Eur J Nutr (2013) 52:975–983
DOI 10.1007/s00394-012-0404-7

ORIGINAL CONTRIBUTION

Effects of L-arginine supplementation on blood flow, oxidative stress status and exercise responses in young adults with uncomplicated type I diabetes

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Received: 30 November 2011 / Accepted: 12 June 2012 / Published online: 6 July 2012

Abstract
Background and aims Vascular disease is the principal cause of death and disability in patients with diabetes, and endothelial dysfunction seems to be the major cause in its pathogenesis. Since L-arginine levels are diminished in conditions such as type 1 and type 2 diabetes, in this work we aimed to verify the effects of L-arginine supplementation (7 g/day) over the endothelial function and oxidative stress markers in young male adults with uncomplicated type 1 diabetes. We also investigated the influences of L-arginine administration on vascular/oxidative stress responses to an acute bout of exercise.
Methods Ten young adult male subjects with uncomplicated type 1 diabetes and twenty matched controls volunteered for this study. We analysed the influence of L-arginine supplementation (7 g/day during 1 week) over lower limb blood flow (using a venous occlusion plethysmography technique), oxidative stress marker (TBARS, Carbonyls), anti-oxidant parameters (uric acid and TRAP) and total tNOx in rest conditions and after a single bout of submaximal exercise (VO_2 at 10 % below the second ventilatory threshold). Data described as mean ± standard error (SE). Alpha level was $P < 0.05$.
Results Glycaemic control parameters were altered in type 1 diabetic subjects, such as HbA1c (5.5 ± 0.03 vs. 8.3 ± 0.4 %) and fasted glycaemia (94.8 ± 1.4 vs. 183 ± 19 mg/dL). Oxidative stress/damage markers (carbonyls and TBARS) were increased in the diabetic group, while uric acid was decreased. Rest lower limb blood flow was lower in type 1 diabetic subjects than in healthy controls (3.53 ± 0.35 vs. 2.66 ± 0.3 ml 100 ml^{-1} min^{-1}). L-Arginine supplementation completely recovered basal blood flow to normal levels in type 1 diabetics' subjects (2.66 ± 0.3 to 4.74 ± 0.86 ml 100 ml^{-1} min^{-1}) but did not interfere in any parameter of redox state or exercise.
Conclusion Our findings highlight the importance of L-arginine for the improvement of vascular function in subjects with diabetes, indicating that L-arginine supplementation could be an essential tool for the treatment for the disease complications, at least in non-complicated diabetes. However, based on our data, it is not possible to draw conclusions regarding the mechanisms by which L-arginine therapy is inducing improvements on cardiovascular function, but this important issue requires further investigations.

Electronic supplementary material The online version of this article (doi:10.1007/s00394-012-0404-7) contains supplementary material, which is available to authorized users.

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Keywords L-Arginine · Type 1 diabetes · Blood flow · Oxidative stress

Introduction

Vascular disease is the principal cause of death and disability in patients with diabetes [1], being endothelial dysfunction one of the major causes in its pathogenesis [2–4]. The normal vascular endothelium plays an important role in maintaining vessel wall homoeostasis, through synthesizing substances such as prostacyclin and the free radical nitric oxide (NO·), which modulate vascular tone, prevent thrombosis, and stimulate smooth muscle growth [5]. Strategies aiming to maintain/restore a normal vascular function are important for vascular diseases prevention in diabetes. Rather than the use of therapeutic drugs for the control of cholesterol, hyperglycaemia and other risk factors related to atherogenesis process, blood flow restoration seems to be essential.

Endothelial dysfunction, which is defined by decreased endothelium-dependent vasodilation, is the hallmark of cardiovascular complications in diabetes. A potent vasodilator, nitric oxide (NO·), is found reduced in diabetic subjects, compromising their vascular tonus control [6]. Reductions in the NO· availability may occur when the levels of its precursor L-arginine are decreased [6].L-Arginine, considered a semi-essential or conditionally essential amino acid [7, 8], has been used as a strategy to improve endothelial function [9], insulin secretion and pancreatic beta cell protection [10, 11], besides adiposity control in obesity and diabetes [12].

