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## Beneficial effects of a long-term oral L-arginine treatment added to a hypocaloric diet and exercise training program in obese, insulin-resistant type 2 diabetic patients

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**Lucotti, Pietro, Emanuela Setola, Lucilla D. Monti, Elena Galluccio, Sabrina Costa, Emilia P. Sandoli, Isabella Fermo, Giovanni Rabaiotti, Roberto Gatti, and PierMarco Piatti.** Beneficial effects of a long-term oral L-arginine treatment added to a hypocaloric diet and exercise training program in obese, insulin-resistant type 2 diabetic patients. *Am J Physiol Endocrinol Metab* 291: E906–E912, 2006. First published June 13, 2006; doi:10.1152/ajpendo.00002.2006.—Because chronic L-arginine supplementation improves insulin sensitivity and endothelial function in nonobese type 2 diabetic patients, the aim of this study was to evaluate the effects of a long-term oral L-arginine therapy on adipose fat mass (FM) and muscle free-fat mass (FFM) distribution, daily glucose levels, insulin sensitivity, endothelial function, oxidative stress, and adipokine release in obese type 2 diabetic patients with insulin resistance who were treated with a combined period of hypocaloric diet and exercise training. Thirty-three type 2 diabetic patients participated in a hypocaloric diet plus an exercise training program for 21 days. Furthermore, they were divided into two groups in randomized order: the first group was also treated with L-arginine (8.3 g/day), and the second group was treated with placebo. Although in the placebo group body weight, waist circumference, daily glucose profiles, fructosamine, insulin, and homeostasis model assessment index significantly decreased, L-arginine supplementation further decreased FM ( $P < 0.05$ ) and waist circumference ( $P < 0.0001$ ), preserving FFM ( $P < 0.03$ ), and improved mean daily glucose profiles ( $P < 0.0001$ ) and fructosamine ( $P < 0.03$ ). Moreover, change in area under the curve of cGMP (second messenger of nitric oxide;  $P < 0.001$ ), superoxide dismutase (index of antioxidant capacity;  $P < 0.01$ ), and adiponectin levels ( $P < 0.02$ ) increased, whereas basal endothelin-1 levels ( $P < 0.01$ ) and leptin-to-adiponectin ratio ( $P < 0.05$ ) decreased in the L-arginine group. Long-term oral L-arginine treatment resulted in an additive effect compared with a diet and exercise training program alone on glucose metabolism and insulin sensitivity. Furthermore, it improved endothelial function, oxidative stress, and adipokine release in obese type 2 diabetic patients with insulin resistance.

obesity; type 2 diabetes; endothelium; lean body mass; L-arginine

TYPE 2 DIABETES AND OBESITY are frequently clustered with other metabolic conditions such as insulin resistance, hypertriglyceridemia, low HDL cholesterol, hypertension, visceral obesity, and deregulated adipokine release, characterizing the metabolic syndrome with a high risk for the development of cardiovascular disease (19). In this context, an exercise pro-

gram associated with diet therapy is able to reduce body weight and ameliorate insulin sensitivity (17) and endothelial function in patients with chronic heart failure (14) as well as in obese children (34).

Moreover, both in vitro and in vivo studies have demonstrated that L-arginine, a precursor of nitric oxide (NO), can be used to revert endothelial dysfunction and insulin resistance. In particular, Hambrecht et al. (13) showed that L-arginine supplementation improved endothelium-dependent vasodilation to a similar extent as physical exercise, whereas the association of L-arginine with physical activity displayed an additive effect. In addition, a chronic administration of L-arginine improved glucose levels, insulin induced-hepatic glucose production, and insulin sensitivity in type 2 diabetic patients (26).

Patients with the metabolic syndrome show a defect of NO bioavailability (27) associated with an increased endothelin-1 activity (3). Recently, it was found that a chronic endothelin-1 treatment induced insulin resistance in humans (33), whereas a chronic exposure to endothelin-1 dramatically decreased the stimulatory effect of insulin on adiponectin secretion (20), suggesting a relationship between endothelial function and adiponectin release. Consistent with these findings, a chronic inhibition of NO release by  $N^G$ -nitro-L-arginine methyl ester administration in rats induced a state of hypo adiponectinemia, suggesting that NO per se might modulate adiponectin release (15).

Our group previously showed that chronic L-arginine administration improved insulin sensitivity in lean type 2 diabetic patients (26), but results on the beneficial metabolic and endothelial effects of long-term oral L-arginine treatment were not confirmed in other patients with higher degree of insulin resistance, i.e., obese type 2 diabetic patients. To achieve this goal, in the present study, L-arginine supplementation was added to a hypocaloric regime and exercise training program to obtain additive beneficial effects on metabolic and endothelial function, as reported by Hambrecht et al. (13).

