Effects of low molecular weight procyanidin rich extract from french maritime pine bark on cardiovascular disease risk factors in stage-1 hypertensive subjects: randomized, double-blind, crossover, placebo-controlled intervention trial

Short title: Procyanidin rich extract effects on HDL and BP

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Abbreviations: ACE, angiotensin converting enzyme; ADO, Available Data Only ; Apo A-1, Aplipoprotein A-1; Apo B-100, Aplipoprotein B-100; ATP III, Adult Treatment Panel III; BMI, body mass index; BOCF, Baseline Observation Carried Forward; BP, Blood Pressure; CTNS, Nutrition and Health Technology Centre; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; FMPB, Frech Maritime Pine Bark; GSH, reduced glutathione; GSSG, oxidized glutathione; HDL, High Density Lipoprotein; hsCRP, high sensitivity C-reactive protein; HSUJ, Hospital Universitari Sant Joan; ICAM-1, Intercellular Adhesion Molecule type 1; ICH GCP, International Conference of Harmonization Good Clinical Practice; ITT, Intention to Treat population; LDL, Low Density Lipoprotein; OP, Oligopin; OPC, oligomeric procyanidins; ox-LDL, oxidized LDL; PP, Protocol population; SD, standard deviation; TG, triglycerides; VCAM-1, Vascular Cell Adhesion Molecule type 1; VEGF, vascular endothelial growth factor; WC, waist circumference

Abstract

Background: Oligopin® (OP) is a quantified extract from French Maritime Pine bark (FMPB) with low molecular weight procyanidins. The cardioprotective effects of OP need to be tested in human clinical intervention trials with an appropriate design.

Purpose: The aim of the present study was to assess the effect of subchronic consumption of OP on cardiovascular disease risk factors such as lipid profile, systolic blood pressure (BP) and oxidized-Low Density Lipoprotein (ox-LDL) in stage-1-hypertensive subjects.

Methods: Between February 14 and May 31, 2014, eligible subjects were recruited from the outpatient clinics of Hospital Universitari Sant Joan (Reus, Spain). A total of 24 participants (mean age ± DS; 57.36±11.25; 17 men) with stage-1-hypertension who were not receiving BP-lowering medication and LDL cholesterol< 4.88 mmol/Lwere randomized in a double-blind, placebo-controlled, crossover study. The subjects received 2 capsules/day with 75 mg of OP or placebo for 5-weeks.

Results: At 5-weeks, compared to the placebo, OP raised High Density Lipoprotein-cholesterol (HDL-c) by 14.06% (p=0.012) and apolipoprotein A-1 by 8.12% (p=0.038) and reduced the ratio of apolipoprotein B-100/A-1 by 10.26% (p=0.046). Moreover, at 5-weeks, compared to the baseline, OP reduced the systolic BP by 6.36 mmHg (p=0.014), and decreased ox-LDL concentrations by 31.72U/L (p=0.015).

Conclusion: At 5-weeks, the consumption of 150 mg/day of OP improve lipid cardiovascular profile and represents one of the scarce ways to increase HDL-c in stage-1-hypertensive subjects.

Trial Registration: ClinicalTrials.gov: NCT02063477

56 **Key words:** systolic blood pressure, HDL-c, cardiovascular biomarkers, low weight
57 molecular procyanidin

Introduction

Hypertension is a leading risk factor for cardiovascular disease (CVD) , which is the major cause of premature death in the world (James et al., 2014; Perk et al., 2012). The reduction of blood pressure (BP) levels in hypertensive subjects is associated with a decrease in cardiovascular events (James et al., 2014; Perk et al., 2012). According to the Lifestyle Work Group, recommendations of lifestyle modifications have a potential role in improving BP (Reidlinger et al., 2015), specially, in early stages of hypertension or in subjects with grade-1-hypertension (James et al., 2014).

In the approach to reduce BP in subjects with hypertension, other CVD risk factors such as lipids or emergent CVD risk factors as oxidized-Low Density Lipoprotein (ox-LDL) should be lowered additionally to BP. In this line, phenolic compounds present in a healthy dietary pattern may have a beneficial impact on all these CVD risk factors (Rosa Cde et al., 2015).

Among phenolic compounds, procyanidins (condensed oligomeric catechin and epicatechin) are proanthocyanidin biopolymers classified in the flavonoid subgroup. These bioactive compounds have been related to different beneficial health properties, such as hypolipidemic, antihypertensive, anti-inflammatory and antioxidant effects, thus improving different CVD risk factors (Gonzalez-Abuin et al., 2015). The French Maritime Pine (*Pinus pinaster*) bark (FMPB) extract is a natural extract rich in specific oligomeric procyanidins and other phenolic compounds that have been related to beneficial CVD risk factors effects (Schoonees et al., 2012). Pycnogenol® is one of the most studied quantified FMPB extracts, and it has been observed to inhibit the activity of angiotensin-converting enzyme (ACE), decreasing BP levels (Hosseini et al., 2001; Liu et al., 2004). The consumption of 150 mg/day of Pycnogenol® during a 6-month period has also been observed to improve other CVD risk factors in subjects with metabolic syndrome, such as waist circumference (WC), glucose and triglycerides (TG) levels and high density lipoprotein cholesterol (HDL-c) levels, compared to the baseline (Belcaro et al., 2013). These interesting results need to be

verified, as no control product was consumed in Belcaro et al., 2013 study thus representing a methodological limitation (Belcaro et al., 2013). Moreover, two different placebo-controlled studies reported no improvement in BP or other CVD risk factors at 6 and 12 weeks following FPBE intake (Drieling et al., 2010; Enseleit et al., 2012). Therefore, the beneficial health effects of FMPB extract, including its possible hypotensive effect, its ability to modulate plasma lipids levels, or antioxidant lipid activity are inconsistent, and methodological aspects are hindering the interpretation. Recent systematic reviews stated that the current evidence of FMPB extract value is insufficient, and that well-designed, high quality and adequately powered trials are needed (Sahebkar, 2014; Schoonees et al., 2012).