Indeed, L-arginine administration in vivo (using animal models) resulted in protection against the effects of many diabetic agents, such as alloxan and streptozotocin [10, 13–16]. Interestingly, a single administration of watermelon, rich in L-citrulline (L-arginine precursor), was able to reduce the serum concentrations of cardiovascular risk factors such as homocysteine, improved glycaemic control and ameliorated vascular dysfunction in obese insulin-resistant animals [17]. In addition, several studies have identified L-arginine supplementation in humans as a tool for the treatment for cardiovascular complications and diabetes, improving endothelial function, reducing oxidative stress markers and increasing NO· availability [9, 18–24].

The beneficial effects of aerobic exercise of moderate intensity in diabetes are also well documented regarding glycaemic control and multiple CVD (cardiovascular disease) risk factors. Improvements in glycaemic control included improvements in Hb1Ac (glycated haemoglobin), insulin sensitivity and AUC (area under the curve) during an OGTT (oral glucose tolerance test). Enhanced vascular function included changes in endothelial function (e.g. flow-mediated dilation), carotid artery intima-media thickness and arterial distensibility. Finally, improvements in metabolic control included glycaemic control, weight loss and amelioration in lipid profile [25]. Although the exercise-induced improvements in the parameters described above are widely accepted, the molecular basis underlying that is still open to debate [6].

Herein, we aimed to describe the effects of a short-term L-arginine supplementation (7 g/day) over the endothelial function and oxidative stress markers in young male adults with uncomplicated type 1 diabetes. In addition, we have also investigated the vascular/oxidative stress responses in type 1 diabetic subjects submitted to an acute bout of exercise before and after L-arginine administration.

Research design and methods

Subject characteristics

Ten young adult male subjects with uncomplicated type 1 diabetes and twenty matched controls volunteered for this study. They provided informed consent after being fully informed about the study protocol. The research design, assessments and protocols were approved by the Research Ethical Committee from the *Hospital de Clínicas of Porto Alegre* (protocol 04-009).

Subjects' age range was between 18 and 30 years old, non-smokers, physically active, but non-athletes (according the criteria used by the American College of Sports Medicine, ACSM—2005) and not using any vitamin supplement were included in this study. All selected diabetic subjects were not undertaking any medication, apart from exogenous insulin. They were also free of clear diabetic complications such as retinopathy, neuropathy, nephropathy or vascular disease at the time of recruitment. Patients presenting any of the previous complications, more than 10 years of disease or HbA1c higher than 10 %, were excluded from the study. All subjects were recruited and screened in the Pathology Laboratory [Hospital de Clinicas de Porto Alegre (HCPA)—Brazil) for diagnostics of diabetes and biochemical variables such as fasted glycaemia, lipid profile (LDL, HDL, total cholesterol and triglycerides), HbA1c (glycated haemoglobin) and urea, using an automated system analyser (Mega Bayer, USA).

A prospective sample size power calculation was performed using according to the calculation using Eplinfo software version 6.0. General subjects' characteristics are listed in Table 1. Sample calculation was based on the study results of Palloshi et al. (2004) where L-arginine supplementation (6 g/day) increased blood flow in 31 % of

Table 1 Subjects' anthropometric, biochemical and physiological characteristics

	Controls ($n = 20$)	Type I diabetics ($n = 10$)	P
Age (years)	23.4 ± 0.59	23.3 ± 1.73	0.958
Time diagnosed (years)	–	8.5 ± 5.94	–
Body mass (Kg)	75.2 ± 2.54	72.3 ± 4.25	0.574
Height (cm)	177.6 ± 1.81	173.7 ± 2.18	0.281
BMI (kg/m^2)	23.7 ± 0.43	23.9 ± 1.43	0.883
Body Fat (%)	17.8 ± 1.20	19.06 ± 2.92	0.661
SBP (mmHg)	120.6 ± 2.53	126.6 ± 2.71	0.256
DBP (mmHg)	80.0 ± 2.54	85.0 ± 3.02	0.109
HR_{max} (bpm)	186.7 ± 2.19	179.7 ± 1.83	0.023*
VO_{2max} (ml kg^{-1} min^{-1})	45.4 ± 1.75	37.1 ± 2.28	0.013*
Fast glycaemia (mg/dl)	94.8 ± 1.42	183.1 ± 19.13	0.000*
HbA_{1C} (%)	5.5 ± 0.03	8.3 ± 0.43	0.000*
Total cholesterol (mg/dl)	146.7 ± 5.09	145.2 ± 7.37	0.863
HDL (mg/dl)	42.8 ± 1.76	46.6 ± 2.58	0.236
Triglycerides (mg/dl)	113.6 ± 22.79	61.7 ± 16.44	0.153

