Metabolic and vascular effect of a new standardized bergamot phytocomplex: a three-arm, placebo-controlled, double-blind clinical trial

Federica Fogacci[1,2], Marina Giovannini[1,2], Edigio Imbalzano[3], Elisa Grandi[1], Elisabetta Rizzoli[1], Sergio D'Addato[1], Arrigo F.G. Cicero[1,2]

[1]Hypertension and cardiovascular risk research center, Medical and Surgical Sciences Department, University of Bologna, Bologna, Italy
[2]Italian Nutraceutical Society (SINut), Bologna, Italy
[3]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

Corresponding author
Prof. Arrigo F.G. Cicero
Hypertension and
Cardiovascular Risk
Research Center
Medical and Surgical
Sciences Department
University of Bologna
Bologna, Italy
Phone: +39 3498558017
E-mail: arrigo.cicero@unibo.it

Abstract

Introduction: In addition to healthy lifestyle and balanced diet, nutraceutical supplementation may be useful to maintain overall metabolic wellness. The aim of the present study was to assess the metabolic and vascular effects of a new, highly standardized bergamot phytocomplex supplement in healthy volunteers with the features of metabolic syndrome.

Material and methods: We carried out a double-blind, randomized, placebo-controlled, three-arm, parallel-group clinical trial in 90 adult subjects (30 per group) treated for 12 weeks with two different dosages of a highly standardized bergamot phytocomplex (Endoberg by AKHYNEX and Kalita by Giellepi) or placebo. Lipid plasma levels, glycemia, plasma hs-CRP, HOMA-IR, body fat, endothelial reactivity and fatty liver index were assessed at baseline, after 6 weeks and at the end of treatment in all subjects.

Results: At the end of treatment, both tested bergamot extract doses were able to significantly improve atherogenic dyslipidemia and insulin sensitivity ($p < 0.05$) compared to placebo. In the high-dose treated group, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), non high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) improved versus placebo ($p < 0.05$) just after 6 weeks of treatment. After 12 weeks of treatment, TC decreased by 13.2 ±2.1%, LDL-C by 17.7 ±3.2%, non-HDL-C by 17.5 ±3.1%, TG by 16.6 ±3.3%, TG/HDL-C by 22.5 ±4.4%, homeostasis insulin resistance index (HOMA-IR) by 12.2 ±2.1%, γ-glutamyl transferase (GGT) by 22.2 ±4.6%, and high-sensitivity C-reactive protein (hs-CRP) by 17.9 ±3.4% versus baseline ($p < 0.05$) and vs. placebo ($p < 0.05$). Percentage body fat and endothelial reactivity improved versus baseline, but not versus placebo, in the high-dose treatment only ($p < 0.05$).

Conclusions: The tested bergamot phytocomplex was able to significantly improve glucose and lipid metabolism, as well as inflammation, and might represent a novel multi-target approach to control metabolic syndrome.

Key words: bergamot, metabolic syndrome, randomized clinical trial, TG/HDL index, C-reactive protein.

Introduction

Metabolic syndrome is a highly prevalent and complex disease affecting around 24% of the general population in Europe [1]. It is characterized by the contemporaneous presence of different cardiometabolic risk factors such as overweight, suboptimal blood pressure levels, atherogenic dyslipidemia (low high-density lipoprotein cholesterol (HDL-C) and/or high triglycerides (TG)), dyslgycemia/insulin resistance, and/or systemic inflammation. However, recovering from the condition of metabolic syndrome to a healthy state can significantly reduce the risk of cardiovascular disease [2].

Timely implementation of dietary recommendations and lifestyle modifications is crucial for preventing the development of metabolic syndrome. Maintaining a healthy diet and regular exercise [3, 4] are fundamental to maintain overall metabolic wellness. Nevertheless, supplements properly formulated with scientifically backed ingredients may represent an effective and convenient strategy to support a busy lifestyle. Recent evidence has highlighted a strong correlation between foods rich in flavonoids and cardiometabolic risk reduction [5]. Nutraceutical administration may be useful for controlling altered metabolic parameters in subjects exhibiting features of metabolic syndrome [6, 7]. Among the nutraceuticals and plant extracts believed to be able to modulate metabolism, bergamot is attracting the attention of many researchers for its effectiveness, safety and natural origin [8]. Bergamot and bergamot extracts have been shown to exert a wide range of positive metabolic effects in both preclinical models and clinical tests. In particular, its mechanism of action has been characterized and it has been found that bergamot is able to modulate different metabolic pathways [9]. Therefore, its supplementation may represent a natural multi-target approach for metabolic health. Indeed, bergamot is known to improve cardiometabolic parameters altered in subjects with features of metabolic syndrome such as unbalanced plasma lipid levels, insulin resistance, and systemic inflammation [10].

Most of the studies focus on the purified fraction of bergamot flavonoids [11, 12]. Nevertheless, the whole bergamot phytocomplex is clinically interesting because the different bioactive molecules work in synergy, exerting a stronger effect when compared to the single active fraction.

The aim of our study was to compare the metabolic and vascular effects of 12-week supplementation with two different doses of a new, highly standardized bergamot phytocomplex versus placebo in healthy subjects with unbalanced metabolic parameters.

