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Abstract

Background:
Cardiovascular diseases are currently the commonest cause of death worldwide. Different strategies for their primary prevention have been planned, taking into account the main known risk factors, which include an atherogenic lipid profile and visceral fat excess.
Methods:
The study was designed as a randomized, parallel, single-center study with a nutritional intervention duration of 12 weeks. Whole soy foods corresponding to 30 g/day soy protein were given in substitution of animal foods containing the same protein amount.
Results:
The soy nutritional intervention resulted in a reduction in the number of MetS features in 13/26 subjects. Moreover, in the soy group we observed a significant improvement of median percentage changes for body

	weight (−1.5 %) and BMI (−1.5 %), as well as for atherogenic lipid markers, namely TC (−4.85 %), LDL-C (−5.25 %), non-HDL-C (−7.14 %) and apoB (−14.8 %). Since the majority of the studied variables were strongly correlated, three factors were identified which explained the majority (52 %) of the total variance in the whole data set. Among them, factor 1, which loaded lipid and adipose variables, explained the 22 % of total variance, showing a statistically significant difference between treatment arms ($p = 0.002$). *Conclusions:* The inclusion of whole soy foods (corresponding to 30 g/day protein) in a lipid-lowering diet significantly improved a relevant set of biomarkers associated with cardiovascular risk.
Keywords (separated by '-')	Soy protein - Lipids - Metabolic syndrome and obesity
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ORIGINAL CONTRIBUTION

Effect of soy on metabolic syndrome and cardiovascular risk factors: a randomized controlled trial

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Abstract

Background Cardiovascular diseases are currently the commonest cause of death worldwide. Different strategies for their primary prevention have been planned, taking into account the main known risk factors, which include an atherogenic lipid profile and visceral fat excess.

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Conclusions The inclusion of whole soy foods (corresponding to 30 g/day protein) in a lipid-lowering diet significantly improved a relevant set of biomarkers associated with cardiovascular risk.

Keywords Soy protein · Lipids · Metabolic syndrome and obesity

Abbreviations

ApoB	Apolipoprotein B
ApoA-I	Apolipoprotein A-I
BMI	Body mass index
BIA	Bioelectrical impedance analysis
CRP	High-sensitivity C-reactive protein
CVD	Cardiovascular diseases
DBP	Diastolic blood pressure
FPG	Fasting plasma glucose
HC	Hip circumference
HDL-C	High-density lipoprotein cholesterol
HOMA	Homeostatic model assessment
HR	Heart rate
LDL-C	Low-density lipoprotein cholesterol
Lp(a)	Lipoprotein (a)
N	Number
PCSK9	Proprotein convertase subtilisin/kexin 9

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SBP	Systolic blood pressure
sICAM-1	Soluble intercellular adhesion molecular 1
TC	Total cholesterol
TG	Triglycerides
VFR	Visceral fat rating
WC	Waist circumference

Introduction

Cardiovascular diseases (CVD) are currently the commonest cause of death worldwide (WHO January 2015; [1]), and different strategies for their primary prevention have been planned, taking into account the main known risk factors, which include an atherogenic lipid profile and abdominal/visceral fat excess [2–4]. Total abdominal adipose tissue may be subdivided into subcutaneous-abdominal compartment and intra-abdominal compartment. This latter, also referred to as 'visceral fat,' is associated with insulin resistance and the specific features of the metabolic syndrome (MetS) [5], which also includes the combination of dyslipidemia, hyperglycemia or type 2 diabetes mellitus and hypertension, in association with insulin resistance and systemic inflammation [6, 7].

The biochemical factors involved in increased primary CVD risk associated with these features include elevated free fatty acid flux to the liver, altered adipokine production and altered HDL level and distribution into different subclasses [3], resulting in a proatherogenic environment. In particular, MetS has been associated with increased small HDL-3 and reduced large HDL-2 particles [8]. All these risk factors can be a consequence of dietary habits and may therefore be influenced by diet and lifestyle modifications. Functional foods [9] and nutraceuticals [10] have been assessed in several clinical studies, and meta-analytical reports have indicated these as effective approaches for the management of primary CVD risk in the MetS [11, 12]. Within this context, numerous randomized controlled trials (RCTs) and some meta-analyses [13, 14] have shown that a regular consumption of soy protein improves circulating lipid parameters. More specifically, the inclusion of purified soy protein in the range of 15–40 g/day into the diet of adults with normal or moderately elevated total cholesterol (TC) resulted in a significant reduction in TC (at least −4 %) and LDL-cholesterol (LDL-C, about −6 %) [13, 15–17]. In addition, dietary intake of soy protein reduced body weight in overweight and obese subjects, compared to diets containing animal protein [18, 19], although data on soy protein impact on overall fat mass reduction and abdominal adipose changes [20], as well as on circulating adipokine levels [21], are scanty and controversial.

The majorities of published studies on soy protein have evaluated the effect of purified protein included in the daily diet, without changes of the percent caloric intake from protein.

It should be, however, pointed out that patients do not eat nutrients such as purified soy protein; thus, an approach based on whole soy foods, possibly commercially available, appears to be most desirable [22]. It must be, however, noted that in other conditions, the whole soy food approach has shown differences in effects when compared to the isolated components [23]. To our knowledge, the effects of commercially available whole soy foods on the cardiometabolic parameters of the metabolic syndrome have never been evaluated. The present study, with an RCT design, aimed to assess the effects of a low-lipid diet with whole soy foods, on abdominal adipose tissue and related adipokines, lipid/lipoprotein profiles and glucose metabolism, and to compare them with the effects of standard low-lipid diet with animal protein.

Materials and methods

Ethical issues

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and its later amendments. The study was approved by the ethics committee of A.S.S.T Grande Ospedale Metropolitano Ospedale Niguarda (approval no 170_04/2012).

