METFORMIN, ARTERIAL FUNCTION, INTIMA-MEDIA THICKNESS, AND NITROXIDATION IN THE METABOLIC SYNDROME

The MEFISTO Study

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Summary

Introduction. Metabolic syndrome (MS) is one of the greatest public health problems in Mexico, where more than 75% of adults in urban populations are overweight or obese. MS has several comorbidities, which yield to a high cardiometabolic risk. Some of the vasopathogenic phenomena in MS are caused by nitroxidant stress, secondary to cardiometabolic disarrangements. The action of metformin (Met) in MS is still a matter of debate. Patients and methods. Sixty patients with at least 3 diagnostic criteria for MS in two groups received similar dietary counseling, but one group received 850 mg of metformin daily. The studied variables were: body mass index (BMI), waist circumference, systolic and diastolic blood pressures (SAP, DAP), total cholesterol (TC), high and low-density lipoprotein cholesterol (HDL-C, LDL-C), triglycerides (TG), fasting glucose (FG), nitroxidant metabolites (free carbonyls, MDA, dityrosines, and protein oxidation advanced products, AOPP), NO, carotid vascular stiffness, carotid IMT and CRP. Results. After one year of follow up, both groups experienced weight loss, waist circumference, SAP and DAP decreased. Met patients displayed reductions in TC, IMT and evoked striking modifications of nitroxidation: carbonyls, dityrosines and AOPP were reduced, and NO was increased expressing better endothelial function. Decreased CRP values were observed in the Met patients. Conclusions. Met has a deep beneficial effect on nitroxidation, endothelial function and IMT in patients with MS.

Key words: Metabolic syndrome, nitroxidation, metformin, intima-media thickness, arterial stiffness

Introduction

There is an increasing problem of abdominal obesity in most industrialized countries,1 as well as in many nations with emerging economies like Mexico.2 Aside from its growing prevalence, abdominal obesity has paramount clinical
and epidemiological implications, as its associated comorbidities confer significant cardiometabolic risk. Very often, abdominal obesity, which has been pathologically linked to insulin resistance and hyperinsulinism, accompanied by pre-hypertension or hypertension, atherogenic dyslipidemia, and abnormal fasting glucose constitute the metabolic syndrome (MS), which is frequently associated with a wider constellation of cardiovascular and metabolic damage factors and markers of metabolic, hemodynamic, oxidative, inflammatory, prothrombotic and atherogenic nature. The true relevance of MS resides in the associated long-term cardiometabolic complications, and cardiovascular morbidity and mortality.

Prevention of MS complications is based primarily on weight loss and the treatment of all comorbidities. A proper low-calorie diet and the practice of frequent dynamic exercise are the cornerstones of both MS treatment and prevention. However, some anti-hyperglycemic agents like thiazolidinediones (pioglitazone, rosiglitazone) and biguanides (metformin) have shown their ability to prevent type 2 diabetes mellitus (DM2) and correct some of the cardiometabolic abnormalities of MS.

Metformin has been used widely as an anti-diabetic drug, but also is indicated in insulin-resistant conditions like polycystic ovary syndrome (PCOS), acanthosis nigricans, in the prevention of DM2 and in patients with MS. Inflammation and endothelial dysfunction are outstanding features of MS that contribute to its pathogenic cardiovascular profile. Until recently, most studies on metformin focusing on oxidative stress, inflammation or endothelial dysfunction have been conducted in animal experiments, in patients with DM2, or in women with PCOS. Limited and conflicting reports about the effect of metformin on several inflammatory and endothelial dysfunction indices in MS with or without DM2 exist. Some studies show an improvement in several markers of endothelial function and/or inflammation, while others show amelioration of some markers of endothelial activation and coagulation but without any effects on inflammatory markers.

The purpose of this work was to study the effect of a moderate dose of metformin in adults with MS, who do not meet the criteria for diabetes mellitus, focusing on several standard clinical and metabolic variables, as well as data on carotid intima-media thickness, arterial stiffness, and some markers of oxidative stress and inflammation.

**Patients and methods**

All procedures were approved by institutional ethics and research committees and all patients signed an informed consent.

