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Reduced venous endothelial responsiveness after oral lipid overload in healthy volunteers

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Abstract

The aim of this study was to investigate endothelial venous function, inflammatory markers, and systemic oxidative stress after an oral lipid overload (OLO). We studied 18 healthy adults (9 men; age, 29.2 ± 0.9 years; body mass index, 22.3 ± 0.4 kg/m^2). Blood samples were collected in the fasting state and 3, 4, and 5 hour after the OLO (1000 kcal, 58% fat) for metabolic variables, oxidative stress, inflammatory markers, adiponectin, and resistin. Changes in vein diameter to phenylephrine, acetylcholine, and sodium nitroprusside (dorsal hand vein technique) were measured before and after the OLO. Oral lipid overload increased triglycerides (61 ± 6 vs 134 ± 17 mg/dL, $P < .001$), insulin (7.2 ± 0.8 vs 10.7 ± 1.3 μU/mL, $P < .05$), and resistin (5.38 ± 0.5 vs 6.81 ± 0.7 ng/mL, $P < .05$) and reduced antioxidant capacity (plasma total antioxidant capacity: 186.7 ± 56 vs 161.8 ± 50 U Trolox per microliter plasma, $P < .01$), vascular reactivity (171.3 ± 85 vs 894.4 ± 301 ng/mL, $P < .001$), and maximum acetylcholine venodilation (105.9% ± 9% vs 61.0% ± 7%, $P < .05$). No changes were observed for sodium nitroprusside. Post-OLO triglycerides were positively correlated with phenylephrine dose ($\rho = 0.38$, $P < .05$) and resistin ($\rho = 0.43$, $P < .01$) and negatively correlated with the maximum acetylcholine venodilation ($\rho = -0.36$, $P < .05$). In conclusion, an OLO impaired venoconstriction responsiveness in healthy subjects, probably because of a reduction in the antioxidant capacity.

1. Introduction

Fasting hypertriglyceridemia has an established role in the genesis of atherosclerosis [1]. However, modern man lives in the postprandial state most of the day, when sustained high levels of triglyceride-rich lipoproteins can cause arterial endothelial dysfunction [2], nitric oxide is less available, and higher levels of postprandial oxidative stress are generated [3]. Clinical data have associated the decrease of the postprandial metabolism of triglyceride-rich lipoproteins with coronary artery disease, even in the presence of normal fasting lipid levels [4].

Arterial postprandial endothelial dysfunction can be attenuated by antioxidants [5,6], suggesting that free radicals could mediate this process. Lipid hydroperoxides from the diet contribute to the pro-oxidant load [2], stimulating formation of free radicals that are related to increased triglyceride-rich lipoproteins, low-density lipoprotein cholesterol (LDL-C) oxidation, and deposition in the subendothelial space [2,7,8]. Postprandial endothelial changes are observed in normolipemic individuals [9] and in those with dyslipidemia [10] and diabetes mellitus [2]. The higher the postprandial lipemia, the more vascular dysfunction is induced [10,11], a process associated with the transient rise in proinflammatory cytokines, adhesion molecules, and

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pro-oxidative activity [12]. Continuous exposure of the vessel wall to these inflammatory and oxidative processes could promote vascular endothelium injury and atherogenesis. Recent work from our group showed that rosiglitazone, a drug that reduces insulin resistance and has anti-inflammatory properties, could raise adiponectin, but not resistin, restoring arterial endothelial function in subjects with the metabolic syndrome [13].

The studies cited above evaluated arterial endothelial function. There is no information on capacitance vessels in the postprandial state, even though these are important in circulatory homeostasis. Indeed, arteries and veins have different biological activities concerning the endothelium, probably by marked regional and segmental heterogeneity in vascular endothelial function [14]. The aim of this study was to investigate endothelium-dependent and endothelium-independent venous function, inflammatory markers, adipocytokines, and oxidative stress after an oral lipid overload (OLO) in healthy volunteers.