Oligopin® (OP) is a quantified extract, commercially available, with a specific selective extraction and purification process of FMPB, and its effects on CVD risk factors have not previously been evaluated in human trials (Assoud et al., 2007). OP is characterized by a practical absence of tannins (<1%) and a high content in low molecular weight oligomeric procyanidins (OPC >70%; dimers about 20%), a distinctive feature of other proanthocyanidin-rich extracts such as Pycnogenol® which contained about 5% of dimers (Assoud et al., 2007). Furthermore, although the insoluble products in water are low in both products, lower and more adequate concentrations are present in OP (OP: typically 2 to 4% to a maximum at 5% versus Pycnogenol®: 6% - 8.1%). The degree of polymerization of OPC can determine its absorption across cell membranes and, as it has been observed in rats, only a certain OPC of a lower degree of polymerization is absorbed during transit in the gut (Cheah et al., 2014). Consequently, the effect of FMPB extract could be determined, in part, by the quality of its OPC. In this context, we hypothesized that OP that contain an originally low weight molecular procyanidin rich quantified extract from FMPB can improve, in hypertensive patients, not only BP, but also other CVD risk factors such as lipid profile or ox-LDL. The aim of the present study was to assess the effect of subchronic consumption of OP on several CVD risk factors, such as lipid profile, systolic BP and ox-LDL in stage-1-hypertensive subjects.

Materials and Methods

Design

The study was randomized, double-blind, placebo-controlled and crossover. After a run-in week for dietary stabilization of all participants, they were randomly assigned to placebo or OP periods of 5-weeks each, with a 3 week washout period between the first and second periods of the study to test possible interactions between treatment and sequence order (carryover effect). Thus, the duration of the study was 14-weeks (1+5+3+5 weeks).

The study was approved by the Clinical Research Ethical Committee of *Hospital Universitari Sant Joan* (HUSJ) de Reus (Spain) on May 13, 2013. The protocol and the trial were conducted in accordance to the Helsinki Declaration and good clinical practice guidelines of the International Conference of Harmonization (ICH GCP) and reported as CONSORT criteria. The trial was registered with Clinical-Trials.gov: number NCT02063477. There have not been any deviations from the study protocol. We declare that there are no restrictions on the sharing of data and/or materials.

Participants and recruitment

Between February 14 and May 31, 2014, eligible patients were recruited from the outpatient clinics of HUSJ Reus. The follow-up of the participants was conducted on the Nutrition and Health Technology Centre (CTNS) and the HUSJ and lasted until September 30, 2014. The trial registration was completed on February 13, 2014. The end of study was October 31, 2014 for the final data collection for primary outcome measure. All the participants provided written informed consent prior to participation in the study.

The participants were community-dwelling men and women >18 years of age with stage 1 hypertension (systolic BP ≥ 140 and ≤ 159 mm Hg) and/or diastolic BP ≥ 90 and ≤ 99 mm Hg and were not receiving BP-lowering medication. The exclusion criteria were Body Mass Index (BMI) > 30 kg/m^2, consumption of antihypertensive medications, smoking, persons with a self-reported history of clinical CVD, cancer, chronic kidney disease (or a serum creatinine ≥ 1.7 mg/dL for men and ≥ 1.5 mg/dL for women), hypercholesterolemia (LDL-c ≥4.88 mmol/L), diabetes mellitus (or serum glucose ≥126 mg/dL), consumption of more than 14 drinks of alcoholic beverages per week, pregnancy, or with the intention to breastfeed or become pregnant.

Participant eligibility or exclusion was assessed by the attending physician and was based on review of clinical records, followed by a screening visit.

Randomization and intervention

The randomization allocation sequence was generated by a statistician with SAS 9.2 (Cary, NC: SAS Institute Inc.) statistical software PROC PLAN. The statistician responsible for the randomization did not participate in the study. Because all participants received both interventions (OP and placebo), restrictions such as blocking were unnecessary. Participant assignment to treatment or placebo arm was at a ratio of 1:1. The sequence number for the subject and treatment assignment was allocated through an interactive electronic response system hosted by the Nutrition and Health Technology Centre (CTNS). Subjects complying with selection criteria were assigned a randomization number taken from a randomization list following the chronological order by which they were included, after verifying compliance with inclusion and exclusion criteria. The participant enrollment was conducted by a researcher, and participants' assignment to interventions according to the random sequence was done by a physician. The randomization list remained closed until the end of the experimental intervention and the data registering had finished.

151 The randomized patients receive a placebo (250 mg maltodextrine plus 30 mg magnesium stearate:
152 280 mg of total content per capsule; 2 times a day) or OP quantified extract (75 mg Oligopin® plus
153 175 mg maltodextrine plus 30 mg magnesium stearate: 280 mg of total content per capsule; 2 times
154 a day) for 5 weeks each. During the intervention, patients consume one capsule of placebo or OP in
155 the morning and the other one in the afternoon, being indifferent if taken before, during or after
156 meals as the product is soluble in water so its biodisponibility is not affected by food consumption.
157 These products were provided by Les Derivés Résiniques & Térpeniques (DRT); 40105 DAX
158 CEDEX – FRANCE. Pine bark extract from Pinus pinaster is positively listed in annex 1 of the
159 French Plant decree (Arrêté du 24 juin 2014 établissant la liste des plantes, autres que les
160 champignons, autorisées dans les compléments alimentaires et les conditions de leur emploi). By
161 mutual recognition it should be authorized in Spain unless a specific legislation applies. We are not
162 aware of any restriction in Spain. The polyphenol composition of Oligopin® is described in
163 Supplemental Table 1.

164 Blinding was maintained using matching placebo capsules that did not differ from the OP with
165 respect to appearance or any other physical characteristics. A total of 45 capsules were presented in
166 opaque bottle plastic packaging, and 2 bottles were delivered face-to-face at the beginning of each
167 intervention period by the physician.