Study design and population

The study was performed at the Centro Dislipidemie (A.S.S.T Grande Ospedale Metropolitano Ospedale Niguarda, Milan, Italy) in the period March 2013–June 2015 following a randomized, parallel, controlled, single-center design. Study subjects were followed at the Centro Dislipidemie and were used to consume a lipid-lowering diet. Inclusion criteria were: males and postmenopausal females aged between 45 and 75 years; BMI within the 25–30 kg/m^2 range; and LDL-C levels in the 130–190 mg/dL range. Additionally, volunteers had to fulfill 3/5 features of the metabolic syndrome criteria, namely waist circumference (WC) ≥102 cm (M) or ≥88 cm (F); blood pressure (BP) ≥130/85 mmHg; fasting glycemia (FPG) ≥100 mg/dL; triglycerides (TG) ≥150 mg/dL; HDL-C <40 mg/dL (M); and <50 mg/dL (F) [24]. All study subjects fulfilled 3 or 4 MetS criteria; none met all 5 criteria.

The exclusion criteria were: the presence of chronic liver disease, renal disease or severe renal impairment, untreated arterial hypertension, obesity (BMI ≥30 kg/m^2), any past history of cerebro-vascular accident or coronary events, including unstable angina, myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary

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artery bypass graft; subjects affected by any kind of food allergy; any concomitant therapy known to alter any of the parameters to be assessed; history of or current alcohol or drug abuse; any clinically significant medical condition that could interfere with the conduct of the study; known or suspected diagnosis of hepatitis or HIV infection; subjects unable or unwilling to comply with the protocol requirements, or deemed by the investigator to be unfit for the study; and patients who were enrolled in another research study in the last 90 days. All patients were in primary prevention, were free from liver/kidney disorders potentially affecting the response to treatment and did not take any drug affecting lipid/lipoprotein or glycemic profile, including thiazolidinediones or corticosteroids. Concomitant medications are reported in Table 1.

Clinical evaluations

Clinical and biochemical evaluations were performed at the beginning and at the end of the treatment period. At all visits, patients underwent a fasting venous blood sampling and a full clinical examination, including the evaluation of height, body weight, heart rate and arterial blood pressure. The ViScan device (Tanita Inc., Tokio, Japan) is a validated tool to assess waist circumference (WC) [25] and abdominal fat mass [26, 27] by bioelectrical impedance analysis (BIA). WC was measured by the ViScan device (supine position, WC_{ViScan}) and by means of a non-stretchable tape at the umbilical level (standing position, WC). Hip circumference (HC) was assessed by tape. ViScan was also used to evaluate bioelectrical impedance analysis (BIA) % and visceral fat rating (VFR) %. The reproducibility of ViScan was measured with a rigid human phantom (waist 65 cm, hip 90 cm).

All visits were performed by the same investigator (PM), and all ViScan analyses were conducted by the same operator (RB). Plasma samples were prepared by low-speed centrifugation, and aliquots were immediately stored at −20 °C for subsequent assays. Safety and compliance information were collected at each visit, also by means of 24-h dietary recalls, relative to 3 non-consecutive days for each month of nutritional intervention. Data retrieval, analysis and manuscript preparation were solely the responsibility of the authors.

Table 1 Concomitant medications (unchanged over the entire study duration)

Medication	Patients (%)
ACE-I/ARB	1.9
Beta blockers	9.4
Diuretics	22.6
Calcium antagonists	1.9
Allopurinol	1.9
Proton-pump inhibitors	13.2
Other drugs	47.2

Intervention

After enrollment, they were instructed to follow a normocaloric/low-lipid diet, designed according to the Mediterranean diet criteria [28], with three main meals and two snacks and adapted to individual preferences in order to improve patient compliance. Extra virgin olive oil in moderate quantity was suggested as topping. Dietary plans were defined with the aid of a dedicated software (Dietosystem, DS Medica srl, Milan, Italy). Diet composition was different for male and female subjects as shown in Table 2. Subjects were then randomly assigned to receive either the experimental diet, containing whole soy foods corresponding to 30 g/day soy protein in substitution of animal foods containing the same amount of protein, or the control diet containing the animal foods, for 12 weeks (Fig. 1). The total daily amount of protein was 1 g/kg for all diets. In order to have a constant total energy intake over the intervention period, personalized recommendations were given to each participant during each visit, according to three 24-h dietary recalls.

Table 2 Energy and macronutrient content of the soy food diet and the control diet used in the study

Variable	Soy		Control	
	Male	Female	Male	Female
Energy (kcal/d)	1809.2	1520.5	1770.4	1493.0
Carbohydrate (g/d)	261.4 (54.2 %)	209.4 (51.6 %)	270.5 (57.3 %)	223.8 (56.2 %)
Protein (g/d)	77.1 (17.0 %)	56.7 (14.9 %)	70.6 (15.9 %)	62.6 (16.8 %)
Total fat (g/d)	55.8 (27.8 %)	54.5 (32.3 %)	52.8 (26.8 %)	44.8 (27 % %)
Saturated fat (g/d)	7.5	8.5	10.4	7.3
Unsaturated fat (g/d)	17.2	15.8	6.7	5.3
Monounsaturated fat (g/d)	25.8	26.5	30.7	28.7
Cholesterol (mg/d)	21.2	7.6	100.5	113.4

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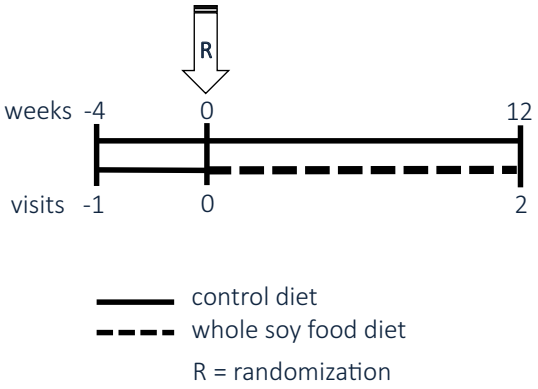

Fig. 1 Schematic representation of the trial design

Characteristics of the soy diet

The soy diet was composed by different commercial soy foods from a portfolio including soy nuggets, soy burgers, soy desserts (different flavorings) and soy drinks (different flavorings), all provided by Alpro (Belgium). The composition of these products is shown in supplementary Table S1. In order to reach the necessary daily intake of soy foods, corresponding to 30 g soy protein, the subjects should consume 3–4 servings per day, distributed in different meals as indicated in supplementary Table S2 for a better compliance. At the beginning of each month, each subject received at home a bag containing all the soy foods necessary for the following 30 days.

