Berberis aristata/Silybum marianum fixed combination (Berberol®) effects on lipid profile in dyslipidemic patients intolerant to statins at high dosages: A randomized, placebo-controlled, clinical trial

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ABSTRACT

Aim: to evaluate the efficacy of *Berberis aristata/Silybum marianum* (Berberol®) in a sample of dyslipidemic patients intolerant to statins at high dosages in a randomized, double blind, placebo-controlled clinical trial.
Methods: we enrolled 175 euglycemic, dyslipidemic subjects, intolerant to statins at high dosages. During the run-in period, statins were stopped for 1 month, then they were re-introduced at the half of the previously taken dose. After that, patients were randomized to placebo or Berberol®, 1 tablet during the lunch and 1 tablet during the dinner, for 6 months. Anthropometric, metabolic and inflammatory parameters were assessed at randomization, at 3 and 6 months.
Results: fasting plasma glucose, insulin, and HOMA-index levels were reduced by Berberol®, but not by placebo; moreover they were lower than the ones recorded with placebo. Total cholesterol, LDL-C, triglycerides, and myeloperoxidase did not change after 6 months since the reduction of statin dosage and the introduction of Berberol®, while they increased in the placebo group, and were higher compared to the ones obtained with active treatment. No patients had serious adverse events in both groups.
Conclusions: our study displays the rationale of the combination of Berberol® and a reduced dosage of statin for the treatment of hyperlipidemia in patients intolerant to statins at high dosage.

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Introduction

Dyslipidemia, especially high low-density lipoprotein cholesterol (LDL-C) level, is the key risk factor leading to coronary heart disease (CHD) (Natarajan et al. 2010). It has been clearly documented that statins, also known as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, are effective medications for reducing the risk of death and future cardiovascular disease in those with known coronary heart disease (Vijan et al. 2004). Although many people treated with statins do well, no drug is without potential for adverse effects. The best recognized and most commonly reported adverse events of statins include muscle pain with creatine phosphokinase (CPK) elevation, fatigue and weakness as well as rhabdomyolysis, and liver transaminases increase (McClure et al. 2007). Adverse events are more common at higher dosages of statins, and often contribute to patients' low adherence to treatment. One strategy to reduce the risk of statin-induced adverse events includes using a lower dosage, however this consequently leads to a lower benefit on lipid profile. In the latest years relatively large number of dietary supplements and nutraceuticals have been studied for their supposed or demonstrated ability to reduce cholesterolemia in humans (Cicero et al. 2008, 2012). These supplements include soluble fibers, phytosterols, soy proteins (Cicero et al., 2004), omega 3 polyunsaturated fatty acids (Derosa et al., 2011, 2012a), red yeast rice (Cicero et al., 2013), policosanols, berberine both alone (Derosa et al., 2012b, 2013a) or in combination with *Silybum marianum* (Derosa et al., 2013b,c), and garlic extracts. In particular, *Berberis aristata* acts up-regulating LDL-receptor (LDL-R) expression independent of sterol regulatory element binding proteins, but dependent on extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinase (JNK) activation leading to total cholesterol (TC) and LDL-C reduction of about 30 and 25%, respectively (Kong et al. 2004). *Berberis aristata* is available in combination with *Silybum marianum*, a potential P-gp inhibitor, traditionally used as liver protectant (Luper 1998; Kidd and Head 2005) and with a potential beneficial effect on improving glycemic profile in type 2 diabetic patients (Huseini et al. 2006), even if the mechanism underlying the glucose lowering effect of *Silybum marianum* is not clear.

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Table 1a
Dosage of statins taken at the study enrollment in the group treated with Berberol®.

Dosage	Lovastatin	Pravastatin	Simvastatin	Atorvastatin	Rosuvastatin
10 mg (n)				5	5
20 mg (n)	5	6	10	12	8
40 mg (n)		5	9	12	4

n: number of patients.

Table 1b
Dosage of statins taken at the study enrollment in the group treated with placebo.

