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Improvement in Circulation and in Cardiovascular Risk Factors With a Proprietary Isotonic Bioflavonoid Formula OPC-3[®]

Maria R. Cesarone, MD, Andrea Di Renzo, Silvia Errichi, MD, Frank Schönlau, PhD, James L. Wilmer, PhD, and Julian Blumenfeld, MD

This study investigated the efficacy of isotonic bioflavonoid supplementation, OPC-3 on 61 individuals presenting with risk factors meeting the criteria for metabolic syndrome. Subjects were supplemented with a proprietary isotonic bioflavonoid OPC-3 or placebo over 2 months. Plasma oxidative stress status was significantly lowered by 10.1% with OPC-3. All major cardiovascular risk factors were improved with blood pressure, total cholesterol, and fasting blood glucose lowered. OPC-3 significantly improved endothelial function as evaluated by increased vasorelaxation in reactive hyperemia and enhanced diastolic carotid artery flow. Cardiac

ultrasound scanning revealed a significant increase of left ventricular ejection fraction. Skin microcirculation was enhanced, and better tissue perfusion led to significantly increased transcutaneous oxygen partial pressure and decreased $p\text{CO}_2$. With OPC-3 a dramatic and significant plasma C-reactive protein decrease by 52.1% occurred. Individuals may improve key cardiovascular risk factors by daily supplementation with the bioflavonoid OPC-3 as an important part of a healthier lifestyle.

Keywords: bioflavonoid; OPC-3; metabolic syndrome; isotonic; cardiovascular risk factors; Pycnogenol

Cardiovascular risks and metabolic syndrome represent major health problems in all industrialized nations. Inadequate and poor nutrition in combination with stress and sedentary living have contributed to poor general health, an obesity epidemic, and unprecedented health care costs.¹

The importance of ingesting foods and supplements high in antioxidants is becoming more valued as oxidative stress is being uncovered as a common pathologic mechanism to many chronic and degenerative diseases. Oxidative stress, the damage caused by excessive and uncontrolled reactive oxygen species to proteins, lipids, and DNA, has been implicated as a critical mechanism in the initiation and promotion

of cardiovascular disease, cancer, neurodegenerative disorders, retinopathies, cataracts, and autoimmune diseases, to name a few.²

As Americans are ingesting well below the daily fresh fruit and vegetable guidelines of the National Cancer Institute and the US Department of Agriculture, the use of high-quality supplementation is becoming widespread. Many clinicians are now recommending and offering efficacious antioxidant supplementation more frequently since recognizing the real and deficient eating habits of their patients and the growing science behind oxidative stress and antioxidants.

In recent years, the constituents in fruits and vegetables predominantly responsible for the health benefits have been identified as flavonoids. These molecules are synthesized by essentially all plants as secondary metabolites, providing them with protection from oxidative and microbial damage. More than 4000 structurally unique flavonoids have been identified in plant sources.³ Various efforts have been made to subdivide flavonoids into different

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categories. Presently, they are usually subdivided according to common molecular structures into flavonols, anthocyanins, flavones, flavanones, flavan-3-ols, and proanthocyanidins.⁴

All flavonoids represent potent antioxidants, yet their health benefits may further be attributed to properties well beyond antioxidation such as the modulation of cell signaling pathways and modulation of enzyme activities.^{3,5} Despite the wide distribution of flavonoids in the human diet, research showing the nutritional value and versatility for disease prevention has increased sharply only since 1995. Meanwhile, clinical research suggests that the health benefits are as diverse as the flavonoid species themselves.⁶

Citrus flavonoids (flavanones) were among the first discovered flavonoids and were shown to support capillary wall integrity by the Nobel Laureate Albert Szent-Györgi.⁷ Citrus flavonoids today are widely used for relieving edema such as in pathologies related to venous insufficiency. The molecular structures of citrus flavonoids inhibit dietary cholesterol absorption, resulting in cholesterol lowering in clinical trials.⁸

Bilberries represent a particularly rich source for the flavonoid group of anthocyanins, which are chemically unique due to the cationic molecular nature. Anthocyanins are characterized by specific health effects, predominantly in ophthalmologic disorders. The most prominent use dates back to Royal Air Force pilots who used bilberry for improving nighttime vision during World War II. In addition to improved nighttime visual acuity, a faster adjustment to darkness and a faster restoration of visual acuity after exposure to glare was described.⁹ Pharmacologic studies showed enhanced rhodopsin regeneration with anthocyanins, whereas other tested flavonoids were ineffective.¹⁰ Interestingly, bilberry was traditionally used for the treatment of diabetes mellitus, and anthocyanins are reported to inhibit digestive enzymes, particularly α -glucosidase.¹¹ Biochemical investigations have suggested anti-inflammatory activities of anthocyanins.¹²