Therefore, the aim of the study was to evaluate the effects of a long-term oral L-arginine treatment added to a program of hypocaloric diet and aerobic plus resistance exercise training on fat and lean mass distribution, mean daily glucose levels, insulin levels and sensitivity, endothelial function, and adipokine release in severely obese type 2 diabetic patients.

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## METHODS

*Patients' characteristics, diet and exercise training program and L-arginine or placebo treatment.* Thirty-three middle-aged ( $56.4 \pm 1.4$  yr patients; 25 women and 8 men) gave informed consent to participate in the study that was approved by the local Ethics Committee. All subjects were affected by visceral obesity (body mass index:  $39.1 \pm 0.5$  kg/m<sup>2</sup>; waist circumference:  $116 \pm 1.3$  cm) and type 2 diabetes associated with the metabolic syndrome according to ATP III.

The choice to study type 2 diabetic patients not receiving any medication for treatment of diabetes other than diet was related to the need to evaluate the specific effect of L-arginine on glucose metabolism independent of possible interaction with other hypoglycemic agents. Diet was controlled not only for carbohydrate but also for cholesterol content, and statin and fibrate therapies were withdrawn 1 wk before the start of the study to avoid any possible interaction with L-arginine therapy. For hypertension therapy, patients received standard treatments with angiotensin-converting enzyme inhibitors,  $\beta$ -blockers and treatments were matched in L-arginine and placebo groups. In addition, all subjects showed a normal kidney function.

All patients were hospitalized for the duration of the study program of 21 days and randomized to receive in double blind one of two treatments: L-arginine treatment (8.3 g/day, L-arginine; Bioargina, Farmaceutici DAMOR, Naples, Italy; first group) or placebo therapy (Placebo; second group). In the first group, there were 16 patients (12 women and 4 men), whereas in the second group, there were 17 patients (13 women and 4 men). In addition, all patients were submitted to a similar hypocaloric diet and exercise training program, and all completed the study period. No adverse events were reported in both groups. The hypocaloric diet regime consisted of 1,000 kcal/day with 55% carbohydrate, 25–30% fat, and 15–20% protein, subdivided as follows: 15% for breakfast, 50% for lunch, and 35% for dinner, administered under a daily supervision of a dietician. The 3-wk exercise training program consisted of 45-min twice a day session of whole body exercise for 5 days/wk. Each training session consisted in 30 min of aerobic exercise divided into row ergometer (15 min) and bicycle ergometer (15 min) followed by nine resistance exercises for 15 min. Patients exercised under the supervision of a physician. The training program was performed at 70% of their individual age-predicted maximal heart rate (HR<sub>max</sub>) according to Tanaka et al. (32).

*Experimental protocol.* All subjects were evaluated at baseline and 21 days after hypocaloric diet, exercise training program, and L-arginine/placebo double-blind therapy. After an overnight fast, vital signs and anthropometric measurements were evaluated after at least 30 min of rest in the supine position by the same examiner in climatized temperature conditions at 25°C. The waist circumference was used as the best anthropometric correlate of the distribution of visceral adipose tissue. Fat mass (FM) and fat-free mass (FFM) were measured by bioimpedentiometry using TANITA body fat analyzer, which applies the principle of bioelectrical impedance measurements of voltage drop from foot to foot when a small alternative current is applied through contact with two metal foot pads. Previous studies showed a high correlation between bioimpedance analysis and dual-energy X-ray absorptiometry results (29).

After this period, a 20-gauge plastic cannula (Abbot T, Abbot Ireland, Sligo, Ireland) was inserted in an antecubital vein of the arm for blood sampling and an exercise test was started, which consisted of a 6-min ergometric walking test at 85% of age-predicted HR<sub>max</sub> (18, 32).

Basal samples for glucose, insulin, lipids, and adipokines were withdrawn immediately before the exercise test, whereas those for endothelin-1, nitrate/nitrite (NOx), cGMP, and extracellular superoxide dismutase (ec-SOD) were withdrawn during the ergometric walking test at *time 0*, 6 min (end of the exercise), and 11 min (recovery).

The day before the start of the study during isocaloric diet and at the end of each week of hypocaloric and exercise treatment period, patients underwent a capillary glycemic profile before breakfast (8 AM), before lunch (12 PM), 3 h after lunch (3 PM), before dinner (6 PM), 3 h after dinner (9 PM), and at midnight (12 AM). Glucose profile data were collected by a research nurse.

