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Journal Name	European Journal of Nutrition	
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Schedule Received 23 June 2016  
Revised  
Accepted 12 October 2016

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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.

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Keywords (separated by '-') Soy protein - Lipids - Metabolic syndrome and obesity

Footnote Information **Electronic supplementary material** The online version of this article (doi:10.1007/s00394-016-1333-7) contains supplementary material, which is available to authorized users.

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

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6 **Cesare R. Sirtori**<sup>4</sup> · **Paolo Magni**<sup>2</sup>

7 Received: 23 June 2016 / Accepted: 12 October 2016  
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11 **AQ1** commonest cause of death worldwide. Different strategies  
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**Keywords** Soy protein · Lipids · Metabolic syndrome and 38  
obesity 39

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**Abbreviations** 40

ApoB	Apolipoprotein B	41
ApoA-I	Apolipoprotein A-I	42
BMI	Body mass index	43
BIA	Bioelectrical impedance analysis	44
CRP	High-sensitivity C-reactive protein	45
CVD	Cardiovascular diseases	46
DBP	Diastolic blood pressure	47
FPG	Fasting plasma glucose	48
HC	Hip circumference	49
HDL-C	High-density lipoprotein cholesterol	50
HOMA	Homeostatic model assessment	51
HR	Heart rate	52
LDL-C	Low-density lipoprotein cholesterol	53
Lp(a)	Lipoprotein (a)	54
N	Number	55
PCSK9	Proprotein convertase subtilisin/kexin 9	56

57	SBP	Systolic blood pressure
58	sICAM-1	Soluble intercellular adhesion molecular 1
59	TC	Total cholesterol
60	TG	Triglycerides
61	VFR	Visceral fat rating
62	WC	Waist circumference

## 63 Introduction

64 Cardiovascular diseases (CVD) are currently the com-  
65 monest cause of death worldwide (WHO January 2015;  
66 [1]), and different strategies for their primary prevention  
67 have been planned, taking into account the main known  
68 risk factors, which include an atherogenic lipid profile and  
69 abdominal/visceral fat excess [2–4]. Total abdominal adi-  
70 pose tissue may be subdivided into subcutaneous-abdomi-  
71 nal compartment and intra-abdominal compartment. This  
72 latter, also referred to as ‘visceral fat,’ is associated with  
73 insulin resistance and the specific features of the metabolic  
74 syndrome (MetS) [5], which also includes the combination  
75 of dyslipidemia, hyperglycemia or type 2 diabetes mellitus  
76 and hypertension, in association with insulin resistance and  
77 systemic inflammation [6, 7].

78 The biochemical factors involved in increased primary  
79 CVD risk associated with these features include elevated  
80 free fatty acid flux to the liver, altered adipokine produc-  
81 tion and altered HDL level and distribution into different  
82 subclasses [3], resulting in a proatherogenic environment.  
83 In particular, MetS has been associated with increased  
84 small HDL-3 and reduced large HDL-2 particles [8]. All  
85 these risk factors can be a consequence of dietary habits  
86 and may therefore be influenced by diet and lifestyle modi-  
87 fications. Functional foods [9] and nutraceuticals [10] have  
88 been assessed in several clinical studies, and meta-analyti-  
89 cal reports have indicated these as effective approaches for  
90 the management of primary CVD risk in the MetS [11, 12].  
91 Within this context, numerous randomized controlled trials  
92 (RCTs) and some meta-analyses [13, 14] have shown that  
93 a regular consumption of soy protein improves circulating  
94 lipid parameters. More specifically, the inclusion of puri-  
95 fied soy protein in the range of 15–40 g/day into the diet  
96 of adults with normal or moderately elevated total chole-  
97 sterol (TC) resulted in a significant reduction in TC (at least  
98 –4 %) and LDL-cholesterol (LDL-C, about –6 %) [13,  
99 15–17]. In addition, dietary intake of soy protein reduced  
100 body weight in overweight and obese subjects, compared  
101 to diets containing animal protein [18, 19], although data  
102 on soy protein impact on overall fat mass reduction and  
103 abdominal adipose changes [20], as well as on circulating  
104 adipokine levels [21], are scanty and controversial.

105 The majorities of published studies on soy protein have  
106 evaluated the effect of purified protein included in the daily

107 diet, without changes of the percent caloric intake from  
108 protein.

