

# Evaluation of the Effects of a Supplement Composed by Quercetin, Rutin, Bromelain and L-Carnosine in Patients with Borderline Uricemia

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**Abstract** The aim of this study was to evaluate if a nutraceutical containing quercetin, rutin, bromelain and L-carnosine (Uricemin®) can reduce uric acid levels in subjects with values between  $\geq 6$  and  $< 7$  mg/dl after 3 months of therapy. Patients were randomized to Uricemin® or placebo for three months. We evaluated body weight, fasting plasma glucose (FPG), lipid profile, uricemia (UA), high-sensitivity C-reactive protein (Hs-CRP) at baseline and after 3 months since the study start. Treatment tolerability was assessed evaluating transaminases, and creatinine, and all adverse events were recorded. A total of 116 patients were enrolled in the trial. Of these, 59 were randomized to the nutraceutical and 57 to placebo. One hundred and seven subjects completed the study, and were included in the analysis. We did not record any change in glycemia, or lipid profile in neither group. We observed a reduction of uricemia, and Hs-CRP in the group treated with Uricemin®, compared to baseline and to placebo ( $p < 0.05$  for both). The results of this study suggested that a combination of quercetin, rutin, bromelain and L-carnosine can be useful in reducing uricemia in patients with borderline uricemia.

**Keywords:** bromelain, hyperuricemia, L-carnosine, quercetin, rutin

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## 1. Introduction

Hyperuricemia is defined as the presence of uric acid levels in the blood above 7 mg/dl [1,2,3], which represents the saturation point of monosodium uric acid at physiological levels of temperature and pH [4]. In presence of hyperuricemia, intra-articular deposition and in surrounding tissues of monosodium urate crystals is responsible for the genesis of acute gout attack and chronic arthropathy [5]. Uric acid, mainly synthesized at hepatic level by the enzyme xanthine dehydrogenase, represents the final product of the metabolism of endogenous purines, deriving from the metabolism of nucleic acids, and exogenous purines, derived mainly from the intake of meat, fruit, fish, and alcoholic beverages. Uric acid is a powerful anti-oxidant agent capable of acting as an intra-cellular scavenger of excess free oxygen radicals (ROS), which are present in conditions of increased oxidative stress [6]. However, in case of hyperuricemia, uric acid is able to perform pro-oxidant actions, inducing an increased production of ROS

and a reduced production of nitric oxide, leading to progressive endothelial damage [7]. Many epidemiological and observational studies have documented the presence of a significant association between hyperuricemia and a wide variety of cardio-metabolic diseases, including hypertension, obesity, dyslipidemia, metabolic syndrome, type 2 diabetes mellitus, coronary heart disease, vascular dementia, pre-eclampsia and chronic kidney disease, when uricemia value  $\geq 6$  mg/dl [6,8,9,10,11]. The currently available hypouricemic drugs are very effective in reducing circulating levels of uric acid; it has been observed that long-term hypouricemic treatment can reduce both cardiovascular risk and progression of chronic kidney disease [12]. However, also food supplements may represent a valid option to the conventional pharmacological treatment of hyperuricemia. One of these products is Uricemin®, a food supplement, for oral use, indicated for the treatment of hyperuricemia, containing quercetin, rutin, bromelain and L-carnosine. On these basis, the aim of this study was to evaluate if the Uricemin® supplementation can reduce uric acid levels in subjects with values between  $\geq 6$  and  $< 7$  mg/dl (borderline uricemia) after 3 months of therapy.

## 2. Materials and Methods

### 2.1. Study Design

This 3-months, double-blind, randomized, placebo-controlled, clinical trial was conducted at the Centre of Diabetes and Metabolic Diseases, Department of Internal Medicine and Therapeutics, University of Pavia and Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy.

The study protocol was approved by institutional review board and was conducted in accordance with the 1994 Declaration of Helsinki [13], and its amendments and the Code of Good Clinical Practice. All patients provided written informed consent to participate in this study after a full explanation of the study. ClinicalTrials.gov Identifier: NCT04161872.

### 2.2. Patients

We enrolled patients with uric acid levels between  $\geq 6$  and  $< 7$  mg/dl, not taking hypouricemic agents (both pharmaceuticals or nutraceutical agents), without previous gout attack. Suitable patients, identified from review of case notes and/or computerized clinic registers, were contacted by the investigators in person or by telephone.

