

Soy reduces bone turnover markers in women during early menopause – a randomized controlled trial

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Disclosure summary

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Abstract

Menopausal estrogen loss leads to an increased bone loss. Soy isoflavones can act as selective estrogen receptor modulators, their role in bone turnover is unclear. The primary outcome was assessing changes in plasma bone turnover markers. The secondary outcomes were assessing changes in cardiovascular risk markers including insulin resistance, blood pressure and lipid profile. We performed a double blind randomised parallel study where 200 women within 2 years after the onset of their menopause were randomised to 15 g soy protein with 66mg isoflavone (SPI) or 15 g soy protein alone (SP), daily for 6 months.

There was a significant reduction in type I collagen crosslinked Beta C-telopeptide (β CTX) (bone-resorption marker) with SPI supplementation (0.40 ± 0.17 vs. $0.15 \pm 0.09\mu\text{g/L}$; $p<0.01$) compared to SP supplementation (0.35 ± 0.12 vs. $0.35 \pm 0.13\mu\text{g/L}$; $p=0.92$) after 6 months. There was also a significant reduction in type I procollagen-N-propeptide (P1NP) (bone-formation marker) with SPI supplementation (50.5 ± 25.0 vs. $34.3 \pm 17.6\mu\text{g/L}$; $p<0.01$), more marked between 3 and 6 month.

Following SPI there was a significant reduction in fasting glucose, fasting insulin, insulin resistance and systolic blood pressure whereas no significant changes in these parameters was observed with SP. There were no significant changes in fasting lipid profile and diastolic blood pressure with either preparation. There was a significant increase in TSH and reduction in free thyroxine ($p<0.01$) with SPI supplementation though free tri-iodothyronine was unchanged.

In conclusion, soy protein with isoflavones may confer a beneficial effect on bone health, analogous to the mode of action of anti-resorptive agents albeit to a less magnitude. There was a significant improvement of cardiovascular risk markers, but a

significant increase in TSH and reduction in free thyroxine after SPI supplementation indicating a detrimental effect on thyroid function.

Background

Postmenopausal estrogen loss leads to osteoporosis that is a skeletal disorder characterized by compromised bone strength, resulting in nearly 9 million fractures annually worldwide.(1) The annual rates and cumulative amounts of bone mineral density (BMD) loss is greater one year prior to its onset and through two years after the final menstrual period (transmenopause) than those occurring between two and five years after the final menstrual period (postmenopause).(2)

Epidemiological studies generally suggest a positive association between soy consumption and BMD,(3) but it is not known if this is due to the soy phytoestrogens that have effects similar to estrogen, due to the soy protein alone, or requires a combination of both. Phytoestrogens are a broad group of compounds that consist of isoflavones, lignans and coumestans. Of these, isoflavones are heterocyclic phenols the main constituents of which are genistein, daidzein and glycitein. They have a similar structure to 17 beta estradiol and have been shown to have biological activity exerting estrogen-like effects both in vitro and in vivo. Estrogen increases BMD and has a protective effect on bones in females.

Previous studies evaluating the effects of soy isoflavones on bone health have shown conflicting results likely due to differences in the soy preparations and the comparators used, the study population chosen and the use of bone densitometry as an outcome measure that is unable to detect early or small treatment effects. Bone turnover markers (BTMs) are biomarkers for fracture risk that have been used for the diagnosis and evaluation of therapy effects on osteoporosis, and include both bone resorption markers (e.g. type I collagen crosslinked Beta C-telopeptide (β CTX)) and bone formation markers (e.g. type I procollagen-N-propeptide (PINP)).

It was hypothesised by us that any soy effect would be apparent within the first two

years of the menopause when there was maximal bone loss, and therefore this randomised, double blind, parallel study was undertaken during this timeframe in women within two years of their last menstrual period using the BTM outcome measures of β CTX and PINP. BTMs rather than BMD were utilized as it has previously been shown that it can take two years before conventional anti-resorptive treatment leads to a change in BMD sufficient to exceed the precision error of the test or least significant change.(4)

Materials and methods

Two hundred Caucasian women within two years of the onset of their menopause (FSH greater than 20 mU/L and amenorrhoea for one year) were recruited after screening 334 women who responded to newspaper advertisements. Subjects were taking either no medication or on stable medications for at least three months and did not smoke. Women who were taking medication that could interfere with bone metabolism including steroids, bisphosphonates, thyroxine or hormone replacement therapy were excluded. Women with significant hepatic or renal impairment, who were allergic to soy products and those who had antibiotic exposure in the three months prior to the study were also excluded.

They were randomised into groups and either administered SPI (15 g soy protein with 66 mg of isoflavones) (100 women) or SP (15 g soy protein alone, isoflavone free) (100 women) daily for a period of six months.