*Assays.* Blood glucose, HDL cholesterol, total cholesterol and triglycerides were measured with spectrophotometric methods adapted to Cobas MIRA using commercial kits (ABX, Montpellier, France). Free fatty acid levels were measured using automated enzymatic spectrophotometric techniques adapted to Cobas MIRA using commercial kits (NEFA C, Wako Chemicals, Neuss, Germany). Serum insulin levels were assayed with a microparticle enzyme immunoassay (IMX, Abbott Laboratories).

NOx levels were evaluated through the measurement of metabolic end products, i.e., NOx, using enzymatic catalysis coupled with Griess reaction. Endothelin-1 samples were extracted on Sep-Pack C<sub>18</sub> mini-column (Amrep, Amersham International, Buckinghamshire, UK) and assayed by a RIA kit (NEN Life Science Products, Boston, MA). cGMP levels were measured with radioimmunoassay kits (NEN Life Science Products).

Human ec-SOD levels were assayed with ELISA kits (Bender MedSystems, Vienna, Austria). Human leptin and adiponectin levels were assayed with an ELISA kit and a RIA kit (LINCO Research, St. Charles, MO), respectively.

L-arginine levels were assayed by high-performance liquid chromatography after extraction of plasma samples by cation-exchange Strata SCX 100-mg columns (Phenomenex).

*Statistical analysis.* The sample size of patients for each group was estimated taking advantage from previous studies performed in type 2 diabetic patients and in patients with chronic heart failure in whom plasma cGMP levels were considered an index of L-arginine treatment on endothelial function and correlated with endothelial vasodilation and amelioration of glucose metabolism (26, 13). Therefore, we have designed the study to be large enough to be able to detect, with a  $\beta = 20\%$  and  $\alpha = 5\%$ , an increase in cGMP concentrations from 10% with placebo plus diet and physical activity to 70% with L-arginine plus diet and physical activity treatment, and the final sample for each group was evaluated with 16 patients. All values are expressed as means  $\pm$  SE at each time interval. Incremental areas for each parameter were calculated by the trapezoidal rule. Two-way ANOVA with repeated measurements of one factor was used to analyze the effects of treatment time on the different variables and the interaction between changes in different variables and different treatments. Unpaired Student's *t*-test was used to compare differences between groups during each 24-h glycemic profile. Pearson and Spearman correlation coefficients were also calculated, where appropriate.

## RESULTS

*Clinical and anthropometric evaluation, insulin sensitivity, and glucose metabolism measurements.* In Table 1, anthropometric measurements, vital signs, and insulin sensitivity indexes are reported. The measurements of the L-arginine plasma levels showed a significant increment only in L-arginine group (from  $81.8 \pm 12.3$  to  $131.8 \pm 16.5$   $\mu$ mol/l;  $P < 0.001$ ), whereas no changes were found in the placebo group (from  $82.6 \pm 7.5$  to  $79.4 \pm 9.1$   $\mu$ mol/l; not significant), as expected. After 21 days of hypocaloric diet and exercise training, both L-arginine and placebo therapy caused a significant decrement in whole body weight and FM, but compared with placebo, the L-arginine group showed a statistically significant reduction compared with that observed in placebo group in FM ( $F = 4.0$ ,  $P < 0.05$ ) and in waist circumference ( $F = 45.8$ ,  $P < 0.0001$ ). In particular, in the L-arginine group, FM accounted for 100% of the total weight loss without any changes in FFM, whereas

Table 1. Changes in anthropometric and metabolic variables before and after L-arginine and placebo therapy

	L-Arginine		Placebo		P Value	
	Before	After	Before	After	Time effect	Interaction effect
Weight, kg	105.8±3.1	102.8±3.0	102.1±3.6	98.4±3.0	<0.0001	<0.35
Fat mass, kg	49.3±2.2	46.3±2.3	46.8±2.3	44.7±2.4	<0.0001	<0.05
Free-fat mass, kg	56.5±2.0	56.5±1.8	55.3±2.2	53.6±2.2	<0.02	<0.03
Waist, cm	121.1±3.0	112.8±2.6	116.7±2.2	113.5±2.4	<0.0001	<0.0001
Systolic blood press, mmHg	151±3	128±3	149±2	149±2	<0.0001	<0.0001
Diastolic blood press, mmHg	90±2	78±2	89±2	88±3	<0.0001	<0.0002
Fructosamine, μmol/l	274.6±15.6	220.4±8.6	280.8±9.4	257.8±8.7	<0.0001	<0.02
Fasting insulin, mU/l	21.8±3.1	13.6±1.8	18.6±1.7	15.6±1.4	<0.0002	<0.04
Fasting triglycerides, mmol/l	1.95±0.23	1.34±0.11	2.06±0.16	1.49±0.11	<0.0001	<0.09
Fasting FFA, mmol/l	0.76±0.6	0.69±0.05	0.77±0.06	0.68±0.03	<0.08	<0.73