Patients of any gender, aged 35 to 60 years old, were selected from the Risk Factors Outpatient Clinic of our institution, if they fulfilled at least three of the five diagnostic criteria of MS. The sample size was calculated according to the reported changes of serum malondialdehyde (MDA), an oxidative molecule which expresses lipoperoxidation with the following formula:

$$N = \frac{(Z_+Z_-)^2(2)(Sp)^2}{d^2} = 12.7 \_ 13 \text{ patients}$$

Where $Sp = 1.8 \text{ nmol MDA/100 mg of protein}$ and $d = 2.0 \text{ nmol MD/100 mg of protein}$
The estimation allowed for an alpha level of 0.05 and a potency of 80%. Patients were randomly assigned to two groups of 30 individuals. The study was conducted following the procedures of Good Clinical Practices and in accordance with international and Mexican official regulations for human clinical investigation.

Patients with overt diabetes, atherosclerotic coronary, cerebral or peripheral diseases, other forms of structural cardiac diseases, severe systemic diseases, malignancies, present or past episodes of heart failure, drug or alcohol abuse, acute or chronic inflammatory diseases, or any other condition likely to interfere with the conduction of the trial were excluded. Elimination criteria included consent withdrawal, the lack of compliance with trial requirements, severe complications, and insurmountable intolerance to metformin.

Weight, height, and abdominal waist circumference were measured, body mass index (BMI) was calculated. Systolic, diastolic and pulse blood pressures (SBP, DBP, PP) were obtained by means of a mercury sphygmomanometer. In the fasting period, a venous blood sample was obtained to measure fasting glucose, creatinine, liver function tests, total cholesterol (TC), HDL-cholesterol (C-HDL), and triglycerides (TG). LDL-cholesterol (C-LDL) was estimated by the Friedewald formula. When the concentration of TG was over 400 mg/dl, instead of C-LDL, the non-HDL cholesterol was estimated (TC – C-HDL).

Analytical procedures.

The concentration of several substances which express diverse stages and substrates of biomolecules oxidation were determined. Free carbonyls.

Quantification of free carbonyls was performed using the method proposed by Dalle-Donne. One-hundred μL of plasma were mixed with 1 mL of 2,4-dinitrofenilhidrazine (DNPH) 10 mM in HCl 2.5 M. Samples were incubated at room temperature in darkness and stirred every 15 minutes for one hour. Then, they were precipitated with a 20% solution of trichloroacetic acid (TCA), centrifuged for ten minutes at 3500 rpm, and the washed again with 10% solution of TCA for the collection of precipitated protein. The samples were centrifuged for ten minutes at 3500 rpm and washed again with a 10% solution of TCA for the collection of precipitated protein. Finally, the precipitate was washed with a 3 mL solvent mixture (1:1) of ethanol plus ethyl acetate to eliminate excessive DNPH. The product was centrifuged again and the new precipitate was dissolved in 1 mL of guanidine 6M in a potassium phosphate solution 20 nM, and incubated for ten minutes at 37°C. The sample was analyzed spectrophotometrically at a wavelength of 370 nm. The coefficient of molar extinction of DNPH is M-1 cm-1 = 22,000/106 nmoL/mL, and i.e. was used to calculate the concentration of free carbonyls, expressed in osazone/mL plasma, corrected for mg of protein and quantified according with Lowry’s method.

Malondialdehyde (MDA).

Malondialdehyde was measured according to the Yagi method, based on the quantification of reactive compounds to thiobarbituric acid (TBARS), which are markers of lipid peroxidation. The procedure was performed by mixing 400 μL of
buffer Tris-preset 7.2 mM at a pH of 8.0 with 100 µL of plasma and 1 mL of acid thiobarbituric (TBA) 0.375% in 0.2N HCl. The mixture was warmed at 90°C for 15 minutes. Later, 0.5 ml of 0.2 N HCl were added and the solution was analyzed spectrophotometrically at 532 nm wavelength in a Beckman UV/VIS spectrophotometer using 1,1,3,3-tetramethoxipropylamine as standard.

