

# Pharmacological approach to cardiovascular risk in metabolic syndrome

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**Metabolic syndrome is not a discrete entity with a single pathogenesis, but different complex mechanisms, especially those inducing oxidative stress, play a major role in the genesis of this condition. This consideration suggests that treatment of recognized cardiovascular risk factors alone cannot be enough to prevent cardiovascular events in patients with a diagnosed metabolic syndrome. However, it has been reported that oxidative stress is involved in the transduction of the effects of haemodynamic and metabolic pathological conditions. Thus, drugs acting on the renin-angiotensin system [angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers], or on the glucose or lipid metabolism as substrate of oxidative mechanisms (statins and nutraceuticals) in association with a dietary restriction may be taken in account, because they**

**play a synergistic effect in preventing functional and structural changes responsible for the high cardiovascular risk in metabolic syndrome.**

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## Introduction

The metabolic syndrome is characterized by a constellation of interrelated pathologic conditions of metabolic or haemodynamic nature (abdominal obesity, atherogenic dyslipidaemia, impaired glucose control, or elevated blood pressure levels) that appear to directly promote the development of cardiovascular disease.

According to American Heart Association/National Heart, Lung and Blood Institute guidelines,<sup>1</sup> the primary endpoint in management of the metabolic syndrome is to mitigate the modifiable factors underlying cardiovascular risk (obesity, physical inactivity and atherogenic diet) through lifestyle changes. Then, if the absolute risk remains high, consideration can be given to incorporating drug therapy to the regimen. The priorities of drug therapy are elevation of high-density lipoprotein cholesterol (HDL-C), reduction of blood pressure and plasma glucose: current guidelines for their management should be followed. For instance, when there is hypertension, pharmacologic treatment should start with a drug unlikely to facilitate onset of type 2 diabetes mellitus (T2DM), such as a blocker of the renin-angiotensin system (RAS), followed, if needed, by the addition of a calcium antagonist or a low-dose thiazide diuretic.

However, it is noteworthy to remark that metabolic syndrome is not a discrete entity known to be caused by a single pathogenesis. Thus, treatment of only detectable well-defined risk factors cannot be enough to prevent cardiovascular events in patients with a diagnosed metabolic syndrome.

As support to this hypothesis, it is interesting to note that, in large clinical trials performed to verify the efficacy of specific therapies [statins for dyslipidaemia or angiotensin-converting enzyme (ACE) inhibitors and AT1-receptor blockers (ARBs) for hypertension] in patients with high cardiovascular risk, the reduction of incidence of major cardiovascular events (ranged between 12 and 20%) was constantly greater than expected for the simple risk factor control.<sup>2–4</sup>

This leading article aims to identify pharmacological treatments more prone to interfere with pathogenetic mechanisms underlying metabolic syndrome and, for this reason, more useful to synergistically prevent cardiovascular risk linked to this condition.

## Pharmacological approach to hypertension in metabolic syndrome

It is well known<sup>5</sup> that chronic hypertension induces oxidative stress in brain tissues, provoking neuronal injury. In particular, it has been demonstrated, in a mouse model, that transverse aortic coarctation (TAC) between the two carotid arteries, which imposes on the right brain hemisphere a dramatic increase in blood pressure, is able to evoke selective brain damage through formation of reactive oxygen species (ROS).

At 1 day after TAC, the hippocampus and cortex showed an increase in dihydroethidium (DHE) fluorescent signal as compared with that observed in sham mice, thus reflecting an increased superoxide production in both brain areas. This effect was also present at 7 days from TAC. Notably, DHE fluorescence signal increased

to a similar extent in both cerebral hemispheres. The analysis of brain nitrotyrosine, a marker of oxidative stress action, showed similar results.

Consistent with these findings, analysis of astrocyte activation (an inflammation index of the brain) through glial fibrillary acidic protein (GFAP) immunostaining showed an increase in the immunoreactivity of GFAP-positive cells in the hippocampus and cortex of TAC compared with sham-operated mice. TAC induced a clear-cut activation of these cells whereby phenotypic changes from the quiescent to the activated state became apparent by the enlargement of cell bodies and thickening of processes.