2. Material and methods

The study was performed at the Institute of Cardiology of Rio Grande do Sul/University Foundation of Cardiology. A group of 18 healthy subjects (9 men, 24-36 years old) was recruited. A medical history and physical examination were performed for each subject. Subjects with diabetes mellitus (personal history or use of antihyperglycemic drugs), fasting hypertriglyceridemia (>150 mg/dL), hypercholesterolemia (>200 mg/dL), inflammatory or cardiovascular disease, psychiatric problems, alcohol abuse; those treated with lipid-lowering agents, aspirin, glucocorticoids, antineoplastic agents, vitamin supplements, thiazides, oral contraceptives, and β-blockers; and subjects who had smoked within the last 3 months before the present study were excluded. Women were studied randomly with respect to their menstrual cycle. Each subject gave written informed consent to participate in this study, which was approved by the ethics committee of the institution.

After the selection, individuals attended the Institute of Cardiology of Rio Grande do Sul/University Foundation of Cardiology on 12-hour fasting. They were instructed not to perform any physical activity during the last 72 hours before evaluation and not to use alcoholic beverages and caffeine in the day before the examination. The blood pressure (average of 2 readings), weight, height, and waist and hip circumferences were obtained. Blood samples were drawn from an antecubital vein with a 19-gauge needle without venous stasis in the fasting state and 3, 4, and 5 hours after the oral intake of a fat meal. Endothelial venous function was evaluated in the fasting state and 2 to 4 hours after the OLO. The OLO consisted of a mixed meal with 1000 kcal, 27% carbohydrates, 15% proteins, and 58% lipids (20 g saturated fat, 22 g monounsaturated, 20 g polyunsaturated, 300 mg cholesterol) as proposed by us previously [15].

Plasma glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides were determined by automated enzymatic commercial kits (Roche, Mannheim, Germany); serum insulin was determined by enzyme immunoassay commercial kits (Abbot-Murex, Park, IL); and glycated hemoglobin was determined by immunoturbidimetry (Roche). Low-density lipoprotein cholesterol was calculated by the formula of Friedewald. Insulin resistance was assessed by the homeostasis model assessment of insulin resistance (HOMA-IR) [16]. Fibrinogen was evaluated on Fibrintimer II (Dade Behring, Newark, DE) and processed in the autoanalyzer (CA-540; Sysmex, Roche, Mannheim, Germany), and C-reactive protein (CRP) was evaluated by nephelometry (Nephelometer BN100; Dade Behring). These variables were analyzed in the fasting state and 3, 4, and 5 hours after the OLO.

Plasminogen activator inhibitor 1 (PAI-1) (Immunogenetics, Zwijnaarde, Belgium) was evaluated by enzyme-linked immunosorbent assay. Intra- and interassay coefficients of variation in all assays were lower than 7% and 14%, respectively. Adiponectin and resistin were measured using specific radioimmunoassay commercial kits (Linco Research, St Louis, MO). Intra- and interassay coefficients of variation in both assays were <4% and <8%, respectively. These variables were analyzed in the fasting state and 3 hours after the OLO.

The dorsal hand vein technique was described by Aellig [17], and our group recently published a study using this technique [14,18]. In brief, a 23-gauge butterfly needle was inserted into a vein on the back of the hand with the arm positioned at an upward angle of 30°; and a continuous infusion of physiologic saline solution (rate, 0.3 mL/min) was started. A tripod holding a linear variable differential transformer (LVDT) (model 025 MHR; Shaevitz Engineering, Pennsauken, NJ) was mounted on the hand with the central aperture of the LVDT that contained a movable metallic core at a distance of 10 mm downstream from the tip of the needle. The signal output of the LVDT, which is linearly proportional to the vertical movement of the core, gave a measurement of the diameter of the vein. Readings are taken at a congestive pressure of 40 mm Hg by inflation of a blood pressure cuff placed on the upper portion of the arm under study. Results are presented as normalized dose-response curves. The diameter of the vein during saline infusion with the cuff inflated was defined as 100% relaxation. This technique has been found to be highly reproducible as a means of studying venous responses: intersubject coefficient of variation of ED80 ranges from 0.9% to 6.7%, and no correlation was observed between phenylephrine responses and basal vein diameter [17-20].

Afterward, the vein was preconstricted by infusing increasing doses (7 minutes each) of the α1-adrenergic selective agonist phenylephrine (37-25 000 ng/mL) until the dose that produced approximately 70% constriction of the vein ($ED_{70\%}$) was found. This dose of phenylephrine was the reference for the subsequent study of the vein response.