Dityrosines
The quantification of dityrosines was performed according to the Lehrer method using a plasma sample resuspended in a 6M urea solution in NaHCO₃ 0.1 M, pH 9.8, incubated at 23°C for 30 minutes. The excitation spectrum for the fluorescence of dityrosines was 280 to 370 nm wavelength. A spectrofluorometer PTI (Photon Technology International) registered the emission at 405 nm.

Advanced oxidative protein products (AOPP)
AOPP were determined using the technique of Capeillére-Blandin. 200 µL of plasma (1:5 dilution in polybenzamine solution, PBS) was mixed with 20 µL of glacial acetic acid. The mixture was stirred for 2 minutes and an optical density was read at 340 nm.

Nitric Oxide
Nitric oxide serum concentration was indirectly quantified measuring nitrites using the Griess reaction. To convert nitrates to nitrites, a 1775 E coli strain was used as a source of nitrate reductase. 250 µL of plasma was incubated with 40 µL of E. coli culture for one hour at 37°C with constant agitation. The sample was centrifuged at 3000 rpm for 5 minutes. 250 µL of the supernatant was mixed with 250 µL of N- (1-naftil) ethylenediamine hydrochloride (0.1g in 100 mL of distilled water) and 250 µL of sulfanilamide (1g in 100 mL of orthophosphoric acid 5%) for one minute. Optical density was read at 540 nm.

C - reactive protein
High sensitivity C-reactive protein (hsCRP) was measured in plasma in duplicate using an ELISA method.

Intima-media thickness (IMT)
IMT of the carotid wall was measured by high resolution carotid artery B-mode echographic scans (Phillips Sonos 500 ultrasonograph) using a vascular transducer (7.5 to 10 mHz), which provided an axial resolution of 0.1 mm. Using a bi-dimensional approach, a segment 1 cm below the carotid bifurcation was selected. Using a bidimensional approach, a segment 1 cm below the carotid bifurcation was selected and the image was freezeed in a place free of gross atheromatosis. Two measurements were performed manually using electronic calipers from the intima-lumen to the intima media interphases in both near (GIM A) and far (GIM B) walls. Also, one cm below the bifurcation, the internal systolic (CSD) and diastolic diameters (CDD) of the main right carotid artery were measured with the electronic caliper using a reference electrocardiographic signal to define both systole and diastole. Carotid stiffness was calculated as follows:

Pulse pressure (PP) or differential pressure (∆P) was determined by subtracting CDD from CSD (S Carotid diameter). Stiffness was estimated using a modified and previously validated formula.
Carotid stiffness = \( \frac{P}{\text{Carotid diameter}} \)

Patients were randomly assigned to one of two groups, one served as the control (C) and the other served as the experimental group and received metformin (Met). All patients received similar nutritional advice. Dietary treatment was supervised by a registered nutritionist. A Mediterranean style diet was prescribed with a caloric content of 20 kcal per “ideal weight” (“ideal weight” \([\text{kg}] = \text{height} \text{[cm]} – 100\)). According to the comorbidities identified in every patient, they received the usual anti-hypertensive and lipid-lowering therapies, using calcium antagonists, ACE inhibitors, or angiotensin II antagonists, and statins to attain the standard goals for blood pressure and lipid profile in accordance with international recommendations.\(^{22,36}\) While patients in the C group were treated in the described way, those in the Met group received 850 mg of metformin once daily. All patients were followed every three months. During every visit, BP measurements, anthropometric variables and secondary unwanted effects were registered. Laboratory tests and ultrasonographic studies were repeated at the beginning and at the end of the study.

Statistical analysis.

Data were analyzed using SSPS13 for Windows. For every numerical variable, the mean value and standard deviation was calculated. A Student t-test was used to compare mean values and standard deviations. Statistical significance was set at a \(p\) value less than 0.05.

Results

Thirty nine patients finished the study (29 women and 10 men). 17 patients belonged to the C group and 22 belonged to the Met group. The mean patient age was 49 ± 8 years in the C group and 49 ± 9.5 years in the Met group. Table 1 shows the basal data of both groups and noticed that there were not significant differences in anthropometric, BP or laboratory variables, indicating homogeneity in both trial groups.

