± 7.4	131 ± 4.9
T4 (ng/dL)	1.3 ± 0.05	1.4 ± 0.05	1.2 ± 0.07	1.4 ± 0.09
TSH (uIU/mL)	1.3 ± 0.13	1.3 ± 0.11	1.07 ± 0.35	0.64 ± 0.09

Values expressed as mean ± SEM.

CL, casein and lactalbumin diet; SOY, isolated soy protein diet; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone