

Soy isoflavones improve insulin sensitivity without changing serum leptin among postmenopausal women

P. Llana, C. González*, J. Fernández-Iñarra†, A. Alonso*, F. Díaz* and F. R. Pérez-López‡

Department of Obstetrics and Gynecology, Asturias Central University Hospital, Universidad de Oviedo, Oviedo; *Department of Functional Biology, Physiology Area, Universidad de Oviedo, Oviedo; †Department of Obstetrics and Gynecology, Cabueñes Hospital, Gijón; ‡Department of Obstetrics and Gynecology, Universidad de Zaragoza, Facultad de Medicina, Hospital Clínico, Zaragoza, Spain

Key words: ISOFLAVONES, LEPTIN, INSULIN, EXERCISE, DIET, POSTMENOPAUSAL WOMEN

ABSTRACT

Objective To investigate the effect of a soy isoflavone extract over insulin sensitivity and plasma leptin levels.

Methods Eighty postmenopausal women were randomly assigned to participate for 24 months either to a physical exercise and Mediterranean diet program (Control group: CG) or this intervention plus a daily oral intake of a soy isoflavone extract (Soy isoflavone group: SIG). Anthropometry, body composition analysis, blood biochemistry, menopausal symptoms and health-related quality of life were assessed at baseline and every 6 months.

Results Sixty-five women completed the protocol with no differences found among groups at baseline in age and time since the menopause. At month 24, body mass index (BMI) was lower in the SIG as compared to the CG. Fat mass, glucose, insulin, HOMA-IR, tumor necrosis factor- α (TNF- α), Kupperman Index and Cervantes Scale values significantly decreased in the SIG as compared to baseline and to CG values. Kupperman scores and serum TNF- α levels significantly decreased in both studied groups. No changes in plasma leptin levels were observed after 24 months within and between groups. When analysis was stratified according to BMI values, changes in the aforementioned parameters displayed a similar trend; however, the impact over glucose, insulin and HOMA-IR values was more evident among obese women assigned to the SIG.

Conclusion Diet, physical exercise and a daily oral intake of soy isoflavones exerted a beneficial effect on the homeostatic model in postmenopausal women which was not related to significant changes in plasma leptin levels, despite a decrease in TNF- α , fat mass and Kupperman values.

INTRODUCTION

The female menopausal phase exerts a number of changes that may trigger and maintain weight gain. The higher risk of developing obesity during the postmenopausal period has been related to hormonal changes, cultural, environmental, family-related and individual factors such as excessive energy intake, sedentary behavior, attitudes, beliefs, and biological and genetic predispositions. The menopausal transition is associated with the accumulation of centrally located and, in

particular, intra-abdominal fat^{1,2}. Hence, the metabolic syndrome may occur in 40% of postmenopausal women and insulin sensitivity may be altered in those^{3–5}.

Adipose tissue, once thought to be a static organ, is now widely acknowledged as an active promoter of cell generation and apoptosis. Similar to bone cells, adipocytes suffer the ‘turn over’ process. In this sense, fat tissue is also viewed as a secretory organ, synthesizing and secreting several proteins and cytokines such as leptin or tumor necrosis factor- α (TNF- α) that modulate various biological functions and play

Correspondence: Dr P. Llana, Asturias Central University Hospital, Department of Obstetrics and Gynecology, C/Celestino Villamil s/n, 33006 Oviedo, Spain

major roles in human metabolic regulation and vascular biology^{6–8}. Circulating leptin levels primarily reflect the amount of stored energy in the fat and secondarily acute changes in caloric intake⁹. An association between fasting leptin levels and insulin sensitivity has been reported. In addition, it has been suggested that fasting plasma leptin levels probably serve as an endogenous response to environment insulin resistance and may provide a surrogate measure of insulin action¹⁰.

Due to the fact that Asian countries display a lower frequency of obesity and related diseases, the Asian diet has gained attention. This diet consists largely of soy and soy-based products. In fact, soy isoflavones are relatively potent agonists of the β -isoform of the estrogen receptor, and dose-dependent effects on adipocyte differentiation and function have been reported¹¹. We have previously reported that plasma leptin levels were lower in ovariectomized rats in comparison to estradiol-treated counterparts, suggesting a sex steroid–leptin interaction¹². Two recent studies of our research group found that soy isoflavones, together with physical exercise and a Mediterranean diet, reduced insulin resistance in postmenopausal women with abnormal homeostasis model assessment–insulin resistance (HOMA-IR) values¹³, and decreased serum leptin and TNF- α levels after 6 months among those who were obese¹⁴.

Despite circumstantial evidence pointing to the fact that soy isoflavones could improve insulin sensitivity, little information is available in relation to the potentially involved mechanisms of action. We therefore hypothesized that changes in the insulin sensitivity, induced by soy isoflavones, physical exercise and diet, would relate to changes in plasma leptin levels and other inflammatory markers. Hence, the aim of the present study was to investigate the effect of a daily oral intake of a soy isoflavone extract over insulin sensitivity and leptin levels among postmenopausal women participating in a 24-month program of physical exercise and Mediterranean diet.

METHODS

Study design and participants

This longitudinal study was carried out at the Asturias Central University Hospital (Oviedo) and the Cabueñes Hospital (Gijón), both affiliated to the Oviedo University, Spain. A total of 80 postmenopausal women who were attending their annual gynecological check-up at the outpatient clinics of these hospitals were asked to participate. Participants were aged 50–64 years (mean \pm standard deviation, 56.7 ± 3.5 years), had an intact uterus and ovaries, were 6.5 ± 3.1 years since menopause onset, were sexually active and were not on hormone therapy (HT). Postmenopausal status was defined as an amenorrhea of at least 1 year. Body mass index (BMI) was categorized as follows: <20 kg/m² as underweight, 20–24.9 kg/m² as normal weight, 25–29.9 kg/m² as overweight, and ≥ 30 kg/m² as obese. BMI ranged from

22.5 to 43.5 kg/m², with more than 95% being overweight or obese.

Women were randomly assigned to participate for 24 months either to a program of physical exercise and Mediterranean diet (Control group: CG) or this intervention plus a daily oral intake of a soy isoflavone extract (Soy isoflavone group: SIG). Diet and physical activity were monitored during the study through compliance interviews and motivational and support counseling sessions. Blood pressure, anthropometry, body composition analysis, menopausal symptom intensity and quality of life were assessed and recorded at baseline and at 6-month intervals. In addition, a fasting blood sample was withdrawn for biochemical analysis at the proposed intervals. Information regarding health status was obtained from the women's medical charts and physical examination.

Five participants in the CG refused participation in the study after signing the consent form. Consequently, 75 postmenopausal women (40 in SIG and 35 in CG) comprised the final study sample. This sample size was determined to be appropriate as it provides a 95% power for variance analysis using a two-sided significance level of 0.05, assuming a mean plasma leptin value of 37.9 ng/dl (standard deviation, 26.7 ng/dl)¹⁵ and a difference change equal to one half of a standard deviation.

Ethical considerations

The research protocol of the study was reviewed and approved by the Asturias Ethical Committee, Oviedo, Spain. All women were informed about the research (purposes and used tools) and written consent obtained.

Menopausal symptom and health-related quality of life assessment

Menopausal symptom intensity was assessed with the Kupperman Index, a tool composed of 11 items¹⁶. The Cervantes Scale was used to assess menopause health-related quality of life. This scale is a 31-item, self-reporting questionnaire composed of four domains: the 'Menopause and health' domain with 15 items (including three sub-domains: vasomotor symptoms, health, and aging); the 'Psychical' domain with nine items; the 'Sexuality' domain with four items; and the 'Couple relationship' domain with three items. Higher domain, sub-domain or global scores are indicative of worse menopause health-related quality of life. For the purpose of this research, only global or total Cervantes Scale scores were analyzed at proposed intervals (maximum achievable score is 155)^{13,16–18}.

Blood pressure and anthropometric assessment

Trained personnel measured blood pressure (calibrated sphygmomanometer), weight and height (wall-mounted

Table 1 Baseline characteristics and changes observed throughout the study for both groups (all women). Data are given as mean \pm standard deviation

	<i>Baseline</i>	<i>6 months</i>	<i>12 months</i>	<i>18 months</i>	<i>24 months</i>
SBP (mmHg)					
CG	130 \pm 14.6	131.2 \pm 17.1	130.1 \pm 13.7	121.4 \pm 19.5	129.8 \pm 16.8
SIG	131.0 \pm 16.3	127.1 \pm 12.8	126.1 \pm 11.2	130.9 \pm 16.1*	132.2 \pm 13.6
DBP (mmHg)					
CG	78.3 \pm 7.2	84.2 \pm 16.8	81.2 \pm 8.9	74.6 \pm 8.8	76.4 \pm 11.5
SIG	76.6 \pm 9.7	77.5 \pm 8.2*	78.3 \pm 7.6	78.3 \pm 9.6	81.1 \pm 4.7*
BMI (kg/m ²)					
CG	30.6 \pm 4.7	30.7 \pm 4.7	30.6 \pm 4.3	31.0 \pm 4.3	30.6 \pm 4.8
SIG	30.5 \pm 4.2	29.2 \pm 4.5	29.6 \pm 4.6	28.6 \pm 4.6*	27.9 \pm 2.8*
AC (cm)					
CG	94.0 \pm 10.4	93.3 \pm 10.3	91.5 \pm 15.8	92.8 \pm 9.1	93.4 \pm 9.3
SIG	90.8 \pm 13.2	90.8 \pm 8.6	92.5 \pm 8.4	91.1 \pm 8.1	92.8 \pm 8.1
Lean mass (kg)					
CG	39.9 \pm 6.1	43.2 \pm 8.6	43.6 \pm 7.9	43.9 \pm 5.3	42.9 \pm 6.9
SIG	40.9 \pm 6.3	39.4 \pm 5.8	43.1 \pm 5.5	45.3 \pm 5.9	43.0 \pm 6.9
Lean mass (%)					
CG	54.1 \pm 7.7	57.5 \pm 9.3	60.0 \pm 8.7	59.1 \pm 9.1	58.7 \pm 6.8
SIG	56.6 \pm 7.6	57.4 \pm 10.6	59.3 \pm 7.8	59.1 \pm 7.4	57.5 \pm 7.9
Fat mass (kg)					
CG	30.5 \pm 8.6	32.8 \pm 7.6	30.2 \pm 7.4	32.3 \pm 10.5	35.8 \pm 5.7
SIG	31.1 \pm 7.7	30.3 \pm 5.0	34.3 \pm 7.0	26.7 \pm 8.9	26.3 \pm 5.2 ^{†,*}
Fat mass (%)					
CG	45.9 \pm 7.8	42.5 \pm 9.3	42.1 \pm 7.7	40.3 \pm 7.6	42.7 \pm 7.5
SIG	43.3 \pm 7.5	42.6 \pm 10.6	40.3 \pm 7.5	39.7 \pm 6.1	39.0 \pm 4.8 ^{†,*}
Kupperman					
CG	24.3 \pm 8.7	16.5 \pm 8.0	13.9 \pm 8.0	12.3 \pm 6.8	12.4 \pm 6.4 [†]
SIG	22.0 \pm 10.4	12.9 \pm 6.2*	13.4 \pm 7.6	11.7 \pm 7.3	9.7 \pm 5.9 [†]
Cervantes					
CG	66.0 \pm 20.4	60.5 \pm 22.7	59.2 \pm 24.4	61.1 \pm 21.7	60.6 \pm 20.1
SIG	75.3 \pm 22.7	61.6 \pm 24.3	63.5 \pm 23.6	61.8 \pm 29.3	53.7 \pm 19.8 [†]
Glucose (mg/dl)					
CG	101.8 \pm 9.6	97.4 \pm 10.3	99.7 \pm 17.9	99.8 \pm 16.1	98.1 \pm 13.4
SIG	101.9 \pm 9.3	94.4 \pm 9.6	96.2 \pm 9.1	98.9 \pm 10.5	93.6 \pm 5.1 [†]
Insulin (mU/l)					
CG	13.1 \pm 3.4	12.6 \pm 5.1	13.2 \pm 7.1	11.8 \pm 5.2	12.7 \pm 6.8
SIG	14.6 \pm 8.6	10.6 \pm 4.4	12.1 \pm 3.9	11.0 \pm 4.1	12.2 \pm 3.1 [†]
HOMA-IR					
CG	3.2 \pm 0.9	3.1 \pm 1.3	3.3 \pm 2.1	3.0 \pm 1.8	3.7 \pm 1.7
SIG	3.3 \pm 1.1	2.5 \pm 1.2	2.9 \pm 1.2	2.7 \pm 1.1	2.6 \pm 0.8 ^{†,*}
Cholesterol (mg/dl)					
CG	218.4 \pm 30.9	228.2 \pm 46.1	223.8 \pm 37.9	216.9 \pm 33.0	212.7 \pm 34.4
SIG	237.7 \pm 32.1*	227.4 \pm 28.3	224.6 \pm 38.1	230.1 \pm 38.2	233.1 \pm 52.3
HDL-C (mg/dl)					
CG	65.5 \pm 16.3	62.2 \pm 15.1	62.5 \pm 15.5	64.9 \pm 31.1	60.1 \pm 14.9
SIG	64.0 \pm 15.4	63.2 \pm 13.5	59.5 \pm 11.5	57.5 \pm 10.9	59.6 \pm 12.0
LDL-C (mg/dl)					
CG	134.2 \pm 31.3	147.2 \pm 39.8	141.6 \pm 36.1	133.3 \pm 33.0	131.4 \pm 33.2
SIG	148.9 \pm 31.3*	142.3 \pm 27.2	138.8 \pm 37.0	147.4 \pm 37.3	150.5 \pm 43.4
Triglycerides (mg/dl)					
CG	103.8 \pm 53.6	105.9 \pm 45.7	100.9 \pm 54.1	117.0 \pm 81.0	110.6 \pm 57.1
SIG	124.2 \pm 57.6	116.6 \pm 50.4	119.8 \pm 48.7	120.4 \pm 48.6	133.1 \pm 65.4

(Continued)

Table 1 (Continued)

	Baseline	6 months	12 months	18 months	24 months
Leptin (ng/ml)					
CG	25.2 ± 9.3	23.9 ± 9.4	23.7 ± 8.4	25.9 ± 7.8	21.1 ± 6.9
SIG	21.3 ± 8.0	22.7 ± 8.0	23.8 ± 7.9	24.0 ± 8.5	25.6 ± 9.0
TNF- α (pg/ml)					
CG	8.7 ± 5.9	5.7 ± 1.2	5.5 ± 1.4	5.0 ± 1.0	5.5 ± 1.4 [†]
SIG	7.9 ± 1.5	6.5 ± 1.6*	6.1 ± 1.4	5.5 ± 1.4	4.6 ± 0.8 ^{†,*}
Homocysteine (μ mol/l)					
CG	9.9 ± 2.7	9.8 ± 2.3	9.4 ± 2.7	8.4 ± 2.8	10.0 ± 2.7
SIG	9.6 ± 2.8	9.5 ± 2.1	9.9 ± 3.4	9.9 ± 2.3*	10.5 ± 3.6
C-reactive protein (mg/dl)					
CG	0.3 ± 0.2	0.4 ± 0.5	0.6 ± 1.9	0.4 ± 0.3	0.3 ± 0.2
SIG	0.3 ± 0.2	0.4 ± 0.4	0.4 ± 0.3	0.7 ± 1.2	0.3 ± 0.2
Estradiol (pg/ml)					
CG	9.3 ± 3.9	10.8 ± 3.9	9.1 ± 4.1	10.5 ± 5.2	10.5 ± 3.9
SIG	9.6 ± 4.5	12.3 ± 5.6	10.3 ± 5.9	11.4 ± 7.2	11.6 ± 10.0

SIG, soy isoflavone group; CG, control group; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; AC, abdominal circumference; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TNF- α , tumor necrosis factor α ; [†], $p < 0.05$ for analyzed group throughout the study; *, $p < 0.05$ between groups

stadiometer) among subjects in a standing position wearing light clothes and no shoes. Waist or abdominal circumference was measured with an anthropometric tape placed directly on the narrowest point between the lower rib margin and the iliac crest, on a plane perpendicular to the long axis of the body, while the subject stood balanced on both feet, approximately 20 cm apart, and with both arms hanging freely. A waist circumference greater than 88 cm was used to define abdominal obesity¹⁹.

Body composition was evaluated by bioelectrical impedance analysis. Measurements were carried out with the subject lying in a supine position on a flat, non-conductive bed using a multi-frequency tetrapolar technique (QuadScan 4000; Bodystat, Douglas, UK). This method adequately correlates with dual-energy X-ray absorptiometry²⁰, and its coefficient variation of 1.8%.

Biochemical blood analysis

Blood samples were taken at proposed intervals between 09.00 and 10.00 after an overnight fast and immediately sent to the hospital's chemistry laboratory and the University's Physiology laboratory. Leptin and homocysteine were measured in plasma, and the remaining analytes in serum. Two blinded researchers measured biochemical circulating marker levels, using radioimmunoassay kits (DGR Instruments kit GMBH, Marburg, Germany) for leptin, enzyme-linked immunosorbent assay kits (DRG Instruments GmbH, Marburg, Germany) for TNF- α , latex particle-enhanced immunoturbidimetric assay for C-reactive protein, an enzymatic method with hexokinase for glucose, the Trinder method for lipid profile (total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol and

triglycerides), the ICMA method for homocysteine and the ECLIA method for insulin and estradiol. All samples were analyzed in duplicate, maintaining a quality management system fulfilling ISO 9001:2000 standard requirements. Intra- and inter-assay coefficient variations were less than 8.2% for all tests.

Homeostatic model assessment-insulin resistance

The HOMA-IR was used to assess insulin resistance among studied groups at baseline and proposed intervals. HOMA-IR is calculated by multiplying insulin and glucose levels and then dividing this product by a constant. This model has correlated well with estimates using the euglycemic clamp method and has been extensively tested against other measures of insulin resistance²¹.

Soy isoflavone treatment

The soy isoflavone extract contained 200 mg of Glycine max, corresponding to 80 mg of isoflavones (60.8 mg genistein, 16 mg daidzein and 3.2 mg glicitein) (Fisiogen[®], Zambon Pharmaceutical Group, Barcelona, Spain). This compound is registered by the Spanish Health Authority, fulfilling all quality control regulations.

Educational sessions

Educational sessions were carried out once women were included in the study, in which they received verbal and written instructions regarding the health benefits of increasing

Table 2 Baseline characteristics and changes observed throughout the study for both groups, non-obese women only. Data are given as mean \pm standard deviation

	Baseline	6 months	12 months	18 months	24 months
SBP (mmHg)					
CG	136.9 \pm 16.1	128.0 \pm 12.0	127.0 \pm 11.6	124.7 \pm 12.8	127.1 \pm 17.4
SIG	129.2 \pm 15.3	128.0 \pm 13.7	124.4 \pm 13.2	135.0 \pm 15.3*	130.5 \pm 14.3
DBP (mmHg)					
CG	80.4 \pm 7.7	81.3 \pm 8.9	81.3 \pm 9.1	72.8 \pm 9.3	75.0 \pm 12.0
SIG	76.2 \pm 9.2	79.0 \pm 7.5	75.6 \pm 8.2	81.0 \pm 7.9	82.0 \pm 4.1*
BMI (kg/m ²)					
CG	27.0 \pm 1.9	27.0 \pm 1.9	27.4 \pm 1.7	27.8 \pm 1.7	27.7 \pm 1.6
SIG	28.1 \pm 1.6	26.8 \pm 2.1	26.4 \pm 1.6	26.3 \pm 2.0*	25.9 \pm 1.5 [†] *
AC (cm)					
CG	87.9 \pm 5.8	87.2 \pm 6.1	84.2 \pm 17.1	87.9 \pm 5.6	88.4 \pm 5.7
SIG	86.1 \pm 9.4	87.5 \pm 6.7	88.1 \pm 6.1	87.6 \pm 5.6	85.4 \pm 8.1
Lean mass (kg)					
CG	39.2 \pm 4.7	41.2 \pm 7.8	44.2 \pm 9.9	44.2 \pm 5.9	42.7 \pm 7.3
SIG	40.8 \pm 7.0	38.7 \pm 5.2	43.2 \pm 5.9	45.1 \pm 6.1	43.6 \pm 6.5
Lean mass (%)					
CG	58.3 \pm 6.8	60.2 \pm 7.0	61.5 \pm 8.8	64.0 \pm 5.3	60.0 \pm 6.6
SIG	58.3 \pm 6.9	60.1 \pm 9.8	62.3 \pm 6.5	60.8 \pm 7.6	60.1 \pm 8.5
Fat mass (kg)					
CG	35.4 \pm 6.5	28.7 \pm 6.3	30.3 \pm 8.4	29.3 \pm 8.5	33.1 \pm 5.9
SIG	33.4 \pm 6.2	34.3 \pm 11.3	28.9 \pm 9.9	31.2 \pm 7.3	26.1 \pm 7.2 [†] *
Fat mass (%)					
CG	44.1 \pm 6.6	39.8 \pm 7.0	39.1 \pm 8.1	36.0 \pm 5.3	40.3 \pm 6.0
SIG	41.6 \pm 6.8	39.9 \pm 9.8	37.7 \pm 6.5	38.0 \pm 5.8	37.5 \pm 4.9 [†]
Kupperman					
CG	24.0 \pm 5.9	15.9 \pm 7.3	33.0 \pm 15.5	13.3 \pm 6.8	12.9 \pm 5.6 [†]
SIG	22.6 \pm 11.4	12.4 \pm 6.2	40.0 \pm 14.4	10.7 \pm 7.4	9.1 \pm 5.4 [†]
Cervantes					
CG	67.8 \pm 22.2	61.9 \pm 20.9	114.0 \pm 58.1	59.8 \pm 18.4	62.0 \pm 19.9
SIG	73.5 \pm 25.1	59.8 \pm 24.9	94.0 \pm 56.3	56.1 \pm 27.6	51.8 \pm 19.0 [†]
Glucose (mg/dl)					
CG	98.2 \pm 7.3	94.2 \pm 9.5	97.4 \pm 12.2	97.2 \pm 9.2	94.1 \pm 10.5
SIG	100.9 \pm 9.5	93.6 \pm 10.1	95.5 \pm 9.8	98.8 \pm 10.6	93.7 \pm 5.7
Insulin (mU/l)					
CG	12.1 \pm 2.5	10.8 \pm 4.2	11.4 \pm 5.0	10.5 \pm 4.7	10.4 \pm 4.0
SIG	14.0 \pm 10.6	9.1 \pm 3.3	10.9 \pm 3.1	10.5 \pm 4.1	11.9 \pm 3.5
HOMA-IR					
CG	2.9 \pm 0.5	2.6 \pm 0.9	2.6 \pm 1.0	2.6 \pm 1.0	3.4 \pm 1.1
SIG	3.1 \pm 0.9	2.1 \pm 0.9	2.8 \pm 1.0	2.6 \pm 1.0	2.5 \pm 0.6*
Cholesterol (mg/dl)					
CG	223.3 \pm 22.0	237.2 \pm 28.1	217.7 \pm 32.2	215.4 \pm 37.5	222.8 \pm 30.4
SIG	237.6 \pm 30.4	227.5 \pm 23.7	229.5 \pm 29.2	228.5 \pm 34.0	233.0 \pm 56.2
HDL-C (mg/dl)					
CG	73.1 \pm 12.9	69.5 \pm 14.3	63.7 \pm 17.3	72.8 \pm 40.0	65.0 \pm 14.3
SIG	63.2 \pm 17.4	64.6 \pm 14.6	62.2 \pm 12.0	57.8 \pm 11.9	60.4 \pm 12.2
LDL-C (mg/dl)					
CG	136.6 \pm 28.3	148.4 \pm 32.7	135.7 \pm 28.4	127.9 \pm 34.8	137.7 \pm 33.5
SIG	149.4 \pm 30.4	141.4 \pm 23.7	141.3 \pm 29.6	145.8 \pm 33.3	153.0 \pm 43.7
Triglycerides (mg/dl)					
CG	81.3 \pm 38.1	94.8 \pm 46.9	97.6 \pm 62.6	119.9 \pm 18.9	108.2 \pm 63.5
SIG	126.9 \pm 66.9	112.0 \pm 42.5	112.5 \pm 27.1	117.6 \pm 43.4	128.5 \pm 64.8

(Continued)

Table 2 (Continued)

	Baseline	6 months	12 months	18 months	24 months
Leptin (ng/ml)					
CG	22.1 ± 4.5	21.0 ± 7.3	20.4 ± 6.8	22.1 ± 7.3	19.7 ± 7.1
SIG	18.9 ± 5.6	21.3 ± 6.3	21.8 ± 7.9	22.1 ± 8.4	23.0 ± 9.7
TNF- α (pg/ml)					
CG	7.1 ± 3.4	5.7 ± 1.2	5.6 ± 1.6	5.2 ± 1.1	5.4 ± 1.7 [†]
SIG	7.9 ± 3.0	6.4 ± 1.3	6.3 ± 1.6	5.5 ± 1.6	4.3 ± 0.8 ^{†,*}
Homocysteine (μ mol/l)					
CG	10.0 ± 2.7	9.2 ± 1.9	8.9 ± 2.3	8.1 ± 3.0	9.5 ± 2.5
SIG	9.0 ± 2.6	9.6 ± 2.1	10.1 ± 2.6	9.9 ± 2.4*	9.9 ± 3.5
C-reactive protein (mg/dl)					
CG	0.2 ± 0.2	0.2 ± 0.2	2.5 ± 0.5	0.3 ± 0.4	0.2 ± 0.1
SIG	0.3 ± 0.2	0.3 ± 0.4	0.3 ± 0.3	0.5 ± 0.7	0.4 ± 0.3
Estradiol (pg/ml)					
CG	9.7 ± 3.4	10.3 ± 3.1	9.2 ± 3.8	9.5 ± 4.3	9.8 ± 3.3
SIG	10.0 ± 4.8	12.5 ± 5.8	8.7 ± 4.3	11.7 ± 8.0	12.6 ± 12.1

SIG, soy isoflavone group; CG, control group; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; AC, abdominal circumference; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TNF- α , tumor necrosis factor α ; [†], $p < 0.05$ for analyzed group throughout the study; *, $p < 0.05$ between groups

their physical activity to at least 30 min of moderate walking or aerobic exercising (biking, jogging, dancing, swimming, etc.) five times a week, and shifting to a Mediterranean diet in which meat and pastries, cakes, and sweets are decreased while intake of virgin olive oil, nuts, vegetables, legumes, oily fish and fruits is increased^{13,22}.

Statistical analysis

Statistical analysis was performed using SPSS statistical package (Version 18.0 for Windows, SPSS Inc, Chicago, Illinois, USA). Data are presented as mean \pm standard deviation. The Kolmogorov–Smirnov test was used to determine the normality of data distribution. Means between groups were compared with non-paired Student's *t*-test. Changes throughout the study within each group were compared with paired Student's *t*-test (baseline vs. 24th month) or Tukey's *post-hoc* multiple comparisons (for the trend). When appropriate, Pearson's coefficients were calculated to determine correlation between variables. For all calculations, a *p* value of < 0.05 was considered as statistically significant.

RESULTS

After 24 months, 65 women completed the protocol (33 in the SIG and 32 in the CG). Hence, the drop-out rate was 17.5% for the SIG and 8.6% for the CG. No differences were determined between studied groups at baseline in terms of age and time since menopause onset. Baseline characteristics and changes observed in analyzed parameters throughout the study for both groups (all women, $n = 65$) are shown in Table 1. For the whole sample, 5.4% of participants had a normal

BMI, whereas 52% were overweight and 42.6% obese. No differences were observed between studied groups for all parameters, except for serum total cholesterol and LDL cholesterol levels, which were significantly higher in the SIG. Several significant intergroup differences were found in certain parameters at months 6 and 18 of the study.

BMI values were lower at month 24 in the SIG as compared to the CG. Fat mass (expressed as % or kg), glucose, insulin, HOMA-IR, TNF- α , Kupperman Index and Cervantes Scale values significantly decreased in the SIG as compared to baseline. These values were significantly lower than those of the CG for fat mass, HOMA-IR and TNF- α . Interestingly, the Kupperman Index and serum TNF- α values significantly decreased in both studied groups. No changes in plasma leptin levels were observed after 24 months within and between groups. Significant correlations were observed at month 24 in the SIG between serum TNF- α levels and fat mass and between Cervantes scores and BMI and abdominal circumference values.

As the BMI in the SIG was significantly lower at month 24 as compared to that in the CG, analysis was stratified according to BMI values (Tables 2 and 3). Table 2 shows baseline characteristics and changes observed in studied parameters throughout the study for non-obese women (SIG and CG) ($n = 38$). Baseline characteristics between groups (SIG vs. CG) did not differ among non-obese women. Intergroup differences were observed at 18 (systolic blood pressure and BMI values) and 24 months (diastolic blood pressure values). BMI, fat mass, serum TNF- α , Kupperman Index and Cervantes Scale values significantly decreased at month 24 in the SIG. These values were significantly lower than those found in the CG, only for BMI, fat mass (kg), and TNF- α . Kupperman Index and TNF- α values also decreased at month 24 in non-obese women (SIG and CG).

Table 3 Baseline characteristics and changes observed throughout the study for both groups, obese women only. Data are given as mean \pm standard deviation

	Baseline	6 months	12 months	18 months	24 months
SBP (mmHg)					
CG	125 \pm 10.9	135.0 \pm 21.4	133.8 \pm 15.5	118.0 \pm 24.8	135.0 \pm 15.0
SIG	134.0 \pm 18.1	125.0 \pm 10.7	128.2 \pm 8.2	120.0 \pm 13.2	135.5 \pm 12.1
DBP (mmHg)					
CG	76.4 \pm 6.3	87.7 \pm 22.7	81.2 \pm 9.1	76.7 \pm 8.1	79.1 \pm 10.6
SIG	77.3 \pm 10.8	74.1 \pm 9.1	81.8 \pm 5.4	71.1 \pm 10.4	79.5 \pm 5.6
BMI (kg/m ²)					
CG	34.6 \pm 3.5	30.4 \pm 3.8	34.4 \pm 3.2	34.5 \pm 3.3	36.3 \pm 3.7
SIG	34.7 \pm 4.2	34.8 \pm 3.3	34.1 \pm 3.6	35.1 \pm 3.6	31.0 \pm 1.0 ^{†,*}
AC (cm)					
CG	100.4 \pm 10.4	99.5 \pm 10.1	100.3 \pm 8.1	98.1 \pm 9.3	103.0 \pm 7.0
SIG	98.9 \pm 14.9	98.5 \pm 8.0	99.0 \pm 6.9	101.1 \pm 4.9	100 \pm 6.2
Lean mass (kg)					
CG	40.6 \pm 7.3	45.4 \pm 9.2	43.0 \pm 5.0	43.4 \pm 4.1	43.5 \pm 6.3
SIG	41.1 \pm 5.2	40.9 \pm 6.9	45.4 \pm 9.2	46.0 \pm 5.8	42.2 \pm 7.6
Lean mass (%)					
CG	52.3 \pm 8.6	54.8 \pm 10.7	58.3 \pm 8.4	53.9 \pm 9.5	56.4 \pm 6.9
SIG	53.9 \pm 8.0	51.3 \pm 10.1	55.2 \pm 7.8	54.2 \pm 4.3	53.5 \pm 4.7
Fat mass (kg)					
CG	30.5 \pm 8.6	32.8 \pm 7.6	30.2 \pm 7.4	32.3 \pm 10.5	35.8 \pm 5.7
SIG	31.1 \pm 7.7	28.7 \pm 6.3	34.3 \pm 7.0	29.3 \pm 8.5	26.4 \pm 5.2 [†]
Fat mass (%)					
CG	47.7 \pm 8.6	45.2 \pm 10.7	45.8 \pm 5.3	44.8 \pm 7.1	47.2 \pm 8.1
SIG	46.1 \pm 8.0	48.7 \pm 10.1	43.9 \pm 7.5	44.6 \pm 4.1	41.3 \pm 3.7 ^{†,*}
Kupperman					
CG	24.5 \pm 11.2	17.0 \pm 8.8	11.9 \pm 8.4	11.2 \pm 6.7	11.4 \pm 7.9 [†]
SIG	21.0 \pm 8.8	14.0 \pm 6.2	12.0 \pm 6.9	14.6 \pm 6.4	10.6 \pm 6.7 ^{†,*}
Cervantes					
CG	64.1 \pm 18.7	59.1 \pm 24.8	60.5 \pm 21.6	62.6 \pm 25.3	57.8 \pm 21.1
SIG	78.2 \pm 18.7	65.7 \pm 23.4	74.1 \pm 19.5	78.0 \pm 29.6	56.6 \pm 21.4
Glucose (mg/dl)					
CG	105.7 \pm 10.3	100.6 \pm 10.3	102.4 \pm 23.1	102.6 \pm 21.0	105.6 \pm 15.3
SIG	103.8 \pm 9.1	96.2 \pm 8.4	97.4 \pm 8.1	99.2 \pm 10.6	93.4 \pm 4.1 ^{†,*}
Insulin (mU/l)					
CG	14.2 \pm 3.9	14.3 \pm 5.3	15.4 \pm 5.0	13.1 \pm 5.4	17.0 \pm 9.0
SIG	15.6 \pm 5.5	13.8 \pm 4.7	13.9 \pm 4.5	12.6 \pm 2.3	12.6 \pm 2.3*
HOMA-IR					
CG	3.7 \pm 1.0	3.6 \pm 1.5	4.0 \pm 2.7	3.5 \pm 2.3	4.4 \pm 2.5
SIG	3.8 \pm 1.2	3.3 \pm 1.1	3.5 \pm 1.4	3.1 \pm 1.2	2.9 \pm 1.0*
Cholesterol (mg/dl)					
CG	213.2 \pm 38.2	219.1 \pm 58.4	217.7 \pm 32.2	218.6 \pm 28.6	193.5 \pm 35.3
SIG	237.6 \pm 30.4	227.3 \pm 38.2	229.5 \pm 29.2	234.6 \pm 50.0	233.4 \pm 47.8
HDL-C (mg/dl)					
CG	57.5 \pm 15.5	55.0 \pm 12.4	61.1 \pm 13.4	56.6 \pm 12.5	50.8 \pm 11.5
SIG	65.4 \pm 11.7	60.0 \pm 10.3	55.6 \pm 9.7	56.8 \pm 8.5	58.4 \pm 12.0
LDL-C (mg/dl)					
CG	136.6 \pm 28.3	145.7 \pm 46.9	148.9 \pm 43.4	139.2 \pm 31.0	119.5 \pm 30.7
SIG	148.1 \pm 33.7	144.4 \pm 35.2	135.7 \pm 45.8	152.1 \pm 48.7	146.7 \pm 44.4
Triglycerides (mg/dl)					
CG	127.7 \pm 58.2	117.1 \pm 42.8	105.0 \pm 43.4	113.9 \pm 36.3	115.0 \pm 44.8
SIG	119.7 \pm 39.2	127.1 \pm 66.2	130.3 \pm 68.8	128.1 \pm 63.1	140.3 \pm 68.1

(Continued)

Table 3 (Continued)

	Baseline	6 months	12 months	18 months	24 months
Leptin (ng/ml)					
CG	28.5 ± 11.8	26.4 ± 11.3	27.7 ± 8.6	30.0 ± 6.2	24.0 ± 5.7
SIG	25.4 ± 9.7	26.8 ± 8.3	26.8 ± 7.2	29.5 ± 6.3	29.5 ± 6.2
TNF- α (pg/ml)					
CG	10.4 ± 7.7	5.7 ± 1.2	5.5 ± 1.1	4.7 ± 0.8	5.8 ± 2.2 [†]
SIG	7.8 ± 1.5	6.6 ± 2.0	5.8 ± 0.9	5.1 ± 0.8	5.1 ± 0.6 [†]
Homocysteine (μ mol/l)					
CG	9.8 ± 2.7	10.5 ± 2.5	10.0 ± 3.0	8.9 ± 2.6	11.2 ± 3.0
SIG	10.8 ± 2.7	9.2 ± 2.0	9.7 ± 4.2	10.0 ± 2.1	11.5 ± 3.5
C-reactive protein (mg/dl)					
CG	0.4 ± 0.2	0.6 ± 0.7	0.4 ± 0.2	0.4 ± 0.3	0.5 ± 0.3
SIG	0.3 ± 0.1	0.5 ± 0.5	0.5 ± 0.4	1.2 ± 2.0	0.3 ± 0.1
Estradiol (pg/ml)					
CG	9.0 ± 4.4	11.4 ± 4.7	9.1 ± 4.6	11.6 ± 6.1	12.1 ± 4.6
SIG	9.1 ± 3.9	11.7 ± 5.3	12.6 ± 7.1	10.8 ± 4.6	10.2 ± 5.6

SIG, soy isoflavone group; CG, control group; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; AC, abdominal circumference; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TNF- α , tumor necrosis factor α ; [†], $p < 0.05$ for analyzed group throughout the study; *, $p < 0.05$ between groups

Changes in the aforementioned parameters displayed a similar trend when only obese women were analyzed ($n = 27$, BMI ≥ 30 kg/m², Table 3); however, impact at month 24 over HOMA-IR, glucose and insulin levels was more clearly observed among obese women assigned to the SIG.

