Abstract
The short-term lipid-lowering activity of some nutraceuticals is well known, however there is a lack of knowledge about their long-term effect, especially regarding insulin-resistance parameters and biomarkers of vascular health. This study is a 12-month follow-up randomised clinical trial carried out on 269 non-smoker hyperlipidaemic patients in primary prevention for cardiovascular disease; 214 of them (129 men and 85 women) completed the trial with good compliance. None of the subjects were diabetics or had been treated with antihyperlipidaemic drugs. Seventy-nine normoweight subjects (Group 1) were treated with a combined nutraceutical monakolin-berberine-policosanol (MBP) mixture associated with a standardised therapeutic lifestyle (TLS) (as defined in the third Adult Treatment Panel of the National Cholesterol Education Program), 85 overweight subjects were treated with MBP-TLS (Group 2) and 50 overweight subjects only with intensified TLS (Group 3). Efficacy parameters (Body Mass Index, fasting plasma glucose, fasting plasma insulin, Homeostasis Model Assessment index, metalloproteinase-2 (MMP-2), MMP-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), TIMP-2, total cholesterol, low-density lipoprotein-C, high-density lipoprotein-C, triglyceride) were evaluated every 4 months. Educational reinforcements were also planned every 4 months. This study demonstrated the long-term efficacy and safety of a combined nutraceutical added to a TLS in overweight and normoweight dyslipidaemic subjects. Berberine- and red yeast rice-based nutraceuticals showed efficacy in body weight reduction and insulin-sensitivity promotion in non-diabetic hyperlipidaemic patients. Favourable effects of a combined nutraceutical in combination with a TLS were observed on biomarkers of vascular remodelling in overweight and normoweight dyslipidaemic subjects.

Introduction
Increasing evidence supports the antihyperlipidaemic efficacy of some nutraceuticals [1]. However, the use of full-dose nutraceuticals entails some tolerability concerns, because they could have statin- or metformin-like side effects. The use of a combination of nutraceuticals with different but synergic mechanisms of action at lower and safer dosages appears to be an interesting alternative. In particular, recent data support the efficacy of a MBP mixture as a lipid-
lowering drug. The MBP mixture was developed and clinically tested after evaluation of the lipid-lowering efficacy of different mixtures of its components: monakolins and policosanols [2, 3] and berberine and monakolins [4]. Finally, MBP consumed in conjunction with a standard Mediterranean healthy diet seems to be able to reduce low-density lipoprotein (LDL)-C and triglyceride (TG) by a mean of 20% in different kinds of patients [5]. This effect has obvious positive considerations for metabolic syndrome management and patients with high risk of cardiovascular disease [6]. On the other hand, MBP has been proven to have some direct protective vascular effects, similar to pharmacological lipid-lowering agents, such as improvement of endothelial dysfunction [7]. The MBP tolerability has also been confirmed in elderly hypercholesterolaemic subjects [8] and in patients previously intolerant to more than one statin [9]. However there is no published data on its long-term effects on lipid parameters and other parameters related to cardiovascular disease risk. In this context we planned a 12-month trial to evaluate the long-term efficacy of the MBP mixture and its effect on insulin-resistance-related parameters.

**Methods**

This is a 12-month, partially randomised clinical trial. We consecutively enrolled 269 non-smoker, non-diabetic, pharmacologically untreated hyperlipidaemic patients in primary prevention for cardiovascular disease.

Group 1 included 79 normoweight subjects treated with a combined nutraceutical containing berberine 500 mg, policosanols 10 mg and monakolins 3 mg/dose (MBP) combined with a standardised therapeutic lifestyle (TLS, as suggested by the third Adult Treatment Panel of the National Cholesterol Education Program); 135 overweight patients were randomised to be treated with MBP-TLS (Group 2) or with placebo-TLS (Group 3).

The efficacy parameters were evaluated every 4 months. Educational reinforcements were also planned every 4 months. Systolic (1st phase) and diastolic (5th phase) blood pressure readings were measured to the nearest even digit using a standard mercury manometer (Erkameter 3000, ERKA, Bad Tolz, Germany), with a large cuff size where the arm circumference was greater than 33 cm. Three readings were recorded on the right arm of the seated participant following a minimum of 10 minutes rest in a quiet room and the average of the second and third readings was defined as the subject’s blood pressure. Measurements were always taken by the same investigator in the morning before daily drug intake (i.e., ~24 h after dosing).

All plasma parameters were determined after a 12-h overnight fast. Venous blood was taken between 08:00 and 09:00 and samples were kept on ice prior to spinning. Plasma was obtained by addition of Na2-EDTA, 1 mg/ml, and centrifuged at 3000 g for 15 min at 4°C. Immediately after centrifugation plasma samples were frozen and stored at –80°C for no more than 3 months. Plasma glucose was assayed using the glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and inter-assay coefficients of variation (CsV) <2% [10]. Plasma insulin was assayed with a Phadiaseph Insulin RIA (Pharmacia, Uppsala, Sweden) using a second antibody to separate the free and antibody-bound 125I-insulin (intra- and inter-assay CsV 4.6 and 7.3%, respectively) [11]. Total cholesterol (TC) and TG levels were determined using a fully enzymatic technique on a clinical chemistry analyser (HITACHI 737; Hitachi, Tokyo, Japan) [12, 13]. Intra- and inter-assay CsV were 1.0 and 2.1 for TC determination, and 0.9 and 2.4 for TG determination, respectively. High-density lipoprotein (HDL)-C level was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid; intra- and inter-assay CsV were 1.0 and 1.9, respectively. LDL-C level was calculated using the Friedewald formula [14, 15]. Homeostasis Model Assessment (HOMA) index was calculated using the formula fasting plasma insulin (FPI) (mcU/ml)×fasting plasma glucose (FPG) (mmol/l)/22.5, as described by Matthews and coworkers [16].