Effect of Soy in Men With Type 2 Diabetes Mellitus and Subclinical Hypogonadism: A Randomized Controlled Study

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Context: Isoflavones found in soy products have a chemical structure similar to estrogen, leading to concerns of an adverse estrogenic effect in men, particularly in those with type 2 diabetes mellitus (T2DM) who have low testosterone levels due to hypogonadism.

Objective: The primary outcome was change in total testosterone levels. The secondary outcomes were the changes in glycemia and cardiovascular risk markers.

Design: This was a randomized double-blind parallel study.

Setting: This study occurred in a secondary care setting in United Kingdom.

Participants: Two hundred men with T2DM and a total testosterone level ≤12 nmol/L were included.

Intervention: Fifteen grams of soy protein with 66 mg of isoflavones (SPI) or 15 g soy protein alone without isoflavones (SP) daily as snack bars for 3 months were administered.

Results: There was no change in either total testosterone or in absolute free testosterone levels with either SPI or SP. There was an increase in thyrotropin (TSH) and reduction in free thyroxine (fT4; $P < 0.01$) after SPI supplementation. Glycemic control improved with a significant reduction in hemoglobin A1c (-4.19 [7.29] mmol/mol, $P < 0.01$) and homeostasis model of assessment - insulin resistance after SPI. Cardiovascular risk improved with a reduction in triglycerides, C-reactive protein, and diastolic blood pressure (DBP; $P < 0.05$) with SPI *vs* SP supplementation. There was a 6% improvement in 10-year coronary heart disease risk after 3 months of SPI supplementation. Endothelial function improved with both SPI and SP supplementation ($P < 0.01$), with an increased reactive hyperemia index that was greater for the SPI group ($P < 0.05$).

Conclusions: Testosterone levels were unchanged and there was a substantial improvement in glycaemia and cardiovascular risk markers with SPI compared with SP alone over 3 months. There was also a substantial increase in TSH and a reduction in fT4. (*J Clin Endocrinol Metab* **102: 425–433, 2017**)

Abbreviations: AI, augmentation index; CVD, cardiovascular disease; DBP, diastolic blood pressure; FRTL, Fischer rat thyroid cell; FSH, follicle-stimulating hormone; fT4, free thyroxine; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LC-MS/MS, liquid chromatography tandem mass spectrometry; LDL-C, low-density lipoprotein cholesterol; LLOQ, lower limit of quantification; LS, luteinizing hormone; RHI, reactive hyperemic index; SBP, systolic blood pressure; SD, standard deviation; SHBG, sex hormone–binding globulin; SP, soy protein alone without isoflavones; SPI, soy protein with isoflavones; T2DM, type 2 diabetes mellitus; UKPDS, UK Prospective Diabetes Study.

Production and consumption of soy foods within Western countries have increased dramatically in the past decade, with the postulated health benefits including improvement in bone health, relief of menopausal symptoms, and reduced risk of certain types of cancers (1). In addition, habitual intake of soy isoflavones has also been associated with a reduced risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (2), which is of particular relevance given the increasing global prevalence of diabetes.

However, there are concerns because isoflavones have a chemical structure similar to estrogens and can transactivate estrogen receptors, exerting estrogen-like effects *in vitro* and *in vivo* (3). Isoflavones have been shown to affect reproductive health in animals. Male rats exposed to maternal dietary isoflavones through gestation and lactation exhibited a decrease in the anogenital distance, testis size, and serum testosterone levels (4). Dietary feeding of isoflavones in male mice resulted in reduced plasma testosterone concentrations, atrophy of seminiferous epithelium and accessory sex glands, and squamous metaplasia of seminal vesicles (5). When fed with soy, infant marmoset monkeys showed an increase in testicular size and lower testosterone levels, suggesting a potential "compensated Leydig cell failure" (6). This has raised concerns regarding possible adverse effects of isoflavones in men, including feminization and infertility (7). Testosterone levels in men with T2DM are lower from a combination of factors, including insulin resistance and obesity, which may make these individuals more susceptible to isoflavone-mediated adverse effects (8). Therefore, given the estrogenic effect of phytoestrogens, we hypothesized that a detrimental estrogenic effect of soy with and without isoflavones on testosterone levels would be exaggerated in men who had low testosterone levels; therefore, this randomized double-blind, parallel study was undertaken in men with T2DM and compensated hypogonadism.

