

Improved Endothelial Function With Metformin in Type 2 Diabetes Mellitus

Kieren J. Mather, MD,* Subodh Verma, MD, PhD,† Todd J. Anderson, MD‡
Indianapolis, Indiana; and Toronto and Calgary, Canada

- OBJECTIVES** This study was designed to assess the effect of metformin on impaired endothelial function in type 2 diabetes mellitus.
- BACKGROUND** Abnormalities in vascular endothelial function are well recognized among patients with type 2 (insulin-resistant) diabetes mellitus. Insulin resistance itself may be central to the pathogenesis of endothelial dysfunction. The effects of metformin, an antidiabetic agent that improves insulin sensitivity, on endothelial function have not been reported.
- METHODS** Subjects with diet-treated type 2 diabetes but without the confounding collection of cardiovascular risk factors seen in the metabolic syndrome were treated with metformin 500 mg twice daily (n = 29) or placebo (n = 15) for 12 weeks. Before and after treatment, blood flow responses to intraarterial administration of endothelium-dependent (acetylcholine), endothelium-independent (sodium nitroprusside) and nitrate-independent (verapamil) vasodilators were measured using forearm plethysmography. Whole-body insulin resistance was assessed on both occasions using the homeostasis model (HOMA-IR).
- RESULTS** Subjects who received metformin demonstrated statistically significant improvement in acetylcholine-stimulated flows compared with those treated with placebo (p = 0.0027 by 2-way analysis of variance), whereas no significant effect was seen on nitroprusside-stimulated (p = 0.27) or verapamil-stimulated (p = 0.40) flows. There was a significant improvement in insulin resistance with metformin (32.5% reduction in HOMA-IR, p = 0.01), and by stepwise multivariate analysis insulin resistance was the sole predictor of endothelium-dependent blood flow following treatment (r = -0.659, p = 0.0012).
- CONCLUSIONS** Metformin treatment improved both insulin resistance and endothelial function, with a strong statistical link between these variables. This supports the concept of the central role of insulin resistance in the pathogenesis of endothelial dysfunction in type 2 diabetes mellitus. This has important implications for the investigation and treatment of vascular disease in patients with type 2 diabetes mellitus. (J Am Coll Cardiol 2001;37:1344-50) © 2001 by the American College of Cardiology

Vascular disease is a major cause of morbidity and mortality among patients with diabetes mellitus (DM), and these patients account for a significant proportion of all patients with cardiovascular disease (1). Abnormalities in endothelium-dependent vascular responses, perhaps the earliest demonstrable alteration in vascular health, have been found among patients with type 2 DM (2,3). The origins of these alterations remain poorly understood. The contributions of a number of potentially important variables have been examined, including hyperglycemia (4), altered antioxidant balance (5,6), and alterations in circulating lipid composition including elevated free fatty acids (FFA) (7).

More recently, the question of the contribution of insulin

resistance (IR) to vascular abnormalities in DM has been raised. Insulin resistance is felt to be central to the pathogenesis of the metabolic syndrome (Syndrome X), a clustering of traditional cardiovascular risk factors in patients with type 2 DM (8). Furthermore, IR per se has a direct impact on endothelial function, distinct from effects related to unfavorable changes in lipid composition or blood pressure (9). Therefore, IR presents an important variable both with regard to understanding the pathogenesis of cardiovascular disease and as a potential therapeutic target.

The question arises as to the effect of therapies targeting IR on endothelial function in particular and cardiovascular disease in general. Metformin, a medication of the biguanide family, is now widely available. Its mechanism of action is poorly understood, but includes a reduction in whole-body IR (10). The effects of altering IR with metformin on endothelial function in humans have not been previously reported. In a placebo-controlled study, we measured forearm resistance vessel endothelial function in patients with type 2 (insulin-resistant) DM, but without other classical cardiovascular risk factors, before and after three months' treatment with metformin.