109 It should be, however, pointed out that patients do not  
110 eat nutrients such as purified soy protein; thus, an approach  
111 based on whole soy foods, possibly commercially availa-  
112 ble, appears to be most desirable [22]. It must be, however,  
113 noted that in other conditions, the whole soy food approach  
114 has shown differences in effects when compared to the iso-  
115 lated components [23]. To our knowledge, the effects of  
116 commercially available whole soy foods on the cardiometab-  
117 olic parameters of the metabolic syndrome have never  
118 been evaluated. The present study, with an RCT design,  
119 aimed to assess the effects of a low-lipid diet with whole  
120 soy foods, on abdominal adipose tissue and related adi-  
121 pokines, lipid/lipoprotein profiles and glucose metabolism,  
122 and to compare them with the effects of standard low-lipid  
123 diet with animal protein.

## 124 Materials and methods

### 125 Ethical issues

126 The study was conducted in accordance with the guide-  
127 lines of the Declaration of Helsinki and its later amend-  
128 ments. The study was approved by the ethics commit-  
129 tee of A.S.S.T Grande Ospedale Metropolitano Ospedale  
130 Niguarda (approval no 170\_04/2012).

### 131 Study design and population

132 The study was performed at the Centro Dislipidemie  
133 (A.S.S.T Grande Ospedale Metropolitano Ospedale  
134 Niguarda, Milan, Italy) in the period March 2013–June  
135 2015 following a randomized, parallel, controlled, single-  
136 center design. Study subjects were followed at the Centro  
137 Dislipidemie and were used to consume a lipid-lowering  
138 diet. Inclusion criteria were: males and postmenopausal  
139 females aged between 45 and 75 years; BMI within the  
140 25–30 kg/m<sup>2</sup> range; and LDL-C levels in the 130–190 mg/  
141 dL range. Additionally, volunteers had to fulfill 3/5 features  
142 of the metabolic syndrome criteria, namely waist circum-  
143 ference (WC)  $\geq 102$  cm (M) or  $\geq 88$  cm (F); blood pressure  
144 (BP)  $\geq 130/85$  mmHg; fasting glycemia (FPG)  $\geq 100$  mg/  
145 dL; triglycerides (TG)  $\geq 150$  mg/dL; HDL-C  $< 40$  mg/dL  
146 (M); and  $< 50$  mg/dL (F) [24]. All study subjects fulfilled 3  
147 or 4 MetS criteria; none met all 5 criteria.

148 The exclusion criteria were: the presence of chronic liver  
149 disease, renal disease or severe renal impairment, untreated  
150 arterial hypertension, obesity (BMI  $\geq 30$  kg/m<sup>2</sup>), any past  
151 history of cerebro-vascular accident or coronary events,  
152 including unstable angina, myocardial infarction, percu-  
153 taneous transluminal coronary angioplasty, or coronary



154 artery bypass graft; subjects affected by any kind of food  
155 allergy; any concomitant therapy known to alter any of the  
156 parameters to be assessed; history of or current alcohol or  
157 drug abuse; any clinically significant medical condition  
158 that could interfere with the conduct of the study; known or  
159 suspected diagnosis of hepatitis or HIV infection; subjects  
160 unable or unwilling to comply with the protocol require-  
161 ments, or deemed by the investigator to be unfit for the  
162 study; and patients who were enrolled in another research  
163 study in the last 90 days. All patients were in primary pre-  
164 vention, were free from liver/kidney disorders potentially  
165 affecting the response to treatment and did not take any  
166 drug affecting lipid/lipoprotein or glycemic profile, includ-  
167 ing thiazolidinediones or corticosteroids. Concomitant  
168 medications are reported in Table 1.

### 169 Clinical evaluations

170 Clinical and biochemical evaluations were performed at  
171 the beginning and at the end of the treatment period. At all  
172 visits, patients underwent a fasting venous blood sampling  
173 and a full clinical examination, including the evaluation of  
174 height, body weight, heart rate and arterial blood pressure.  
175 The ViScan device (Tanita Inc., Tokio, Japan) is a validated  
176 tool to assess waist circumference (WC) [25] and abdomi-  
177 nal fat mass [26, 27] by bioelectrical impedance analysis

(BIA). WC was measured by the ViScan device (supine 178  
position,  $WC_{ViScan}$ ) and by means of a non-stretchable tape 179  
at the umbilical level (standing position, WC). Hip circum- 180  
ference (HC) was assessed by tape. ViScan was also used 181  
to evaluate bioelectrical impedance analysis (BIA) % and 182  
visceral fat rating (VFR) %. The reproducibility of ViScan 183  
was measured with a rigid human phantom (waist 65 cm, 184  
hip 90 cm). 185