This degree of preconstriction was defined as 0% dilation. The vasodilation produced by acetylcholine (endothelium dependent) and sodium nitroprusside (endothelium independent) was analyzed (3 minutes each dose) and was calculated as a percentage of the range between 100% and 0% vasodilation. The individual effects were analyzed as the percentage of minimum (E_{min}), medium (E_{med}), and maximum (E_{max}) venodilation of infused acetylcholine doses (12-12 000 ng/mL). Drugs were infused with a Harvard infusion pump (Harvard Apparatus, South Natick, MA). Blood pressure and heart rate were monitored in the opposite arm with a sphygmomanometer. Ambient temperature was kept constant (24°C-27°C).

Oxidative stress was evaluated by one measurement of oxidative damage (carbonyls) and 2 antioxidant defense measurements (superoxide dismutase and total radical trapping antioxidant potential). These variables were analyzed in the fasting state and 3 hours after the oral fat tolerance test and centrifuged for 10 minutes at 1000*g* (Sorval RC 5b-rotor SM 24; Du Pont Instruments, Norwalk, CT), and plasma was kept apart. Red blood cells were washed out with saline solution (NaCl 0.9%) and used for further antioxidant enzyme activity.

Plasma samples were used to determine carbonyls and were incubated with 2.4 dinitrophenylhydrazine (10 mmol/L) in 2.5 mol/L HCl solution for 1 hour at room temperature, in the dark. Samples were vortexed every 15 minutes. Afterward, 20% trichloroacetic acid (TCA) (wt/vol) solution was added in tube samples, left in ice for 10 minutes, and centrifuged for 5 minutes at 1000*g* to collect protein precipitates. Another wash was performed with 10% TCA. The pellet was washed 3 times with ethanol–ethyl acetate (1:1) (vol/vol). The final precipitates were dissolved in 6 mol/L guanidine hydrochloride solution, left for 10 minutes at 37°C, and read at 360 nm [21]. The results were expressed as nanomoles per milligram of protein.

The superoxide dismutase enzyme (SOD) metabolizes superoxide anion. Activity is based on the inhibition of superoxide radical reaction with pyrogallol [22]. The SOD activity was determined by measuring the velocity of oxidized pyrogallol formation. Reagent medium contained Tris buffer (50 mmol/L, pH 8.20), pyrogallol (24 mmol/L), and catalase (30 mmol/L). Absorbance changes were observed at 420 nm for 2 minutes. This activity was determined from a standard curve of commercially available SOD and reported as units of SOD per milligram of protein.

Plasma total antioxidant capacity (TRAP) was measured by luminescence using 2,2′-azo-bis(2-amidinopropane) (a source of alkyl peroxyl free radicals) and luminol. A mixture consisting of 50 μmol/L 2,2′-azo-bis(2-amidinopropane), 40 μmol/L luminol, and 50 mmol/L sodium phosphate buffer (pH 7.4) was incubated; and a steady-state luminescence arose from the free radical–mediated luminol oxidation. This emission was almost completely quenched by the addition of Trolox (water-soluble vitamin E, Sigma Aldrich, EUA), yielding induction times linearly related to the free radical scavenger concentration added. A calibration curve was obtained by using 0.2 to 1 μmol/L Trolox. The addition of plasma samples instead of Trolox elicits an induction time related to the initial amount of sample added [23]. Luminescence was measured with a scintillation counter in the out-of-coincidence mode, and the results are reported as millimoles Trolox per liter per milligram of protein.

Data are presented as the mean ± SEM. Paired and unpaired *t* tests were used for parametrically distributed data. For data obtained on more than 2 occasions, repeated-measure analysis of variance (ANOVA) followed by the Bonferroni post hoc test was applied. The dose–percentual venodilation maximum response curves in each acetylcholine dose were analyzed by 2-factor ANOVA. For nonparametrically distributed data, Mann-Whitney and Wilcoxon tests were used. The Spearman correlation coefficient was used to evaluate the correlation of the variables. $P < .05$ was considered statistically significant.

2.1. Experimental results

Table 1 shows clinical characteristics for men and women together. Participants were 29.2 ± 0.9 years old and had normal body mass index and other anthropometric measurements, blood pressure levels, plasma lipids, and glucose, as expected. All of them had HOMA-IR lower than 2.71, the valid threshold for insulin resistance in the Brazilian population [24].

