

# Blueberry Extract Reduces Oxidative Stress in Patients with Metabolic Syndrome

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**Abstract:** Oxidative stress is a health condition that could potentially harm the patient, and it is defined as the lack of balance between the production of oxygen free radicals, which rises, and antioxidant defenses, which are in decrease. Metabolic syndrome implies a variety of risk factors that are based on abdominal obesity and insulin resistance. Moreover, the importance of metabolic syndrome is emphasized due to the fact that it presents an increase in oxidative stress, which is produced by the sum of alterations that each risk factor produces within the metabolic syndrome pathology. Reducing oxidative stress in these patients is currently one of the most interesting challenges of cardiovascular and metabolic therapeutics, because it is a molecular biology alteration that is not generally diagnosed and, therefore, not treated. The increasing incidence of overweight and obesity cause an increase in the incidence of metabolic syndrome, thus turning into a huge problem that keeps growing at alarming proportions. This syndrome's incidence oscillates between 20 and 40%, depending on the gender, age, ethnic group, and diagnostic criteria used for the definition of the disease. Prospective studies show that metabolic syndrome doubles the risk of cardiovascular disease and causes a five-fold increase in the risk of developing type 2 diabetes. With the aim of decreasing the oxidative stress caused by metabolic syndrome, we investigated the effect of antioxidant protection in DNA repair and cell membranes through the use of blueberry extract, which is the fruit with the highest antioxidant capacity, in patients with metabolic syndrome. Thirty patients were studied for a period of 6 months of intervention, and it could be demonstrated that they showed a highly significant decrease in the damage produced to the DNA, which was measured by the urinary excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and the damage caused to the vascular endothelium and cell membranes, which was measured through the urinary excretion of F2-isoprostane. Based on our knowledge, this investigation is the first one to show that lyophilized blueberry extract (BlueKing®) as a dietary supplement, with meals, is an additional therapeutic tool of great value for the treatment of oxidative stress through DNA and cell membrane protection in patients with metabolic syndrome.

**Key words:** Metabolic syndrome, urinary biomarkers of oxidative stress, blueberries, antioxidants.

## 1. Introduction

Metabolic syndrome (MS) causes multiple metabolic disorders [1] that in turn cause a five-fold increase in the risk of developing type 2 diabetes (T2D) and a 2-to-3-fold increase in the prevalence of cardiovascular diseases (CVD). MS is considered as a very prevalent (20 to 40%) and relevant pathology in the current epidemics of T2D and CVD, reason why it became a public health issue of importance all over

the world. The World Health Organization (WHO) and other programs, such as the National Cholesterol Education Program (NCEP) and Adults Treatment Program III (ATP III), determined [2, 3] that in order to make a diagnosis of MS, the patient must present at least three of the following five variables: a) increase in waist circumference, b) low levels of high-density lipoprotein cholesterol (HDL), c) hypertriglyceridemia, d) high blood pressure, and e) hyperglycemia in fasting state. Besides the described pathologies, there may also be a significant degree of chronic inflammatory activity and oxidative stress in obesity,

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in T2D, in high blood pressure, and in atherosclerosis. One of the main deficiencies in the current definition of MS is the lack of inclusion of proinflammatory state and oxidative stress for the diagnosis of the disease [4, 5]. These are variables that may provide useful information for the quantification of oxidative and inflammatory deterioration caused by MS. There are several research studies that correlate oxidative damage caused to the DNA and to the endothelium and cell membranes in general in MS, with biomarkers that can be detected in blood and urine, such as 8(OH)dG and F2-isoprostanes, respectively. The only resource that may lower the negative impact of oxidative stress in these patients is the intake of antioxidants by means of a diet rich in fruits and vegetables, or in a more concentrated manner by means of dietary supplements. Polyphenols are antioxidant molecules that synthesize plants from which foods of usual intake are obtained, such as tea, cocoa beans, blueberries, grapes, and that have many beneficial effects over health and against oxidative stress. Taking into account that the blueberry is the fruit that has the most powerful antioxidant effect and that such bioactivity is caused by its high content of polyphenols, our hypothesis is that a concentrated blueberry extract could reduce the amount of oxidative damage caused to the DNA and the vascular endothelium in patients with MS [6-14].