168 Compliance treatment monitoring was measured with a questionnaire filled-in by patients at a
169 clinical interview in all visits, and the capsule bottles were returned afterwards. Consumption of >
170 80% was considered an acceptable level of adherence.

171 The stabilization diet had a 13% of saturated fatty acid content. During the intervention, dietary
172 recommendations were disseminated according to the guidelines of the Adult Treatment Panel
173 (ATP) III (Stone et al., 2014) and Dietary Approaches to Stop Hypertension (DASH) diet (Saneei et
174 al., 2014). At the basal level and at the end of each intervention, dietary compliance was monitored

using 3-day dietary records and was confirmed in interviews with the dietician. In addition, the follow-up of the dietary recommendations was verified through a 24-h record by trained dieticians in each follow-up visit.

Measurements

The systolic BP was defined as a primary outcome measure. BP was measured twice after subjects respite 2-5 minutes seated, with a 1-min interval in between, using an automatic sphygmomanometer (OMRON HEM-907; Peroxfarma, Barcelona, Spain) by a physician. The mean values were employed in the statistical analyses.

The anthropometric measurements, lipid profile, ox-LDL and other CVD risk biomarkers were defined as secondary outcome measures.

Screening chemistries and haemograms were performed with appropriate clinical chemistry quality controls in the HUSJ. A fasting blood sample was obtained at 0 and after 5 weeks of each intervention. Samples were stored at -80ºC in the central laboratory's Biobanc (biobanc.reus@iispv.cat) until required for batch analyses.

Total cholesterol, HDL-c, TG, Apolipoprotein A-1 (Apo A-1), Apolipoprotein B-100 (Apo B-100) and glucose were measured in serum by standardized enzymatic automated methods in a PENTRA-400 autoanalyzer (ABX-Horiba Diagnostics, Montpellier, France). LDL-c was calculated by the Friedewald formula (Friedewald et al., 1972)**.** High sensitivity C-reactive protein (hsCRP) was determined by standardized methods in a Cobas Mira Plus autoanalyzer (Roche Diagnostics Systems, Madrid, Spain). Insulin was measured using a specific ELISA kit (Mercodia AB, Uppsala, Sweden).

EDTA plasma ox-LDL was measured with an ELISA kit (Mercodia AB, Uppsala, Sweden), and Heparin-lithium plasma GSH and GSSG were analyzed by fluorimetric methods.

198 Endothelin-1, nitric oxide, ACE, vascular endothelial growth factor (VEGF), Intercellular Adhesion
199 Molecule type 1 (ICAM-1), Vascular Cell Adhesion Molecule type 1 (VCAM-1) and e-Selectin,
200 which were measured in serum using ELISA kits (R&D Systems, Minneapolis, USA).

201 Standard anthropometric data were obtained while participants were wearing lightweight clothing
202 and no shoes at each visit. Trained dieticians measured weight and body composition using a body
203 composition analyzer (Tanita SC 330-S; Tanita Corp., Barcelona, Spain) and height using a well-
204 mounted stadiometer (Tanita Leicester Portable; Tanita Corp., Barcelona, Spain). WC was
205 measured midway between the lowest rib and the iliac crest using an anthropometric tape. All
206 participants were advised to maintain their usual physical activity throughout the study.

207 Safety

208 Adverse events were coded according to the Medical Dictionary for Regulatory Activities (MedDra
209 dictionary; version 16.1) and described per subject, including their characteristics, listed by visit and
210 study intervention. Relationship to the intervention was also listed in those adverse events
211 catalogued as serious. The number of individuals with at least one adverse event and the number of
212 adverse events per study intervention were analyzed.

213 Sample size

214 To detect differences between the two interventions (OP and placebo) of 10 mm Hg under an
215 α=0.05 bilateral significance level, a power of 80% and assumption that the common standard
216 deviation (SD) was 12.15 mm Hg, the sample size was 24 participants.

217 Statistical analyses

Descriptive results were expressed as the mean ± SD or percentages, according to the type of variable. The efficacy analysis was evaluated by paired Student's t-test using employing the Baseline Observation Carried Forward (BOCF) approach on the Intention to Treat population (ITT). The analysis was performed using the Available Data Only (ADO) approach on the same population. The primary efficacy variable was also analyzed using the Per Protocol population (PP) to test the robustness of the results with both approximations (BOCF and ADO). The Kolmogorov–Smirnov test was used to verify the distributions of the variables. Carryover effect determined by a period-by-treatment interaction was discarded. Student's T-Test and Mann-Whitney's U were applied according to the variables' nature. Exploratory analysis was determined by the primary outcome by gender on ITT population using ADO approach.

The possible interaction between the treatments and the treatment sequence (carryover effect) was discarded in all variables of the study. The level of statistical significance was set at $p<0.05$. Data were analyzed using the SAS software package version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of subjects

From the 45 eligible volunteers, 25 were randomized, and finally, data from 24 were analyzed by ITT (Figure 1). The baseline characteristics from the 24 participants (17 men and 7 women) included in the study are described in Supplemental Table 2. No relevant differences between sequences were observed at the baseline.

Blood pressure

The changes in BP are shown in Table 1. At 5-weeks, systolic BP had been significantly reduced by 6.36 mm Hg (p=0.014) compared with its baseline during the OP intervention. However, this decrease was not significantly different when compared to the placebo intervention, because the placebo also reduced systolic BP levels by 3.98 mm Hg (p=0.426) after 5-weeks. Moreover, after OP intervention and stratifying by gender, the female participants significantly reduced their systolic BP by 14.75 mm Hg (p=0.002) compared to the placebo intervention. Additionally, diastolic BP had been reduced by 1.82 mm Hg compared with its baseline during the 5-weeks OP intervention, although this reduction did not reach the significance level (p=0.259). Likewise, this decrease was not significantly different when compared to the placebo intervention, because the placebo also reduced diastolic BP levels by 1.74 mm Hg (p=0.805).