Patients were excluded if they had previous gout attack, impaired hepatic function (defined as plasma aminotransferase and/or gamma-glutamyl transpeptidase ( $\gamma$ -GT) level higher than the three times the upper limit of normal [ULN] for age and sex), impaired renal function (defined as serum creatinine level higher than the ULN for age and sex), endocrine (included type 1 and 2 diabetes mellitus), or gastrointestinal disorders; current or previous evidence of ischemic heart disease, heart failure, or stroke; weight change of  $> 3$  Kg during the preceding 3 months; malignancy, and significant neurological or psychiatric disturbances, including alcohol or drug abuse. Excluded medications (within the previous 3 months) included hypouricemic agents, laxatives,  $\beta$ -agonists (other than inhalers), cyproheptadine, anti-depressants, anti-serotonergics, phenothiazines, barbiturates, oral corticosteroids, and anti-psychotics. Women who were pregnant or breastfeeding or of childbearing potential and not taking adequate contraceptive precautions were also excluded.

### 2.3. Treatments

Patients were randomized to take placebo or Uricemin® for 3 months, in a randomized, double-blind, placebo-controlled design. Uricemin® and placebo were self-administered once a day, 1 tablet during the breakfast (Table 1).

Both Uricemin® and placebo were supplied as identical, opaque, tablets in coded bottles to ensure the blind status of the study. Randomization was done using a drawing of envelopes containing randomization codes prepared by a statistician. Medication compliance was assessed by counting the number of pills returned at the time of specified clinic visits. Throughout the study, we instructed patients to take their first dose of new medication on the day after they were given the study medication. At the

same time, all unused medication was retrieved for inventory. All medications were provided free of charge.

Table 1. Composition of Uricemin® and placebo

Uricemin®	
Ingredients	Daily intake
Quercetin 95%	200 mg
Rutin 95%	100 mg
Bromelain	100 mg
L-carnosine	50 mg
Silicon Dioxide	q.s.
Magnesium Stearate	q.s.
Dicalcium Phosphate	q.s.
Microcrystalline Cellulose	q.s.
Coating agents	q.s.
Placebo	
Ingredients	Daily intake
Silicon Dioxide	q.s.
Magnesium Stearate	q.s.
Dicalcium Phosphate	q.s.
Microcrystalline Cellulose	q.s.
Coating agents	q.s.

q.s: quantum sufficit.

### 2.4. Assessments

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, vital signs (blood pressure and heart rate), a 12-lead electrocardiogram, measurements of height and body weight, calculation of body mass index (BMI), assessment of fasting plasma glucose (FPG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (Tg), uricemia (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, high-sensitivity C-reactive protein (Hs-CRP).

All parameters were assessed at baseline and after 3 months since the study start.

All plasmatic variables were determined after a 12-hour overnight fast. Venous blood samples were drawn by a research nurse for all patients between 8:00 AM and 9:00 AM. We used plasma obtained by addition of  $\text{Na}_2\text{-EDTA}$ , 1 mg/mL, and centrifuged at 3000g for 15 minutes at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at -80°C for  $\leq 3$  months. Laboratory technicians drew blood samples and the biologist responsible for the laboratory performed the assays. All measurements were performed in a central laboratory.

Body mass index was calculated by the investigators as weight in kilograms divided by the square of height in meters.

Plasma glucose was assayed using a glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and interassay coefficients of variation (CsV)  $< 2\%$  [14].

TC and Tg levels were determined using fully enzymatic techniques [15,16] on a clinical chemistry analyzer (Hitachi 737; Hitachi, Tokyo, Japan); intra- and interassay CsV were 1.0% and 2.1% for TC measurement, and 0.9%

and 2.4% for Tg measurement, respectively. HDL-C level was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid [17]; intra- and interassay CsV were 1.0% and 1.9%, respectively. LDL-C level was calculated using the Friedewald formula [18].

Uric acid, GOT, GPT, creatinine were sampled with standardized methods [19].

High sensitivity C-reactive protein was measured with use of latex-enhanced immunonephelometric assays on a BN II analyser (Dade Behring, Newark, Delaware, USA). The intra- and interassay CsV were 5.7% and 1.3%, respectively [20].

