

Applied nutritional investigation

A nutraceutical with *Citrus bergamia* and *Cynara cardunculus* improves endothelial function in adults with non-alcoholic fatty liver disease



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ABSTRACT

Objective: Polyphenol intake may prevent hepatic steatosis and cardiovascular disease by potentially improving endothelial function. The purposes of this study are to investigate the association between fatty liver disease and endothelial dysfunction and to test the effects of a nutraceutical containing extracts made from *Citrus bergamia* and *Cynara cardunculus* on peripheral vascular endothelial function in adults with liver steatosis.

Methods: We analyzed data from 32 individuals with hepatic steatosis and endothelial dysfunction (reactive hyperemia index ≤ 1.67). Sixteen subjects took 1 capsule/d (300 mg/d) containing *Cynara cardunculus* extract and bergamot polyphenol fraction, while the other 16 subjects matched for age, sex, and body mass index took 1 capsule/d of placebo (maltodextrin) for 12 wk. All anthropometric parameters were assessed at baseline and after 12 wk as were lipids, glucose, and reactive hyperemia index using an EndoPAT 2000.

Results: The mean age was 52 ± 9 y. The mean reactive hyperemia index was 1.15 ± 0.4 . After 12 wk, we found a greater increase in reactive hyperemia index in the participants taking the nutraceutical rather than placebo (0.58 ± 0.5 versus 0.13 ± 0.5 ; $P = 0.02$, respectively). The stepwise multivariable analysis confirmed a positive association between reactive hyperemia index change and the nutraceutical treatment ($B = 0.38$; $P = 0.025$) and negative association with reactive hyperemia index values at baseline ($B = -0.81$; $P < 0.001$). No association was found between the reduction in the amount of intrahepatic fat and the improvement of endothelial function ($B = 0.002$; $P = 0.56$).

Conclusions: A nutraceutical containing bergamot and artichoke extracts improves peripheral vascular endothelial function in adults with hepatic steatosis and early phase of atherosclerosis.

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The protocol of the study was approved by the Local Ethic Committee (no. 219/2018/CE, approved September 24, 2018) and registered in the ISRCTN registry with reg. no. ISRCTN12833814.

Informed consent was obtained from all subjects involved in the study.

Data sets generated for this study are available on request to the corresponding author.

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Introduction

It is well accepted that non-alcoholic fatty liver disease (NAFLD) is associated with several atherosclerotic risk factors, such as hypertension, diabetes, and dyslipidemia [1,2], although the underlying mechanisms linking these conditions are still not fully understood. Insulin resistance plays a pivotal role in developing NAFLD-related complications as well as in increasing the risk of premature cardiovascular diseases, a leading cause of morbidity and mortality in patients with NAFLD [3]. Endothelial dysfunction

(ED) can be considered a marker of cardiovascular disease [4]. ED is characterized by an increase of endothelium-derived contracting molecules and by a reduction of the bioavailability of vasodilators, such as nitric oxide (NO) [5]. ED is generally evaluated by invasive methods, such as quantitative coronary angiography and coronary Doppler flow measurements, by intravascular infusions of endothelium-dependent vasodilators. At present, the main non-invasive techniques to evaluate ED are ultrasound techniques [6–9].

It has been found that NO-mediated vasodilation is abnormal in patients with insulin resistance [10,11]. At present, few data are available about the association between NAFLD and endothelium-dependent vasodilation assessed by non-invasive procedures [12,13]. However, a case-control study found that reactive hyperemia index (RHI), obtained by reactive hyperemia–peripheral artery tonometry [14], was associated with histologic markers of liver disease in subjects affected by NAFLD [15]. Several studies have found that intake of dietary polyphenols from fruits and vegetables may help prevent cardiovascular disease by potentially affecting endothelial function [16–18].

Along these lines, we performed a secondary analysis of a double-blind, placebo-controlled, randomized controlled trial (RCT) to investigate the association between fatty liver disease and ED, also testing the effects of Bergacyn [19], a nutraceutical containing extracts made from *Citrus bergamia* and *Cynara cardunculus*, on peripheral vascular endothelial function in adults with liver steatosis.

Materials and methods

Study design

This is a secondary analysis of a RCT that took place at the Clinical Nutrition Unit of the “Mater Domini” University Hospital, in Catanzaro, Italy, between February 11 and June 24, 2019. The trial protocol and the main results were previously published [19].