Material and methods

This was a monocentric, randomized, double-blind, placebo-controlled, three-arm, parallel-group clinical trial conducted on overall healthy volunteers aged 20–75 years with typical features of metabolic syndrome according to the harmonized criteria proposed by the International Atherosclerosis Society [13]. The study was carried out between January and December 2022. In particular, subjects with estimated cardiovascular risk higher than 5% (ESC SCORE) [14], low-density lipoprotein cholesterol (LDL-C) > 190 mg/dl, body mass index (BMI) > 30 kg/m², with diabetes mellitus, with personal history of atherosclerosis-related cardiovascular disease (CVD) (coronary artery disease, cerebrovascular disease, carotid atherosclerosis diagnosed by ultrasound), myopathy, renal failure, chronic liver disease, or with any other serious or invalidating disease reducing the subjects' ability to comply with the full protocol, were excluded from the clinical trial. Subjects who were under pharmacological treatment with lipid-lowering drugs, taking medication that affects lipid metabolism, or consuming any food supplements that may potentially impact cholesterol, triglycerides, blood glucose, blood pressure, and liver markers were excluded from the randomization process.

The study was conducted in accordance with the Declaration of Helsinki and with Guidelines for Good Clinical Practice (GCP). The study protocol was approved by the Ethical Committee of the University of Bologna. All volunteers signed a written informed consent document before starting any study related activity.

The study included a 2-week run-in period of diet standardization. After the run-in period, the volunteers were randomized to 3 different groups depending on the treatment with high-dose (700 mg/day) or low-dose (350 mg/day) bergamot extract or to placebo.

The investigational product (produced by AKHYNEX Srl, Polistena, RC, Italy, and commercially available with the tradename of Endoberg by AKHYNEX and as Kalita by Giellepi S.p.A., Milan, Italy) is a water extract obtained from bergamot (*Citrus bergamia* Risso) fruit cultivated in the Calabrian region, Southern Italy.

The product is produced by, firstly, washing bergamot fruits in steel tanks with water. Then, once peeled, fruits are pressed using a screw press, yielding juice and solid residue (pulp). The pulp obtained is extracted several times with pure water, then dried in rotary kilns and finally sent for pectin extraction for industrial use. The juice obtained by the extraction of the pulp is concentrated in an evaporator in order to obtain a highly concentrated juice suitable for easier storage and

transportation. After the addition of arabic gum and adjusting the pH, the concentrated juice is dried in a spray drier, obtaining a powder, referred to below as bergamot phytocomplex.

The water extraction process is capable of preserving all biologically active molecules that make up the phytocomplex, including polysaccharides, amino acids, and polyphenols. These components work synergistically with each other, resulting in an effective extract that does not require further concentration. The product obtained exhibits a unique quali-quantitative profile and contains the full spectrum of bergamot bioactive components, despite not being a highly purified plant extract.

All patients were recommended to take the tested products regularly, i.e. every day. Participants' adherence was assessed by counting the number of tablets returned at the time of specified clinic visits. At the end of the study all unused tablets were retrieved for inventory.

At enrolment, patients were given standard behavioral and qualitative dietary suggestions to correct their unhealthy habits. In particular, subjects were strongly recommended to follow for the entire duration of the study the general indications of a Mediterranean diet, avoiding an excessive intake of dairy and red meat derived products and reducing dietary excesses, in order to maintain an overall balanced diet. With the advice of a specialist physician, volunteers had to aim to obtain in their diet approximately 50% of calories from carbohydrates, 30% from fat (6% saturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/day and 35 g/day of fibers. Furthermore, the enrolled volunteers were asked to maintain a constant intake of fruits, vegetables, olive oil and wine, in order to reduce the variability in the dietary content of fibers and polyphenols. Individuals were also encouraged to increase their physical activity by engaging in brisk walking or cycling for at least 20 min 3 to 5 times per week [15].

Nutrient intake was estimated from the 4-day records before the randomization and at the end of the intervention period. The nutritional evaluation was performed by an expert nutritionist biologist using Italian Food Composition databases [16].

Assessments

Patients underwent anamnestic evaluations, physical examinations, and laboratory analyses at three time points: baseline, after 6 weeks of treatment, and at the trial's end (week 12). These measurements followed standardized protocols and were conducted by specially trained staff.

The patients' personal history was evaluated paying particular attention to CVD and other dis-

eases, assessment of dietary and smoking habits (both evaluated with validated semiquantitative questionnaires) [17], physical activity [18] and pharmacological treatment.

Waist circumference (WC) was measured at the end of a normal expiration, in a horizontal plane at the midpoint between the inferior margin of the last rib and the superior iliac crest. Height and weight were measured to the nearest 0.1 cm and 0.1 kg respectively, with subjects standing erect with eyes directed straight, wearing light clothes, and with bare feet. Then, BMI was calculated as body weight in kilograms, divided by height squared in meters (kg/m^2).