From the *Division of Endocrinology and Metabolism, Indiana University, Indianapolis, Indiana; †Division of Cardiac Surgery, University of Toronto, Toronto, Canada; and the ‡Division of Cardiology, University of Calgary, Calgary, Canada. This study was supported by the Alberta Heart and Stroke Foundation, Edmonton, Alberta, Canada. We gratefully acknowledge the financial support of the Dr. Howard MacEwan Diabetes Center Research Fund. At the time of this study, Dr. Mather was supported by a Clinical Research Fellowship from the Alberta Heritage Fund for Medical Research (AHFMR). Dr. Verma was also supported by the AHFMR. Dr. Anderson is an AHFMR Scholar.

Manuscript received August 11, 2000; revised manuscript received November 17, 2000, accepted December 21, 2000.

Abbreviations and Acronyms

Ach	=	acetylcholine
ANCOVA	=	analysis of covariance
ANOVA	=	analysis of variance
BMI	=	body mass index
CV	=	coefficient of variation
DM	=	diabetes mellitus
FBF	=	forearm blood flow
FFA	=	free fatty acid
HOMA-IR	=	homeostasis model assessment of insulin resistance
IR	=	insulin resistance
LDL	=	low density lipoprotein
SNP	=	sodium nitroprusside
VER	=	verapamil

METHODS

Patients. Patients with stable weight and diet-controlled type 2 DM (according to American Diabetes Association criteria, namely a fasting serum glucose >7.0 mmol/L on two occasions, a casual glucose >11.1 mmol/l with symptoms, or a 2-h post-oral glucose tolerance test (75 g glucose load) glucose of >11.1 mmol/l) were recruited from our regional outpatient diabetes day care center. Weight and fasting glucose levels were stable over a minimum of four weeks prior to recruitment. To minimize the confounding effects of other known cardiovascular risk factors on measures of endothelial function, and in particular the potential for changes related to metformin treatment, we studied patients with DM but without the clustering of risk factors seen in the metabolic syndrome. Both men and women were recruited, as diabetes is known to abrogate the relatively preserved vascular responses otherwise evident among women (11). Exclusion criteria included known coronary or peripheral vascular disease, hypertension (blood pressure $>130/85$), current treatment with vasoactive medications, hypercholesterolemia (total cholesterol >6.2 mmol/l), hypertriglyceridemia (triglycerides >2.3 mmol/l), a history of smoking within three months of enrollment, micro- or macroalbuminuria, known diabetic retinopathy, age at diagnosis of diabetes ≤ 25 years, current participation in another clinical trial and contraindications to metformin therapy, including renal or hepatic impairment and known intolerance to metformin.

This trial was designed and performed in accordance with the principles of the Declaration of Helsinki and was approved by our University's Ethical Review Board. Written informed consent was obtained after the purpose, nature and potential risks of the interventions were explained to the subjects.

Protocol. Forty-four subjects underwent vascular response measurements using forearm plethysmography (described in the following text) before and after three months' treatment with metformin 500 mg orally twice daily or placebo. Patients were assigned in a 2:1 ratio to metformin or matching placebo. End point measures including blood flow

data analyses were blinded to patient name, treatment assignment and treatment timing.

On the pretreatment study day, blood work confirming normal hepatic and renal function as well as a spot urine determination of the albumin:creatinine ratio was obtained. If a cholesterol profile was not available from ≤ 3 months before the study date, this was measured concurrent with determinations of fasting serum glucose and insulin levels, hemoglobin A1c, free fatty acids and total homocysteine. Cuff blood pressure, hip and waist circumferences, height and weight were determined before the vascular study. These parameters were measured again following three months' therapy with metformin or placebo. Compliance with study medication was assessed with pill counts at the end of the treatment period.

Vascular responses. All studies were performed in a quiet clinical laboratory maintained at 21 to 23°C. Subjects were asked to refrain from drinking alcohol- or caffeine-containing beverages for at least 12 h before the study. Aspirin and any prescribed or over-the-counter nonsteroidal anti-inflammatory medications were stopped at least five days before each plethysmography session. Studies were performed following an overnight (10 to 12 h) fast. For the posttreatment study day, the last study medication tablet was taken with the evening meal on the day before the study.

