Long-Term Oral L-Arginine Administration Improves Peripheral and Hepatic Insulin Sensitivity in Type 2 Diabetic Patients

PierMarco Piatti, MD[1]
Lucilla D. Monti, MD[2]
Gianpietro Valsecchi, PhD[2]
Fulvio Magni, MD[3]
Emanuela Setola, MD[1]
Federica Marchesi, MD[2]
Marzia Galli-Kienle, MD[4]
Guido Pozza, MD[5]
K. George M.M. Alberti, FRCP[5]

OBJECTIVE — The aim of this study was to evaluate whether long-term administration of L-arginine acting through a normalization of NO/cyclic-guanosine-3′,5′-cyclic monophosphate (cGMP) pathway was able to ameliorate peripheral and hepatic insulin sensitivity in 12 lean type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — A double-blind study was performed for 3 months. In the first month, patients were treated with their usual diet. Then they were randomly allocated into two groups. In group 1, patients were treated with diet plus placebo (orally three times per day) for 2 months. In group 2 patients were treated for 1 month with diet plus placebo (orally, three times per day) and then for 1 month with diet plus L-arginine (3 g three times per day). At the end of the first and the second month of therapy, patients underwent a euglycemic-hyperinsulinemic clamp combined with [6,6-$^2H_2$]glucose infusion. A total of 10 normal subjects underwent the same test as control subjects.

RESULTS — In group 1, no changes in basal cGMP levels, systolic blood pressure, forearm blood flow, glucose disposal, and endogenous glucose production were observed throughout. In group 2, L-arginine normalized basal cGMP levels and significantly increased forearm blood flow by 36% and glucose disposal during the clamp by 34%, whereas it decreased systolic blood pressure and endogenous glucose production by 14 and 29%, respectively. However, compared with normal subjects, L-arginine treatment was not able to completely overcome the defect in glucose disposal.

CONCLUSIONS — L-Arginine treatment significantly improves but does not completely normalize peripheral and hepatic insulin sensitivity in type 2 diabetic patients.

Diabetes Care 24:875–880, 2001

Contradictory results have been found concerning the influence of insulin on nitric oxide (NO), a potent molecule with vasodilatory function. Baron et al. (1,2) showed that insulin-mediated vasodilation is largely dependent on the action of insulin on NO release, whereas Petrie et al. (3) have shown that endothelial NO synthesis and insulin sensitivity are positively correlated in healthy individuals. In addition, in obese patients and patients with type 2 diabetes, the insulin-mediated vasodilatory response seems blunted (4). However, Yki-Jarvinen et al. (5) were unable to find any correlation between insulin action and increment in blood flow in normal and obese subjects, although all agree that methacoline-induced vasodilation is impaired in insulin-resistant subjects.

L-Arginine is a precursor for NO, and both in vitro and in vivo studies have demonstrated that L-arginine can augment vascular dilation under certain conditions (6). Experimental studies in cholesterol-fed rabbits have shown that dietary supplementation with L-arginine causes attenuation of endothelial dysfunction with increased NO activity, resulting in reduced platelet activation (7), monocyte adhesion (8), and a marked reduction in aortic and coronary atherosclerosis (9). Moreover, in young hypercholesterolemic adults, the administration of L-arginine results in significant improvement in endothelium-dependent dilatation and positively effects the atherogenetic process after 4 weeks (10). Although the effect of acute administration of L-arginine on insulin secretion in humans is well known (11), little is known about the effect of long-term administration of L-arginine on insulin sensitivity in type 2 diabetic patients.

Therefore, the aim of this study was to evaluate whether long-term administration of L-arginine acting through a normalization of NO/cyclic-guanosine-3′,5′-cyclic monophosphate (cGMP) pathway was able to ameliorate peripheral and hepatic insulin sensitivity in 12 lean type 2 diabetic patients with good metabolic control.

RESEARCH DESIGN AND METHODS

— The protocol of the study was approved by the local ethics committee, and informed consent was obtained from each volunteer. A total of 12 lean type 2 diabetic patients (age 58 ± 3 years, BMI 25.3 ± 0.9 kg/m^2) with good metabolic control (glycated hemoglobin

From the [1]Metabolic Diseases Unit; the [2]Medicine I Division; the [3]Mass-Spectrometry Unit Cattedra di Clinica Medica Generale e Terapia Medica, Università Vita-Salute, IRCCS H. San Raffaele; the [4]University of Milano-Bicocca, Faculty of Medicine, Milan, Italy; and the [5]Department of Diabetes and Metabolism, School of Clinical Medical Sciences, University of Newcastle, Newcastle upon Tyne, U.K.

Address correspondence and reprint requests to PierMarco Piatti, Metabolic Diseases Unit, Medicine I Division, IRCCS H. San Raffaele, Via Olgettina 60, 20132 Milan, Italy. E-mail: piermarco.piatti@hsr.it.

Received for publication 19 December 2000 and accepted in revised form 6 February 2001.

Abbreviations: cGMP, cyclic-guanosine-3′,5′-cyclic monophosphate; CV, coefficient of variation; NO, nitric oxide; NOS, NO synthase.

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

Table 1—*Basic characterization of type 2 diabetic patients and normal subjects*

	Type 2 diabetic patients submitted to L-arginine or placebo treatment	Pool of type 2 diabetic patients	Normal subjects
Sex (M/F)	8/4	14/11	22/18
Age (years)	57.8 ± 3.2	53.8 ± 2.0	56.3 ± 1.2
Body weight (kg)	67.0 ± 4.7	70.2 ± 2.3	70.0 ± 1.6
BMI (kg/m^2)	25.3 ± 0.9	26.1 ± 0.7	25.1 ± 0.4
Systolic blood pressure (mmHg)	124 ± 3	129 ± 2	129 ± 2
Diastolic blood pressure (mmHg)	70 ± 2	80 ± 4*	74 ± 2
Glycated hemoglobin (%)	5.7 ± 0.1	7.2 ± 0.3*	4.9 ± 0.2†
Fasting plasma glucose (mmol/l)	7.2 ± 0.2	7.9 ± 0.3*	5.6 ± 0.1†

Data are means ± SEM, unless otherwise indicated. *$P < 0.05$ vs. type 2 diabetic patients submitted to L-arginine or placebo therapy; †$P < 0.05$ vs. both groups of type 2 diabetic patients.

5.7 ± 0.1%) and who were treated with diet alone were admitted to the study. The inclusion criteria were as follows: normal glycated hemoglobin associated with stable body weight 6 months before the start of the study; absence of diabetic complications, except for background retinopathy; diet treatment; normal systolic and diastolic blood pressure in the absence of antihypertensive treatment; normal electrocardiogram at rest; no history of ischemic heart disease; and normal renal and liver function.

To establish values of fasting circulating cGMP in a large population of type 2 diabetic patients and in normal subjects, 25 type 2 diabetic patients treated with diet and metformin and/or sulfonylureas and 40 normal control subjects matched for age, sex, body weight, and BMI were studied.

Table 1 shows the clinical and metabolic characteristics of all subjects included. No differences were found for systolic blood pressure, whereas a significant increase in fasting plasma glucose and glycated hemoglobin were found in type 2 diabetic patients compared with normal control subjects. Fasting plasma glucose levels, glycated hemoglobin, and diastolic blood pressure were lower in patients submitted to placebo or L-arginine treatment compared with the other type 2 diabetic patients.

Research design

The study design (Fig. 1) consisted of a double-blind study for a total duration of 3 months. In the first month, all patients were treated with usual diet, then they were randomly allocated into two groups of six subjects each. The first group was treated with diet plus placebo (orally three times per day) for 2 months. The second group was treated with diet plus placebo (orally three times per day) for 1 month and with diet plus L-arginine (L-arginine aspartate, orally 3 g three times per day) during the second month. The dosage of L-arginine was the lowest one possible in order to create endothelial effects without changing insulin secretion. Both the active drug and the placebo were prepared as identical powder formulations, which were mixed with water and 200 mg of aspartame.

Every 14 days, patients were reviewed by a dietitian to maintain a constant body weight throughout the study.

At the end of the first and second month of therapy, patients underwent a euglycemic-hyperinsulinemic clamp (insulin infusion 25 mU · kg^{-1} · h^{-1}) (12). The 3 days before the test, all subjects kept a constant diet to control for differences in food intake. In particular, animal- and vegetable-nitrogen daily intakes were maintained identical to rule out the role of nitrogen intake with diet in NO production and clearance, which could represent a bias in the study.

After an overnight fast and 12 h after the last administration of placebo or L-arginine, a 20-gauge plastic cannula (Abbocath T; Abbocath, Ireland LTD, Sling, Ireland) was inserted in a dorsal vein of one hand in retrograde position, and the hand was maintained at 55°C for intermittent sampling of arterialized blood. Another 20-gauge plastic cannula used for 20% dextrose infusion was placed in a large antecubital vein.

Insulin sensitivity was also evaluated in 10 normal subjects who underwent an identical euglycemic-hyperinsulinemic clamp.

Hormonal and metabolic evaluations

Baseline samples for plasma glucose, serum insulin, cGMP (second messenger of NO), and potassium levels were withdrawn before the start of the euglycemic clamp.

During both euglycemic clamps, samples for blood glucose were taken every 5 min from the start of the test, and samples for insulin were withdrawn every 30 min.

To evaluate the changes in insulin sensitivity, we used the *M* value and the rate of disappearance, which was evaluated isotopically by means of a primed (5 mg/kg) continuous (0.05 mg · kg^{-1} · min^{-1}) infusion of [6,6-$^2H_2$]glucose, as previously reported in (13–16).

Hemodynamic measurements

On the morning of the test, forearm blood flow (17) and systolic and diastolic blood pressure were measured at the beginning of the euglycemic-hyperinsulinemic clamp after at least 30 min of rest with the patients in the supine position and at the end of the euglycemic-hyperinsulinemic clamp.

Forearm vascular resistance was calculated as mean blood pressure divided

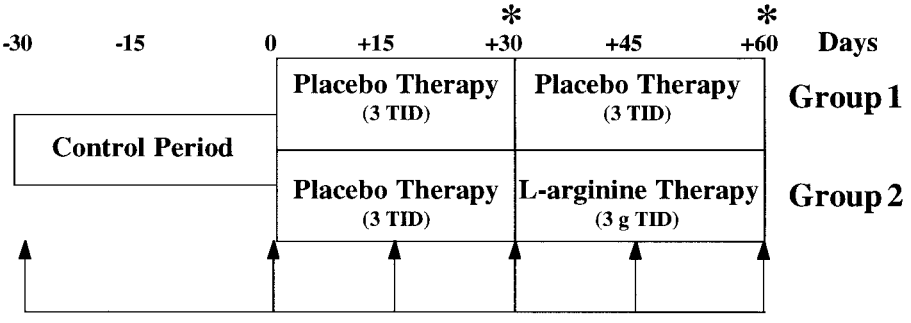

Figure 1—*Research design. ↑, Dietician visit; *euglycemic-hyperinsulinemic clamp.*

Table 2—*Clinical and anthropometric data after placebo or L-arginine therapy in groups 1 and 2*

	Group 1		Group 2	
	Placebo 1st month	Placebo 2nd month	Placebo 1st month	L-Arginine 2nd month
Body weight (kg)	68.2 ± 6.0	68.0 ± 6.1	74.1 ± 5.7	74.3 ± 5.9
Systolic blood pressure (mmHg)	120 ± 4	120 ± 5	128 ± 4	110 ± 3*
Diastolic blood pressure (mmHg)	71 ± 3	67 ± 3	70 ± 3	67 ± 2
Heart rate (pulse/min)	67 ± 3	71 ± 4	70 ± 2	66 ± 3
Glycated hemoglobin (%)	5.8 ± 0.1	5.8 ± 0.2	5.6 ± 0.2	5.5 ± 0.2
Serum potassium (mmol/l)	4.12 ± 0.15	4.13 ± 0.15	3.98 ± 0.11	4.01 ± 0.08

Data are means ± SEM. *$P < 0.05$ vs. placebo 1st month.

by forearm blood flow and expressed in arbitrary units.

Assays

Plasma glucose was measured with a glucose oxidase–based analyzer (Yellow Springs Instrument, Yellow Springs, OH). Glycated hemoglobin was assayed using a commercial kit (Unimate, Roche). Serum insulin levels (intra-assay coefficient of variation [CV] 3.0%, inter-assay CV 5.0%) were assayed with a microparticle enzyme immunoassay (IMX; Abbott Laboratories, Abbott Park, IL). Serum C-peptide (intra-assay CV 2.5%, interassay CV 5.0%) were assayed with a radioimmunoassay kit (Medical System, Genoa, Italy). cGMP was assayed with a radioimmunoassay kit (Amersham International, Buckinghamshire, U.K.). Isotopic enrichment of [6,6-$^2H_2$]glucose was determined using a gas chromatography–mass spectrometry method, as previously reported (15).

Calculation and statistical analysis

All results are expressed as the mean ± SEM at each time interval. Comparisons within groups were performed by Student's *t* test for paired data. Comparisons among groups were performed by analysis of variance followed by the Scheffe *F* test when appropriate. Linear regression analyses were also used as appropriate. A two-tailed probability level <0.05 was considered statistically significant.

RESULTS — Table 2 shows that there were no changes in body weight, glycated hemoglobin, serum potassium, diastolic blood pressure, and heart rate in both groups. In contrast, systolic blood pressure remained unchanged in group 1, whereas it significantly decreased after L-arginine therapy in group 2 (128 ± 4 vs. 110 ± 3 mmHg; $P < 0.05$) (Table 2).

Preclamp values

In group 1, basal blood glucose, serum insulin (Fig. 2), serum C-peptide (from 0.68 ± 0.04 to 0.70 ± 0.09 nmol/l; NS), endogenous glucose production, plasma cGMP, blood flow, and peripheral vascular resistance remained unchanged (Table 3) during the 2 months of placebo therapy.

In group 2, compared with placebo, L-arginine had no effect on blood glucose, serum insulin levels (Fig. 2), and serum C-peptide (from 0.71 ± 0.09 to 0.62 ± 0.06 nmol/l; NS). Endogenous glucose production decreased by 9.3% (from 18.0 ± 1.3 to 16.4 ± 0.9 μmol · kg^{-1} · min^{-1}; $P = 0.07$) (Table 3). During the same period, plasma cGMP significantly increased by 60.5% (from 2.76 ± 0.35 to 4.43 ± 0.54 nmol/l; $P < 0.03$), and baseline blood flow increased by 36% (from 2.19 ± 0.26 to 3.01 ± 0.22 ml · 100 ml^{-1} · min^{-1}; $P < 0.02$) (Table 3). Correspondingly, peripheral vascular resistance decreased from 43.0 ± 3.9 to 27.6 ± 1.9 UI ($P < 0.01$) (Table 3).

Basal cGMP levels were positively correlated with basal blood flow ($r = 0.58$, $P < 0.003$; data not shown) and negatively correlated with basal endogenous glucose production ($r = -0.41$, $P < 0.05$; data not shown).

The analysis of differences between the two treatments demonstrated that in group 2 there was a significant increase in cGMP levels ($P < 0.001$) and forearm