## 2.5. Safety Measurements

Treatment tolerability was assessed at each study visit using an accurate interview of patients by the investigators, and comparisons of clinical and laboratory values with baseline levels. Safety monitoring included physical examination, vital sign assessment, weight, electrocardiogram, adverse events, and laboratory tests. Liver and renal function were evaluated by measurement of transaminases [AST, ALT] and creatinine, respectively, and all adverse events were recorded.

## 2.6. Statistical Analysis

An intention-to-treat (ITT) analysis was conducted in patients who had received  $\geq 1$  dose of study medication and had a subsequent efficacy observation. Patients were included in the tolerability analysis if they had received  $\geq 1$

dose of trial medication after randomization and had undergone a subsequent tolerability observation. The null hypothesis that the expected mean uricemia change from the end of the study did not differ significantly between placebo, and Uricemin® was tested using analysis of variance and analysis of covariance (ANCOVA) models [21]. Similar analyses were applied to the other variables. The statistical significance of the independent effects of treatments on the other variables was determined using ANCOVA. A 1-sample *t* test was used to compare values obtained before and after treatment administration; 2-sample *t* tests were used for between-group comparisons. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 21.0 (SPSS Inc., Chicago, Illinois, USA). Data are presented as mean (SD). For all statistical analyses,  $p < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Study Sample

A total of 116 patients were enrolled in the trial. Of these, 59 were randomized to the nutraceutical and 57 to placebo. One hundred and seven subjects completed the study, and were included in the analysis; there were 6 patients who did not complete the study (lost to follow-up), and 3 patients not included in the analysis due to low-compliance to treatment (Figure 1). The characteristics of the patient population at study entry are shown in Table 2.

Table 2. Baseline, and 3 months data of patients during Uricemin® treatment and placebo

Parameters	Uricemin®		Placebo	
	Baseline	3 months	Baseline	3 months
Patients (n)	59	55	57	52
M/F	29/30	26/29	26/31	24/28
Age (years)	56.4 ± 8.1	-	57.1 ± 8.5	-
Smoking status (M/F)	13/15	12/15	14/13	13/12
Height (cm)	1.69 ± 0.04	1.69 ± 0.04	1.70 ± 0.05	1.70 ± 0.05
Weight (Kg)	80.1 ± 6.9	80.7 ± 7.2	81.3 ± 7.5	81.9 ± 8.1
BMI (Kg/m <sup>2</sup> )	28.3 ± 2.4	28.2 ± 2.2	28.1 ± 2.1	28.3 ± 2.4
FPG (mg/dl)	91.2 ± 8.0	89.6 ± 7.4	90.5 ± 7.7	91.8 ± 8.5
TC (mg/dl)	218.6 ± 17.2	212.4 ± 15.1	220.7 ± 18.3	225.1 ± 18.9
LDL-C (mg/dl)	151.8 ± 15.7	145.4 ± 14.8	154.8 ± 16.4	156.7 ± 16.9
HDL-C (mg/dl)	43.5 ± 3.8	44.5 ± 4.6	43.1 ± 3.5	44.8 ± 4.9
Tg (mg/dl)	116.3 ± 30.5	112.5 ± 28.2	114.2 ± 30.1	118.2 ± 31.3
AST (U/l)	19.2 ± 8.8	18.7 ± 8.4	19.9 ± 9.6	19.2 ± 8.8
ALT (U/l)	23.2 ± 11.1	23.6 ± 11.4	25.7 ± 12.3	26.2 ± 12.6
Creatinine (mg/dl)	0.8 ± 0.4	0.7 ± 0.3	0.9 ± 0.5	0.9 ± 0.5
UA (mg/dl)	6.5 ± 0.4	5.9 ± 0.3* <sup>^</sup>	6.6 ± 0.5	6.7 ± 0.6
UA (6.5-7.0 mg/dl) (n; %)	15/18 (55.9)	0/0	17/14 (54.4)	15/14 (55.8)
UA (6.0-6.4 mg/dl) (n; %)	14/12 (44.1)	19/18 (67.3)	9/17 (45.6)	9/14 (44.2)
UA (5.5-5.9 mg/dl) (n; %)	0/0	7/11 (32.7)	0/0	0/0
Hs-CRP (mg/l)	1.1 ± 0.5	0.6 ± 0.3* <sup>^</sup>	1.0 ± 0.4	1.1 ± 0.5

Data are expressed as mean ± SD

M: males; F: females

\*  $p < 0.05$  vs baseline; <sup>^</sup>  $p < 0.05$  vs placebo.