Biochemical and immunometric assays

In each blood sample, total cholesterol (TC), TG, HDL-C, lipoprotein(a) (Lp(a)), apolipoprotein (apo)A-I, apoB, C-reactive protein (CRP), fasting glycemia (FPG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT) and creatine phosphokinase (CPK) were measured according to standard clinical procedures. LDL-C was calculated according to the Friedewald equation. Non-HDL-C was calculated as TC minus HDL-C [29]. Commercial enzyme-linked immunosorbent assay (ELISA) kits were used according to manufacturer's specifications and previously published protocols to quantify plasma leptin [30], adiponectin, soluble intercellular adhesion molecule-1 (sICAM-1), PCSK9 (all from R&D System [31], Minneapolis, MN) and insulin (Mercodia, Uppsala, Sweden). The Homeostasis Model Assessment of Insulin Resistance (HOMA) index was calculated. The plasma concentration of HDL particles containing only apoA-I (LpA-I) and of particles containing both apoA-I and apoA-II (LpA-I:A-II) was determined by electroimmunodiffusion in agarose gel using a commercial kit (Sebia, Lisses, France) [32]. The content of discoidal prebeta-migrating HDL was evaluated by non-denaturing two-dimensional electrophoresis followed by immunodetection against human apoA-I [33]. The content of prebeta-HDL was calculated as percentage of total apoA-I signal. HDL subclass distribution according to particle size was determined by non-denaturing polyacrylamide gradient gel electrophoresis (4–30 %) of the $d < 1.21$ g/mL plasma total lipoprotein fraction; the protein-stained gels were scanned with an imaging densitometer to determine particle size and HDL were divided into small (diameter 7.2–8.2 nm), medium (diameter 8.2–8.8 nm) and large (diameter 8.8–12.7 nm) particles [32]. Densitometric analyses were performed with the GS-690 Imaging Densitometer and the Multi-Analyst software (Bio-Rad Laboratories, Hercules, CA).

Chemicals for isoflavones analysis

Daidzein (97 % purity) and genistein (97 % purity) were from Lancaster Synthesis (Morecambe, UK); deuterated daidzein (2′,3′,5′,6′-d4, 98 % purity), deuterated genistein (2′,3′,5′,6′-d4, 98 % purity) and equol (≥98 % purity) were from Cayman Chemicals (Milan, Italy). Dihydrogenistein (DHG, 98 % purity) was from Alfachem (Milan, Italy). The hydrolytic enzyme mixture containing sulfatase and *β*-glucuronidase from *Helix pomatia* (glucuronidase activity 400 units/g, sulfatase activity less than 40 units/g), sodium citrate, ammonium bicarbonate and methanol was from Sigma-Aldrich (Milan, Italy).

Isoflavone extraction from human serum and HPLC-CHIP ESI–MS analysis

The extraction of isoflavones and their metabolites was performed according to our published method [34]. The quantitative analysis was performed using an Agilent 1200 Series Nanoflow LC system. The Agilent HPLC-Chip/MS was interfaced to an Agilent SL series ion trap (Agilent, CA). The intra-assay variations reported as RDS % were within the range 1.8–6.7 % (Table 6). For more details, see Supplementary Materials and Methods.

Sample size and statistical analysis

Results are presented as median and interquartile ranges (Q1 and Q3) for all parameters. The differences from treatment arms at baseline were assessed by Wilcoxon-rank sum test. Chi-square test was applied to evaluate the difference in frequencies among arms. The difference by treatment arms as changes from baseline [12-week treatment–baseline (0 week)] was evaluated by ANCOVA adjusted for baseline, age and sex. Data were also

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expressed as median of changes between [12-week treatment and baseline (0 week)] and quartiles (supplementary Table S3). Residuals from full models, investigating factors variations, were checked to assess normal distribution. Principal components analysis was performed, and the scree plot of ordered eigenvalues of a correlation matrix was used to decide the appropriate number of factors extracted. Only variables with loading ≥0.40 were considered for interpretation. Finally, we checked whether the scores of factors obtained were significantly different between the two treatment arms. Statistical analysis was performed by using the SAS software v. 9.2 (SAS Inc., Cary, NC). A group sample size of 26 per arm achieves 80 % power to detect a difference of 20 mg/dL in absolute changes (12–0 week) in LDL levels (mg/mL) between the null hypothesis that both arms means of change in LDL are 10 mg/mL and the alternative hypothesis that the mean of change in LDL in the treatment arms is −10.0 mg/mL with estimated group standard deviations of 25.0 mg/mL per arm and with a significance level of 5 % using a two-sided two-sample *t* test.

Results

Study population

After a run-in period of 4 weeks on a balanced low-lipid diet, only subjects showing changes in total cholesterol <10 % were recruited for the study. Sixty-two subjects (32 M, 30 F) were assessed for eligibility, 6 were excluded, and 56 (29 M, 27 F) were enrolled into the study and randomly allocated to either the soy diet ($N = 28$; 14 M, 14 F) or the control diet ($N = 28$; 15 M, 13 F), for a total intervention duration of 12 weeks. Of them, 27/28 subjects completed the control diet arm and 26/28 completed the soy diet arm (Fig. 2). Wilcoxon-rank sum test indicates that at baseline all clinical and biochemical values, including lipids, adipokines and inflammatory markers, were similar between the two treatment arms (Table 3). Fifty-three volunteers, gender and age matched, were included, and 13.2 % of them were smokers. Men were 55 % in the control arm and 50 % in the soy arm; median age was 60 years in both arms. As reported in Table 4, BMI (27.3 kg/m^2 in the AQ3

