Metformin Improves Endothelial Vascular Reactivity in First-Degree Relatives of Type 2 Diabetic Patients With Metabolic Syndrome and Normal Glucose Tolerance

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OBJECTIVE — Endothelial dysfunction is an early marker of atherosclerosis seen in type 2 diabetic subjects. Metformin is commonly used in the treatment of type 2 diabetes and has known vasculoprotective effects beyond its hypoglycemic ones. We aimed to investigate the vascular effects of metformin in first-degree relatives with metabolic syndrome of type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — The study included 31 subjects (age 38.3 ± 7.6 years and BMI 36.3 ± 5.2 kg/m^2), who were first-degree relatives of type 2 diabetic patients and who had metabolic syndrome and normal glucose tolerance. The subjects were randomly assigned 1:1 in a double-blind fashion to receive placebo (n = 15) or metformin (n = 16). Endothelial function was assessed by venous occlusion plethysmography, measuring forearm blood flow (FBF) and vascular resistance responses to three intra-arterial infusions of endothelium-dependent (acetylcholine 7.5, 15, and 30 μg/min) and independent (sodium nitroprusside 2, 4, and 8 μg/min) vasodilators. Weight, BMI, systolic and diastolic blood pressure, waist, and laboratory parameters (lipid profile and fasting plasma glucose [FPG]) were assessed at baseline and after treatment.

RESULTS — The metformin and placebo groups did not differ in anthropometric, clinical, laboratory, and vascular measurements at baseline. The metformin group had decreased weight, BMI, systolic blood pressure, and FPG and improved lipid profile. Endothelium-dependent FBF responses were also improved, without any effect on endothelium-independent responses. There was no correlation between the improvement on FBF responses and the observed changes on anthropometric, clinical, and laboratory parameters.

CONCLUSIONS — We concluded that metformin improved vascular endothelial reactivity in first-degree relatives with metabolic syndrome of type 2 diabetic patients, independently of its known antihyperglycemic effects.

Diabetes Care 29:1083–1089, 2006

The precocious and accelerated atherosclerosis seen in type 2 diabetes raised the question about pathogenetic factors that initiate the development of vascular derangements in the prediabetic population. Metabolic syndrome, a pre-diabetic state, comprises an array of cardiovascular risk factors such as abdominal obesity, dyslipidemia, hypertension, impaired glucose tolerance, and insulin resistance. Insulin resistance, the central abnormality for the pathogenesis of metabolic syndrome, is considered an independent risk factor for cardiovascular mortality in general (1) and in the diabetic population (2) in particular.

The endothelium is an important locus for the control of vascular function. It actively regulates vascular tone, permeability to leukocytes and macromolecules, the balance between coagulation and fibrinolysis, composition of the subendothelial matrix, and proliferation of vascular smooth muscle cells. The great variety of beneficial functions attributed to the endothelium is mainly associated with nitric oxide (NO) bioavailability. In experimental studies of atherogenesis, the damage to the endothelium is the initiating event. An early marker of endothelial dysfunction is the reduction of endothelium-dependent vasodilation due to reduced bioavailability of NO (3).

Endothelial dysfunction precedes and predicts clinical macrovascular disease (4) and should be considered as a target for different therapeutic interventions. Endothelial function can be assessed, for clinical and research purposes, by measuring vascular reactivity to acetylcholine (ACh), which directly stimulates NO production via muscarinic receptors. Abnormal vasomotor responses occur in the presence of traditional risk factors, such as hypercholesterolemia, hypertension, smoking, diabetes, a low HDL cholesterol level, and hyperhomocysteinemia (5,6). Insulin resistance, obesity (7), and visceral fat accumulation (8) in nonobese men were associated with endothelial dysfunction, which was also observed in first-degree relatives of type 2 diabetic patients (9,10). Several mechanisms that could impair endothelial function are associated with insulin resistance. There is increasing evidence that hyperglycemia is only a worsening factor for endothelial dysfunction and not the triggering one. It is hypothesized that endotheliopathy precedes type 2 diabetes (11). Therefore, comprehension of the mechanisms responsible for impaired insulin action are fundamental to understand possible favorable effects of insulin sensitizers.

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Received for publication 4 November 2005 and accepted in revised form 28 January 2006.

