The Effect of Soy Phytoestrogen Supplementation on Thyroid Status and Cardiovascular Risk Markers in Patients with Subclinical Hypothyroidism: A Randomized, Double-Blind, Crossover Study

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Context: There is concern whether soy phytoestrogens may affect thyroid function. If true, soy phytoestrogens may be expected to have a greater impact in subjects with subclinical hypothyroidism.

Objective: The primary aim was to determine the effect of soy phytoestrogen supplementation on thyroid function, with a secondary aim of assessing the effects on cardiovascular risk indices in patients with subclinical hypothyroidism.

Design and Setting: We conducted a randomized, double-blind, crossover study in a tertiary care setting.

Participants: Sixty patients with subclinical hypothyroidism participated in the study.

Intervention: Patients were randomly assigned to either low-dose phytoestrogen (30 g soy protein with 2 mg phytoestrogens, representative of a Western diet) or high-dose phytoestrogen (30 g soy protein with 16 mg phytoestrogens, representative of a vegetarian diet) supplementation for 8 wk, then crossed over after an 8-wk washout period.

Main Outcome Measures: The primary outcome was progression to overt hypothyroidism, with secondary outcome measures of blood pressure, insulin resistance, lipids, and highly sensitive C-reactive protein (hsCRP).

Results: Six female patients in the study progressed into overt hypothyroidism with a standardized rate ratio of 3.6 (95% confidence interval, 1.9, 6.2) after 16-mg phytoestrogen supplementation. Both systolic and diastolic blood pressure decreased with 16 mg phytoestrogens, whereas systolic pressure alone decreased with 2 mg phytoestrogens. Insulin resistance (homeostasis model assessment of insulin resistance, 3.5 ± 0.09 vs. 2.6 ± 0.08; $P < 0.02$) and hsCRP (4.9 ± 0.04 vs. 3.9 ± 0.03; $P < 0.01$) decreased with 16 mg phytoestrogens. Lipid profile remained unchanged.

Conclusion: There is a 3-fold increased risk of developing overt hypothyroidism with dietary supplementation of 16 mg soy phytoestrogens with subclinical hypothyroidism. However, 16-mg soy phytoestrogen supplementation significantly reduces the insulin resistance, hsCRP, and blood pressure in these patients. (*J Clin Endocrinol Metab* **96:** 1442–1449, 2011)

Abbreviations: fT$_4$, Free T$_4$; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, highly sensitive C-reactive protein; TPO, thyroid peroxidase.

Soy foods have played an important role in the diets of many Asian countries for centuries, and in recent years they have become increasingly popular in many non-Asian countries as a result of their suggested health effects. Postulated benefits of soy foods particularly due to their phytoestrogen components include protection against coronary heart disease (1, 2), breast and prostate cancer (3–5), and osteoporosis (6), and alleviation of hot flushes (7). Public interest in the health benefits of phytoestrogens has led to the development of phytoestrogen supplements and the fortification of foods with these soybean constituents (8, 9). However, concern has been expressed that soy may adversely affect thyroid function in susceptible individuals (10, 11). *In vitro* studies have demonstrated that phytoestrogens inhibit thyroid peroxidase (TPO), an enzyme involved in the synthesis of T_3 and T_4 (12, 13).

Subclinical hypothyroidism has a worldwide prevalence ranging from 1 to 10%; the highest age- and sex-specific rates are in women older than 60 yr of age, approaching 20% in some reports (14, 15). Studies have suggested that it is an independent risk factor for coronary heart disease (16). Subclinical hypothyroidism during pregnancy is also associated with adverse maternal and neonatal outcomes (17–19). In this population group, the potential impact of soy phytoestrogens on thyroid could be of concern, although at the same time it could have benefits in terms of improving cardiovascular risk.