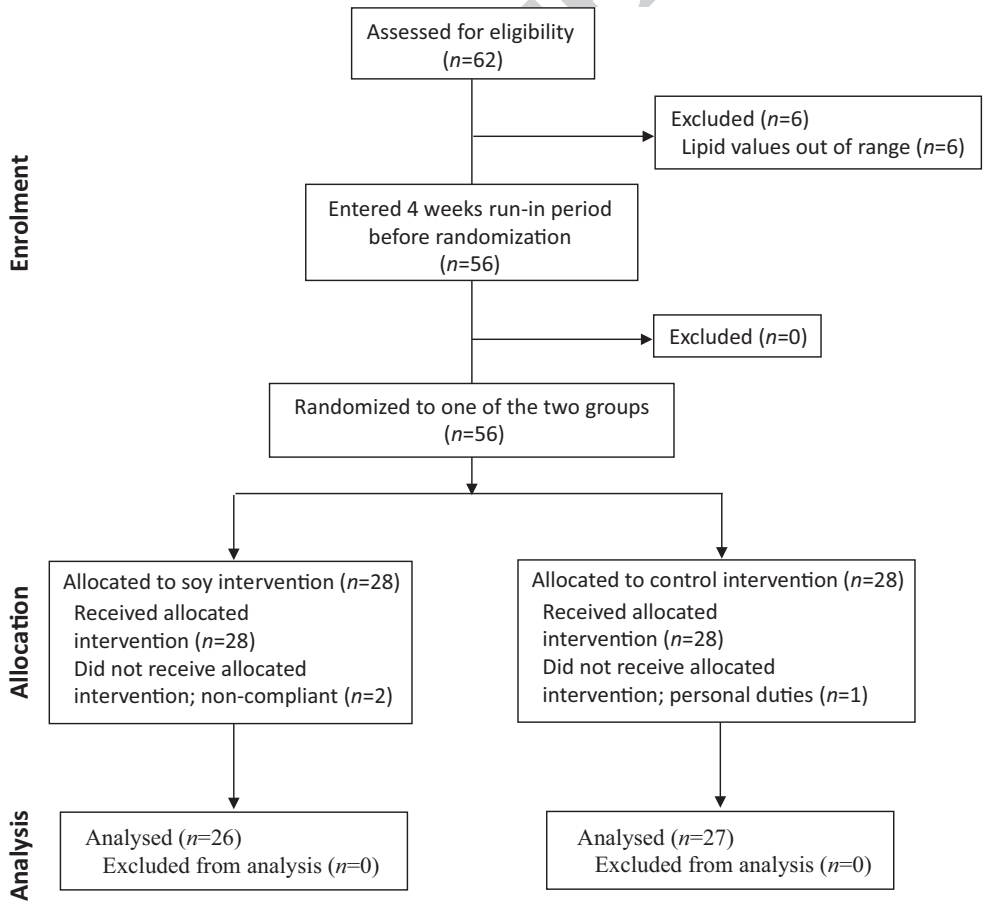

Fig. 2 Consort flow diagram

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Table 3 Main baseline clinical and biochemical characteristics of the study population

Parameter	Value	*p* value
No. of participants (men/women)	53 (28/25)	–
Smokers, *n* (%)	7 (13.2)	–
Age, years	58.9 (55.5, 66.3)	0.89
SBP (mmHg)	125 (120, 132.5)	0.20
DBP (mmHg)	80 (77.5, 87.5)	0.85
HR (bpm)	66 (64, 74)	0.97
Weight (kg)	76.0 (69, 81)	0.51
BMI (kg/m^2)	27.8 (25.8, 29.6)	0.31
WC_{TAPE} (cm)	97.0 (93.5, 103.5)	0.26
HC_{TAPE} (cm)	99.0 (94, 102)	0.56
WC_{TAPE}:HC_{TAPE}	1.0 (0.9, 1.0)	0.31
WC_{VSCAN} (cm)	104.5 (9.7, 113)	0.98
BIA (%)	40.6 (34, 45.8)	0.67
VFR (%)	13.0 (11.3, 17.0)	0.86
Leptin (ng/mL)	14.2 (6.8, 22.4)	0.31
Adiponectin (μg/mL)	5.9 (4.4, 9.7)	0.68
Leptin:adiponectin	2.1 (1.2, 3.3)	0.09
TC (mg/dL)	254.2 (227.5, 274.6)	0.42
LDL-C (mg/dL)	168.0 (141.8, 186.5)	0.57
HDL-C (mg/dL)	45.6 (38.5, 50.5)	0.52
Non-HDL-C (mg/dL)	208.1 (186.1, 231.2)	0.36
Lp(a) (mg/dL)	16.0 (6.0, 25.0)	0.61
TG (mg/dL)	193.0 (143.3, 240.4)	0.37
ApoB (mg/dL)	155.0 (141.5, 172)	0.11
ApoA-I (mg/dL)	115.0 (110, 123.5)	0.63
PCSK9 (ng/mL)	289.6 (243.6, 333.6)	0.66
ApoB:apoA-I	1.3 (1.2, 1.5)	0.36
ApoB:PCSK9	0.54 (0.4, 0.6)	0.06
FPG (mg/dL)	94.0 (87.3, 104.1)	0.37
Insulin (mU/L)	7.66 (6.2, 14.0)	0.61
HOMA-IR	1.9 (1.4, 3.8)	0.48
sICAM-1(ng/mL)	260.2 (230, 294.)	0.36
CRP (mg/dL)	0.187 (0.1, 0.3)	0.81

Values are expressed as median (interquartile range, Q1 and Q3). *p* values were assessed by Wilcoxon-rank sum test and represent differences between median values at baseline between the two arms

SBP Systolic blood pressure, *DBP* diastolic blood pressure, *HR* heart rate, *BMI* body mass index, *WC* waist circumference, *HC* hip circumference, *BIA* bioelectrical impedance analysis, *VFR* visceral fat rating, *TC* total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TG* triglycerides, *Lp(a)* lipoprotein (a), *apoB* apolipoprotein B, *apoA-I* apolipoprotein A-I, *FPG* fasting plasma glucose, *sICAM-1* soluble intercellular adhesion molecular 1, *CRP* high-sensitivity C-reactive protein

control group and 28.2 kg/m^2 in the soy group) indicated that study subjects were overweight with a relevant abdominal adiposity, as evaluated by WC, WC_{Viscan} (104 cm in the control group and 105 cm in the soy group), HC and WC:HC ratio (1.00 in both arms). Subjects also had a moderate dyslipidemia and met 3 or 4 out of 5 MetS criteria. No signs of hypertension (SBP, 125 mmHg in both arms) and of relevant systemic low-grade inflammation (CRP, 0.2 mg/dL in both arms) were detected.

