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A nutraceutical approach (Armolipid Plus) to reduce total and LDL cholesterol in individuals with mild to moderate dyslipidemia: Review of the clinical evidence

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Abstract

Compelling evidence supports the effectiveness of the reduction of total and LDL cholesterol (TC and LDL-C) in primarily preventing cardiovascular events, within the framework of life-long prevention programs mainly consisting in lifestyle changes. Pharmacological treatment should be introduced when lifestyle changes, including use of nutraceuticals, have failed. ESC/EAS guidelines list a number of nutraceutical compounds and functional foods which have been individually studied in randomized, controlled clinical trials (RCTs). To date only a proprietary formulation of three naturally occurring substances with putative complementary lipid-lowering properties − red yeast rice, policosanol and berberine − combined with folic acid, astaxanthin, and coenzyme Q10 (Armolipid Plus®) has been extensively investigated in several RCTs, 7 of which were placebo-controlled, 2 were ezetimibe comparators and 4 were "real life" studies comparing diet and Armolipid Plus to diet alone. The trials included mostly patients with mild to moderate dyslipidemia, treated for 6−48 weeks. The trials also included special populations and patients in whom statins were contraindicated or who could not tolerate them. Armolipid Plus has proved to be able to achieve significant reductions in TC (11−21%) and in LDL-C (15−31%) levels, which is equivalent to expectations from low dose statins. In patients intolerant to statins, who do not achieve their therapeutic target with ezetimibe, Armolipid Plus can achieve a further 10% improvement in TC and LDL-C. The safety and tolerability of Armolipid Plus were excellent, thought likely due to the intentional combination of low doses of its active ingredients: low enough not to be associated with untoward effects, but high enough to exert therapeutic effects in combination with other complementary substances. Consequently, in the event of intolerance to statins, Armolipid Plus offers an effective alternative, which is devoid of the safety risks associated with

Abbreviations: AMPK, AMP-activated protein kinase; AP, Armolipid Plus; AUClast, area under the concentration−time curve; CKD, chronic kidney disease; CPK, creatine phosphokinase; CYP, cytochrome P-450; CVD, Cardiovascular disease; DALY, disability-adjusted life-year; EAS, European Atherosclerosis Society; ESC, European Society of Cardiology; GP, general practitioner; HDL-C, High-density lipoprotein cholesterol; HMG-CoA, Hydroxyl-methylglutaryl coenzyme A; hsCRP, high sensitivity C reactive protein; HOMA-IR, homeostatic model assessment for insulin resistance; LC-MS/MS, liquid chromatography−tandem mass spectrometry; LDL-C, Low-density lipoprotein cholesterol; MetS, metabolic syndrome; PL, placebo; RCT, randomized clinical trial; ROS, reactive oxygen species; TC, Total cholesterol; ULN, upper limit of normal.

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synthetic pharmacological therapy. In conclusion Armolipid Plus, in addition to dietary measures, could be a rational choice for individuals with mild to moderate hyperlipidemia and for all dyslipidemic patients in whom statins are not indicated or who cannot tolerate them.

Keywords: Hypercholesterolemia; Lifestyle change; Nutraceuticals; Functional foods; Armolipid plus

Coronary heart disease still is the leading cause of mortality worldwide, both in men and women. Stroke is also an important cause of mortality, as well as a major cause of disability in our society. Atherosclerosis plays an important part in both of these diseases and reference is made to the "total burden" of atherosclerotic cardiovascular disease (CVD). It has been estimated that in Europe CVD accounts for 42% and 38% of all deaths before the age of 75 years in women and men respectively, and that worldwide it will be responsible for the loss of approx. 150 million disability-adjusted life-years (DALYs) by 2020. In 2008 the economic cost of CVD in the European Union was reported to amount to approx. € 192 billion in terms of direct and indirect healthcare costs [1,2].

Prevention programs designed to correct modifiable risk factors, such as smoking, hypertension and hypercholesterolemia, have proved to be effective and account for more than 50% of the reduction in coronary heart disease deaths achieved in the 1980s and 1990s.

A growing body of evidence shows that prevention should be a life-long effort, continuing up to advanced age. Programs should include everyone, not only middle-aged or elderly subjects with established CVD (secondary prevention) or people at high risk of experiencing a first cardiovascular event based on risk factor scores (primary prevention according to the high-risk approach), since atherosclerosis develops very slowly, starting at an early age, making a healthy lifestyle in youth most likely the best method to address the worldwide CVD pandemic. However, the ESC (European Society of Cardiology) guideline warns that the beneficial effects of CVD preventive measures in terms of reduction in the incidence of fatal or non-fatal cardiovascular events and related disability, should always be weighed against the potential harm that such measures may cause in the long-term, including side effects of drugs [1].

One of the key modifiable risk factors is raised total cholesterol (TC). Compelling evidence exists supporting the effectiveness of the reduction of TC and low-density lipoprotein-cholesterol (LDL-C) in preventing CVD events [1,2], and concerns have been raised over trans fatty acid-rich diets [3]. The Cholesterol Treatment Trialists' Collaboration meta-analysis of trials including more than 170,000 patients confirmed the dose-dependent reduction in CVD events achieved by lowering LDL-C, every 1.0 mmol/L (40 mg/dl) reduction being associated with a 22% reduction in CVD morbidity and mortality [4].

The recommended target level for TC is less than 5 mmol/l (190 mg/dl) in all patients, whereas the recommended LDL-C varies according to ESC/EAS guidelines [2] as follows:

- less than 3.0 mmol/l (115 mg/dl) in subjects at moderate risk ($\geq 1 < 5\%$).
- less than 2.5 mmol/l (100 mg/dl) in subjects at high risk ($\geq 5 < 10\%$)
- less than 1.8 mmol/l (70 mg/dl) in subjects at very high total cardiovascular risk ($\geq 10\%$). Should target achievement not be feasible, the aim should be to reduce LDL-C levels by at least 50%

The recommended target level should be achieved primarily by lifestyle changes: research investigating the CV protective effect of use of functional foods within diet-based interventional trials is ongoing. A clinically proven safe and effective nutraceutical, as appropriate, would appear a useful therapeutic tool unless the CV risk is high and LDL-C levels are above 2.5 mmol/l (100 mg/dl) or the CV risk is very high and LDL-C levels are above 1.8 mmol/l (70 mg/dl), in which cases medication intervention is recommended. In all the other subjects pharmacological treatment should be introduced when repeated attempts have failed to achieve target levels through lifestyle changes and the use of safe and clinically proven nutraceuticals [2].

The most effective lifestyle changes designed to reduce TC and LDL-C levels, increase high density lipoprotein cholesterol (HDL-C) levels and reduce triglyceride levels, relate to the reduction in dietary saturated fat, trans fat and mono-/di-saccharides, use of functional foods enriched with phytosterols, reduction in excessive body weight, a moderate alcohol intake and increase in habitual physical activity [2]. In Western society, due to a combination of insufficient physical activity with dietary habits including large quantities of animal-fat rich and/or refined carbohydrate-rich food, a large proportion of the population may fail to achieve LDL-C targets through lifestyle changes alone. Hence, in view of the recommendations for patients at very high and high risk, and the difficulty that subjects at moderate risk may experience in reaching targets, a large number of patients may become candidates for life-long lipid-lowering drug therapy.