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HRmax* maximal heart rate, *HDL* high-density lipoprotein

* $P < 0.05$ when compared diabetic with control group

individuals with endothelial dysfunction [26]. The statistical power was 80 % and the confidence interval of 95 %.

Study design and experimental groups

Subjects underwent four assessment sessions. In session one, prior to participation, subjects completed a heath history questionnaire, PAR-Q and also a dietary intake assessment. Blood pressure and body composition were verified. Participants' VO_{2max}, ventilatory thresholds and maximum heart rate (HR_{max}) were determined through an incremental exercise test further described. In session two, following 12 h fast, antecubital venous blood and urine were collected for baseline biochemistry measurements and subjects' characterization related to lipid profile, glycaemia and urea. In session three, lower limb blood flow was assessed, followed by a submaximal exercise test, also further explained. All subjects were instructed to feed themselves in a minimum of 60 min and a maximum of 90 min prior to the session. Blood samples were taken before and immediately after the exercise bout. After the first exercise bout, subjects received the L-arginine (or placebo) supplementation treatment (double-blind) and all the instructions for oral administration. After 7 days under supplementation, subjects returned to the laboratory for session four, when they repeated the same submaximal exercise bout performed in session three. Again, blood flow, antecubital venous blood and urine samples were collected before and after the exercise.

L-Arginine supplementation consisted in oral ingestion of identical pills containing either amide compound (as placebo) or 7 g of L-arginine-hydrochloride (for the L-arginine group). The treatment consists in 7 g per day of L-arginine or placebo for seven consecutive days. The subjects were assessed for baseline values and then randomly distributed in four groups: *Group 1* Control Placebo (CP), *Group 2* Control L-arginine (CA), *Group 3* Diabetic Placebo (DP) and *Group 4* Diabetic L-arginine (DA). Our decision for the use of 7 g of arginine/day was based on previous studies that have shown positive improvements on the endothelial and metabolic function [27–30]. Placebo consisted in an amide-based formulation in the same quantity, colour and taste.

Anthropometric measurements

Standing height was measured using a Stainless Steel Stadiometer, with the participants' shoes off and head at the Frankfort horizontal plane. Body mass was assessed using a Filizola Scale. A Lange calliper was used to measure 7 skinfold thickness (abdominal, iliac crest, biceps, triceps, suprailiac, chest, thigh and subscapular), and a protocol from Jackson et al. [31] was used to calculate the percentage of body fat mass.

Dietary intake assessment

Subjects' dietary intake was assessed 1 week before the trial, using a three-day dietary record, to identify any differences in the general diet, enabling quantification of total energy (kilocalories), carbohydrate, protein and total fat (g). All subjects were instructed to fill the dietary record correctly. For these analyses, we used the software *Programa de Apoio do Centro de Informatica em Saude da Escola Paulista de Medicina* (CIS-EPM) from the Federal University of Sao Paulo, version 2.5.

Maximal exercise test

Ventilatory and respiratory parameters were measured by indirect calorimetry, using a gas analyser (*MedGraphics Cardiorespiratory Diagnostic Systems* model MGC/CPX-D USA) calibrated prior and after each test. Participants who had never previously performed an exercise test in a cycle ergometer underwent a familiarization before the exercise sessions. VO_2, gas carbonic production (VCO_2), ventilation (VE), respiratory exchange rate (RER) and HR were continuously measured during the test. VO_{2max} and individual's ventilatory threshold (*Tvent*) were determined in the following plots: VE and ventilatory equivalents of oxygen uptake and carbonic gas (VE/VO_2 and VE/VCO_2, respectively), as a function of the VO_2.