## 2. Purpose and Design

The primary objective of this investigation was to assess the effects of the intake of chewable tablets of lyophilized blueberry extract over urinary biomarkers of oxidative stress in patients with MS. These tablets have chemical properties that are very similar to the ones of the fruit itself; each tablet is equivalent to 15 blueberries with 1,200 mg of anthocyanins (BlueKing®). The secondary objectives of this study were the assessment of the tolerability of the studied product and the reporting of adverse events. The design was based on treating patients with MS with 2

tablets of blueberries per day with meals for a period of 6 months, comparing pretreatment data at baseline with the data after 3 and 6 months of treatment. This means that each patient was its own control subject. The clinical trial recruitment was carried out by the University of Buenos Aires at different medical sites of *Hospital de Clínicas “José de San Martín”*.

## 3. Materials and Methods

### 3.1 Patients

62 patients with metabolic syndrome were included in the study. Forty-four of them complied with inclusion criteria, 30 of which completed the trial (20 females and 10 males) (Table 1).

The study protocol and the informed consent were approved by the Ethics Committee and the Division of Teaching and Research of *Hospital de Clínicas “José de San Martín”* of the University of Buenos Aires. After signing the informed consent for their participation in the study, the subjects attended an appointment at *Centro de Diagnóstico Molecular* for a first consultation, and they had to take with them a 24-hour sample of urine. The first blood drawn in fasting state was performed at that consultation too. The volunteers were selected based on their homogenous life habits. They all had at least three of the following variables that define MS: a) waist circumference: equal or greater than 102 cm in men, and equal or greater than 88 cm in women; b) blood pressure > 130/85 mmHg and, if the patient was receiving treatment for high blood pressure, they must be receiving a stable dose of antihypertensive agents with no changes within the last 3 months; c) fasting glycemia: equal or greater than 100 mg/dl; d) triglycerides greater than 150 mg/dl; e) HDL cholesterol less than 40 mg/dl; f) LDL cholesterol greater than 130 mg/dl. All participants were willing to maintain their current daily level of physical activity throughout the whole study. All patients had a body mass index (BMI) > 30. Volunteers did not have a history of liver or kidney disease nor disorders of psychiatric origin. A clinical

**Table 1** Baseline variables in patients at recruitment.

Variables	Baseline
MS patients—n	30 (20 F-10 M)
Age—years	55 ± 11
Systolic blood pressure—mmHg	135 ± 7
Diastolic blood pressure—mmHg	90 ± 14
Weight—Kg	93.5 ± 39
Height—cm	164.5 ± 9
Waist circumference—cm	111 ± 25.45
Body mass index	33.9 ± 10.6

record was prepared for each participant and the following clinical biochemistry tests were requested: full blood count, erythrocyte sedimentation rate, glycemia, uremia, blood uric acid, plasma ionogram, hepatogram, HDL cholesterol, LDL cholesterol, triglycerides, prothrombin time, KPTT, ultra-sensitive reactive C protein, glycosylated hemoglobin, insulinaemia, pregnancy test in women of childbearing potential and 24-hour urine for the determination of oxidative stress biomarkers. Oxidative damage to the DNA was measured through the excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and the damage caused to cell membranes was measured through 24-hour urinary F2-isoprostanes. All the analyses were done at *Centro de Diagnóstico Molecular* at baseline, and after 3 and 6 months of treatment.

The exclusion criteria were as follows: having undergone a gastrointestinal surgery (except for appendectomy) within three months before the initiation of the study (e.g. gastric by-pass, etc.); having donated blood within the three previous months; anticoagulated patients; patients affected by a known addiction; volunteers that during the period of this study had to initiate a treatment that was not compatible with the study treatment (e.g. multivitamins or antioxidants); history of allergies or intolerance to certain drugs or pharmacotherapy for weight loss; untreated or chronic disease of the intestine, thyroid gland, liver, or kidney; pregnancy or willingness to become pregnant during the study;

history of allergies to blueberries; any known pathologic condition that may have altered the white blood cell count within the last 6 months; exposure to drugs that are known to cause mutations; radiation or vaccination within the last 6 months; alcohol intake greater than 2 drinks/day in men and 1 drink/day in women; smoking habit.