The biochemical analyses were carried out on venous blood withdrawn early in the morning from the basilica vein. Subjects fasted for at least 12 h before sampling. The biochemical measurements were centrally performed in our department's laboratory. Serum used was centrifuged at 3000 RPM for 15 min at ambient temperature. All of the laboratory analyses were performed by trained personnel immediately after centrifugation, in accordance with the standardized methods described in detail elsewhere [19]. The following parameters were obtained or calculated through the appropriate formulae: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), non-HDL cholesterol (non-HDL-C), low-density lipoprotein cholesterol (LDL-C), serum uric acid (SUA), fasting plasma glucose (FPG), fasting plasma insulin (FPI), homeostatic model assessment of insulin resistance (HOMA-IR), hs-CRP, creatinine (Cr), γ-glutamyl transpeptidases (γ-GT), alanine transaminase (ALT), aspartate transaminase (AST), fatty liver index (FLI), and creatine kinase (CK). LDL-C was obtained by the Friedewald formula (LDL-C= TC − HDL-C − TG/5 expressed in mg/dl) [20]. Non-HDL-C resulted from the difference between TC and HDL-C. HOMA-IR was calculated as the product of FPG and FPI (respectively expressed in mmol/l and U/ml) divided by 22.5 [21]. FLI was obtained by dividing ($e^{0.953 \times log_e(TG) + 0.139 \times BMI + 0.718 \times log_e(GGT) + 0.053 \times WC − 15.745}$) by ($1 + e^{0.953 \times log_e(TG) + 0.139 \times BMI + 0.718 \times log_e(GGT) + 0.053 \times WC − 15.745}$) and then by multiplying by 100, where TG are reported in mg/dl and GGT in U/l [22].

Systolic (SBP) and diastolic blood pressure (DBP) were measured in each participant while they were supine and at rest. To do so, a validated oscillometric device was used with cuffs of the appropriate size applied to the right upper arm. Three BP readings were sequentially obtained at a 1-min interval in order to implement detection accuracy protocols. The first one was discarded, and the average between the other two was considered as the study's variable.

Endothelial function was evaluated through Endocheck (BC Biomedical Laboratories Ltd., Vancou-

ver, BC, Canada), a method embedded within the Vicorder device that guarantees good intra- and inter-operator reliability. The measurement was carried out with patients in supine position and in abstinence from cigarette smoking and caffeinated beverages for at least 12 h. After a 10-min rest, the brachial pulse volume (PV) waveforms were recorded at baseline for 10 s and during reactive hyperemia. The BP cuff was inflated to 200 mm Hg for 5 min and PV waveforms were recorded for 3 min after the cuff was released. Endothelial reactivity (ER) was calculated as change in the PV waveform area, comparing waveforms before and during hyperemia through the equation $\sqrt{PV2/PV1}$, where PV1 represents PV at the baseline and PV2 represents PV during hyperemia [23, 24].

Safety and tolerability were evaluated through the continuous monitoring of treatment-emergent adverse events, clinical safety laboratory findings, vital sign measurements and physical examinations. A blind, independent expert clinical event committee was appointed by the principal investigator in order to categorize the adverse events that could possibly be experienced during the trial as not related, unlikely related, possibly related, probably related, or definitely related to the study treatment.

Statistical analysis

Data were analyzed using intention to treat by mean of SPSS Statistics version 26.0 (IBM Corporation, Armonk, NY, USA) for Windows. The primary outcome of the study was the change in TG/HDL-C as a marker of atherogenic dyslipidemia in metabolic syndrome. A sample size of 28 subjects per group was needed to detect a mean treatment difference in TG/HDL of 5%, with a power of 0.90 and an α error of 0.05. As per protocol, we decided a priori to check the efficacy of treatments in subjects who had taken at least 90% of the tested product's doses as per trial design. Normally distributed baseline characteristics of the population were compared using Student's t test and the χ^2 test followed by Fisher's exact test for categorical variables. Between-group differences were assessed by ANOVA followed by Tukey's post-hoc test. Data were expressed as mean values with their corresponding standard deviations. All tests were 2-sided. A p level of < 0.05 was considered significant for all tests.

Results

Ninety patients were enrolled and assigned via randomization to receive high-dose (n = 30), low-dose (n = 30) or placebo (n = 30) treatment. All participants completed the trial according to the study design (Figure 1) and no patient experienced any subjective or laboratory adverse event (dropout rate = 0). The adherence to the treatment neared 100% in all groups.

Enrolled subjects maintained similar dietary habits from the randomization until the end of the study, without significant changes in total energy, salt intake or coffee and alcohol consumption (Table I).

Baseline clinical features and laboratory analyses were similar between groups (Tables II and III).