Research Design and Methods

Two hundred men aged between 45 to 75 years with an early morning total testosterone level <12 nmol/L (normal range, 12 to 25 nmol/L) (at least on 2 different occasions) with normal gonadotrophins and hemoglobin A1c (HbA1c) <9% (normal: <6.5%) were recruited after screening 412 Caucasian male patients with T2DM. These men were randomized either to 15 g soy protein with 66 mg of isoflavones (SPI) per day or 15 g soy protein alone without any isoflavones (SP) per day for 3 months.

Studies of isoflavones intakes in Western countries indicate an average daily intake of approximately 2 mg isoflavones; vegetarians have a higher daily isoflavone intake of 16 mg and Asian populations consuming a high soy diet or people consuming soy supplements have a daily isoflavone intake of around 50 to 90 mg (9). The isoflavone concentrations used in this study reflected the daily intake of an Asian population consuming a high soy diet or people consuming soy supplements (10).

SP contained less than 300 parts per billion of isoflavones, achieved by serial alcohol washing (Dishman Ltd, India) and confirmed analytically by FERA (Sand Hutton, York, United Kingdom). Subjects were on stable medications for T2DM, hyperlipidemia, and hypertension for at least 3 months before the study. Subjects with significant hepatic or renal impairment, who were allergic to soy products, receiving a testosterone replacement, or who had received antibiotics 3 months before the study were excluded.

The primary outcome of this study was a change in testosterone levels. The secondary outcomes for this study were changes in glycemic control and cardiovascular risk markers, including insulin resistance, lipid profile, high-sensitivity C-reactive protein (hsCRP), and endothelial function.

At randomization and during study visits, 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 isoflavone levels to ensure adherence. Adherence with study preparation was calculated by counting the returned snack bars. All subjects gave their written informed consent. The study was given ethical approval by the Research Ethics Committee (East Yorkshire & North Lincolnshire Research Ethics Committee (reference 09/H1304/45).

Study product

A bar containing 7.5 g isolated soy protein powder (Solcon F; Solbar Industries, Ashdod, Israel) with 33 mg of isoflavones (Solgen 40, Solbar Industries) or 7.5 g of SP was consumed twice daily for 3 months. The soy protein and phytoestrogens were supplied by Solbar Industries from a single batch that was designated for the study. The trial product was packaged by Halo Food Ltd, Swindon, United Kingdom. Randomization based on a computer-generated randomization list was performed by Essential Nutrition Ltd, Brough, United Kingdom, who held the randomization codes.

Study measurements

At the beginning and end of the study, following an overnight fast, weight and blood pressure were measured and blood samples were collected. Blood pressure was measured after the subjects had been seated quietly for at least 5 minutes 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 minute apart and the average of the readings was taken. Waist circumference was measured using a specific abdominal circumference tape measure. The tape measure was wrapped around the participant's waist at the midway point between the bottom of the ribs and the top of the iliac crest. The participants were encouraged to breath naturally during the procedure, relax their abdominal muscles, and not hold their breath. Fasting venous blood samples were collected, separated by centrifugation at 2000*g* for 15 minutes at 4°C, and the aliquots stored at −80°C within 1 hour of collection. Plasma glucose was measured using a Synchron LX20 analyzer

(Beckman-Coulter, High Wycombe, United Kingdom), and serum insulin was assayed using a competitive chemiluminescent immunoassay performed using the DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, United Kingdom). 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 the homeostasis model of assessment-insulin resistance (HOMA-IR) (insulin $\times$ glucose)/22.5) (11).

Blood samples for testosterone levels were taken before 9 AM. Serum total testosterone was measured by isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS). Serum free testosterone was measured using equilibrium dialysis by adding a tracer amount of tritium-labeled testosterone to serum and then dialyzed against a buffer whose ionic composition was similar to that of serum. The percent of tracer that crossed the dialysis membrane at equilibrium was taken as percent free fraction. Absolute free testosterone was calculated by multiplying total testosterone with percent free testosterone.

Total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) levels were measured enzymatically using a Synchron LX20 analyzer (Beckman-Coulter). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. Serum hsCRP was measured by the high-sensitivity method on a Beckman DXC analyzer. All thyroid assays were performed on an Abbott Architect i4000 immunoassay analyzer (Abbott Diagnostics Division, Maidenhead, United Kingdom).