Forearm blood flow (FBF) responses were determined using standard techniques of forearm strain-gauge plethysmography. Mercury-in-silastic strain gauges (Hokanson, Inc., Seattle, Washington) were connected to plethysmographs (Model EC-4, Hokanson, Inc.) calibrated to measure percent change in volume, expressed as flow in ml per 100 ml tissue per minute. Both arms were supported at heart level. Starting 10 s before each set of measurements, circulation to the hand was prevented by inflation of a wrist cuff to 160 mm Hg. For each measurement, a cuff placed on the upper arm was inflated to 40 mm Hg to occlude venous egress. This was achieved by rapidly inflating a cuff (Inflator model E10, Hokanson, Inc.) for 10 of every 20 s.

On each study day a standard dose-response profile was obtained for brachial arterial infusions of the endothelium-dependent vasodilator acetylcholine (Ach) (3, 10 and 30 $\mu\text{g}/\text{min}$) (Iolab, Claremont, California), the endothelium-independent vasodilator sodium nitroprusside (SNP) (1, 3 and 10 $\mu\text{g}/\text{min}$) (Roche, Basel, Switzerland) and the nitrate-independent vasodilator verapamil (VER) (1, 10 and 100 $\mu\text{g}/\text{min}$) (Abbott Laboratories, Montreal, Canada). All solutions were infused at 1.0 ml/min (Harvard Apparatus, South Natick, Massachusetts) into the brachial artery of the nondominant arm via an epidural catheter (Concord Portex, Keene, New Hampshire) sealed with dental wax to a 27-gauge dental needle (Sherwood Medical, St. Louis, Missouri).

A baseline measurement of flow was obtained at least 10 min after placement of the intraarterial needle. The sequence of Ach and SNP was randomized on the pretreat-

ment day for each patient and repeated on the second study day. Because of its long-lasting vasodilating effect, VER was infused last on both days. Each dose was infused for 6 min. Vasodilator infusions were separated from each other by infusions of normal saline for at least 18 min to allow flow to return to baseline, with flow measurements performed during the last 3 min of each infusion. Measurements were performed simultaneously in both the infused and contralateral arms. Flow data analysis was performed at a later date by investigators blind to patient name, study day and treatment assignment. Forearm blood flow was taken as the mean of the last five flow measurements at a given drug dose. Data were expressed as the percent increase in flow relative to the immediately preceding baseline measurement.

Laboratory. Fasting blood work was collected following the flow measurements. All assays were performed in our local hospital's clinical laboratory. Standard methodologies for glucose, hemoglobin A1c, cholesterol and triglyceride measurements were used. Hemoglobin A1c measurements have an upper limit of normal of 6.2% in our laboratory. Insulin levels were measured using a double-antibody radioimmuno assay (Pharmacia & Upjohn Inc., Mississauga, Ontario, Canada), with an observed intra-assay coefficient of variation (CV) 2.4% at 51 pmol/l and 6.1% at 741 pmol/l. Interassay CV is reported at 5.8% across the range of standards. Insulin measurements were performed in duplicate. Whole-body IR was assessed using the homeostasis model approximation (homeostasis model IR [HOMA-IR]) (12). This tool has excellent correlations with more formal and cumbersome techniques for the measurement of IR (12,13), particularly when logarithmically transformed (14-16). Free fatty acid levels were determined using spectrophotometric quantitation (Wako Diagnostics, Richmond, Virginia) following oxidation/peroxidation (17); normal range 0.10 to 0.70 mmol/l. Homocysteine levels were determined by HPLC (Hewlett-Packard, Palo Alto, California) (18); normal range 7.1 to 15.1 μ mol/l.