Values are means ± SE. FFA; free fatty acids.

in the placebo group the loss of FM was 57% and the loss of FFM was 43% of the total weight loss. Our group previously demonstrated that 3 wk of a similar hypocaloric diet treatment without exercise training resulted in a 51% decrease of FM and a 49% decrease of FFM (28). Taking all these data together, it seems possible to speculate that L-arginine significantly spares lean body mass. This is strengthened by statistical evaluation of the interaction between changes in FFM and the different treatments ( $F = 5.1$ ,  $P < 0.03$ ; Table 1).

Systolic and diastolic blood pressure, fructosamine, insulin, and triglyceride levels significantly decreased after both L-arginine and placebo treatments, but compared with placebo, L-arginine group showed a more significant reduction in systolic ( $F = 43.5$ ,  $P < 0.0001$ ) and diastolic blood pressure ( $F = 18.5$ ,  $P < 0.0002$ ), fructosamine ( $F = 6.1$ ,  $P < 0.02$ ), and insulin ( $54 \pm 4$  vs.  $32 \pm 8\%$ ;  $F = 4.25$ ,  $P < 0.04$ ). In addition, the decrement in homeostasis model assessment index was almost twofold after L-arginine compared with placebo (L-arginine: from  $8.7 \pm 1.6$  to  $3.4 \pm 0.4$  vs. placebo from  $7.7 \pm 1.1$  to  $4.3 \pm 0.3$ ;  $P < 0.02$ ). Furthermore, total cholesterol (L-arginine: from  $196 \pm 12$  to  $156 \pm 8$  vs. placebo from  $199 \pm 8$  to  $173 \pm 11$  mg/dl;  $P < 0.01$ ) and LDL cholesterol (L-arginine: from  $121 \pm 9$  to  $90 \pm 8$  mg/dl vs. placebo from  $113 \pm 8$  to  $96 \pm 8$  mg/dl;  $P < 0.01$ ) similarly decreased in both groups, whereas HDL cholesterol remained unchanged in both groups.

In Fig. 1, the capillary glycemic profiles before and at the end of each week of therapy are represented. Daily glucose profiles were similar between the two treatments groups at baseline (mean daily glucose levels,  $163 \pm 8$  and  $156 \pm 8$  mg/dl for L-arginine and placebo groups, respectively) with inadequate postprandial glucose control observed after each meal. At the end of the first week of therapy, patients in treatment with L-arginine had lower glucose levels at fasting, whereas at the end of the second week of therapy L-arginine significantly decreased glucose levels at fasting, before lunch, and after lunch compared with placebo. At the end of the third week of therapy mean daily glucose profiles significantly decreased in both groups at baseline ( $106 \pm 3$  and  $123 \pm 4$  mg/dl;  $P < 0.001$ ), although L-arginine therapy was able to further decrease the mean daily glucose profiles compared with placebo ( $F = 48.9$ ,  $P < 0.0001$ ). The latter effect was mainly determined by a near normalization of postprandial glucose excursions throughout the study (time effect  $F = 14.1$ ,  $P < 0.001$ ; interaction between changes in postprandial glucose

levels and the different treatments  $F = 9.2$ ,  $P < 0.01$ ) compared with the glucose profiles obtained in 10 normal subjects (represented in Fig. 1 as shaded areas).

**Endothelial function, basal oxidative stress, and adipokine release.** Table 2 shows endothelial parameters and adipokine release. After 21 days of therapy, only L-arginine treatment induced a reduction of endothelin-1 levels by 30% (interaction effect  $F = 38.5$ ,  $P < 0.0001$ ) and an increase of cGMP levels by 35% (interaction effect  $F = 14.8$ ,  $P < 0.001$ ) and ec-SOD by 35% (interaction effect  $F = 5.5$ ,  $P < 0.03$ ), whereas after placebo these variables remained unchanged. At the end of the study, mean daily glucose profiles significantly correlated with cGMP ( $r = 0.41$ ,  $P < 0.02$ ; Fig. 2A), ec-SOD ( $r = 0.32$ ,  $P < 0.05$ ; Fig. 2B), and endothelin-1 levels ( $r = 0.52$ ,  $P < 0.002$ ; Fig. 2C).

Interestingly, adiponectin levels significantly increased after L-arginine treatment, whereas they remained unchanged after placebo (interaction effect  $F = 5.4$ ,  $P < 0.03$ ). On the contrary, leptin levels decreased after both treatments to the same extent (time effect  $F = 9.2$ ,  $P < 0.01$ ).