All visits were performed by the same investigator 186  
(PM), and all ViScan analyses were conducted by the same 187  
operator (RB). Plasma samples were prepared by low- 188  
speed centrifugation, and aliquots were immediately stored 189  
at  $-20\text{ }^{\circ}\text{C}$  for subsequent assays. Safety and compliance 190  
information were collected at each visit, also by means of 191  
24-h dietary recalls, relative to 3 non-consecutive days for 192  
each month of nutritional intervention. Data retrieval, anal- 193  
ysis and manuscript preparation were solely the responsi- 194  
bility of the authors. 195

### 196 Intervention

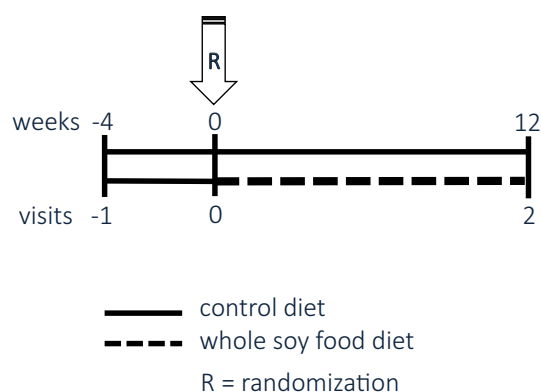
197 After enrollment, they were instructed to follow a nor- 198  
mocaloric/low-lipid diet, designed according to the Medi- 199  
terranean diet criteria [28], with three main meals and two 200  
snacks and adapted to individual preferences in order to 201  
improve patient compliance. Extra virgin olive oil in mod- 202  
erate quantity was suggested as topping. Dietary plans were 203  
defined with the aid of a dedicated software (Dietosystem, 204  
DS Medica srl, Milan, Italy). Diet composition was dif- 205  
ferent for male and female subjects as shown in Table 2. 206  
Subjects were then randomly assigned to receive either the 207  
experimental diet, containing whole soy foods correspond- 208  
ing to 30 g/day soy protein in substitution of animal foods 209  
containing the same amount of protein, or the control diet 210  
containing the animal foods, for 12 weeks (Fig. 1). The 211  
total daily amount of protein was 1 g/kg for all diets. In 212  
order to have a constant total energy intake over the inter- 213  
vention period, personalized recommendations were given 214  
to each participant during each visit, according to three 215  
24-h dietary recalls.

**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

**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



**Fig. 1** Schematic representation of the trial design

## 216 Characteristics of the soy diet

217 The soy diet was composed by different commercial soy  
 218 foods from a portfolio including soy nuggets, soy burgers,  
 219 soy desserts (different flavorings) and soy drinks (different  
 220 flavorings), all provided by Alpro (Belgium). The composi-  
 221 tion of these products is shown in supplementary Table S1.  
 222 In order to reach the necessary daily intake of soy foods,  
 223 corresponding to 30 g soy protein, the subjects should con-  
 224 sume 3–4 servings per day, distributed in different meals  
 225 as indicated in supplementary Table S2 for a better compli-  
 226 ance. At the beginning of each month, each subject received  
 227 at home a bag containing all the soy foods necessary for the  
 228 following 30 days.

## 229 Biochemical and immunometric assays

230 In each blood sample, total cholesterol (TC), TG, HDL-  
 231 C, lipoprotein(a) (Lp(a)), apolipoprotein (apo)A-I, apoB,  
 232 C-reactive protein (CRP), fasting glycemia (FPG), aspar-  
 233 tate aminotransferase (AST), alanine aminotransferase  
 234 (ALT), gamma-glutamyl transpeptidase (GGT) and creatine  
 235 phosphokinase (CPK) were measured according to stand-  
 236 ard clinical procedures. LDL-C was calculated according  
 237 to the Friedewald equation. Non-HDL-C was calculated  
 238 as TC minus HDL-C [29]. Commercial enzyme-linked  
 239 immunosorbent assay (ELISA) kits were used according  
 240 to manufacturer's specifications and previously published  
 241 protocols to quantify plasma leptin [30], adiponectin, solu-  
 242 ble intercellular adhesion molecule-1 (sICAM-1), PCSK9  
 243 (all from R&D System [31], Minneapolis, MN) and insu-  
 244 lin (Mercodia, Uppsala, Sweden). The Homeostasis Model  
 245 Assessment of Insulin Resistance (HOMA) index was cal-  
 246 culated. The plasma concentration of HDL particles con-  
 247 taining only apoA-I (LpA-I) and of particles containing  
 248 both apoA-I and apoA-II (LpA-I:A-II) was determined by  
 249 electroimmunodiffusion in agarose gel using a commercial