The phytoestrogens in serum were extracted and analyzed by LGC, Fordham, Cambridgeshire, United Kingdom, using isotope-dilution LC-MS/MS (12). LC-MS/MS was conducted using a Sciex 4000 Qtrap (Singapore) with separation achieved using a C18 column and mobile phases of water and acetonitrile, both containing acetic acid. No column switching was used. The calibration range for all analytes was 0.5 ng/mL to 200 ng/mL, with quality control samples prepared at low (1.5 ng/mL), medium (80 ng/mL), and high (150 ng/mL) concentrations, and analyzed to confirm the assay performance. Incurred quality control samples of serum were also run in the sample batches. The assay sensitivity for equol, daidzein, and genistein were all 0.5 ng/mL. The interassay coefficient of variations were <6.8% for daidzein, <6.1% for genistein, and <7.4% for equol. The intra-assay coefficient of variations were < 7.2% for daidzein (3.9% at the lower limit of quantification [LLOQ]), <3.6% for genistein (7.5% at the LLOQ), and <8.0% for equol (8.9% at the LLOQ).

Reactive hyperemia peripheral arterial tonometry to assess peripheral microvascular endothelial function was measured using an EndoPat 2000 (Itamar Ltd, Caesarea, Israel) according to the manufacturer's instructions. The reactive hyperemic index (RHI), which is a measure for endothelial function, and the augmentation index (AI), which is a measure for arterial stiffness, was also assessed using the EndoPat 2000 device. Both measures were calculated using a computerized automated algorithm (software version 3.1.2) provided with the device. The subjects were in the supine position for a minimum of 20 minutes before measurements, in a quiet, temperature-controlled (22°C) room with dimmed lights. They were asked to remain as still as possible and silent during the entire measurement period. Each recording consisted of 5 minutes of baseline measurement, 5 minutes of occlusion measurement, and 5 minutes postocclusion measurement (hyperemic period).

Occlusion of the brachial artery was performed on the nondominant upper arm. The occlusion pressure was at least mm Hg60 mm Hg above the systolic blood pressure (SBP). This technique determines functional endothelial change by measuring changes in digital pulse volume during reactive hyperemia in an operator-independent manner. RHI is the ratio of the average pulse wave amplitude measured over 60 seconds, starting 1 minute after cuff deflation, to the average pulse wave amplitude measured at the baseline. The other arm served as a control and the ratio was corrected for changes in the systemic vascular tone. The AI is an indirect measure of arterial stiffness and is calculated as augmentation pressure divided by pulse pressure $\times$100 to give a percentage.

Cardiovascular risk was estimated by using the UK Prospective Diabetes Study (UKPDS) Risk Engine, which is a T2DM-specific risk calculator based on 53,000 patients years of data (13).

Breast ultrasound was undertaken and assessed for each subject before and after the study by a consultant radiologist who was blinded to treatment; this was a safety measure to assess enlargement of breast tissue because excess estrogen can stimulate breast enlargement in males.

Statistical analysis

Baseline continuously distributed data are presented as median (25th/75th centiles); categorical data by n (%). Based on the variability of the testosterone (14), for a 1 nmol change in total testosterone, it was calculated that 150 subjects would be required to give 90% power and an alpha value <0.0l. Assuming a dropout rate of 33%, 200 patients were recruited for this study. Given randomization to treatment, baselines were not compared statistically (15–17). Within-group differences (difference between 12-week values and baseline values) are shown for each treatment group separately by a mean and a standard deviation (SD). Between-group comparisons were performed using the independent sample t test. The t test assumes equal variance between groups, which is reported for the 2 within-treatment groups separately. For all statistical analyses, a 2-tailed $P < 0.05$ was considered to indicate statistical significance. Bonferroni corrections were not applied (18). Statistical analysis was performed using the STATA statistical computer package (StataCorp, 2013release 13; College Station, TX).