Leptin-to-adiponectin ratio, a new atherogenic index (31), significantly decreased in both groups after the diet and exercise training program (time effect  $F = 13.6$ ,  $P < 0.001$ ),

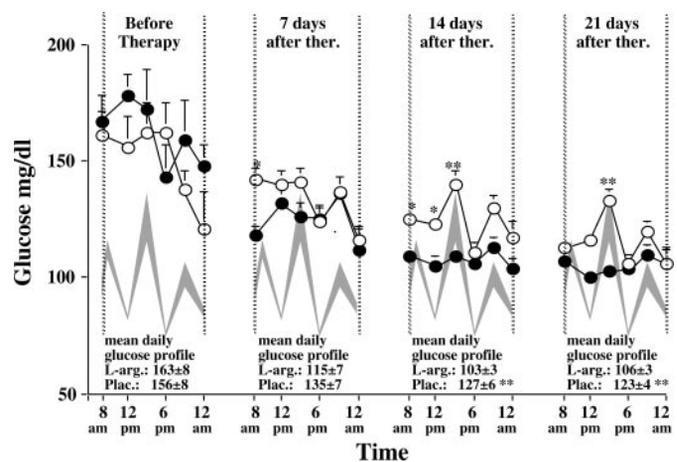


Fig. 1. Daily capillary glucose profile levels before and after 1–3 wk of treatment with hypocaloric diet plus exercise training program [○; placebo (Plac)] or associated with L-arginine supplementation (●; L-Arg) in obese, insulin-resistant type 2 diabetic patients. Shaded areas represent mean ± SD of daily capillary glucose profiles in 10 normal subjects. \* $P < 0.05$  vs. L-arginine ther. \*\* $P < 0.01$  vs. L-arginine.

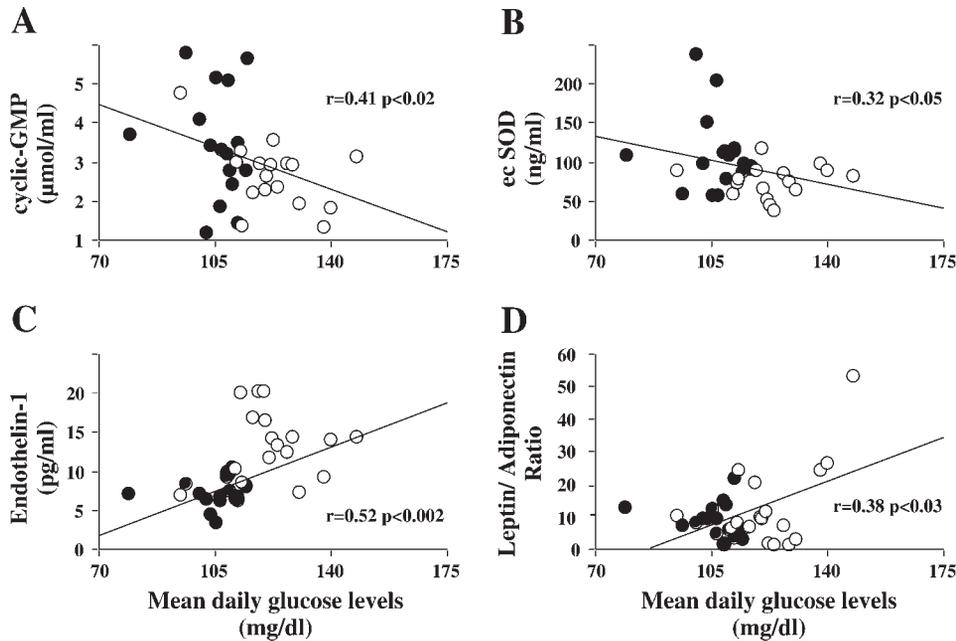


Fig. 2. Relationship between mean capillary daily glucose profiles and cGMP (A), extracellular superoxide dismutase (ec-SOD; B), endothelin-1 (C), and leptin-to-adiponectin ratio (D) at the end of 21-days period with hypocaloric diet plus exercise program (○) or associated with L-arginine supplementation (●) in obese, insulin-resistant type 2 diabetic patients.

although in L-arginine group the decrement was more pronounced (interaction effect:  $F = 3.2$ ,  $P < 0.05$ ). Taking the two group together, at the end of the study, the leptin-to-adiponectin ratio significantly correlated with mean daily glucose profiles ( $r = 0.38$ ,  $P < 0.03$ ; Fig. 2D)

**Ergometric walking test.** After the period of diet and exercise training program, the ergometric walking test demonstrated a significant and similar increase in meters that were walked (from  $492 \pm 25$  to  $533 \pm 20$  m with L-arginine; from  $481 \pm 24$  to  $543 \pm 25$  m with placebo; time effect  $F = 28.0$ ,  $P < 0.0001$ ).

