

Effects of a nutraceutical multicomponent including bergamot (*Citrus Bergamia Risso*) juice on metabolic syndrome: A pilot study

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Abstract.

BACKGROUND: The role of lipid-lowering and hypoglycemic nutraceuticals in cardiovascular disease prevention is the focus in recent years. The most studied compounds and plants are sterols, soy, red fermented rice, policosanols, artichoke, berberine. Epidemiological and experimental evidences suggest that dietary polyphenols, especially flavonoids, might play a role in preventing atherosclerosis, owing to their pleiotropic metabolic, anti-inflammatory and antioxidant effects. Recent studies have shown that bergamot juice and albedo (*Citrus Bergamia Risso et Poiteau*), an endemic plant growing in a limited part of the Ionian coast of Calabria (Italy) has a unique content of flavonoids and glycosides, such as neoeriocitrine, neoesperidine, naringenine, rutin, neodesmine, polyphenol and poncirine.

OBJECTIVE: The aim of this study was to investigate the effects of a phytocomplex from bergamot fruit (EP3116520A1) as dietary supplement to a Mediterranean diet on body weight, body mass index (BMI), waist circumference, plasmatic lipid fractions, glucose and C – reactive protein (CRP) in subjects with the metabolic syndrome (MetS; according to NCEP-ATP III criteria) without pharmacological treatment, except for basic treatment.

METHODS: 80 overweight adults (54% females, 46% males) with the diagnosis of Metabolic Syndrome (MetS), aged 45 ± 5 years, were enrolled and randomized to 2 groups: group A) followed a personalized low calorie Mediterranean diet (control group) and group B) enriched the same diet therapy with 1 tablet of a phytocomplex from bergamot fruit per day for 6 months (intervention group).

RESULTS: After 6 months patients in the intervention group showed a significant reduction of total cholesterol (–15%), LDL-Cholesterol (–22%), triglycerides (–23%), blood glucose (–15%), CRP (–40%) and a significant increase in the HDL-Cholesterol (+ 14%) levels compared to the control group (diet alone) where the changes were not significant, with not much significance in reduced body weight.

CONCLUSION: Our findings suggest that bergamot supplementation improves significantly all aspects of metabolic profile in patients with MetS and is superior to diet alone.

Keywords: Diet, bergamot, cholesterol, blood glucose

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1. Introduction

Lipid and glucose metabolism impairments are the most important modifiable risk factors for atherosclerotic vascular diseases such as coronary heart disease (CHD), cerebrovascular disease (e. g. stroke) and peripheral vascular disease [1, 2].

The control of these risk factors is crucial both for primary and secondary prevention of coronary heart disease (CHD) events. The most important conventional risk factors are high total cholesterol levels including high levels of low-density lipoprotein (C-LDL) and low levels of high-density lipoprotein (C-HDL); cigarette smoking, obesity, hypertension, diabetes mellitus, age (male ≥ 45 , female ≥ 55 or premature menopause without estrogen replacement therapy) and family history of premature CHD events (definite as myocardial infarction or sudden death (before 55 years in male and before 65 years in female) in first-degree relatives) [3].

The lipid pattern consisting of moderate hypertriglyceridemia, low HDL cholesterol, predominance of small dense LDL particles and mild to moderate increase in LDL cholesterol (so-called “atherogenic dyslipidemia”), is strongly related to pathogenesis of atherosclerosis [4].