Results

Two hundred patients with T2DM and low testosterone levels were recruited after screening 412 patients (Fig. 1). Adherence of completed patients by counting returned bars was 98%. Twenty-nine patients dropped out (15 patients in the SPI group and 14 patients in the SP group) because of gastrointestinal intolerance to the bars (19 patients [SPI, 10; SP, 9]), nonadherence (6 patients [SPI, 3; SP, 3]), the need to take antibiotics for concurrent illness (3 patients [SPI, 2; SP, 1]); and 1 patient (SP, 1) was started on testosterone replacement for his erectile dysfunction. The baseline anthropometric, hormonal, and biochemical parameters and isoflavone levels of the 2 groups are given in Table 1.
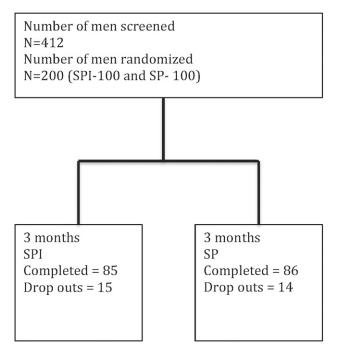
Number of men screened
N=412
Number of men randomized
N=200 (SPI-100 and SP- 100)

3 months
SPI
Completed = 85
Drop outs = 15

3 months
SP
Completed = 86
Drop outs = 14

Figure 1. Flow diagram of participants through the study.

There were no changes in weight, body mass index, or waist circumference with either SPI or SP supplementation. There was a substantial reduction of DBP after both SPI and SP supplementation, but there were no important differences between groups. There was no change in SBP in either group. There was a substantial increase (improvement) in RHI with SPI supplementation compared with SP supplementation alone (Table 2).

The UKPDS cardiovascular risk engine showed a significant 6% reduction in nonfatal and fatal coronary heart disease risk, a significant 9% reduction in fatal coronary heart disease risk, and suggested a significant reduction in fatal stroke risk with 3 months of SPI supplementation (Table 3).

There were substantial reductions in triglycerides and hsCRP with SPI compared with SP supplementation; however, there were no changes in total cholesterol, LDL-C, or HDL-C with SPI supplementation compared with SP supplementation (Table 2).

There were no changes in serum total testosterone, absolute free testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), or estradiol with either SPI or SP supplementation (Table 2). There was a substantial reduction in HbA1c, fasting glucose, fasting insulin, and HOMA-IR with SPI vs SP supplementation (Table 2).

There was a substantial increase in TSH with 3 months of SPI supplementation (mean [SD]), 1.81 [0.92] vs 3.23 [1.03] mU/L) compared with SP supplementation (1.82 [0.93] vs 1.96 [1.11] mU/L). There was also an important decrease in free thyroxine (fT4) with 3 months of SPI supplementation (12.68 [1.90] vs 11.09 [2.00] pmol/L)

compared with 3 months of SP supplementation (13.06 [1.74] vs 12.74 [1.62] pmol/L. However, there were no relevant changes in free tri-iodo thyronine with 3 months of either SPI or SP supplementation.

There was a substantial increase in TSH and a reduction in fT4 after SPI vs SP supplementation, but this did not reflect in any changes in free tri-iodo thyronine after either SPI or SP supplementation (Table 2).

There were important increases in daidzein, genistein, and equol with SPI supplementation confirming adherence, whereas no changes from baseline were seen with SP supplementation (Table 4).

Discussion

There were no substantial changes in testosterone measured by the gold standard techniques of MS/MS and absolute free testosterone measured by equilibrium dialysis, by SP with and without isoflavones after 3 months treatment. Previous studies on soy supplementation have shown conflicting results (19); however, the previous studies were not designed to investigate testosterone as the primary end point (19), and most used immunoassay (20) rather than gold standard testosterone measurements.

There was also no substantial change in sex hormone–binding globulin (SHBG) or albumin with either SPI or SP supplementation. Testosterone is bound to SHBG and albumin in circulation. There were also no changes in either FSH or LH after both preparations suggesting that there was no important alteration of hypothalamic-pituitary-gonadal axis with either preparation. There were also no substantial changes in estrogen levels in either group. Testosterone is converted into estrogen in various tissues, including adipose tissue by aromatase enzyme (21) and could potentially cause gynecomastia. There were no changes in volume of breast tissue in either group.