Anthropometric variables

Despite dietary counsel and the strict supervision of a nutritionist, patients of both groups attain a similar and modest weight loss (table 2). BMI was also marginally diminished. The waist circumference was reduced significantly, 3.36 cm in the Met group, and 1.72 cm in the control group. The difference was highly significant in the intra-group (before and after), but not in the inter-group comparison.

Blood pressure

No significant differences were observed in blood pressure values between both groups (Table 3). However, some intra-group differences were significant. SBP decreased significantly in both groups (6 and 7 mm Hg respectively). DBP also was reduced significantly in both groups (5.7 and 4.4 mm Hg respectively), while PP did not change.

Biochemical variables

No significant differences were observed in the inter-group analysis, but there were some in the intra-group comparison (table 4). Fasting glycemic values were not increased significantly in both groups. TC value was reduced 8% (22 mg/dL) in the Met group and unchanged in the control subjects. Although HDCL-C was increased in both groups, the difference only reached significance
in the C group (+17 vs. +10%, respectively). TG values increased in the Met, and decreased in the C group, but those changes were not significant. Also, LDL-C values, which increased slightly in the control group and decreased slightly in patients treated with metformin, were not significantly changed. However, non HDL-C was reduced significantly in both groups (about 20 mg/dL, 12% from the base).

Carotid structural and functional changes
In both groups, combined IMT values were greater than the normal values for this variable (0.6 mm) (Table 5). With respect to the A measurement, a significant reduction of 0.099 mm (9%) in the Met group and slight reduction of 0.021 mm in the control group was observed. At point B, IMT followed a similar trend: IMT diminished slightly but significantly 0.05 mm (4.8%) in the Met group but remained unchanged in the control group. Carotid stiffness increased in the Met group (35 mmHg/mm, +24.5%) and diminished the control (45.3, -26.7%), but these changes did not reach significant values in the inter- or intra-group comparisons.

Nitroxidative stress
Figures 1 to 6 depict the markers of nitroxidative stress, inflammation and oxide nitrite availability, with the latter serving as an indirect sign of endothelial function.
Free carbonyls (figure 1) decreased significantly in the Met group (-51%, p<0.01) while the reduction in the control group was not significant (-3.76%). Malondialdehyde (figure 2) increased in both groups, but the increment was less marked in the Met group (+35%) relative to the control patients (+59%). Nevertheless, the differences between groups and intra-groups were not significant.
Figure 3 shows the behavior of dityrosine, a marker of intermediate nitroxidation. Although the marker decreased in both, the change reached statistical significance in the Met group.
Figure 4 shows AOPP, a marker of advanced nitroxidation. Patients treated with metformin showed a significant marked reduction in AOPP concentrations (-49%), while in the control patients, no considerable change was observed.
Figure 5 shows the differences observed in the concentration of nitrites. While this concentration remained unchanged in the control group, it increased significantly in the experimental group.
Finally, figure 6 shows the concentration of high sensitivity CRP, a nonspecific marker of inflammation. Basal values were less than 0.6 mg/dl, the lower limit of normal. At the end of the study, the mean CRP value decreased significantly in the Met group, but not in the control.

Discussion
Abdominal obesity is often associated with a cluster of comorbidities like high blood pressure, abnormalities in carbohydrate metabolism, the so-called atherogenic dyslipidemia, and very frequently with a wider constellation of metabolic, hemodynamic, humoral, nitoxidative, inflammatory, prothrombotic and endothelial factors or conditions, which altogether compose the metabolic syndrome (MS). Also, MS has several mechanisms of direct vascular damage secondary to
nitroxidative stress, inflammation and reparative tissue healing mechanisms, which are causes and effects of endothelial dysfunction, the common vasopathogenic route of vascular lesions.\textsuperscript{39} Endothelial dysfunction is characterized by a diminished capacity to produce nitric oxide and other molecules implicated in the adequate functioning and structure of endothelium. Among other multiple causes, the reduced capacity of NO generation, nitroxidative stress and the diminishing production of hyperpolarizing endothelial factor are some of the main mechanisms of endothelial dysfunction.\textsuperscript{40} When this happens, dysfunctional endothelium produces many molecules, like proinflammatory and proapoptotic cytokines, adhesion proteins, and monocytes chemoattractant molecules, while the prothrombotic factor PAI-1 is activated.