Inflammation, insulin resistance and endothelial dysfunction are closely interconnected; genetic and environmental factors contribute to determine these three pathophysiological alterations occurring in a complex association. Inflammation and insulin resistance precede and aggravate the clinical manifestation of important pathological conditions such as type 2 diabetes mellitus (T2DM) and cardiovascular disease. Many studies [5] have demonstrated that non-pharmacological treatments can correct these metabolic alterations; it emphasizes the importance of an early detection and treatment of subjects at risk of developing T2DM. Hyperglycemia is not isolated but is a part of a cluster of metabolic abnormalities, defined as metabolic syndrome (MetS): it includes “classical” components such as dyslipidemia, arterial hypertension and central obesity; they are necessary for its definition and diagnosis. However, also “new” components such as alterations in the haemocoagulatory system, endothelial dysfunction, and low chronic inflammation have been identified and can represent an useful diagnostic tool to define the individual risk assessment and to tailor the therapy. Increasing evidence suggests that inflammation, insulin resistance and endothelial dysfunction may interact with each other in “dangerous crossroads” of pathogenetic factors responsible for the T2DM onset and its cardiovascular complications [6]. One of the first studies that supports a close relationship between inflammation and insulin resistance is the observation that interleukin (IL) –6 and C – Reactive Protein (CRP) circulating levels increase in subjects with impaired glucose tolerance – IGT and T2DM but not in subjects with normal glucose metabolism or patients with impaired fasting glucose – IFG (CATAnzaro METabolic RIsk Factors Study – CATAMERIS, an observational study focused on the identification of cardio-metabolic risk factors in a Caucasian population resident in Calabria) [7]. Both IL-6 and CRP levels are inversely related to insulin sensitivity, evaluated by the euglycemic-hyperinsulinemic clamp technique or by oral glucose tolerance test derived indices, such as Matsuda index [8]. Genetic studies have shown a role of inflammatory factors in the insulin resistance pathogenesis. The C-174G polymorphism in the promoter of the IL-6 gene is able to enhance its transcription. *In vitro* experiments [9] have demonstrated that the allele G has an increased transcriptional activity both under baseline conditions and in response to inflammatory stimuli, such as lipopolysaccharides or IL-1 cytokines. In another study [10] conducted on a sample of non-diabetic subjects the association of this polymorphism with an increased IL-6 circulating levels (higher in subjects carrying genotype GG) was demonstrated. GG genotype carriers presented reduced sensitivity to insulin (measured by euglycemic-hyperinsulinemic clamp) compared to allele C carriers, even after adjustment for age, sex, and body mass index (BMI). Various evidences indicate that adipose tissue is one of the main sources of the circulating IL-6; to evaluate the influence of the polymorphism on the expression of IL-6 in adipose tissue, Cardellini et al. studied 77 obese subjects after gastric banding intervention. GG genotype carriers showed an increase in IL-6 mRNA expression evaluated by the technique of real-time PCR, which was associated with an increase in insulin resistance, measured by HOMA index (homeostasis Model assessment).

Both genetic pattern and environmental factors (sedentary lifestyle, high-calorie diet rich in saturated fatty acids and cholesterol) contribute to the development of MetS [11].

The first therapeutic strategy to reduce the vascular risk in MetS, effective in many patients over a long period of time, is a lifestyle intervention based on physical activity increase, weight control and dietary habits improvement, adopting a Mediterranean Diet. These therapeutic steps have a great efficacy and a lower cost for national health care systems than pharmacotherapy. If drugs are unneeded, there are numerous effective options; pharmacological treatment of dyslipidemia consists in these following drug classes: statins (inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase), bile acids sequestrants (resins), nicotinic acid, fibrates and cholesterol absorption inhibitor (ezetimibe). Other drugs may also be necessary: metformin, antihypertensive agents, acetylsalicylic acid, etc. All these agents can cause side effects, also serious in many circumstances. The role of lipid-lowering and hypoglycemic supplements based on nutraceutical and herbal remedies (phytochemicals) agents in cardiovascular disease prevention is still on debate; their clinical use is very common and popular and the efficacy of many compounds is supported by good quality scientific evidence; nevertheless the use of food components (including polyphenols, glucosinolates, PUFAs, fibers, and friendly bacteria) by patients on conventional pharmacological therapy should be carefully assessed due to the possibility of food-drug interactions; even if several food compounds may exert a prophylactic function within the human body, their bioavailability and bioactivity have high interindividual variability and the mechanisms of biological action of food extracts and bioactive compounds remain to be elucidated [12].

The traditional management of metabolic diseases involves pharmacological intervention if diet and lifestyle modifications are not sufficient to achieve therapeutic goals. In the last years, many Guidelines and Recommendations [13] suggest to consider in selected patients at low-intermediate risk the “pharma-nutrition interface” before introducing high-cost therapy with potential adverse effects: it is represented by functional foods, characterized by the presence of bioactive compounds and by nutraceuticals, the same compounds in a drug form, suitably extracted titrated and often added with bio-enhancers to allow a standardized pharmacological effect.

The most studied and utilized compounds and plants are vegetable sterols, soy, red fermented rice, policosanols, artichoke, berberine [14]. Epidemiological and experimental evidences suggest that dietary polyphenols, especially flavonoids, might play a role in preventing atherosclerosis, owing to their pleiotropic metabolic, anti-inflammatory and antioxidant effects [15].