There was a substantial improvement in glycemic control as evidenced by a reduction in HbA1c after 3 months of SP and 66 mg isoflavone supplementation. There was also an important reduction of fasting glucose and insulin after SPI, which was reflected by a 65% reduction in HOMA-IR in this group of men with T2DM and low testosterone levels. In vitro studies have suggested several mechanisms for a direct pharmacological action of soy on glycemic control, including inhibiting intestinal brush border uptake of glucose, having α-glucosidase inhibitor actions and tyrosine kinase inhibitory actions, changes in insulin receptor numbers and affinity, intracellular phosphorylation, and alterations in glucose transport (22). Estrogen has been suggested to participate in glucose homeostasis by modulating the expression of genes that are involved in insulin sensitivity and glucose uptake (23). Further, estrogen is a major regulator of

Table 1.   Baseline Parameters Between the SPI and SP Groups

Parameters	SP1 Group (n = 100)	SP Group (n = 100)
Age, y	52.0 (50.0, 55.0)	52.0 (50.0, 55.0)
Weight	100.1 (88.5, 112.3)	98 (85.7, 111.9)
Body mass index, kg/m²	31.8 (28.8, 34.7)	31.6 (29.2, 35.0)
Duration of diabetes, y	7.3 (4.2, 8.8)	7.9 (4.4, 9.1)
HbA1c, mmol/mol	56 (52, 60)	58 (53, 64)
Total testosterone, nmol/L	10.0 (8.6, 11.0)	9.4 (7.9, 11.0)
% free testosterone	2.7 (2.2, 3.0)	2.6 (2.3, 3.2)
Absolute free testosterone, nmol/L	0.209 (0.183, 0.236)	0.202 (0.167, 0.238)
SHBG, nmol/L	29.0 (23.0, 40.0)	31.0 (21.3, 39.0)
TSH, mU/L	1.6 (1.2, 2.4)	1.6 (1.2, 2.5)
fT4, pmol/L	12.0 (12.0, 14.0)	13.0 (12.0, 14.0)
fT3, pmol/L	4.6 (4.2, 5.0)	4.6 (4.2, 4.9)
Fasting glucose, mmol/L	139.5 (118.8, 160.7)	135.9 (115.2, 154.4)
Fasting insulin, μIU/mL	16.5 (9.9, 25.3)	18.0 (10.4, 28.6)
HOMA-IR	5.6 (3.6, 9.0)	6.2 (3.8, 9.7)
hsCRP, mg/L	2.1 (0.8, 3.9)	1.9 (0.9, 4.1)
TC, mmol/L	3.9 (3.4, 4.6)	3.8 (3.4, 4.5)
LDL-C, mmol/L	2.0 (1.7, 2.9)	2.0 (1.6, 2.7)
HDL-C, mmol/L	1.1 (0.9, 1.3)	1.0 (0.9, 1.2)
Triglycerides, mmol/L	1.4 (1.0, 2.1)	1.3 (0.9, 2.0)
FSH, IU/L	5.7 (4.0, 9.4)	6.0 (4.8, 8.5)
LH, IU/L	3.7 (2.4, 5.6)	3.8 (3.0, 5.0)
Estradiol, pmol/L	89.0 (72.0, 110.0)	81.5 (69.0, 99.3)
Daidzein, ng/mL	1.4 (0.6, 2.7)	1.9 (0.7, 4.3)
Genistein, ng/mL	2.6 (0.7, 5.7)	2.9 (1.3, 7.2)
Equol, ng/mL	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)

Values are provided as medians (25th/75th centiles). 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.

Abbreviations: fT3, free tri-iodo thyronine; HbA1c, glycated hemoglobin; TC, total cholesterol; TG, triglycerides; TSH, thyroid-stimulating hormone.

adipocyte development and adipocyte number and inhibits lipogenesis by reducing the activity of lipoprotein lipase, an enzyme that regulates lipid uptake by adipocytes (24). Isoflavones may also affect glucose metabolism by non-estrogen receptor-mediated mechanisms. For example, isoflavones have been reported to have an antidiabetic effect through activation of PPARs, nuclear receptors that participate in cellular lipid homeostasis and insulin action (25).

There were no changes in weight after 3 months of either SPI or SP alone, indicating that insulin resistance decreased independently of a change in weight that has been suggested by others (26). This is supported by epidemiological data showing that, compared with Japanese in Tokyo on a traditional soy diet, Japanese-Americans have a higher prevalence of T2DM and insulin resistance despite similar body mass index levels (27).