M: males; F: females; BMI: body mass index; FPG: fasting plasma glucose; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; Tg: triglycerides; AST: aspartate aminotransferase; ALT: alanine aminotransferase; UA: uric acid; Hs-CRP: high-sensitivity C-reactive protein.



### CONSORT 2010 Flow Diagram

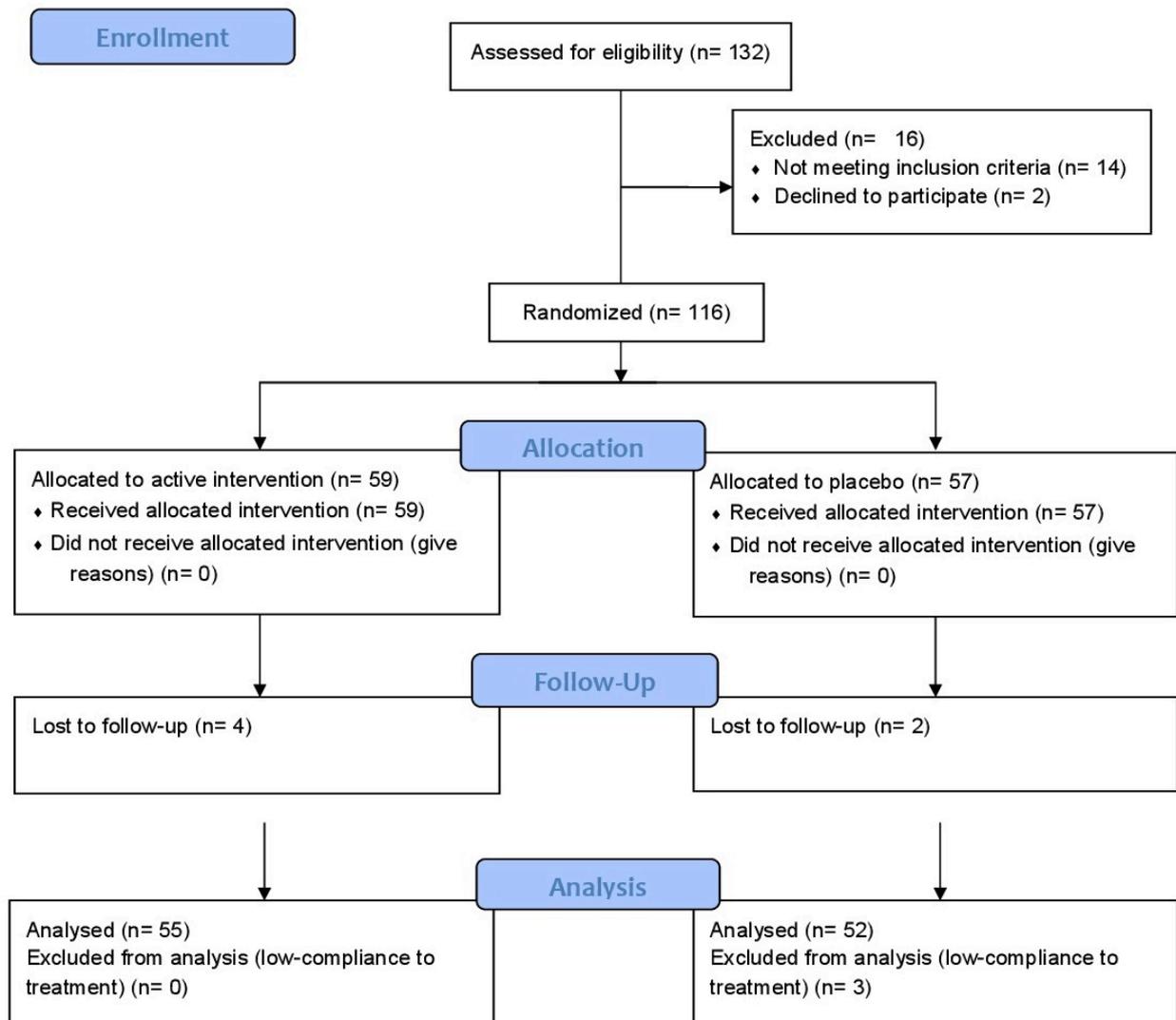


Figure 1. Consort Flow Diagram

### 3.2. Glycemia

We did not record any change in glycemia in neither group.