Statistics. Data were expressed as mean (SEM). Statistical analyses were performed using StatView 5.0 (SAS Corporation, Carey, North Carolina), with statistical significance set at a two-sided p value of 0.05. Comparisons between groups prior to treatment were performed using unpaired t tests. The end point for statistical analyses was the percent increase in flow observed across the dose-response range of each vasodilator. Two-way repeated-measures analysis of variance (ANOVA) was used to compare the response to treatment between the two study groups, with analysis of covariance (ANCOVA) used to assess the interaction effects of multiple variables. Linear regression analysis was used to assess the dependence of vascular responses on various metabolic variables, followed by forward stepwise regression analysis incorporating these variables.

Forearm blood flow measurements have a coefficient of variation of ~22% (standard deviation ~2.4 at flow responses 12 to 15 ml/100 ml/min) in our laboratory. We

Table 1. Pretreatment Patient Characteristics

Parameter	Placebo (n = 15)	Metformin (n = 28)
Age (yrs)	54.8 (2.6)	50.7 (1.8)
Gender (M/F)	11/4	15/14
SBP (mm Hg)	124 (4)	126 (2)
DBP (mm Hg)	78 (1)	80 (1)
BMI (kg/m ²)	33.1 (1.9)	32.1 (1.3)
Hb A1c (%)	7.2 (0.5)	6.8 (0.2)
Glucose (mmol/l)	7.43 (0.55)	7.00 (0.23)
Insulin (pmol/l)	86.9 (9.2)	107.8 (13.2)
HOMA-IR	4.1 (0.6)	4.8 (0.6)
Cholesterol (mmol/l)	4.59 (0.22)	5.38 (0.18)
LDL-C (mmol/l)	2.68 (0.20)	3.36 (0.15)*
HDL-C (mmol/l)	1.18 (0.08)	1.15 (0.06)
FFA (pmol/l)	0.51 (0.06)	0.74 (0.05)
Homocysteine (pmol/l)	9.27 (0.62)	9.45 (0.70)

*p = 0.049.

BMI = body mass index; DBP = diastolic blood pressure; FFA = free fatty acids; Hb A1c = hemoglobin A1c; HDL-C = high density lipoprotein cholesterol; HOMA-IR = homeostasis model assessment insulin resistance; LDL-C = low density lipoprotein cholesterol; SBP = systolic blood pressure.

anticipated a ≥ 2.5 ml/100 ml/min improvement in flow response. From these values, we calculated a sample size of ~20 per paired treatment group in order to demonstrate this difference with $\alpha = 0.05$ and $\beta = 0.80$. The 29:15 patient assignment of the overall study was chosen with the aim of minimizing the error of the treatment effect estimate while maintaining statistical power.

RESULTS

Demographics. Pretreatment subject characteristics are detailed in Table 1. None of the subjects had previously received metformin therapy. Subjects assigned to metformin and placebo were well matched for degree of obesity and level of glycemic control, and both the fasting insulin level and the calculated index of IR indicated equivalent degrees of IR. The level of low density lipoprotein (LDL) cholesterol was significantly lower in the group who received placebo. A borderline difference (p = 0.065) in FFA levels, also favoring the placebo group, was also present. No baseline differences in metabolic or vascular parameters attributable to gender were evident (data not shown).

Medication compliance was >95% by pill count. Four subjects withdrew from the study before the second set of measurements, two of whom had been assigned to receive metformin therapy. One from each group withdrew because of concerns over medication side effects, specifically gastrointestinal side effects. Four other subjects noted mild gastrointestinal discomfort (three on metformin, one on placebo) intermittently throughout the treatment period.