kit (Sebia, Lisses, France) [32]. The content of discoidal 250  
 prebeta-migrating HDL was evaluated by non-denaturing 251  
 two-dimensional electrophoresis followed by immunode- 252  
 tection against human apoA-I [33]. The content of prebeta- 253  
 HDL was calculated as percentage of total apoA-I signal. 254  
 HDL subclass distribution according to particle size was 255  
 determined by non-denaturing polyacrylamide gradient gel 256  
 electrophoresis (4–30 %) of the  $d < 1.21$  g/mL plasma total 257  
 lipoprotein fraction; the protein-stained gels were scanned 258  
 with an imaging densitometer to determine particle size 259  
 and HDL were divided into small (diameter 7.2–8.2 nm), 260  
 medium (diameter 8.2–8.8 nm) and large (diameter 8.8– 261  
 12.7 nm) particles [32]. Densitometric analyses were per- 262  
 formed with the GS-690 Imaging Densitometer and the 263  
 Multi-Analyst software (Bio-Rad Laboratories, Hercules, 264  
 CA). 265

## 266 Chemicals for isoflavones analysis

267 Daidzein (97 % purity) and genistein (97 % purity) were 268  
 from Lancaster Synthesis (Morecambe, UK); deuterated 269  
 daidzein (2',3',5',6'-d<sub>4</sub>, 98 % purity), deuterated genistein 270  
 (2',3',5',6'-d<sub>4</sub>, 98 % purity) and equol ( $\geq 98$  % purity) were 271  
 from Cayman Chemicals (Milan, Italy). Dihydrogenistein 272  
 (DHG, 98 % purity) was from Alfachem (Milan, Italy). 273  
 The hydrolytic enzyme mixture containing sulfatase and 274  
 $\beta$ -glucuronidase from *Helix pomatia* (glucuronidase activ- 275  
 ity 400 units/g, sulfatase activity less than 40 units/g), 276  
 sodium citrate, ammonium bicarbonate and methanol was 277  
 from Sigma-Aldrich (Milan, Italy).

## 278 Isoflavone extraction from human serum 279 and HPLC-CHIP ESI-MS analysis

280 The extraction of isoflavones and their metabolites was 281  
 performed according to our published method [34]. The 282  
 quantitative analysis was performed using an Agilent 1200 283  
 Series Nanoflow LC system. The Agilent HPLC-Chip/MS 284  
 was interfaced to an Agilent SL series ion trap (Agilent, 285  
 CA). The intra-assay variations reported as RDS % were 286  
 within the range 1.8–6.7 % (Table 6). For more details, see 287  
 Supplementary Materials and Methods.

## 288 Sample size and statistical analysis

289 Results are presented as median and interquartile ranges 290  
 (Q1 and Q3) for all parameters. The differences from 291  
 treatment arms at baseline were assessed by Wilcoxon- 292  
 rank sum test. Chi-square test was applied to evaluate the 293  
 difference in frequencies among arms. The difference by 294  
 treatment arms as changes from baseline [12-week treat- 295  
 ment–baseline (0 week)] was evaluated by ANCOVA 296  
 adjusted for baseline, age and sex. Data were also

297 expressed as median of changes between [12-week treat-  
 298 ment and baseline (0 week)] and quartiles (supplemen-  
 299 tary Table S3). Residuals from full models, investigating  
 300 factors variations, were checked to assess normal distri-  
 301 bution. Principal components analysis was performed,  
 302 and the scree plot of ordered eigenvalues of a correlation  
 303 matrix was used to decide the appropriate number of fac-  
 304 tors extracted. Only variables with loading  $\geq 0.40$  were  
 305 considered for interpretation. Finally, we checked whether  
 306 the scores of factors obtained were significantly different  
 307 between the two treatment arms. Statistical analysis was  
 308 performed by using the SAS software v. 9.2 (SAS Inc.,  
 309 Cary, NC). A group sample size of 26 per arm achieves  
 310 80 % power to detect a difference of 20 mg/dL in absolute  
 311 changes (12–0 week) in LDL levels (mg/mL) between the  
 312 null hypothesis that both arms means of change in LDL  
 313 are 10 mg/mL and the alternative hypothesis that the mean  
 314 of change in LDL in the treatment arms is  $-10.0$  mg/mL  
 315 with estimated group standard deviations of 25.0 mg/mL  
 316 per arm and with a significance level of 5 % using a two-  
 317 sided two-sample *t* test.