Abbreviations: ACh, acetylcholine; FBF, forearm blood flow; FFA, free fatty acid; FPG, fasting plasma glucose; SNP, sodium nitroprusside.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

DOI: 10.2337/dc05-2146

Table 1—*Anthropometric, clinical, and laboratory characteristics of both groups at baseline and after treatment*

	Placebo		Metformin	
	Baseline	After treatment	Baseline	After treatment
Sex (female/male)	9/6		13/3	
Age (years)	37 (32–41)		40 (33.5–50)	
Known hypertension	7 (46.7)		8 (50)	
Antihypertensive drug use	5 (33.3)		6 (37.5)	
Weight (kg)	100.3 (84.6–109.9)	102.2 (86.2–111.6)†	83.5 (78.6–96.7)	82.5 (77.5–96.4)*
BMI (kg/m^2)	36.7 (34.3–40.2)	37.2 (33.9–41.1)†	34.2 (29.8–39.5)	33.6 (30.1–38.3)*
Waist (cm)	104 (98–112)	105 (96–113)	97.5 (90.5–104.5)	97.5 (92.5–105.5)
Systolic BP (mmHg)	140 (126–149)	140 (124–145)	143 (123.5–149.5)	133.5 (124–141.5)*
Diastolic BP (mmHg)	84 (75–91)	83 (71–88)	80.5 (73–87.5)	78 (72–87.5)
FPG (mmol/l)	4.88 (4.71–5.21)	5.05 (4.77–5.38)	5.21 (4.80–5.43)	4.96 (4.49–5.24)*
Postload plasma glucose (mmol/l)	5.55 (4.94–6.05)		6.13 (5.55–6.82)	
Total cholesterol (mmol/l)	4.78 (4.24–5.27)	5.12 (4.70–5.89)	5.37 (4.93–5.76)	5.06 (4.59–5.67)*
LDL cholesterol (mmol/l)	3.10 (2.76–3.80)	3.46 (3.07–3.82)	3.36 (2.97–4.00)	3.09 (2.58–3.62)‡
HDL cholesterol (mmol/l)	0.93 (0.90–1.13)	1.00 (0.87–1.21)	1.03 (0.93–1.30)	1.21 (0.89–1.38)*
Triglycerides (mmol/l)	1.38 (1.19–2.21)	1.33 (1.10–1.83)	1.83 (1.28–2.32)	2.09 (1.22–2.53)

Data are *n*, median (first to third quartile), or *n* (%). *$P < 0.05$; ‡$P < 0.01$, comparisons in metformin group; †$P < 0.05$, comparisons in placebo group. BP, blood pressure.

Metformin exerts an antihyperglycemic effect, with minimal risk of hypoglycemia, and has been recently used to prevent type 2 diabetes with a 31% reduction in incidence (12). Moreover, in overweight type 2 diabetic patients, metformin was associated with a decrease in macrovascular morbidity and mortality, which appears to be independent of the improvement in glycemia control (13). This observation suggests that this drug may affect the risk of atherothrombotic disease through mechanisms other than lowering glycemia, possibly by vasculoprotective effects. Our aim was to elucidate whether metformin had vasculoprotective effects when used in subjects at risk for developing type 2 diabetes, such as individuals with metabolic syndrome who are the first-degree relatives of type 2 diabetic patients.

RESEARCH DESIGNS AND METHODS

All subjects were selected at the Cardiometabolic Clinic for outpatient care of the State University of Rio de Janeiro. First-degree relatives with metabolic syndrome of type 2 diabetic patients were selected according to National Cholesterol Education Program Adult Treatment Panel III criteria (14); those with glucose intolerance or type 2 diabetes were excluded. Fifty subjects were recruited for the study (11 men and 39 women). Nineteen subjects were excluded because they did not have adequate compliance ($n = 9$), decided not to be subjected to another endothelial function test ($n = 9$), or had severe diarrhea ($n = 1$). The eligible 31 subjects were divided into placebo ($n = 15$) and metformin ($n = 16$) groups (Table 1). At baseline, the study sample had the same anthropometric, clinical, laboratory, and vascular parameters as the dropouts.

All subjects gave their written informed consent. The local ethics committee approved the protocol.