The drugs of first choice, and the "gold standard" in patients with hypercholesterolemia or combined hyperlipidemia are statins, which reduce synthesis of cholesterol in the liver by inhibiting HMG-CoA reductase activity competitively. The reduction in intracellular cholesterol concentration induces LDL receptor expression on the hepatocyte cell membrane, resulting in increased extraction of LDL-C from the blood and reduction in LDL-C plasma concentrations. They can be used either alone or in combination with other agents with different mechanisms of action, such as fibrates, bile acid sequestrants or nicotinic acid. Statins have proved to be able to reduce cardiovascular morbidity and mortality both in primary and secondary prevention trials [2]. Moreover, they appear to stop and even induce regression of coronary atherosclerosis [2]. These are notable benefits that outweigh the risks associated with the well-known side effects of these drugs, mainly myopathy (incidence of less than 1 in 1000), which may very rarely progress to rhabdomyolysis, renal failure and death; and initial impairment of liver function (increase in transaminases), which is dose-dependent [2]. However, as information accumulates on their long term usage, additional risks have been identified that should be taken into consideration [5–10].

An alternative to drug therapy included in the guidelines is the use of innovative nutritional strategies, based on the consumption of specifically targeted "health" functional foods and/or dietary supplements – the so-called nutraceuticals – which can be used, together with dietary measures, either as alternatives or in addition to lipid-lowering drugs so as not to have to increase their dose. The ESC/EAS guidelines [2] mention the following:

- *phytosterols*, which occur naturally in vegetable oils and vegetables, fresh fruit, grains and legumes
- *Soy* protein from the soybean, which can be used as a plant protein substitute for animal protein foods
- *dietary fiber*, contained mainly in whole grains, vegetables and fruits. The water soluble variety (e.g. oat bran) is the most effective
- *n-3 unsaturated fatty acids*, contained in fish oil, chestnuts, some vegetables and some seed oils
- *policosanol*, which occurs naturally in sugar cane, rice and wheat germ
- *red yeast rice*, which has been used in China as a food dye and flavor enhancer for centuries
- *berberine*, which is isolated from many kinds of medicinal plants and which has a long history of use in traditional Chinese medicine

In view of the interest for nutraceuticals as natural therapy that can offer efficacy without the artificial effects of the synthetic compounds in medicinal products, a proprietary formulation of six naturally occurring substances in plants called Armolipid® Plus[1] has been devised for the management of dyslipidemic subjects. The recommended posology is 1 tablet/day; each tablet contains three of the nutraceuticals mentioned in the guidelines, namely red yeast extract (200 mg, corresponding to 3 mg of monacolin), policosanol (10 mg) and berberine (500 mg), in addition to folic acid (0.2 mg), astaxanthin (0.5 mg), and coenzyme Q10 (2 mg).

The criterion for the selection of these ingredients was that they should counteract factors that contribute to the formation of atherosclerotic plaque starting from its early stages.

1. Folic acid

The starting point for the formation of an atherosclerotic plaque is chronic endothelial injury, which may for instance be induced by arterial hypertension and eventually results in endothelial dysfunction [11]. An independent cardiovascular risk factor that is believed to be involved in the development of endothelial dysfunction is hyperhomocysteinemia. The 2012 ESC guidelines acknowledge it as a lesser cardiovascular risk factor [1]. It may be the result of a diet poor in folic-acid rich food, namely leafy vegetables, liver and eggs; as well as of deficiencies in B6 and B12 vitamins. Folic acid, also known as vitamin B9, is essential for many biochemical functions, such as remethylation of homocysteine to methionine, whereby it is able to reduce homocysteine plasma levels [12], although clear evidences showing that supplementation of folate may actually reduce the CV risk are still missing.

2. Lipid-lowering compounds

Endothelial dysfunction results in increased permeability, which permits entry of LDL into the subendothelial space of the intima, where it accumulates, especially when LDL blood levels are high [11]. This is why it is imperative to control TC and LDL levels, which may be reduced by a combination of red yeast extract, policosanol and berberine.

2.1. Red yeast rice

Red yeast rice is a product of fermentation of the fungus *Monascus purpureus* grown on rice; this fungus produces a number of substances, including a red pigment (hence the name "red yeast rice") and the active ingredient monacolin K, also called lovastatin, which modulates the lipid profile by reducing TC and LDL-C via a statin-like mechanism i.e. by competitively inhibiting HMG-CoA reductase, the rate-controlling enzyme of the mevalonate pathway. Of note, the affinity of monacolin K for this enzyme is about 2000 times higher than the affinity for the endogenous substrate [13,14]. A meta-analysis [15] of 13 randomized, placebo-

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controlled clinical trials including a total of 804 dyslipidemic subjects consuming red yeast rice for periods ranging from 4 weeks to 12 months revealed significant weighted mean improvements in the lipid profile as compared to placebo (TC −0.97 (95%CI −1.13, −0.80) mmol/l $p < 0.001$; LDL-C −0.87 (95%CI −1.03, −0.71) mmol/l $p < 0.001$). No serious adverse events were reported. The improved lipid profile resulted in secondary cardiovascular prevention in 5000 Chinese patients who had experienced a myocardial infarction and consumed a partially purified extract of red yeast rice or a placebo for 4.5 years. Major coronary events (nonfatal myocardial infarction and death from coronary heart disease) were reduced by 45% in the red yeast rice arm (5.7% vs 10.4% in the placebo arm), as well as cardiovascular mortality by 30% and total mortality by 33% [16].

2.2. Policosanol

Policosanol is a natural mixture of long-chain saturated primary aliphatic alcohols that occur in beeswax, sugar cane, rice bran and a number of vegetables. This compound may have the capability to mildly inhibit the synthesis of HMG-CoA reductase, the enzyme responsible for the conversion of HMG-CoA to mevalonate. Studies have also shown that it reduces intracellular cholesterol, increasing cholesterol uptake by the liver from circulating lipoproteins [17,18]. At doses ranging between 5 mg and 40 mg daily, it has been reported to lower cholesterol in a series of placebo-controlled or active-reference controlled clinical trials versus simvastatin, pravastatin, lovastatin [19–25]; however, a more recent randomized, double-blind cross-over placebo-controlled trial failed to confirm the capability of policosanol, when used alone at 10 mg/day dosage, to significantly reduce LDL-C and improve all tested parameters, with the exception of a minor reduction in plasma total cholesterol [26].

2.3. Berberine

Berberine is an alkaloid that occurs naturally in the bark of *Berberis aristata*, a shrub that grows in the Himalayas and Nepal. According to studies in human hepatocytes, berberine may reduce expression of PCSK9, and consequently lower LDL receptor degradation [27]. The increase in LDLR expression in the liver stimulates hepatic uptake of plasma cholesterol, thus promoting clearance of LDL-C from the bloodstream [28,29]. Interestingly, this mechanism is not dependent on intracellular cholesterol levels. In other words, the efficacy of berberine is not diminished by other cholesterol-lowering substances.

Extensive in vitro and in vivo studies have shown that berberine possesses additional properties. It stimulates AMP-activated protein kinase (AMPK), which is an enzyme involved in the inactivation of acetyl-CoA carboxylase (ACC), an enzyme that catalyzes the irreversible carboxylation of acetyl CoA to produce malonyl CoA – a rate limiting step in the synthesis of fatty acids. Hence, it is able to reduce the synthesis of triglycerides [30].