Many other substances produced locally like angiotensin II and endothelin-1 could have deleterious effects in the arterial wall and nearby myocytes and extracellular matrix.

Other cellular and biochemical mechanisms are involved in the rather complex phenomenon of endothelial dysfunction, such as the accumulation of asymmetric dimethylarginine (ADMA), a guanidine analogue of L-arginine, which competitively inhibits the endothelial NO synthase (eNOS) and therefore interferes with normal NO generation.\textsuperscript{41} The enzyme dimethylarginine dymethylaminohydrolase (DDAH) metabolizes ADMA, which preserves endothelial function. DDAH activity diminishes associated to nitroxidation and endothelial dysfunction due to the fact that oxidation inhibits the enzyme, modifying the sulphydrilic portion of its chemical structure.\textsuperscript{42} In conditions like dyslipidemia, SAH, hyperinsulinism, advanced age, and renal insufficiency, DDAH activity decreases, ADMA concentration increases, and endothelial function decrease. In both MS and DM2, significant nitroxidation occurs.\textsuperscript{43}

The results of the present study identify several important issues about the clinical and therapeutic management of MS. Our data confirms the relative failure of dietary treatment, even if it is tightly supervised, as it was in our study. As has been established in other clinical studies, dietary counsel promotes disappointing results in the vast majority of patients.\textsuperscript{44} The treatment for obesity places too much emphasis on psychological and behavioral aspects;\textsuperscript{45} instead, it should focus on the essential biological aspects of the disease, which are the biochemistry and molecular failure of appetite control.\textsuperscript{46} In this respect, despite the known fact that metformin causes weight loss, no effect on weight and BMI was observed in patients treated with metformin in this study. Although, both groups had a reduced waist circumference at the end of the study. Second, our data indicate that this slight weight decrease was associated with a modest, but significant, BP reduction in both groups, as expected. So, the alleged antihypertensive effect of metformin was not evident in this group.\textsuperscript{47} In the literature, conflicting and controversial evidences about this matter exist.\textsuperscript{48} In spite of several anti-hypertensive mechanisms described: less Ca\textsuperscript{++} entrance into vascular myocyte, the stimulation of the endothelium derived hyperpolarizing factor, the antagonizing effect of insulin pressor activity, the lessening of noradrenergic flow, the amelioration of endothelium function and a greater NO
availability, among other probable causes on clinical grounds, it has been difficult to establish the real effect of the drug on human BP. No effect on fasting glycemia was observed in both groups. Less than 50% of our patients had FG above 100 mg/dL and the hypoglycemic effect of metformin was less obvious, as typically observed in this kind of patients without overt dysglycemia. The modification of blood lipids was very discreet in both study groups. TC descended more in the Met group relative to control patients, while HDL-C, although increased in both groups, showed a greater increase in the control group. TG increased marginally in the metformin group while diminished non-significantly in the control group. LDL-C did not change, but non HDL-C was reduced significantly to the same extent in both groups. These subtle changes seemed to be secondary to the weight reduction as compared to the pharmacological effect of metformin. These observations on the scarce effect of metformin on lipids are in general agreement with other previous studies. In the group treated with metformin, a significant reduction of carotid IMT in the two selected points below the bifurcation was observed. Previous reports have described the anti-atherosclerotic effect of glitazones, secondary to the enhancement of insulin tissue action, the reduction of hyperinsulinism, the interruption of the transcription of proinflammatory signals, and the betterment of endothelial function. The unfolding of IMT was observed in patients with MS, but the effect of metformin on this variable has been described only in patients with MS associated with POCS. Carotid IMT is a sensitive marker of subclinical atherosclerosis with a remarkable power to predict the development of atherosclerotic plaques, and cardiovascular outcomes, mainly myocardial infarction, and stroke. The halt or reduction of IMT is a consequence of the amelioration of blood pressure, lipids, dysglycemia and nitrooxidative stress. In our patients, a reduction in oxidative stress seems to be the main explanation for this anti-atherogenic effect of metformin.