Effect of soy diet

At the end of the treatment period, 50 % (13/26) of subjects on soy food showed a reduction in the number of MetS features. In the control group, 26 % (7/27) of subjects showed a reduction in MetS feature number. The difference in frequencies (Chi-square test) among arms was $p = 0.094$. A significant reduction in weight (median percentage change: −1.5 %; $p = 0.005$) and BMI (median percentage change: −1.5 %; $p = 0.05$), after adjustment for age and sex, was noted in the soy food arm (Table 4). No differences were instead recorded between the two groups for abdominal adipose tissue variables (WC, HC, WC:HC, BIA % and VFR %) and related adipokines, namely leptin and adiponectin. This lack of significant changes in visceral adipose and related biomarkers was paralleled by unaffected glucose metabolism (FPG, insulin and HOMA) and inflammation (sICAM-1 and CRP) parameters.

A 12-week (wk) lipid/lipoprotein changes were characterized by significantly reduced TC ($p = 0.002$), LDL-C ($p = 0.01$) and non-HDL-C ($p = 0.007$) in the soy food group versus the control group, with median percentage changes for TC = −4.85 %, LDL-C = −5.25 % and non-HDL-C = −7.14 %. These were not linked to BMI changes as assessed by ANCOVA adjusted for the confounding factors; p values were 0.10 for TC, 0.45 for LDL-C and 0.08 for non-HDL-C. Conversely, these lipid markers showed a percentage median increment (TC = 4.6 %, LDL-C = 5.7 % and non-HDL-C = 4.2 %) in the control group. Overall, results were not influenced by median changes recorded during the 4-week run-in period (−4 and 0 week). Specifically, these were +4.2 mg/dL ($p = 0.78$) for TC; −1.7 mg/dL ($p = 0.57$) for LDL-C; and +4 mg/dL ($p = 0.88$) for non-HDL-C. ApoB, apoAI and LpA-I levels were also significantly modified by soy food consumption. Percentage changes of these parameters were −14.8 % (apoB; $p = 0.019$), −5 % (apoAI; $p = 0.008$) and −3.8 % (LpA-I; $p = 0.02$). No significant differences were found between the two groups for TG, Lp(a) and PCSK9 values.

Plasma levels of HDL-C and HDL subclass distribution (discoidal pre-migrating HDL, small, medium and large HDL, HDL2, HDL3), as well as that of apoA-I-containing HDL subclass LpA-I:A-II, were not modified by soy food consumption (supplementary Table S4).

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Table 4 Summary of primary and secondary end points

	Soy diet						Control diet						Difference between treatment arms
	Basal			12 weeks			Basal			12 weeks			
	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	*p* value
SBP (mmHg)	125.0	120.0	135.0	120.0	120.0	130.0	125.0	110.0	130.0	120.0	120.0	130.0	0.28
DBP (mmHg)	80.0	75.0	85.0	80.0	80.0	85.0	80.0	80.0	90.0	80.0	80.0	90.0	0.80
HR (bpm)	67.0	60.0	72.0	66.0	60.0	72.0	64.0	64.0	76.0	68.0	64.0	72.0	0.39
WC (cm)	99.5	95.0	106.0	97.5	94.0	103.0	97.0	93.0	102.0	97.0	93.0	102.0	0.35
WC_{vscan} (cm)	105.0	98.0	112.0	101.0	97.0	109.0	104.0	97.0	102.0	103.0	97.0	110.0	0.45
HC (cm)	99.5	94.0	102.5	97.7	95.0	103.0	98.0	94.0	101.0	98.0	95.0	101.0	0.76
WC_{Tape}:HC_{Tape}	1.0	0.9	1.0	1.0	0.9	1.0	1.0	0.9	1.0	1.0	0.9	1.0	0.39
BIA (%)	41.6	36.0	45.4	40.9	33.0	46.4	39.1	34.0	46.3	39.5	32.3	45.0	0.37
VFR (%)	12.5	11.5	16.5	12.7	11.0	15.5	13.0	11.0	17.5	12.5	10.0	16.0	0.42
Weight (Kg)	77.5	70.0	81.0	75.5	70.0	80.0	75.0	68.0	81.0	76.0	66.0	82.0	**0.005**
BMI (Kg/m^2)	28.2	26.4	29.8	27.2	25.8	29.2	27.3	25.4	29.4	27.1	25.7	29.1	**0.05**
Leptin (ng/mL)	16.5	8.26	29.0	13.5	5.1	25.8	12.8	5.86	21.8	13.2	6.9	18.1	0.38
Adiponectin (mg/dL)	5.59	4.26	10.2	6.3	4.3	9.7	6.57	4.95	9.23	7.9	4.9	9.8	0.99
Leptin:Adiponectin	2.72	1.60	3.97	2.3	1.3	3.5	1.85	1.16	2.63	1.9	0.9	2.6	0.88
TC (mg/dL)	256.0	231.8	276.0	236.7	221.0	251.8	251.9	221.0	272.0	250.0	234.0	271.6	**0.002**
LDL-C (mg/dL)	169.4	139.4	189.0	154.7	138.6	173.4	164.4	144.2	181.6	171.0	148.0	190.1	**0.01**
HDL-C (mg/dL)	44.3	40.0	47.0	44.0	38.8	49.0	47.0	37.5	52.0	46.0	40.1	51.0	0.26
Non-HDL-C	213.5	186.2	234.0	186.5	177.0	212.4	208.0	185.4	219.0	199.0	188.5	230.1	**0.007**
Lp(a) (mg/dL)	16.5	6.0	25.0	17.0	9.0	30.0	11.0	5.0	25.0	11.0	6.0	24.0	0.23
PCSK9 (ng/dL)	297.6	236.6	334.6	268.9	228.9	334.6	285.3	256.6	330.7	305.6	245.9	336.3	0.52
ApoB (mg/dL)	159.5	148.0	185.0	139.5	127.0	153.0	155.0	127.0	165.0	139.0	133.0	165.0	**0.019**
ApoB:PCSK9	0.6	0.5	0.6	0.5	0.4	0.6	0.52	0.42	0.58	0.5	0.4	0.6	0.76
apoAI (mg/dL)	114.0	111.0	125.0	108.0	101.0	115.0	115.0	107.0	122.0	115.0	109.0	121.0	**0.008**
ApoB:apoAI	1.4	1.3	1.5	1.3	1.1	1.4	1.3	1.1	1.5	1.2	1.1	1.5	0.86
TG (mg/dL)	206.5	162.0	243.7	169.6	120.0	233.4	190.0	121.4	237.0	146.0	123.5	213.1	0.94
FPG (mg/dL)	97.0	88.0	104.1	93.4	86.5	109.3	92.0	86.5	100.4	96.0	87.9	106.0	0.23
Insulin (UI/L)	8.20	6.3	14.7	7.7	5.6	10.7	7.50	6.10	11.5	7.5	6.0	10.7	0.90
HOMA	2.1	1.6	4.1	2.1	1.4	2.5	1.9	1.4	2.6	1.9	1.4	3.3	0.85
CRP (mg/dL)	0.2	0.1	0.3	0.2	0.1	0.3	0.2	0.1	0.3	0.2	0.1	0.3	0.59
sICAM	255.9	221.9	289.8	238.4	223.4	274.4	226.9	239.7	299.2	263.4	247.7	287.1	0.43