Interestingly, mice treated with ARB candesartan showed a reduction of pressure-induced oxidative stress detected by DHE in the hippocampus, at 1 and 7 days from TAC, compared with mice treated with hydrochlorothiazide, although both treatments produced a similar reduction of blood pressure levels. Furthermore, reduction of pressure-induced oxidative stress in the hippocampus was translated into an improvement of neuronal survival, assessed by neuronal count.

Similarly, in humans, essential hypertension evoked a significant neuronal degeneration in the hippocampus and cortex, evaluated by analysis of N-acetyl aspartate/creatine (NAA/Cr) ratio, which is a reliable marker of neuronal integrity, non-invasively measured by proton nuclear magnetic resonance spectroscopy.

These data suggest that acute, as well as chronic, hypertension induces injury in brain tissues through an increase in oxidative stress, which is successfully antagonized by reduction of superoxide production obtained by ARB therapy. Thus, because RAS blocking decreases ROS production, ARBs may play a key role in prevention of cardiovascular risk dependent on hypertension in the metabolic syndrome.

These experimental findings seem to be corroborated by results of clinical studies. In TROPHY (Treatment Of Pre-Hypertension) trial,<sup>6</sup> the administration of an ARB (candesartan cilexetil 16 mg) to a population with prehypertension (SBP values between 120 and 139 mmHg and DBP values between 80 and 89 mmHg) was able to prevent the onset of hypertension and of cardiovascular events compared to treatment with placebo in a control group. The endpoints reached by ARB treatment in the TROPHY study were also better when compared with those obtained with dietary restriction and physical exercise in Trials of Hypertension Prevention.<sup>7</sup> Furthermore, it has been demonstrated that tissue RAS blockade with ARBs antagonizes the effects of increased angiotensin II (Ang II) synthesis in the myocardium of hypertensive patients, blunting the mechanisms accounting for left-ventricular hypertrophy<sup>8</sup> and improving diastolic ventricular function (Table 1).<sup>9</sup>

## Pharmacological approach to insulin resistance in metabolic syndrome

Insulin resistance is defined as a less than expected biological response to a given concentration of the hormone.<sup>10</sup> For many years, the term insulin resistance has been associated exclusively with an inadequate effect of insulin on glucose metabolism and did not address other aspects of insulin action.

Over the past decade, it became evident that insulin resistance is not merely a problem of impaired glucose homeostasis but a complex and multifaceted syndrome that can affect different organs or tissues with different pathophysiological consequences, such as obesity,<sup>11</sup> dysregulation of vascular tone<sup>12,13</sup> and inhibition of anti-oxidant mechanisms.<sup>14,15</sup> In fact, insulin resistance has been described in several diseases that increase cardiovascular risk and mortality, including metabolic syndrome.<sup>16</sup>

Dysregulation of sympathetic nervous system and RAS, resulting in enhanced stimulation of both adrenergic receptors and angiotensin II receptors, seems to be the principal feature accounting for insulin resistance in several cardiovascular diseases.

It has been documented that stimulation of  $\beta$  adrenergic receptors is able to induce insulin resistance through the activation of different serine/threonine kinases that blunt the insulin signal by phosphorylating insulin receptor or insulin receptor substrate-1 with different mechanisms and kinetics depending upon the receptor subtype expressed. For instance,  $\beta_3$  adrenergic receptor stimulation in adipocytes impairs insulin signalling within a few minutes,<sup>17</sup> whereas in cardiac myocytes, stimulation of  $\beta_1$  adrenergic receptors has a biphasic effect on insulin-stimulated glucose uptake, with an initial additive, followed by an inhibitory, action.<sup>18</sup>