Lipid profile

At 5-weeks with OP intervention, the HDL-c concentrations significantly increased by 14.06% (0.22 mmol/L; p=0.011) and Apo A-1 concentrations by 8.12% (p=0.038); the Apo B-100/Apo A-1 ratio was significantly reduced by 10.25% (p=0.046) compared with the placebo intervention. The changes in lipid profiles are described in Table 2.

Anthropometric variables and other CVD risk biomarkers

The changes in anthropometric variables and other CVD risk biomarkers analyzed are shown in Supplemental Table 3. At 5 weeks, ox-LDL was significantly reduced by 31.72 U/L (-29.4%) (p=0.015) compared with its baseline during the OP intervention. Moreover, a reduction trend in ox-LDL values was also observed when compared with the placebo intervention group (p=0.077). After OP intervention and stratifying by gender, the male participants' trend for ox-LDL was significantly reduced by 33.96 U/L (p=0.059) compared with the placebo intervention.

BMI and waist circumference of the participants remain stable during the study. No significant changes were observed between the OP and placebo interventions for anthropometric variables and other CVD risk biomarkers.

Dietary intake

Supplemental Table 4 summarizes the dietary intake of study participants. The intake of energy, macronutrients, dietary cholesterol, fiber, sodium, potassium, magnesium and calcium did not change after 5-weeks between interventions, except toward a lower alcohol consumption in the placebo intervention by -5.23 g (p=0.025) compared to the OP intervention.

Adverse events and product tolerance

There were no statistically significant differences between the 2 interventions with respect to the adverse events reported. The OP product was well tolerated.

Discussion

The present study is the first randomized, double-blind, placebo-controlled and crossover study performed with OP in stage-1-hypertensive subjects. The present results reveal that the consumption of 150 mg/day of OP during 5-weeks, compared to the control group, produced beneficial effects on CVD risk factor, not only by the significant clinical increase of HDL-c (0.22 mmol/L, 14.06%), but also by the increase of its main apolipoprotein Apo A-1 (11.1 mg/dL, 8.12%), and the reduction of the Apo B-100/A-1 ratio (10.26%). Moreover, at 5-weeks and compared to baseline, OP significantly reduced systolic BP by 6.36 mm Hg, and decreased ox-LDL concentrations by 31.72 U/L in stage-1-hypertensive subjects.

Our results are in line with the results of Belcaro et al. (2013) (Belcaro et al., 2013), who also observed an increase in HDL-c levels after Pycnogenol® consumption. Moreover, the increment observed in our OP study (14%) was more intense than those observed in the METS-GREECE study, in which an increment of 7% was described after a 3-year therapy with atorvastatin (Athyros et al., 2004). HDL-c levels have long been inversely associated with the risk of coronary heart disease, being a key component in predicting CVD risk. Gordon et al. suggested in 1989 (Gordon et al., 1989) that for each 1 mg/dL of HDL-c increase, a reduction of 3% in coronary heart disease risk was expected. The increment of Apo A-1 observed after OP intervention could lead to the possible improvement of HDL functionality by enhancing HDL-c efflux and HDL antioxidative properties, because Apo A-1 is the major HDL component involved in these activities (Rached et al., 2014). Thus, HDL particle is a major target for novel therapeutic approaches to decrease atherosclerosis. The increase in HDL-c levels and Apo A-1 could occurs due to a mechanism of action of the components present in the OP quantified extract, particularly, the low molecular weight procyanidins. In this line, procyanidin B2 and procyanidin C1, present in cacao, have been shown to influence the regulation of Apo A-1 in HepG2 and Caco2 cells by increasing their mRNA expression and consequently, Apo A-1 protein levels. This mechanism has been suggested as a possibility by which HDL-c levels become elevated after cocoa intake, and it could also be the mechanism by which the OP quantified extract produced the increase in HDL-c and its major Apo lipoprotein (Sarriá et al., 2015). The Apo B-100/Apo A-1 ratio could predict cardiovascular heart disease and stroke risk more accurately than conventional lipid measurements such as total cholesterol or LDL-c levels (Solá et al., 2011). Moreover, an Apo B-100/Apo A-1 ratio value less than 1 has been recommended to improve the lipid cardiovascular profile (McQueen et al., 2008). In the present study, after 5-weeks of OP intervention, compared to placebo, the Apo B-100/Apo A-1 ratio was significantly reduced after 5-weeks of OP intervention by 10.26 % with a final value of 0.75.

305 The systolic BP reduction of 6.46 mm Hg observed after OP consumption compared to the baseline
306 is similar to that observed after the DASH diet, which is based in the consumption of fruits,
307 vegetables, whole grains, low fat products, low sodium and low total and saturated fats; it produced
308 a reduction in systolic BP of 6.74 mm Hg in healthy and hypertensive subjects (Saneei et al., 2014).
309 Similarly, a Mediterranean diet also significantly reduced systolic BP by 7.8 mm Hg, as described
310 in the meta-analysis of Rees, K et al. (2013) (Rees et al., 2013). High polyphenol intake has been
311 associated with an improvement in systolic and diastolic BP (Medina-Remón et al., 2016).
312 Systematic reviews indicate that dietary intakes of polyphenol-rich foods, herbs and beverages,
313 specifically rich in flavonoids, including flavonols (cocoa or tea), anthocyanidins (berry),
314 oligomeric proanthocyanidins (red wine or FMPB), flavones (thyme), flavanones (citrus fruits),
315 isoflavones (soy) and flavan-3-ols (berry and green tea), significantly decrease the risk of
316 hypertension (Hügel et al., 2016). Moreover, the evidence resulted from several recent reviews
317 summarizing the effect of polyphenols and polyphenol-rich foods on BP has resulted in one of the
318 few current European Food Safety Authority (EFSA) allowed health claims on maintenance of
319 normal BP and related to cocoa flavanols (EFSA, 2010). The magnitude of systolic BP reduction
320 observed after OP quantified extract intervention was the same as that achieved by hypotensive
321 drugs (Morgan et al., 2001). However, no significant differences were observed when compared to
322 the placebo, as after taking the placebo, volunteers also obtained a small reduction (-3.98 mm Hg)
323 in systolic BP. This surprising reduction in systolic BP after placebo consumption can be explained
324 by the psychological reactivity of subjects included in an intervention study that produces
325 modifications as a result of knowing that they are being studied and not in response to the
326 experimental intervention (Grufferman, 1999). The placebo effect on systolic BP has been
327 previously described and quantified, that is, approximately 6.5 ± 11.1 mm Hg in mild-to-moderate
328 hypertension subjects (Asmar et al., 2001).