Anthropometric, clinical, and laboratory measurements

The same trained examiner collected anthropometric measurements in duplicate at baseline and after the treatment period: waist at its smallest point with the abdomen relaxed and weight using a digital scale (Filizola, São Paulo, SP, Brazil). BMI was defined as weight in kilograms divided by the square of height in meters. Blood pressure was also measured twice in the supine position with a 5-min interval of rest between the measurements, using an automated apparatus (multiparameter patient monitor, Lifewindow LW6000; Digicare Biomedical Technology, West Palm Beach, FL).

Study design

At the first clinical visit, patients were subjected to a physical examination and then underwent a 75-g oral anhydrous glucose tolerance test (fasting and 2 h) and a lipid profile determination after a 10- to 12-h fast. All subjects enrolled had normal glucose tolerance test results according to American Diabetes Association criteria (15) and at least three criteria for metabolic syndrome according to the National Cholesterol Education Program Adult Treatment Panel III (14). The main exclusion criteria were pregnancy, type 2 diabetes, smoking, major illnesses, a history of previous myocardial infarction or angina pectoris, postmenopausal status, use of oral contraceptives, and a triglyceride level >600 mg/dl.

Subjects were randomly assigned in a double-blinded fashion, 1:1, comparing metformin to placebo. Pills were taken for at least 90 days. During the 1st week of treatment, only the dinner pill was taken to minimize gastrointestinal side effects. After this period, pills were administered at lunch and dinnertime. Metformin pills were prepared with 850 mg/pill by Merck-Santé (Lyon, France). Compliance was certified every 30 days. All subjects were asked to maintain their usual diet and physical activity. Except for antihypertensive drugs, which were not changed during the study, any other drugs were not accepted for use without previous communication.

Endothelial function assessment

Endothelial function was evaluated by measuring forearm blood flow (FBF) responses to intra-arterial infusions of three increasing doses of endothelium-dependent (ACh; 7.5, 15, and 30 μg/min) and independent (sodium nitroprusside

[SNP]; 2, 4, and 8 μg/min) vasodilators in the left arm, using venous occlusion plethysmography (Hokanson EC6; D.E. Hokanson, Bellevue, WA) with a mercury-in-Silastic strain gauge. The study was performed with the subject in the supine position, after an 8- to 10-h overnight fast, in the morning in a quiet, temperature-controlled room (20–22°C), and after the subject had emptied his or her bladder.

Forearm length (medial epicondyle of humerus to ulnar styloid) and maximal circumference were measured using a flexible tape. To avoid underestimation of FBF measurements, the forearm circumference was required to be <28 cm in all subjects. A 27-gauge steel spinal needle (Becton Dickinson, Juiz de Fora, MG, Brazil) was inserted into the left brachial artery anterior to the elbow under sterile conditions and local anesthesia.

After this procedure, FBF, blood pressure, and heart rate were obtained and followed during a 20-min rest period. Noninvasive blood pressure was measured on the right arm in each period.

The mercury-in-Silastic strain gauge was placed on the upper third of the forearm, close to the point of maximal circumference. This method gives values of FBF in milliliters per minute per 100 ml tissue. The arm-collecting cuff pressure was 40 mmHg, and the wrist cuff occlusion pressure was 200 mmHg. The wrist cuff was inflated 1 min before each flow measurement. By blocking venous efflux with the upper arm cuff, the slope of change in forearm volume expresses FBF.

Baseline FBF was recorded after a 20-min infusion of 0.9% NaCl at a rate of 0.8 ml/min controlled by an infusion pump (Harvard Apparatus, Holliston, MA). ACh (Acetilcolina; USP, São Paulo, SP, Brazil) and SNP (NPS; Lebon, Porto Alegre, RS, Brazil), dissolved in 0.9% NaCl, were infused at rates of 0.2, 0.4, and 0.8 ml/min to assess different drug concentrations. For practical purposes, we decided to change the infusion rates of ACh and SNP to achieve different drug concentrations because it is well described that until the intra-arterial infusion reaches 1 ml/min, there is no detectable change in the blood flow measurement (16). ACh and SNP were infused at progressive doses of 7.5, 15, and 30 and 2, 4, and 8 μg/min, respectively, for 5 min each. The blood flow, measured at the last 2 min for each dose, was recorded for 10 s in every 15 s. An interval of 20 min was allowed between each drug, using a 0.9% NaCl infusion at a rate of 0.8 ml/min.