DISCUSSION

Obesity is a common situation in mid-aged women and related to several factors. Present data confirm the high prevalence of overweight and obesity in postmenopausal women aged 50–64 years attending annual gynecological examination at our medical centers. Regular exercise and nutrition play important roles in the prevention and treatment of obesity and related consequences. Despite this, it is not easy for menopausal women to change their lifestyle^{23,24}. Behavioral interventions, especially when extreme changes in diet or physical exercise are required, tend to have very high drop-out rates. Indeed, this was the case in the present research in which the main cause of withdrawal was difficulties in following the diet and physical exercising program. Pharmacological approach could be considered for women unable to achieve weight loss, despite their best intentions to improve lifestyle changes in terms of diet, physical activity and behavioral changes. In this sense, soy isoflavones could have an important role²⁵. Indeed, the present data showed that, after 24 months, BMI decreased in the SIG which was significantly lower than values observed for the CG. This trend was observed even when women were stratified as obese and non-obese.

Changes in glucose, insulin and HOMA-IR observed in the SIG confirm previous data reporting significant soy isoflavone-related glucose homeostasis improvement^{13,26,27}. Our results do not support data of other studies finding no changes in these biochemical parameters, perhaps owing to a

shorter follow-up period²⁵. In this context, it seems likely that a longer treatment period is required in order to improve glucose metabolism in the adipose tissue. Interestingly, when analysis was stratified according to BMI, improvement was more clearly observed for obese women assigned to the SIG.

Basic science research has suggested that, under certain circumstances, leptin resistance may ensue at the central nervous system. Hence, leptin, as an important signal linking fat cell and brain, is decreased, leading to an increase in food intake and a reduction in energy expenditure which appear to be linked to the onset of insulin resistance^{28,29}. The present study failed to find a link between leptin levels and insulin. Indeed, leptin levels did not change in the SIG at the end of month 24 despite the improvement of HOMA-IR values. This is in contradiction with another study reporting a fall in serum leptin levels after 2 months of incorporating 50 mg of soy food to a very low fat diet²⁶. Despite this, it is important to bear in mind that, as opposed to the ingestion of an oral standard isoflavone compound, soy food could have many other components which could have favored the positive effects found over leptin levels in the study by Wu and colleagues²⁶. However, our results are consistent with data of a premenopausal sample in which, after 2 years of dietary intervention with 50 mg of soy isoflavones, no changes in leptin levels were encountered³⁰. The 2-year length of our study adds consistency to the results and should be taken into account for comparison purposes.

The present study encountered at month 24 a significant decrease in circulating TNF- α levels in both arms of the study. Nevertheless, levels were found to be significantly lower in the SIG. These changes were related to fat mass decrease and are consistent with other studies reporting a decrease in circulating levels of TNF- α in postmenopausal women after soy isoflavone consumption³¹. A possible link between TNF- α

and insulin resistance has been suggested by functional studies³²; however, changes in serum TNF- α levels found in the present study were not related to changes in plasma leptin levels.

Situations promoting chronic stress like unemployment, poor health perception, depressed mood or menopause-related factors such as hot flushes may intensify age-associated increase of cortisol which promotes central fat deposition³³. In this context, the Kupperman Index and the Cervantes Scale are useful tools to evaluate a possible chronic stress associated with menopausal symptoms and changes in health-related quality of life. A significant fall in Kupperman Index values was detected in both arms of the study, reflecting perhaps the fact that changes in lifestyles, rather than isoflavones, may have a positive impact on menopausal symptom intensity. Contrary to the Kupperman Index, at month 24, the global Cervantes Scale score significantly decreased only in the SIG and this change was correlated to changes in BMI and abdominal circumference, supporting the idea of a maladaptive response to stress as a predisposing factor of ectopic fat deposition³⁴, and a positive impact over health-related quality of life of the intervention program (diet and physical exercise) complemented with soy isoflavone supplementation. However, it was not possible to detect this positive effect over health-related quality of life in the SIG when only obese women were analyzed. More research is warranted in this regard.

Our study has some limitations. First, serum or urine levels of soy isoflavones were not measured and hence it is uncertain how much of the soy protein was actually absorbed. Despite this, diet was similar in both groups and well controlled throughout the study and the soy isoflavone dose is a widely used one in clinical practice. Second, the sample was obtained from the gynecological outpatient clinic and consisted of mid-aged women with intact uterus and ovaries, sexually

active, and non-HT users, a population which could in fact differ from the general menopausal population, leading to overstated and misleading results. Finally, although the results of comparing women according to BMI values were interesting, one must bear in mind that non-obese women were in fact overweight and that small numbers per group could have decreased statistical power. In any case, more research is warranted in this regard.

Despite these limitations, our data showed that changes in glucose homeostasis, induced by a program encouraging lifestyle changes through physical exercise and dieting in addition to a daily oral isoflavone intake, were not associated with changes in plasma leptin levels. The decreases in circulating levels of serum TNF- α , fat mass and Kupperman Index in the SIG could have, in fact, played a possible role in the positive changes observed in insulin sensitivity. Further studies aimed at an improved understanding of these changes are necessary. Prospective trials with larger sample size and longer follow-up are required to further confirm our results and to determine the efficacy and safety of these interventions.

CONCLUSIONS

Diet, physical exercise and a daily oral soy isoflavone intake exerted a beneficial effect on the homeostatic model in postmenopausal women which was not related to significant changes in plasma leptin levels, despite a decrease in serum TNF- α , fat mass and Kupperman Index values.

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

Source of funding Nil.

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