However, when we stratified the results by gender, we could observe a strong significant decrease in systolic BP (-14.75 mm Hg) compared to the placebo in female participants after 5-weeks of intervention with OP. This preliminary result suggests a sex-dependent response to the FMPB extract present in OP, which must be explored in future studies with a large female sample size.

Consumption of 200 mg/day of Pycnogenol® during 8-weeks has also been observed in another study to significantly decrease (-7 mm Hg) systolic BP compared to placebo consumption and was more effective in subjects with higher pressure (Hosseini et al., 2001). In the context of the same commercial FMPB, Liu X, et al. (2004) (Liu et al., 2004) showed that Pycnogenol® has a significant antihypertensive effect in subjects with mild hypertension compared to the placebo, as they could reduce their hypotensive drug treatment dose after consuming 100 mg/day of Pycnogenol® during 12-weeks. They suggest that a lowered concentration of plasma endothelin could contribute to this antihypertensive effect; however, we did not observe changes in endothelin concentrations after intervention with the OP standardize extract.

Subjects under OP quantified extract intervention also show a significant decrease in ox-LDL compared to the baseline, indicating a protective antioxidant effect. Similarly, a cocoa powder rich in procyanidins has been related to a reduction in ox-LDL in hypercholesterolemic subjects (Baba et al., 2007). The low molecular weight procyanidin rich extract from FMPB could contribute to the resistance of LDL to oxidation, such as evidence that reported catechin and quercetin may be incorporated onto the surface of LDL particles, producing an increase of resistance of ox-LDL by either scavenging chain-initiating oxygen radicals or chelating transitional metal ions (Hayek et al., 1997).

The changes detected in lipid profile and in systolic BP after OP quantified extract consumption cannot be attributed to dietary modifications as no significant differences were observed between the placebo and OP intervention at 5-weeks. Moreover, the differences between the basal and final

353 interventions were due to dietary recommendations given to the participants. Besides, as the
354 anthropometric parameters (BMI and waist circumference) were unchanged during the study, the
355 results observed can be specifically attributed to OP consumption.

356 One of the strengths of the present study is its design as a randomized, placebo-controlled, clinical
357 trial that is able to provide the first level of scientific evidence using a product without FMPB as a
358 placebo. In addition, the crossover design, in which each subject acts as the corresponding control,
359 minimizes the interference of possible confounding variables.

360 One potential limitation of the study is the unknown FMPB extract bioavailability, which was
361 supported by the significantly increased antioxidant capacity of plasma in OP intervention, the same
362 extract used in the present study, compared with grape seed extract or a high-degree polymerized
363 pine bark extract consumed during 8 weeks in rats (Busserolles et al., 2006).

364 In conclusion, at 5-weeks, the consumption of 150 mg/day of OP improve lipid cardiovascular
365 profile and represents one of the scarce ways to increase HDL-c in stage-1-hypertensive subjects. In
366 addition, OP also tends to improve systolic BP and LDL oxidation. Moreover, as no significant
367 differences were reached compared to placebo, further studies are needed to elucidate this trend and
368 to ensure systolic BP and oxidation improvement after OP consumption.

369

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380

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Table 1. Changes in blood pressure through the study.

Variable		Baseline	Final	Change at 5 weeks relative to baseline				Treatment difference			
		Mean (SD)	Mean (SD)	Change from Baseline	[95% CI]	% Change from Baseline	P	Change from Placebo	[95% CI]	% Change from Placebo	P
Systolic Blood Pressure (mm Hg)	Placebo	145.76 (13.97)	141.78 (12.90)	-3.98	[-10.2; 2.2]	-2.73%	0.1974	-2.98	[-10.6; 4.7]	-2.01%	0.4264
	Oligopin	150.20 (12.41)	143.84 (12.26)	-6.36	[-11.3; -1.4]	-4.23%	0.0138				
Diastolic Blood Pressure (mm Hg)	Placebo	82.63 (10.72)	80.89 (8.83)	-1.74	[-5.6; 2.1]	-2.11%	0.3588	-0.57	[-5.3; 4.2]	-0.69%	0.8048
	Oligopin	83.70 (8.05)	81.89 (9.65)	-1.82	[-5.1; 1.4]	-2.17%	0.2594				

Data calculated on the per-protocol (PP) population (n=21). Results from the ANCOVA model. Abbreviations: SD, standard deviation.

Table 2. Changes in lipid profile variables through the study.