French maritime pine bark extract Pycnogenol® consists of oligomeric procyanidins (OPCs) consisting of catechin and epicatechin subunits (flavan-3-ols) as well as monomeric catechin, taxifolin, and phenolic acids in accordance with the United States Pharmacopoeia.¹³ Pycnogenol had been investigated in more than 40 double-blind, placebo-controlled, clinical trials to improve all major cardiovascular risk factors.¹⁴ Pycnogenol lowered blood pressure in hypertensive patients, lowered blood glucose in type II

diabetic patients, improved cholesterol, and decreased platelet activity for prevention of thrombosis.¹⁵ Pycnogenol was demonstrated to possess general anti-inflammatory potency in humans.¹⁶

Grapes are particularly rich in bioactive flavonoids, the highest quantities are present in the seeds and skin.¹⁷ These flavonoids are predominantly OPCs, oligomers of catechin, epicatechin, and epicatechin-3-gallate, as well as the individual monomers. The composition of grape seeds and skin differs considerably. Particularly in the skin of red grapes, large amounts of anthocyanins and resveratrol are found.¹⁸ OPC-3 contains grape seed extract as well as red wine extract, and thus all abovementioned grape flavonoid species are present. Because epidemiologic data showed an inverse correlation between wine consumption and cardiovascular disease (the "French Paradox"), significant evidence has emerged on the health benefits of grape flavonoids. These benefits include reduced oxidative stress, decreased low-density lipoprotein (LDL), platelet activation inhibition, and lowered plasma tumor necrosis factor- α .^{19,20}

The currently available research suggests that supplemental flavonoids should be derived from diverse plant sources, rather than relying on a single species, as this will allow for broader and potentially synergistic health benefits in humans. Therefore, we chose to investigate a widely used, commercially available, and comprehensive proprietary formulation of bioflavonoids, OPC-3. OPC-3 consists of well-studied flavonoids derived from extracts of bilberries, citrus fruit, French maritime pine bark (Pycnogenol), red wine, and grape seeds. Furthermore, this particular product, OPC-3, is available in an isotonic solution (when mixed with water according to label directions). Delivery of nutrients in isotonic solution can contribute to the rapid onset of gastrointestinal absorption, and the solution is easily administered and well tolerated.

The aim of our study was to identify and validate health effects in people presenting with early stage metabolic syndrome and borderline cardiovascular risk factors.

Patients and Methods

Subjects

The enrolled subjects had to meet the criteria for metabolic syndrome, including borderline cardiovascular health risks, with blood pressure, lipid profile, and fasting glucose. However, none of the subjects

was on a medical intervention, and only a risk management program, including diet and an exercise, had been recommended.

The inclusion criteria for subjects were blood pressure >130/85 mm Hg and <140/95 mm Hg, high-density lipoprotein (HDL) cholesterol <40 mg/dL, total cholesterol >200 mg/dL and <240 mg/dL. Fasting blood glucose had to be in the range >100 mg/dL and <125 mg/dL, which is considered prediabetic according to the American Diabetes Association.

Exclusion criteria included any metabolic conditions or any other clinical conditions requiring drug treatment. Severely handicapped people and pregnant or nursing women were excluded from the trial.

Materials and Methods

A randomized, double-blind, placebo-controlled, parallel group study was conducted. All patients were provided with a 2-month supply of OPC-3 (nutraMetrix, Division of Market America, Greensboro, NC). The nutrient powder was supplied in a sealed foil pouch with matching placebo pouches. Each pouch contained 10 g of powder and was dissolved in 180 mL water before consumption, which will yield an isotonic solution of all solubilized constituents. One pouch was consumed each day over a period of 2 months. The active components of an OPC-3 pouch are 400 mg of flavonoids, consisting of equal amounts of extracts derived from French maritime pine bark (Pycnogenol), grape seed, bilberry, citrus, and red wine. A base of inactive ingredients, including fructose, glucose, citric acid, potassium bicarbonate, silica, calcium sulfate, and pectin, contributes to the tonicity of the solution once water is added, acts as a pH buffer, covers the bitter and astringent tastes of the extracts, and improves the uniformity of powder blending. The placebo consisted of fructose, citric acid, potassium bicarbonate, maltodextrin, silica, calcium sulfate, apple fiber, FD&C Red #40, and FD&C Blue #1. The placebo pouches were matched in appearance and contained powder with equivalent taste and color, but were without flavonoids.