Recent studies have demonstrated that bergamot juice and albedo (*Citrus Bergamia*) (Risso et Poiteau), an endemic plant growing in a limited part of the Ionian coast of Calabria, Italy, has a unique content of flavonoids and glycosides, such as neoeriocitrine, neoesperidine, narigin, rutin neodesmine, polyphenol and poncirine [16, 17]. In addition, the bergamot is rich in 3-hydroxy-3-methylglutaryllicexexidosides of esperidine (bruteridine) and naringenine (melitidine) [18]. These substances inhibit HMG-CoA reductase, both in animal models with diet induced hyperlipidemia, and in patients with MetS, demonstrating a favorable effect on total cholesterol, C-LDL, C-HDL, triglycerides and glucose levels [19–25]. Several studies describe the cholesterol lowering effect of plant sterols and plant stanols by reduction of exogenous cholesterol intestinal absorption [26–36]. The same effect is also seen with artichoke extract, a natural compound traditionally used for jaundice and hepatic insufficiency [37]. Quantitative measurements have demonstrated that the artichoke extract inhibits cholesterol biosynthesis in a dose-dependent mechanism [38]. In particular some authors have identified cynarine (1,5-dicaffeoyl-D-chinic acid) – the main active component [39] in addition to lutein as cholesterol synthesis inhibitors which reduce the inflammation. Bergamot juice is rich in brutieridine and melitidine, two flavanone derivatives selectively inhibiting the HMG-CoA reductase enzyme [40, 41]. Even though there is a growing interest for the pleiotropic metabolic and anti-inflammatory effects of bergamot juice, there are not many studies on the effects *in vivo* of this nutraceutical on the plasmatic lipid fractions and glucose.

Aim of this study is to investigate the effects of a nutritional supplement composed of bergamot and other added bioactive compounds such as phytosterols, artichoke, vitamin C, in association with diet, on lipid fractions and glucose levels and inflammatory markers [42] in subjects with MetS.

2. Methods

We planned a pilot study designed as a randomized clinical trial to evaluate the short-term effects of a dietary supplementation with bergamot in association with a personalized dietary plan compared to the diet alone. The trial was conducted from sept. 2016 to march 2017 in the Diabetes Unit, “San Camillo – Forlanini” Hospital in Rome, Italy.

80 overweight adults aged 45 ± 5 years (43 females and 37 males) were recruited according to the criteria of National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III 2001 for Metabolic Syndrome (MetS). According to the NCEP – ATP III definition (2001), MetS is present if three or more of the following five criteria are met: waist circumference >102 cm in men or >88 cm in women; systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg; fasting triglyceride (TG) levels ≥ 150 mg/dl; fasting HDL cholesterol <40 mg/dl in men or <50 mg/dl in women; and fasting blood glucose ≥ 110 mg/dl [43].

The major exclusion criterion was the use of lipid lowering medications (statins, fibrates, omega-3), metformin or other nutraceutical compounds active in metabolism. The study was approved by the local ethics committee and all participants gave a written informed consent.

After the screening first visit the study participants were randomly assigned to 2 groups: group A ($n=40$) followed an hypocaloric personalized diet (control group); group B ($n=40$) supplemented the same diet therapy with a bergamot based phytocomplex provided by ESSERRE PHARMA srl over a period of 6 months (intervention group), each tablet includes: 200 mg bergamot juice dry extract, 120 mg phytosterols, 80 mg artichoke leaf extract and 20 mg vitamin C (commercial preparation). Group B received one tablet daily.

Mediterranean hypocaloric diet: with kcal 25/kg/ of ideal body weight (IBW)/die; CHO 50%, Fiber 15 g/1000 kcal, Protein 20%,

Fat 30% (monounsaturated $>14\%$, saturated $<10\%$, polyunsaturated $<10\%$) [44, 45].

The presence of smoking habits, hypertension, use of diuretics, ACE inhibitors and Ca antagonists, body weight, body height, waist circumference and blood samples, obtained after an overnight fasting, were registered at baseline (T0) and at months 6 (T1). Total cholesterol (TC), HDL-C, LDL-C, triglycerides, fasting blood glucose (FBG), C-reactive protein (CRP), creatine phosphokinase (CPK), glutamico oxalacetic transpherase (GOT) and glutamic pyruvate transpherase (GPT) were analyzed.