Table 2 presents the metabolic responses to the OLO. Triglycerides peaked 4 hours after the OLO, reaching levels approximately 2.7 times the fasting levels. They began to diminish at the 5-hour OLO evaluation, although not statistically different from the 3-hour levels ($P = 1$). Although insulin levels were elevated 3, 4, and 5 hours

Table 1
Clinical and fasting metabolic characteristics of the studied subjects

Characteristic	Mean ± SEM
Age (y)	29.2 ± 0.9
BMI (kg/m^2)	22.3 ± 0.4
Body surface (m^2)	1.77 ± 0.04
Waist circumference (cm)	76.7 ± 2.9
Waist/hip	0.83 ± 0.01
DBP (mm Hg)	69.4 ± 1.5
SBP (mm Hg)	111.7 ± 1.3
Hemoglobin (g/dL)	13.9 ± 0.4
Plasma glucose (mg/dL)	80.2 ± 1.8
Insulin (μU/mL)	7.22 ± 0.8
HbA_{1c} (%)	5.32 ± 0.16
Cholesterol (mg/dL)	149.3 ± 4.7
HDL-C (mg/dL)	52.9 ± 2.8
Triglycerides (mg/dL)	61.1 ± 5.9
VLDL-C (mg/dL)	12.2 ± 1.2
LDL-C (mg/dL)	84.1 ± 4.6
HOMA-IR	1.45 ± 0.18

BMI indicates body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; HbA_{1c}, glycated hemoglobin; VLDL-C, very low-density lipoprotein cholesterol.

Table 2
Metabolic variables at baseline and 3, 4, and 5 hours after the OLO

Variables	Fasting	3 h after OLO	4 h after OLO	5 h after OLO	*P*
Cholesterol (mg/dL)	149.3 ± 4.7	143.9 ± 5.2	145.7 ± 4.3	144.7 ± 4.4	.131
HDL-C (mg/dL)	52.9 ± 2.8	47.9 ± 2.5 *	49.1 ± 2.4 *	49.2 ± 2.7 *	<.001
Triglycerides (mg/dL)	61.1 ± 5.9	146.6 ± 20.2 *	160.4 ± 15.0 *	134.2 ± 16.8*,#	<.001
LDL-C (mg/dL)	84.1 ± 4.6	66.6 ± 4.6 *	64.9 ± 4.3 *	69.4 ± 4.9*,#	<.001
Plasma glucose (mg/dL)	80.2 ± 1.8	83.3 ± 1.3	83.3 ± 1.4	84.44 ± 1.3	.742
Insulin (μU/mL)	7.2 ± 0.8	11.7 ± 1.3 *	10.9 ± 1.4 *	10.7 ± 1.3 *	<.001

Data are mean ± SEM. Repeated-measure ANOVA was used, followed by Bonferroni post hoc test.
* $P < .05$: 3, 4, and 5 hours after OLO vs fasting.
# $P < .05$: 5 hours after OLO vs 4 hours after OLO.

after the OLO (~1.5 times the fasting levels), plasma glucose did not change. The LDL-C and HDL-C levels were significantly lower (~22% and 8%, respectively) 4 and 5 hours after the OLO as compared with fasting levels.

Inflammatory markers did not change after the OLO, as evaluated by CRP (0.16 ± 0.04, 0.14 ± 0.03, 0.15 ± 0.03, and 0.15 ± 0.03 mg/dL at fasting and 3, 4, and 5 hours after the OLO, respectively; $P = .117$), fibrinogen (317.8 ± 24, 294.7 ± 27, 296.8 ± 25, and 283.9 ± 23 mg/dL at fasting and at 3, 4, and 5 hours after the OLO, respectively; $P = .201$), and PAI-1 (5.49 ± 0.48 and 5.59 ± 0.56 ng/mL at fasting and 3 hours after the OLO, respectively; $P = .850$). Adiponectin also did not change (10.95 ± 1.24 vs 9.95 ± 1.15 μg/mL, $P = .156$); however, resistin increased significantly after the OLO (5.38 ± 0.55 vs 5.66 ± 0.57 ng/mL, $P = .02$).