The modulation of the lipid profile by berberine has been documented by randomized, placebo-controlled clinical trials in patients with mild to moderate mixed hyperlipidemia with and without concomitant type 2 diabetes mellitus, in patients with hypercholesterolemia who cannot tolerate more than one statin, and in patients with hyperlipidemia and hepatitis B and C or cirrhosis. Mean reductions in LDL-C and triglyceride levels have been reported in the range 20–25% and 13–30%, respectively [31].

The properties of berberine extend beyond the modulation of the lipid profile [32]. The stimulation of AMPK also increases the expression of insulin receptors and their sensitivity, thus potentially improving glucose homeostasis and reducing insulin resistance. This property is of considerable interest, because many subjects with dyslipidemia also have diabetes mellitus.

Studies in healthy human volunteers have documented improvement in endothelial function with berberine, and trials in patients with congestive heart failure have shown that berberine is able to exert important inotropic and antiarrhythmic effects. Once again, these properties are important, since many dyslipidemic patients also have cardiovascular disease. In particular, one randomized, placebo-controlled 8-week clinical trial indicated that berberine can improve cardiac function, and reduce ventricular premature complexes and mortality in patients with congestive heart failure [33].

The three lipid-lowering compounds in Armolipid Plus, namely red yeast rice, policosanol and berberine, have complementary mechanisms of action, as shown in Fig. 1.

The first two both target the HMG-CoA enzyme reducing cholesterol synthesis, albeit in different ways: the active ingredient of red yeast rice, monacolin K, inhibits HMG-CoA reductase activity competitively, whereas policosanol decreases HMG-CoA reductase synthesis. Berberine was added to achieve comprehensive effects on the lipid profile, targeting also triglyceride synthesis and LDL liver uptake.

3. Antioxidants

After entering into the subendothelial space of the intima, LDL is oxidized by ROS and transformed into pro-atherogenic particles [11]. This may be prevented by increasing antioxidant stores through the administration of antioxidants, such as astaxanthin and coenzyme Q10.

3.1. Astaxanthin

Astaxanthin is a red-orange carotenoid obtained from microalgae *Haematococcus pluvialis*, which is one of the most potent naturally occurring antioxidants (100–500 times more potent than vitamin E). Astaxanthin inhibits LDL peroxidation preventing lipoproteins from being converted into pro-atherogenic particles [32,33].

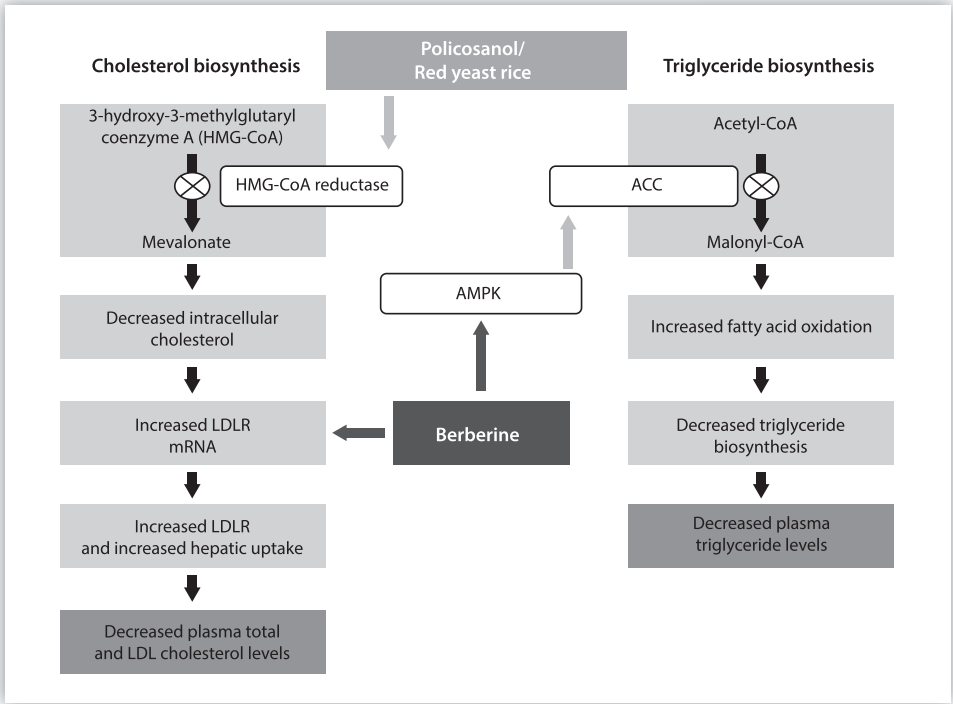

Fig. 1. Complementary mechanisms of Armolipid Plus containing red yeast rice, policosanol, berberine, folic acid, astaxanthin, and coenzyme Q10.

3.2. Coenzyme Q10

Coenzyme Q10 is one of the main antioxidant defenses of the human body, which is depleted by HMG-CoA reductase inhibitor therapy. Adequate stores of coenzyme Q10 are essential for oxide reduction involved in ATP synthesis and to prevent LDL peroxidation [34–36], although clinical evidence supporting use of coenzyme Q10 supplementation in reducing the CV risk are still missing.

4. Interaction potential and bioavailability

The interaction potential of Armolipid Plus and bioavailability of the main active ingredients of Armolipid Plus i.e. berberine and monacolin (lovastatin lactone) were assessed in healthy male volunteers aged 20–45 years [37]. They received a five-probe drug cocktail (one for each CYP enzyme that could be influenced by Armolipid Plus) alone or in combination with Armolipid Plus at the recommended dose. Plasma concentrations of each probe were determined by validated LC-MS/MS methods; blood samples were drawn before administration and up to 48 h post-dose. Results (Tables 1 and 2) show that clinically relevant drug interactions due to metabolic inhibition can be excluded when Armolipid Plus is administered at the recommended dose with medications metabolized by CYP1A2, CYP2D6, CYP2C19, CYP3A4, CYP2C9 [37].

Table 1
Summary of PK parameters for each probe drug, administered during reference (cocktail alone) and test (AP + cocktail). AP = Armolipid Plus [37].

DRUG	Treatment	C_{max} (ng/ml)	T_{max} (h)	AUC $_{last}$ (ng/ml*h)	$T_{1/2}$ (h)
Caffeine	Cocktail alone	2949 (834.8)	0.75 (0.5–1.5)	26,791 (11,928)	7.4 (4.1)
	AP + cocktail	2624 (560.8)	1.0 (0.5–2.0)	27,831 (11,495)	5.7 (2.3)
Metoprolol	Cocktail alone	160.3 (89.3)	1.5 (1.0–3.0)	882 (702)	3.5 (1.0)
	AP + cocktail	140.8 (80.5)	1.75 (1.0–3.0)	788 (704)	3.5 (0.9)
Omeprazole	Cocktail alone	221.3 (163.4)	2.0 (1.0–6.0)	429 (277)	0.9 (0.1)
	AP + cocktail	225.8 (132.6)	2.5 (1.0–4.0)	408 (266)	0.9 (0.1)
Midazolam	Cocktail alone	10.4 (3.6)	1.0 (0.5–1.0)	27.7 (7.1)	4.3 (1.4)
	AP + cocktail	9.9 (4.2)	0.5 (0.5–2.0)	27.1 (10.3)	3.7 (1.2)
S-warfarin	Cocktail alone	543.7 (153.7)	2.0 (1.0–6.0)	24.843 (9.609)	120.9 (99.8)
	AP + cocktail	621.2 (227.1)	2.0 (0.5–4.0)	25.119 (9.352)	91.5 (28.2)
R-warfarin	Cocktail alone	537.9 (131.3)	2.0 (1.0–6.0)	36.474 (7.204)	83.6 (37.4)
	AP + cocktail	576.9 (182.7)	3.0 (0.5–12.0)	36.301 (5.656)	67.2 (14.9)

Table 2
PK parameters point estimate (90% CI) for each probe drug [3].