Variable		Baseline	Final	Change at 5 weeks relative to baseline				Treatment difference			
		Mean (SD)	Mean (SD)	Change from Baseline	[95% CI]	% Change from Baseline	P	Change from Placebo	[95% CI]	% Change from Placebo	P
Cholesterol (mmol/L)	Placebo	5.44 (0.65)	5.27 (0.58)	-0.16	[-0.4; 0.1]	-2.94%	0.1913	0.07	[-0.3; 0.5]	1.27%	0.7287
	Oligopin	5.56 (0.77)	5.43 (0.69)	-0.14	[-0.5; 0.2]	-2.52%	0.4219				
LDL-c (mmol/L)	Placebo	3.28 (0.45)	3.16 (0.54)	-0.12	[-0.3; 0.1]	-3.66%	0.1637	-0.08	[-0.3; 0.2]	-2.39%	0.5454
	Oligopin	3.41 (0.63)	3.18 (0.57)	-0.23	[-0.5; 0.0]	-6.74%	0.0659				
HDL-c (mmol/L)	Placebo	1.58 (0.25)	1.47 (0.19)	-0.11	[-0.2; 0.0]	-6.96%	0.0731	0.22	[0.1; 0.4]	14.06%	0.0119
	Oligopin	1.55 (0.29)	1.62 (0.32)	0.07	[-0.0; 0.2]	4.52%	0.2092				
Triglycerides (mmol/L)	Placebo	1.27 (0.72)	1.43 (0.83)	0.16	[-0.1; 0.4]	12.60%	0.1929	-0.16	[-0.5; 0.2]	-12.17%	0.3026
	Oligopin	1.36 (0.74)	1.40 (0.95)	0.04	[-0.1; 0.2]	2.94%	0.6692				
Apolipoprotein A-1 (mg/dL)	Placebo	137.35 (18.36)	131.91 (14.57)	-5.43	[-12.7; 1.8]	-3.95%	0.1351	11.10	[0.7; 21.5]	8.12%	0.0380
	Oligopin	135.91 (21.88)	139.64 (19.76)	3.73	[-3.4; 10.9]	2.74%	0.2914				
Apolipoprotein B-	Placebo	101.26 (15.02)	100.04 (16.97)	-1.22	[-6.1; 3.7]	-1.20%	0.6106	-2.67	[-10.9; 5.6]	-2.58%	0.5079
	Oligopin	105.82 (21.22)	101.09 (16.07)	-4.73	[-11.8; 2.3]	-4.47%	0.1773				

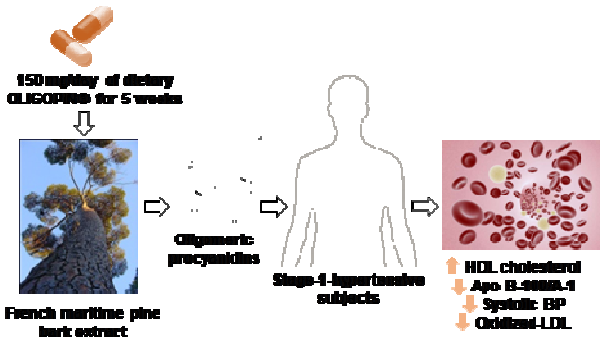
100 (mg/dL)											
Ratio Apolipoprotein B-100 A-1	Placebo	0.75 (0.17)	0.77 (0.19)	0.02	[-0.0; 0.1]	2.67%	0.3515	-0.08	[-0.2; -0.0]	-10.26%	0.0460
	Oligopin	0.81 (0.25)	0.75 (0.21)	-0.06	[-0.1; 0.0]	-7.41%	0.0538				

Data calculated on the per-protocol (PP) population (n=21). Results from the ANCOVA model. Abbreviations: SD, standard deviation; LDL-c, low density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol.

FIGURE CAPTIONS

Figure 1. Flow of participants through the study. ITT: intention-to-treat; PP: per protocol.

TOC Graphic.


150mg/day of dietary
for 5 weeks
Oligomeric
procyanidins
Stage-1-hypertensive
subjects
HDL cholesterol
Systolic BP
French maritime pine
bark extract

Supplemental Table 1. Polyphenol composition of Oligopin® (dry extract)

Polyphenols :	**Average content (mass %)**
Flavonoid oligomeric proanthocyanidins	
Oligomeric Proanthocyanidins (also named Procyanidolic Oligomers; OPC), providing a subtype of procyanidins (85% condensed oligomeric catechin and epicatechin)	67 – 75
Dimers	15-20
Trimers	15-20
Tetramers-hexamers	30-40
Flavonoids monomers	
Catechin	4 - 10
Dihydroquercetin (taxifoliol)	0.5 - 4
Flavonoid Glucosides derivatives	
3'–O–b-glucoside taxifoliol	3 - 8
Phenolic acid derivatives	
Ferulate glucoside	4 - 10
Phenolic Acids	
Gallic acid	0.1 - 1
Protocatechic acid	0.5 - 3
Caffeic acid	0.5 - 3
p-coumaric acid	0.3 - 2
Ferulic acid	1 - 5

Supplemental Table 2. Baseline characteristics of study participants

Variable	Total (n=24)
Gender, m/f	17/7
Age, years	57.36 ±11.25
Weight, kg	75.13 ±12.82
BMI, kg/m^2	27.03 ±2.81
Waist circumference, cm	94.81 ±10.30
Systolic BP, mm Hg	149.65 ±6.72
Diastolic BP, mm Hg	87.81 ±7.67
Glucose, mg/dL	96.13 ±13.32
LDL-c, mg/dL	132.13 ±22.90
HDL-c, mg/dL	56.46 ±12.30
Triglycerides, mg/dL	125.29 ±64.54

Values expressed as mean ± standard deviation (SD). No significant differences were observed between intervention groups. Data calculated on intention-to-treat (ITT) population. Abbreviations: OP, Oligopin®; BMI, body mass index; BP, blood pressure; LDL-c, cholesterol of low density lipoprotein; HDL-c, cholesterol of high density lipoprotein

Supplemental Table 3. Changes in anthropometric variables and other cardiovascular disease risk biomarkers through the study.