The first flow measurement was always excluded from the analysis. The mean of four measurements in each recording period was used for the analysis, and their coefficients of variation varied from 8.44 to 15.41% in both groups at baseline and after treatment.

Forearm vascular resistance was calculated by dividing mean blood pressure by FBF in each recorded period. Heart rate was measured continuously using lead II of the electrocardiogram. The venous occlusion plethysmograph was connected to an analog-to-digital converter (PowerLab/8SP; AD Instruments, Castle Hill, Australia), and data were analyzed by PowerLab software (8SP; AD Instruments) on an IBM-compatible PC.

Laboratory analysis

All laboratory measurements were performed in duplicate, after a 10- to 12-h fast using an automated method (Modular Analytics PP; Roche, Basel, Switzerland). Fasting plasma glucose (FPG), total cholesterol, triglycerides, and HDL cholesterol were measured by enzyme-colorimetric GOD-PAP (interassay coefficient of variation [IECV] = 1.09%), enzymatic GPO-PAP (IECV = 2.93%), enzymatic GPO-PAP (IECV = 1.29%), and enzyme-colorimetric without pretreatment (IECV = 3.23%), respectively. Plasma LDL cholesterol was calculated according to the Friedewald equation.

Statistical analysis

Data were analyzed by Statistic 6.0 software (STATSOFT, Tulsa, OK). Comparisons between groups at baseline and after the treatment period were done using a Mann-Whitney *U* test and Wilcoxon matched pair test, respectively. Frequency comparisons by tables 2×2 were made by Yates corrected χ^2 tests. Correlation analysis was performed by a Spearman rank-order test. Significant differences were assumed to be present at $P < 0.05$. All group data are reported as median (first to third quartiles).

RESULTS — There was no difference between the number of days of treatment for the placebo and metformin groups (median 109 [range 101–112] vs. 102.2 [97–112.5] days, $P = 0.21$). Mean/maximal durations of treatment for placebo and metformin were 106.7/116 and 102.5/116 days, respectively. In both groups ~50% of subjects has a known history of hypertension, and there was no difference between them. Anthropometric, clinical, laboratory, and vascular reactivity measurements at baseline were not different between the groups (Table 1). Basal FBF at baseline correlated with basal FBF after treatment ($r = 0.58$, $P < 0.001$), an index of good reliability of the method.

Anthropometric, clinical, and laboratory changes

There were no changes in clinical and laboratory measurements in the placebo group, except for increased weight ($P < 0.05$) and BMI ($P < 0.05$). In the metformin group, weight ($P < 0.05$), BMI ($P < 0.05$), systolic blood pressure ($P < 0.03$), total cholesterol ($P = 0.01$), LDL cholesterol ($P < 0.01$), and FPG ($P = 0.01$) decreased and HDL cholesterol increased ($P < 0.05$) (Table 1).

Vascular changes

In the pooled group, basal FBF measurements before ACh and SNP infusions correlated at baseline and after the treatment period ($r = 0.47$, $P = 0.01$ and $r = 0.72$, $P < 0.01$). There were no significant differences between basal FBF at baseline and after the treatment period in the placebo and metformin groups (2.84 [1.77–3.55] vs. 2.89 [1.67–3.47] ml · min^{-1} · 100 ml tissue^{-1}, $P = 0.36$, and 2.37 [1.83–2.67] vs. 2.13 [1.53–2.72] ml · min^{-1} · 100 ml tissue^{-1}, $P = 0.31$).

Endothelium-dependent and -independent vasodilations were kept unchanged in the placebo group. In contrast, metformin was associated with an improvement in endothelium-dependent FBF by 111% at the middle dose (114 [76–243] vs. 241 [78–416]%, $P = 0.01$) (Fig. 1) and by 49% at the highest dose (217 [132–335] vs. 323 [266–586]%, $P < 0.03$), with no change in endothelium-independent responses (Fig. 2).