Regarding the primary outcome of the study, the TG/HDL-C ratio improved in all treatment groups vs. baseline after 6 weeks of treatment
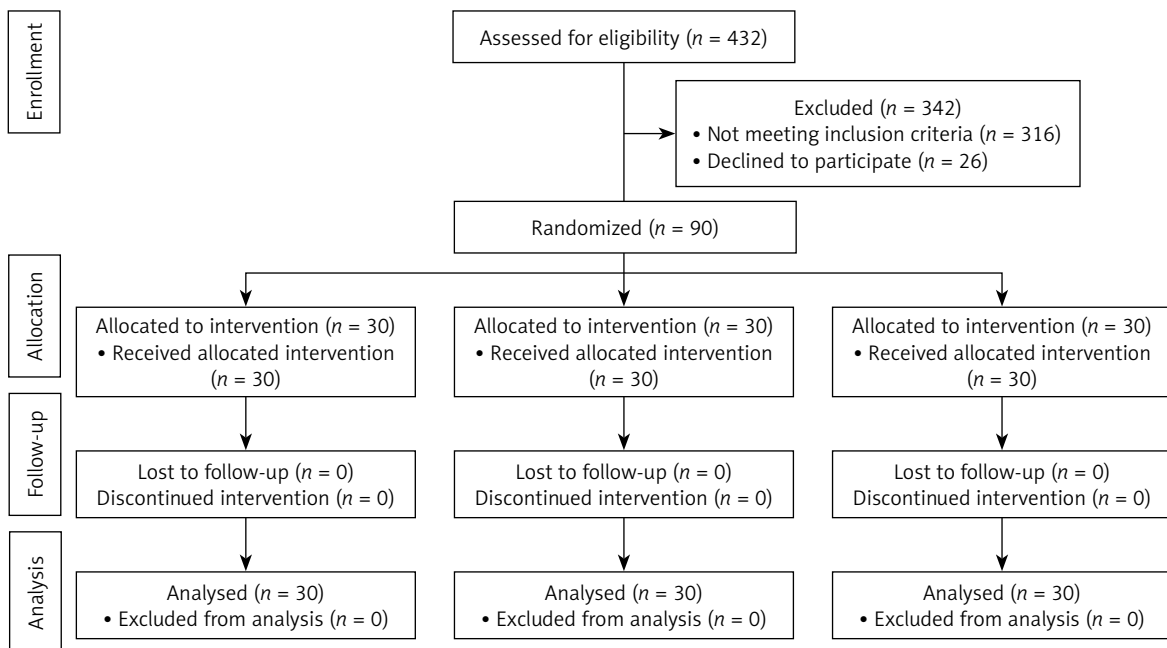

Figure 1. Trial selection and design according the study protocol

Federica Fogacci, Marina Giovannini, Edigio Imbalzano, Elisa Grandi, Elisabetta Rizzoli, Sergio D'Addato, Arrigo F.G. Cicero

Table I. Daily energy and nutrient intakes assessed by patient food diaries, before and after the treatment periods, expressed as mean ± standard deviation

Variables	Placebo arm (n = 30)		Low-dose arm (n = 30)		High-dose arm (n = 30)	
	Baseline	Post-treatment	Baseline	Post-treatment	Baseline	Post-treatment
Energy value [kJ/kcal]	1489 ±132	1492 ±148	1474 ±149	1482 ±136	1486 ±143	1499 ±139
Carbohydrate (% of energy)	53.4 ±3.6	54.2 ±3.7	53.4 ±2.8	54.3 ±2.8	53.4 ±2.7	52.3 ±2.9
Protein (% of energy)	18.4 ±1.9	18.0 ±1.8	18.6 ±1.8	18.1 ±1.7	18.3 ±1.9	17.1 ±1.8
Animal protein (% of energy)	10.5 ±1.7	10.4 ±1.4	10.8 ±0.9	10.6 ±1.0	10.6 ±0.8	9.8 ±0.7
Vegetal protein (% of energy)	6.7 ±0.8	6.7 ±1.0	6.9 ±0.9	6.8 ±0.9	6.8 ±0.8	7.2 ±1.9
Total fat (% of energy)	28.2 ±1.6	29.1 ±1.8	29.4 ±2.1	28.7 ±2.4	29.2 ±2.1	28.7 ±2.5
Saturated fatty acids (% of energy)	9.5 ±1.9	9.3 ±0.9	8.8 ±1.0	8.9 ±1.2	9.1 ±0.9	9.3 ±1.1
MUFA (% of energy)	12.9 ±1.3	12.5 ±1.4	12.5 ±1.2	12.7 ±1.1	12.0 ±1.2	12.4 ±1.1
PUFA (% of energy)	6.7 ±0.5	6.7 ±0.8	6.9 ±0.9	6.7 ±1.0	6.7 ±0.8	6.7 ±0.6
Dietary fiber [g]	18.2 ±2.5	18.0 ±1.9	17.8 ±2.3	17.9 ±2.4	17.9 ±2.3	18.3 ±2.4
Cholesterol [mg]	193.7 ±13.9	189.9 ±14.3	195.7 ±13.7	193.2 ±14.5	188.7 ±12.3	188.7 ±11.3

MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

Table II. Trend in anthropometric and hemodynamic parameters during the trial in the different treatment groups