In-vivo and in-vitro studies have shown that Ang II stimulation also induces insulin resistance. Interventional studies have documented that ACE inhibitors<sup>19</sup> and ARB<sup>20</sup> reduce the incidence of T2DM (Table 1). Therefore, overactivity of the RAS observed in cardiovascular diseases is likely to impair insulin signalling and contributes to insulin resistance. Actually, Ang II acting through the AT1 receptor inhibits the actions of insulin in vascular tissue, in part, by interfering with insulin signalling through phosphoinositide 3-kinase and downstream Akt signalling pathways via generation of ROS by nicotinamide adenine dinucleotide phosphate oxidase.<sup>21</sup>

The results of these experimental studies focus on the utility of  $\beta$ -blockers and RAS-blocking agents in clinical treatment of insulin resistance in patients with metabolic syndrome. Abnormalities of glucose, insulin, and lipid and carbohydrate metabolism have been reported frequently during treatment with conventional  $\beta$ -blockers.<sup>22</sup>

**Table 1 Indications for drug use in metabolic syndrome (MS)**

	Hypertension	Insulin resistance/T2DM	Dyslipidaemia	HDL	Obesity	TOD
ACE-I	+++	++	-	-	-	+
AT1 blockers	+++	++	-	-	-	+++
$\alpha$ 1/ $\beta$ 1 blockers	++	+	+	-	-	-
Metformin/TZDs	-	+++	-	+	+	+
Physical activity	+	+	+	+	+	-

The table summarizes indications for the use of discussed pharmacological classes in the treatment of pathologic conditions in patients affected by MS, according to a qualitative scale. -, not effective; +, quite effective; ++, effective; +++, very effective; ACE-I, angiotensin-converting enzyme inhibitor; HDL, high-density lipoprotein; T2DM, type 2 diabetes mellitus; TOD, target organ damage; TZDs, thiazolidinediones.

In a recent and comprehensive network meta-analysis of 22 clinical trials, mostly in patients with hypertension, Elliott and Meyer<sup>23</sup> documented the odds ratio of new-onset T2DM compared with placebo to be highest with diuretics and  $\beta$ -blockers (included studies using predominantly atenolol or metoprolol) and lowest with ARBs and ACE inhibitors.

Conversely, clinical trial data suggest that vasodilating  $\beta$ -blockers, such as carvedilol, a  $\beta$ 1/ $\beta$ 2-antagonist with  $\alpha$ 1-blocking activity, and nebivolol, a highly  $\beta$ 1-selective agent with nitric oxide-mediated vasodilatory effects,<sup>24,25</sup> have neutral or even beneficial metabolic effects, and thus can be potentially useful in patients with hypertension who have or are at risk for the cardiometabolic syndrome or T2DM. The Glycemic Effects in Diabetes Mellitus: Carvedilol–Metoprolol Comparison in Hypertensives (GEMINI) trial<sup>26</sup> compared carvedilol with the nonvasodilating agent metoprolol in 1235 patients with hypertension and T2DM. All patients received background therapy with a RAS blocker. Results showed that the addition of carvedilol, compared with metoprolol, had a favourable effect on glycaemic control, insulin resistance, microalbuminuria and body weight. The highly  $\beta$ 1-selective agent nebivolol has vasodilatory properties that are mediated by its stimulation of nitric oxide release from endothelial cells.<sup>27,28</sup> Studies comparing nebivolol with atenolol<sup>29</sup> and with metoprolol<sup>30</sup> showed neutral effects of nebivolol on insulin sensitivity, whereas both  $\beta$ -blockers increased insulin resistance. That the differences between metoprolol and carvedilol were observed in the presence of an ACE inhibitor or an ARB is important, as it suggests that the metabolic effects of  $\beta$ -blockers remain clinically significant even in the context of metabolically salutary RAS blockade.<sup>31</sup> Thus, combination antihypertensive therapy, even with RAS agents, has the potential to cause further favourable metabolic responses (Table 1).

### Pharmacological approach to type 2 diabetes mellitus in metabolic syndrome

We can distinguish two different pharmacological approaches to treatment of T2DM in metabolic syndrome. The first one aims to antagonize the oxidative burst produced by high plasma concentrations of glucose; the second one to directly reduce hyperglycaemia.