Variable		Baseline		Final		Change at 5 weeks relative to baseline				Treatment difference			
		Mean	SD	Mean	SD	Change from Baseline	[95% CI]	% Change from Baseline	P	Change from Placebo	[95% CI]	% Change from Placebo	P
Weight (Kg)	Placebo	73.91	12.95	73.58	12.87	-0.33	[-0.8;0.2]	-0.45%	0.1783	0.00	[-0.5;0.5]	0.00%	0.9849
	Intervention	73.32	10.76	73.09	10.79	-0.23	[-0.6;0.1]	-0.31%	0.1456				
BMI (Kg/m^2)	Placebo	26.53	2.69	26.43	2.81	-0.10	[-0.3;0.1]	-0.38%	0.2693	-0.03	[-0.2;0.2]	-0.11%	0.7603
	Intervention	26.52	2.76	26.42	2.76	-0.10	[-0.2;-0.0]	-0.38%	0.0490				
Waist Circumference (cm)	Placebo	93.02	10.08	92.93	9.47	-0.09	[-1.6;1.4]	-0.10%	0.9067	-0.14	[-2.5;2.2]	-0.15%	0.9015
	Intervention	94.27	9.39	94.09	9.94	-0.18	[-1.5;1.1]	-0.19%	0.7751				
High-sensitivity C-reative protein (mg/dL)	Placebo	1.45	1.39	2.00	2.20	0.54	[-0.3; 1.4]	37.24%	0.1818	-0.91	[-2.1; 0.2]	-60.87%	0.1165
	Intervention	1.54	1.24	1.25	1.04	-0.29	[-0.7; 0.1]	-18.83%	0.1718				
Glucose (mg/dL)	Placebo	99.91	11.94	99.91	13.04	0.00	[-4.2; 4.2]	0.00%	1.0000	-0.43	[-7.2; 6.4]	-0.43%	0.8966
	Intervention	100.86	13.38	100.91	10.90	0.05	[-4.1; 4.2]	0.05%	0.9819				
Insulin (pmol/L)	Placebo	47.08	13.77	44.53	6.74	-2.55	[-7.7; 2.6]	-5.42%	0.3175	4.24	[-4.9; 13.4]	9.09%	0.3448
	Intervention	46.23	7.02	47.50	12.00	1.27	[-2.6; 5.1]	2.75%	0.5030				
Oxidized- LDL (U/L)	Placebo	97.85	49.44	104.00	46.74	1.90	[-26.9; 30.7]	1.94%	0.8926	-33.61	[-71.1; 3.9]	-32.70%	0.0765
	Intervention	107.74	43.06	76.02	32.15	-31.72	[-56.5; -6.9]	-29.44%	0.0147				
	Placebo	48.99	21.77	51.24	35.18	2.25	[-6.9; 11.4]	4.59%	0.6161	-6.44	[-18.8; 6.0]	-13.40%	0.2916

Intracellular adhesion molecule-1 (ng/mL)	Intervention	47.15	24.21	44.40	20.63	-2.75	[-7.6; 2.1]	-5.83%	0.2510				
Nitrates (uM)	Placebo	26.50	22.84	21.66	13.04	-4.84	[-12.4; 2.7]	-18.26%	0.1989	3.69	[-9.5; 16.9]	14.07%	0.5665
	Intervention	25.97	13.37	24.55	23.29	-1.42	[-13.2; 10.3]	-5.47%	0.8044				
E-Selectin (ng/mL)	Placebo	41.04	29.87	45.18	31.17	4.14	[-5.4; 13.7]	10.09%	0.3774	-5.72	[-15.2; 3.7]	-13.77%	0.2221
	Intervention	42.05	25.51	40.09	22.45	-1.96	[-5.2; 1.3]	-4.66%	0.2267				
Vascular cell adhesion molecule-1 (ng/mL)	Placebo	613.38	262.43	696.73	286.78	83.35	[-56.7; 223.4]	13.59%	0.2302	-130.42	[-345.8; 85.0]	-19.57%	0.2211
	Intervention	719.65	348.84	675.90	365.31	-43.75	[-222.6; 135.1]	-6.08%	0.6162				
GSH in plasma (nmol/mL)	Placebo	10.93	3.27	11.79	3.11	0.70	[-1.3; 2.7]	6.40%	0.4713	0.02	[-2.9; 3.0]	0.18%	0.9901
	Intervention	10.80	2.75	12.54	6.03	0.80	[-1.4; 3.0]	7.41%	0.4656				
GSSG in plasma (nmol/mL)	Placebo	50.20	10.15	50.49	8.52	0.81	[-4.4; 6.0]	1.61%	0.7519	-3.08	[-11.2; 5.1]	-5.98%	0.4372
	Intervention	52.88	10.84	51.24	11.21	-1.11	[-6.6; 4.4]	-2.10%	0.6768				
Ratio GSH/GSSG in plasma	Placebo	0.23	0.09	0.24	0.07	0.01	[-0.0; 0.0]	4.35%	0.7173	0.02	[-0.0; 0.1]	9.09%	0.5112
	Intervention	0.21	0.06	0.26	0.16	0.02	[-0.0; 0.1]	9.52%	0.2943				
Human ACE (ng/mL)	Placebo	99.30	16.42	98.40	15.89	-0.49	[-5.6; 4.6]	-0.49%	0.8454	-2.25	[-10.2; 5.7]	-2.30%	0.5615
	Intervention	96.74	14.78	94.92	12.58	-1.82	[-6.5; 2.9]	-1.88%	0.4294				
Vascular Endotelial growth factor (pg/mL)	Placebo	141.24	158.21	141.99	134.19	0.76	[-21.8; 23.3]	0.54%	0.9452	-19.61	[-68.1; 28.9]	-11.30%	0.4088
	Intervention	205.94	201.09	180.84	147.40	-25.10	[-76.8; 26.6]	-12.19%	0.3240				
Endothelin-1 (pg/mL)	Placebo	5.16	1.74	5.70	1.63	0.52	[-0.5; 1.6]	10.08%	0.3197	-0.40	[-2.0; 1.2]	-7.46%	0.6142
	Intervention	5.57	2.10	5.55	1.76	-0.03	[-1.0; 0.9]	-0.54%	0.9557				

Data calculated on the per-protocol (PP) population (n=21). Results from the ANCOVA model. Abbreviations: SD, standard deviation; BMI, body mass index; LDL, low density lipoprotein; GSH, reduced glutathione; GSSG, oxidized glutathione; ACE, angiotensin converting enzyme.

Supplemental Table 4. Composition of participant's diet during the study.