At the time of recruitment and again after 2 months consumption of OPC-3 or placebo, the following parameters were recorded.

Blood parameters. Samples of venous blood were placed in tubes containing sodium EDTA (1 mg/mL) and in polystyrene tubes without anticoagulant. EDTA-containing tubes were chilled in an ice bath

followed by separation of plasma by centrifugation. Serum was separated by centrifugation at room temperature. Samples were stored at -80°C until assayed. Routine chemical methods were used to determine serum concentrations of total cholesterol, HDL, LDL, and glucose.

Fasting blood was tested for blood cell count (erythrocytes, platelets, lymphocytes, neutrophils, leukocytes, eosinophils, basophils, monocytes), and indices of liver (glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, γ -glutamyltransferase, alkaline phosphatase) and kidney (creatinine) function. Investigation of fibrinogen concentration and prothrombin time were carried out with plasma specimen.

The oxidative stress status of patients was investigated by quantifying direct reactive oxygen metabolites (D-ROM) using the Free Radical Analytical System (FRAS; Diacron, Grosseto, Italy). In brief, the assay estimates hydroperoxides in a small blood sample (20 μL) after incubation in buffer solution together with a chromogenic agent. Photometric analysis provides oxidative stress status in Carr units, with 1 Carr unit corresponding to 80 $\mu\text{g H}_2\text{O}_2/\text{dL}$. Values greater than 300 Carr units suggest oxidative stress.²¹

Plasma C-reactive protein (CRP) was measured using an automated analysis system CRP 200 (Roche, Basel, Switzerland).

Macrovascular and heart parameters. Blood pressure and heart rate were measured, followed by more detailed investigations on reactive hyperemia as well as carotid artery velocities of systolic and diastolic blood flow components.

Forearm blood flow was measured using strain-gauge plethysmography during reactive hyperemia to test for endothelium-dependent vasodilatation. Forearm blood flow was measured using a mercury-filled Silastic strain-gauge plethysmograph (Hokanson Inc., Bellevue, WA). In brief, forearm blood flow was occluded by inflating a cuff over the left upper arm to a pressure of 280 mm Hg for 5 minutes. Following release of the ischemic cuff occlusion forearm, the peak flow was measured for 3 minutes and the values were set as a ratio to flow at baseline.

Diastolic and systolic components of blood flow of carotid arteries were investigated by means of high-resolution duplex ultrasonography scanning using an ATL 5000 HDI (Advanced Technology Laboratories, Seattle, WA).

Left ventricular ejection fraction was measured by cardiac ultrasound using a modified Simpson rule according to standards of the American Society of Cardiac Ultrasound and the American Heart Association.²² The analysis of the left ventricle included measurements of the left ventricular volume in end-diastole and end-systole in milliliters and the evaluation of the ejection fraction from the 4-chamber apical projection.²³

Microcirculatory parameters. All microcirculatory measurements were carried out in rooms at 21°C to 22°C before 10 AM to avoid any artifacts resulting from prolonged standing during the day. For acclimatization patients rested for 30 minutes in a supine position.

Laser Doppler flowmetry was carried out using a TSI Vasamedics instrument (TSI, St. Paul, MN). The skin flux at the perimalleolar region was measured at rest and after standing as described previously.²⁴

Transcutaneous respiration was investigated using a Kontron pO_2 - pCO_2 analyzer (Zurich, Switzerland) with a combi-sensor for simultaneously measuring both parameters following adaptation of the skin at 42°C for 20 minutes.

Statistical Analysis

Results are presented as the mean \pm standard deviation (mean \pm SD). Statistical analysis of blood pressure and blood glucose was performed using Student's *t* test. Heart rate and CRP data were analyzed using Tukey's test. Blood lipids and parameters characteristic for liver and kidney function were calculated using analysis of variance (ANOVA). Statistical analysis of oxidative stress testing, the diastolic component of carotid artery flow, Laser Doppler testing of microcirculation and transcutaneous partial pressures was performed with the Mann-Whitney *U* test. Values of $P < 0.05$ were considered significant.

Results

A total of 61 subjects were recruited and completed this study (Table 1). They were randomized to either the group receiving OPC-3 or to the control group receiving a placebo product.