Studies of phytoestrogen intakes in Western countries indicate a typical average daily intake of around 2 mg phytoestrogens (20, 21), whereas vegans consume an average of 16 mg (22). Phytoestrogens are found in high quantities in soy products, but other dietary sources include vegetables, fruits, and nuts in lesser amounts. This double-blind, crossover trial was undertaken to determine the effect of soy phytoestrogens on thyroid function and cardiovascular disease risk markers in patients with subclinical hypothyroidism. Soy protein was supplemented with 16 mg phytoestrogens or 2 mg phytoestrogens as a powder to be mixed with their daily dietary preparations.

Patients and Methods

Patients with subclinical hypothyroidism (TSH value between 5 and 15 mU/liter; normal range, 0.5–4.7 mU/liter) with a normal free T_4 (fT_4) were recruited after identification through routine biochemical testing performed at Hull Royal Infirmary over a 12-month period. The screening thyroid function tests were done 4–8 wk after the initial biochemical testing. A total of 75 patients were initially identified, but 15 were excluded during screening: 10 patients could not tolerate the soy preparation due to its unpalatability at taste testing before undertaking the study, and in five patients thyroid function had returned to normal at screening, suggesting transient thyroiditis. Exclusion criteria included patients taking drugs that may interfere with thyroid function; antihypertensive, insulin-sensitizing agents; lipid-lowering medications, as well as antibiotic use within the previous 6 months. Women who were contemplating pregnancy were excluded. At randomization and each subsequent visit, subjects were instructed to maintain their level of physical activity throughout the study. In addition, subjects were required to avoid food products containing soy, alcohol, vitamin or mineral supplementation, and over-the-counter medications. Dietary reinforcement was undertaken at each visit, together with measurement of plasma phytoestrogen levels to ensure compliance. A 24-h urine collection was made during the second phase to measure urine iodine excretion.

Study design

A randomized, double-blind, crossover study was undertaken. A total of 60 patients (age range, 44–70 yr) were randomized for the study; 30 patients initiated with the 2 mg phytoestrogen with 30 g soy protein powder, and 30 patients on the 16 mg phytoestrogen with 30 g soy protein powder per day for 8 wk to be mixed with their daily dietary preparations (first phase). After an 8-wk washout period, the participants received the alternative supplementation for 8 wk (second phase).

An 8-wk time period was chosen for each arm because this was the minimum time for phytoestrogen exposure that might have been expected to affect thyroid function, given that the half-life of T_4 is around 7 d.

Each box of supplements that patients received contained the number of sachets required for 8 wk of supplementation plus six sachets marked as reserve in case of trial material loss or any delay in their final clinic attendance.

After completing the baseline tests, the subjects were randomly assigned to either 16- or 2-mg phytoestrogen supplementation based on a computer-generated randomization list. Each randomization number corresponded with one of the two possible interventions, and labeling of the identical boxes of study preparation was done by personnel not involved in the trial. Compliance was calculated by counting the returned sachets.

The primary outcome of the study was progression to overt hypothyroidism, whereas secondary outcome measures were blood pressure, homeostasis model assessment of insulin resistance (HOMA-IR), lipids, and highly sensitive C-reactive protein (hsCRP). Overt hypothyroidism was defined as a combination of TSH greater than 4.7 mU/liter and fT_4 less than 9 pmol/liter.

Of the 60 patients randomized, six female patients withdrew during the high-dose phytoestrogen supplementation due to progression to overt hypothyroidism, and another six female patients withdrew while on low-dose phytoestrogen supplementation [four with loose stools (ages 64, 52, 66, and 68 yr), one with use of antibiotics (age, 55 yr), and one lost to follow-up (age, 63 yr)]; however, the data from withdrawals were included as part of intention-to-treat analysis.

Phytoestrogen composition

The low-dose phytoestrogen preparation consisted of 30 g of soy protein concentrate (70% proteins) containing 2 mg of phytoestrogens, and the high-dose preparation contained 16 mg of phytoestrogens. Analysis showed the composition of the dose materials to be 54% genistein, 35% daidzein, and 12% glycitein as aglycones and further confirmed that 90% of phytoestrogens were in the primary glucoside form, with the remaining 10% as

aglycones or acetyl and malonyl glucosides. The soy protein (Sol-con F) and the phytoestrogens (Solgen 40) were supplied by Sol-bar Industries Ltd. (Ashdod, Israel) and prepared by Essential Nutrition, Ltd. (Brough, UK), who randomized the sachets.