The RCT was performed in accordance with the Declaration of Helsinki guidelines. The protocol of the study was registered in the ISRCTN registry with reg. no. ISRCTN12833814 and approved by the Local Ethic Committee (no. 219/2018/CE; approved September 24, 2018). This project was financed by the Italian Ministry for Universities and Research (Nutramed Project, PON 03PE000_78_1). All participants were given and signed an informed consent.

Population and randomization

For the RCT, we enrolled a population of both sexes with liver steatosis between the ages of 30 and 75. Diagnosis of NAFLD was made based on the values of controlled attenuation parameter (CAP) >216 dB/m assessed by vibration-controlled transient elastography, as previously described [20]. We excluded individuals with liver steatosis due to other causes. In particular, we were excluded subjects with past and current alcohol abuse (>20 g of alcohol/d; 350 mL [12 oz] of beer, 120 mL [4 oz] of wine, and 45 mL [1.5 oz] of hard liquor, each containing 10 g of alcohol) and individuals with chronic hepatitis B and/or C virus infection, autoimmune or cholestatic liver disease, liver cirrhosis, pregnancy, nephrotic syndrome, chronic renal failure, gastroesophageal reflux, or cancer. We were also excluded patients were taking dietary supplements, functional foods and nutraceuticals to treat NAFLD, amiodarone, antiretroviral agents, corticosteroids, methotrexate, tamoxifen, or valproate, as ascertained from their clinical records [19]. All patients with allergies to nutraceutical compounds, or maize, with diabetes [21] or with serum triacylglycerols levels >250 mg/dL were also excluded [19].

One hundred and two adults with hepatic steatosis were randomized in a 1:1 ratio to take active treatment (Bergacyn) or placebo for 12 wk [19]. Participants were randomized using an Excel random number generator (Microsoft, Redmond, WA) to receive the nutraceutical or placebo. Thus, for this secondary analysis, we only included participants who had an ED assessment and completed data on all parameters analyzed. We performed diagnosis of ED based on the value of RHI ≤ 1.67 [21,22], evaluated by reactive hyperemia–peripheral artery tonometry. The intervention group was treated for 12 wk with 1 capsule/d of Bergacyn containing bergamot polyphenol fraction (BPF), *Cynara cardunculus* extract (CyC) plus excipients (i.e., polyunsaturated fatty acid and a mixture of bergamot pulp and albedo derivative). The placebo group matched by sex, age, and body mass index (BMI) to the intervention group took 1 capsule/d of placebo that contained 600 mg of

maltodextrin. A total of 32 participants were included for this analysis, 16 subjects for each group.

Outcomes

The primary endpoint of this study was a change of RHI after 12 wk of intervention in participants with NAFLD and ED. Detailed definitions of the RCT's primary and secondary endpoints were summarized in the original report [19]. All participants received oral and written recommendations to follow a Mediterranean-type diet without energy intake restriction [19]. The participants and investigators were unaware of who received the active treatment or placebo.

Dietary supplements

The specification sheet with the most relevant active ingredients of Bergacyn is reported in a previous study [19]. Briefly, bergamot juice was obtained by industrial pressing and clarified by ultrafiltration after depletion of oil fraction. Polyphenols were subsequently isolated, eluted by alkaline solution, and filtrated through a cationic resin and pH was neutralized. Polyphenol content as well as the toxicologic profiles of the extracts were assessed by high-performance liquid chromatography. In addition, standard microbiologic tests proved the absence of mycotoxins and bacteria. The extractive protocol, discussed previously, was also used for CyC extract.

Fibers obtained by bergamot albedo were used micronized and cogrinded with plant extracts as excipients for final formulations. All vegetables were provided by H&D (Bianco, Italy).

The final formulation contained 5% w/w of cynaropicrin. Both capsules were stored in bottles containing 56 capsules each (sufficient for a 4-wk treatment). A plastic bag containing four bottles each was provided to the participants with the aim of supplying a 12-wk treatment. All procedures have been performed according to the European Union guidelines concerning dietary supplements as previously reported [19].