The primary outcome of this study was assessing any change in plasma β CTX and PINP. The secondary outcome for this study was assessing any change in cardiovascular risk markers including insulin resistance, lipids, and hsCRP since in some studies soy has been shown to have a beneficial effect on these markers. There

is some evidence to show that soy could have an effect on thyroid function hence changes in thyroid function tests were assessed although was not planned apriori.

During study visits (baseline, three months and six months), subjects were instructed to maintain their normal 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 serum isoflavone concentrations to ensure compliance. There was telephone contact by study personnel, six and 18 weeks after study visits to ensure compliance. Analysis of compliance with the study preparation was undertaken by counting the returned sachets. All subjects gave their written informed consent for this study that had been approved by the Research Ethics Committee (East Yorkshire & North Lincolnshire Research Ethics Committee, ref: 09/1304/45).

Study product

A snack bar either containing 7.5 g isolated soy protein powder (Solcon F, Solbar Industries, Israel) with 33 mg of isoflavones (SPI) (Sogen 40, Solbar Industries, Israel) given twice daily between meals (15 g soy protein and 66 mg of isoflavones per day), or 7.5 g of the isolated soy protein alone given twice daily (15 g soy protein per day without isoflavones per day) as control (isoflavone of less than 300 parts per billion following serial alcohol extraction, Dishman Ltd, India; assayed by FERA, Sand Hutton, UK) (SP). The snack bars were to be eaten twice daily between meals for 6 months. The soy protein and the isoflavones were from a single batch that was designated for the study. The study bars were specifically prepared and packaged by Halo foods, Swindon, UK. Soy bars were identical and had similar macronutrient content.

Randomisation

The randomisation was performed by Essential Nutrition Ltd, UK. A computer generated randomisation sequence was used to provide balanced blocks of patient numbers for each of the two treatment groups. A one-to-one treatment allocation was used. Blinding was performed at manufacture and the randomisation code was held by Essential Nutrition Ltd. All investigators, study staff, and subjects were blinded to the treatment assignment throughout the entire study. Empty wrappers and uneaten bars were returned and counted by the study team. If compliance was less than 75% then the subject was withdrawn from the study.

Study measurements

During the baseline, three months and six month study visits, following an over-night fast, anthropometric parameters were measured and blood samples collected. Blood pressure was measured after the patients had been seated quietly for at least five 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 one minute apart and the average of the readings was taken. Fasting venous blood samples were collected, separated by centrifugation at 2000 g for 15 min at 4°C, and the aliquots stored at -80°C within one hour of collection. Plasma glucose was measured using a Synchron DxC analyzer (Beckman-Coulter, UK), and serum insulin was assayed using an ultra-sensitive chemiluminescent one-step immunoenzymatic ‘sandwich’ assay performed on a Unicel DXi Immunoassay system (Beckman-Coulter, UK). The coefficient of variation (CV) 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 x glucose)/22.5)

(5). HOMA is a validated surrogate marker for IR that correlates closely with the gold standard hyperinsulinaemic euglycaemic clamp (5). Total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) levels were measured enzymatically using a Synchron DxC analyzer (Beckman-Coulter, UK). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. Analytical performance in terms of imprecision at discreet levels (%CV, Mean) was total cholesterol (0.7%, 4.9mmol/L), HDL cholesterol (1.6%, 1.9mmol/L) and triglyceride (3.3%, 1.6mmol/L). Serum CRP was measured by the high-sensitivity method on a Beckman DXC analyzer. Analytical performance in terms of imprecision at discreet levels (%CV, Mean) was (0.9%, 9mg/L). All thyroid assays were performed on an Abbott Architect i4000 immunoassay analyser (Abbott Diagnostics Division, UK). Analytical performance in terms of imprecision at discreet levels (%CV, Mean) was free thyroxine (fT4) was (4%, 13.7pmol/L) and that of thyroid stimulating hormone (TSH) was (4.2%, 1.32 pmol/L).

Plasma β CTX was measured using an electrochemiluminescent immunoassay (ECLIA) on a Cobas 1600i analyser (Roche Diagnostics, Lewes, UK). Inter/ Intra assay coefficient of variation of plasma β CTX was <3% between 0.2 and 1.5 μ g/L. The assay sensitivity (replicates of the zero standard) was 0.01 μ g/L. Plasma P1NP was measured using an ECLIA on a Cobas 1600i analyser (Roche Diagnostics, Lewes, UK). Inter/ Intra assay CV of plasma P1NP was <3% between 20-600 μ g/L. The assay sensitivity (replicates of the zero standards) was 8 μ g/L.

The isoflavones in serum were extracted and analysed by Quotient Biosciences, UK using isotope-dilution LC-MS/MS (6). LC-MS/MS was conducted using a Sciex 4000 Qtrap 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 analysed to confirm the assay performance. Incurred QC 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 inter assay CVs were less than 6.8% for daidzein, less than 6.1% for genistein and less than 7.4% for equol.