## Results

### Study population

318  
 319  
 320 After a run-in period of 4 weeks on a balanced low-lipid  
 321 diet, only subjects showing changes in total cholesterol  
 322  $< 10$  % were recruited for the study. Sixty-two subjects (32  
 323 M, 30 F) were assessed for eligibility, 6 were excluded, and  
 324 56 (29 M, 27 F) were enrolled into the study and randomly  
 325 allocated to either the soy diet ( $N = 28$ ; 14 M, 14 F) or the  
 326 control diet ( $N = 28$ ; 15 M, 13 F), for a total intervention  
 327 duration of 12 weeks. Of them, 27/28 subjects completed  
 328 the control diet arm and 26/28 completed the soy diet arm  
 329 (Fig. 2). Wilcoxon-rank sum test indicates that at baseline  
 330 all clinical and biochemical values, including lipids, adi-  
 331 pokines and inflammatory markers, were similar between  
 332 the two treatment arms (Table 3). Fifty-three volunteers,  
 333 gender and age matched, were included, and 13.2 % of  
 334 them were smokers. Men were 55 % in the control arm  
 335 and 50 % in the soy arm; median age was 60 years in  
 both arms. As reported in Table 4, BMI ( $27.3 \text{ kg/m}^2$  in the

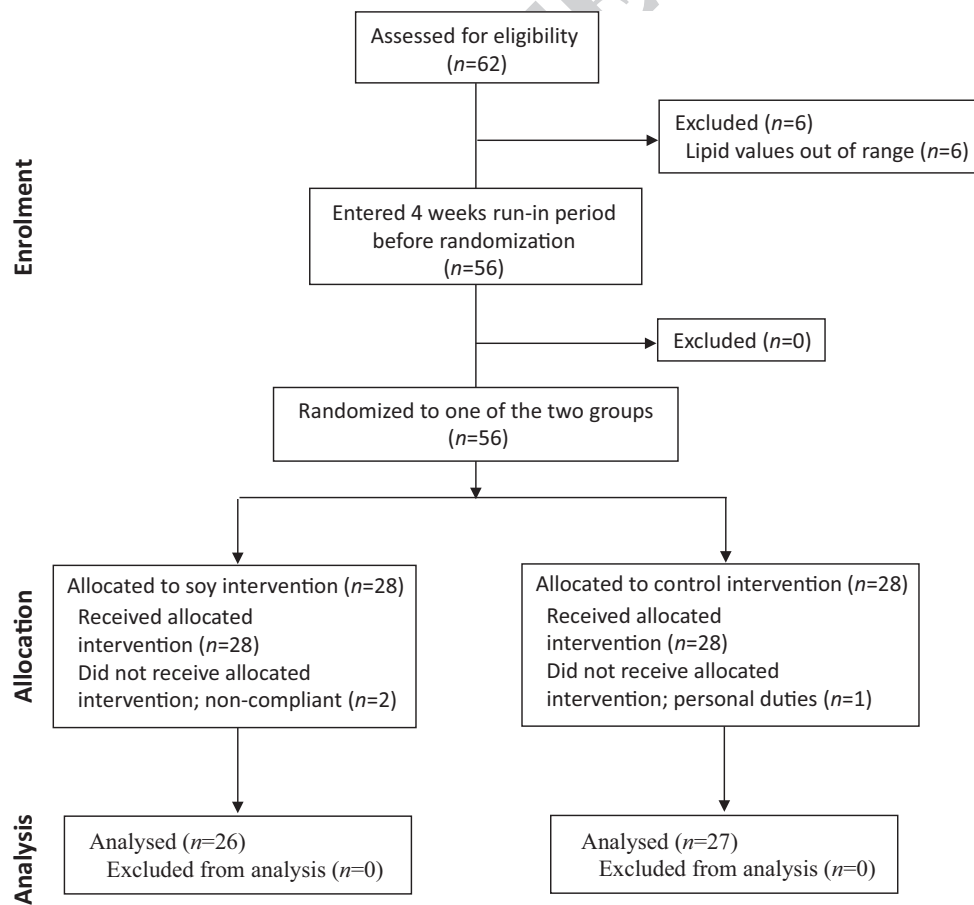


Fig. 2 Consort flow diagram

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

Parameter	Value	<i>p</i> value
No. of participants (men/women)	53 (28/25)	–
Smokers, <i>n</i> (%)	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 <sup>2</sup> )	27.8 (25.8, 29.6)	0.31
WC <sub>TAPE</sub> (cm)	97.0 (93.5, 103.5)	0.26
HC <sub>TAPE</sub> (cm)	99.0 (94, 102)	0.56
WC <sub>TAPE</sub> :HC <sub>TAPE</sub>	1.0 (0.9, 1.0)	0.31
WC <sub>VSCAN</sub> (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