There was an important reduction in DBP of ~2 mm Hg with both SPI and SP for 3 months. Hypertension, estimated to affect ~1 billion individuals worldwide, is a major risk factor for CVD. A 4 to 5 mm Hg reduction in SBP and a 2 to 3 mm Hg reduction in DBP can reduce CVD risk by 8% to 20% (28). Dietary soy isoflavones have been suggested to result in arterial vasodilatation, improvement in endothelial function, and decreased blood pressure, perhaps by a nitric oxide–dependent mechanism in animal experiments (29). A meta-analysis of 11 trials demonstrated that soy isoflavone intake resulted in a mean decrease of 2.5 mm Hg for SBP and 1.5 mm Hg for DBP compared with placebo (30), although there was a significant heterogeneity between the studies; however, the reduction of DBP here is in accord with those reports on a vasodilation mechanism. Such an antihypertensive effect has been suggested to be intrinsic to the amino acid composition of protein, especially arginine in soy, through multiple mechanisms perhaps accounting for the decrease in blood pressure that was independent of isoflavones treatment (31).

The UKPDS risk engine (13) that calculates coronary heart disease and stroke risk in patients with T2DM showed a substantial reduction in nonfatal and fatal coronary heart disease risk reduction of 6% and reduction of fatal coronary heart disease risk of 9% with 3 months of SPI supplementation. There was a statistically relevant reduction of fatal stroke risk with both SPI and SP supplementation, but there were no changes between the 2 groups. This is particularly important in patients with T2DM who are at in increased risk of heart disease and stroke.

Table 2.   Anthropometric, Metabolic, and Hormonal Parameters: Comparison Between SPI and SP Supplementation at End of Study

Parameter	SPI Group		SP Group			
	Paired Difference 3 Mo *vs* Baseline Mean (SD)	P Value	Paired Difference 3 Mo *vs* Baseline Mean (SD)	P Value	Difference of the Difference Between Groups (95% CI)	P Value
Weight, kg	0.60 (6.83)	0.39	0.39 (9.23)	0.30	0.21 (–2.1, 2.6)	0.85
Body mass index, kg/m^2	0.17 (2.03)	0.39	0.15 (3.32)	0.29	0.02 (–0.79, 0.83)	0.86
Waist, cm	0.68 (7.12)	0.36	−0.70 (5.42)	0.50	1.38 (–0.54, 3.32)	0.15
SBP, mm Hg	−2.32 (15.84)	0.09	−4.1 (15.05)	0.07	1.77 (–2.79, 6.34)	0.44
DBP, mm Hg	−2.47 (11.28)	0.03	−1.04 (11.46)	0.05	−1.42 (–4.79, 1.93)	0.40
RHI	0.30 (0.60)	<0.01	−0.11 (0.56)	<0.01	0.19 (0.02, 0.36)	0.02
AI	0.01 (0.14)	0.72	−0.05 (0.12)	0.07	0.05 (–0,01, 0.089)	0.09
HbA1c, mmol/mol	−4.19 (7.29)	0.01	1.63 (7.62)	0.06	−5.82 (8.09, –3.56)	<0.001
Fasting glucose, mmol/L	−1.44 (1.61)	<0.01	0.59 (2.02)	0.09	−2.03 (–2.58, –1.49)	<0.001
Fasting insulin, μIU/mL	−10.96 (13.51)	<0.01	−0.70 (25.78)	0.83	−10.25 (–16.36, –4.14)	0.001
HOMA-IR	−4.42 (5.73)	<0.01	0.78 (17.67)	0.69	−5.21 (–9.1, –1.32)	0.009
TC, mmol/L	−0.05 (0.60)	0.41	0.14 (0.55)	0.02	−0.19 (–0.36, –0.02)	0.08
LDL-C, mmol/L	−0.05 (0.49)	0.34	0.11 (0.47)	0.05	−0.16 (–0.31, –0.77)	0.09
HDL-C, mmol/L	−0.01 (0.13)	0.38	0.02 (0.14)	0.25	−0.03 (–0.07, 0.01)	0.14
Triglycerides, mmol/L	−0.78 (0.80)	<0.01	0.08 (0.82)	0.37	−0.86 (–1.1, –0.62)	<0.001
hsCRP, mg/L	−2.55 (4.35)	<0.01	−0.13 (3.92)	0.74	−2.41 (–3.65, –1.18)	<0.001
Total testosterone, nmol/L	0.05 (0.5)	0.30	0.11 (0.55)	0.06	−0.06 (–0.22, 0.09)	0.42
% free testosterone	−0.01 (0.44)	0.92	0.05 (0.37)	0.12	−0.07 (–0.2, 0.05)	0.22
Absolute free testosterone, nmol/L	0.02 (0.04)	0.81	0.02 (0.06)	0.08	0 (–0.01, 0.02)	0.80
SHBG, nmol/L	−0.26 (5.91)	0.67	−1.10 (4.46)	0.07	0.84 (–0.72, 2.40)	0.29
FSH, IU/L	0.26 (1.59)	0.12	0.09 (1.06)	0.56	0.19 (–0.21, 0.61)	0.34
LH, IU/L	0.34 (1.17)	0.07	0.17 (1.34)	0.26	0.17 (–0.20, 0.54)	0.37
Estradiol, pmol/L	2.15 (23.23)	0.39	0.36 (20.43)	0.80	1.79 (–4.80, 8.38)	0.59
TSH, mU/L	1.42 (0.83)	<0.01	0.16 (0.64)	0.06	1.26 (1.04, 1.49)	<0.001
fT4, pmol/L	−1.53 (2.69)	<0.01	−0.36 (1.98)	0.09	−1.16 (–1.89, –0.44)	0.002
fT3, pmol/L	0.05 (0.71)	0.54	−0.09 (1.05)	0.43	0.14 (–0.13, 0.42)	0.30