The experimental design of the study was developed in two consecutive phases: the first phase or wash-out period that lasted for 2 weeks. Treatments that did not consist of antioxidant or multivitamin dietary supplements had to be maintained. Once the first phase was over, a free-polyphenol diet was indicated for 24 h before blood was drawn. Then, patients had to collect their urine for 24 h. On the day of the first consultation, the clinical record was completed, the laboratory test was done, which consisted of a blood sample in fasting state, and the 24-hour urine sample was received. The second phase of the intervention consisted in the administration of a 1,200 mg tablet of lyophilized blueberry extract (BlueKing®, *Laboratorio Sidus S.A.*) twice a day at the time of both lunch and dinner. The same tests were performed after 3 and 6 months of treatment. At the time of consultation, all the patients received the dose of blueberry extract tablets for the 6 months of treatment. The participants followed a free diet and were supervised by a research team physician. The following vital signs were controlled at these consultations: blood pressure, heart rate, temperature and physical examination.

The observations of potential adverse events were recorded and all the information was included in the clinical record and in the clinical research form (CRF).

### 3.2 Biochemical Analyses

All biochemical analyses were performed at *Centro de Diagnóstico Molecular S.A.* The complete blood count was measured with a hematologic counter and the clinical chemistry analyses were done through an enzymatic spectrophotometric method (Wiener Lab). The 8(OH)dG was measured with the Oxi Select Oxidative DNA Damage ELISA Kit Catalog STA-320 Cayman Chemical [15], and F2-isoprostanes were calculated by means of an 8-Isoprostane EIA Kit Item № 516351, Cayman Chemical [16-23].

### 3.3 Statistical Analysis

The statistical analysis was performed through the

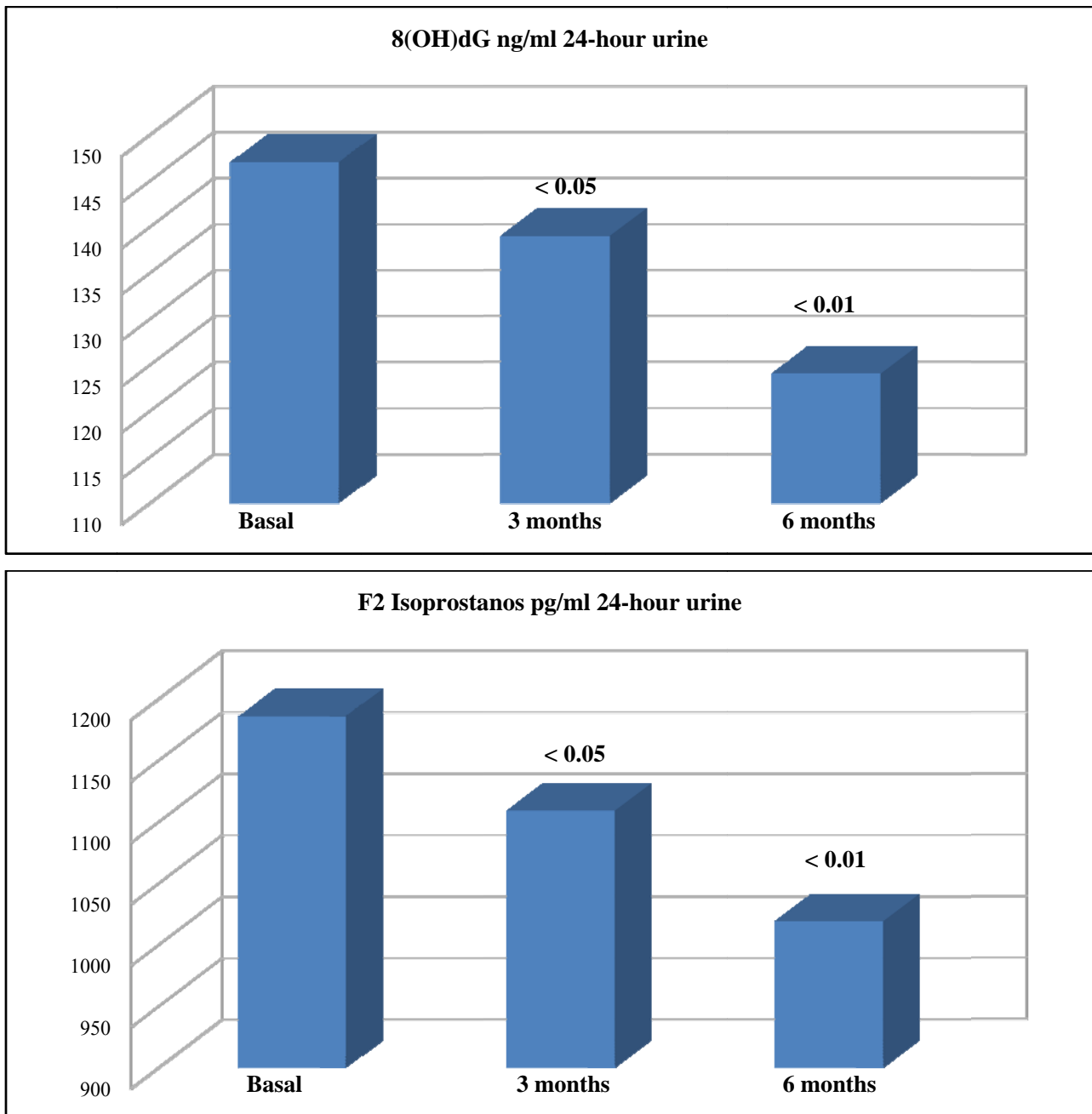
student's *t*-test for parametric data of paired samples.