Maximal effort exercise test was performed in a cycle ergometer (Cybex, The Byke, USA). The incremental protocol started with initial resistance of 25 watts (W), followed by increases of 25 W each 1 min ($W\ min^{-1}$), until subjects' exhaustion (Lucía et al. 2000). Five-minute recovery at 25 W was provided at the end of the test. During the test, participants were instructed to keep their cycling pace in between 60 and 90 rotations per minute (rpm). The test was interrupted when subjects could not maintain at least 60 rpm or when it as requested by themselves.

VO_{2max} was identified by a tendency to a plateau of VO_2 despite an increase in the workload or an increase in the $VO_2 < 1\ ml/kg^{-1}/min^{-1}$ in comparison with the one produced by the previous workload [32]. VO_2 data were continuously measured breath by breath. Other criteria were also considered to verify the maximal effort, such as an attainment of age-predicted maximum heart rate and a respiratory exchange ratio (RER) >1.15 [33].

Criteria considered to determine *Tvent* were (1) attributed to the lowest workload in which VE/VO_2 showed a concomitant increase with VE/VCO_2 [34, 35]; (2) the workload in which the RER related to the time and to the VO_2 attained the value 1 and did not decrease until the end of the exercise [35, 36]; (3) second nonlinear increases in the ventilation's curve during the exercise [34].

Submaximum exercise test

Submaximum exercise test was performed during 45 min in the same cycle ergometer previously mentioned. Individuals' steady-state intensities were adjusted in relation to VO_2 values corresponding to 10 % below VO_2 responses at *Tvent*.

Blood flow measurement

Lower limb blood flow measurement was evaluated using a venous occlusion plethysmography technique according to the methods previously described by Copeland et al. [37]. This technique is a non-invasive method used to study human vascular changes in vivo. Changes in lower limb blood flow/volume were measured by a mercury-in-rubber strain gauge connected to a plethysmograph (Hokanson TL-400, USA) in the left calf.

Uric acid measurement

The non-enzymatic anti-oxidant uric acid was measured using a colorimetric kit (Wiener Lab, AA Rosario, ARG), according to the manufacturer instructions.

Total nitrites/nitrates (tNOx) and plasma protein

A colorimetric assay was used for the measurement of total nitrites and nitrate simultaneously (tNOx) in a 96-well plate. The principle of this assay is the reduction of nitrate by vanadium (III) combined with detection by the acidic Griess reaction that can be read in a microplate reader (Backmark Bio Rad-USA) at 540 nm [38] against a standard nitrite curve. Plasma total protein content was measured using a colorimetric modified Lowry assay [39].

Thiobarbituric-acid-reactive substances (TBARS) assay

As an index to lipoperoxidation, we used the thiobarbituric-acid-reactive species (TBARS) test, as previously described [40]. TBARS consists in an acid-heating reaction of the lipid peroxidation end product, malondialdehyde, with thiobarbituric acid (TBA). TBARS were determined at 532 nm and were expressed as $nmol\ mg\ protein^{-1}$.

Determination of plasma carbonyl content

Oxidative damage to proteins was measured by the determination of carbonyl groups with the addition of DNPH (2,4-dinitrophenylhydrazine) to the plasma sample, as described by Levine et al. [41]. This method is based on the reaction of DNPH with protein carbonyl groups. Results were expressed as $nmol\ of\ carbonyl\ per\ mg\ protein^{-1}$.

Total reactive antioxidant potential (TRAP)

We measure the total reactive antioxidant potential (TRAP) as described by Wayner et al. [42]. We used this test as an index of the non-enzymatic antioxidant capacity on plasma, based on the peroxyl radical (generated by AAPH solution, 2,2′-azobis[2-amidinopropane], with luminol) quenching by sample compounds. Briefly, luminol (system) was added to AAPH solution and incubated for 2 h for stabilization followed by the first reading. After that, the sample was added and incubated for further 30 min for

the second reading. The reading is done by chemiluminescence emission. Counts of photons emitted from each well were measured at 1-min intervals and recorded as counts per minute (cpm). Results were expressed as cpm % using a known standard antioxidant as 100 % of antioxidant capacity.