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Table 4 continued

	Soy diet						Control diet						Difference between treatment arms
	Basal			12 weeks			Basal			12 weeks			
	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	*p* value
LpAI	38.0	35.0	44.0	37.0	33.0	40.0	38.0	31.0	41.0	77.0	70.0	81.0	**0.02**
LpAI:AII	78.0	70.0	87.0	68.5	64.0	77.0	78.0	74.0	89.0	41.0	34.0	45.0	0.10

Values are expressed as median (interquartile range, Q1 and Q3). The differences between treatment arms (median changes) were evaluated by ANCOVA adjusted for baseline, age and sex

SBP Systolic blood pressure, *DBP* diastolic blood pressure, *HR* heart rate, *BMI* body mass index, *WC* waist circumference, *HC* hip circumference, *BIA* bioelectrical impedance analysis, *VFR* visceral fat rating, *TC* total cholesterol, *LDL-C* low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, *TG* triglycerides, *Lp(a)* lipoprotein (a), *apoB* apolipoprotein B, *apoA-I* apolipoprotein A-I, *FPG* fasting plasma glucose, *sICAM-1* soluble intercellular adhesion molecular 1, *CRP* high-sensitivity C-reactive protein

Table 5 Results of factor analysis of all studied subjects

	Factors		
	1	2	3
TC	**0.87**	0.25	−0.09
ApoB	**0.77**	0.26	−0.17
LDL	**0.64**	0.18	0.05
BMI	**0.52**	−0.34	0.31
apoAI	**0.43**	0.27	0.05
WC_{vscan}	**0.41**	−0.22	−0.08
Leptin	**0.40**	−0.09	0.10
Non-HDL-C	**−0.43**	−0.10	0.24
HOMA	0.09	**0.79**	0.20
Insulin	−0.01	**0.72**	0.29
FPG	0.27	**0.40**	−0.17
VFR (%)	**0.44**	**−0.60**	−0.01
BIA (%)	**0.47**	**−0.68**	0.17
WC	0.26	−0.12	**0.71**
HC	0.14	0.03	**0.55**

Factor analysis

Since the majority of the studied variables were strongly correlated, to reduce them to a smaller set of latent or underlying independent factors, factor analyses were applied. Three factors were identified which explained the majority (52 %) of the total variance in the whole data set. As shown in Table 5, the factor with the highest loading scores (≥0.40), which were those describing lipid and adipose features, was the most influential factor explaining the 22 % of the total variance (52 %) (factor 1). In particular, the lipid parameters were described by TC (loading score 0.87), apoB (loading score 0.77), LDL (loading score 0.64) and the adipose ones by BMI (loading score 0.52), total abdominal fat (BIA %, loading score 0.44) and abdominal cavity (VFR %, loading score 0.41). Factor 2 had positive loading of HOMA (0.79) and insulin (0.72) and a negative one of BIA % (−0.68) and VFR % (−0.60). The third factor was characterized by positive loadings for WC (0.71) and HC (0.51) (Table 6). Notably, we found that scores of obtained factors were significantly different between the two treatment arms only for factor 1 ($p = 0.002$, corrected for age and sex) (Fig. 3).

Safety, tolerability and compliance

The nutritional intervention with either soy or control food items for 12 weeks was well tolerated by all participants, and no specific adverse effects were reported. No changes in liver function and thyroid parameters were detected after the nutritional intervention with soy foods, which were

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Table 6 Serum isoflavone metabolites (soy food group)