Sample size calculation

The study was powered for changes in β CTX (SD=0.20 μ g/L, medium effect size=0.67, minimum detectable difference of 0.13 μ g/L) and P1NP (SD=115 μ g/L, minimum detectable difference=76 μ g/L) based on the study of Charatcharoenwitthaya et al (7) using N-query software, for 95% power, 5% significance (2-tailed), 100 women were required per group including a 40% dropout rate.

Statistical analysis

Continuous data was summarised by the median (25th/75th centiles) and categorical data by percentages (%). Given this was a randomised controlled trial, baselines were not compared statistically. Box and whisker plots show data distributions. The line in the middle of the box is the median: the bottom and top of the box are the 25th and 75th centiles respectively, while the dots are observations that lie outside the quartiles. For each group (SPI and SP) separately a paired difference (six-months minus baseline) of means was calculated. These two statistics are summarised as a paired mean across all patients in tables. The two paired means were then compared using an independent t-test the p-value is the probability of the difference of the difference being a false positive. This is referred to the tables as the 'difference of the difference' and 95% confidence interval gives the precision of the difference of the difference in

the tables. The Stata statistical computer package was used to analyse the data (StataCorp. *Stata Statistical Software. Release 13*. College Station, Texas, 2013).

Results

Two hundred Caucasian women within two years of their menopause (FSH greater than 20 mU/L and amenorrhoea for one year) were recruited after screening 334 women who responded to newspaper advertisements. In total 60 women in the SPI group and 60 women in the SP group completed six months of the study, with an overall dropout rate of 39% (Figure 1). Their characteristics and reasons for drop outs are described in Table 1.

The baseline anthropometric, metabolic, plasma isoflavone levels and BTMs were comparable between the two groups (Table 2).

The mean β CTX was significantly lower after 6 months of SPI supplementation compared to SP supplementation ($p < 0.01$: Figure 2a). There was also a significant reduction of P1NP with SPI supplementation for 6 months compared to SP supplementation ($p < 0.01$: Table 2) and was more pronounced between 3 and 6 months (Figure 2b).

There was a significant reduction in the mean metabolic parameters including fasting glucose, fasting insulin and HOMA-IR in the SPI group compared to the SP group after six months (Table 3). There were no significant changes observed in lipid parameters (total cholesterol, LDL-C, HDL-C and triglycerides) and hsCRP between the SPI and SP administered groups. There was a significant reduction in systolic blood pressure with six months of SPI supplementation but no change with SP supplementation. There were no changes in diastolic blood pressure after either SPI or SP supplementation. (Table 3)

There was a significant increase in mean TSH (mean (SD) (1.58 (0.93) vs. 2.61 (1.24) mU/L, $p < 0.01$) and corresponding significant reduction in mean fT4 (13.5 (2.2) vs. 11.2 (1.8) pmol/L, $p < 0.01$) from baseline to three months. There were no significant changes in TSH and fT4 between three and six months. There were also no significant changes in the fT3 between both preparations (Table 3).

There was a significant increase in mean daidzein, genistein and equol in the SPI administered group compared to the SP group (Table 3).

There were no significant changes in endometrial thickness before and after either SPI or SP supplementation.

Discussion

This study showed that soy with isoflavones had a significant beneficial effect on both BTMs and on cardiovascular risk factors including systolic blood pressure, fasting glucose, fasting insulin and insulin resistance compared to isoflavone-free soy protein in women whose bone loss was maximal in the first 2 years of their menopause. There was also a detrimental effect on thyroid function with a decrease in serum T4 and an increase in serum TSH with a combination of soy protein and isoflavones in this group of women. This study is the first to use a soy preparation that was truly isoflavone free that could determine the contribution to any effect by solely the soy protein. There were no significant treatment effects attributed to soy protein alone at either at 3 or 6 months compared to baseline, suggesting that the soy protein alone is inactive on these parameters.

An initial effect on osteoclast function as seen by the changes in P1NP followed by decreased osteoblast function, as seen by the changes in β CTX, found in the current study suggests isoflavones may have beneficial effects on bone health. There is an early phase of predominantly trabecular bone loss following menopause associated

with the decrease in oestrogen (8). The annual rates and cumulative amounts of bone mineral density loss is greater one year prior through two years after the final menstrual period (transmenopause) than those occurring between two and five years after the final menstrual period (postmenopause) (2). The rapid bone loss during early postmenopausal years is associated with marked increases in biochemical markers of bone resorption and due to the coupling of bone resorption and bone formation, bone formation also increases over time (9, 10). The effect of hormone replacement therapy on postmenopausal women is mainly a reduction in bone resorption and a secondary decrease in bone formation, reflecting a decrease in the activation frequency of new bone-remodelling units (11, 12). Soy isoflavones have a chemical structure similar to oestrogen, bind to and transactivate estrogen receptors (ER) (13-15) and exert oestrogen-like effects both in vitro and in vivo (16). The significant early reduction in bone resorption markers followed by later reduction in bone formation markers in the current study suggests the mechanism of soy protein and isoflavones in bone could be similar to oestrogen replacement therapy. A similar pattern of initial reduction in bone resorption markers followed by bone formation markers is also seen with the potent anti-resorptive agents bisphosphonates and denosumab, although to a lesser magnitude.(17)