Hyperglycaemia-induced endothelial oxidative stress plays a crucial role in the development of vascular damage in T2DM.<sup>32,33</sup> Among the different sources of ROS reported to be activated by hyperglycaemia, NADPH oxidase<sup>34–36</sup> and Rac-1,<sup>37,38</sup> a small G protein upstream of it, play a major role. In fact, it has been demonstrated that selective Rac-1 inhibition protects from T2DM-induced vascular injury.<sup>39</sup> In particular, aortas from different experimental murine models of T2DM showed higher ROS production, associated with a higher NADPH oxidase and an increased Rac-1 activity, as compared with non-diabetic controls. Furthermore, Rac-1 was mainly localized on the cell membrane in these animals, whereas in vehicle-treated mice, Rac-1 was in the cytosolic fraction. Interestingly, carotid arteries infected with an adenoviral vector carrying a dominant negative Rac-1 mutant (AdN17) were protected against NADPH oxidase activation induced by T2DM. In contrast, vessels infected with an empty adenovirus still showed enhanced NADPH oxidase activation and ROS production. More importantly, analysis of vascular function revealed that in vessels from diabetic mice only infection with AdN17 was able to rescue acetylcholine-induced vasorelaxation, suggesting that selective Rac-1 inhibition significantly improves endothelial function in T2DM. These results were confirmed in endothelial cells. In fact, high glucose levels were able to enhance ROS production as compared with cells exposed to low glucose levels and high glucose-induced ROS generation was blunted by selective inhibition of NADPH oxidase.

Given these findings, we can consider Rac-1 as an important target in preventing endothelial damage mediated by high glucose concentrations. In this regard, a more recent experimental study showed that inhibition of Rac-1 might represent the mechanism by which atorvastatin exerts beneficial vascular effects in T2DM.<sup>40</sup> In endothelial cells exposed to high glucose levels, atorvastatin prevented oxidative stress, and this protection was associated with impaired Rac-1 activation. This effect was also observed in a murine model of T2DM. More importantly, the addition of geranylgeranyl pyrophosphate (GGPP) blocked the effects of atorvastatin in both glucose-exposed endothelial cells and diabetic vessels. Atorvastatin failed to afford protection against

vascular abnormalities in the presence of a constitutively active mutant of Rac-1. The results of this study demonstrated that the vascular antioxidant effect of atorvastatin in T2DM is mediated through inhibition of Rac-1 via a reduction in GGPP. Thus, selective Rac-1 inhibition should be considered in the design of novel pharmacological strategies to reduce the impact of T2DM on vascular function, and statins could play a pivotal role to address this aim. In particular, it has to be mentioned that statins are molecules usually administered to reduce plasma cholesterol concentrations; therefore they could represent an important pharmacological tool to obtain both inhibition of hyperglycaemic and hypercholesterolaemic-induced vascular injury (Table 1).

Concerning specific hypoglycaemic drugs, we have to consider metformin and thiazolidinediones (TZDs). The primary antihyperglycaemic action of metformin results from improved insulin sensitivity, primarily in the liver and secondarily in skeletal muscle.<sup>41,42</sup> Metformin is an antihyperglycaemic rather than a hypoglycaemic drug, since it has little or no effect in normoglycaemic states. Its early use in the treatment algorithm<sup>43</sup> is supported by lack of weight gain, low risk of hypoglycaemia, favourable safety and efficacy profile and by its potential benefits on cardiovascular risk. In this regard, in a clinical study involving 5102 newly diagnosed T2DM patients, largely free of prior major cardiovascular events and followed for an average of 11 years, overweight patients receiving metformin benefited from reductions in the risk of all-cause death, T2DM-related death, myocardial infarction and other adverse combined T2DM-related endpoints when compared to patients receiving other hypoglycaemic therapy regimens.<sup>44</sup> Notably, the benefit of metformin was independent of its glucose-lowering activity. A post-trial monitoring confirmed a long-term benefit in the risk reduction for myocardial infarction and all-cause death after metformin therapy among overweight diabetic patients (Table 1).<sup>45</sup>