Dosage	Lovastatin	Pravastatin	Simvastatin	Atorvastatin	Rosuvastatin
10 mg (n)				5	4
20 mg (n)	6	7	8	10	10
40 mg (n)		5	10	12	5

n: number of patients.

Table 2
Type of adverse events recorded for enrolled patients at baseline.

Statins	Lovastatin	Pravastatin	Simvastatin	Atorvastatin	Rosuvastatin
n	11	23	37	56	36
ALT increase $\geq 3 \times$ ULN (n)	4	3	7	9	6
ALT increase $\geq 5 \times$ ULN (n)	1	1	2	3	2
AST increase $\geq 3 \times$ ULN (n)	2	3	6	7	7
AST increase $\geq 5 \times$ ULN (n)	0	1	2	2	2
ALT and AST increase $\geq 3 \times$ ULN (n)	1	5	3	10	6
ALT and AST increase $\geq 5 \times$ ULN (n)	0	0	1	2	2
CPK increase $\geq 3 \times$ ULN (n)	4	5	5	12	6
CPK increase $\geq 10 \times$ ULN (n)	0	0	0	0	0
Asthenia (n)	1	5	4	7	5
Myalgia (n)	1	4	9	6	4
Rhabdomyolysis (n)	0	0	0	1	0

AST: alanine aminotransferase; AST: aspartate aminotransferase; CPK: creatinine phosphokinase; ULN: upper limit of normal; m ± SD: means ± standard deviation.

Under the circumstances mentioned above, combination therapy of statins with other kinds of lipid-altering agents could be a valid choice to reach an enhanced lipid-lowering effect, a reduced dosage of statins, and a decreased risk of adverse effects (Brown 2002; Davidson 2002). In particular, the primary endpoint of our study was to evaluate the effects of a combination of *Berberis aristata/Silybum marianum* (Berberol®) on lipid profile, in dyslipidemic patients intolerant to statins at high dosages. The secondary endpoint was to evaluate the incidence of adverse events with Berberol or placebo.

Methods

Study design

This 6-months, double-blind, randomized, placebo-controlled, clinical trial was conducted at the Centre for the Cure of Diabetes and Metabolic Disease, at the Department of Internal Medicine and Therapeutics, University of Pavia (Pavia, Italy). The study protocol was approved by the institutional review board and was conducted in accordance with the Declaration of Helsinki (The Council for International Organisation of Medical Sciences, 1982) 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 had been given.

Patients

From May 2013 to March 2014, 175 Caucasian outpatients, aged ≥18, of either sex were enrolled in this study. Patients were eligible for inclusion if they had a condition of euglycemia (fasting plasma glucose <100 mg/dl), hypercholesterolemia, according to National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III) criteria (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). We enrolled patients whose LDL cholesterol levels were not adequately controlled, and that were found intolerant to statins at high dosages. The list of statins dosage taken by patients at the enrollment is listed in Tables 1a and 1b. Subjects were considered intolerant if, on actual statin dosage, they have experienced: an increase of CPK >3 until 10 times the upper limits of the laboratory (ULN), and/or a rise in the value of transaminases >3 until 5 times the ULN, and/or the onset of asthenia, myalgia or rhabdomyolysis. Type of adverse events recorded before enrolment for patients by subgroup of statins are listed in Table 2. Patients were overweight (World Health Organization 1997), and also normotensive according to the World Health Organization criteria (Systolic Blood Pressure [SBP] <140 mmHg and Diastolic Blood Pressure [DBP] <90 mmHg) (Guidelines Subcommittee 1999). Furthermore, they had normal thyroid function, none of the selected subjects was taking diuretics or β-blockers.