## 4. Results

The intervention with blueberry extract was well tolerated. No side effects or adverse events that led to treatment discontinuation were reported. Patients showed a general improvement in vital signs and in self-esteem due to the fact that they received a more intensive health care. Clinical chemistry analyses (Table 2) have shown significant variations in: uric acid, which improved after 6 months of treatment; hemoglobin, which increased; and ultra-sensitive C-reactive protein, which decreased, whereas HDL cholesterol increased after 3 months of treatment and then decreased after 6 months. The urinary biomarkers of oxidative stress 8(OH)dG and F2-isoprostanes significantly decreased after 3 and 6 months of treatment, this last reduction being highly significant (Fig. 1).

**Table 2 Results of the intervention over clinical blood analyses and over the urinary biomarkers of oxidative stress, 8(OH)dG and F2-isoprostanes.**

Intervention	Baseline	3 months	<i>P</i>	6 months	<i>P</i>
Hemoglobin	13.18 ± 1.2	13.76 ± 1.6	< 0.05	13.77 ± 1.4	< 0.001
WBC/mm <sup>3</sup>	8,333 ± 6,284	8,480 ± 7,116	NS	8,987 ± 7,129	NS
ESR—mm	17.56 ± 12.43	16.5 ± 9.05	NS	18.23 ± 10.43	NS
Glycemia mg/dl	121.03 ± 44.06	116.67 ± 60.42	NS	119.2 ± 55.93	NS
Urea mg/dl	31 ± 11	34 ± 10	NS	33 ± 8.5	NS
Uric acid mg/dl	4.72 ± 1.46	4.23 ± 0.98	NS	4.24 ± 1.19	< 0.05
Total cholesterol mg/dl	194.56 ± 38.55	198.96 ± 38.65	NS	196.06 ± 40.49	NS
HDL cholesterol mg/dl	50.81 ± 11.80	57.06 ± 13.83	< 0.05	50 ± 12.86	NS
LDL cholesterol mg/dl	111.13 ± 10.60	106 ± 30.40	NS	111.50 ± 33.71	NS
Triglycerides mg/dl	167.96 ± 66.46	175.03 ± 146.90	NS	172.66 ± 108.55	NS
U-S CRP mg/L	4.78 ± 13.08	5.47 ± 5.37	NS	3.17 ± 2.24	< 0.05
Glycosylated hemoglobin %	5.93 ± 2.91	6.39 ± 1.21	< 0.05	6.17 ± 1.53	NS
Insulinaemia mU/mL	20.59 ± 26.36	16.66 ± 9.20	NS	16.41 ± 9.35	Trend
8(OH)dG ng/ml	147 ± 46.7	139 ± 73.7	< 0.05	124.96 ± 74.70	0.000032
F2-isoprostanes pg/mL	1,186.17 ± 926.31	1,109.33 ± 375.96	< 0.05	1,019.83 ± 380.01	0.000002