Very few randomised controlled trials on soy isoflavones effects on bone have contained data for β CTX and P1NP, and those reported have generally not detected significant effects of soy isoflavone supplements on these bone turn over markers.(18)

The heterogeneity of trial results are likely to be a combination of underpowered clinical trials and differences in habitual dietary intake of soy isoflavones,(19) the variable interval since the onset of menopause(20) as well as the chemical forms and proportions of individual soy isoflavones.(21) The current study sought to minimize

these variables by investigating a homogeneous large study population of women who were likely to be at maximal bone turnover by being within the first two years of their menopause. Bone densitometry was not performed as it is not able to detect early or small treatment effects with soy isoflavones over such a short period since the margin of error with bone densitometry is high and would require larger numbers to pick up significant changes and a longer duration of treatment.

There was a significant improvement in glucose metabolism with a reduction in both insulin and glucose after combined soy protein and isoflavone supplementation. These data are in accord with the reported pharmacological action of soy on glycaemic control in diabetes, including the inhibition glucose uptake at the intestinal brush border through a α -glucosidase inhibitor action, tyrosine kinase inhibitory actions, changes in insulin receptor numbers and affinity, intracellular phosphorylation, and alterations in glucose transport.(22) The effect on insulin may be mediated by an estrogenic effect on insulin sensitivity and glucose uptake and through activation of the nuclear peroxisome Proliferator-Activated Receptors (PPAR) that may modulate insulin action;(23) PPAR agonists have also been shown to modulate osteoclast function that may have contributed to the BTM changes reported.(24) In addition, soy has been shown to inhibit insulin secretion from pancreatic β cells.(25)

This study is the first to use a control soy preparation that was truly isoflavone free and as it showed no treatment effects it suggests soy protein alone is inert in relation to bone health. There were no changes in body weight after 24 weeks of soy, suggesting that the reduction in insulin resistance was independent of the action of soy fiber that might enhance satiation and hence induce weight loss.

There were no changes in any of the lipid parameters by the soy preparations, a finding that was discrepant to some(26) but not in all studies.(25) A recent meta-

analysis suggested that soy isoflavones significantly reduced total cholesterol and LDL-C but do not change HDL-C.(27) These differences may be attributed to the basal lipid profile or different study designs and isoflavones composition with glucoside instead of aglycone forms. There was a significant reduction in systolic blood pressure within six months of SPI supplementation but no change with SP supplementation. There were no changes in diastolic blood pressure with either after SPI or SP supplementation.

There was a significant increase in TSH and reduction in fT4 with 12 weeks of combined soy protein and isoflavone supplementation where as no significant changes between three and six months. This suggests that the maximal effect of SPI was in the first three months, which then plateaued, rather than a continual reduction. There were no significant changes in TSH and fT4 with SP supplementation. These findings are in accord with an observational cohort showing high consumption of soya was associated with elevated TSH concentration in women.(28) Soy consumption is associated with thyroid disorders such as hypothyroidism, goitre, and autoimmune thyroid disease.(29) *In vitro* studies have demonstrated that isoflavones inhibit thyroid peroxidase (TPO), and inhibit iodide.(30) However, other studies using different preparations for up to 3 years have not shown changes in thyroid hormones.(31) In patients with subclinical hypothyroidism, the soy protein and isoflavone combination has been shown to increase the risk of developing overt hypothyroidism.(32) These data suggests that a synergistic matrix of soy protein and isoflavones is needed to maximise the effects rather than either soy protein or isoflavone alone.(25, 33) In the current study, where all patients had normal thyroid function tests at baseline, none of the patients developed subclinical or overt hypothyroidism. There was also no significant change in fT3 with either SPI or SP supplementation. The statistically

significant differences in the concentrations of most markers between zero and six months were present within the first three months of the study with the exception of PINP where the change was only apparent at six 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 population consuming high soy diet or people consuming soy supplements have a daily isoflavone intake of around 50-90 mg.(34) The isoflavone concentrations used in this study reflected the daily intake of an Asian population consuming high soy diet or people consuming soy supplements.

As anticipated the drop out was around 40% so that the power of study was not compromised. The most frequent reasons for participant withdrawal in both groups are attributable to non-compliance due to palatability, comparable to other nutrition and medication trials in patients with osteoporosis.(35) The group who dropped out was not significantly different from the group who completed the study. There was a significant increase in plasma isoflavone concentrations with SPI confirming compliance and isoflavone absorption, whilst there was no change from baseline in plasma isoflavones in the SP excluding exogenous isoflavones ingestion.