P values from independent *t* test. Within-pair SD in parentheses. To convert HbA1c from mmol/mol to %: (x/10.929) + 2.15; to convert HbA1c from % to mmol/mol: (y − 2.15) × 10.929; to convert glucose from mmol/L to mg/dL: (x * 18); to convert glucose from mg/dL to mmol/L: (y/18).

Abbreviations: CI, confidence interval; DHEAS, dehydroepiandrostenedione sulfate; fT3, free tri-iodo thyronine.

There was a substantial increase in the RHI with SPI supplementation compared with SP supplementation. Endothelial dysfunction is an early predictor of CVD (32) and can be measured using EndoPat, which is nonoperator-dependent. In the Framingham study, an important inverse relation was observed between endothelial function as determined by the EndoPat (RHI) and multiple cardiovascular risk factors (33). The RHI was reported to be significantly decreased in patients with coronary artery disease, hypertension, hyperlipidemia and diabetes (34), and several studies have demonstrated an improvement in endothelial function as a result of lifestyle modification including diet (35). In a small study, soy nuts were shown to have a modest effects on attenuating endothelial dysfunction over 4 weeks in adults with features of metabolic syndrome (36). In the current study on patients with T2DM, there was a substantial increase in RHI, suggesting potential favorable effects of SPI combination on endothelial function and cardiovascular risk.

There was an important reduction in triglycerides with SPI compared with the SP group. There were no substantial changes in total cholesterol, LDL-C, and HDL-C with SPI supplementation compared with SP supplementation. Isoflavones were shown to be hypolipidemic in both cynomolgus monkeys (37) and humans (38) in some, but not all, studies (39). Ingestion of ethanol extract rich in isoflavones increases the abundance of hepatic messenger RNA for cholesterol 7α-hydroxylase (40) and LDL receptors in rats and play important roles in cholesterol catabolism. A recent meta-analysis indicated that soy isoflavones significantly reduced total cholesterol and LDL-C, but do not change HDL-C (41). However, a positive association between isolated soy isoflavones and HDL-C has been documented in postmenopausal women (42). On the other hand, SP enhances the expression of the LDL receptor in hypercholesterolemic patients with T2DM (43) and in animal studies (44). Moreover, some previous studies have demonstrated that SP rather than isoflavones contributes to the lipid-lowering and hypocholesterolemic properties of soy (45). These discrepancies could be attributed to basal lipid profile or different study designs and treatment composition, such as the use of mixed isoflavones of poor purity, glucoside

Table 3.   10-Y Cardiovascular Risk Reduction Using UKPDS Risk Engine After 3 Months of SPI Supplementation and SP Alone Supplementation