Metabolic parameters. Table 2 reports the outcome of a subset of metabolic parameters following treatment. Metformin produced a significant 32.5% drop in HOMA-IR, whereas no change was seen in the patients receiving placebo. Treatment effects on glucose (0.5 mmol/l reduction) and weight (1.5 kg) were small and did not achieve statistical significance. The pretreatment differences in LDL

Table 2. Metabolic and Anthropomorphic Changes Following Treatment

Parameter	Placebo		Metformin		p Value
	Baseline	Treated	Baseline	Treated	
HOMA-IR	4.07 (0.63)	4.08 (0.67)	4.77 (0.66)	3.22 (0.38)	0.01
Glucose (mmol/l)	7.43 (0.55)	7.39 (0.68)	7.00 (0.23)	6.50 (0.20)	0.11
Hb A1c (%)	7.2 (0.5)	6.9 (0.3)	6.8 (0.2)	6.5 (0.2)	0.83
Insulin (pmol/l)	86.9 (9.2)	87.6 (9.9)	107.8 (13.2)	77.7 (7.2)	0.60
BMI (kg/m ²)	33.0 (2.2)	33.0 (2.3)	32.8 (1.3)	32.1 (1.3)	0.13
Cholesterol (mmol/l)	4.59 (0.22)	4.64 (0.24)	5.38 (0.18)	5.41 (0.16)	0.85
LDL-C (mmol/l)	2.68 (0.20)	2.80 (0.23)	3.36 (0.15)	3.37 (0.11)	0.11
HDL-C (mmol/l)	1.18 (0.08)	1.19 (0.09)	1.15 (0.06)	1.20 (0.07)	0.39
FFA (pmol/l)	0.51 (0.06)	0.56 (0.08)	0.74 (0.05)	0.63 (0.05)	0.019

BMI = body mass index; FFA = free fatty acids; Hb A1c = hemoglobin A1c; HDL-C = high density lipoprotein cholesterol; HOMA-IR = homeostasis model assessment insulin resistance; LDL-C = low density lipoprotein cholesterol. P values are reported for comparison by two-way analysis of variance, comparing differences attributable to treatment.

cholesterol persisted following treatment, but without any change attributable to metformin treatment. The significant reduction in FFA is an increasingly recognized effect of this medication.

Blood flow responses. For all results presented, parallel analyses using the measures in the infused arm and the ratio of infused to noninfused arm were carried out, and because the two approaches provided equivalent results, only those of the infused arm are presented. Baseline FBF in the infused arm did not differ within or between study days ($p = 0.43$), with values in both groups of 2.8 ± 0.3 ml/100ml/min. Forearm blood flow in the contralateral arm showed the variation inherent to this technique, but did not change within or between study days ($p = 0.83$). Figures 1 to 3 present data for the infused arm as percent increase in FBF from the immediately preceding baseline measurement.

The metformin-treated subjects experienced a statistically significant improvement in endothelium-dependent vasodilator response compared with the placebo-treated group (Fig. 1, $p = 0.0027$ by two-way ANOVA), owing principally to improved FBF responses in the metformin-treated subjects compared to baseline (Fig. 1). The slightly diminished endothelium-dependent responses in the placebo

group at the end of the study (Fig. 1) were not statistically different from this group's baseline responses.

Baseline endothelium-independent nitrate responses were unexpectedly higher in the group who received placebo, but at the end of treatment this difference was abolished (Fig. 2). Although the change in response at peak dose was statistically significant for metformin-treated subjects compared with their baseline ($p = 0.01$), across all doses there was no statistically significant treatment effect relative to placebo ($p = 0.27$). Verapamil-induced, nitrate-independent flows were not different between groups either before or after treatment ($p = 0.40$, Fig. 3).

Predictors of endothelial function. At baseline, univariate ANOVA revealed no effect of any metabolic or anthropomorphic parameters on endothelial responses, and similarly by ANCOVA there were no significant interactions of these effects on baseline responses. After therapy with metformin, univariate ANOVA revealed an effect of logHOMA-IR ($p = 0.06$) and of body mass index (BMI) ($p = 0.007$) on endothelial responses. Again, ANCOVA revealed no interactions, suggesting that the chance imbalances between groups, particularly with regard to LDL cholesterol, did not influence the results. Among the subjects who received