On the other hand, carotid stiffness was reduced in control group, while it was increased (although non-significantly) in the Met group. It has been informed that the drug raises vascular stiffness, function regulated by a very complex interaction of structural and dynamic factors: hemodynamic forces, anatomic composition of the middle arterial tunic, sodium and calcium content in both, vascular wall and interstitium, adrenergic tone, angiotensin II and other substances and hormones actions, and the oxidative stress, among other factors. In any case, the true action of metformin on arterial stiffness remains highly speculative.

The more striking differences between both study groups were the values of the nitrooxidative stress molecules which were analyzed. In comparison with controls, patients treated with metformin show a significant improvement of oxidation, nitrationation, inflammation and NO availability.

In physiological conditions, there is a constant production of intermediate metabolites of oxygen metabolism. Reactive oxygen species (ROS) are obligatorily produced in all tissues, where they play a critical role as molecular signals with the capacity to activate diverse local defense systems. One of the main ROS is a superoxide anion (O₂
produced mostly by the action of NADPH-oxidases. In normal endothelium, the production of NO exceeded the production of O$_2$ and the latter is rapidly neutralized by antioxidant enzymes. Oxidative stress is developed sequentially after the crucial and early phenomenon of endothelial dysfunction occurs, as was described before. When the endothelial cell is dysfunctional, the production of O$_2$ is greater than the production of NO, superoxide oxidizes the NO forming peroxinitrite (ONOO$^-$), starting a cascade of ROS production, free radicals or not, capable of oxidizing several biomolecules like carbohydrates, lipids, proteins and DNA. The metabolite ONOO$^-$ oxidizes sulphhydryl radicals, which initiates the lipoxidation of membranes. The end products of the oxidation, peroxinitroso acid, OH$^-$ radicals and nitrogen dioxide, which are extremely reactive and toxic to all tissues, also initiate lipoxidation. Subproducts of lipoxidation, such as malondialdehyde (MDA) and 4-hidroxynonenal (HNE), are the result of oxidation of polyunsaturated fatty acids, which are a structural part of the external cap of LDL, MDA, its subproduct lysine-MDA-lysine and the advanced glycation endproduct, carboximethyl-lysine (CML) are increased during LDL oxidation, indicating that MDA and its analogues are markers of lipoxidation as well as advanced glycation. Tissue damage observed in dysglycemic conditions is due to the accumulation of reactive carbonyl species (RCS), especially the compounds dicarbonyl, highly oxidants, which are not neutralized by common antioxidants, but for the specific enzymatic system of glioxalase. The oxidation of lipids and the reduction of sugars and aminoacids form dicarbonyl compounds, such as methylgloxal and gioxal, extremely reactive glycant agents, which are involved in the formation of advanced glycation end-products (AGEs), and therefore implicated in protein oxidation, cell and extracellular matrix (EMC) damage. Guanidine compounds, like metformin, inhibit AGE formation reacting with dicarbonyl groups. Carboxyls express autoxidation of carbohydrates and lipids and are the precursors of AGE and advanced lipoxidation end-products (ALEs). When the aminoacid tyrosine is oxidized, it forms nitrotyrosine and dityrosine, markers of an early stage of protein oxidation and degradation. AOPP are oxidized plasma proteins (especially albumin) without oxidant capacities but which are excellent markers of advanced nitrooxidative damage. AOPP concentrations are closely correlated with dityrosine and AGE concentrations, but not with lipoxidation markers. Metformin, aside from its well known anti-hyperglycemic effects (increment of peripheral insulin sensitivity, decreased production of hepatic glucose, and interference with intestinal absorption of sugar) has multiple cellular actions, some of which are incompletely understood. For example, although there are conflicting reports, some authors have found that metformin inhibits the sympathetic system, as well as the expression of multiple transcription factors.
and the inflammatory response of human vascular cells mediated by proinflammatory cytokines, IL-1, IL-6 e IL-8. Also, metformin is involved in the liberation of _-endorphin, a substance that and stimulates hepatic glycogen synthesis on the skeletal muscle, which decreases the levels of glucose, independent of insulin levels. The anti-inflammatory effects of metformin in humans have not been widely studied. Recent observations have revealed that in patients with glucose intolerance, metformin decreases the magnitude of endothelial activation markers, while inflammatory markers remain unchanged.