Statistical analyses

Data were described as mean ± standard error (SE). Alpha level was set at $p < 0.05$. Shapiro–Wilk normality test was applied previously to all analyses. Independent Student's t test was used to compare diabetic and control group regarding age, diet profile, body composition, VO_{2max}, VT2, level of physical activity, fasting blood glucose, total cholesterol, HDL, triglycerides, HbA1c, urea, endothelial function and oxidative stress parameters. Dependent Student's t test was applied to verify the differences in endothelial function and oxidative stress variables before and after acute exercise for each group, previously to the supplementation period. The effects of arginine supplementation for each group and between them were verified by two-way ANOVA, followed by post hoc Tukey test.

Results

Anthropometric measurements, VO_{2max} and dietary intake

As identified in Table 1, no differences were found regarding anthropometric characteristics between both groups of subjects. As expected, all the parameters related to glycaemic control were altered in type 1 diabetic subjects, such as HbA1c and fasted glycaemia. Mean dietary intake for total energy (kcal) and nutrients did not differ between the groups (dietary intake data are represented in Table 4). Type 1 diabetic subjects' VO_{2max} and HR_{max} were lower when compared with non-diabetic subjects.

Nitric oxide metabolites (tNOx) and hemodynamic measures

Lower limb blood flow measurement was evaluated using a venous occlusion plethysmography technique according to the methods previously described by Copeland et al. [37]. Rest lower limb blood flow was lower in type 1 diabetic subjects than in healthy controls (Fig. 1). Although lower blood flow values in the diabetic group, blood flow was increased in all groups after an acute exercise bout (Table 2), suggesting that the diabetic subjects are able to raise blood flow levels in response to exercise, however, in lower magnitude. Most importantly,

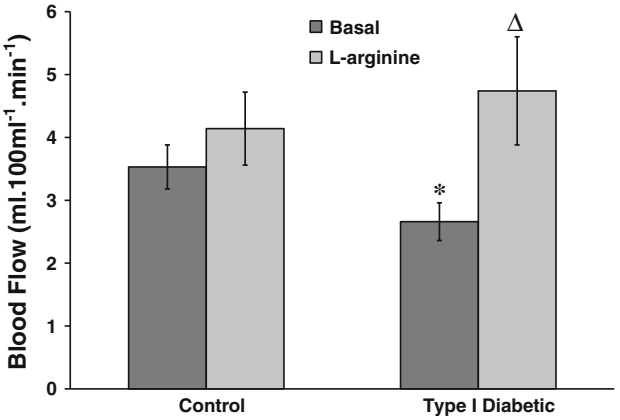

Fig. 1 Baseline comparison of lower limb blood flow (ml 100 ml^{-1} min^{-1}), measured by a venous occlusion plethysmography. *$P < 0.05$ when compared diabetic with control group; Δ $P < 0.05$ when compared before and after L-arginine supplementation

L-arginine supplementation completely recovered basal blood flow to normal levels in type 1 diabetics' subjects (Table 3). L-Arginine did not change the post-exercise blood flow in any experimental group. In our hands, tNOx concentrations did not change in any circumstance, in response neither to exercise nor to L-arginine supplementation for all the groups studied. Moreover, no difference was found in relation to basal tNOx concentration between diabetic and non-diabetic subjects.

Oxidative stress parameters

As expected, all markers for oxidative stress/damage (carbonyls and TBARS) were found increased in the diabetic group, while the antioxidant marker (uric acid) was decreased in the same group (Table 2). TRAP values were lower in diabetic subjects (Fig. 2 supplementary data). The acute bout of exercise and the L-arginine supplementation did not cause any change in these parameters (Tables 2, 3).