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 secondary dyslipidemia, impaired renal function (defined as serum creatinine level higher than the ULN for age and sex); gastrointestinal disorders, weight change of >3 kg during the preceding 3 months, malignancy, and significant neurological or psychiatric disturbances, including alcohol or drug abuse. Patients with serious cardiovascular disease (CVD) (e.g., New York Heart Association classes I–IV congestive heart failure or a history of myocardial infarction or stroke) or cerebrovascular conditions within 6 months before study enrollment were also
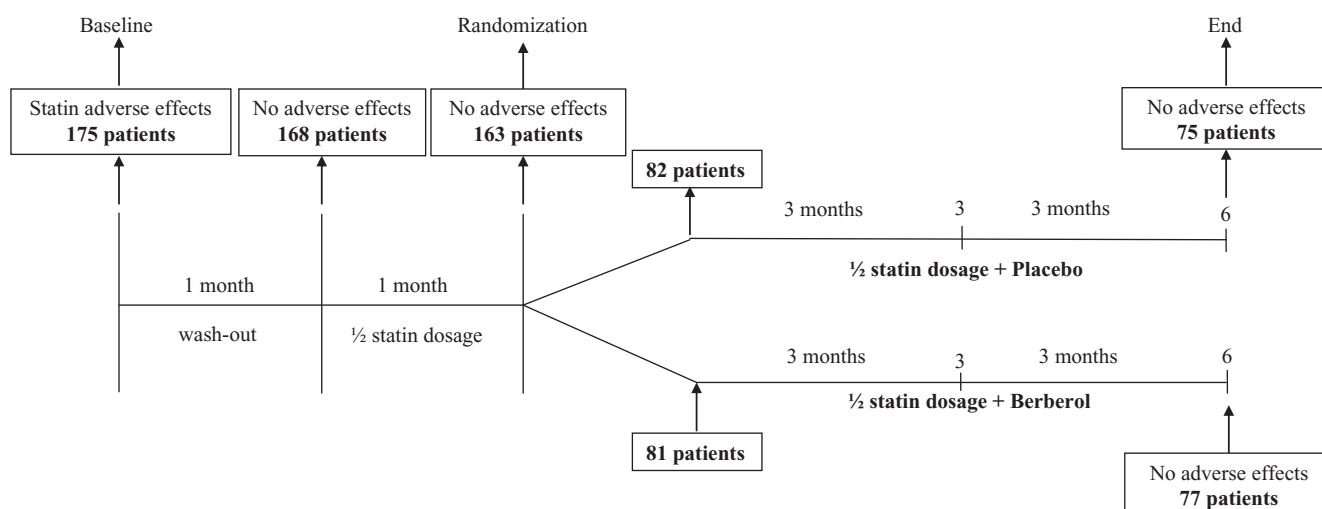
Fig. 1. Design of the study.

excluded. Excluded medications (within the previous 3 months) were anorectic agents, laxatives, β-agonists (other than inhalers), cyproheptadine, anti-depressants, anti-serotoninergics, 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.

Diet and physical activity

During the study all patients followed an adequate diet and practiced physical activity. The controlled-energy diet (~600 kcal daily deficit) was based on NCEP-ATP III recommendations (NCEP 2002), that contained 50% of calories from carbohydrates, 30% from fat ($<7\%$ saturated, up to 10% polyunsaturated, and up to 20% monounsaturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/d and 35 g/d of fiber. Standard diet advice was given by a dietitian and/or specialist physician. Dietitians and/or specialists provided instruction on dietary intake-recording procedures as part of a behavior-modification program and then used the patients' food diaries for counseling. Individuals were also encouraged to increase their physical activity and we standardized the same physical aerobics exercise program by riding a stationary bicycle for 20–30 min, 3–4 times per week.

Treatment

During the run-in period, patients underwent a 1 month wash-out period during which statins were stopped. At the end of the wash-out period, if safety parameters returned into the normal range, statins were re-introduced at the half of the previously taken dose. After 1 month since the statins re-introduction, patients who did not experience adverse events were randomized to take placebo or Berberol®, 1 tablet during the lunch and 1 tablet during the dinner, for 6 months, in a double-blind, randomized, placebo-controlled design (Fig. 1). Berberol is a patented nutraceutical association in tablet form (Berberol®, EP 2149377, traded in Italy by PharmExtracta, Pontenure, Italy). The product, in agreement with the Italian law number 169/2004, has been notified to the Minister of Health in 2010 (Registration number: E10 40753Y) and registered as food supplement being both its actives (*Berberis aristata* and *Silybum marianum* standardized extracts) belonging to the positive list of botanicals admitted as nutraceuticals and its excipients all food grade.