	Gender	Equol (μM)	RSD (%)	Daidzein (μM)	RSD (%)	DHD (μM)	RSD (%)	Genistein (μM)	RSD (%)	DHG (μM)	RSD (%)
A	M	n.d.	–	<LOQ	–	n.d.	–	<LOQ	–	n.d.	–
B	F	<LOQ	–	0.151 ± 0.049	4.5	<LOQ	–	<LOQ	–	n.d.	–
B	M	<LOQ	–	0.079 ± 0.035	3.6	<LOQ	–	<LOQ	–	<LOQ	–
B	M	<LOQ	–	0.253 ± 0.063	3.7	<LOQ	–	<LOQ	–	n.d.	–
B	F	<LOQ	–	0.063 ± 0.007	5.1	<LOQ	–	<LOQ	–	n.d.	–
C	M	n.d.	–	0.343 ± 0.022	2.0	n.d.	–	0.007 ± 0.001	4.1	<LOQ	–
C	F	n.d.	–	0.080 ± 0.015	1.9	n.d.	–	0.192 ± 0.093	2.7	<LOQ	–
C	M	<LOQ	–	0.722 ± 0.038	4.6	<LOQ	–	0.051 ± 0.012	3.8	n.d.	–
C	M	<LOQ	–	0.097 ± 0.023	2.4	<LOQ	–	0.036 ± 0.009	4.7	n.d.	–
C	F	n.d.	–	0.063 ± 0.014	1.8	n.d.	–	0.328 ± 0.183	4.8	<LOQ	–
C	M	n.d.	–	0.074 ± 0.018	3.0	<LOQ	–	0.039 ± 0.012	5.1	n.d.	–
C	F	n.d	–	0.082 ± 0.030	2.6	n.d.	–	0.034 ± 0.016	3.0	n.d.	–
C	F	<LOQ	–	0.103 ± 0.041	5.3	<LOQ	–	0.005 ± 0.001	5.0	<LOQ	–
C	F	<LOQ	–	0.068 ± 0.027	6.7	<LOQ	–	0.255 ± 0.168	5.1	<LOQ	–
C	M	<LOQ	–	0.096 ± 0.023	6.4	<LOQ	–	0.050 ± 0.010	4.7	n.d.	–
C	M	<LOQ	–	0.010 ± 0.003	5.4	<LOQ	–	0.077 ± 0.012	4.3	n.d.	–
C	M	< LOQ	–	0.090 ± 0.020	3.7	n.d.	–	0.190 ± 0.057	3.0	n.d.	–
C	M	<LOQ	–	0.090 ± 0.036	3.4	<LOQ	–	0.102 ± 0.041	3.9	<LOQ	–
C	F	<LOQ	–	0.075 ± 0.023	5.9	<LOQ	–	0.016 ± 0.005	4.8	<LOQ	–
C	F	n.d.	–	0.069 ± 0.040	3.4	n.d.	–	0.032 ± 0.007	4.0	n.d.	–
	F	<LOQ	–	0.072 ± 0.011	6.1	<LOQ	–	0.127 ± 0.015	5.6	<LOQ	–
	M	n.d.	–	0.070 ± 0.013	5.6	n.d.	–	0.014 ± 0.008	5.0	<LOQ	–
	F	n.d.	–	0.072 ± 0.187	6.4	n.d.	–	0.034 ± 0.009	4.6	n.d.	–
	F	<LOQ	–	0.087 ± 0.007	4.3	0.066 ± 0.013	4.9	0.102 ± 0.011	6.1	n.d.	–
	M	n.d.	–	0.022 ± 0.009	4.7	n.d.	–	0.231 ± 0.087	6.4	0.124 ± 0.051	2.9
	F	n.d.	–	0.068 ± 0.013	3.2	<LOQ	–	0.094 ± 0.012	3.4	0.070 ± 0.01	3.2

n.d., lower than the limit of detection; < LOQ, lower than the limit of quantification; RSD, intra-assay variations

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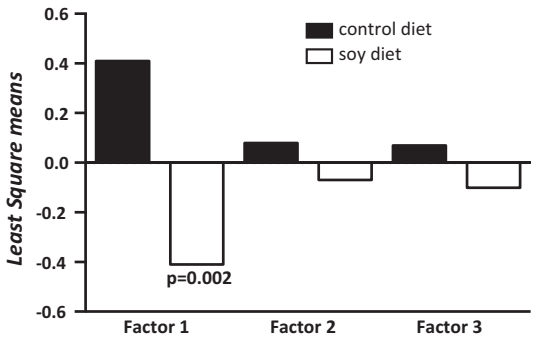

Fig. 3 Effect of soy treatment on the scores of the obtained factors

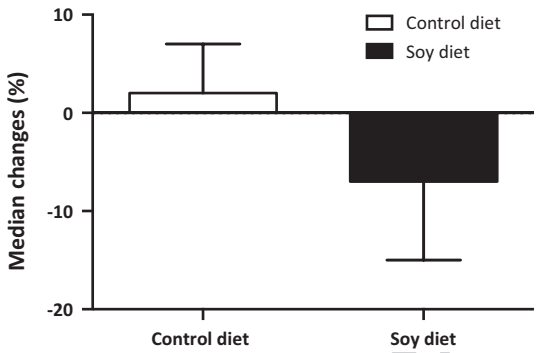

Fig. 4 Percentage median changes of non-HDL-C. Data are expressed as median with range

well accepted by all subjects. The compliance toward both diets was well above 95 %, according to food diary analysis. To assess the compliance, circulating isoflavones and their metabolites were quantified in the soy food group subjects. Isoflavone concentrations at baseline were under the LOD or LOQ of the analytical method (data not shown). At the end of the dietary intervention, quantifiable amounts of daidzein were detected in 25 subjects, of genistein in 22 subjects, of DHG in 2 subjects and of DHD in 1 subject, whereas equol remained always under the limit of quantification (Table S5). Hence, patients were clustered according to different metabolic pathways. One male (cluster A) did not show any quantifiable metabolite, since even daidzein and genistein, the two main isoflavones, were under the LOQ. In subjects (2 M and 2 F) included in cluster B, only daidzein was quantifiable with concentrations ranging between 0.063 and 0.253 μM, whereas in subjects (9 M and 9 F) included in cluster C it was possible to detect either daidzein, in the range from 0.010 to 0.722 μM, or genistein, in the range between 0.007 and 0.328 μM. One female (cluster D) besides daidzein and genistein showed also DHD, a metabolite of daidzein (0.066 μM), while in 2 subjects (1 M and 1 F; cluster E), serum contained DHG, a metabolite of genistein, at concentrations of 0.070–0.124 μM as well as daidzein and genistein (Table 6).

Discussion

The reduction in metabolic and consequent CV risks by an appropriate nutritional approach has been widely addressed in the last decades. Different strategies, such as the implementation of traditional habits (Mediterranean diet [28] or Far East traditional diets [35]), novel functional foods [36] and nutraceuticals [10], have been described. The association of soy protein consumption with reduced CV risk, mainly by way of TC and LDL-C lowering, is well established [13, 37] and has led to a health claim approval by the FDA for coronary heart disease risk reduction [38].