Study measurements

At the beginning and end of each phase, after an overnight fast, weight and blood pressure were measured, and blood samples were collected. Blood pressure was measured after the patients had been seated quietly for at least 5 min with the right arm supported at heart level. Blood pressure measurements were performed using an automated device (NPB-3900; Nellcor Puritan Bennett, Pleasanton, CA) during each study visit. Two readings were obtained at the beginning of each visit at least 1 min apart. If there was more than a 5-mm Hg difference in systolic blood pressure between the two readings, a third reading was obtained. Fasting venous blood samples were collected and separated by centrifugation at 2000 × *g* for 15 min at 4 C, and the aliquots were stored at −80 C within 1 h of collection. Plasma glucose was measured using a Synchron LX20 analyzer (Beckman Coulter, Buckinghamshire, UK), and serum insulin was assayed using a competitive chemiluminescent immunoassay performed using the DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, UK). The coefficient of variation of this method was 8%, calculated using duplicate study samples. The analytical sensitivity was 2 μU/ml. Insulin resistance was calculated using HOMA-IR (insulin × glucose)/22.5 (23). Total cholesterol, triglycerides, and high-density lipoprotein cholesterol levels were measured enzymatically using a Synchron LX20 analyzer (Beckman Coulter). Low-density lipoprotein cholesterol was calculated using the Friedewald equation. All thyroid assays were performed on an Abbott Architect i4000 immunoassay analyzer (Abbott Diagnostics Division, Berkshire, UK). The reference range for TSH was 0.5–4.7 mU/liter, fT_4 was 9–24 pmol/liter, and free T_3 was 2.5–5.3 pmol/liter. Urinary iodine measurements in 24-h urine collections were undertaken by inductively coupled plasma–mass spectrometry to monitor for iodine repletion, and plasma phytoestrogen measurement was undertaken by liquid chromatography–tandem mass spectrometry (Central Science Laboratory, Sand Hutton, UK).

Statistical analysis

For a significant reduction in fT_4, a sample size of 50 patients in a crossover design was calculated, giving 80% power to detect a mean decrease of 0.4 nmol/liter of free T_4, with a two-sided α error of 0.05 (24).

Intention-to-treat analysis was performed. Wilcoxon's signed-rank test was used to compare thyroid function tests before and after supplementation with 2 and 16 mg phytoestrogens, as well as phytoestrogen data that violated the assumptions of normality when tested using the Kolmogorov-Smirnov test. The period and the carryover effect that may have occurred from the crossover design were also tested using the appropriate Student's *t* test. Independent samples *t* test was used to compare plasma phytoestrogen levels after washout and after 2 mg/16 mg phytoestrogens. Statistical analysis was performed using SPSS 14.0 (SPSS Inc., Chicago, IL). An arbitrary level of 5% statistical significance (two-tailed) was assumed. The data are reported as mean ± SEM. All subjects gave their written informed consent, and the protocol was approved by the Hull and East Riding local research ethics committee.

Results

The mean age of patients was 57.2 ± 13.8 yr. There were eight males and 52 female subjects with subclinical hypothyroidism. TPO antibodies, the marker for autoimmune thyroid dysfunction, were positive (>75 U/ml) in 38 (63.3%) patients. Compliance was 98% in both groups.

Six patients (10%) developed overt hypothyroidism after high-dose phytoestrogen, and none after low-dose phytoestrogen supplementation. Of these six patients who developed overt hypothyroidism, three had high-dose phytoestrogen supplementation before low-dose phytoestrogen, and three had high-dose phytoestrogen supplementation after low-dose phytoestrogen supplementation (Fig. 1). All of the subjects who withdrew from the study came back to clinic for an end-of-study visit where all of the outcomes were measured. All six subjects were diagnosed as having overt hypothyroidism during their end-of-study visit after the 8-wk high-dose phytoestrogen supplementation period, when they were found to have raised TSH of more than 10 mU/liter and a low fT_4 of less than 9 pmol/liter, *i.e.* overt hypothyroidism.