Parameter	Placebo arm (n = 30; F = 14, M = 16)			Low-dose arm (n = 30; F = 15, M = 15)			High-dose arm (n = 30; F = 14, M = 16)		
	Baseline	Week-6 follow-up	Week-12 follow-up	Baseline	Week-6 follow-up	Week-12 follow-up	Baseline	Week-6 follow-up	Week-12 follow-up
WC [cm]	88.2 ±4.2	87.1 ±3.9	87.2 ±4.0	89.3 ±4.4	88.2 ±4.0	87.1 ±3.8	87.4 ±3.8	85.8 ±3.4	85.2 ±3.3
BMI [kg/m²]	27.0 ±1.2	26.8 ±1.1	26.7 ±1.3	26.8 ±1.3	26.7 ±1.2	26.5 ±1.3	26.8 ±1.4	26.7 ±1.3	26.0 ±1.1
BF (%)	32 ±3	31 ±4	31 ±2	33 ±4	32 ±4	32 ±4	34 ±4	33 ±4	31 ±3*
SBP [mm Hg]	128 ±4	129 ±3	128 ±5	129 ±4	126 ±3	127 ±4	125 ±4	124 ±5	123 ±5
DBP [mm Hg]	82 ±2	83 ±3	83 ±2	82 ±2	85 ±3	84 ±2	84 ±2	83 ±3	83 ±3
Endothelial reactivity (%)	1.38 ±0.21	1.31 ±0.19	1.35 ±0.20	1.33 ±0.24	1.37 ±0.21	1.39 ±0.19	1.36 ±0.17	1.39 ±0.22	1.44 ±0.15*

$*P < 0.05$ vs. baseline. WC – waist circumference, BMI – body mass index, BF – body fat, SBP – systolic blood pressure, DBP – diastolic blood pressure.

($p < 0.05$), while it improved versus placebo in both the low-bergamot dose and high-bergamot dose groups ($p < 0.05$).

In the placebo group, only plasma TG significantly decreased versus baseline values after 6 and 12 weeks ($p < 0.05$), and TG/HDL-C ratio after 6 weeks (but not after 12 weeks). No other parameter changed during the observation period.

In the low-dose treated group, after 6 weeks plasma LDL-C and TG significantly decreased versus baseline values ($p < 0.05$). After 12 weeks, TC, non-HDL-C, TG and HOMA-index improved both versus baseline and placebo values (all, $p < 0.05$).

In the high-dose treated group, after 6 weeks TC, LDL-C, non-HDL-C, TG, γGT and hs-CRP significantly decreased versus baseline values ($p < 0.05$). TC, LDL-C, non-HDL-C, and TG also decreased versus placebo values ($p < 0.05$).

After 12 weeks, TC, LDL-C, non-HDL-C, TG, FPG, FPI, HOMA-Index, GGT FLI, and hs-CRP significant-

ly decreased versus baseline values ($p < 0.05$), while HDL-C increased. TC, LDL-C, non-HDL-C, TG, HOMA index, GGT, and hs-CRP values significantly decreased versus placebo, as well ($p < 0.05$).

Percentage body fat decreased by 9.7 ±1.1% after 12 weeks of treatment versus baseline only in the high-dose treated group ($p < 0.05$). Similarly, endothelial reactivity (ER) improved by 0.08 ±0.02% after 12 weeks of treatment versus baseline only in the high-dose treated group ($p < 0.05$).

None of the other anthropometric or hemodynamic parameters exhibited a significant difference compared to the baseline or placebo (p always > 0.05).

Discussion

There is an increasing interest in clinically tested nutraceuticals with positive effects on human metabolic health, in particular when they can si-

Table III. Trend in laboratory parameters during the trial in the different treatment groups

Parameter	Placebo arm (n = 30; F = 14, M = 16)			Low-dose arm (n = 30; F = 15, M = 15)			High-dose arm (n = 30; F = 14, M = 16)		
	Baseline	Week-6 follow-up	Week-12 follow-up	Baseline	Week-6 follow-up	Week-12 follow-up	Baseline	Week-6 follow-up	Week-12 follow-up
TC [mg/dl]	248 ±14	244 ±11	243 ±12	244 ±12	233 ±11	228 ±9*°	250 ±13	223 ±10*°	217 ±9*°
LDL-C [mg/l]	164 ±9	163 ±9	163 ±10	162 ±9	154 ±7*	158 ±6	169 ±9	145 ±8*°	139 ±7*°
HDL-C [mg/dl]	45 ±4	46 ±3	44 ±3	44 ±2	45 ±3	46 ±3	45 ±3	47 ±3	48 ±4*
Non HDL-C [mg/dl]	203 ±11	198 ±10	199 ±10	200 ±11	188 ±10	182 ±10*°	205 ±12	176 ±11*°	169 ±9*°
TG [mg/dl]	195 ±12	177 ±13*	179 ±11*	189 ±13	170 ±14*	167 ±13*°	181 ±14	156 ±15*°	151 ±13*°
TG/HDL-C	4.3 ±1.1	3.8 ±1.2*	4.1 ±1.0	4.3 ±1.2	3.8 ±1.3*	3.6 ±1.2*°	4.0 ±1.3	3.3 ±1.4*°	3.1 ±1.2*°
FPG [mg/dl]	90 ±8	88 ±6	87 ±7	87 ±5	85 ±4	84 ±4	90 ±7	87 ±5	85 ±5*
FPI [µU/ml]	18.1 ±3.2	17.7 ±2.8	17.5 ±2.9	18.5 ±3.6	18.3 ±3.4	17.3 ±3.6	18.4 ±3.5	17.8 ±3.0	16.4 ±2.4*
HOMA-IR	4.1 ±0.8	3.9 ±0.7	3.9 ±0.6	3.9 ±0.9	3.8 ±0.7	3.5 ±0.6*°	4.1 ±0.7	3.9 ±0.8	3.6 ±0.7*°
AST [U/l]	22 ±5	23 ±4	22 ±4	19 ±5	18 ±3	18 ±4	22 ±6	20 ±4	21 ±4
ALT [U/l]	24 ±6	24 ±5	23 ±6	22 ±4	21 ±4	21 ±5	23 ±5	22 ±4	23 ±5
γ-GT [U/l]	29 ±7	27 ±7	26 ±8	26 ±6	23 ±5	25 ±5	27 ±8	22 ±6*	21 ±4*°
FLI	58 ±10	57 ±9	56 ±9	56 ±8	54 ±7	54 ±8	58 ±10	54 ±8	53 ±8*
SUA [mg/dl]	5.3 ±0.8	5.1 ±0.9	5.3 ±0.7	5.2 ±0.8	5.3 ±0.8	5.1 ±0.7	5.1 ±1.0	5.2 ±1.0	5.0 ±0.8
CK [U/l]	131 ±22	141 ±38	157 ±36	144 ±41	124 ±36	137 ±29	139 ±36	141 ±38	143 ±22
hs-CRP [mg/l]	2.86 ±0.13	2.84 ±0.19	2.88 ±0.15	2.80 ±0.15	2.66 ±0.11	2.61 ±0.13	2.91 ±0.12	2.61 ±0.09*	2.39 ±0.08*°