The primary outcome of the study was to evaluate the effects of combined therapy (diet + supplementation) vs. diet therapy alone on lipid parameters, plasma glucose and inflammation markers in patients with MetS without any additional pharmacological treatment (except the basic therapy) over a period of 6 months. The secondary endpoint was to verify the effect of the supplementation on body weight. Baseline (T0) and month 6 (T1) data are presented as mean \pm SD (standard deviation). Paired *t*-test was applied to compare all data at the time of measurement. *P*-value <0.05 was considered significant.

3. Results

Baseline characteristics of the participants are shown in Table 1. A total of 80 individuals (43 women, 37 men) were randomized in 2 groups. There were no differences in age, weight and BMI, waist circumference between the groups at baseline.

After 6 months of treatment, patients in Group B (supplemented group) showed a significant reduction in total cholesterol (-15%), LDL-C (-22%), triglycerides (-23%), glucose (-15%), CRP (-40%) and a significant increase in HDL-C ($+14\%$) in comparison to the control group where the reduction of total cholesterol was -3% , LDL – cholesterol -2% , triglycerides -5% , blood glucose -2.5% , CRP -4.5% and also in HDL – cholesterol there was a reduction by -9.5% . Changes from baseline to month 6 are shown in Table 2.

In addition, nobody presented any adverse event such as myalgia, increase of serum creatine phosphokinase or transaminase. There was a slight, no significant reduction in weight and body mass index, waist circumference, with no differences between the groups.

Table 1
Baseline characteristics, results are expressed as mean values \pm SD, ns = not significant

PARAMETERS	GROUP A (control, n = 40)	GROUP B (supplemented, n = 40)	p-value
Age (years)	44 \pm 3	45 \pm 5	ns
Sex (M/F)	19/21	18/22	ns
Weight (kg)	88 \pm 18	87 \pm 19	ns
BMI (kg/m ²)	31 \pm 3	30 \pm 6	ns
Waist Circumference (cm)	109 \pm 11	108 \pm 12	ns
Hypertension	24	22	ns
Smoke	11	10	ns
Diuretics	1	1	ns
Ca antagonists	2	1	ns
ACE inhibitors	9	8	ns

Table 2
Variation of metabolic parameters of inflammation and anthropometrics after 6 months of treatment, results are expressed as mean values \pm SD, ns = not significant

PARAMETERS	GROUP A T0	GROUP A T1	p-value	GROUP B T0	GROUP B T1	p-value
TC (mg/dl)	228 \pm 14	221 \pm 15	ns	224 \pm 33	190 \pm 30	$p < 0.00005$
LDL-C (mg/dl)	141 \pm 12	138 \pm 10	ns	145 \pm 35	113 \pm 26	$p < 0.00005$
HDL-C (mg/dl)	42 \pm 13	38 \pm 15	ns	42 \pm 11	49 \pm 11	$p < 0.00005$
Triglycerides (mg/dl)	189 \pm 9	179 \pm 10	ns	195 \pm 63	149 \pm 45	$p < 0.00005$
FBG (mg/dl)	118 \pm 8	115 \pm 6	ns	114 \pm 16	105 \pm 15	$p < 0.00005$
CRP (mg/dl)	2.2 \pm 1	2.1 \pm 1	ns	2 \pm 1	1.21 \pm 1	$p < 0.05$
GOT (U/L)	22.4 \pm 4	23 \pm 5	ns	21.5 \pm 4	23.5 \pm 4	ns
GPT (U/L)	24 \pm 3	25 \pm 4	ns	25 \pm 3	25.5 \pm 4	ns
CPK (U/L)	86 \pm 19	87 \pm 15	ns	89 \pm 13	87 \pm 20	ns
Weight (kg)	88 \pm 18	86 \pm 16	ns	87 \pm 19	85 \pm 17	ns
BMI (kg/m ²)	31 \pm 3	30 \pm 3	ns	30 \pm 5	29 \pm 5	ns
Waist Circumference (cm)	108 \pm 10	106 \pm 9	ns	107 \pm 11	105 \pm 12	ns

4. Discussion

Each MetS component represents a modifiable cardiovascular risk factor and its control is a goal in the prevention of cardiovascular disease and in the evolution towards type 2 diabetes mellitus. The high costs of these pathological conditions justify their surveillance and the implementation of effective preventive measures.