Dietary intake

We evaluated participants' dietary intake using a food frequency questionnaire both at the start of the study and after 12 wk. In this RCT, all individuals were provided with written and oral advice by a registered dietitian to follow a Mediterranean diet, as previously detailed [19]. The macronutrient profiles were in the ~50% to 55% range for carbohydrates, 20% to 30% for fats, and 15% to 20% for protein, with a protein recommendation of 1 g/kg of ideal body weight. The dietitian encouraged the consumption of a diverse array of aliments from four food categories (vegetables and fruits, grain products, dairy and its derivatives, and fish and meat), promoted the inclusion of fresh and minimally processed foods, emphasized the use of extra virgin olive oil as the primary source of dietary fat, and discouraged the consumption of refined products.

Anthropometric parameters

A stadiometer (wall-mounted) was used to measure the body height. Using a calibrated digital scale (BC-418MA; Tanita, Manchester, UK) with an accuracy of 0.1 kg, the body weight was assessed before breakfast after a 12-h overnight fast, with the participants lightly clothed (clothing weight was subtracted). BMI was derived from height and weight measurements. A diagnosis of obesity was performed on the value of BMI ≥ 30 kg/m². From patient interviews and clinical records, we investigated on the existence of traditional cardiovascular risk factors (i.e., hyperlipidemia, smoking, and hypertension) [19]. We measured blood pressure at baseline and after 12 wk [19,23,24].

Vibration-controlled transient elastography

The FibroScan (Echosens; Paris, France) is an instrument capable of quantifying liver fibrosis and fatty liver content by non-invasive analysis, which is vibration-controlled transient elastography [25]. In detail, the CAP score is a parameter representing the fatty liver content; it is measured in decibels per meter and ranges from 100 to 400 dB/m. A CAP score between 216 and 252 dB/m suggests early-grade steatosis liver, with liver parenchyma characterized by fatty change between 11% and 33% [26]. CAP values ≤ 296 dB/m represent mild-grade steatosis liver, with parenchymal lipids change capable of reaching 66% [26]. CAP scores >296 dB/m describe severe-grade steatosis liver, with lipids change in $\geq 67\%$ of parenchymal tissue [26]. A diagnosis of NAFLD usually is based on a CAP score >216 dB/m [19,27]. All measurements were performed using a 3.5-MHz standard M probe, as previously described [20].

Evaluation of peripheral vascular endothelial function

Endothelial function was assessed using the EndoPAT 2000 device (Itamar Medical, Caesarea, Israel), which measures the peripheral arterial tone signal by a

non-invasive examination of arterial tone changes in peripheral arterial beds and estimates this parameter in terms of RHI (arbitrary units). In detail, subjects were allowed to rest lied in a 45° inclined chair for 20 min before data were acquired. Subjects' non-dominant arm was used as a study arm and equipped with a blood pressure cuff on an armchair support. The study arm was in a comfortable position with fingers capable of hanging freely. The dominant arm of each subject acted as respective control. For data acquisition, EndoPAT probes were placed on the tip of each index finger of both hands. Probes were preserved from touching skin and surfaces before analyses, signals were continuously acquired during the test, and baseline pulse amplitude was based on 5-min acquisition. Probes connected to the experimental arm had arterial flow interrupted by the rapid application of 200 mm Hg pressure, to be sure it exceeded both diastolic and systolic blood pressure. After 5-min occlusion, the cuff pressure was rapidly deflated, and postocclusion data acquired for further 5 min both for experimental arm as well as the control [28,29].

Biochemical analysis

Venous blood was collected after fasting overnight into vacutainer tubes (Becton, Dickinson; Plymouth, England) and centrifuged in ≤ 4 h. Serum total cholesterol, high-density lipoprotein cholesterol, triacylglycerols, glucose, insulin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, and creatinine were measured by chemiluminescent immunoassay on cobas 8000 (Roche; Rotkreuz, Switzerland), according to the manufacturer's instructions. Low-density lipoprotein cholesterol concentration was calculated by the Friedewald formula [30,31]. The homeostatic model assessment (HOMA) index was calculated for assessing IR and β -cell function from fasting glucose and insulin concentrations [32,33].