DRUG	Parameter	Ratio estimate (90% CI)	CV_{intra} %
Caffeine	AUC_{last}	1.04 (0.86,1.27)	27.1
	C_{max}	0.90 (0.78, 1.04)	19.8
Metoprolol	AUC_{last}	0.90 (0.77,1.06)	22.1
	C_{max}	0.89 (0.76, 1.05)	22.3
Omeprazole	AUC_{last}	0.94 (0.71,1.25)	39.0
	C_{max}	1.07 (0.73, 1.57)	54.2
Midazolam	AUC_{last}	0.94 (0.75,1.17)	30.8
	C_{max}	0.94 (0.73, 1.21)	35.0
S-warfarin	AUC_{last}	1.01 (0.93,1.11)	11.6
	C_{max}	1.11 (1.00, 1.24)	14.4
R-warfarin	AUC_{last}	1.00 (0.93,1.08)	9.9
	C_{max}	1.06 (1.00, 1.12)	7.1

In terms of bioavailability, the berberine plasma concentration–time profile (Fig. 2A) indicated that this ingredient was absorbed with average peak plasma concentrations of 0.11 ng/ml at 4 h post-dose. The berberine plasma concentrations decreased thereafter and were still measurable 24 h after dosing in 6/12 subjects. Multiple peaks in the plasma concentration–time profile indicated the presence of enterohepatic circulation for berberine, in agreement with previous pre-clinical and clinical studies [38–40]. Monacolin K, assessed as lovastatin hydroxy acid (Fig. 2B), was absorbed, with peak plasma concentrations averaging 3.06 ng/ml at 2.5 h. The mean AUClast values for berberine and lovastatin were 1.0 and 10.8 ng/ml*h, respectively [38].

5. Efficacy in modulating the lipid profile

5.1. Placebo-controlled studies on top of low-fat diet

Five double-blind and two single-blind randomized, placebo controlled studies [41–47] in over 500 dyslipidemic patients have consistently shown that Armolipid Plus is able to reduce TC and LDL-C levels significantly. The mean reductions in TC and LDL-C levels ranged between 10 and 20% and between 15 and 31%, respectively. The main reasons for the variability likely were the differences in the severity of dyslipidemia, since reductions in these parameters may be proportionate to baseline values (Table 3).

The first study [41] was performed in 50 dyslipidemic patients of all ages (range: 18–70 years) from the general dyslipidemic population. The patients were allocated to Armolipid Plus or placebo while continuing a low-fat diet that had been introduced during a 4-week run-in period. Blinded treatment continued for 6 weeks. Important mean differences were recorded between Armolipid Plus and placebo, showing evident effects of Armolipid Plus on the lipid profile (−16.8% for TC ($p < 0.001$), −22.3% for LDL-C ($p < 0.001$), +1.3% for HDL-C (NS) and −12.1% for triglycerides (NS)). A similar study, but with longer treatment period [49], was performed in 60 patients with low-moderate risk hypercholesterolemia, treated with Armolipid Plus or placebo for 24 weeks: results confirm the lipid lowering effect of the nutraceutical formulation (−24.6% $p < 0.01$ for TC and −23.7%, $p < 0.01$ for LDL-C) (Table 3).

Similar results were obtained in particular subsets of the dyslipidemic population: the elderly (>75 years) with statin intolerance who were not willing to be treated with other medicinal products [42]; and patients with metabolic syndrome [44,46]. A comparison between normoweight and overweight patients indicated that Armolipid Plus is effective independent of body weight [43].

A recent study [47] in 102 patients with mild to moderate dyslipidemia of all ages (>18 years) allocated to Armolipid Plus or placebo after a 1-week run-in for 12 weeks showed significant differences in the reduction of TC, LDL-C and triglycerides vs placebo, but their extent was lower (mean reductions: TC −10.5%, LDL-C −14.9%, triglycerides −0.6%) (Table 3), likely because this study recruited subjects at low cardiovascular risk, with moderate dyslipidemia at baseline.

Hence, Armolipid Plus has consistently proved to be effective independent of a number of cardiovascular risk

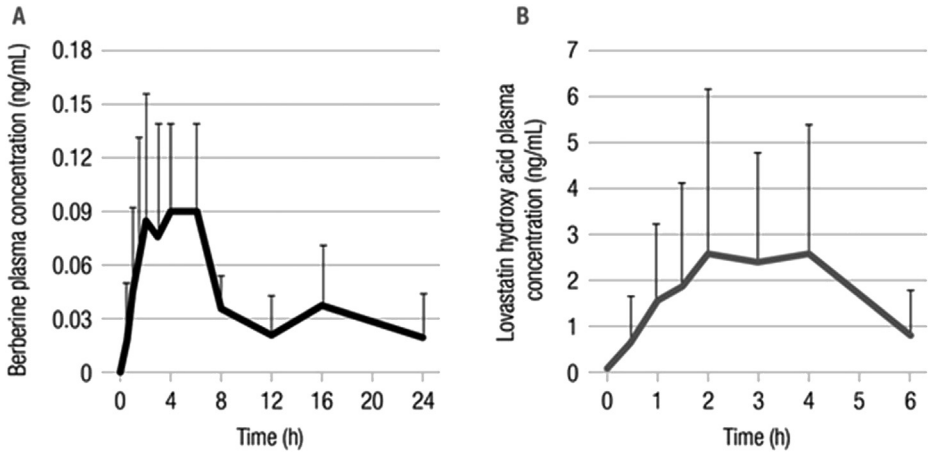

Fig. 2. Mean plasma concentrations for berberine (A) and lovastatin hydroxyl acid (B). SD are shown with vertical bar [38, modified].

Table 3
Efficacy of Armolipid Plus in modulating the lipid profile: blinded, placebo-controlled clinical trials (the p-value is for the between-group comparison).