In particular, each tablet of Berberol contains:

- 105 mg of hydro-ethanolic extract from fruits of *Silybum marianum* L. Gaertn. (Indena, Milan, Italy) standardized as 60–80% flavanolignans calculated as silybin by high-pressure liquid chromatography (HLPC). For details of the analytical method see what reported in European Pharmacopoeia 6.0 (European Pharmacopoeia, 2008);
- 588 mg of hydro-ethanolic extract from cortex arboris of *Berberis aristata* DC. (SIIT, Trezzano S/N, Milan, Italy) standardized as 85% of berberine calculated as berberine by HPLC. For details of the analytical method see what reported in previously published papers (Chen et al. 2011; Weber et al. 2003).

Both Berberol® and placebo were supplied as identical, opaque, white capsules in coded bottles to ensure the blind status of the study. The batches of dispensed nutraceutical were TJ808 (containing Silymarin batch 20120604TH and *Berberis aristata* batch 01206168) and LJ665 (containing Silymarin batch 20120609TH and *Berberis aristata* batch 01201037).

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.

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), abdominal circumference (Abd. Cir.), waist circumference (Waist Cir.), and hip circumference (Hip Cir.), assessment of fasting plasma glucose (FPG), fasting plasma insulin (FPI), HOMA index, TC, LDL-C, high density lipoprotein-cholesterol (HDL-C), triglycerides (Tg), high sensitivity C-reactive protein (Hs-CRP), myeloperoxidase (MPO).

Changes in lipid profile were the primary efficacy factors. Anthropometric, metabolic and inflammatory parameters were assessed at randomization, at 3 and 6 months.

All plasmatic variables were determined after a 12-h 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 Na_2-EDTA, 1 mg/ml, and centrifuged at 3000 g for 15 min at 4 °C. Immediately after centrifugation, the plasma samples were frozen and stored at 80 °C for ≤3 months. 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. Waist circumference was measured midway between the lateral lower rib margin and the iliac crest and its reduction was determined with a Gulick anthropometric spring-loaded tape measure (Model 5829, Bell Medical Services, Neptune, NJ, USA).

Plasma glucose was assayed using a glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and interassay coefficients of variation (CsV) <2% (European Diabetes Policy Group 1999).

Plasma insulin was assayed with Phadiaseph Insulin radioimmunoassay (RIA) (Pharmacia, Uppsala, Sweden) by using a second antibody to separate the free and antibody-bound 125 I-insulin (intra- and interassay CsV: 4.6 and 7.3%, respectively) (Heding 1972).

The HOMA-IR was calculated as the product of basal glucose (mmol/l) and insulin levels (μU/ml) divided by 22.5 (Matthews et al. 1985; Wallace et al. 2004). Total cholesterol and Tg levels were determined using fully enzymatic techniques (Klose and Borner 1978; Wahlefeld 1974) 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 (Havel et al. 1955); intra- and interassay CsV were 1.0% and 1.9%, respectively. LDL-C level was calculated using the Friedewald formula (Friedewald et al. 1972).

High sensitivity C-reactive protein was measured with use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, DE, USA). The intra- and inter-assay CsV were 5.7% and 1.3%, respectively (Rifai et al. 1999).

Myeloperoxidase was assessed using commercially available ELISA kits according to the manufacturer's instructions (R&DSystems). The intra- and inter-assay CsV were 7.7% and 8.3%, respectively (Morishita et al. 1987).

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 muscle function were evaluated by measurement of transaminases [aspartate aminotransferase (AST), alanine aminotransferase (ALT), and CPK], and all adverse events were recorded.