Discussion

The main finding of this study is that L-arginine supplementation (7 g/day) can elicit significant improvements in lower limb vascular function (i.e. blood flow) in type 1 diabetic subjects. Improvements in blood flow and cardiovascular function are related to a diminution in oxidative stress [43]. In the present report we found that, in accordance with other studies [44], diabetic subjects present higher levels of ROS damage as indicated by the results of TBARS and carbonyls. In this regard, supplementation with L-arginine has failed to reverse these parameters; then, it is unlikely that the improvements in blood flow caused by L-arginine administration are related to any concurrent

Table 2 Biochemical oxidative stress markers and blood flow measurement before L-arginine supplementation

	Control subjects ($n = 20$)		Type I diabetic subjects ($n = 10$)	
	Basal	Post-exercise	Basal	Post-exercise
Blood flow (ml.100 ml^{-1} min^{-1})	3.53 ± 0.35	5.46 ± 0.65†	2.66 ± 0.33*	3.77 ± 0.36†
tNox (μM)	11.02 ± 3.92	10.77 ± 3.93	11.39 ± 3.7	11.57 ± 3.75
TBARS (nmol MDA mg $protein^{-1}$)	1.09 ± 0.41	0.83 ± 0.68	2.44 ± 0.46*	2.40 ± 0.19
Carbonyls (fM mg $protein^{-1}$)	0.148 ± 0.019	0.171 ± 0.016	1.48 ± 0.205*	1.26 ± 0.227
Uric acid (mg/dL)	44.55 ± 2.06	41.93 ± 1.76	28.1 ± 1.57*	28.69 ± 1.87

† $P < 0.05$ when compared basal versus post-exercise within the same group. * $P < 0.05$ when compared diabetic with control group

up-regulation of anti-oxidant mechanisms as was previously suggested [9, 10] (Table 4).

Several studies have been conducted in order to investigate the effects of L-arginine supplementation in conditions likely to reduce nitric oxide availability [10–13, 15–17]. Results have consistently shown that L-arginine ameliorates several metabolic parameters such as lipid oxidation [12], reduction of the polyol pathway [15], eNOS activation [45] and ameliorated vascular dysfunction [17]. Different from our results, most of the reports attribute the L-arginine effects to the activation of NO· production and nitrite/nitrate increments. In our hands, the L-arginine supplementation used (7 g/day/1 week) did not change the plasma levels of tNOxs (nitric oxide metabolites nitrite and nitrate) in any circumstance or group studied. NO· have a high binding affinity for glycosilated proteins such as haemoglobin [46]. Thus, protein glycosylation can alter nitric oxide binding affinity of haemoglobin and plasma proteins, hence reducing nitric oxide availability and causing NO· metabolism alterations [46]. This process seems to be enhanced when the level of HbA1c rises dramatically above 8 % [46], condition found in our type 1 diabetes subjects. Hence, the fact that we did not found increments in nitric oxide levels even with L-arginine supplementation could be explained by the rapidly binding of nitric oxide to blood proteins, preventing the appearance of its metabolite tNOx in the plasma; however, this is only a speculative possibility.

In basal conditions, we found that patients with diabetes present lower blood flow than their controls (Fig. 1). Since L-arginine supplementation was able to partially recover this cardiovascular deficit, we suggest that this amino acid administration could induce other mechanism of vasodilation rather than nitric oxide synthesis. It was previously demonstrated that some effects of L-arginine are known to be independent of NO· production [47]. L-Arginine is an inhibitor of angiotensin-converting enzyme (ACE) [48], reducing plasma angiotensin II levels and thus amplifying its hypotensive effect. Thus, our finding that L-arginine increases blood flow in basal conditions may be attributed to this function of the amino acid.

Even though L-arginine supplementation increases blood flow in basal conditions, the amino acid did not change this variable after exercise. This could indicate that during exercise, other mechanisms of vasodilation in the microcirculation of active muscles may be involved, rather than NO production. For instance, prostaglandin activity could fill this vasoactive function. As previously mentioned, the normal vascular endothelium plays an important role in maintaining vessel wall homoeostasis, synthesizing substances such as prostacyclin, which modulate vascular tone, prevent thrombosis, and influence smooth muscle growth [5]. There is evidence that vasodilatory prostanoids may be important in determining responses to acetylcholine (Ach) in both diabetic [4] and non-diabetic subjects [49, 50], being their effects mediated through an increase in cyclic AMP. Recent findings [5] suggest that vasodilatory prostanoids are important in determining endothelial response to Ach in diabetic and non-diabetic subjects. Increased prostaglandin-mediated vasodilation may compensate for attenuated responses to NO· previously reported in diabetic subjects. These reports partly explain the conflicting reports of endothelial dysfunction in patients with type 1 diabetes.