Thiazolidinediones are a novel class of drugs that directly decrease insulin resistance by enhancing insulin action in skeletal muscle, liver and adipose tissue.<sup>46</sup> Currently, two compounds – rosiglitazone and pioglitazone – are approved for the treatment of hyperglycaemia in T2DM, as monotherapy or in combination with other oral hypoglycaemic agents. There is evidence suggesting that TZDs can exert potentially beneficial effects on several cardiovascular risk factors.<sup>46</sup> TZDs have favourable effects on blood pressure, systemic inflammation, oxidative stress, endothelial dysfunction and haemostasis. TZDs may also exert a beneficial effect on plasma lipids by increasing HDL-C and decreasing low-density lipoprotein cholesterol (LDL-C) and triglycerides.<sup>46</sup> TZDs have additional beneficial effects on cardiovascular intermediate markers, such as carotid intima-media thickness and coronary atherosclerosis (Table 1).<sup>46</sup>

However, TZDs have not been definitively shown to reduce the risk of future cardiovascular events in T2DM. The PROactive study provided evidence for a beneficial effect on a secondary composite endpoint (all-cause mortality, non-fatal myocardial infarction or stroke) compared with placebo in T2DM patients at high risk for cardiovascular events, followed for an average of 3 years.<sup>47</sup> Lincoff *et al.*<sup>48</sup> confirmed a moderate benefit of pioglitazone therapy on all-cause death, myocardial infarction and stroke in a large meta-analysis. The effects of rosiglitazone on cardiovascular morbidity and mortality are even more controversial. In a meta-analysis including 42 studies of at least 6 months duration, rosiglitazone was associated with a significant increase in the risk of nonfatal myocardial infarction and with a borderline increase in the risk of cardiovascular mortality.<sup>49</sup>

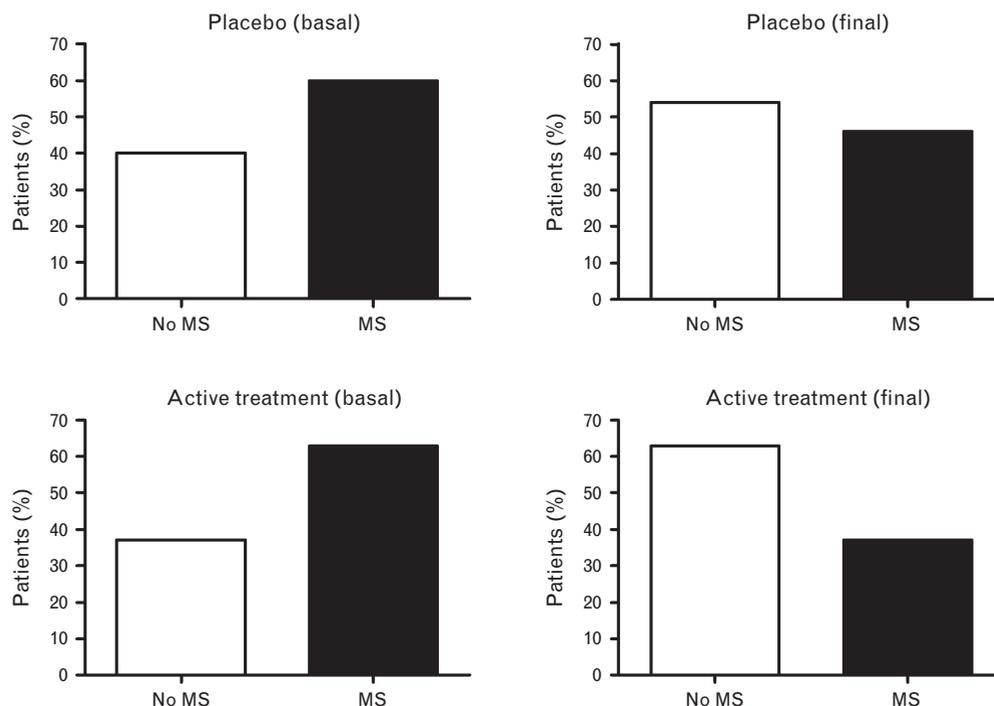
### Pharmacological approach to dyslipidaemia in metabolic syndrome

Together with statins, which have been previously shown to play a role in the reduction of oxidative stress burden,<sup>40</sup> we have to consider a new generation of drugs acting on dyslipidaemia. Natural substances (policosanols, red yeast, berberine, folic acid, coenzyme Q10, astaxanthin) are now available for control of lipid disorders. These molecules, acting on different steps of the pathway of endogenous cholesterol production,<sup>50–55</sup> reduce plasma content of total cholesterol, LDL-C and very low density lipoprotein cholesterol (VLDL-C), decreasing vessel injury derived from oxidation of these components (Table 1).