Statistical analysis

An intention-to-treat (ITT) analysis was conducted in patients who had received ≥1 dose of study medication and had a subsequent efficacy observation. Patients were included in the tolerability analysis if they had received ≥1 dose of trial medication after randomization and had undergone a subsequent tolerability observation. The null hypothesis that the expected mean TC, LDL-C, HDL-C, and Tg change from randomization would not differ significantly between placebo, and Berberol® was tested using analysis of variance (ANOVA) (Winer 1971). Continuous variables were tested using a one-way repeated measure ANOVA to assess overall differences within groups. A 1-sample t test was used to compare values obtained before and after

Table 3
Data of the screened population at randomization and after placebo.

	Randomization	3 months	6 months
N	82	77	75
Sex (M/F)	41/41	38/39	37/38
Age (years)	58.3 ± 13.5	–	–
Height (m)	1.67 ± 0.05	–	–
Weight (kg)	82.5 ± 9.6	82.1 ± 9.4	81.8 ± 9.2
BMI (kg/m²)	29.6 ± 1.3	29.4 ± 1.1	29.3 ± 1.0
Abd. Cir. (cm)	94.9 ± 3.6	94.6 ± 3.5	94.1 ± 3.3
Waist Cir. (cm)	89.5 ± 2.4	89.5 ± 2.4	89.4 ± 2.3
Hip Cir. (cm)	101.9 ± 2.5	100.9 ± 2.1	100.4 ± 1.8
AST (U/l)	25.1 ± 12.8	25.9 ± 13.2	25.3 ± 12.9
ALT (U/l)	18.5 ± 7.8	19.3 ± 8.7	19.6 ± 9.0
CPK (U/l)	155.9 ± 40.2	159.4 ± 43.5	162.4 ± 43.6
Hs-CRP (mg/l)	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.3
MPO (ng/ml)	402.3 ± 139.5	405.6 ± 142.7	461.3 ± 154.7*

Data are expressed as mean ± standard deviations (SD).
Abd. Cir.: abdominal circumference; Waist Cir.: waist circumference; Hip Cir.: hip circumference; BMI: body mass index; AST: alanine aminotransferase; AST: aspartate aminotransferase; CPK: creatinine phosphokinase; Hs-CRP: high sensitivity C-reactive protein; MPO: myeloperoxidase.
* $p < 0.05$ vs randomization

treatment administration. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 11.0 (SPSS Inc., Chicago, Illinois, USA). Data are presented as mean (SD). For all statistical analyses, $p < 0.05$ was considered statistically significant.

Results

Study sample

A total of 175 patients were enrolled in the trial. Of these, 168 completed the 1 month wash-out period, and 163 completed the 1 month period with half dosage of statin without adverse events. At randomization, 81 (49.7%) patients were randomized to Berberol® and 82 (50.3%) to placebo. One hundred and fifty-two subjects completed the study; there were 11 patients (6 males and 5 females) who did not complete the study and the reasons for premature withdrawal included: lost to follow-up (2 males in Berberol group), myalgia (2 females in Berberol group taking rosuvastatin 20 mg, 2 males and 2 females in placebo group taking atorvastatin 20 mg) and ALT increase ≥3 × ULN (1 female in placebo group taking rosuvastatin 20 mg, and 2 males in placebo group taking atorvastatin 80 mg).

Anthropometric parameters

We did not record any significant change of body weight, BMI or waist, abdominal and hip circumferences after 6 months of treatment in neither groups (Tables 3 and 4).

Metabolic parameters

Fasting plasma glucose levels were reduced by Berberol® ($p < 0.05$ compared to baseline), but not by placebo. Moreover, FPG obtained with active treatment was lower than the one recorded with placebo ($p < 0.05$). Fasting plasma insulin and HOMA-IR were reduced by Berberol®, both compared to randomization and to placebo that did not affect these parameters ($p < 0.05$ for all). Total cholesterol, LDL-C, and Tg did not change after 6 months since the reduction of statins dosage and the introduction of Berberol®, while they increased in the placebo group ($p < 0.05$ for all). Total cholesterol, LDL-C and Tg values recorded with placebo were significantly higher compared to the ones obtained with active treatment (Figs. 2 and 3). No variations of Hs-CRP were recorded, while there was an increase of MPO with

Table 4
Data of the screened population at randomization and after Berberol®.