There was no difference in FBF after metformin use between those who were taking ($n = 6$) or not taking ($n = 9$) antihypertensive drugs. Improvement in endothelium-dependent FBF was noted only when both groups were pooled. We did not observe any direct relationship between the improvement in endothelium-dependent FBF at the middle dose and observed changes for weight ($r =$

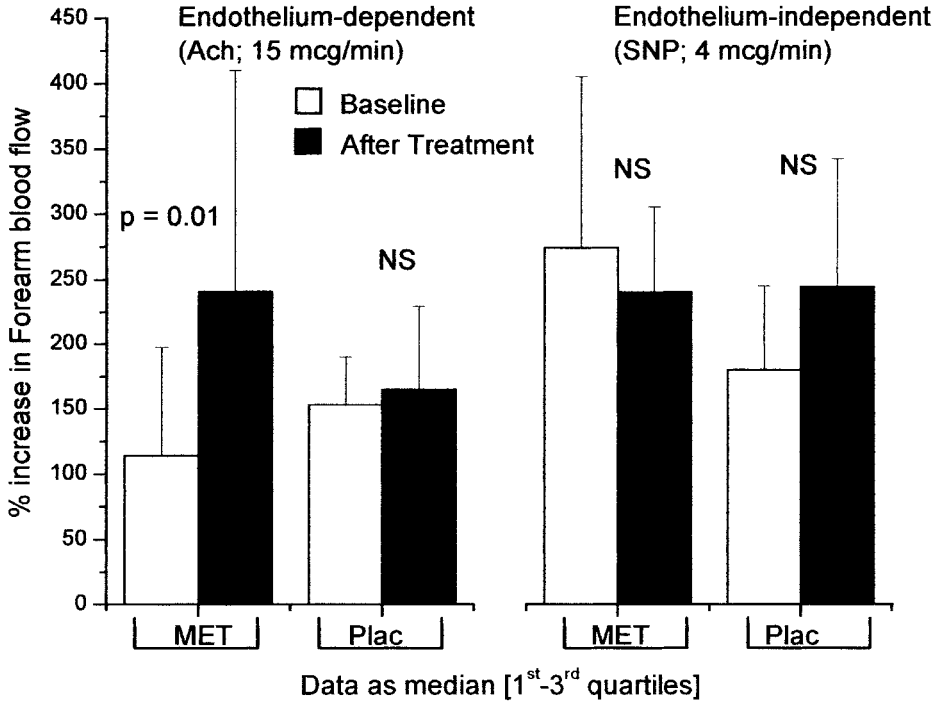

Figure 1—*Endothelium-dependent and -independent vasodilation at middle dose, at baseline, and after treatment in both groups. Met, metformin; Plac, placebo.*

0.39, $P = 0.80$), BMI ($r = 0.04$, $P = 0.86$), systolic blood pressure ($r = 0.37$, $P = 0.17$), FPG ($r = -0.44$, $P = 0.08$), total cholesterol ($r = -0.27$, $P = 0.32$), LDL cholesterol ($r = 0.11$, $P = 0.67$), and HDL cholesterol ($r = -0.12$, $P = 0.65$).

CONCLUSIONS— The benefits of metformin have been well demonstrated in type 2 diabetes. In the U.K. Prospective Diabetes Study (UKPDS), the metformin-treated group showed reduced diabetes-related end points (13). The progression of intima-media thickening in the carotid artery, a marker of atherosclerosis, was reduced by the use of metformin associated with a sulfonylurea for 3 years compared with the use of a sulfonylurea alone (17).