The six patients who developed overt hypothyroidism started on levothyroxine on the basis of their biochemical testing alone and continued to receive levothyroxine supplementation after 6 months.

All six patients who developed overt hypothyroidism were females, *i.e.* 11.5% of females in this study developed overt hypothyroidism. All six patients who withdrew from study were females, reflecting that 13% of females who completed the study developed overt hypothyroidism. Only one patient who developed overt hypothyroidism during the study had TPO antibody positivity.

Although six patients receiving 16-mg phytoestrogen supplementation advanced from subclinical to overt hypothyroidism and no patients advanced while receiving 2-mg phytoestrogen supplementation, the mean TSH, fT_4, and free T_3 levels were not statistically different between the two groups. There were also no statistically significant changes in any of these parameters when the two groups (*i.e.* the group who had 2 mg phytoestrogen first, followed by the 16-mg phytoestrogen supplementation, and the group who had 16 mg phytoestrogen first followed by the 2-mg phytoestrogen supplementation) were analyzed separately. There were no period effects or carryover effects in any of the parameters. Thyroid antibody positivity did not alter before or after either supplement. This suggests that neither baseline thyroid function tests nor thyroid antibody positivity predicts progression of patients with subclinical hypothyroidism to overt hypothyroidism with high-dose phytoestrogen supplementation.
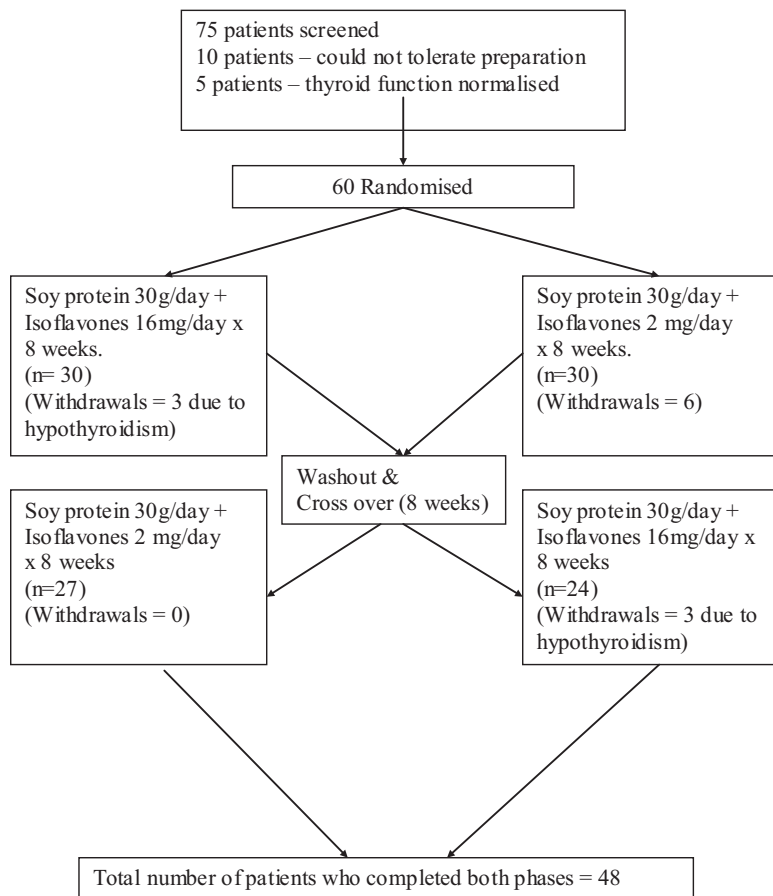
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┌─────────────────────────────────────────┐
│ 75 patients screened                    │
│ 10 patients – could not tolerate preparation │
│ 5 patients – thyroid function normalised │
└─────────────────────────────────────────┘
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          ┌─────────────────────┐
          │   60 Randomised     │
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Soy protein 30g/day +
Isoflavones 16mg/day x
8 weeks.
(n= 30)
(Withdrawals = 3 due to
hypothyroidism)