	SPI			SP			SPI-SP Difference
Parameters	Pre *vs* Post SPI	% Change	*P* Value	Pre *vs* Post SP	% Change	*P* Value	*P* Value
Nonfatal and fatal CHD risk (%	21.14 ± 9.34 *vs* 19.86 ± 8.84	−6 ± 12	0.01	22.13 ± 7.65 *vs* 22.24 ± 7.86	0.02 ± 0.19	0.82	0.04
Fatal CHD risk, %)	14.07 ± 7.18 *vs* 12.86 ± 6.95	−9 ± 0.11	<0.01	16.82 ± 17.27 *vs* 16.89 ± 18.22	0.02 ± 0.22	0.86	0.03
Nonfatal and fatal stroke risk, %	10.02 ± 5.67 *vs* 9.84 ± 5.65	−2 ± 0.11	0.13	10.53 *vs* 4.35 *vs* 10.27 *vs* 4.14	−0.01 ± 0.10	0.07	0.98
Fatal stroke risk, %	1.60 ± 1.12 *vs* 1.51 *vs* 1.04 ±	−0.06 ± 0.10	0.04	1.69 ± 0.88 *vs* 1.55 ± 0.77	−0.04 ± 0.23	0.05	0.76

Abbreviation: CHD, coronary heart disease.

instead of aglycone forms, and the concomitant presence of proteins.

There was a substantial reduction of hsCRP that is an important cardiovascular risk marker with 3 months of combined SPI preparation compared with SP supplementation. The effect of soy on CRP is variable. SPI supplementation significantly improved CRP in postmenopausal women with T2DM (2), whereas isoflavone supplementation alone was ineffective (39), suggesting a potential matrix effect (*i.e.*, the combination of both soy protein and isoflavones rather than either alone) between SP and isoflavones on CRP.

There was a substantial increase in TSH and reduction in fT4 with 3 months of combined SPI supplementation. Soy consumption is associated with thyroid disorders such as hypothyroidism, goiter, and autoimmune thyroid disease (46). *In vitro* studies have demonstrated that isoflavones inhibit thyroid peroxidase, an enzyme involved in the synthesis of T_3 and T_4 (47). In a study examining the effects of soy isoflavones, Fischer rat thyroid cells (FRTLs) were treated with a combination of SPI; it was found that there was a dose-dependent suppression of iodide uptake in FRTLs, whereas isoflavone alone was ineffective. SPI combination and isoflavone (genistein) alone increased the 40-kDa thyroglobulin fragment (a known autoimmunogen) and nonglycosylated sodium/iodide symporter in the FRTLs that might contribute to the higher incidence of thyroid dysfunction (48). In patients with subclinical hypothyroidism, SPI combination has shown to increase the risk of developing overt

hypothyroidism (49). In women in early menopause, SPI supplementation has been shown to significantly increase TSH and reduce fT4, suggesting a detrimental effect on thyroid function (50). In the current study, none of the patients developed subclinical or overt hypothyroidism; however, all patients had normal thyroid function tests at baseline.

The dropout rate during the intervention was less than expected (14.5%), with the most frequent reason for participant withdrawal in both groups attributable to palatability; however, these numbers are comparable to other nutrition trials in patients with T2DM (51). There was a substantial rise in plasma isoflavone levels with SPI, confirming adherence, whereas plasma isoflavone levels did not differ from baseline in the SP group, confirming that they had not taken any exogenous isoflavones during the study.

In conclusion, SP with and without 66 mg isoflavone per day for 3 months did not have an effect on testosterone levels in men with T2DM, confirming its safety. In addition, there were substantial improvements in both glycemic control and cardiovascular risk markers, including DBP, triglycerides, and hsCRP. These were reflected in a significant improvement in the calculated coronary heart disease risk with SPI supplementation, whereas there were no changes seen with SP supplementation. However, there was an important increase in TSH with a reduction in free T4 after high-dose isoflavone in combination with SP supplementation, suggesting a potential adverse effect of soy on the thyroid.

Table 4.   Comparison of Plasma Phytoestrogen Levels With SPI and SP Supplementation

	SPI Group		SP Group			
Parameter	Paired Difference 3 Mo *vs* Baseline Mean (SD)	*P* Value	Paired Difference 3 Mo *vs* Baseline Mean (SD)	*P* Value	Difference of the Difference Between Groups (95% CI)	*P* Value
Daidzein, ng/mL	6.61 (7.24)	<0.001	0.91 (3.28)	0.34	7.24 (4.48, 17.70)	<0.001
Genistein, ng/mL	13.19 (9.77)	<0.001	0.90 (3.59)	0.26	14.58 (8.11, 27.66)	<0.001
Equol, ng/mL	1.69 (3.52)	<0.001	1.03 (1.25)	0.20	1.65 (1.26, 2.15)	<0.001

Daidzein, genistein, and equol are natural log-transformed.

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