Statistical analysis

Data were expressed as mean \pm SD or percentage. The prevalence between treatment groups was analyzed by a χ^2 test, and an independent unpaired sample *t* test was used to compare the difference between means. Specifically, we calculated the changes in variables and compared the means of these changes between treatment groups after 12 wk. The change in RHI of the participants after 12 wk of treatments was compared by paired sample *t* tests. Pearson's correlation was performed to identify the variables correlated with RHI change (absolute value). Finally, a stepwise multivariable linear regression analysis was used to test the association between absolute RHI change and all of the potentially confounding factors. The potential confounders were all those variables correlated with RHI change in the Pearson's correlation with $P < 0.1$. In particular, we assessed the relationship between RHI change and treatment (BPF + CyC or placebo), dyslipidemia, antihypertensive drugs, RHI, HOMA-IR, and gamma-glutamyl transferase at baseline and change of systolic blood pressure and CAP score after 12 wk. Significant differences were assumed to be present at $P < 0.05$ (two-tailed). SPSS version 25.0 for Windows (IBM Corp., Armonk, NY) was used to perform all comparisons.

Results

Participants' demographic and clinical characteristics

Baseline clinical characteristics of participants according to the treatment are listed in Table 1. In the population analyzed, 69% of them were male and 37% suffered from obesity. In addition, 44% of the patients had dyslipidemia and 34% of them were suffering from hypertension. The mean age was 52 ± 9 y. Mean RHI was 1.15 ± 0.4 . At baseline, there were no statistically significant differences in the parameters analyzed between the two groups (Table 1). Nutrient intake assessments are reported in Supplemental Table 1. At baseline there were no statistically significant differences on dietary intake between two groups.

Changes in clinical features at follow-up

The main outcome of this study is illustrated in Figure 1. After 12 wk, a nutraceutical containing BPF + CyC determined a greater increase in the RHI (0.58 ± 0.5 versus 0.13 ± 0.5 ; $P = 0.02$; 95% versus + 30%, respectively) (Fig. 1A). Figure 1B indicates the prevalence of improvement of liver steatosis grade according to the treatments.

Table 1
Baseline characteristics of participants according to the treatments

Variables	Placebo (n = 16)	BPF + CyC (n = 16)	P value
Age (aa)	52 \pm 11	51 \pm 8	0.82
BMI (kg/m ²)	29.6 \pm 3	29 \pm 3	0.56
SBP (mm Hg)	111 \pm 17	108 \pm 14	0.61
DBP (mm Hg)	73 \pm 14	70 \pm 9	0.54
Glucose (mg/dL)	91 \pm 7	93 \pm 9	0.57
HOMA-IR	2.1 \pm 2	2.3 \pm 1	0.64
TC (mg/dL)	182 \pm 38	192 \pm 32	0.45
TG (mg/dL)	111 \pm 48	96 \pm 33	0.30
LDL-C (mg/dL)	113 \pm 38	120 \pm 30	0.55
Creatinine (mg/dL)	0.80 \pm 0.09	0.83 \pm 0.1	0.55
AST (IU/L)	19 \pm 5	19 \pm 3	0.69
ALT (IU/L)	27 \pm 14	20 \pm 4	0.09
γ GT (UI/L)	25 \pm 15	26 \pm 17	0.78
RHI	1.18 \pm 0.4	1.11 \pm 0.4	0.62
CAP score (dB/m)	290 \pm 47	286 \pm 35	0.72
IQR	10 \pm 5	10 \pm 6	0.76
Prevalence			
Sex (male, %)	69	69	1
Smokers (%)	6	25	0.33
Physical activity (%)	50	31	0.47
Hypertension (%)	31	37	1
Antihypertensive drugs (%)	25	31	1
Hyperlipidemia (%)	37	50	0.72
Lipid-lowering agents (%)	6	12	1
Liver steatosis, grade S1 (%)	19	12	
Liver steatosis, grade S2 (%)	44	56	1
Liver steatosis, grade S3 (%)	37	31	

γ GT, gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low density lipoprotein cholesterol; RHI, reactive hyperemia index; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglycerols; aa, years.

We found that individuals taking nutraceutical capsules had a significantly greater improvement in the stage of liver steatosis compared with placebo (69% versus 31%; $P = 0.03$, respectively). Individual variations of the RHI in the two treatment groups are indicated in Figure 2. We find, in individuals with hypertension ($n = 11$), a statistically difference between placebo and nutraceutical (0.20 ± 0.07 and 0.86 ± 0.6 , respectively; $P = 0.045$; data not shown); Due to the limited sample size, statistical analysis is not performed for other subgroups.