A clinical study<sup>56</sup> has been recently conducted to test the effect of a formulation containing the above cited molecules on control of cardiovascular risk and metabolic syndrome in patients with alteration of cholesterol and triglycerides. The study recruitment included patients with levels of total cholesterol above 190 mg/dl, LDL-C above 150 mg/dl, triglycerides above 150 mg/dl and in which a therapy with statin or fibrate was not indicated or contraindicated. Two groups of patients were randomized to diet alone and diet + formulation of alimentary integrators (1079 vs. 1223 patients), respectively, and were followed for 16 weeks.

Statistical analysis performed at the end of the study showed a positive, but not significant ( $P=0.09$ ), trend in reduction of metabolic syndrome prevalence (according to National Cholesterol Education Program criteria)<sup>56</sup> for patients in therapy with diet alone, whereas patients undergoing treatment with diet + formulation of alimentary integrators showed a significant decrease in metabolic syndrome ( $P<0.001$ ; Fig. 1). Furthermore, the calculation of cardiovascular risk, obtained by using global cardiovascular risk chart of the CUORE Project,<sup>57</sup> showed a more significant migration from higher to lower cardiovascular risk classes for patients in combined therapy compared with those treated with diet alone

Fig. 1



Comparison of diet and nutraceuticals on metabolic syndrome (MS) regression. Patients with alteration of cholesterol and triglycerides, in which therapy with statin or fibrate was not indicated or contraindicated, were recruited and followed for 16 weeks. Evaluation of MS prevalence was assessed according to National Cholesterol Education Program criteria. Patients undergoing a diet restriction showed a positive, but not significant ( $P=0.09$ ), trend in reduction of MS prevalence, whereas patients treated with diet + formulation of alimentary integrators showed a significant decrease in MS ( $P<0.001$ ).

(Fig. 2). These data allow the conclusion that combination of diet and natural integrators with hypolipidaemic power may represent a useful choice in order to prevent or control metabolic syndrome in patients with contraindication to classic pharmacological therapy.

### Physical activity in prevention and treatment of metabolic syndrome

Physical inactivity lowers insulin sensitivity in skeletal muscles and magnifies the postprandial fluctuations of plasma glucose and triglycerides. Postprandial triglycerides and glucose have been shown to be lowered substantially by walking for 90 min<sup>58</sup> or even by light-intensity exercise,<sup>59</sup> whereas long cumulative sedentary time was associated with higher 2-h glucose levels.<sup>59</sup> Improvements in lipoprotein profile, including in lipoprotein particle sizes, in 111 sedentary overweight adults, were related to the amount (jogging 20 miles a week) of physical activity rather than to its intensity or improvement in fitness.<sup>60</sup> Thus, exercise exerts beneficial effect by improving inflammation by both lowering postprandial metabolic alterations as well as by avoiding the accumulation of abdominal fat (Table 1).<sup>59</sup> In a systematic review of the literature on the influence of physical activity on abdominal fat, 7 out of 10 controlled

trials using imaging techniques to measure change in abdominal fat in overweight or obese patients reported significant reductions compared with controls.<sup>61</sup>