Reference	Study design	Patient population	Treatment	TC (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)
Affuso et al. (2010) [41]	4-week run-in on low fat diet randomized, double-blind, placebo controlled	50 patients Age 18–70 TC > 5.68 mmol/l LDL-C > 3.36 mmol/l	Armolipid Plus (AP) + diet or placebo (PL) +diet for 6 weeks	AP −1.14 ± 0.88 PL −0.03 ± 0.78 p < 0.001	AP −1.06 ± 0.75 PL −0.04 ± 0.54 p < 0.001	AP +0.03 ± 0.28 PL +0.01 ± 0.16 NS	AP −0.19 ± 0.45 PL +0.05 ± 0.64 NS
Marazzi et al. (2011) [42]	randomized, single-blind, placebo controlled	80 patients age > 75 TC > 4.1 mmol/l LDL-C > 3.36 mmol/l mostly in secondary prevention, intolerant to statins	AP or PL for 12 months	AP From 6.51 ± 0.59 to 5.19 ± 0.67 PL From 6.54 ± 0.49 to 6.59 ± 0.72 p = 0.008	AP From 4.44 ± 0.41 to 3.07 ± 0.54 PL From 4.47 ± 0.25 to 4.52 ± 0.64 p = 0.002	AP From 1.13 ± 0.31 to 1.26 ± 0.28 PL From 1.13 ± 0.20 to 1.16 ± 0.20 NS	AP From 2.02 ± 0.54 to 1.83 ± 0.37 PL From 2.02 ± 0.56 to 1.99 ± 0.55 NS
Cicero et al. (2012) [43]	partially randomized allocation of overweight patients, placebo controlled	135 patients TC > 5.64 mmol/l LDL-C > 3.51 mmol/l	AP + diet (N = 85) or PL + diet (N = 50) for 12 months	AP −1.08 ± 0.44 PL −0.59 ± 0.44 p < 0.05	AP −0.70 ± 0.54 PL −0.16 ± 0.57 p < 0.05	AP +0.13 ± 0.16 PL +0.05 ± 0.16 p < 0.05	AP −1.16 ± 0.61 PL −1.09 ± 0.59 NS
Affuso et al. (2012) [44]	randomized, double-blind, placebo controlled	64 patients age 18–65 TC > 5.09 mmol/l LDL-C > 3.05 mmol/l + metabolic syndrome	AP + diet or PL + diet for 18 weeks	AP −0.82 ± 0.76 PL −0.13 ± 0.60 p < 0.001	AP −0.82 ± 0.68 PL −0.13 ± 0.55 p < 0.001	AP +0.00 ± 0.13 PL −0.08 ± 0.21 NS	AP −0.01 ± 0.89 PL +0.17 ± 1.03 NS
Gonnelli et al. (2015) [45]	randomized, double-blind, placebo controlled	60 patients TC > 6.16 mmol/l LDL-C > 4.19 mmol/l	AP + diet or PL + diet for 24 weeks	AP −24.6% PL −2% p < 0.01	AP −23.7% PL +2% p < 0.01	AP −1% PL −1% NS	AP −22.9% PL −4.1% NS
Ruscica et al. (2013) [46]	2-week run-in on low fat diet randomized, double-blind, crossover, placebo controlled,	30 patients age >18 TC > 6.11 mmol/l LDL-C > 3.83 mmol/l + metabolic syndrome	AP + diet or PL + diet for 8 weeks	AP From 6.2 ± 0.8 to 5.4 ± 0.7 PL From 6.1 ± 1 to 6.3 ± 0.9 p < 0.001	AP From 3.91 ± 0.62 to 3.09 ± 0.66 PL From 3.88 ± 0.76 to 3.73 ± 0.87 p < 0.001	AP From 1.04 ± 0.23 to 1.09 ± 0.25 PL From 1.07 ± 0.19 to 1.07 ± 0.24 NS	AP From 2.44 to 2.21 PL From 2.60 to 2.42 NS
Solà et al. (2014) [47]	1-week run-in on low fat diet randomized, double-blind, placebo controlled,	102 patients age >18 TC > 6.28 mmol/l LDL-C > 4.02 mmol/l	AP + diet or PL + diet for 12 weeks	AP −10.5% PL −5.5% p = 0.01	AP −14.9% PL −8% p = 0.029	AP +0.4% PL −3.6% NS	AP −0.63% PL +4.9% NS

factors, namely advanced age, metabolic syndrome and increased body weight.

5.2. Real life studies as add-on therapy to low-fat diet

The next step was to assess the efficacy of Armolipid Plus in general practice, in conditions that resembled usual clinical practice more closely than the artificial setting of a double-blind, randomized clinical trial at specialized tertiary clinics, i.e. in large real life (open label) parallel group, multicenter trials assessing the effect of the nutraceutical on top of a dietary regimen in comparison to the continuation of the diet alone [48,49] (Table 4).

A large multicenter trial [48,49] was carried out in dyslipidemic patients of all ages (18–80 years of age) with inclusion criteria restricted to the level, not to the cause of

Table 4
Efficacy of Armolipid Plus in modulating the lipid profile: randomized, controlled clinical trials vs. dietary regimen alone (the p-value is for the between-group comparison).

Reference	Study design	Patient population	Treatment	TC (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)
Trimarco et al. (2010) [48]	2-week dietary run-in, partially randomized, controlled, parallel group	1751 patients age 18–80 TC > 5.17 mmol/l LDL-C > 3.87 mmol/l	Armolipid Plus (AP) + diet (N = 933) or diet-only (D) (N = 818) for 16 weeks	AP −19.1% D −9.4% p < 0.001	AP −23.5% D −10.8% p < 0.001	AP +11.6% D +4.0% p < 0.001	AP −17.9% D −11.3% p < 0.001
Pirro et al. (2013) [63]	Randomized, controlled,	70 patients TC > 6.7 mmol/l LDL-C > 4.53 mmol/l never previously treated	AP + D or D for 8 weeks	AP From 6.69 ± 0.75 to 5.77 ± 0.72 D From 6.72 ± 0.70 to 6.65 ± 0.67 P < 0.001	AP From 4.53 ± 0.75 to 3.62 ± 0.78 D From 4.60 ± 0.80 to 4.55 ± 0.52 P < 0.001	AP From 1.45 ± 0.26 to 1.45 ± 0.31 D From 1.50 ± 0.36 to 1.47 ± 0.31 NS	AP From 1.25 to 1.25 D From 1.34 to 1.38 NS
Pirro et al. (2016) [60]	Randomized, controlled	100 patients, TC > 5.4 mmol/l LDL-C > 3.4 mmol/l with hsCRP > 2 mg/l	AP + D or D for 3 months	AP From 5.46 ± 0.44 to 4.78 ± 0.44 D From 5.43 ± 0.62 to 5.43 ± 0.65 P < 0.001	AP From 3.46 ± 0.36 to 2.72 ± 0.39 D From 3.39 ± 0.41 to 3.41 ± 0.47 P < 0.001	AP From 1.32 ± 0.39 to 1.40 ± 0.34 D From 1.32 ± 0.31 to 1.34 ± 0.28 NS	AP From 1.30 to 1.23 D From 1.24 to 1.23 NS
Mazza et al. (2015) [71]	Controlled study, parallel groups, age and gender match	132 patients TC > 5.95 mmol/l LDL-C > 4.27 mmol/l with grade I essential hypertension	AP + D (N = 66) or D (N = 66) for 6 months	AP −19.2% D No change p < 0.001	AP −17.4% D No change p < 0.001	AP No change D No change NS	AP −16.3% D No change p < 0.001

the dyslipidemia (TC > 4.91–5.17 mmol/l and/or LDL-C >3.87 mmol/l and/or TG > 3.87 mmol/l), who did not require immediate intervention with lipid-lowering agents, were intolerant to them or refused them. Hence, the results regard all dyslipidemic patients in general. The study included more than 1700 patients recruited by 248 GPs and treated for 16 weeks. The results were consistent with those of the double-blind studies in terms of the extent in the reduction in TC (18–19%) and LDL-C (23–24%) and increase in HDL-C (7–11%), whereas the reduction in triglycerides was slightly more pronounced (approx 18%).