337 control group and 28.2 kg/m<sup>2</sup> in the soy group) indicated  
338 that study subjects were overweight with a relevant abdomi-  
339 nal adiposity, as evaluated by WC, WC<sub>Vscan</sub> (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 mod-  
erate 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 sub-  
jects on soy food showed a reduction in the number of  
MetS features. In the control group, 26 % (7/27) of sub-  
jects showed a reduction in MetS feature number. The dif-  
ference in frequencies (Chi-square test) among arms was  
*p* = 0.094. A significant reduction in weight (median per-  
centage 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, insu-  
lin and HOMA) and inflammation (sICAM-1 and CRP)  
parameters.

A 12-week (wk) lipid/lipoprotein changes were charac-  
terized 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 percent-  
age 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 con-  
founding 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 param-  
eters 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).

**Table 4** Summary of primary and secondary end points

	Soy diet				Control diet				Difference between treatment arms		
	12 weeks				12 weeks				p value		
	Basal	Median	Q1	Q3	Basal	Median	Q1	Q3			
SBP (mmHg)	125.0	120.0	120.0	130.0	125.0	110.0	110.0	130.0	120.0	130.0	0.28
DBP (mmHg)	80.0	80.0	80.0	85.0	80.0	80.0	80.0	90.0	80.0	90.0	0.80
HR (bpm)	67.0	66.0	66.0	72.0	64.0	64.0	64.0	76.0	64.0	72.0	0.39
WC (cm)	99.5	97.5	97.5	106.0	97.0	93.0	93.0	102.0	93.0	102.0	0.35
WC <sub>visceral</sub> (cm)	105.0	101.0	101.0	112.0	104.0	97.0	97.0	102.0	97.0	110.0	0.45
HC (cm)	99.5	97.7	97.7	102.5	98.0	94.0	94.0	101.0	95.0	101.0	0.76
WC <sub>Tape</sub> :HC <sub>Tape</sub>	1.0	1.0	1.0	1.0	1.0	0.9	0.9	1.0	0.9	1.0	0.39
BIA (%)	41.6	40.9	40.9	46.4	39.1	34.0	34.0	46.3	32.3	45.0	0.37
VFR (%)	12.5	12.7	12.7	15.5	13.0	11.0	11.0	17.5	10.0	16.0	0.42
Weight (Kg)	77.5	75.5	75.5	80.0	75.0	68.0	68.0	81.0	66.0	82.0	<b>0.005</b>
BMI (Kg/m <sup>2</sup> )	28.2	27.2	27.2	29.8	27.3	25.4	25.4	29.4	25.7	29.1	<b>0.05</b>
Leptin (ng/mL)	16.5	13.5	13.5	25.8	12.8	5.86	5.86	21.8	6.9	18.1	0.38
Adiponectin (mg/dL)	5.59	6.3	6.3	9.7	6.57	4.95	4.95	9.23	4.9	9.8	0.99
Leptin:Adiponectin	2.72	2.3	2.3	3.5	1.85	1.16	1.16	2.63	0.9	2.6	0.88
TC (mg/dL)	256.0	236.7	236.7	251.8	251.9	221.0	221.0	272.0	250.0	271.6	<b>0.002</b>
LDL-C (mg/dL)	169.4	154.7	154.7	173.4	164.4	144.2	144.2	181.6	171.0	190.1	<b>0.01</b>
HDL-C (mg/dL)	44.3	44.0	44.0	49.0	47.0	37.5	37.5	52.0	46.0	51.0	0.26
Non-HDL-C	213.5	186.5	186.5	212.4	208.0	185.4	185.4	219.0	199.0	230.1	<b>0.007</b>
Lp(a) (mg/dL)	16.5	17.0	17.0	30.0	11.0	5.0	5.0	25.0	6.0	24.0	0.23
PCSK9 (ng/dL)	297.6	268.9	268.9	334.6	285.3	256.6	256.6	330.7	305.6	336.3	0.52
ApoB (mg/dL)	159.5	139.5	139.5	153.0	155.0	127.0	127.0	165.0	139.0	165.0	<b>0.019</b>
ApoB:PCSK9	0.6	0.5	0.5	0.6	0.52	0.42	0.42	0.58	0.5	0.6	0.76
apoAI (mg/dL)	114.0	108.0	108.0	115.0	115.0	107.0	107.0	122.0	115.0	121.0	<b>0.008</b>
ApoB:apoAI	1.4	1.3	1.3	1.4	1.3	1.1	1.1	1.5	1.2	1.5	0.86
TG (mg/dL)	206.5	169.6	169.6	233.4	190.0	121.4	121.4	237.0	146.0	213.1	0.94
FPG (mg/dL)	97.0	93.4	93.4	104.1	92.0	86.5	86.5	100.4	96.0	106.0	0.23
Insulin (UI/L)	8.20	7.7	7.7	10.7	7.50	6.10	6.10	11.5	7.5	10.7	0.90
HOMA	2.1	2.1	2.1	2.5	1.9	1.4	1.4	2.6	1.9	3.3	0.85
CRP (mg/dL)	0.2	0.2	0.2	0.3	0.2	0.1	0.1	0.3	0.2	0.3	0.59
sICAM	255.9	238.4	238.4	274.4	226.9	239.7	239.7	299.2	263.4	287.1	0.43