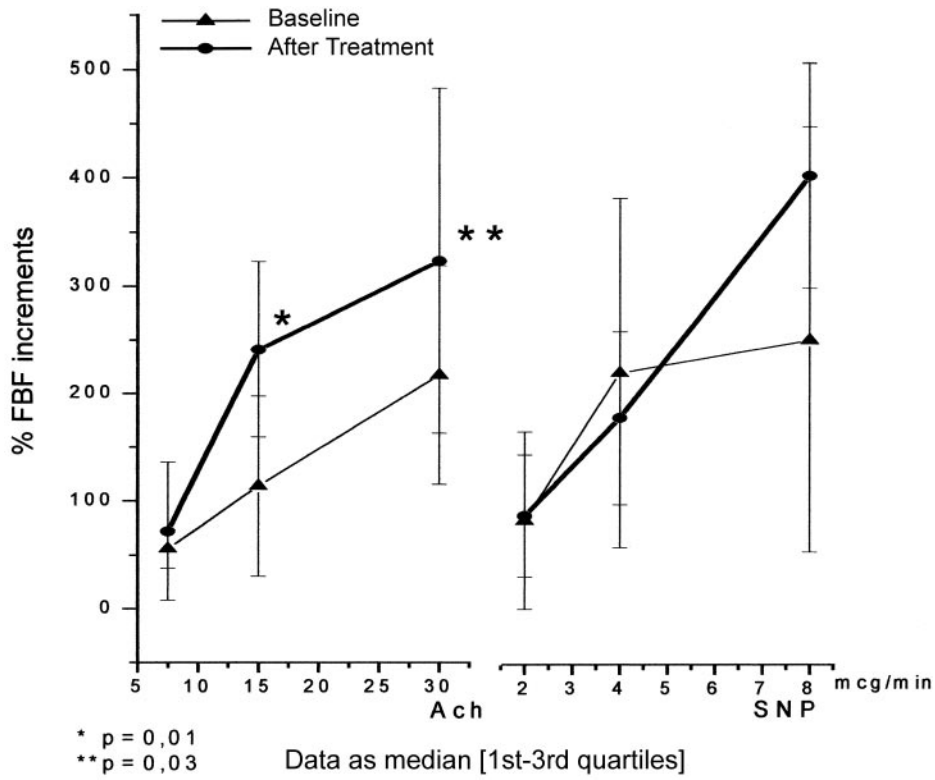

Figure 2—*Percent increase in endothelium-dependent and -independent FBF at baseline and after treatment with metformin.*

Two key features in the pathophysiology of atherothrombosis are endothelial dysfunction and low-grade inflammation of the vascular wall. There is a strong association between markers of endothelial dysfunction, chronic low-grade inflammation, and increased risk of atherothrombotic disease (18). In type 2 diabetic patients, short-term use of metformin improved markers of endothelial dysfunction and inflammatory activity, such as plasma von Willebrand factor, soluble vascular adhesion molecule 1, soluble E-selectin, tissue-type plasminogen activator, and plasminogen activator inhibitor 1, which were largely unrelated to changes in glycemia control (19). Considering that metformin in diabetic patients has vasculoprotective effects and that endothelial dysfunction is the first manifestation of the atherosclerotic process, which is well established in insulin resistance states (5,7), perhaps early interventions would be of value in subjects at risk of developing type 2 diabetes.

We had a high percentage of dropouts (38%) for many reasons, but at the end of the study we had similar groups according to the number of subjects studied and also to clinical, laboratory, and vascular parameters observed at the baseline in each group. The study sample represents the whole group because they had the same baseline characteristics as the dropouts.

Impaired measurements of coronary endothelial function predicted long-term atherosclerotic disease progression and increased cardiovascular event rates (4). Additionally, the impairment of the endothelium-dependent vasodilation was also associated with future cardiovascular events in patients surviving an episode of an acute coronary syndrome (20).

All subjects maintained the same patterns of diet, physical activity, and antihypertensive drugs during the study. In first-degree relatives of patients with type 2 diabetes without any degree of glucose intolerance, not only did clinical and laboratory parameters improve but endothelium-dependent vasodilation also improved after metformin treatment. This is the first known study in which the short-term vascular benefits of metformin in subjects at risk for type 2 diabetes with a normoglycemic state observed after muscarinic receptor stimulation in forearm resistance vessels were prospectively evaluated.

Some authors observed impaired endothelium-dependent vasodilation in first-degree relatives of type 2 diabetic patients even in a normoglycemic state, although the group studied had higher BMI and waist-to-hip ratios than control subjects (10), which is consistent with features of insulin resistance. Almost the same findings were presented in another study that showed reduced vascular reactivity in the normoglycemic state, when only insulin resistance was present (9). Both studies also indicated that when the hyperglycemic state is well established, there is greater reduction in endothelium-dependent vasodilation. Such findings suggest that endothelial dysfunction is an early alteration in the atherogenic process observed in subjects at risk for developing of type 2 diabetes, and perhaps interventions for insulin resistance and endothelial dysfunction, even in subjects without any degree of glucose intolerance, could retard the atherogenic process and possibly reduce the cardiovascular risk.

The most important tissues involved in the pathogenesis of insulin resistance are muscle and adipose tissue. Controversy exists as to whether free fatty acids (FFAs) are or glucose is the primary fuel source in the overnourished muscle and adipose tissue. In either case, an influx of substrates in the citric acid cycle activity generates an excess of mitochondrial NADPH and subsequent production of reactive oxygen species, resulting in oxidative stress. It is hypothesized that the same mechanism may also occur in endothelial cells (21).