No protein damage was observed after the OLO, as evaluated by carbonyls (5.94 ± 0.45 and 5.48 ± 0.37 nmol/mg protein in the fasting state and 3 hours after the OLO, respectively; $P = .349$). Superoxide dismutase, the first step in the reactive oxygen species detoxification, also did not change (3.32 ± 0.19 and 3.65 ± 0.15 U SOD per milligram of protein in the fasting state and 3 hours after the OLO, respectively; $P = .181$). There was a significant reduction of the TRAP at 3 hours after the OLO (186.7 ± 13 and 161.8 ± 14 U of Trolox per microliter of plasma, $P = .011$).

Table 3 presents data concerning the endothelial venous function. The diameter of baseline venodilation remained constant throughout the study. The OLO did not alter the individual percentages of endothelium-dependent (acetylcholine) E_{min}, E_{med}, and endothelium-independent (sodium nitroprussiate) E_{max}; but the endothelial function showed a reduction of the percentage of E_{max} (105.9% ± 9% vs 61.0% ± 6%, $P = .001$) via acetylcholine.

Table 3
Venoconstriction (phenylephrine) and endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) venodilation in percentual

Response	Fasting	2-4 h after OLO	*P*
Basal vein size (mm)	1 ± 0.1	1 ± 0.7	.965
Phenylephrine $ED_{70\%}$	71.9 ± 1.7	73 ± 1.8	.635
Acetylcholine % E_{min}	16.3 ± 5.2	21.5 ± 6	.389
Acetylcholine % E_{med}	58.4 ± 6.2	43.1 ± 6.4	.093
Acetylcholine % E_{max}	105.8 ± 9.5	61.0 ± 6.6	.001
Sodium nitroprusside % E_{max}	146.5 ± 11.1	129.9 ± 8.3	.131

Data are mean ± SEM. Paired *t* test was used for all comparisons.

Table 4 presents the drug doses used for venoconstriction (phenylephrine) and for endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) venodilation. The OLO did not change the doses of acetylcholine and sodium nitroprussiate needed to reach E_{max}, but there was greater need for phenylephrine doses (171.3 ± 66 vs 894.4 ± 301 ng/mL, $P < .001$) to reach $ED_{70\%}$. All individuals required a higher dose of phenylephrine to reach $ED_{70\%}$ after the OLO. Fig. 1 presents the E_{max} response at the 6 acetylcholine doses used (between 12 and 12 000 ng/mL), showing reduction after the OLO ($P < .01$).

No differences were observed between men and women concerning the delta variation of the phenylephrine response (744.7 ± 442 vs 634.9 ± 266 ng/mL, respectively; $P = .835$) at the percentage of maximum dependent venodilation (−32.7 ± 12 vs −57.1 ± 20, $P = .315$) and independent venodilation of the endothelium (−2.1 ± 16 vs −30.9 ± 12.3 ng/mL, $P = .175$).

The triglyceride levels were positively correlated with phenylephrine dose needed to reach $ED_{70\%}$ ($\rho = 0.38$, $P = .02$) and with resistin levels ($\rho = 0.43$, $P = .01$). E_{max} (0 and 3 hours) was positively correlated with the respective timetables of the total antioxidant potential ($\rho = 0.41$, $P = .01$) and negatively correlated with the triglyceride levels ($\rho = -0.36$, $P = .02$) and insulin levels ($\rho = -0.34$, $P = .04$). resistin ($\rho = 0.43$, $P < .01$).

3. Discussion

The present study showed, for the first time, that an OLO in healthy individuals leads to increased resistin levels,

Table 4
Venoconstriction (phenylephrine) and endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) venodilation: different drug concentrations

Drug (ng/mL)	Fasting	2-4 h after OLO	*P*
Phenylephrine $ED_{70\%}$	171.3 ± 66.7	861.1 ± 303.6	<.001
Acetylcholine % E_{max}	5076 ± 1164	4434.6 ± 1216	.875
Sodium nitroprusside % E_{max}	1648.8 ± 86.3	1735.6 ± 119.1	.248

Data are mean ± SEM. Wilcoxon test was used for all comparisons.