Table 4 continued

	Soy diet						Control diet						Difference between treatment arms		
	Basal			12 weeks			Basal			12 weeks			Q1	Q3	p value
	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3			
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	<b>0.02</b>		
LpAII	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, sCAMP-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	<b>0.87</b>	0.25	-0.09
ApoB	<b>0.77</b>	0.26	-0.17
LDL	<b>0.64</b>	0.18	0.05
BMI	<b>0.52</b>	-0.34	0.31
apoAI	<b>0.43</b>	0.27	0.05
WC <sub>vscan</sub>	<b>0.41</b>	-0.22	-0.08
Leptin	<b>0.40</b>	-0.09	0.10
Non-HDL-C	<b>-0.43</b>	-0.10	0.24
HOMA	0.09	<b>0.79</b>	0.20
Insulin	-0.01	<b>0.72</b>	0.29
FPG	0.27	<b>0.40</b>	-0.17
VFR (%)	<b>0.44</b>	<b>-0.60</b>	-0.01
BIA (%)	<b>0.47</b>	<b>-0.68</b>	0.17
WC	0.26	-0.12	<b>0.71</b>
HC	0.14	0.03	<b>0.55</b>

### 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 ( $\geq 0.40$ ), 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

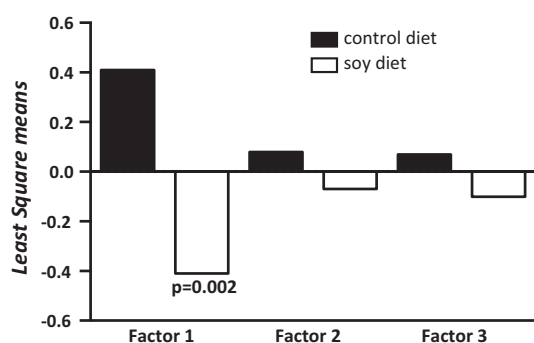
The nutritional intervention with either soy or control food items for 12 weeks was well tolerated by all participants, and no specific adverse effects were reported. No changes in liver function and thyroid parameters were detected after the nutritional intervention with soy foods, which were

**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.	-
C	F	<LOQ	0.072 ± 0.011	6.1	<LOQ	-	0.127 ± 0.015	5.6	<LOQ	-
C	M	n.d.	0.070 ± 0.013	5.6	n.d.	-	0.014 ± 0.008	5.0	<LOQ	-
C	F	n.d.	0.072 ± 0.187	6.4	n.d.	-	0.034 ± 0.009	4.6	n.d.	-
C	F	<LOQ	0.087 ± 0.007	4.3	0.066 ± 0.013	4.9	0.102 ± 0.011	6.1	n.d.	-
C	M	n.d.	0.022 ± 0.009	4.7	n.d.	-	0.231 ± 0.087	6.4	0.124 ± 0.051	2.9
C	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



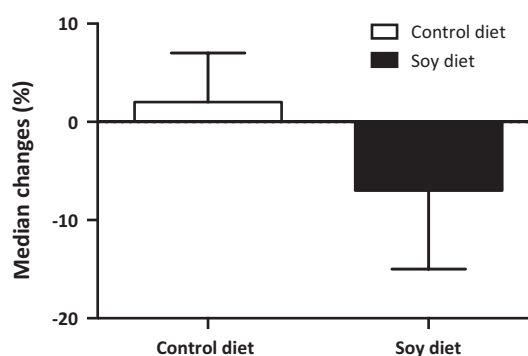


**Fig. 3** Effect of soy treatment on the scores of the obtained factors

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  $\mu\text{M}$ , 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  $\mu\text{M}$ , or genistein, in the range between 0.007 and 0.328  $\mu\text{M}$ . One female (cluster D) besides daidzein and genistein showed also DHD, a metabolite of daidzein (0.066  $\mu\text{M}$ ), 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  $\mu\text{M}$  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].