Systolic blood pressure levels at the end of the ergometric walking test significantly decreased after 21 days of L-arginine, and the net decrement of the systolic blood pressure at the end of the ergometric walking test was doubled after L-arginine than after placebo ( $-22 \pm 5$  vs.  $-11 \pm 3$  mmHg;  $P < 0.05$ ).

Furthermore, endothelin-1 incremental areas significantly decreased by 96%, whereas NOx and cGMP incremental areas increased by 250 and by 263%, respectively, after L-arginine treatment. Two-way ANOVA test demonstrated a highly significant interaction effect between changes in these variables and the two treatments (Table 2).

ec-SOD incremental areas during the ergometric walking test significantly increased after both L-arginine and placebo therapies, but a greater effect was demonstrated after L-arginine treatment (interaction effect  $F = 3.4$ ,  $P < 0.05$ ).

Systolic blood pressure significantly correlated with cGMP incremental areas ( $r = 0.32$ ,  $P < 0.05$ ; Fig. 3A), with ec-SOD incremental areas ( $r = 0.38$ ,  $P < 0.03$ ; Fig. 3B) and with endothelin-1 incremental areas ( $r = 0.54$ ,  $P < 0.001$ ; Fig. 3C).

## DISCUSSION

A relatively short period of changes in lifestyle can improve glucose and insulin levels and endothelial function, and in the present study, for the first time, it was possible to show that a prolonged oral L-arginine supplementation can further ameliorate glucose metabolism and insulin sensitivity, endothelial function, oxidative stress, and adipokine release, saving FFM during a period of hypocaloric diet when associated with an exercise training program in obese, insulin-resistant, type 2 diabetic patients.

The association of a hypocaloric diet with aerobic plus resistance training program was chosen to induce a synergistic

Table 2. Changes in endothelial and hormonal variables before and after L-arginine and placebo therapy

	L-Arginine		Placebo		P Value	
	Before	After	Before	After	Time effect	Interaction effect
Incremental area NOx, $\mu\text{mol/l}\cdot 6$ min	$9.2 \pm 5.0$	$32.8 \pm 5.7$	$9.5 \pm 2.6$	$8.9 \pm 2.7$	$<0.005$	$<0.002$
Fasting cGMP, $\mu\text{mol/ml}$	$2.30 \pm 0.2$	$3.56 \pm 0.3$	$2.25 \pm 0.1$	$2.70 \pm 0.2$	$<0.005$	$<0.001$
Incremental area cGMP, $\mu\text{mol/ml}\cdot 6$ min	$1.50 \pm 0.3$	$5.45 \pm 0.6$	$1.61 \pm 0.2$	$3.67 \pm 0.2$	$<0.0001$	$<0.001$
Fasting ET-1 pg/ml	$10.5 \pm 0.5$	$7.4 \pm 0.4$	$9.5 \pm 0.4$	$13.7 \pm 1.0$	$<0.37$	$<0.0001$
Incremental area ET-1, pg/ml $\cdot 6$ min	$12.4 \pm 2.6$	$0.4 \pm 1.1$	$11.2 \pm 2.6$	$11.1 \pm 2.4$	$<0.01$	$<0.01$
Basal ec-SOD, ng/ml	$84.6 \pm 7.0$	$113.9 \pm 12.3$	$86.8 \pm 7.4$	$77.3 \pm 4.9$	$<0.22$	$<0.03$
Incremental area ec-SOD, mol/ml $\cdot 6$ min	$72.8 \pm 31.8$	$210.5 \pm 20.6$	$37.1 \pm 21.9$	$95.7 \pm 10.4$	$<0.0002$	$<0.05$
Adiponectin, ng/ml	$4.0 \pm 0.7$	$5.6 \pm 0.6$	$4.2 \pm 0.6$	$4.2 \pm 0.5$	$<0.02$	$<0.03$
Leptin, ng/ml	$62.4 \pm 7.7$	$48.0 \pm 7.8$	$53.0 \pm 9.5$	$48.6 \pm 9.7$	$<0.01$	$<0.07$
Leptin-to-adiponectin ratio	$21.8 \pm 3.9$	$9.4 \pm 1.3$	$18.1 \pm 5.5$	$13.7 \pm 3.2$	$<0.001$	$<0.05$

Values are means  $\pm$  SE. NOx, nitrate/nitrite; ET-1, endothelin-1; ec-SOD, extracellular; superoxide dismutase.