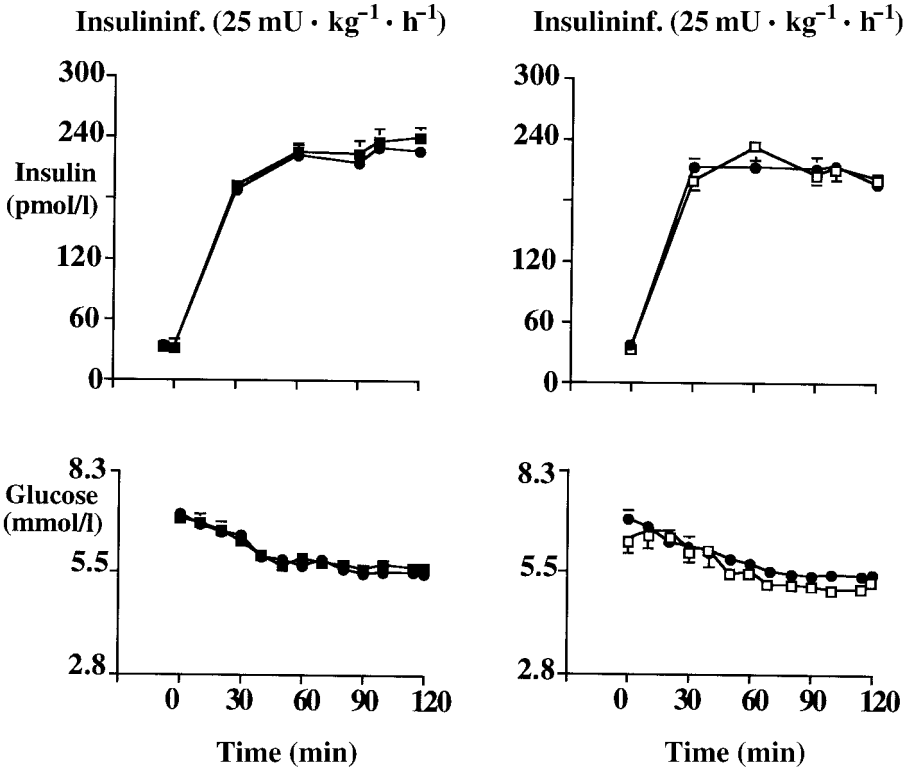

Figure 2—*Insulin and glucose levels during the euglycemic-hyperinsulinemic clamp period in groups 1 (left panels) and 2 (right panels). ●, Placebo therapy (1st month); ■, placebo therapy (2nd month); □, L-arginine therapy.*

Table 3—*Variation of peripheral and hepatic insulin sensitivity and vascular indexes during placebo or L-arginine therapy in both groups of patients compared with normal subjects*

	Group 1			Group 2			
	Placebo 1st month	Placebo 2nd month	Difference*	Placebo 1st month	L-Arginine 2nd month	Difference*	Normal subjects
Basic							
Plasma glucose (mmol/l)	7.3 ± 0.1	7.3 ± 0.2	–0.03 ± 0.15	7.0 ± 0.3	6.4 ± 0.4	–0.52 ± 0.24	5.0 ± 0.2†
Endogenous glucose production (μmol·kg^{-1} · min^{-1})	17.8 ± 1.3	17.8 ± 1.4	+0.2 ± 0.6	18.0 ± 1.3	16.4 ± 0.9	–1.7 ± 0.8	11.3 ± 1.3†
cGMP (nmol/l)	2.43 ± 0.27	2.44 ± 0.25	+0.01 ± 0.07	2.76 ± 0.35	4.43 ± 0.54‡	+1.67 ± 0.52§	4.93 ± 0.33†
Forearm blood flow (ml · 100 ml forearm^{-1} · min^{-1})	2.03 ± 0.26	2.11 ± 0.23	+0.09 ± 0.07	2.19 ± 0.26	3.01 ± 0.22‡	+0.82 ± 0.18‖	2.87 ± 0.24
Peripheral vascular resistance (UI)	46.0 ± 4.9	43.3 ± 5.2	–2.7 ± 3.6	43.0 ± 3.9	27.6 ± 1.9‡	–15.3 ± 3.0‖	31.4 ± 2.8
Euglycemic clamp							
Steady-state plasma glucose	5.7 ± 0.2	5.7 ± 0.3	+0.08 ± 0.04	5.4 ± 0.1	5.2 ± 0.2	–0.30 ± 0.12	5.0 ± 0.1†
Endogenous glucose production (μmol · kg^{-1} · min^{-1})	6.22 ± 1.16	6.77 ± 0.61	+0.50 ± 1.93	6.11 ± 0.33	4.29 ± 0.39‡	–2.09 ± 0.61‖	4.96 ± 0.18†
M value (μmol · kg^{-1} · min^{-1})	11.3 ± 2.0	11.7 ± 1.6	+0.39 ± 1.10	11.3 ± 1.1	15.1 ± 1.3‡	+3.96 ± 0.77‖	24.2 ± 0.7†
Forearm blood flow (ml · 100 ml forearm^{-1} · min^{-1})	2.46 ± 0.26	2.36 ± 0.24	–0.10 ± 0.35	2.80 ± 0.28	3.32 ± 0.24	+0.52 ± 0.11	3.10 ± 0.29
Peripheral vascular resistance (UI)	37.1 ± 3.4	37.7 ± 3.8	+1.19 ± 5.28	32.4 ± 3.5	29.6 ± 5.3	–2.68 ± 3.89	28.3 ± 2.5

Data are means ± SEM. *Negative differences correspond with a decrease, whereas positive differences correspond with an increase of variables after therapy; †$P <$ 0.01 vs. groups 1 and 2; ‡$P < 0.05$ vs. placebo 1 month; §$P < 0.001$ vs. group 1; ‖$P < 0.05$ vs. group 1.

blood flow ($P < 0.02$) (Table 3) compared with group 1.

Circulating cGMP levels were similar in groups 1 and 2 submitted to placebo treatment and in the pool of type 2 diabetic patients and were significantly lower than those in normal subjects (2.60 ± 0.22 and 2.72 ± 0.31 vs. 4.93 ± 0.33 nmol/l, respectively; $P < 0.01$). Interestingly, after L-arginine treatment, cGMP levels increased, reaching the levels found in the normal subjects (4.43 ± 0.54 vs. 4.93 ± 0.33 nmol/l; NS).

Basal endogenous glucose production was significantly higher in both groups than in normal subjects before and after L-arginine treatment (Table 3).

Euglycemic-hyperinsulinemic clamp

During the euglycemic-hyperinsulinemic clamp, blood glucose levels reached the target values (5.0–5.5 mmol/l) within the first hour of the test, and they were successfully maintained throughout the test, while insulin levels reached a plateau of 210–240 pmol/l in both groups (Fig. 2).

In group 1, peripheral and hepatic insulin sensitivity and forearm blood flow were not modified during the study period (Table 3). In group 2, compared with the first month of placebo treatment, L-arginine significantly increased the *M* value by 34% ($P < 0.01$) (Table 3), whereas endogenous glucose production decreased significantly ($P < 0.02$) (Table 3). Forearm blood flow remained significantly higher after L-arginine than after placebo (3.32 ± 0.24 vs. 2.80 ± 0.28 ml · 100 ml^{-1} · min^{-1}; $P < 0.05$) (Table 3).

The analysis of differences between the two treatments demonstrated that in group 2 there was a significant improvement in *M* value ($P < 0.03$) and hepatic insulin sensitivity ($P < 0.05$) compared with group 1 (Table 3) .

The *M* value was significantly lower and endogenous glucose production significantly higher in groups 1 and 2 than in normal subjects before and after treatment.

CONCLUSIONS — The aim of this study was to evaluate whether long-term administration of L-arginine acting through a normalization of the NO/cGMP pathway was able to ameliorate peripheral and hepatic insulin sensitivity in 12 lean type 2 diabetic patients with good metabolic control. Our study has shown for the first time that an increment in NO availability induced by the administration of L-arginine is able to increase insulin sensitivity, even if complete normalization is not achieved.

Although in the present study arginine levels were not measured to confirm the effectiveness of L-arginine supplementation, the significant decrease in vascular resistance (18) and the increase in cGMP (19), a second messenger of NO, strongly supports the positive compliance of patient and therapy. It is currently believed that NO interacts with soluble guanylate cyclase, leading to the elevation of cGMP concentrations (19–21). A close correlation between NO and cGMP has recently been demonstrated by Albert et al (22). The authors demonstrated that inhaled NO produced a threefold elevation in plasma cGMP concentrations (22). It is interesting to note that circulating cGMP levels were significantly lower in type 2 diabetic patients than in normal subjects and that administration of L-arginine completely normalized their levels. Decreased circulating cGMP was previously reported in first-degree relatives of patients with type 2 diabetes independent of the degree of glucose tolerance (23).

A simple approach to explain our re-

sults is to propose that insulin resistance is associated with an impairment in the ability of NO to generate its messenger, leading to a decrease in cGMP generation and a relative decline in insulin's ability to produce vasodilation. Although speculative, this formulation is consistent with the results of this article and with Petrie et al.'s (3) conclusion that there is a relationship between insulin resistance and the endothelial response to inhibition of NO synthesis as well as with evidence that the vasodilatory response is decreased in insulin-resistant individuals (4). The significant correlation between basal cGMP and forearm blood flow supports this hypothesis.

The results of the present study are contradictory to previous reports showing that acute intravenous administration of agents with endothelium-dependent vasodilation activity, such as adenosine or bradykinin, failed to improve glucose utilization, despite a significant increment in local blood flow (24,25). The discrepancy between our data and those shown in the previous studies (24,25) may be the result of different experimental approaches, such as those which acutely infused intravenous bradikynin (24) or adenosine (25) for a few hours. In the present study, L-arginine was administered chronically for 30 days.

On the other hand, it is possible that an increase in NO availability could also improve glucose metabolism independently from its vasodilation activity. In fact, cGMP levels were inversely and significantly correlated with basal endogenous glucose production. In support of this hypothesis, recent studies have shown that NO synthase (NOS) is expressed in skeletal muscle (26) and that NO per se could influence muscle glucose metabolism in studies in animals and in vitro (27). In fact, NOS inhibition by N-monomethyl-L-arginine inhibits glucose transport in incubated skeletal muscle preparations (28), and it has been shown that sodium nitroprusside, an NO donor, increases glucose transport both in the absence and presence of insulin in rat extensor digitorum longus muscle in vitro (29). Insulin-resistant obese Zucker *fa/fa* rats were found to have a defect in the metabolic pathway of NO, and the administration of Zaprinast, a selective cGMP phosphodiesterase inhibitor, increased both cGMP levels and glucose uptake in skeletal muscle (28). In addition, we recently demonstrated that L-arginine is able to enhance glucokinase activity in cultured rat hepatocytes (30).