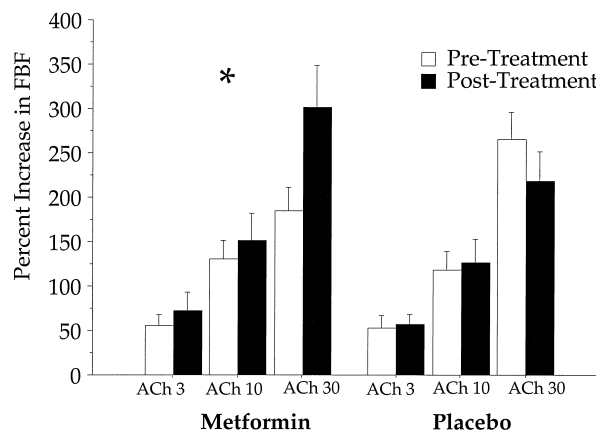


Figure 1. Endothelium-dependent blood flow responses before and after treatment with metformin. Doses are 3, 10 and 30 µg/min. * $p = 0.0027$ by two-way analysis of variance, comparing treatment effects in the two groups. ACh = acetylcholine; FBF = forearm blood flow.

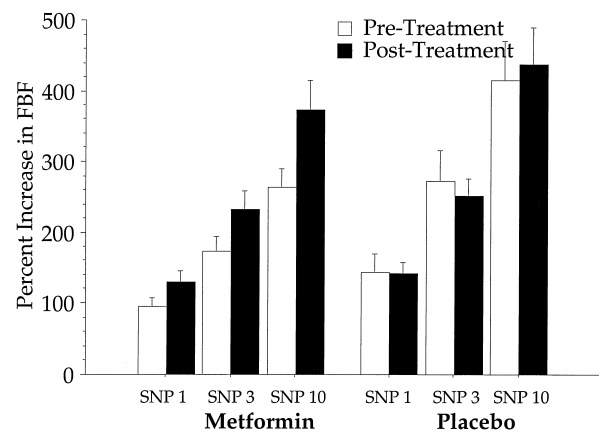


Figure 2. Endothelium-independent blood flow responses before and after treatment with metformin. Doses are 1, 3 and 10 µg/min. $p = 0.27$ by two-way analysis of variance, comparing treatment effects in the two groups. SNP = sodium nitroprusside; FBF = forearm blood flow.

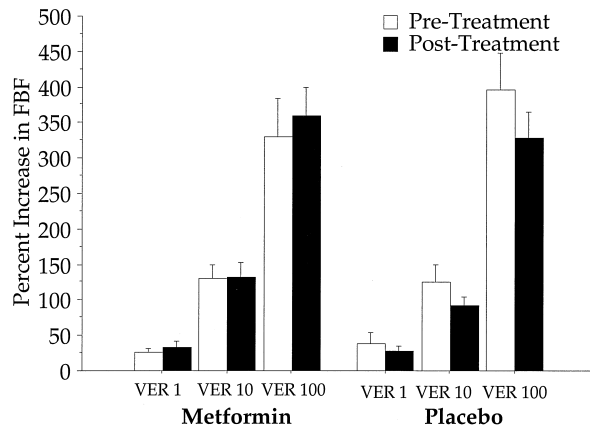


Figure 3. Nitrate-independent blood flow responses before and after treatment with metformin. Doses are 1, 10 and 100 $\mu\text{g}/\text{min}$. $p = 0.40$ by two-way analysis of variance, comparing treatment effects in the two groups. VER = verapamil; FBF = forearm blood flow.

metformin, a relationship of changes in metabolic parameters to changes in endothelium-dependent responses was suggested by trends with $\Delta\log\text{HOMA-IR}$ ($p = 0.12$) and ΔFFA ($p = 0.08$). The inclusion of these variables with the change in BMI and in lipid variables in a multivariate model accounted for >95% of the variation in the change in endothelial response ($p = 0.005$).