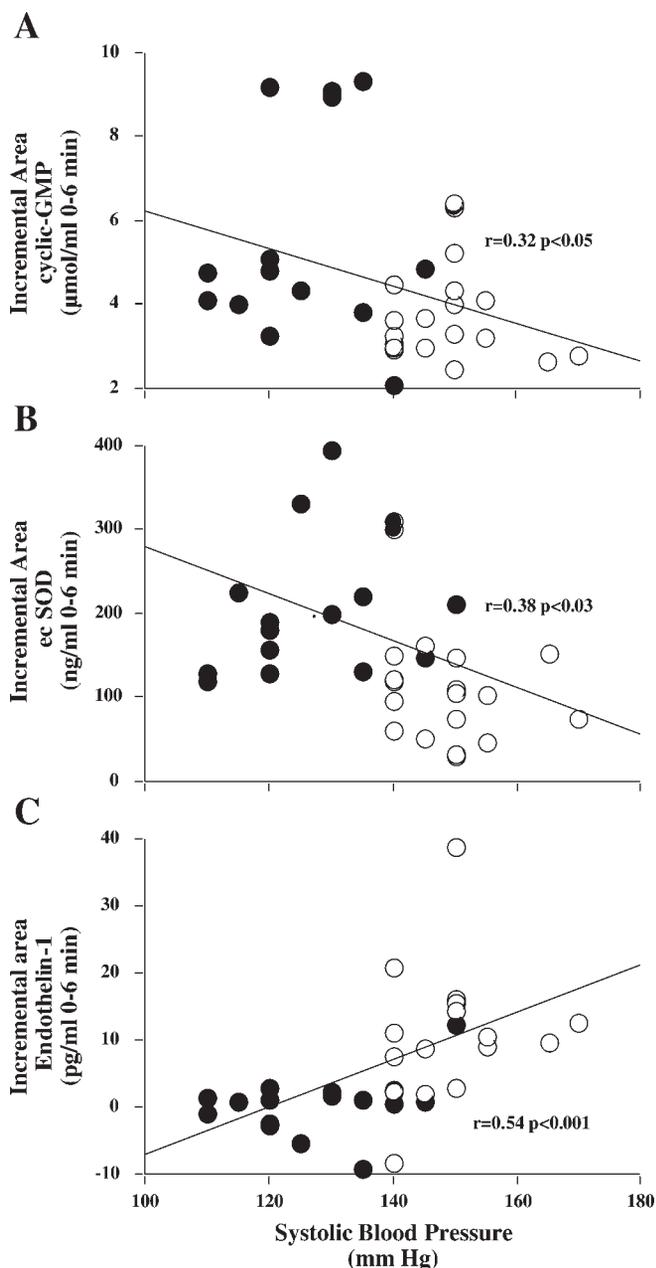


Fig. 3. Relationship between mean systolic blood pressure and incremental areas for cGMP (A), ec-SOD (B), and endothelin-1 (C) at the end of 21-day period with hypocaloric diet plus exercise program (○) or associated with L-arginine supplementation (●) in obese, insulin-resistant type 2 diabetic patients.

effect on glucose metabolism and insulin sensitivity, preserving FFM. It is well known that a hypocaloric diet induces a reduction of FM and FFM in the same amount (28), whereas the association with exercise may save FFM. Indeed, the reduction in FFM, FM, and waist circumference after placebo therapy are in agreement with previous results found in obese type 2 diabetic patients (30). The relative small amount of total weight loss observed in the present study during placebo treatment seems to be due to the influence of physical activity on body composition with preservation of FFM. Interestingly, L-arginine was able to cause a further additive effect, nearly abolishing the loss in FFM and inducing a more important

reduction in FM. In addition, there was a twofold decrement of waist circumference when L-arginine was added to diet and exercise, suggesting a specific effect in decreasing visceral obesity. These findings, to our knowledge first observed in humans, are confirmatory of previous results in animal studies utilizing L-arginine supplementation in Zucker diabetic fatty rats. In fact, L-arginine therapy increased expression of key genes responsible for fatty acid and glucose oxidation in adipose tissue. The final result was a reduction of abdominal (retroperitoneal) and epididymal adipose tissue (11).