In conclusion, this study suggests the consumption of soy with isoflavones may have a beneficial effect on bone health where there was a significant decrease in bone turnover markers of resorption and formation after supplementation with 15 g soy protein with isoflavones for six months. An initial decrease on osteoclast function followed by a decrease in osteoblast function appears to be the mechanism of SPI action though it is unclear if this translates to a clinical benefit. There was no significant change in BTMs with 15 g soy protein alone over six months.

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Table 1
Reasons for withdrawal in each group

	SPI group baseline to 3 months (n=19)	SPI group 3 to 6 months (n=21)	SP group 3 to 6 months (n=25)	SP group 3 to 6 months (n=15)
Age median (25 th /75 th centiles) years	52 (50,55)	52 (50,54)	53 (50,55)	53 (50,55)
Body mass index (kg/m ²)	25.9 (23.9, 30.9)	25.8 (24.2, 31.2)	26.5 (24.9, 31.0)	26.6 (24.3, 31.2)
Reasons for withdrawal				
Lost to follow up	4	10	7	8
Non- compliance	13		11	
Post menopausal bleeding	1		1	
Intolerance to soy bar	1	8	3	5
Antibiotic use		2	3	1
Started hormone replacement therapy		1		1

SPI (15g soy protein with 66mg of isoflavones); SP (15g soy protein alone isoflavone free)

Table 2. Baseline anthropometric, hormonal and biochemical measurements between the soy protein with (SPI) and without (SP) isoflavones.

Parameters (units) (normal range)	SPI (n=100)	SP (n=100)
Age (years)	52 (49, 56)	52 (50, 55)
Body mass index (kg/m ²)	26.3 (24.3, 30.7)	24.6 (22.7, 28.4)
Systolic blood pressure (mmHg)	121 (110, 137)	128 (113, 141)
Diastolic blood pressure (mmHg)	77 (69, 88)	79 (72, 83)
TSH (mU/L) (0.35 - 4.7)	1.5 (0.9, 2.2)	1.6 (0.9, 2.3)
fT4 (pmol/L) (7.8 – 21)	13 (13, 15)	13 (12, 14)
fT3 (pmol/L) (3.8 – 6)	4.6 (4.3, 5.1)	4.7 (4.3, 4.9)
^a Fasting glucose (mg/dL)	90 (86.4, 99.0)	86.4 (82.8, 93.6)
^b Fasting insulin (μIU/mL)	4.6 (3.4, 6.7)	4.4 (3.2, 7.4)
HOMA-IR	1.0 (0.7, 1.5)	0.9 (0.7, 1.6)
hs CRP (mg/L) (0 – 8)	1.3 (0.6, 2.2)	1.3 (0.9, 2.7)
^c TC (mmol/L)	5.98 (5.38, 6.54)	5.66 (4.98, 6.37)
LDL-C (mmol/L)	3.3 (2.9, 3.9)	3.3 (2.7, 3.9)
HDL-C (mmol/L)	1.66 (1.45, 1.88)	1.70 (1.46, 2.10)
^d Triglycerides (mmol/L)	1.08 (0.85, 1.36)	1.08 (0.84, 1.33)
βCTX (μg/L) (0.1 – 0.5)	0.38 (0.28, 0.48)	0.34 (0.25, 0.46)
P1NP (μg/L) (19-69)	49.4 (34.7, 57.7)	41.4 (31.5, 50)
Daidzein (ng/mL)	0.73 (0.49, 2.37)	0.82 (0.49, 2.65)
Genistin (ng/mL)	1.43 (0.56, 4.2)	1.66 (0.71, 6.98)
Equol (ng/mL)	0.49 (0.47, 0.51)	0.49 (0.46, 0.54)
FSH (IU/L)	77 (57, 97)	71 (49, 89)
LH (IU/L)	32 (25, 42)	29 (27, 38)

SPI (15 g soy protein with 66 mg of isoflavones); SP (15 g soy protein alone isoflavone free)

Data given as Mean (SEM)

^aTo convert values for glucose to milligrams per deciliter, divide by 0.056.

^bTo convert values for insulin to picomoles per liter, multiply by 6.

^cTo convert values for cholesterol to milligrams per deciliter, divide by 0.0259.

^dTo convert values for triglycerides to milligrams per deciliter, divide by 0.0113.

TC - Total cholesterol; LDL-C - LDL-cholesterol; HDL-C - HDL cholesterol; TG-Triglycerides

HOMA-IR – Homeostasis model of assessment – insulin resistance

hs CRP – highly sensitive C-reactive protein

TSH – thyroid stimulating hormone

fT4 – free thyroxine

fT3 – free tri-iodo thyronine

βCTX - collagen type 1 cross-linked Beta C-telopeptide

P1NP - propeptide of type I collagen, FSH – follicle stimulating hormone, LH – Luteinising hormone

Table 3. Comparison between SPI and SP supplementation at end of study of metabolic and hormonal factors.