After 12 wk, only the group who took the nutraceutical had a significant increase in the RHI ($P = 0.001$) in comparison with baseline values (Supplementary Fig. 1).

All other changes in clinical parameters at the follow-up of the subject analyzed according to the intervention group are listed in Table 2. After the 12-wk treatment, there were no statistically significant differences in changes in BMI, HOMA-IR, lipid, and serum transaminase values as well as in other parameters evaluated between two groups (Table 2). Furthermore, at follow-up, there was not significant changes in dietary intake, physical activities in the two groups (Supplementary Table 1).

From the Pearson's correlation analysis, we found that RHI change (absolute value) correlated with the treatment ($r = 0.38$; $P = 0.028$); dyslipidemia ($r = 0.43$; $P = 0.013$); antihypertensive drugs ($r = 0.29$; $P = 0.097$); RHI ($r = -0.59$; $P < 0.001$); gamma-glutamyl transferase ($r = 0.35$; $P = 0.046$); HOMA-IR ($r = 0.40$; $P = 0.023$) at baseline and with the change of systolic blood pressure at follow-up ($r = -0.33$; $P = 0.062$).

Finally, in the multivariate stepwise analysis, the RHI change remained positively associated with the treatment ($B = 0.38$; $P = 0.025$) and negatively associated with RHI values at baseline ($B = -0.81$; $P < 0.001$) (Table 3). In addition, the multivariate linear regression analysis does not indicate a statistically significant

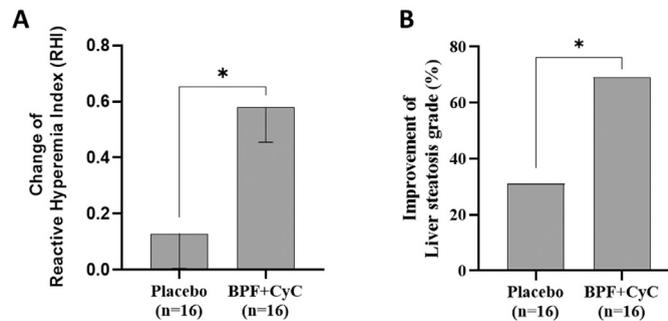


Fig. 1. Change in (A) RHI and (B) prevalence of subjects who had an improvement of liver steatosis grade in the population according to treatment after 12 wk. BPF, bergamot polyphenol fraction; CyC, *Cynara cardunculus* extract; RHI, reactive hyperemia index.

relationship between the reduction in the amount of intrahepatic fat (CAP score) and the improvement of endothelial function (absolute RHI change) ($B = 0.002$; $SE = 0.003$; $P = 0.56$).

Discussion

This is a secondary analysis of a previous RCT [19] in which we found that supplementation with an innovative nutraceutical containing BPF and CyC improves peripheral vascular endothelial function in adult individuals with NAFLD and ED. Specifically, 12-wk treatment with BPF + CyC determined a greater increase in the RHI by 95% in comparison with placebo group (Fig. 1). In previous studies, it was reported that this nutraceutical is safe and effective in reducing fatty liver content [34], inflammation, serum uric acid [35], and markers of oxidative stress [19,29].

To the best of our knowledge, this is the first study reporting the positive association between the improvement of endothelial function (RHI change) and the treatment with innovative nutraceutical. We hypothesized systemic effects of the nutraceutical, not only on the liver. These results are interesting, because, for the first time, we have found that this nutraceutical is also effective for improving endothelial function with a lower dosage and with a shorter treatment period in apparently healthy subjects. Furthermore, we did not find a statistically significant association between the change in RHI and CAP. We thus hypothesize an effect of the nutraceutical on endothelial function that is independent of the change in amount of intrahepatic fat. However, individuals taking our nutraceutical had a significantly greater improvement in the stage of liver steatosis compared with placebo (69% versus 31%;

$P = 0.03$). A larger sample size might have resulted in better results with CAP as well.