Conclusions and perspectives

This study illustrates that 1 week of L-arginine supplementation (7 g/day) is sufficient to improve endothelial function (i.e. blood flow) in basal conditions in young adult subjects with non-complicated type 1 diabetes. The observed changes in blood flow in rest with administration of the amino acid were not associated with changes in redox state or antioxidant defences since there were no significant changes in oxidative stress markers. However, based on our data, it is not possible to draw conclusions regarding the mechanisms by which L-arginine therapy could induce improvements in cardiovascular and metabolic function, but this important issue requires further investigations. Nonetheless, our findings highlight the importance of L-arginine for the improvement of blood

Table 3 Biochemical oxidative stress markers and blood flow measurement after supplementation

	Control subjects				Type I diabetic subjects			
	Placebo ($n = 10$)		L-arginine ($n = 10$)		Placebo ($n = 5$)		L-Arginine ($n = 5$)	
	PreEx	PostEx	PreEx	PostEx	PreEx	PostEx	PreEx	PostEx
Blood flow (ml 100 ml^{-1} min^{-1})	3.19 ± 0.38	5.40 ± 0.88†	4.14 ± 0.58	6.26 ± 0.87†	2.41 ± 0.21	3.09 ± 0.49†	4.74 ± 0.86‡	5.4 ± 0.81†
tNOx (μM)	11.02 ± 3.9	11.7 ± 4.5	11.4 ± 4.1	11.79 ± 4.4	11.3 ± 3.7	11.5 ± 3.7	12.8 ± 4	12.33 ± 4.2
TBARS (nmolMDA mg protein^{-1})	0.76 ± 0.06	0.71 ± 0.07	0.81 ± 0.11	0.66 ± 0.07	3.28 ± 0.94	2.74 ± 0.4	3.03 ± 0.65	2.63 ± 0.5
Carbonyls (fM.mg protein^{-1})	0.238 ± 0.03	0.147 ± 0.02	0.105 ± 0.02	0.150 ± 0.03	1.44 ± 0.23	2.14 ± 0.88	2.05 ± 0.71	2.01 ± 0.87
Uric acid (mg/dL)	46.27 ± 3.1	44.96 ± 3.8	41.3 ± 3.15	42.2 ± 3.8	28.07 ± 4.46	29.18 ± 5.37	28.29 ± 4.46	28.36 ± 5.4

† $P < 0.05$ when compared basal versus post-exercise within the same group. ‡ $P < 0.05$ when compared before vs. after L-arginine supplementation

Table 4 Dietary intake data

	Control ($n = 20$)	Diabetics ($n = 10$)	P
Energy requirement	2,628.97 ± 250.83	2,532.15 ± 274.98	0.427
Caloric ingestion	2,874.36 ± 173.89	2,655.21 ± 280.61	0.354
% Carbohydrates	56.29 ± 1.51	52.75 ± 0.78	0.785
(g) CHO/kg	5.23 ± 0.28	4.92 ± 0.68	0.857
% Proteins	16.29 ± 0.60	17.60 ± 0.70	0.963
(g) PTN/kg	1.49 ± 0.07	1.54 ± 0.14	0.876
% Lipids	27.43 ± 1.37	29.65 ± 0.08	0.835
(g) LIP/kg	1.12 ± 0.07	1.20 ± 0.15	0.768
L-Arginine (g)	4.82 ± 1.42	4.47 ± 1.89	0.578

flow in subjects with type 1 diabetes, indicating that L-arginine supplementation is an essential tool for the treatment for the disease.

Acknowledgments This work was supported by the National Council for Scientific and Technological Development (CNPq, Brazil).

Conflict of interest The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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