Parameter	SPI			SP			Difference of the difference (95% CI)	p-value
	Baseline Mean (SD)	6 months Mean (SD)	Difference (6 mo – baseline)	Baseline Mean (SD)	6 months Mean (SD)	Difference (6 mo – baseline)		
Anthropometric parameters								
Body mass index (kg/m ²)	27 (4.6)	27.3 (4.4)	0.37	26.7 (7.0)	27 (6.9)	0.15	0.2 (-0.09,0.53)	0.17
Systolic blood pressure (mmHg)	125 (20.2)	121.2 (14.9)	-3.2	124.6 (18.8)	123.4 (16)	-0.8	-2.5 (-4.2,-1.9)	<0.01
Diastolic blood pressure (mmHg)	77 (13.8)	76.8 (9.4)	-0.6	77.2 (10.9)	77.4 (11.6)	0.2	-0.8 (-5.2,3.4)	0.68
Lipid parameters								
TC (mmol/L)	5.8 (0.9)	5.8 (0.9)	0	5.8 (0.8)	5.7 (0.8)	-0.15	0.2 (-0.07,0.47)	0.15
LDL-C (mmol/L)	3.65 (0.7)	3.6 (0.6)	-0.15	3.65 (0.9)	3.57 (0.75)	-0.10	-0.16 (-0.65,0.72)	0.47
HDL-C (mmol/L)	1.68 (0.94)	1.62 (0.36)	-0.05	1.78 (0.42)	1.65 (0.39)	-0.23	-0.37 (-1.28,0.52)	0.39
Triglycerides (mmol/L)	1.16 (0.54)	1.22 (0.71)	0.09	1.18 (0.57)	1.27 (0.91)	0.09	-0.12 (-0.31,0.06)	0.20
hs CRP (mg/L)	1.65 (1.55)	0.69 (0.92)	-0.96	2.65 (4.49)	2.1 (2.27)	-0.5	-0.46 (-1.6,0.58)	0.38
Glycemic parameters								
Fasting glucose (mmol/L)	5.2 (0.7)	4.4 (0.5)	-0.7	5.1 (1.6)	5.0 (0.9)	-0.10	-0.7 (-1,-0.4)	<0.01
Fasting insulin (μIU/mL)	5.78 (3.59)	2.64 (1.89)	-3.1	5.65 (3.74)	5.82 (3.7)	0.11	-3.25 (-4.-2.43)	<0.01
HOMA-IR	1.39 (1.03)	0.52 (0.4)	-0.86	1.43 (1.77)	1.37 (1.42)	-0.04	-0.82 (-1.07,-0.56)	<0.01

Bone turn over markers								
βCTX (μg/L)	0.40 (0.17)	0.15 (0.09)	-0.25	0.35 (0.12)	0.35 (0.13)	0.01	-0.26 (-0.31,-0.21)	<0.01
P1NP (μg/L)	50.5 (25)	34.3 (17.6)	-16.4	43.3 (15.9)	45.7 (17.7)	2.3	-18.8 (-25.2,-1.2)	<0.01
Thyroid function tests								
TSH (mU/L)	1.58 (0.93)	2.62 (1.26)	1	1.80 (1)	1.80 (1.15)	0	1 (0.6,1.3)	<0.01
fT4 (pmol/L)	13.5 (2.2)	11.5 (1.4)	-2.2	13.5 (1.8)	12.6 (2.4)	-0.8	-1.4 (-2.1,-0.6)	<0.01
fT3 (pmol/L)	4.9 (1.8)	5 (2.5)	0.10	4.6 (0.5)	4.5 (0.5)	-0.11	0.12 (-0.25,0.51)	0.5
Plasma isoflavone levels								
Daidzein (ng/mL)	4.49(18.2)	17.8(23.7)	13.3	5.4(19.8)	2.8(6.1)	-2.6	15.(6.3,25.6)	<0.01
Genistein (ng/mL)	9.2(32.4)	91.2(107.8)	82	10.1(30)	5.4(12.2)	-4.7	86.7(56.6,116.8)	<0.01
Equol (ng/mL)	0.54(0.36)	5.85(11.9)	5.3	0.85(1.69)	0.75(1.29)	-0.1	5.4(2.3,8.4)	<0.01

Paired difference=6-months-baseline. Difference of the difference is an unpaired t-test of the paired differences.