However, even if L-arginine treatment totally normalized NO activity (measured through cGMP levels), it was not able to completely overcome the defect of insulin sensitivity in type 2 diabetic patients; this suggests that the pathogenesis of insulin resistance in these patients is multifactorial and that other genetic, environmental, or metabolic factors need to be taken into account.

The results of the present study are in agreement with in vitro and in vivo studies in animals showing that although NO is a mediator of glucose-induced cGMP production in islets, the resulting increase in cGMP is not correlated with insulin secretion (31). Moreover, Pueyo et al. (19) have shown that the administration of *N*ω-nitro-L-arginine methyl ester in vivo markedly diminished NO-dependent cGMP production without affecting insulin secretion .

Because the 1-month period with L-arginine therapy was subsequent to 1 month on diet alone, it could be argued that the improvement in insulin sensitivity was caused by prolonged diet therapy. However, in group 1, no significant effect of prolonged diet treatment was demonstrated. In addition, body weight did not change during the 6 months before and throughout the study, and a dietitian visit was performed every 2 weeks to maintain a constant diet during the three study periods.

In conclusion, the present study shows that long-term L-arginine treatment significantly improves peripheral and hepatic insulin sensitivity in type 2 diabetic patients. Further studies in which this treatment is prolonged are necessary to confirm these preliminary results.

References

1. Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G: Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J Clin Invest* 96:786–792, 1995
2. Baron AD, Steinberg H, Brezchtel G, Johnson A: Skeletal muscle blood flow independently modulates insulin-mediated glucose uptake. *Am J Physiol* 266:E248–E253, 1994
3. Petrie JR, Ueda S, Webb DJ, Elliott HL, Connell GMC: Endothelial NO production and insulin sensitivity: a physiological link with implications for pathogenesis of cardiovascular disease. *Circulation* 93: 1331–1333, 1996
4. Laakso M, Edelman SV, Brechtel G, Baron AD: Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 41:1076–1083, 1992
5. Yki-Jarvinen H, Utriainen T: Insulin-induced vasodilatation: physiology or pharmacology? *Diabetologia* 41:369–379, 1998
6. Jun T, Wennmalm A: NO-dependent and -independent elevation of plasma levels of insulin and glucose in rats by L-arginine. *Br J Pharmacol* 113:345–348, 1994
7. Tsao PS, Theilmeier G, Singer AH, Leung LL, Cooke JP: L-Arginine attenuates platelet reactivity in hypercholesterolemic rabbits. *Arterioscler Thromb* 14:1529–1533, 1994
8. Tsao PS, McEvoy LM, Drexler H, Butcher EC, Cooke JP: Enhanced endothelial adhesiveness in hypercholesterolemia is attenuated by L-arginine. *Circulation* 89: 2176–2182, 1994
9. Cooke PJ, Singer AH, Tsao PS, Zera P, Rowan RA, Billingham ME: Anti-atherogenic effects of L-Arginine in the hypercholesterolemic rabbit. *J Clin Invest* 90: 1168–1172, 1992
10. Clarkson P, Adams MR, Powe AJ, Donald AE, McCredie R, Robinson J, McCarthy SN, Keech A, Celermajer DS, Deanfield JE: Oral L-arginine improves endothelium-dependent dilation in hypercholesterolemic young adults. *J Clin Invest* 97: 1989–1994, 1996
11. Palmer JP, Walter RM, Ensinck JW: Arginine-stimulated acute phase of insulin and glucagon secretion in normal men. *Diabetes* 24:735–740, 1975
12. De Fronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
13. Steel R: Influence of glucose loading and of injected insulin on hepatic glucose output. *Ann N Y Acad Sci* 82:420–430, 1959
14. Cobelli C, Toffolo G, Bier DM, Nosadini R: Models to interpret kinetic data in stable isotope tracer studies. *Am J Physiol* 253:E551–E564, 1987
15. Magni F, Monti LD, Brambilla P, Poma R, Pozza G, Galli-Kienle M: Determination of plasma [6.6-$^2H_2$]glucose enrichment by a simple and accurate gas chromatographic-mass spectrometric method. *J Chromatogr* 573:127–131, 1987
16. Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic-euglycemic clamp: comparison of unlabelled and labeled exogenous glucose infusates. *Diabetes* 36:914–924, 1987
17. Piatti PM, Monti LD, Pacchioni M, Ponti-