A large number of data are available on the effects of purified soy protein on lipid parameters, generally obtained from human studies substituting 25–30 g/day protein from animal sources with an equal amount of soy protein provided in model foods [13], whereas the effects of commercially available whole soy foods have not been fully evaluated. Today, it appears of growing importance moving from studies on isolated nutrient effects toward RCTs evaluating whole foods [39]. Further, scanty data are available on the impact of soy-based dietary plans on novel metabolic and CV risk factors [40], such as body size variables (body weight and abdominal fat), insulin resistance biomarkers and adipokines.

The present 3-month intervention study, designed following the FDA recommended intake of soy protein (25–30 g/day) [38], specifically evaluated commercially available whole soy foods. The study was conducted in subjects with moderate dyslipidemia and MetS carriers, also attempting to replicate the large number of data on isolated soy proteins in a seldom studied patient population.

The soy food diet significantly improved the plasma lipid profile, regardless of age, sex and baseline values, with significant median reductions in TC (−4.8 %), LDL-C (−5.2 %), non-HDL-C (−7.1 %) and apoB (−14.8 %), in line with most clinical trials evaluating the effect of the use of soy protein concentrates or isolates [13, 14, 17]. Moreover, these changes were not correlated with those of BMI, thus indicating that the lipid-lowering effect is independent of weight loss.

Of note, both apoB and non-HDL-C have been reported to be superior to LDL-C as markers of CV risk [29]. Being apoB synthesized by the liver and reflecting the total number of chylomicrons, VLDL, intermediate density lipoprotein and LDL particles, it better reflects the total atherogenic burden than LDL-C [33]. Similarly, non-HDL-C accounts for all atherogenic lipoproteins and recent data from a large series of studies confirmed it to be a better CV risk predictor than LDL-C in both primary and secondary

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prevention [23, 34]. Remarkably, a significant median reduction in non-HDL-C (−7.1 %, Fig. 4) occurred in the soy group. The apoB:apoA1 ratio was instead unchanged. A reduction in the CV/metabolic risk by the soy food diet may also be secondary to the median body weight and BMI changes (−1.5 % for both).

It is well known that MetS criteria take into account WC, which, along with other anthropometric measures (e.g., WHR, waist-to-height ratio, sagittal depth), better reflects the amount of visceral adipose tissue [41], although BMI in itself is a strong predictor of CV risk and overall mortality [42]. In order to understand the effects of soy foods on the classical MetS features, a factor analysis was applied. This represents a multivariate correlation technique which reduces a large number of interrelated variables to a smaller set of latent or underlying independent factors. Thus, the factor analysis has the potential to clarify the complex pathophysiological and statistical interactions underlying the MetS. Loadings are continuously distributed correlations, higher loadings indicating stronger associations between measured variables and associated factors [43]. In our cohort, among the three factors behind the overall correlation amidst risk variables, we found that adiposity parameters, either general or central, loaded on all three factors, implying that obesity is the link that unifies the MetS. Interestingly, our data are in accordance with those reported by Anderson [44] describing how, in a cohort of different ethnicity (Hong Kong Chinese subjects), adiposity (both central and general) was the common link between the major facets of MetS. Since the MetS is a condition also characterized by increased visceral fat accumulation, it can be hypothesized that an imbalance in the secretion of adipokines may be related to some of the metabolic abnormalities. In our cohort, leptin was highly correlated only with factor 1 (related to lipid and adipose features) characterized by high positive loadings for BMI and WC_{ViScan}. This finding is in line with previous studies indicating that leptin is positively correlated with BMI, but does not link features of MetS [45–47].

Along with the well-known effect of soy on lipid parameters, a recent meta-analysis on randomized controlled studies [48] failed to show a significant body weight reduction in MetS patients. Our nutritional intervention led to an important improvement of factor 1 (lipid and adiposity features), describing 22 % of the total variance. This indicates that this nutritional approach can improve, in MetS subjects, both lipid and adiposity parameters, and, to a lesser extent, glucometabolic indices (FPG, insulin and HOMA), as described by factor 2 (Fig. 2). Further, in the soy group, a reduction in MetS feature numbers was observed in 50 % of the subjects, thereby lowering their overall cardiometabolic risk.

A satisfactory compliance to the dietary intervention was supported by the isoflavone analyses. It is well known that in soybean and in unfermented soy foods, isoflavones are present as β-glucosides [49], not absorbed at the intestinal level. After ingestion, however, the glycosidic bond is hydrolyzed by the microbiota to release free aglycones, which may be either absorbed or further metabolized, mostly by microbiota. These metabolic steps include the conversion of daidzein to dihydrodaidzein (DHD), equol and O-desmethylangolensin (O-DMA), whereas genistein is converted to dihydrogenistein (DHG) [50]. After absorption, the aglycones are again conjugated with glucuronic acid and, to a smaller extent, sulfate, or bound to plasma proteins, such as albumin. Conjugated forms follow the enterohepatic circulation and may be excreted, primarily in urines [51]. Isoflavones and their metabolites were detected in sera of all soy group participants, with only one exception. This does not rule out soy food consumption by this subject: many variables influence absorption and metabolism of isoflavones and inter-individual variations in gut microbiota have a major role in formation, absorption and/or metabolism of free aglycones [50, 51].

Major limitations of the present study were the intrinsic impossibility to implement a double-blind design, in order to avoid personal preferences toward the different foods, and the fact that the selected women were all postmenopausal, at greater metabolic and CV risk. The major strength, instead, was the validation of a dietary approach based on commercially available whole soy foods, allowing to achieve a better compliance and providing positive outcomes on some metabolic risk biomarkers.

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Author contributions MR wrote the paper and performed ELISA experiments; PM wrote the paper and coordinated the study; AN, LC and CS conceived the study and critically revised the manuscript; CP selected the patients and acted as clinical monitor; SG performed all the statistical analyses; BM and CM performed biochemical analysis; MG and CV performed analysis on HDL; GA performed HPLC analysis; and RB was the dietician. All authors reviewed the results and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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