SPI (15g soy protein with 66mg of isoflavones); SP (15g soy protein alone isoflavone free)

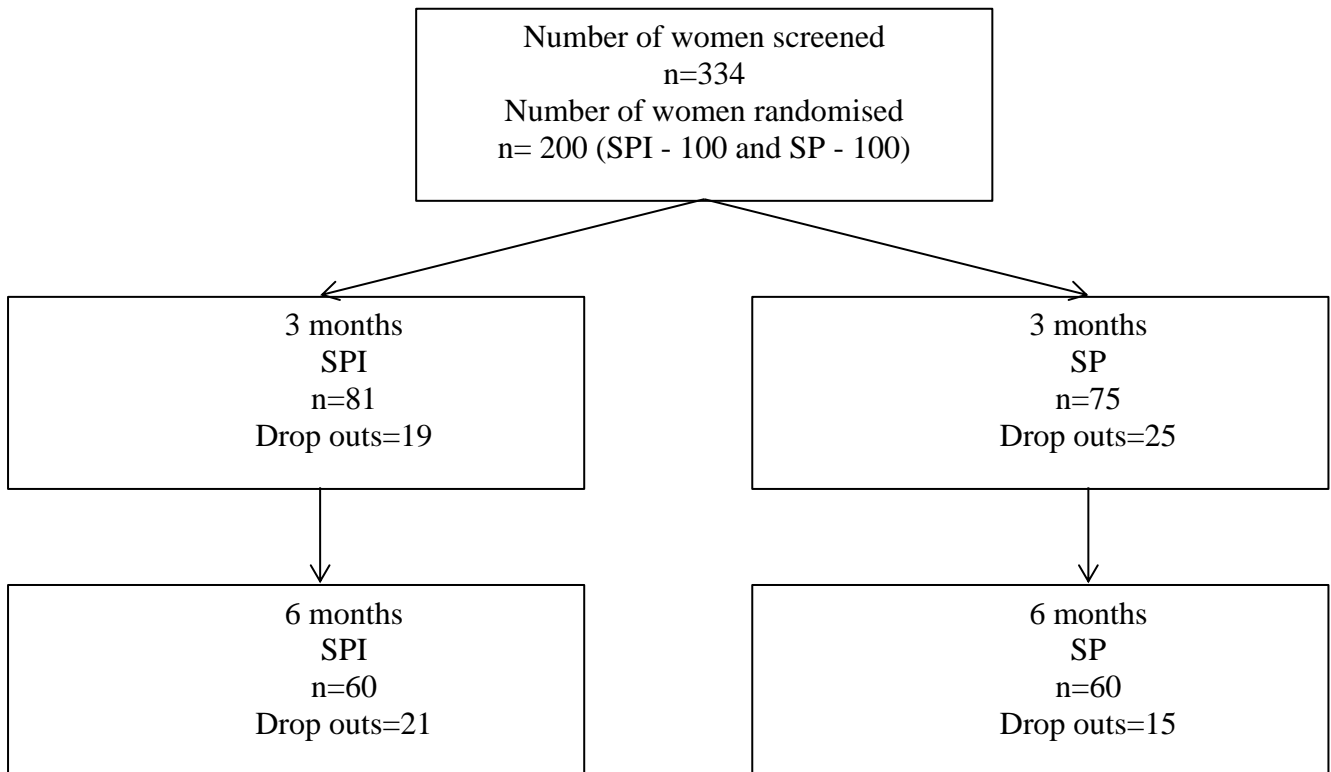
HOMA-IR – Homeostasis model of assessment – insulin resistance; TC - Total cholesterol; LDL-C - LDL-cholesterol; HDL-C - HDL cholesterol; TG- Triglycerides; hs CRP – highly sensitive C reactive protein

TSH – thyroid stimulating hormone; fT4 – free thyroxine; fT3 – free tri-iodo thyronine

Reference ranges: fasting glucose (<7mmol/L), TSH (0.35-4.7mU/L), fT4 (7.8-21pmol/L), fT3 (3.8-6pmol/L)