**Fig. 1** In the bar graph, we can see that there is a decrease that can be interpreted as a highly significant improvement of the 2 most important biomarkers of oxidative stress over DNA and vascular endothelium, which are in both cases determined in 24-hour urine samples after 3 and 6 months of treatment with 2 tablets of blueberry extract (BlueKing®) per day. The US Food and Drug Administration (FDA) defines the biomarkers as a factor that can be measured objectively and that may be evaluated as an indicator of normal or pathogenic biological processes, or of pharmacological responses to a therapeutic intervention. In this case, both are biomarkers of effect of oxidative stress, i.e. biomarker of effects: biochemical, physiological or any other measurable type of alteration of the organism that, based on its magnitude, may be identified as an established or potential health disorder or disease.

## 5. Comments and Conclusions

There are mechanisms that could explain biochemical results. Regarding hemoglobin, it is probable that blueberry extract increases the absorption of iron due to a higher level of acidity. Regarding uric acid, there could have been an improvement of the kidney function due to the protection provided by the antioxidant. The decrease in U-S C-reactive protein was a clear anti-inflammatory effect that is directly related to the results over the biomarkers of oxidative stress. The 8-hydroxy-2'-deoxyguanosine molecule is produced by the oxidative damage caused to the DNA (nuclear and mitochondrial) by the reactive oxygen and nitrogen species, including hydroxyl radical and peroxynitrite. This is an important biomarker that serves as a measure of oxidative stress in biological systems [24-27].

The fact that 8(OH)dG significantly decreased with the use of blueberry extract is an important evidence of the role that antioxidant protection has over one of the most sensitive areas of the cell, which is the DNA. In patients with metabolic syndrome, the elevation of urinary excretion of F2-isoprostanes is the result of the destruction of arachidonic acid, a polyunsaturated omega-6 fatty acid that constitutes an essential structure of cell membranes [28]. This is why the quantification of F2-isoprostanes provides a unique opportunity for the research of lipid peroxidation in human diseases and it also provides an interesting biomarker for the monitoring of antioxidant doses [28]. The reduction of the damage caused to the structure of cell membranes, mainly to the endothelium, is one the most relevant effects of the antioxidant protection of blueberry extract. MS is a complex group of anthropometric, cardiovascular and metabolic abnormalities in which oxidative stress is the common denominator, which could also be the cause that feeds back from the origin of the conditions that compose it, not only in diabetes but also in high blood pressure,

obesity or dyslipoproteinemia. In fact, a large clinical trial that was carried out for a period of 7.5 years and that included 5,220 adult patients provided results that supported the intake of food rich in antioxidants in order to reduce the risk of metabolic syndrome [29]. This trial also found that antioxidant dietary supplements were efficient in reducing the risk of metabolic syndrome. Antioxidants also inhibit the initiation or the spreading of oxidation chain reactions that cause oxidative damage to lipids, proteins and nucleic acids [30]. A study that was designed in order to measure the neutralizing capacity of oxygen free radicals among 24 studied fruits showed that blueberries have the highest Total Antioxidant Capacity (TAC) per portion: 13,427 TAC/portion [31]. Blueberries, due to their high antioxidant capacity, act as neuroprotectors and it has been shown that they avoid and invert the loss of cognitive performance [32-34]. These polyphenols reduce oxidative stress by sweeping free radicals away, thus acting as transition metals chelators that inhibit antioxidant enzymes [35]. Also, in a cell culture essay, blueberries showed the highest cellular antioxidant activity [36]. However, the number of investigations in volunteers is still limited regarding the study of the effects of blueberries over the damage produced by oxidative stress in patients with MS. To the best of our knowledge, this is the first investigation that showed that the intake of a dietary supplement of lyophilized blueberry tablets (BlueKing®), with meals, in patients with MS decreases the inflammation and, in a highly significant manner, it also reduces the damage produced by oxidative stress over nuclear DNA, mitochondrial DNA, and cell membranes.

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