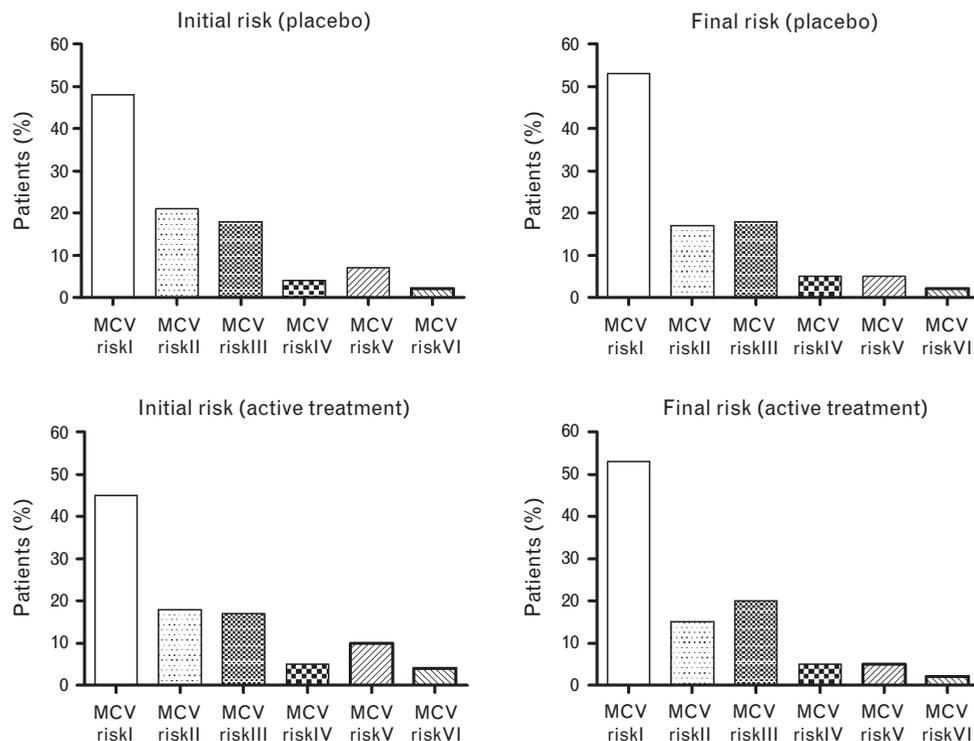
Exercise results in a fall in the markers of inflammation such as plasma C-reactive protein (CRP) level.<sup>62</sup> Although its mechanism is not clear, the development of T2DM is clearly prevented and the risk is reduced among prediabetic people by exercise.<sup>63</sup>

### Conclusions

Unlike other conditions at high cardiovascular risk, it is not possible to distinguish a precise pathophysiology for metabolic syndrome, but it seems that several complex mechanisms are involved in this context. In particular, pathways underlining oxidative stress play a major role in this syndrome. Therefore, in metabolic syndrome, more importantly than in other cardiovascular diseases, it is fundamental to have a drug approach that, blunting at different steps of ROS production, may reduce the burden of vascular damage.

In this regard, molecules acting on RAS (ACE inhibitors and ARBs), NADPH oxidase or other mechanisms of ROS production in glucose and lipid disorders (statins and nutraceuticals, in association with lifestyle changes

Fig. 2



Regression of cardiovascular risk in dyslipidaemic patients after dietetic and combined treatment (diet + nutraceuticals). Major cardiovascular risk was evaluated by using global cardiovascular risk chart of the CUORE Project. The different bars represent the percentage of patients for each major cardiovascular risk class. Major cardiovascular (MCV) risk class I (<5% in 10 years for men, <1% in 5 years for women); major cardiovascular risk class II (between 5 and 10% for men, between 1 and 3% for women), major cardiovascular risk class III (between 10 and 15% for men, between 3 and 5% for women), major cardiovascular risk class IV (between 15 and 20% for men, between 5 and 7% for women), major cardiovascular risk class V (between 20 and 30% for men, between 7 and 10% for women), major cardiovascular risk class VI (>30% for men, >10% for women). Results of this clinical study showed a more significant migration from higher to lower cardiovascular risk classes for patients in combined therapy compared with those treated with diet alone.

and antihyperglycaemic drugs) may be taken into account, since they play a synergistic effect in preventing onset of characteristic alterations responsible for cardiovascular risk in metabolic syndrome.

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