Following therapy, linear regression analyses revealed dependencies of endothelium-dependent responses following therapy on BMI ($r = -0.43$, $p = 0.007$), $\log\text{HOMA-IR}$ ($r = -0.486$, $p = 0.017$) (Fig. 4) and high density lipoprotein cholesterol ($r = 0.34$, $p = 0.036$). Of note, no relationship with LDL cholesterol, ambient glucose, Hb A1c or FFA level was evident. Stepwise multiple regression analysis produced a persistent relationship only with IR ($\log\text{HOMA-IR}$), regardless of its entry position in the model. The final model gave an r value of -0.659 and an adjusted r^2 of 0.434 ($p = 0.0012$). In other words,

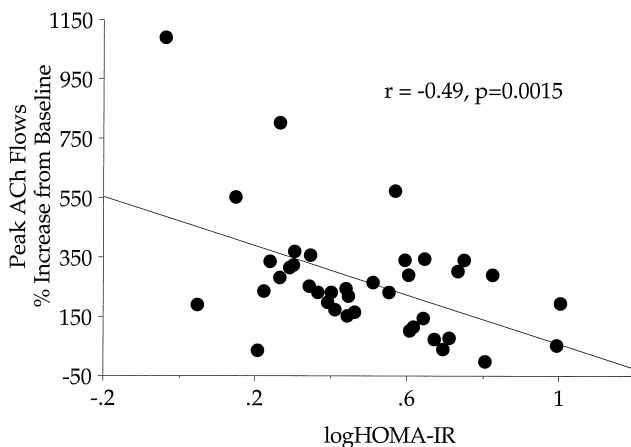


Figure 4. Regression analysis showing a dependence of endothelium-dependent flows on insulin resistance following treatment. ACh = acetylcholine; FBF = forearm blood flow; $\log\text{HOMA-IR}$ = logarithmic transformation of the homeostasis model assessment insulin resistance index.

following treatment the degree of IR was the single best predictor of endothelial response.

DISCUSSION

In patients with IR and type 2 DM, three months of treatment with metformin improved endothelium-dependent vascular responses, whereas vascular responses of patients treated with placebo remained unchanged. Metformin improved insulin sensitivity, as measured by HOMA-IR, and by stepwise multivariate analysis the degree of IR following treatment was the sole predictor of endothelium-dependent responses.

Patients with cardiovascular risks other than type 2 DM were specifically excluded in an attempt to separate the effects of alterations in IR per se on the endothelium from the effects secondary to changes in the clustered metabolic parameters. Metformin treatment resulted in statistically significant improvements only in HOMA-IR and FFA levels compared to placebo, without significant changes in body mass index, blood pressure or cholesterol levels. Of note, no relationship of endothelial responses with either acute or chronic glycemic control was seen, nor was there an evident interaction with FFA levels or LDL cholesterol.

Unexpectedly, pretreatment responses to sodium nitroprusside differed between groups, and this difference was no longer present at the end of treatment. This may simply represent regression to the mean from an anomalous initial reading. The alternative explanation, that treatment resulted in improved nitrate responses, remains possible but cannot be stated from our data.

Metformin and vascular responses. Favorable changes in the overall cardiovascular risk factor profile with metformin are well recognized (19,20). Few studies of metformin's effect on vascular responses in humans have been published. Favorable effects have been reported on hemodynamic and rheologic responses to L-arginine infusion in patients with fasting hyperglycemia (21), basal and postoral glucose tolerance test forearm blood flow in obese patients with type 2 DM (22) and reduced blood pressure response to vasoconstrictor stimuli in patients with DM (23). A beneficial effect to improve postischemic blood flow responses has been reported in patients with symptomatic peripheral vascular disease (24). Our data confirm and extend these previously observed effects of metformin on blood flow in a population of insulin-resistant diabetic subjects.

Insulin resistance and endothelial function. The question of a relationship between IR and endothelial function has been of considerable interest in recent years. In cross-sectional studies, strong correlations have been found in patients with type 2 DM (25), obesity/IR (9), and essential hypertension (26) as well as in healthy subjects (27). Other researchers have not found a relationship in similar patient groups (28-30). The existence of this relationship remains controversial.