ED is known to be one of the first events in pathogenic process leading to atherosclerotic disorders [19], and in subjects with NAFLD it could be indicative of increased cardiovascular risk [11]. A Framingham cohort study revealed a strong correlation between the RHI and cardiovascular risk factors, underscoring its potential to identify high-risk patients [36]. Furthermore, every 0.1 decrease in the RHI was associated with a 20% increase in the risk of cardiovascular events [37]. This implies that cardiovascular risk does not increase in stable participants (without reduction in RHI). This finding, in itself, is clinically significant; those with an increase in RHI achieve a significant reduction in cardiovascular risk. In our study, RHI increases on average by 0.60; therefore, it appears clinically significant.

In literature, studies found that the nutraceutical contains bioactive molecules, such as naringin, neohesperidin, neohesperidin, brutieridin, and melitidin, derived from *Citrus bergamia*, and cynaropicrin, luteolin, and chlorogenic acid, derived from *Cynara cardunculus* [19,38]. Furthermore, a high consumption of fruit and vegetables rich in polyphenols is associated with a lower risk of hepatic steatosis [34,39] and cardiovascular disease [16–18,40,41].

In particular, polyphenols improve endothelial function [41,42], and NO concentration [42,43]. NO plays a vital role in maintaining healthy endothelial function and vascular tone [44]. In this regard, it has been reported that consumption of lemon extracts and sour orange peels, which contain amounts of antioxidants and flavonoids, might have significant benefits on endothelial function in overweight children and adolescents [45]. Another study finds that hesperidin, a polyphenol contained in citrus fruits, stimulates the

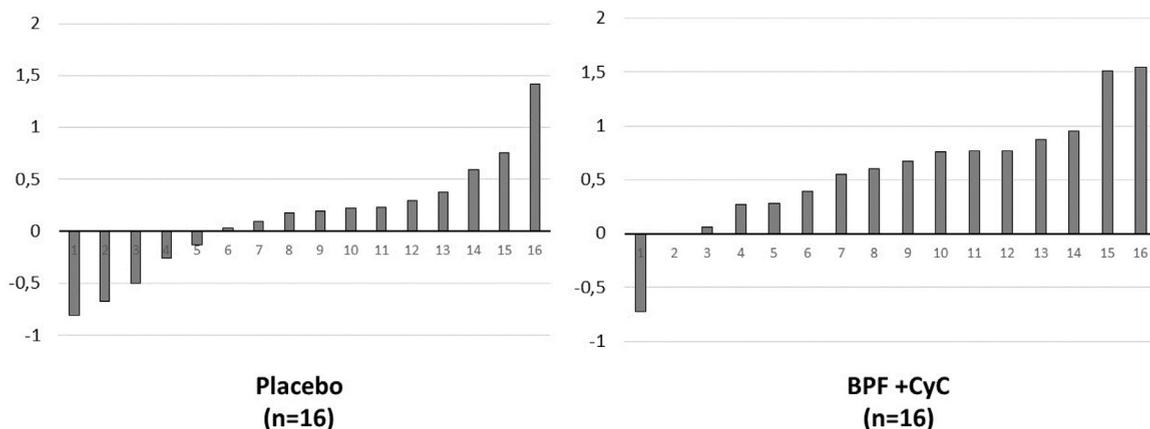


Fig. 2. Individual change of the RHI in the population according to treatments. BPF, bergamot polyphenol fraction; CyC, *Cynara cardunculus* extract; RHI, reactive hyperemia index.

Table 2
Changes in clinical parameters at follow-up according to the treatments

Variable	Placebo (n = 16)	BPF + CyC (n = 16)	P value
BMI (kg/m ²)	-0.9 ± 0.7	-1.2 ± 0.9	0.21
SBP (mm Hg)	-2.4 ± 16	-1.9 ± 13	0.92
DBP (mm Hg)	-5.0 ± 15	-1.2 ± 10	0.41
Glucose (mg/dL)	0.2 ± 7	0.6 ± 16	0.92
HOMA-IR	-0.33 ± 0.8	-0.21 ± 1.3	0.76
TC (mg/dL)	-4.8 ± 19	-10.2 ± 25	0.49
TG (mg/dL)	-10 ± 29	4 ± 30	0.18
LDL-C (mg/dL)	-2.6 ± 17	-8.7 ± 19	0.34
Creatinine (mg/dL)	0.036 ± 0.09	-0.003 ± 0.08	0.21
AST (IU/L)	0.3 ± 4	-2.0 ± 3	0.08
ALT (IU/L)	-4.2 ± 17	5.5 ± 22	0.17
γGT (IU/L)	-2.5 ± 6	-1.1 ± 10	0.64
CAP score (dB/m)	-20.4 ± 30	-29.2 ± 38	0.47