Evaluation of Blood Parameters

As shown in Table 2, the level of plasma oxidative stress was found to be significantly lowered after 2 months

Table 1. Details of the Test Subjects

	Treatment Group	Control Group (Placebo)
Number of patients	31	30
Male/female	19/12	21/9
Average age (years)	49.7	48.3
Age range (years)	45-58	45-62

supplementation with OPC-3 as compared with baseline. In the placebo group, the plasma oxidative stress was found to be only marginally lowered.

Fasting blood glucose and total cholesterol decreased substantially in the OPC-3 treated group, whereas HDL cholesterol showed little change. However, these values were found to be only borderline statistically significant.

The plasma CRP was dramatically lowered in response to supplementation with OPC-3, as compared with minor effects found in the control group. The lowering of CRP was highly significant in the OPC-3 treated group.

Evaluation of Macrovascular Health Parameters

The investigation of macrovascular health characteristics showed a considerable normalization of systolic and diastolic blood pressure in the group supplementing with OPC-3, whereas minimal changes were found in the placebo group (Table 3). The heart rate was slightly, but not significantly, lowered in response to OPC-3 and to a negligible extent in the control group.

Supplementation with OPC-3 was found to significantly improve vasorelaxation as judged by reactive hyperemia. Following occlusion of forearm arteries by means of a cuff, the release of the ischemia showed significantly better blood flow restoration from baseline 25% to 138% after OPC-3 intake for 2 months. In the placebo group, no improved blood vessel response was noted.

The diastolic component of carotid artery blood flow was found to be improved to a relatively limited extent though statistically significant by supplementation with OPC-3, whereas no effect was detected in the placebo group.

The cardiac left ventricular ejection fraction was found to be significantly improved following supplementation with OPC-3, whereas only a marginal effect was seen in the placebo group.

Table 2. Analysis of Blood Parameters

	Baseline	2 Months	P ^a
Oxidative stress D-ROM test (Carr units)			
OPC-3	428 ± 24	385 ± 12	<0.025
Control	429 ± 22	418 ± 17	NS
Fasting blood glucose (mg/dL)			
OPC-3	118 ± 9	108 ± 7	NS
Control	119 ± 8	115 ± 8	NS
Total cholesterol (mg/dL)			
OPC-3	233 ± 11	218 ± 8	NS
Control	228 ± 12	223 ± 9	NS
HDL cholesterol (mg/dL)			
OPC-3	37 ± 3	39 ± 4	NS
Control	38 ± 4	37 ± 5	NS
LDL cholesterol (mg/dL)			
OPC-3	41 ± 3.1	38 ± 2.2	NS
Control	38.1 ± 2.2	39 ± 2.0	NS
C-reactive protein (µg/L)			
OPC-3	238 ± 23	114 ± 12	<.025
Control	228 ± 29	199 ± 26	NS

NOTES: D-ROM = direct reactive oxygen metabolites; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

^aStatistical significance is given for treatment as compared with baseline. NS indicates that difference did not reach statistical significance.

Evaluation of Microcirculatory Parameters

As presented in Table 4, laser Doppler measurements of capillaries at the skin surface of the lower legs showed a pronounced improvement of blood microcirculation of subjects supplemented with OPC-3. The increase of microcirculation at rest, when subjects were kept in supine position, was statistically significant in the OPC-3 treated group as compared with placebo. On standing, the increased microcirculation with OPC-3 did not reach statistical significance. The control group did not show any alteration of blood microcirculation.

The improved blood microcirculation at the skin level of the lower legs was found to coincide with significant elevated transcutaneous partial oxygen pressure and significantly decreased partial pressure of carbon dioxide in the OPC-3 supplemented group. No such effect was evident in the placebo group.

Treatment Tolerance and Safety

Liver enzymes were within a healthy range prior to treatment as well as after completion of the trial

Table 3. Cardiovascular Parameters of Subjects

	Baseline	2 Months	P ^a
Blood pressure systolic/diastolic (mm Hg)			
OPC-3	135 ± 5/92 ± 4	128 ± 4/89 ± 3	NS
Control	134 ± 4/93 ± 3	132 ± 3/92 ± 3	NS
Heart rate (beats/min)			
OPC-3	78 ± 7	74 ± 6	NS
Control	79 ± 6	77 ± 4	NS
Diastolic component of carotid artery flow (%)			
OPC-3	16 (11-26)	22 (13-28)	<0.05
Control	16 (12-27)	16 (13-27)	NS
Reactive hyperemia (%)			
OPC-3	25 (11-69)	138 (98-251)	0.05
Control	24 (12-74)	29 (16-81)	NS
Left ventricular ejection fraction (%)			
OPC-3	100	122 (89-144)	<0.05
Control	100	106 (74-123)	NS

^aStatistical significance is given for treatment as compared with baseline. NS indicates that difference did not reach statistical significance.