	Randomization	3 months	6 months
N	81	78	77
Sex (M/F)	39/42	37/41	37/40
Age (years)	59.4 ± 13.8	–	–
Height (m)	1.67 ± 0.05	–	–
Weight (kg)	82.6 ± 9.8	81.5 ± 9.3	81.1 ± 9.1
BMI (kg/m^2)	29.6 ± 1.2	29.2 ± 1.0	29.1 ± 0.9
Abd. Cir. (cm)	94.6 ± 3.8	93.9 ± 3.6	93.5 ± 3.3
Waist Cir. (cm)	89.9 ± 2.6	89.5 ± 2.4	89.6 ± 2.5
Hip Cir. (cm)	102.2 ± 2.8	101.7 ± 2.7	100.5 ± 2.5
AST (U/l)	24.1 ± 12.2	23.9 ± 11.9	24.5 ± 12.5
ALT (U/l)	20.6 ± 9.6	20.1 ± 9.4	21.4 ± 9.9
CPK (U/l)	161.9 ± 44.8	163.9 ± 45.2	164.4 ± 46.1
Hs-CRP (mg/l)	1.1 ± 0.3	1.1 ± 0.3	1.0 ± 0.2
MPO (ng/ml)	410.1 ± 143.7	415.3 ± 145.1	421.4 ± 147.5^

Data are expressed as mean ± standard deviations (SD).
Abd. Cir.: abdominal circumference; Waist Cir.: waist circumference; Hip Cir.:
hip circumference; BMI: body mass index; AST: alanine aminotransferase;
AST: aspartate aminotransferase; CPK: creatinine phosphokinase, Hs-CRP:
high sensitivity C-reactive protein; MPO: myeloperoxidase.
  ^ $p < 0.05$ vs placebo.

placebo, not observed with Berberol®, moreover MPO value recorded with Berberol® was lower compared to placebo ($p < 0.05$ for both) (Tables 3, and 4).

Safety

No patients had serious adverse events in both groups. No patients experienced musculoskeletal system disorders, as myopathy or hepatotoxicity. Safety biochemical measurements included transaminases (AST and ALT), and CPK. We did not observe any worsening of these parameters during the study (Tables 3 and 4).

Discussion

In our study we observed that Berberol® is effective in reducing lipid profile after that statin dosage was reduced due to adverse events. This was seen compared to placebo group, where, instead, there was a worsening of lipid profile after statins dosage reduction. Our results are in line with what reported by Kong et al.: these authors evaluated the effects of a combination of berberine and simvastatin in 63 outpatients diagnosed with hypercholesterolemia (Kong et al. 2008). As compared with monotherapies, the combination showed an improved lipid-lowering effect with 31.8% reduction of serum LDL-C, and similar efficacies were observed in the reduction of TC as well as Tg in patients. The positive effects of *Berberis aristata* on lipid profile were already reported by our group, in a study previously published where Berberol® reduced lipid profile compared to placebo; in particular, we recorded a TC reduction of 23.2% and a LDL-C reduction of 32.2% (Derosa et al. 2013c). This reduction was higher compared to berberine alone that gave a TC reduction of 10.2% and a LDL-C reduction of 14.6% (11%), probably due to a synergic effect with *Silybum marianum*.