*$P < 0.05$ vs. baseline; °$p < 0.05$ vs. placebo. ALT – alanine aminotransferase, AST – aspartate aminotransferase, CK – creatine kinase, FPG – fasting plasma glucose, FLI – fatty liver index, FPI – fasting plasma insulin, γ-GT – γ-glutamyl transferase, HDL-C – high-density lipoprotein cholesterol, HOMA-IR – homeostatic model assessment for insulin resistance, hs-CRP – high-sensitivity C-reactive protein, LDL-C – low-density lipoprotein cholesterol, SUA – serum uric acid, TC – total cholesterol, TG – triglycerides.

multaneously act on different pharmacological targets [6, 25]. Among these nutraceuticals, bergamot extracts seem to be among the most promising [26].

Overall, the treatment tested in the present randomized, double-blind, placebo-controlled, three-arm, parallel-group clinical trial showed a general improvement of metabolic parameters in adults subjects with features of metabolic syndrome. We observed that after 12 weeks of treatment, both tested bergamot doses were able to improve significantly both atherogenic dyslipidemia and insulin sensitivity versus placebo. In the high-dose treated group, TC, LDL-C, non-HDL-C, and TG improved versus placebo already after 6 weeks of treatment. After 12 weeks of treatment, TC decreased by 13.2 ±2.1%, LDL-C by 17.7 ±3.2%, non-HDL-C by 17.5 ±3.1%, TG by 16.6 ±3.3%, TG/HDL-C by 22.5 ±4.4%, HOMA-IR by 12.2 ±2.1%, GGT by 22.2 ±4.6%, and hs-CRP by 17.9 ±3.4% versus baseline ($p < 0.05$) and versus placebo ($p < 0.05$).

The observed effects on lipid metabolism are consistent with those reported in a recent systematic review of randomized clinical trials that evaluated the efficacy of bergamot extract. These trials showed that the extract can lead to significant decreases in TC, ranging from 12.3% to 31.3%, as well as in LDL-C and TG, with reductions ranging from 7.6% to 40.8% and 11.5% to 39.5%, respectively. Notably, the largest reductions were seen at doses double the highest one we tested [27].

Our study found that high-dose treatment with a bergamot phytocomplex led to an improvement in percentage body fat compared to baseline, but not compared to placebo. Additionally, although not statistically significant, a slight reduction in waist circumference of approximately 2.2 cm was observed in the high-dose treatment group. These findings suggest that the insulin sensitivity improving properties of bergamot extract may have contributed to this effect [28].

Endothelial reactivity improved versus baseline in the high-dose treated group, which could be related to a direct positive impact of *Citrus* polyphenols on endothelial function [29].

All of these data are in line with observations reported for the new improved bergamot-based nutraceutical products with higher polyphenol levels recently tested and not yet included in the previously cited meta-analysis [11, 30].

Unlike most of the nutraceutical bergamot products on the market, the extract used in the present investigation is not selectively purified in order to contain high levels of polyphenols, preserving the natural bergamot phytocomplex. In

Federica Fogacci, Marina Giovannini, Edigio Imbalzano, Elisa Grandi, Elisabetta Rizzoli, Sergio D'Addato, Arrigo F.G. Cicero

fact, preliminary pre-clinical data [31] show that the tested bergamot phytocomplex contains multiple bioactive components and in particular soluble fibers, polyphenols and small molecules, such as stachydrine. LC-MS analysis identified 86 compounds, with hesperetin, naringenin, apigenin and eriodictyol glucosides being the main components found. Endoberg/Kalita contains the full spectrum of bergamot bioactive molecules which, acting synergistically [31], may justify the observed efficacy found in both preclinical and clinical studies. We speculate that such efficacy is due to the whole phytocomplex and particularly the pectin contents, which may assist and enhance the bioavailability of active components [32].