Variable		Baseline		Final		Change at 5 weeks relative to baseline				Treatment difference			
		Mean	SD	Mean	SD	Change from Baseline	[95% CI]	% Change from Baseline	P	Change from Placebo	[95% CI]	% Change from Placebo	P
Energy (Kcal)	Placebo	2203.89	616.87	2137.09	601.52	-66.80	[-268.7; 135.1]	-3.03%	0.4997	-77.91	[-442;6; 286.8]	-3.53%	0.6598
	Intervention	2207.03	478.93	2065.74	574.93	-184.87	[-394.7; 24.9]	-8.38%	0.0809				
Proteins (%)	Placebo	16.68	3.43	17.24	2.49	0.56	[-0.9; 2.1]	3.36%	0.4509	0.14	[-2.9; 3.2]	0.83%	0.9251
	Intervention	17.11	2.76	18.09	3.24	0.97	[-0.8; 2.8]	5.67%	0.2734				
Carbohydrates (%)	Placebo	40.47	7.23	42.16	7.89	1.69	[-1.5; 4.8]	4.18%	0.2777	0.70	[-3.9; 5.3]	1.75%	0.7532
	Intervention	39.31	6.52	42.69	7.42	3.32	[-0.4; 7.1]	8.45%	0.0790				
Simple Carbohydrates (%)	Placebo	18.48	6.06	22.43	8.77	3.95	[1.1; 6.8]	21.37%	0.0094	-1.31	[-5.2; 2.6]	-7.17%	0.4910
	Intervention	18.04	6.34	20.55	5.70	2.71	[0.0; 5.4]	15.02%	0.0497				
Complex Carbohydrates (%)	Placebo	21.46	6.90	20.97	5.88	-0.48	[-2.7; 1.8]	-2.24%	0.6591	-0.06	[-3.3; 3.2]	-0.28%	0.9684
	Intervention	21.12	3.69	21.98	5.91	0.59	[-2.3; 3.5]	2.79%	0.6693				
Lipids (%)	Placebo	39.78	6.28	38.34	6.51	-1.44	[-4.6; 1.7]	-3.62%	0.3524	-1.44	[-6.5; 3.7]	-3.59%	0.5604
	Intervention	40.36	5.57	36.34	7.35	-4.08	[-8.0; -0.2]	-10.11%	0.0418				
Monounsaturated Fatty Acids (%)	Placebo	18.78	3.21	19.59	3.83	0.80	[-1.2; 2.8]	4.26%	0.4069	-1.55	[-4.1; 1.0]	-8.08%	0.2247
	Intervention	19.61	2.89	18.27	4.84	-1.40	[-4.0; 1.2]	-7.14%	0.2764				
Saturated Fatty Acids (%)	Placebo	10.86	2.44	9.78	2.32	-1.07	[-2.4; 0.2]	-9.85%	0.1024	-0.77	[-3.1; 1.5]	-6.90%	0.4942
	Intervention	11.46	3.52	9.17	1.72	-2.22	[-3.9; -0.5]	-19.37%	0.0126				

Polyunsaturated Fatty Acids (%)	Placebo	6.25	1.94	5.66	1.33	-0.59	[-1.4; 0.2]	-9.44%	0.1256	0.02	[-1.1; 1.1]	0.32%	0.9730
	Intervention	6.08	1.55	5.53	1.50	-0.62	[-1.4; 0.1]	-10.20%	0.0919				
Cholesterol (mg)	Placebo	319.12	135.38	290.01	135.98	-29.11	[-94.6; 36.4]	-9.12%	0.3667	-76.71	[-175.7; 22.3]	-21.80%	0.1214
	Intervention	384.60	94.06	281.95	91.35	-106.72	[-163.1; -50.4]	-27.75%	0.0008				
Fiber (g)	Placebo	24.43	8.93	31.19	20.97	6.75	[-0.8; 14.3]	27.63%	0.0770	-6.71	[-19.0; 5.6]	-26.05%	0.2685
	Intervention	27.09	11.74	28.51	12.72	0.32	[-5.6; 6.2]	1.18%	0.9103				
Alcohol (g)	Placebo	9.66	9.55	6.64	7.18	-3.02	[-5.5; -0.5]	-31.26%	0.0197	5.23	[0.7; 9.7]	55.34%	0.0252
	Intervention	9.24	8.77	10.51	11.05	1.75	[-2.1; 5.6]	18.94%	0.3523				
Sodium (mg)	Placebo	2642.11	940.49	2485.20	1142.76	-156.91	[-715.1; 401.3]	-5.94%	0.5658	-60.22	[-780.8; 660.4]	-2.30%	0.8630
	Intervention	2592.96	634.16	2517.19	908.88	-153.29	[-494.7; 188.1]	-5.91%	0.3601				
Potassium (mg)	Placebo	3692.87	1015.65	4204.29	1650.94	511.42	[90.7; 932.1]	13.85%	0.0194	-378.01	[-1197.4; 441.4]	-10.07%	0.3464
	Intervention	3811.49	1111.00	4009.97	1019.92	175.44	[-316.0; 666.9]	4.60%	0.4651				
Magnesium (mg)	Placebo	353.93	86.70	364.81	129.77	10.88	[-30.8; 52.6]	3.07%	0.5941	-7.40	[-93.7; 78.9]	-2.06%	0.8594
	Intervention	365.35	112.00	382.44	128.71	3.65	[-48.4; 55.7]	1.00%	0.8852				
Calcium (mg)	Placebo	867.37	263.68	821.17	312.64	-46.20	[-188.2; 95.8]	-5.33%	0.5068	13.67	[-233.0; 260.3]	1.63%	0.9088
	Intervention	812.83	314.47	780.87	232.93	-41.45	[-201.6; 118.6]	-5.10%	0.5951				

Data calculated on the per-protocol (PP) population (n=21). Mixed models for repeated measures was used to compare differences between intervention and placebo groups. Abbreviations: SD, standard deviation.