An alternative approach to this question comes from

studies of the effect of altering IR on blood flow responses. The experimental induction of IR in rats (31) and in humans (7) resulted in reductions in blood flow responses. In the present study, we found that treatment with metformin simultaneously improved IR and endothelial function, and further were also able to demonstrate a significant dependence of endothelium-dependent responses on IR following therapy. This is in contrast, however, to one prior publication (32) in which troglitazone, a peroxisome proliferator-activated receptor (PPAR)-gamma nuclear receptor activator with insulin-sensitizing actions, was given to obese insulin-resistant subjects in a randomized crossover design. Before treatment, there was no evident defect in cholinergic blood flow responses, but there was blunting of insulin-mediated vasodilation. Despite a clear improvement in insulin sensitivity with treatment, no improvement in insulin-mediated or agonist-stimulated vasodilation was seen. This lack of improvement in insulin-stimulated flow despite a demonstrated improvement in insulin-stimulated glucose uptake might suggest that these phenomena are dissociated. This discrepancy cannot be attributed to the agents used because a clear improvement has been seen with troglitazone treatment in a group of insulin-resistant women with polycystic ovarian syndrome (33).

In the present study, the direct correlation between the change in IR and the improvement in endothelial response did not achieve statistical significance, but the combination of changes in IR, lipids, FFAs and BMI was able to account for the vast majority of the change in endothelial response. This is perhaps not surprising, in light of the known metabolic interrelationships of these variables and their independent effects on vascular function. However, an important effect of IR per se on endothelial function is suggested by the fact that a relationship was not evident at baseline but was brought out by metformin therapy, which concurrently improved insulin sensitivity and endothelial function. Furthermore, the relationship persisted through stepwise regression analysis including the relevant known variables.

In summary, there is conflicting cross-sectional evidence regarding the existence of a relationship between IR and endothelial function in humans, but interventions that modulate IR have been found to alter endothelial responses. Ours is the first study to report such an effect of metformin.

Potential mechanisms for metformin's beneficial effect. The mechanism of this effect is uncertain. It is possible that the reduction in IR directly accounts for the endothelial effects, as we originally hypothesized. Given that our patients had only mild elevations in glucose levels, a secondary effect due to reduction of endothelial cell glucose toxicity seems unlikely. Coincident improvements in IR and other aspects of vascular physiology are possible. In fact, metformin has recently been reported to have antioxidant effects (34) in addition to the known favorable effects on circulating lipids (10) and FFAs (35). However, no evidence links alterations in these parameters with improvements in vas-

cular function separate from improvements in IR, and therefore the hypothesis of the central role of IR remains plausible. A direct metformin effect in vascular endothelial cells is also possible, and supported by recent findings in insulin-resistant rats (36). Also, in ex-vivo preparations of vascular smooth muscle, a direct vasodilating action of metformin has been reported, possibly due to altered calcium handling (35,37,38).

Study limitations. We did not undertake any measure of oxidative balance or oxidative stress, nor was LDL particle size measured. These variables have significant impacts on endothelial function and are potentially affected by metformin. Favorable changes could be contributing to the observed improvement in endothelial function but were not measured. This needs to be addressed in future studies.

Conclusions. In our group of patients with type 2 DM and IR but without other metabolic abnormalities, we found that treatment with metformin resulted in improved IR and improved endothelial function. The principal dependence of endothelium-dependent vasodilation on the degree of IR following therapy argues in favor of a central role of IR in vascular pathophysiology. This finding adds to our understanding of endothelial dysfunction in insulin-resistant states, and also suggests a rational choice of antidiabetic agent in patients with type 2 DM and vascular disease.

Acknowledgments

We are indebted to Hoechst Marion Roussel (Canada) for providing the placebo tablets. We also thank Dr. Alun Edwards and his staff at the Calgary Regional Diabetes Education Center for their invaluable assistance with recruitment.

Reprint requests and correspondence: Dr. Todd J. Anderson, Division of Cardiology, Faculty of Medicine, University of Calgary, c849, 1403 29th Street NW, Calgary, Alberta T2N 2T9, Canada. E-mail: todd.anderson@crha-health.ab.ca.

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