Soy protein 30g/day +
Isoflavones 2 mg/day
x 8 weeks.
(n=30)
(Withdrawals = 6)

Washout &
Cross over (8 weeks)

Soy protein 30g/day +
Isoflavones 2 mg/day
x 8 weeks
(n=27)
(Withdrawals = 0)

Soy protein 30g/day +
Isoflavones 16mg/day x
8 weeks
(n=24)
(Withdrawals = 3 due to
hypothyroidism)

Total number of patients who completed both phases = 48

FIG. 1. Flow chart describing the progress of patients through the trial.

For the six patients who developed overt hypothyroidism after high-dose phytoestrogen supplementation, TSH values increased by 57% (8.0 ± 0.8 *vs.* 13.1 ± 0.7 mU/liter; $P < 0.05$), and fT$_4$ values decreased by 25% (12 ± 0.4 *vs.* 8.8 ± 0.1 pmol/liter; $P < 0.05$). In these six patients, TSH values ranged from 12–14 mU/liter, and fT$_4$ values ranged from 8.6–8.9 pmol/liter. When the six patients who developed overt hypothyroidism were compared with the rest of the patients, there were no significant differences in post high-dose phytoestrogen supplementation daidzein levels (26.3 ± 1 *vs.* 25 ± 0.9 ng/ml; $P = 0.5$) or genistein levels (46.1 ± 0.9 *vs.* 48.6 ± 1.2 ng/ml; $P = 0.1$). The urine iodine levels did not differ between patients who developed overt hypothyroidism compared with the remainder of the subjects (median ± interquartile range, 272.5 ± 10.2 *vs.* 234 ± 6.2 μg/d; $P = 0.2$).

There was a significant reduction in systolic blood pressure and diastolic blood pressure after 16-mg phytoestrogen supplementation. There was also a significant reduction in systolic, but not in diastolic, blood pressure after the 2-mg phytoestrogen supplementation. There were no significant changes in weight after either the 16- or 2-mg phytoestrogen supplementation (Table 1).

There was a significant improvement in insulin resistance measured using HOMA-IR after the 16-mg phytoestrogen supplementation but not after the 2-mg phytoestrogen supplementation (Table 1). There was a significant improvement in hsCRP, a marker for inflammation in atherosclerosis, after 16 mg phytoestrogen but not after 2-mg phytoestrogen supplementation. There were no signifi-

TABLE 1. Subject characteristics and effects on cardiovascular risk at the start of the trial and 3 months after supplementation

	2 mg phytoestrogen/ 30 g soy protein/d			16 mg phytoestrogen/ 30 g soy protein/d			P (between-supplementation difference)
	Baseline	3 months	P value	Baseline	3 months	P value	
Weight (kg)	78.4 ± 2.1	78.2 ± 1.8	0.4	79.1 ± 2.3	78.9 ± 1.9	0.4	0.8
BMI (kg/m^2)	29.2 ± 0.6	29.1 ± 0.8	0.4	29.6 ± 0.7	29.4 ± 0.4	0.4	0.8
SBP (mm Hg)	141.4 ± 2.0	136.8 ± 1.8	0.03	140.7 ± 2.4	133.6 ± 2.8	<0.01	<0.01
DBP (mm Hg)	75.8 ± 1.0	74.9 ± 1.4	0.4	76.7 ± 1.8	72.1 ± 1.4	0.02	<0.01
Plasma glucose (mmol/liter)	5.3 ± 0.03	5.2 ± 0.04	0.2	5.4 ± 0.2	4.9 ± 0.05	<0.01	<0.01
Insulin (μIU/ml)	27.5 ± 1.2	26.9 ± 0.9	0.1	27.3 ± 0.9	20.8 ± 1.2	<0.01	<0.01
HOMA-IR	3.5 ± 0.08	3.4 ± 0.06	0.4	3.5 ± 0.09	2.6 ± 0.08	<0.01	<0.01
TC (mmol/liter)	5.5 ± 1.1	5.2 ± 0.9	0.7	5.4 ± 0.8	5.0 ± 0.2	0.3	0.8
LDL (mmol/liter)	3.5 ± 0.2	3.3 ± 0.1	0.2	3.4 ± 0.1	3.2 ± 0.2	0.2	0.8
HDL (mmol/liter)	1.3 ± 0.02	1.4 ± 0.09	0.3	1.2 ± 0.05	1.4 ± 0.03	0.1	0.3
TG (mmol/liter)	1.8 ± 0.05	1.6 ± 0.03	0.1	1.9 ± 0.02	1.6 ± 0.04	0.3	0.2
CRP (mg/liter)	4.8 ± 0.08	4.6 ± 0.06	0.1	4.9 ± 0.04	3.9 ± 0.03	0.02	<0.01

BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoproteins; HDL, high-density lipoproteins; CRP, C-reactive protein. To convert values for glucose to milligrams per deciliter, divide by 0.056. To convert values for insulin to picomoles per liter, multiply by 6. To convert values for cholesterol to milligrams per deciliter, divide by 0.0259. To convert values for triglycerides to milligrams per deciliter, divide by 0.0113.

TABLE 2. Plasma isoflavone levels and thyroid functions at the start of the trial and 3 months after supplementation

	2 mg phytoestrogen/ 30 g soy protein/d			16 mg phytoestrogen/ 30 g soy protein/d			*P* (between-supplementation difference)
	Baseline	**3 months**	***P* value**	**Baseline**	**3 months**	***P* value**	
fT$_3$ (pmol/liter)	4.0 ± 0.06	4.4 ± 0.09	0.2	4.2 ± 0.8	4.3 ± 0.6	0.4	0.6
fT$_4$ (pmol/liter)	11.8 ± 0.1	11.9 ± 0.4	0.1	12.2 ± 0.1	11.3 ± 0.9	0.1	0.4
TSH (mU/liter)	7.8 ± 0.4	7.5 ± 0.1	0.4	7.9 ± 0.3	8.4 ± 0.8	0.3	0.7
Daidzein (ng/ml)	2.1 ± 0.3	11.6 ± 0.2	<0.01	2.2 ± 0.1	26.2 ± 0.1	<0.01	<0.01
Genistein (ng/ml)	2.6 ± 0.7	10.9 ± 0.3	<0.01	2.4 ± 0.2	46.5 ± 0.6	<0.01	<0.01

fT$_3$, Free T$_3$.

cant changes in lipid profile after either the 16- or 2-mg phytoestrogen supplementation (Table 1).

The baseline plasma phytoestrogen values were consistent with the standard United Kingdom diet (21). The levels of phytoestrogens after the washout phase were comparable to the baseline levels (daidzein, 2.1 ± 0.3 *vs.* 2.2 ± 0.1 ng/ml, *P* = 0.8; and genistein, 2.6 ± 0.7 *vs.* 2.5 ± 0.2 ng/ml), confirming compliance of patients with the study protocol and avoidance of extra dietary sources of phytoestrogens. There was a significant increase in plasma phytoestrogens after 16-mg supplementation compared with 2-mg phytoestrogen supplementation (Table 2). The plasma levels of non-soy phytoestrogens such as formononetin, biochanin A, and coumestrol were low and static before and after 2- and 16-mg phytoestrogen supplementations. The 24-h urine iodine estimation showed adequate dietary iodine repletion with a urinary iodine excretion of 262 ± 18 µg/d (median ± interquartile range).