Metformin also has the capacity to reduce oxidative stress in animal experiments, but this effect has not been properly studied in patients with SM.

The effect of metformin on oxidative molecules in this study displays its powerful anti-nitroxidative properties. The real nature of these actions remain partially understood, but it has been shown that the drug is inactivated via the protein kinase C isoform _ 2 (PKC_ -2).

Metformin, which reduces nitroxidation and inflammation (figure 6), can improve endothelial function, as our data indirectly shows. After a year of treatment and in comparison with control patients in whom the nitrate concentration did not change, the increase of these metabolites indicate greater NO availability in metformin treated patients. Our data show that the levels of CRP were not elevated at baseline (less than 1 mg/dL). However, at the end of the study, patients treated with metformin showed significant decreases in this variable, which likely indicates amelioration of a low-grade inflammatory condition. It has been also shown that part of the antioxidant effect of metformin resides in its scavenger properties, resulting in the elimination of free radicals. Metformin cannot be oxidized by superoxide or hydrogen peroxide, but it is the substrate for oxidation by OH- radicals.

Some clinical studies have proved that metformin improves several inflammatory indices like the expression of vascular cells and intercellular adhesion molecules (VCAM-1, ICAM-1), von Willebrand factor, soluble Eselectin among others. Activation of the NF_-B induces the expression of multiple proinflammatory molecules like IL-6, which in turn stimulates the hepatic production of CRP and IL-8, which activates monocyte recruitment and their adhesion to the vascular wall. At the same time, it arouses neutrophil chemotaxis. Metformin inhibits the expression of NF_-B because it inhibits phophatidylinositol-3-kinase (PI3K-Akt) and in that manner decreases the production of proinflammatory cytokines. However, some studies have not shown a significant effect on RCP.

One of the consequences of carbonyl stress is AGE formation in plasma, cells and interstitium, where they exert agonistic stimulation on specific AGE receptors (RAGE) leading to increased production of oxidants, toxic cytokines, and collagen content in the EMC. The remarkable action of metformin on free carbonyl concentration highlights the drug’s effect on methylglyoxal and glyoxal, with which metformin reacts to form molecules of guanidine-carbonyls, thereby decreasing the deleterious consequences of carbonyl stress. The action of metformin on lipoxydation was less robust. MDA increased in both the metformin and control groups, even though the elevation was less conspicuous.
in the metformin group. The relative failure of this drug with respect to the prevention of lipid oxidation relative to thiazolidinediones has been reported. In this study, metformin showed great ability to reduce intermediate and advanced nitroxidation, abating both dityrosine and AOPP. We believe this is the first report of the action of metformin on advanced nitroxidation.

Conclusions
Metformin is an anti-diabetic agent drug with multiple pleiotropic effects, especially suitable for correcting the cardiometabolic abnormalities of insulin resistance syndrome. However, the effects of the drug on weight, fasting glycemia, blood pressure, and lipids were rather modest or absent. On the other hand, the more significant observations of this study were the profound effect of metformin on several nitroxidation molecules and a modest anti-atherogenic effect (IMT).

Our therapeutic strategy should be based on the fact that the oxidation of biomolecules is a sequential cascade phenomenon, which reflects the vascular functional state better than the classical markers of inflammation and endothelial dysfunction. The concentration of several metabolites derived from nitroxidative stress shows the consequences of oxidative attack on different biological substrates: carbohydrates, lipids and proteins. Our data provide evidence that metformin greatly reduces several indices of nitroxidation in MS, leading to improvements in endothelial function and IMT regression in the carotid arteries. These findings support the use of this drug as a powerful therapeutic tool in MS. Metformin not only retards the development of DM2, but also counteracts nitroxidation, inflammation and atherogenesis, which are all factors that promote the vascular catastrophes associated with MS.