5.3. General considerations on lipid-lowering efficacy of Armolipid Plus in the general population

The addition of Armolipid Plus to a low-fat dietary regimen offers the opportunity of achieving significant reductions in TC (11–21%) and in LDL-C (15–31%) levels, as well as improvement in HDL and triglyceride levels. These reductions are within the range of what can be achieved with low potency statins (pravastatin, fluvastatin) or low doses of other statins, such as simvastatin [50–52]. A recent systematic review and meta-analysis of 14 randomized controlled trials performed with Armolipid Plus calculated that the weighted mean differences (WMD) vs. control were: −26.15 mg/dl for TC (p < 0.001); −23.85 mg/dl for LDL-C (p < 0.001); +2.53 mg/dl for HDL-C (p < 0.001) [53].

5.4. Randomized, controlled trials versus active treatment

After the achievement of evidence showing that Armolipid Plus exerts significantly greater lipid lowering effects than placebo and dietary regimens alone, Armolipid Plus was compared to active treatment within the setting of a 6-month randomized, controlled clinical trial [54]. The selected comparator was ezetimibe, a lipid-lowering agent that inhibits the enteric absorption of dietary and biliary cholesterol, as well as the re-absorption of biliary cholesterol by hepatocytes. It is approved for therapeutic use as a lipid-lowering agent both in combination with statins and for monotherapy in patients in whom statins are contraindicated or not tolerated. The outcome of the comparative trial was higher than expected, since Armolipid Plus was actually significantly more effective than ezetimibe in reducing both TC and LDL-C

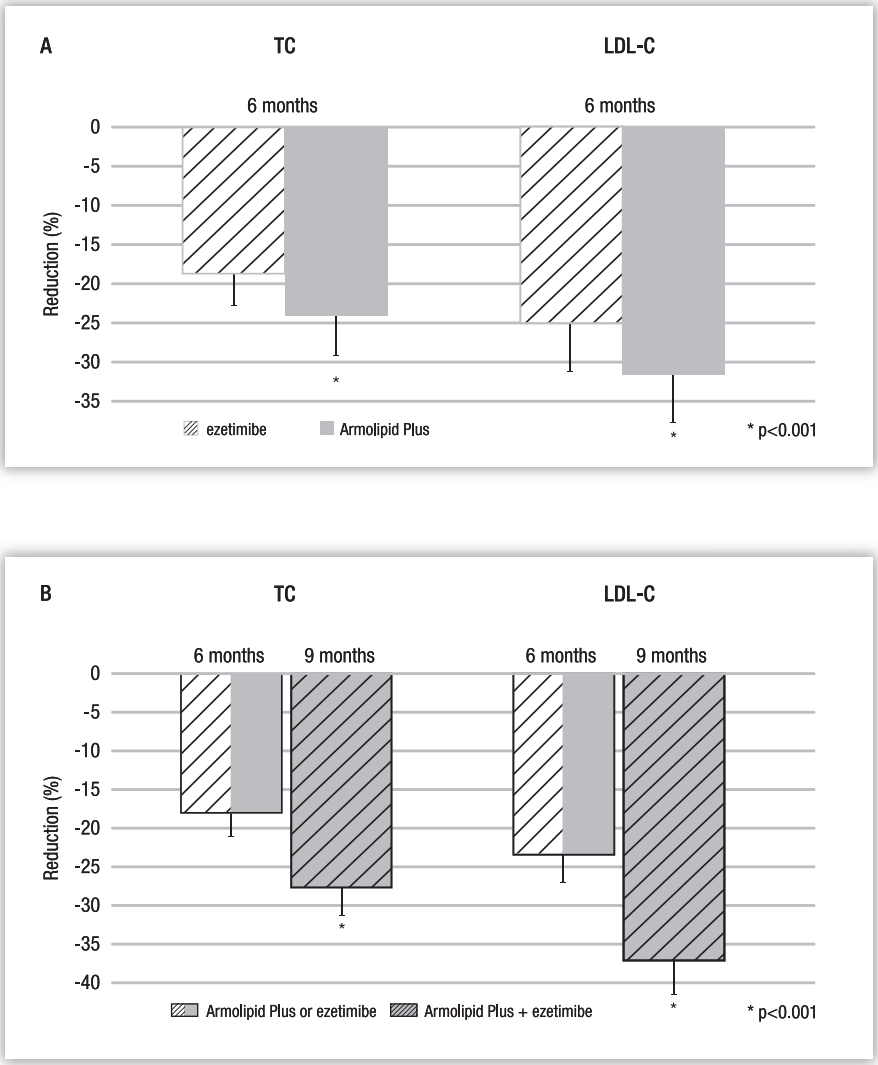

Fig. 3. A. Comparison between Armolipid Plus and ezetimibe (10 mg daily) in 228 patients with primary hypercholesterolemia and intolerance to statins (22%) or refusal to take them (78%) within the setting of a 6-month randomized controlled clinical trial (p-values are for comparison between groups). B. Results of the treatment of 26 patients in whom monotherapy with Armolipid Plus (n = 14) or ezetimibe (10 mg daily) had failed (reduction in LDL-C plasma levels <29%) for another 3 months with the combination of Armolipid Plus + ezetimibe [53] (p-values are for comparison between the association of Armolipid Plus + ezetimibe and the single administration of the 2 compounds).

($p < 0.001$) (Fig. 3A), whereas changes in HDL and in triglycerides were similar in both groups.

A study in 100 dyslipidemic patients [55] with ischemic heart disease, intolerant to statins, treated with percutaneous coronary intervention (PCI), a subgroup of patients particularly in need of secondary prevention, assessed the efficacy of a combined therapy with ezetimibe and Armolipid Plus. According to the protocol, after 3 months of treatment with either ezetimibe or Armolipid Plus, patients who had not reached their targets in terms of TC and LDL control were to add-on the therapy they had not been allocated to. After 3 months, 14/50 patients (28%) in the Armolipid Plus group had reached their target (LDL-C <100 mg/dl), whereas none of the patients in the ezetimibe group had. Consequently, 14 patients continued treatment with Armolipid Plus alone, whereas all the others received the combination of ezetimibe + Armolipid Plus. At the end of treatment, 72.5% of patients had achieved the therapeutic target. No patients discontinued treatment.

The first trial [54] also showed that the effects of Armolipid Plus and ezetimibe are additive, since the addition of Armolipid Plus on top of ezetimibe at the end of the comparative phase i.e. after 6 months of treatment, reduced TC, LDL-C and triglycerides further to a significant degree (by −9.7%, −13.6% and −4.5%, respectively) (Fig. 3B).

6. Studies in special patient populations

6.1. Patients with chronic kidney disease

One study comparing patients with and without chronic kidney disease (CKD) prospectively suggested that renal

function impairment does not affect the lipid-lowering properties of Armolipid Plus [56].

It was a parallel-group study in 40 consecutively enrolled moderately hypercholesterolemic out-patients with mild to moderate CKD and 40 cross-matched hypercholesterolemic patients without CKD. All patients were given Armolipid Plus for 6 months. At the end of this period the improvement in the lipid profile was almost identical in the two groups: mean TC was reduced by 21.1% in CKD patients vs 21.6% in non-CKD patients; mean LDL-C by 23.7% vs 24.2% and triglycerides by 20.4% vs 20.8%. This study shows that CKD does not affect the lipid-lowering effects of Armolipid Plus. Therefore, Armolipid Plus can be a valid alternative for these patients, for whom statin therapy may not be suitable on account of the recently discovered risk of kidney injury [5].