Discussion

This study showed that soy phytoestrogen supplementation at a level found in a vegetarian diet (16 mg) appeared to have a detrimental effect on thyroid status in patients with subclinical hypothyroidism, whereas phytoestrogens at a level equivalent to that seen in a normal Western diet (2 mg) did not. Although some studies have found no differences in thyroid function in vegans compared with omnivores (25, 26), these data are in accord with a report showing that the geometric mean TSH concentration, adjusted for age and body mass index, was 29% higher in vegans than in omnivores in the United Kingdom (27). This is supported by animal studies showing synergism of soy phytoestrogens with iodine deficiency causing hypothyroid effects in rats (28). Indeed, *in vitro* studies have demonstrated that the phytoestrogen inhibits TPO, an enzyme involved in the synthesis of T$_3$ and T$_4$ (12, 13). The subjects in the present study were iodine replete, as seen by the urinary iodine concentration of greater than 100 µg/

liter (29). The minimum daily requirement for iodine is 150 µg/d, which corresponds in general to a urinary iodine concentration of 100–150 µg/d.

Of the 60 patients recruited, six of the 52 female subjects (11.5%) progressed into overt hypothyroidism after the 16-mg phytoestrogen arm of the 6-month period in this study. This suggests that the risk of developing overt hypothyroidism is much higher in females who have subclinical hypothyroidism with high-dose phytoestrogen intake. However, because only 11.5% of the study subjects were male, it was not possible to determine the risk of development of overt hypothyroidism in males. The overt hypothyroidism that developed appeared permanent, with no reversal on phytoestrogen withdrawal, but the underlying mechanism is unclear, although acceleration of the underlying autoimmune process cannot be excluded. In a prospective study looking into the spontaneous course of hypothyroidism in females, the rate of progression of subclinical hypothyroidism to overt hypothyroidism was 5.6%/yr (30). Extrapolating the data from this 8-wk study to give a rate of progression per year, it was expected that in our study population, 3.36 cases per year would progress to overt hypothyroidism; this translates into a standardized rate ratio of 3.6 (95% confidence interval = 1.9, 6.2), *i.e.* supplementation with 16 mg phytoestrogen caused a 3-fold increase in progression from subclinical to overt hypothyroidism in this study. This incidence in our study is higher than the 4.3%/yr risk for progression from subclinical to overt hypothyroidism found in TPO antibody-positive women and the 2.6%/yr risk for subclinical hypothyroid TPO antibody-negative women in the Whickham survey (31). Although TPO antibody positivity is a known risk factor for the progression to overt hypothyroidism and 63% of study participants were TPO antibody positive, only one patient who developed overt hypothyroidism in the present study was TPO antibody positive. This suggests that a relatively higher proportion of patients in this study had TPO-negative autoimmune subclinical hypothyroidism. None of the subjects had prior thyroid surgery or radioiodine therapy. The 24-h

urine iodine estimation showed adequate dietary iodine repletion, which excludes iodine deficiency as the cause for the hypothyroidism seen in this group.

There are currently no data to guide what effect longer periods of exposure would have on thyroid function, with the possibilities that more overt hypothyroidism may result, stabilization may occur, or thyroid function could improve with thyroid adaptation to the phytoestrogen load. Gastrointestinal side effects are recognized adverse effects of soy protein and/or phytoestrogens (32); however, it appears that all the patients who developed gastrointestinal side effects severe enough to withdraw from the study did so during the low-dose phytoestrogen supplementation phase.

A beneficial effect on the cardiovascular risk indices was observed, with a significant reduction of blood pressure, hsCRP, and insulin resistance after the higher 16-mg phytoestrogen supplementation in this study. Epidemiological observations of diet and cardiovascular disease in Japan and China have linked soy-product consumption with a reduced cardiovascular risk (33, 34). Studies of phytoestrogen intakes in Western countries indicate an average daily intake of approximately 2 mg phytoestrogens (20, 21), equivalent to the low-dose phytoestrogen preparation used here. Vegetarians have higher phytoestrogen intake (22), with levels corresponding to the 16-mg phytoestrogen supplementation of this study.

There was a statistically significant reduction in systolic blood pressure of 7.2 ± 2.6 mm Hg and diastolic blood pressure of 4.3 ± 1.2 mm Hg after 16-mg phytoestrogen supplementation in this study. The magnitude of the reduction in blood pressure was as great as that seen in therapeutic trials on hypertension that have shown a marked reduction in cardiovascular risk (35–37), supporting the potential value of such a dietary supplementation.