(data not shown). Kidney function was also not affected. White and red blood cell counts and clotting factors were within the normal range throughout. Subjects were interviewed for possible adverse effects, and none was reported by all subjects.

Discussion

The aim of our study was to investigate and validate an improvement of cardiovascular health with a complex flavonoid supplement in volunteers with early and potential cardiovascular health issues. The significantly lowered oxidative stress level in the OPC-3 group but not in the placebo group demonstrates the bioavailability of the product. A plasma reactive oxidant species value of >300 Carr units is considered indicative for elevated oxidative stress in humans.²¹ Though the average oxidative stress was lowered by a statistically significant degree with OPC-3 by 10%, it did not decrease the value below 300 Carr units suggesting that a longer intake period might be required.

The fasting blood glucose of subjects at baseline was greater than 100 mg/dL, characteristic for metabolic syndrome. OPC-3 lowered fasting glucose from 118 to 108 mg/dL. The blood lipid profile suggests that subjects are borderline hypercholesterolaemic

Table 4. Microcirculatory Parameters of Subjects

	Baseline	2 Months	P ^a
Flux at rest, laser Doppler (flux units)			
OPC-3	1.1 ± 0.1	1.9 ± 0.2	<.05
Control	1.12 ± 0.1	1.1 ± 0.1	NS
Flux on standing, laser Doppler (flux units)			
OPC-3	0.9 ± 0.1	1.1 ± 0.1	NS
Control	1.0 ± 0.15	1.1 ± 0.1	NS
Venoarteriolar response (%)			
OPC-3	34	44	<.05
Control	36	35	NS
Transcutaneous partial O ₂ pressure (mm Hg)			
OPC-3	52 ± 8	58 ± 6	<.05
Control	51 ± 8	50 ± 8	NS
Transcutaneous partial CO ₂ pressure (mm Hg)			
OPC-3	29 ± 3	26 ± 4	<.05
Control	28 ± 4	28 ± 3	NS

^aStatistical significance is given for treatment as compared with baseline. NS indicates that difference did not reach statistical significance.

as judged by the high total cholesterol as well as the low HDL value (<40 mg/dL). These parameters improved but not to a healthy blood lipid profile. Because this was only a 2-month trial, extended periods of daily supplementation may further lower fasting blood glucose and improve blood lipid indices.

Most impressively, the CRP level was significantly decreased to less than half the initial value in response to OPC-3 intake. The evaluation of CRP as inflammatory marker is considered a powerful independent predictor of cardiovascular disease risk.²⁵ Though the average CRP level of our subjects was not at critical levels, which likewise reflects their borderline cardiovascular risk factors, the study demonstrates the potential of OPC-3 for reducing this major cardiovascular risk factor.

OPC-3 was found to reduce borderline hypertension from average systolic blood pressure 135 mm Hg to 128 mm Hg. The obtained results further point to a limited but significant improvement in heart ejection fraction. The significantly improved reactive hyperemia is indicative of better endothelial function.²⁶ The ameliorated endothelial function is believed to have contributed to the lowered blood pressure as it coincides with significantly improved carotid artery flow.

Our findings on improved endothelial function are corroborated by another clinical trial recently

carried out with OPC-3 (T. A. Barringer, L. Hatcher, and H. Sasser, in press, 2008). This study demonstrated by reactive hyperemia that daily intake of OPC-3 for 4 weeks mitigated impaired endothelial function resulting from eating a fat-rich meal, whereas placebo was ineffective.

The significantly enhanced microcirculation, shown in our study by laser Doppler measurements, likewise points to better endothelial function. The elevated tissue perfusion and oxygenation impressively demonstrates an improved vascular function with OPC-3.

The improvement of some parameters did not reach statistical significance in this study. This may be due to the limited health problems the subjects presented at the beginning of the trial. In patients with more advanced health problems, more pronounced improvements with OPC-3 supplementation might be seen. However, it was not the aim of this study to investigate patients with advanced cardiovascular disease.

This comprehensive study by evaluating both macrovascular and microcirculatory parameters demonstrates that a potent flavonoid supplement may be a valid, cost-effective, and clinically efficacious adjunct for people with early health risks, especially when used in conjunction with healthier lifestyle choices.

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