Besides liver LDL-R, a previous study demonstrates that *Berberis aristata* inhibits lipid synthesis in hepatocytes through activation of the adenosine monophosphate-activated protein kinase, which also partially explains the Tg-lowering activity of *Berberis aristata* (Brusq et al. 2006). *Silybum marianum*, besides improving *Berberis aristata* oral bioavailability by a direct antagonism with P-gp (Zhou et al. 2004), could also act inhibiting cholesterol acyltransferase activities, reducing cholesterol absorption and lipoprotein biosynthesis (Sobolová et al. 2006). We also observed a positive effect of Berberol® on FPG and FPI and HOMA index, like already reported by our group (Derosa et al. 2013b), and a reduction of MPO compared to placebo. MPO proved to be an emerging biomarker in cardiovascular risk stratification (Derosa et al., 2012c), and its reduction has a positive effect on cardiovascular risk reduction.
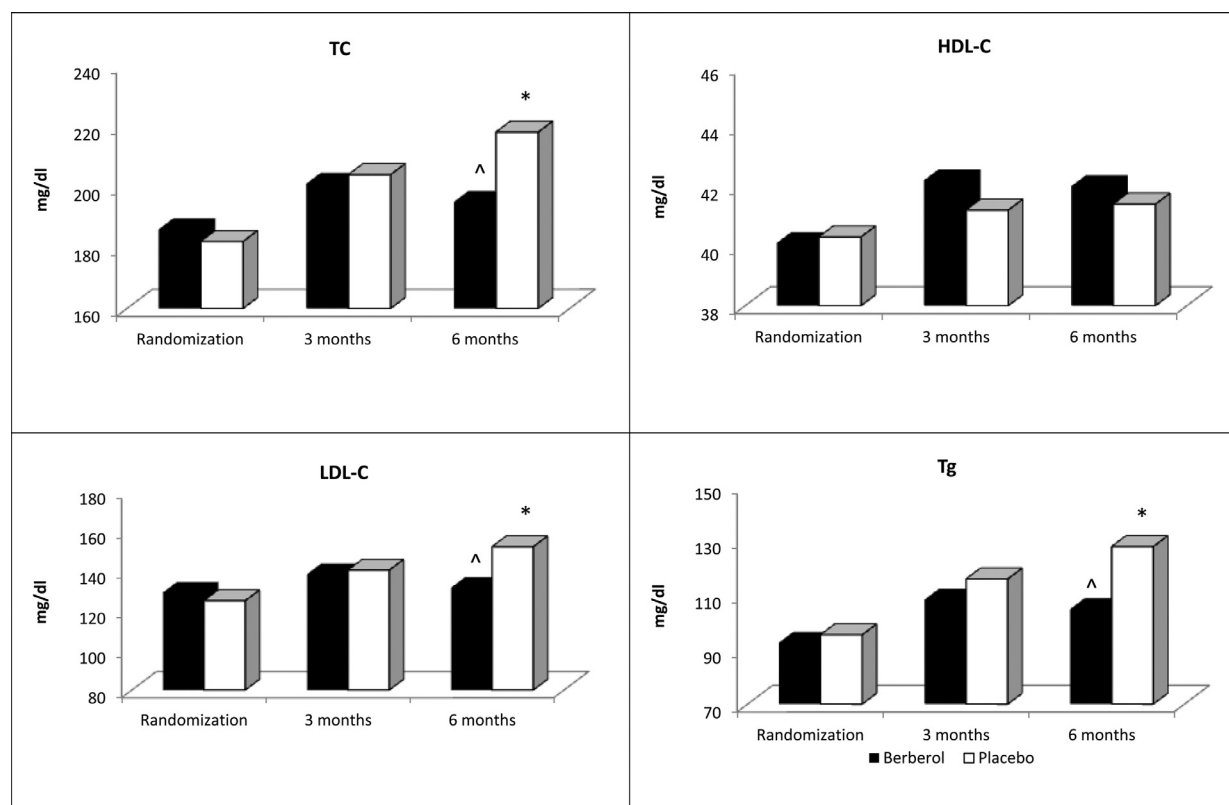