An improvement in systolic pressure alone was seen with 2 mg phytoestrogens in this study. This would suggest that there is a dose-response effect, with the 16-mg phytoestrogen supplementation being more effective. It has been suggested that an antihypertensive effect may be mediated via the action of phytoestrogens on endothelial cell function (38). However, in a study in type 2 diabetes patients treated with 132-mg tablets of phytoestrogens alone without soy protein, there was no antihypertensive effect and there was no effect on cardiovascular disease markers (39). This suggests that a synergistic matrix effect between the soy proteins with the phytoestrogens may be responsible for any cardiovascular disease changes because both supplements contain the same amount of protein. The blood pressure-lowering effects of soy phytoestrogens reported in the literature are conflicting (40–42); however, the studies are difficult to compare because different soy phytoestrogen/placebo/comparator preparations have been used in a diverse range of study populations.

There was a significant improvement in insulin resistance after the 16-mg phytoestrogen supplementation. This is in accord with studies that show soy phytoestrogens improving insulin resistance in postmenopausal women and those with type 2 diabetes (24, 43). Primate studies have suggested that consumption of isoflavone-containing soy protein dose-dependently increased insulin responses to the glucose challenge and decreased plasma adiponectin, whereas isoflavone-depleted soy protein decreased body weight and had no effect on plasma adiponectin concentrations (44). In addition, studies have shown that phytoestrogen alone without soy protein was ineffective in reducing insulin resistance, suggesting a potential matrix effect (42, 45).

There was also a significant improvement of hsCRP after 16 mg phytoestrogen but not after the 2-mg phytoestrogen phase, suggesting that higher phytoestrogen doses may have an antiinflammatory effect in this study. It is now established that hsCRP is an important cardiovascular risk marker (46). In another study, phytoestrogen supplementation alone did not have any effects on hsCRP (47); however, soy protein along with phytoestrogen improved hsCRP in patients having type 2 diabetes with nephropathy (48), again suggesting a potential matrix effect.

There was no significant change in the lipid profile with either high- or low-dose phytoestrogen supplementation in this study. In other studies, higher doses of phytoestrogens (66–166 mg/d) along with soy protein or isolated soy protein are reported to improve the lipid profile (40, 43, 49), but not seen with isolated phytoestrogen supplementation (50, 51). This also supports a potential matrix effect between the protein and the phytoestrogens.

This study suggests that a number of cardiovascular risk factors improved with high-dose phytoestrogens and a number with low-dose phytoestrogens. However, these risk factors may get worse with the progression of subclinical to overt hypothyroidism if left undiagnosed or not treated over time. A beneficial effect of the phytoestrogens on these risk factors over the initial short period of treatment may result, but with deleterious effects resulting from the induction of overt hypothyroidism.

Because all the patients were Caucasians, it will be difficult to generalize to other population groups. The majority of patients in this study were females, but subclinical hypothyroidism is more common in females. Six patients withdrew while they were on low-dose phytoestrogens; however, the dropout rate was better than other studies involving nutritional supplementation. The safety and efficacy of higher doses of phytoestrogens, such as those

found in nutritional supplements, and the potential matrix effect were not ascertained in this study and need future evaluation. This study is a comparative study of two different doses of phytoestrogens within the same soy protein matrix, but it lacks a true placebo arm with a supplementation of soy protein alone that is isoflavone free or an alternative protein such as casein to replace the soy protein. However, the two doses of phytoestrogens studied reflect the typical Western and vegetarian diets.

In conclusion, soy phytoestrogen supplementation at a level reflecting that found in a vegetarian diet appeared to precipitate overt hypothyroidism, especially in female patients with subclinical hypothyroidism. This suggests that female vegetarian patients with subclinical hypothyroidism may need more careful monitoring. Conversely, phytoestrogen supplementation showed a beneficial dose-dependent effect on the cardiovascular risk profiles in this patient population.

Acknowledgments

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