6.2. Patients who cannot tolerate statins

Two randomized, controlled, single-blind trials [42,55] and one open-label trial [57] were carried out in a total of 228 dyslipidemic patients who had not been able to tolerate statin therapy. Intolerance was defined as debilitating muscular pain/myositis/rhabdomyolysis/increase in CPK by more than 10 times the upper limit of normal, and/or gastrointestinal disorder and/or elevated transaminase levels by 2 or 3 times the upper limit of normal and/or sleep disorder and/or dermatological disorder [58].

The first [42 – Table 3] was a placebo-controlled trial that assessed the efficacy and safety of Armolipid Plus in a subgroup of dyslipidemic patients in whom tolerability is known to be low, namely elderly patients aged >75 years. A total of 80 patients were recruited, randomized (40 to Armolipid Plus and 40 to placebo) and completed long-term treatment (12 months). At the end of treatment there were no important changes in the lipid profile in the placebo group, whereas TC and LDL-C were reduced significantly in the Armolipid Plus group (−20% – $p = 0.008$ vs placebo and −31% – $p = 0.002$ vs placebo, respectively). No serious or severe events were reported and no patients discontinued treatment because of intolerance. In particular, no patients experienced increases in transaminases or in CPK.

The second [55], already presented in the above section "randomized controlled trial vs. active treatment", was the single-center trial comparing two alternatives to statins, namely ezetimibe and Armolipid Plus + ezetimibe.

In the open-label study [57] 48 hyperlipidemic patients (mean age 60.7 ± 15 years, mostly female n = 27, n = 19 with metabolic syndrome) took 1 tablet of Armolipid Plus and a yogurt added with 2 g phytostanols for one year. The patients achieved significant decreases in TC, LDL-C and TG (all $p < 0.001$) and at the end of treatment 7 patients no longer met the criteria for metabolic syndrome. All the patients tolerated therapy well, except 4 (8.3%) who discontinued because of asymptomatic increase in CPK >5ULN, n = 2, myalgia, n = 1, and dyspepsia, n = 1. In the other patients no significant changes were recorded in safety parameters; in particular no increases in liver transaminases or CPK levels were recorded.

In addition, one small, randomized, parallel group, single-blind trial was carried out in 50 patients with moderate hypercholesterolemia and subjective intolerance to statins i.e. male patients who had voluntarily interrupted statin treatment because of self-reported erectile dysfunction (ED) [59]. The patients were randomly allocated to ezetimibe 10 mg once daily or Armolipid Plus 1 tablet daily for 12 weeks. Besides the usual measurements to establish the effects on the lipid profile, the investigations included the administration of two questionnaires designed to calculate the International Index of erectile function (IIEF-5) and the Satisfaction profile (SAT-P) before and after treatment. The IIEF-5 score ranges from 5 (severe ED) to 25 (no ED), the SAT-P includes 32 items that address different aspects of daily life, namely physical, psychological, cognitive, social and professional performance; its score ranges from 0 = totally dissatisfied to 100 = completely satisfied. Improvement of the lipid profile was similar and significant versus baseline ($p < 0.05$) in both groups. However, the mean IIEF-5 improved to a significantly greater extent in the AP group than in the ezetimibe group (1.2 ± 0.7 vs 0.7 ± 0.3 $p = 0.04$). The same was true for SAT-P (8 ± 4 vs 6 ± 3 $p < 0.05$); the aspects that improved significantly were the physical, psychological and social performance (all $p < 0.05$ vs baseline).

7. Effects of Armolipid Plus on other established risk factors of cardiovascular mortality and metabolic syndrome

Armolipid Plus has proved to be able to improve not only dyslipidemia, but also other independent predictors of cardiovascular events: reduction of arterial stiffness, which is an independent predictor of all-cause and cardiovascular morbidity, coronary events, and stroke in patients with uncomplicated essential hypertension as well as in the general population [1]. Moreover, reduction of hsCRP in patients with borderline-high levels [60] and improvement in insulin resistance measured by the HOMA index, which is a strong predictor of cardiovascular risk in various kinds of patients [61–64] have also been documented.

7.1. Reduction in vascular stiffness

The effects of Armolipid Plus on vascular stiffness were assessed in 4 studies [41,44,56,65] in a total of 219 patients using two indices of vascular stiffness, namely flow mediated dilation (2 studies) and aortic pulse wave velocity (2 studies).

Armolipid Plus increased flow-mediated dilation to a significantly greater extent than placebo ($p < 0.05$).

Regarding aortic pulse wave velocity, Armolipid Plus on top of a dietary regimen was significantly more effective on this index of vascular stiffness than the dietary regimen alone in 70 out-patients with hypercholesterolemia after 2 months of treatment ($p = 0.005$) [63]. The other study showed that this effect of Armolipid Plus is not affected by impaired renal function [56].

7.2. Reduction in insulin resistance

Insulin resistance was assessed in 6 blinded RCTs [41–43,46,47,49]. The total number of patients investigated was 595, of whom 342 received Armolipid Plus. Insulin resistance was consistently improved to a greater degree with Armolipid Plus than placebo and a statistically significant difference was seen in two of these studies. The improvement ranged from 7.7 to 24.2%. In the placebo groups, insulin resistance remained unchanged or even slightly worsened (by up to 6%), with the exception of one study, where it improved the index by 15% [46], but without reaching statistical significance.

Ezetimibe has been shown to improve insulin resistance [66], whereas statins, on the contrary, do not [67]. Moreover, there is a growing body of evidence suggesting that statins may have a negative impact on glucose metabolism in general [7–9] and on insulin resistance in particular [68,69].

7.3. Improvement in metabolic syndrome

Since Armolipid Plus improves dyslipidemia and insulin resistance, which are important components of the metabolic syndrome, and improves vascular stiffness, which suggests that it may contribute to the amelioration of a third component of the syndrome, namely arterial hypertension, the product could be expected to improve metabolic syndrome. Patients with metabolic syndrome are a patient population at high cardiovascular risk by definition, as they must have a number of cardiovascular risk factors (abdominal obesity, impaired glucose metabolism, dyslipidemia and arterial hypertension) [70]. The effect of Armolipid Plus on metabolic syndrome was assessed in two studies. In a double-blind crossover study [46] in 30 patients with metabolic syndrome, treatment with Armolipid Plus resulted in an overall reduction in MetS criteria in 21 subjects, to such an extent that 10 i.e. one third no longer met the criteria for metabolic syndrome. In the large study in the general dyslipidemic population by Izzo et al. [49] two thirds of the patients had metabolic syndrome (67–68%). After eight weeks of treatment, only 36.1% of patients still had metabolic syndrome versus 48.1% in the placebo group ($p < 0.05$).

In another study [44] the effects of Armolipid Plus on all the important variables contributing to cardiovascular risk were assessed in 64 patients with metabolic syndrome within the context of a prospective 18-week double-blind-placebo-controlled trial. After 18 weeks of treatment, Armolipid Plus, besides reducing TC and LDL-C significantly ($p < 0.001$), determined significant improvements in HOMA-IR index ($p = 0.02$), fasting insulin ($p = 0.04$), mean post-prandial blood glucose ($p = 0.04$) and insulin levels ($p = 0.02$), flow-mediated arterial dilation ($p = 0.03$), and systolic blood pressure ($p = 0.04$) (Fig. 4). Thus, it improved all the individual components of the metabolic syndrome. Quite interestingly, a recent randomized clinical study performed in 132 patients with hypercholesterolemia and grade 1 essential hypertension, treated for 6 months with Armolipid Plus on top of a dietary regimen, was significantly more effective than the dietary regimen alone in reducing TC and LDL-C (−19.2% and −17.4% vs. baseline, respectively, $p < 0.05$) and also in reducing systolic blood pressure and 24-h ambulatory blood monitoring [71].