The main factor associated with vasodilation at the endothelial level is NO. ACh acts to stimulate endogenous endothelial NO production. Our study showed that augmented responses of endothelium-dependent vasodilation after metformin use are possibly due to an increased bioavailability of NO. Such findings could not be duplicated with the use of SNP, an NO donor. Experimental and clinical data obtained with metformin have progressively revealed its mechanism of action to reduce hyperglycemia and insulin resistance (22). Metformin has multiple biological effects, among which are less recognized vascular actions. The 40% reduction in cardiovascular risk observed in metformin-treated type 2 diabetic patients in the UKPDS (13) confirmed previous evidence (23) that this drug has vasculoprotective actions that are largely independent of its known antihyperglycemic effects.

Our findings did not show any correlation between FPG changes with metformin use and improvement in endothelium-dependent vasodilation. Probably, the use of metformin led to better insulin sensitivity, which in turn promoted an additional reduction in FPG. It seems reasonable to suppose that the observed changes in FPG to normal levels would not be the promoting factor for the improvement observed in endothelial vascular reactivity.

Although metformin significantly reduced BMI, weight, and systolic blood pressure and ameliorated the lipid profile, changes that could be considered vasculoprotective actions, we did not observe any correlation between such changes and the improvements observed in endothelial vascular reactivity. Adipose tissue has the capacity to directly trigger endothelial dysfunction by secreting a variety of molecules with local and systemic actions (adipokines) (24). Probably the use of metformin for long-term follow-up in subjects at risk for developing type 2 diabetes would result in beneficial effects in systolic blood pressure, BMI, weight, and lipid profile, leading to an indirect benefit of the vascular function.

Many clinical and experimental data show the beneficial vascular actions of metformin (25,26). It reduces elevated levels of plasminogen activator inhibitor 1 and factor VII, augmenting fibrinolysis. In addition, recent studies indicate that metformin has direct effects on fibrin structure/function and stabilizes platelets, two important components of an arterial thrombus. Metformin has been also shown to improve endothelium-dependent vasodilation in rats (27) and in patients with type 2 diabetes (28). Additionally, it has been recently shown that in subjects with metabolic syndrome, metformin improved flow-mediated vasodilation of the brachial artery (29).

Endothelial cells have the capacity to store triglycerides, which are continuously hydrolyzed to provide an endogenous triglyceride source of FFAs (30). Endothelial triglyceride storage is often elevated in insulin-resistant states, contributing to FFA overexposure. Recently it has been hypothesized that in insulin-resistant states, metformin could exert its vascular effects by its ability to activate endothelial AMP-activated kinase (31), which in turn promotes FFA oxidation in endothelial tissue. It seems reasonable to suppose that this is an additional way to deal with endothelial lipotoxicity, and subjects at risk of developing type 2 diabetes might benefit from the ability of metformin to activate endothelial AMP-

activated kinase. Furthermore, metformin can enhance muscular insulin sensitivity, aid in weight loss, modestly improve lipid profile and hemostatic parameters, and, of particular importance, slow the onset of diabetes (12) or the development of metabolic syndrome (32). Perhaps with long-term use, these improvements could be considered additional beneficial indirect factors acting simultaneously with the direct properties of metformin on endothelial function.

We conclude that in first-degree relatives of type 2 diabetic patients who have metabolic syndrome and normal glucose tolerance, metformin treatment improved forearm endothelial vascular reactivity in resistance vessels despite its antihyperglycemic actions and its capacity to improve clinical and laboratory parameters. Our findings in this patient population may indicate that metformin slows the progression of atherogenic processes and improves cardiovascular outcome, but they will need to be confirmed by additional long-term follow-up studies.

Acknowledgments— This study was supported by grants from Merck-Santé, Lyon, France, and Sociedade de Pesquisas em Microcirculação S/C, Rio de Janeiro, RJ, Brazil.

The authors thank Cristiane Maria Simonato Conde, Francisco Araujo, Rodrigo Bouskela Torres, and Sofia Gama for excellent technical assistance.

Parts of this study were previously presented in abstract form [*Diabetes* 54 (Suppl. 1):A150, 2005].

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