roli AE, Pozza G: Forearm insulin and non–insulin-mediated glucose uptake and muscle metabolism in man: role of free fatty acids and blood glucose levels. *Metabolism* 40:926–933, 1991
18. Higashi Y, Oshima T, Sasaki N, Ishioka N, Nakano Y, Ozono R, Yoshimura M, Ishibashi K, Matsuura H, Kajiyama G: Relationship between insulin resistance and endothelium-dependent vascular relaxation in patients with essential hypertension. *Circulation* 25:898–902, 1995
19. Pueyo ME, Gonzalez W, Pussard E, Arnal JF: Insulin secretion in rats with chronic nitric oxide synthase blockade. *Diabetologia* 37:879–884, 1994
20. Boger RH, Bode-Boger SM, Frolich JC: The L-arginine/nitric oxide pathway: role in atherosclerosis and therapeutic implications. *Atherosclerosis* 127:1–11, 1996
21. Trovati M, Anfossi G, Masucco P, Mattiello L, Costamagna C, Piretto V, Mularoni E, Cavalot F, Bosia A, Ghigo D: Insulin stimulates NO synthesis in human platelets and, through nitric oxide, increases platelet concentrations of both guanosine-3′,5′-cyclic monophosphate and adenosin-3′,5′-cyclic monophosphate. *Diabetes* 46:742–749, 1997
22. Albert J, Wall n NH, Li N, Frostell C, Hjemdahl P: Neither endogenous nor inhaled nitric oxide influences the function of circulating platelets in healthy volunteers. *Clin Sci* 97:345–353, 1999
23. Piatti PM, Monti LD, Zavaroni I, Valsecchi G, Van Phan C, Costa S, Conti M, Sandoli EP, Solerte B, Pozza G, Pontiroli AE, Reaven G: Alterations in nitric oxide/cGMP Pathway in non diabetic siblings of patients with type 2 diabetes. *J Clin Endocrinol Metab* 85:2416–2420, 2000
24. Natali A, Bonadonna R, Santoro D, Galvan AQ, Baldi S, Frascerra S, Palombo C, Ghione S, Ferrannini E: Insulin resistance and vasodilation in essential hypertension: studies with adenosine. *J Clin Invest* 94:1570–1576, 1994
25. Nuutila P, Raitakari M, Laine H, Kirvela O, Takala T, Utriainen T, Makimattila S, Pitkanen OP, Ruotsalainen U, Iida H, Knuuti J, Yki-Jarvinen H: Role of blood flow in regulating insulin-stimulated glucose uptake in humans: studies using bradykinin, [15O]water, and [18F]fluorodeoxyglucose and positron emission tomography. *J Clin Invest* 97:1741–1747, 1996
26. Nakane M, Schmidt HHHW, Pollock GS, Forstermann U, Murad F: Cloned human brain nitric oxide synthase is highly expressed in skeletal muscle. *FEBS Lett* 316: 175–180, 1993
27. Balon TW, Nadler JL: Nitric oxide release is present from incubated skeletal muscle preparations. *J Appl Physiol* 77:2519–2521, 1994
28. Balon TW, Nadler JL: Evidence that nitric oxide increases glucose transport in skeletal muscle. *J Appl Physiol* 82:359–363, 1997
29. Young M, and Leighton B: Evidence for alterated sensitivity of nitric oxide/cGMP signaling cascade in insulin resistant skeletal muscle. *Biochem J* 329:73–79, 1998
30. Monti LD, Valsecchi G, Costa S, Sandoli EP, Phan CV, Pontiroli AE, Pozza G, Piatti PM: Effects of endothelin-1 and nitric oxide on glucokinase activity in isolated rat hepatocytes. *Metabolism* 49:1–9, 2000
31. Jones PM, Persaud SJ, Bjaaland T, Pearson JD, Howell SL: Nitric oxide is not involved in the initiation of insulin secretion from rat islet of Langerhans. *Diabetologia* 35: 1020–1027, 1992