### 3.3. Lipid Profile

No changes in lipid profile were recorded.

### 3.4. Uricemia

We observed a reduction of uricemia in the group treated with Uricemin®, both compared to baseline and to

placebo ( $p < 0.05$  for both). In the group treated with Uricemin®, at baseline 55.9 % of patients had uric acid levels between 6.5 and 7.0 mg/dl vs 0 % after 3 months of treatment. After 3 months, 67.3 % had uric acid levels between 6.0 and 6.4 mg/dl vs 44.1% at baseline and 32.7 % had uric acid levels between 5.5 and 5.9 mg/dl, vs 0 % at baseline. No significant changes were recorded in placebo group (Table 2).

### 3.5. Inflammation

A decrease of Hs-CRP was obtained in the group treated with Uricemin® ( $p < 0.05$  vs baseline), but not

with placebo. High-sensitivity C-reactive protein value recorded with Uricemin® was lower compared to placebo ( $p < 0.05$ ) (Table 2).

## 4. Discussion

In vitro studies showed that quercetin inhibits the activity of the enzyme xanthine oxidoreductase which catalyzes the final reaction of uric acid synthesis [22], suggesting its possible involvement in the reduction of plasma uric acid concentration in humans. In addition, in vivo studies in animal models have shown that quercetin is able to regulate uric acid levels [22,23,24,25]. Although the question of the effect of quercetin on hyperuricemia in humans is still much debated [26,27,28,29,30,31,32], it has recently been observed that the administration for 4 weeks of this compound in healthy male subjects with plasma levels of uric acid  $\geq 6$  mg/dl, resulted in a significant decrease of uricemia without affecting fasting glucose, urinary uric acid excretion and blood pressure [33]. In a recent in vitro study it was shown that rutin, like quercetin, also inhibits the activity of the enzyme xanthine oxidoreductase. Furthermore, this compound has shown itself to be a powerful anti-oxidant agent since it is able to bind to the divalent iron (Fe<sup>2+</sup>) which induces the lipid peroxidation process [34]. The hypouricemic action of rutin was evaluated in a study of patients with asymptomatic hyperuricemia treated for 4 weeks with a combination containing rutin and other natural substances. It has been shown that this nutraceutical, in addition to reducing plasma uric acid levels, improves the lipid profile and simultaneously reduces the overall cardiovascular risk in the absence of adverse events [35]. Bromelain, instead, is primarily known for its analgesic and anti-inflammatory properties as result of its ability to influence prostaglandin synthesis [36,37,38], and has been shown to be useful in the treatment of both rheumatoid arthritis and osteoarthritis, representing an alternative therapy to non-steroidal anti-inflammatory drugs [39,40,41]. As for carnosine, it is an imidazole compound with the ability to alter the pH of body fluids and the ability to alter the activity of the enzymes involved in glycogenolysis [42] and in gluconeogenesis [43]. L-carnosine could alter the reabsorption of uric acid in the glycolytic process, reducing uric acid levels. Recently, the anti-hyperuricemic effect of carnosine was evaluated in a group of males with plasma uric acid levels ranging from 6.5 to 8.0 mg/dl and not affected by gout. The study showed that 4 weeks administration of a food supplement containing carnosine at two different doses significantly reduced uricemia, suggesting that this nutraceutical yields benefits and is safe for individuals with high uric acid plasma levels without complications [44]. The safety of carnosine intake was evaluated in studies on animal models and in humans in which neither adverse events nor abnormalities in routine clinical examinations were reported [45,46]. The reduction of uricemia recorded with the nutraceutical agents, can contribute to reduce the inflammatory status, as suggested by the reduction of Hs-CRP. There is evidence that hyperuricemia and gout are independent risk factors associated with

the development of hypertension, metabolic syndrome, vascular damage, and renal disease. Whether these risk factors are causally related to these important chronic co-morbidities remains uncertain, but inflammation may provide a mechanistic explanation [47].

Of course our study has some limitations, as the short duration of the trial; moreover, we evaluated only some inflammatory markers, focusing our attention only on Hs-CRP. Finally, we did not observe if the effect of nutraceutical agent was reversible after the interruption of the treatment.

## 5. Conclusions

The results of this study suggested that a combination of quercetin, rutin, bromelain and L-carnosine can be useful in reducing uricemia in patients with borderline uricemia.

## 6. Transparency Declaration

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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