After a hypocaloric diet and exercise training program, fasting glucose levels were almost normalized within 3 wk, and insulin and triglycerides levels were reduced confirming that a lifestyle intervention is of benefit in the therapy of type 2 diabetes mellitus. L-Arginine treatment caused not only a faster improvement in fasting glucose levels but also a normalization of postprandial glucose levels, a result not completely achieved during placebo therapy. In agreement with these results, fructosamine levels were significantly lower after L-arginine therapy than placebo. In our opinion, this is one of most striking and interesting result of the present study, because although fasting glucose levels are an important measure of glycemic control, and fasting hyperglycemia is implicated in the chronic complications of diabetes (6), recent studies strongly suggest that postprandial hyperglycemia may actually be more important than fasting glucose levels with respect to overall glycemic control (1, 4, 7). Recent studies have found that management of postprandial blood glucose levels may influence microvascular (9) and possibly cardiovascular (8, 16, 24) risk in patients with type 2 diabetes.

However, the effects of diet, exercise, and L-arginine on endothelial function were already documented. Several authors have found, in obese subjects, that diet and exercise training program improved vascular dysfunction measured by ultrasound-derived brachial artery endothelial function, suggesting that NO-induced endothelial dilation is ameliorated by changes in lifestyle (34). Oral L-arginine supplementation improved endothelial-dependent vasodilation in patients with chronic heart failure with an additional effect compared with exercise alone (13). Although in the present study endothelial-dependent vasodilation was not measured, the measurement of circulating NO end metabolites (i.e., NOx) and the NO second messenger (i.e., cGMP) levels during ergometric walking test confirmed the positive effect of L-arginine supplementation on endothelial function. Associated with an improvement in nitric oxide-induced endothelial function there was a significant decrement in basal and postexercise endothelin-1 levels as previously found after acute and chronic L-arginine therapy (22, 25). As a net effect, during L-arginine therapy, there was a significant decrement in systolic and diastolic blood pressure supporting the potential role of the endothelium on the regulation of blood pressure. In agreement with this hypothesis, systolic blood pressure at the end of the study significantly correlated with cGMP and furthermore with endothelin-1 incremental areas.

In the present study, the dose of 8.3 g/day was chosen because it has been previously demonstrated that 9 g/day is the minimal dose able to increase circulating L-arginine concentration with minimal side effects in healthy subjects (10). The question of the minimal effective dose of L-arginine to be administered is rather important because it has been demon-

strated that high doses of L-arginine supplementation could paradoxically contribute to atherosclerotic lesion formation by mechanisms involving lipid oxidation, peroxynitrite formation, and NO synthase uncoupling (5, 23).

The present study confirms the essential function of the exercise training in the regulation of the antioxidant enzymes activity as found by measuring ec-SOD incremental areas (12). The administration of L-arginine provided supplemental substrate to bolster NO production and further enhance ec-SOD levels. It seems possible to postulate that low L-arginine supplementation provides a direct antioxidant activity, suggesting another mechanism for improving NO bioactivity, but this hypothesis needs further studies.

A new and interesting finding of this study was that L-arginine is effective in modulating adipokine release in vivo. Because in the present study hypocaloric diet and exercise training program was undertaken for only 3 wk, the decrease in FM was relatively small and not sufficient to significantly influence positively or negatively adipokine release during placebo therapy. These results are in agreement with several studies in which it was found that to influence adipokine metabolism it is necessary at least 10 kg of body weight to be lost (2). Surprisingly, L-arginine therapy significantly modified adipokine release, enhancing adiponectin levels. The direct effect of adiponectin on atherosclerosis was recently investigated by Kumada et al. (21), who showed that adiponectin specifically increased tissue inhibitor of metalloproteinase-1, preserving vascular inflammation and in turn delaying the development of atherosclerosis. In this light, the significant decrement in leptin-to-adiponectin ratio after L-arginine treatment compared with placebo is a further element to support the antiatherogenic role of L-arginine therapy. The latter hypothesis is in agreement with a recent study in which the leptin-to-adiponectin ratio was more specific marker of atherosclerosis than the pulse-wave test (31).

Finally, the evidence that daily glucose profiles significantly correlate with cGMP, endothelin-1, ec-SOD levels and with leptin-to-adiponectin ratio at the end of the study period suggests that the improvement in glucose levels, although is mainly influenced by changes in insulin levels, could also be related to other metabolic pathways, including, at least in part, endothelial (for at least 27%) and adipose tissues (for  $\geq 14\%$ ).

In conclusion, a relatively short period of changes in lifestyle can improve glucose and insulin levels and endothelial function. Interestingly, L-arginine therapy seems to further improve several metabolic features characteristic of the metabolic syndrome, such as fasting and postprandial glycemic excursions, hyperinsulinemia, hypertension, visceral obesity, endothelial dysfunction, and unbalance in adipokine release.

However, further prospective studies are necessary to confirm the beneficial effects of L-arginine in reducing risk factors for the development of atherosclerosis and cardiovascular disease.

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