The findings of the present clinical investigation highlight that the bioactive components present in the bergamot phytocomplex work in synergy to enhance clinical effectiveness. We can speculate that while the flavonoid fraction plays a significant role, other classes of molecules and secondary metabolites may also contribute to this effect, suggesting that the active molecules responsible for the observed benefits are diverse and not limited to a single class of compounds. Given the lack of bioactive compounds acting on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, it could be considered in the management of statin intolerant subjects with a low added cardiovascular risk [33].

We have to acknowledge some study limitations. In particular, the trial was short, so we cannot infer that longer intake of the tested dietary supplement could have led to an eventual further improvement of the studied parameters or to a reduction of the positive effect observed. Moreover, the sample size of the study was relatively small, even if adequately powered for the aim of the trial. For this reason, we did not conduct any advanced statistical analysis, such as regression, to identify predictors of better or worse responses to the tested product. Furthermore, as nutraceuticals need to be taken for extended periods, ensuring their safety has become a topic of growing importance [34, 35]. With this in context, the overall middle-term tolerability of the nutraceuticals tested has been largely confirmed by the available literature and by the present trial. Further long-term data on a larger patient sample should be obtained in a new double-blind randomized clinical trial in order to confirm our present findings.

In conclusion, in our study the Endoberg/Kalita bergamot extract was found to significantly improve glucose and lipid metabolism, as well as inflammation, in individuals with metabolic syndrome. These findings suggest that this natural extract could be a promising multi-target solution for enhancing cardiometabolic health.

Conflict of interest

The authors declare no conflict of interest.

References

1. Scuteri A, Laurent S, Cucca F, et al.; Metabolic Syndrome and Arteries Research (MARE) Consortium. Metabolic syndrome across Europe: different clusters of risk factors. Eur J Prev Cardiol 2015; 22: 486-91.
2. Park S, Lee S, Kim Y, et al. Altered risk for cardiovascular events with changes in the metabolic syndrome status: a nationwide population-based study of approximately 10 million persons. Ann Intern Med 2019; 171: 875-84.
3. Dayi T, Ozgoren M. Effects of the Mediterranean diet on the components of metabolic syndrome. J Prev Med Hyg 2022; 63 (2 Suppl 3): E56-64.
4. Kwon DH, Cho YG, Park HA, Koo HS. The difference in the prevalence of metabolic syndrome according to meeting guidelines for aerobic physical activity and muscle-strengthening exercise: a cross-sectional study performed using the Korea National Health and Nutrition Examination Survey, 2014-2019. Nutrients 2022; 14: 5391.
5. Mazidi M, Katsiki N, Banach M. A greater flavonoid intake is associated with lower total and cause-specific mortality: a meta-analysis of cohort studies. Nutrients 2020; 12: 2350.
6. Cicero AFG, Fogacci F, Stoian AP, et al. Nutraceuticals in the management of dyslipidemia: which, when, and for whom? Could nutraceuticals help low-risk individuals with non-optimal lipid levels? Curr Atheroscler Rep 2021; 23: 57.
7. Patti AM, Al-Rasadi K, Giglio RV, et al. Natural approaches in metabolic syndrome management. Arch Med Sci 2018; 14: 422-41.
8. Cicero AF, Colletti A. Role of phytochemicals in the management of metabolic syndrome. Phytomedicine 2016; 23: 1134-44.
9. Palacio TLN, Siqueira JS, de Paula BH, et al. Bergamot (*Citrus bergamia*) leaf extract improves metabolic, antioxidant and anti-inflammatory activity in skeletal muscles in a metabolic syndrome experimental model. Int J Food Sci Nutr 2023; 74: 64-71.
10. Carresi C, Gliozzi M, Musolino V, et al. The effect of natural antioxidants in the development of metabolic syndrome: focus on bergamot polyphenolic fraction. Nutrients 2020; 12: 1504.
11. Fogacci F, Rizzoli E, Giovannini M, et al. Effect of dietary supplementation with Eufortyn® Colesterolo Plus on serum lipids, endothelial reactivity, indexes of non-alcoholic fatty liver disease and systemic inflammation in healthy subjects with polygenic hypercholesterolemia: the ANEMONE Study. Nutrients 2022; 14: 2099.
12. Cicero AFG, Fogacci F, Bove M, Giovannini M, Borghi C. Three-arm, placebo-controlled, randomized clinical trial evaluating the metabolic effect of a combined nutraceutical containing a bergamot standardized flavonoid extract in dyslipidemic overweight subjects. Phytother Res 2019; 33: 2094-101.
13. Alberti KG, Eckel RH, Grundy SM, et al.; International Diabetes Federation Task Force on Epidemiology and Prevention; Hational Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epi-

demiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009; 120: 1640-5.