γGT, gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglycerols.

production of NO in endothelial cells by improving endothelial function [46]. Citrus flavanones are effective at counteracting the negative effect of a sequential double meal on human vascular function, potentially through the actions of flavanone metabolites on NO [47]. Furthermore, Mollace et al. found that administration of the BPF alone improves endothelial function in athletes [48]. It has been reported that artichoke dietary supplementation seems to positively modulate endothelial function in hypercholesterolemia [49]. A diet containing chlorogenic acid reduces oxidative stress and improves the bioavailability of NO by inhibiting the excessive production of reactive oxygen species in the vascular system and leads to the attenuation of ED [50].

On the other hand, it has already been widely reported that both NAFLD and its correlated comorbidity (such as metabolic syndrome and dyslipidemia) are accompanied by reduced NO release by endothelial cells [41–53].

The cause of the ED in metabolic diseases is unknown. However, a clear relationship has been found to exist between increased oxidative stress and impairment of endothelial function in liver diseases [54].

Moreover, the onset of inflammation in the liver tissues alters the mechanism that generates NO at multiple levels due to an altered fat metabolism [51,54].

Therefore, the combination of sesquiterpenes and polyphenols contained in BPF and CyC is likely to improve antioxidant properties, resulting in improved endothelial function and decreased amount of intrahepatic fat in patients with NAFLD treated with the combination of both extracts [19,29], thus suggesting that they could significantly and synergistically influence the release of NO from endothelial cells, which is altered in hepatic steatosis [50].

Table 3
Multivariable linear regression analysis-factors associated with the variation of reactive hyperemia index

Dependent variable ΔRHI	B	SE	P value	95% CI	
				LL	UL
Treatment	0.38	0.16	0.025	0.053	0.724
Baseline RHI	-0.81	0.19	< 0.001	-1.220	-0.404

Δ, change; RHI, reactive hyperemia index; B, unstandardized coefficients; LL, lower limit; UL, upper limit.

Excluded variables: hyperlipidemia, antihypertensive drugs, gamma-glutamyl transferase, and homeostatic model assessment of insulin resistance at baseline and change of systolic blood pressure at follow-up.

These substantial improvements, consequent to the treatment with this nutraceutical (Bergacyn), lead to substantial benefit for better liver protection and for restoring vascular reactivity, which is essential for cardiometabolic risk prevention.

This study has some limitations. It was a secondary analysis of an RCT designed to evaluate the effects of this nutraceutical on fatty liver accumulation and/or on the derived liver damage markers. For this reason, the findings obtained must be interpreted with caution and can only generate hypotheses for future investigations. Furthermore, our analysis found a similar or greater improvement in endothelial function compared with those achieved with exercise and/or dietary intervention [55,56]. However, this improvement was obtained with only one capsule daily, but it is conceivable that a greater increase of RHI could be obtained by increasing the daily dose of this nutraceutical [29]. Another limitation of the investigation is that it did not evaluate the effect of the nutraceutical on the arterial stiffness. We have not evaluated the relationship between the serum concentration of the bioactive components of the nutraceutical and improvement in endothelial function.

However, the study has some strengths, such as analyzing a population of subjects with NAFLD and ED but apparently healthy and evaluating the association between the improvement in endothelial function and possible confounding factors. In addition, the endothelial function was evaluated by a non-invasive, cheap, and operator-independent method [28]. Furthermore, the prognostic value of the cardiovascular events of RH-PAT is comparable with other widely studied methods [56].

In light of these findings, we believe that these results may stimulate future studies to find strategies, with new molecules or with new combinations of bioactive compounds, for the treatment of NAFLD and its complications.

Conclusions

Data confirm that a nutraceutical containing the polyphenolic fraction of bergamot and the extract of artichoke leaves increases reactive vasodilation after 12 wk of treatment in adults with NAFLD and in the early phase of atherosclerosis. The results obtained in this secondary analysis are interesting because they encourage the use of natural molecules with the effects of nutraceuticals for the prevention and management of cardiometabolic risk. However, ad hoc trials are needed to corroborate our findings.

Declaration of Competing Interest

None.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2023.112294.

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