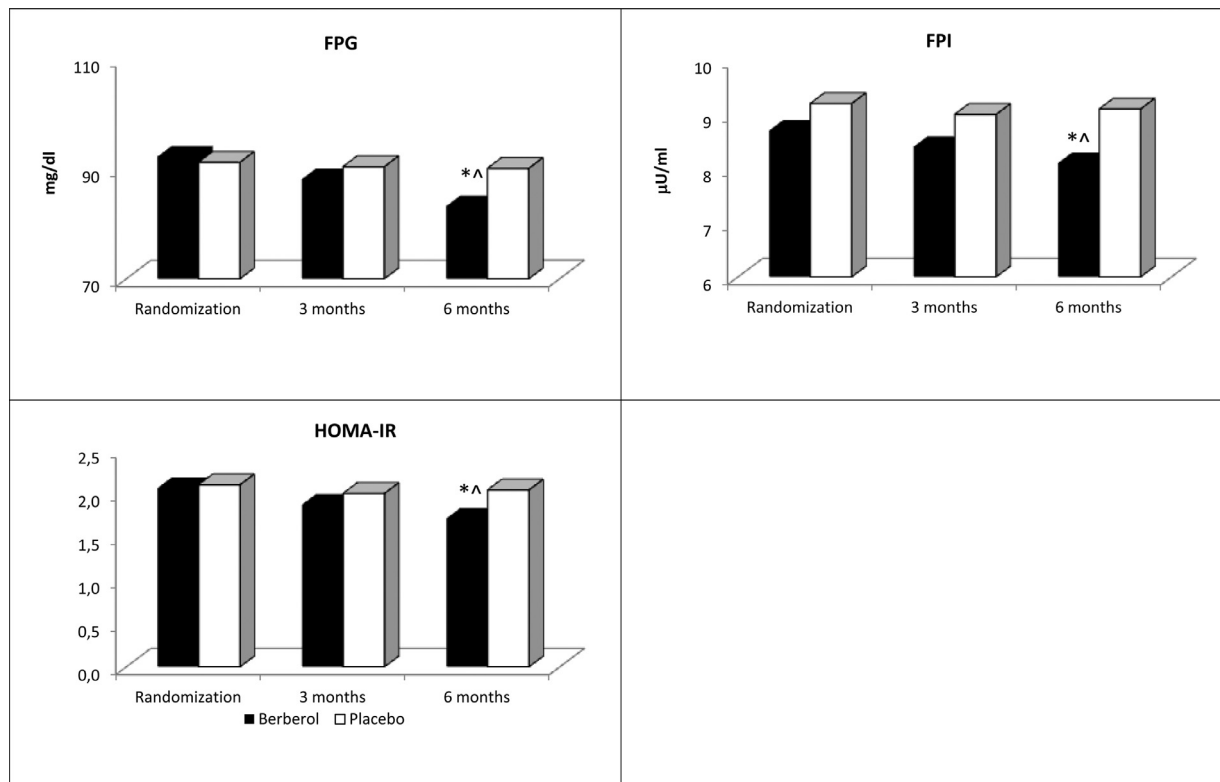**Fig. 2.** Variation of lipid profile during the study. ∗$p < 0.05$ vs randomization; ^$p < 0.05$ vs placebo.

Fig. 3. Variation of glycemia and insulin resistance during the study. $*p < 0.05$ vs randomization; $^\wedge p < 0.05$ vs placebo, FPG: fasting plasma glucose; FPI: fasting plasma insulin.

Of course our study has some limitations; for example we enrolled a heterogeneous group of patients, taking different statins at different dosages, moreover the sample is relatively small and the follow-up period is relatively short. We need to verify if the positive effect of Berberol® will be maintained also in the long term period. However, our study is the first to demonstrate that combining statins treatment with Berberol® can be a valid strategy to help avoiding statins adverse events and obtaining an adequate lipid profile.

Conclusions

Our study displays the rationale, effectiveness, and safety of Berberol® and a reduced dosage of statin for the treatment of hyperlipidemia in dyslipidemic patients intolerant to statins at high dosage.

Conflict of interest

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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