14. Perk J, De Backer G, Gohlke H, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Eur Heart J 2012; 33: 1635-701.

15. Cicero AFG, Fogacci F, Veronesi M, et al. A randomized placebo-controlled clinical trial to evaluate the medium-term effects of oat fibers on human health: the Beta-Glucan Effects on Lipid Profile, Glycemia and in-Testinal Health (BELT) Study. Nutrients 2020; 12: 686.

16. Cicero AFG, Fogacci F, Bove M, Giovannini M, Veronesi M, Borghi C. Short-term effects of dry extracts of artichoke and berberis in hypercholesterolemic patients without cardiovascular disease. Am J Cardiol 2019; 123: 588-91.

17. Cicero AFG, Caliceti C, Fogacci F, et al. Effect of apple polyphenols on vascular oxidative stress and endothelium function: a translational study. Mol Nutr Food Res 2017; 61(11). doi: 10.1002/mnfr.201700373.

18. Cicero AF, D'Addato S, Santi F, Ferroni A, Borghi C; Brisighella Heart Study. Leisure-time physical activity and cardiovascular disease mortality: the Brisighella Heart Study. J Cardiovasc Med 2012; 13: 559-64.

19. Cicero AFG, Sahebkar A, Fogacci F, Bove M, Giovannini M, Borghi C. Effects of phytosomal curcumin on anthropometric parameters, insulin resistance, cortisolemia and non-alcoholic fatty liver disease indices: a double-blind, placebo-controlled clinical trial. Eur J Nutr 2020; 59: 477-83.

20. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.

21. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-9.

22. Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol 2006; 6: 33.

23. Day LM, Maki-Petaja KM, Wilkinson IB, McEniery CM. Assessment of brachial artery reactivity using the endocheck: repeatability, reproducibility and preliminary comparison with ultrasound. Artery Res 2013; 7: 119-20.

24. Cicero AFG, Bove M, Cincione RI, Fogacci F, Veronesi M. Effect of combined lipid-lowering and antioxidant nutraceutical on plasma lipids, endothelial function, and estimated cardiovascular disease risk in moderately hypercholesterolemic patients: a double-blind, placebo-controlled randomized clinical trial. Arch Med Sci Atheroscler Dis 2021; 6: e145-51.

25. Ruscica M, Penson PE, Ferri N, et al.; International Lipid Expert Panel (ILEP) and International Lipid Expert Panel Experts (alphabetically). Impact of nutraceuticals on markers of systemic inflammation: potential relevance to cardiovascular diseases – a position paper from the International Lipid Expert Panel (ILEP). Prog Cardiovasc Dis 2021; 67: 40-52.

26. Osadnik T, Goławski M, Lewandowski P, et al. A network meta-analysis on the comparative effect of nutraceuticals on lipid profile in adults. Pharmacol Res 2022; 183: 106402.

27. Lamiquiz-Moneo I, Giné-González J, Alisente S, et al. Effect of bergamot on lipid profile in humans: a systematic review. Crit Rev Food Sci Nutr 2020; 60: 3133-43.

28. Siqueira JS, Vieira TA, Nakandakare-Maia ET, et al. Bergamot leaf extract treats cardiorenal metabolic syndrome and associated pathophysiological factors in rats fed with a high sugar fat diet. Mol Cell Endocrinol 2022; 556: 111721.

29. Rizza S, Muniyappa R, Iantorno M, et al. Citrus polyphenol hesperidin stimulates production of nitric oxide in endothelial cells while improving endothelial function and reducing inflammatory markers in patients with metabolic syndrome. J Clin Endocrinol Metab 2011; 96: E782-92.

30. Fogacci F, Di Micoli V, Veronesi M, Cicero AF. Comparative effect of a nutraceutical compound based on a flavonoid complex from bergamot on plasma lipids, glucose metabolism and liver enzymes: a 3-arm, double-blind, placebo-controlled, randomized, clinical trial. Arch Med Sci 2023; 19: 1180-5.

31. Della Vedova L, Gado F, Vieira TA, et al. Chemical, nutritional and biological evaluation of a sustainable and scalable complex of phytochemicals from bergamot by-products. Molecules 2023; 28: 2964.

32. Carullo G, Spizzirri UG, Malivindi R, et al. Development of quercetin-DHA ester-based pectin conjugates as new functional supplement: effects on cell viability and migration. Nutraceuticals 2022; 2: 278-88

33. Banach M, Patti AM, Giglio RV, et al.; International Lipid Expert Panel (ILEP). The role of nutraceuticals in statin intolerant patients. J Am Coll Cardiol 2018; 72: 96-118.

34. Banach M, Katsiki N, Latkovskis G, et al. Postmarketing nutrivigilance safety profile: a line of dietary food supplements containing red yeast rice for dyslipidemia. Arch Med Sci 2021; 17: 856-63.

35. Banach M, Burchardt P, Chlebus K, et al. PoLA/CFPiP/PCS/PSLD/PSD/PSH guidelines on diagnosis and therapy of lipid disorders in Poland 2021. Arch Med Sci 2021; 17: 1447-547.