8. Safety

8.1. Exposure and adverse events

To date, data on more than 1600 patients ($n = 1674$) treated with Armolipid Plus for periods ranging from 6 to 48 weeks have been published in the literature. The control groups included a total of 1361 patients; of these 241 were given placebo and 151 ezetimibe. Very few adverse events have been reported in the literature: only 36 in total for Armolipid Plus (2.2% of patients). The rates of patients reporting adverse events were not different from placebo in the blinded trials and were higher with ezetimibe in the comparative trials versus active therapy (4.9%). The most common adverse event reported was constipation with Armolipid Plus ($n = 8$), weakness with placebo ($n = 3$) and gastrointestinal intolerance with ezetimibe ($n = 6$). No life-threatening or other serious adverse events occurred. The very favorable safety profile of Armolipid Plus is likely due to the intentional combination of low doses of its active ingredients: low enough not to be associated with untoward effects, but high enough to exert therapeutic effects in combination with other complementary substances. Hence, the safety profile of Armolipid Plus differs considerably from the safety profile of statins, which may be associated, albeit rarely, with serious untoward effects, such as myopathy and kidney injury [2,5].

9. Conclusions

Armolipid Plus is a nutraceutical that contains low doses of naturally occurring substances, which exert complementary actions designed to prevent the formation of atherosclerotic plaques.

Randomized double-blind clinical trials and a large open-label randomized, controlled clinical trial performed in general practice in a total of more than 3000 patients have shown that the addition of Armolipid Plus to a low-fat

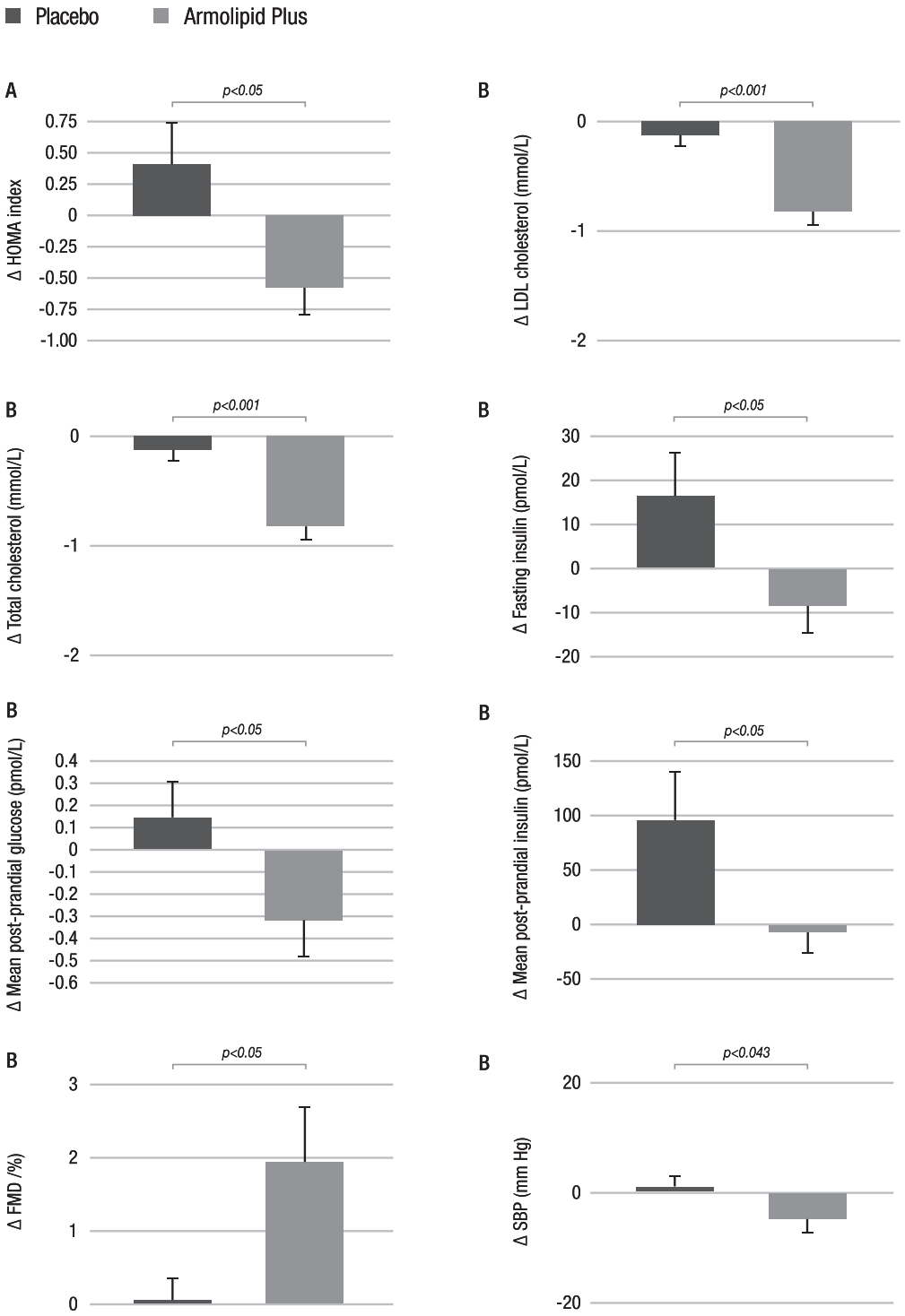

Fig. 4. Variations in the primary endpoint (A) and in secondary endpoints (B) [44]. HOMA: Homeostasis model assessment; FMD: Flow-mediated dilation.

dietary regimen offers the opportunity of achieving significant reductions in TC (11–21%) and in LDL-C (15–31%) levels, equivalent to what can be expected with low-dose statin therapy. Combination with ezetimibe can achieve a further 10% improvement. The results achieved with Armolipid Plus were similar in particular subsets of dyslipidemic patients, such as patients with chronic kidney disease and patients who cannot tolerate statins. Moreover, Armolipid Plus offers additional benefits in terms of improvement of vascular stiffness and insulin resistance, which are independent predictors of cardiovascular events.

The safety and tolerability of Armolipid Plus were excellent likely due to the intentional combination of low doses of its active ingredients: low enough not to be

associated with untoward effects, but high enough to exert therapeutic effects in combination with other complementary substances.

Consequently, in the event of intolerance to statins, Armolipid Plus offers an effective alternative to statin therapy, which is devoid of the safety risks associated with synthetic pharmacological therapy, such as myopathy, kidney injury, and potential onset of diabetes mellitus.

In conclusion Armolipid Plus, in addition to dietary measures, may be an excellent alternative for subjects with mild to moderate hyperlipidemia and for all dyslipidemic patients for whom statins are not indicated. Armolipid Plus may also be useful in combination with other lipid-lowering agents in the attempt to avoid the untoward effects that are often associated with high dose pharmacological therapy.

Conflict of interest

No competing interest for Dr. Escobar, Dr. Burke, Prof. Banach, Prof. Fashing. Dr. Cicero has been international lecturer in the field of nutraceuticals, including Armolipid Plus. Prof. Bruckert is the Investigators' Coordinator of an ongoing clinical study with Armolipid Plus. No known conflicts of interest associated with this review and no significant financial support for this work that could have influenced its outcome.

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