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

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



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

499 It is well known that MetS criteria take into account WC,  
500 which, along with other anthropometric measures (e.g.,  
501 WHR, waist-to-height ratio, sagittal depth), better reflects  
502 the amount of visceral adipose tissue [41], although BMI in  
503 itself is a strong predictor of CV risk and overall mortality  
504 [42]. In order to understand the effects of soy foods on the  
505 classical MetS features, a factor analysis was applied. This  
506 represents a multivariate correlation technique which reduces  
507 a large number of interrelated variables to a smaller set of  
508 latent or underlying independent factors. Thus, the factor  
509 analysis has the potential to clarify the complex pathophysiological  
510 and statistical interactions underlying the MetS. Loadings are  
511 continuously distributed correlations, higher loadings indicating  
512 stronger associations between measured variables and associated  
513 factors [43]. In our cohort, among the three factors behind the  
514 overall correlation amidst risk variables, we found that adiposity  
515 parameters, either general or central, loaded on all three factors,  
516 implying that obesity is the link that unifies the MetS. Interestingly,  
517 our data are in accordance with those reported by Anderson [44]  
518 describing how, in a cohort of different ethnicity (Hong Kong  
519 Chinese subjects), adiposity (both central and general) was the  
520 common link between the major facets of MetS. Since the MetS  
521 is a condition also characterized by increased visceral fat  
522 accumulation, it can be hypothesized that an imbalance in the  
523 secretion of adipokines may be related to some of the metabolic  
524 abnormalities. In our cohort, leptin was highly correlated only  
525 with factor 1 (related to lipid and adipose features) characterized  
526 by high positive loadings for BMI and  $WC_{ViScan}$ . This finding  
527 is in line with previous studies indicating that leptin is positively  
528 correlated with BMI, but does not link features of MetS [45–47].

531 Along with the well-known effect of soy on lipid parameters,  
532 a recent meta-analysis on randomized controlled studies [48] failed  
533 to show a significant body weight reduction in MetS patients. Our  
534 nutritional intervention led to an important improvement of factor  
535 1 (lipid and adiposity features), describing  $22\%$  of the total  
536 variance. This indicates that this nutritional approach can improve,  
537 in MetS subjects, both lipid and adiposity parameters, and, to a  
538 lesser extent, glucometabolic indices (FPG, insulin and HOMA),  
539 as described by factor 2 (Fig. 2). Further, in the soy group,  
540 a reduction in MetS feature numbers was observed in  $50\%$   
541 of the subjects, thereby lowering their overall cardiometabolic  
542 risk.

544 A satisfactory compliance to the dietary intervention was supported  
545 by the isoflavone analyses. It is well

546 known that in soybean and in unfermented soy foods, isoflavones  
547 are present as  $\beta$ -glucosides [49], not absorbed at the intestinal  
548 level. After ingestion, however, the glycosidic bond is hydrolyzed  
549 by the microbiota to release free aglycones, which may be either  
550 absorbed or further metabolized, mostly by microbiota. These  
551 metabolic steps include the conversion of daidzein to dihydrodaidzein  
552 (DHD), equol and O-desmethylangolensin (O-DMA), whereas  
553 genistein is converted to dihydrogenistein (DHG) [50]. After  
554 absorption, the aglycones are again conjugated with glucuronic  
555 acid and, to a smaller extent, sulfate, or bound to plasma  
556 proteins, such as albumin. Conjugated forms follow the enterohepatic  
557 circulation and may be excreted, primarily in urines [51]. Isoflavones  
558 and their metabolites were detected in sera of all soy group  
559 participants, with only one exception. This does not rule out soy  
560 food consumption by this subject: many variables influence  
561 absorption and metabolism of isoflavones and inter-individual  
562 variations in gut microbiota have a major role in formation,  
563 absorption and/or metabolism of free aglycones [50, 51].

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

576 **Acknowledgments** This study has been supported by an unrestricted  
577 grant to Centro Dislipidemie (A.S.S.T. Grande Ospedale Metropolitano  
578 Niguarda, Milano, Italy) from the Alpro Foundation (Gent, Belgium).  
579 The founding sponsors had no role in the design of the study; in  
580 the collection, analyses, or interpretation of data; in the writing of  
581 the manuscript; and in the decision to publish the results.

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

590 **Compliance with ethical standards**

591 **Conflict of interest** The authors declare no conflict of interest.

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