Figure 1

Flow diagram of participants through the study



SPI – Soy protein + isoflavone group

SP – Soy protein without isoflavone group

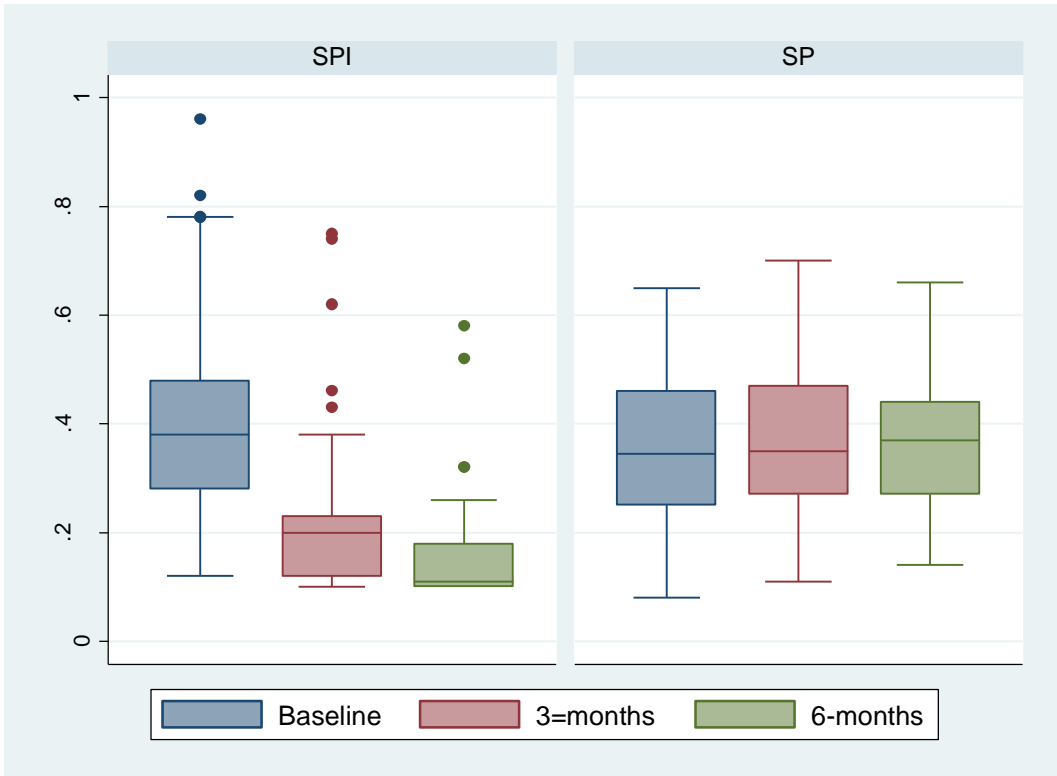


Figure 2A

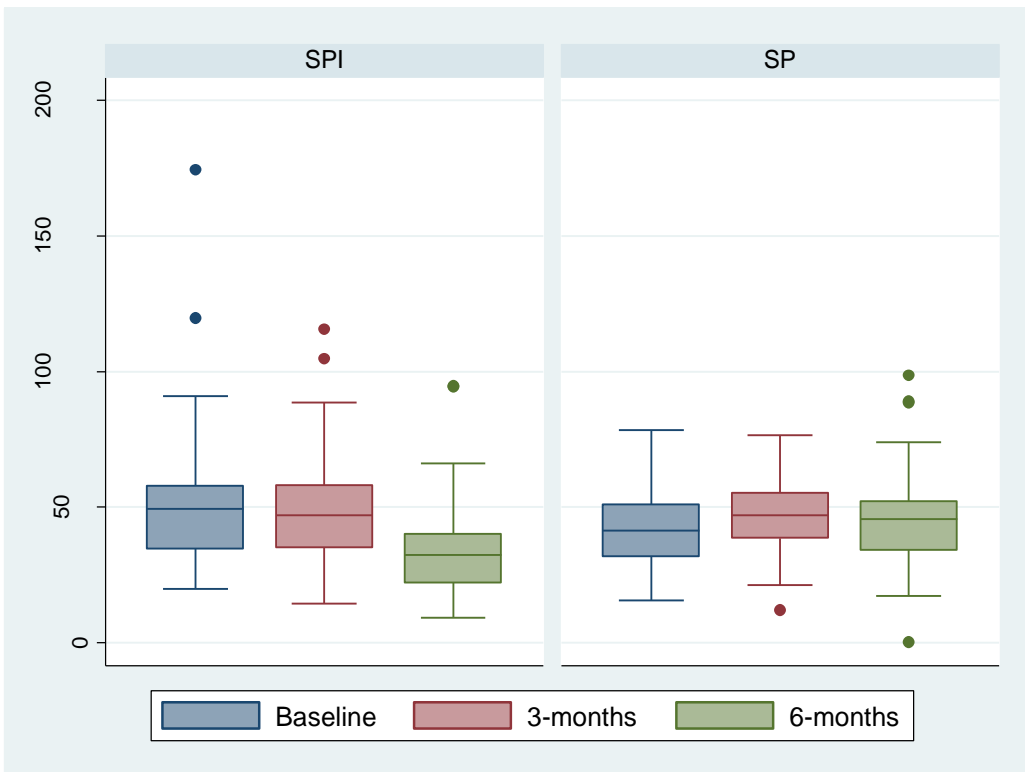


Figure 2B

Figure 2

SPI – Soy protein + isoflavone

SP – Soy protein alone

Outliers to the above (either side of the ‘whiskers’) are show as dots.

Figure 2A.

Box and whisker plot showing plasma levels of β C-terminal telopeptide (CTX) in individuals administered either 30 g soy protein with 66 mg isoflavones (SPI) and without isoflavones (SP), at baseline, 3 and 6 months.

p values

SPI (baseline – 3 months <0.01; 3 months to 6 months = 0.05)

SP (baseline – 3 months=0.72; 3 months to 6 months = 0.84)

Figure 2B.

Box and whisker plot showing plasma levels of propeptide of type I collagen (PINP) in individuals administered either 30 g soy protein with 66 mg isoflavones (SPI) and without isoflavones (SP), at baseline, 3 and 6 months.

p values

SPI (baseline – 3 months =0.92; 3 months to 6 months = <0.01)

SP (baseline – 3 months=0.24; 3 months to 6 months = 0.83)