The current guidelines identify the healthy lifestyle (diet, regular physical activity) as the first line treatment of patients with MetS [43], but generally it is not sufficient to reach the established goals of the individual metabolic parameters, so pharmacological intervention is needed; often it requires multi-drug therapy (statins, ezetimibe, fibrates, metformin, antihypertensives, other insulin-sensitizing agents, orlistat, Acetylsalicylic acid, etc) in a single patient, increasing the risk of side effects. Furthermore, even low-risk patients, whose cholesterol levels do not reach the recommended target, have difficulty to get a metabolic equilibrium.

Nutraceutical titrated and standardized extracts, represent an interface between nutrients and drugs. They are naturally derived bioactive plant compounds with beneficial effects on health; there are more than 40 nutraceuticals with favorable effects on lipid and glucose metabolism reported in the literature; bergamot supplementation is supported by interesting clinical reports: recently Peter et al. 2016 [46] demonstrated in a cohort of 80 patients with MetS the reduction of carotid plaque.

(1.2 ± 0.4 to 0.9 ± 0.1 mm ($p < 0.0001$)) in a 6-months follow-up, in correlation with a significant reduction in LDL, in particular the small and dense fraction.

Our data have shown that a titrated extract of bergamot in association with artichoke extract, phytosterols and vitamin C added to the diet therapy, significantly reduces total cholesterol, LDL and fasting blood glucose, compared to the simple dietary intervention alone.

We wanted to prescribe a personalized dietary plan, avoiding simple nutrition counseling; the effectiveness of nutritional intervention is confirmed by the reduction in body weight in both groups, although not statistically significant: it is remarkable that the dietary model we followed was the Mediterranean one: also in the control group this healthy model, rich in bioactive compounds, induces some beneficial effects on body weight and metabolic parameters, but less relevant than in the intervention group (Mediterranean diet + bergamot extract).

The tested nutraceutical phytocomplex, induces also a significant reduction in CRP to confirm the anti-inflammatory action of the flavonoids contained therein. The role of low-grade inflammation in insulin-resistance pathogenesis is well documented [47] although it is not easy to demonstrate and monitor its presence the parameters currently used in clinical practice.

The inflammatory biomarker CRP, if dosed with high sensitivity kit, is a reliable parameter of inflammation, but because of different standardization methods and elevated costs of its measurement, it is not routinely recommended by the most important guidelines. However, apart from the clear anti-dyslipidemic-hypoglycemic effect of the nutraceuticals used in this study, the mechanism of action of individual substances remains to be clarified.

The strength of this study is represented by the sample size and that is the first study to demonstrate the efficacy of the extract of bergamot juice on metabolic syndrome parameters and on inflammation markers as CRP.

The limitation of this study is the measurement of parameters of routine use in outpatient clinical practice, without the description of the effects of the studied extract on low-grade inflammation and endocrine and paracrine activity of adipose tissue (measurement of TNF, IL, and other inflammatory cytokines). Further studies with longer duration are needed to confirm the effects of nutraceuticals and the study of related mechanisms.

Another point of discussion concerns the effective dose of the bergamot polyphenols: in our study the tested nutraceutical formula demonstrated its efficacy in MetS, but in other clinical settings the results may be different: recently Bruno A. et al. [48] reported the absence of any improvement of the metabolic impairment in patients treated with Antipsychotic agents after a short term treatment with a bergamot derived fraction. No significant effects were demonstrated on body weight, BMI, glucose and lipid plasmatic fractions; it is remarkable that the administered dose in these patients is higher than the one we used; furthermore, as MetS is an heterogeneous condition, it is likely that different genetic and environmental pathogenetic factors (food intake, reduced physical activity) play a different role in specific subsets of patients. Consequently the anti-inflammatory effects of polyphenols and other bioactive compounds may be ineffective.

5. Conclusions

Our data show that a nutraceutical phytocomplex supplementation based on bergamot juice, flavonoids, artichoke extract, phytosterols and vitamin c helps to improve the metabolic and inflammatory profile in patients with metabolic syndrome. Long-term observations are needed to evaluate its effects on the prevention of cardiovascular disease and evolution towards type 2 diabetes.

Conflict of interest

The authors declared no conflict of interest.

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