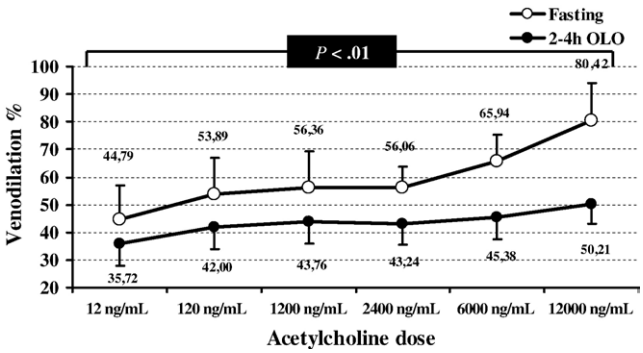

Fig. 1. Average percentual venodilation maximum/acetylcholine dose. Fasting values are represented by black circles; post-oral lipid load values are represented by open circles. Two-factor ANOVA was used for all comparisons. OLO=oral lipid overload.

reduced TRAP, α_1-adrenergic response, and endothelium-dependent venodilation.

The OLO promoted increased triglycerides and insulinemia beginning at the third hour and peaking at the fourth hour; and after 5 hours, these values had not yet returned to the fasting plasma values. These data agree with others presented by us [15] and by other authors [8,25]. There was also a postprandial reduction of LDL-C and HDL-C, which agrees with some authors [11]. Resistin is secreted by macrophages from the white adipose tissue; it increases the expression of the adhesion molecules vascular cell adhesion molecule 1 and intercellular adhesion molecule 1, up-regulates the monocyte chemoattractant chemokine 1, and promotes endothelial cell activation via endothelin 1 release, suggesting an inflammatory role for this cytokine [26] and potentially worsening insulin resistance. The resistin rise was associated with insulin and triglyceride rises, indicating a proatherogenic postprandial lipid profile. Although resistin was reported to be raised by refeeding [27], an evaluation of this cytokine after an OLO in healthy subjects has not yet been described.

Adiponectin is an adipocyte-secreted protein present in the serum and acts to increase insulin sensitivity. Our findings on healthy subjects showed no change of its levels after an OLO, as Peake et al [28] showed before, but were different from the study of Musso et al [29], which showed increased levels. Other authors, testing mixed meals [30], did not disclose any adiponectin rise in the postprandial period.

No changes were observed in the levels of inflammatory markers studied (CRP, fibrinogen, and PAI-1), but our group demonstrated that the CRP is altered in fasting and fibrinogen rises after an OLO in individuals with impaired glucose tolerance [31]. Indeed, Ceriello et al [12] showed that the combination of high fat and a glucose load produced an increase in nitrotyrosine, intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin plasma levels that was even more pronounced than that with either nutrient taken alone in both normal and diabetic subjects. It is likely that the inflammatory markers we used are not sufficiently sensitive to demonstrate the increased inflammatory response already observed by Ceriello et al.

Our results present also a reduction of TRAP after the OLO, which has been described in literature [5,6], without changes in SOD and in protein oxidation. We did not evaluate other forms of damage and defense; therefore, we could not effectively prove that postprandial oxidative stress occurred. Data in the literature describe the increase in free radicals ($O_2^{-\bullet}$ superoxide anion) as related to the meal-induced increase in triglycerides [3,11], although others disagree with this aspect [25]. These data are controversial: there are studies showing the generation of superoxide anion ($O_2^{-\bullet}$) by leukocytes [3] without the occurrence of lipid peroxidation [5]. Tsai et al [11] report a reduction of the glutathione peroxidase enzyme 2 hours after an OLO and the presence of 8-epi-prostaglandin F2α (a product derived from the oxidative modification of arachidonic acid) 4 hours after the OLO.

Several studies [2,6,3] suggest that oxidative stress could be responsible for endothelial damage; but these studies did not take into account the oxidative balance, evaluating only oxidative damage or antioxidant defense. Interestingly, a high-fat meal administered with dietary antioxidants partially restores the previously induced vascular dysfunction [6]. Our results showed that the dose of phenylephrine needed to reach $ED_{70\%}$ was higher after the OLO, characterizing an α_1-adrenergic response alteration, which is demonstrated for the first time in healthy adults. Studies of venous endothelial function in response to the infusion of free fatty acids showed increased α_1-adrenergic reactivity [32] together with an increased pressoric response, probably due to increased intracellular Ca^{2+} activated by phospholipase A_2 [33]. In these studies, increased α_1-adrenergic reactivity was characterized by the lower dose of phenylephrine needed to achieve the vasoconstrictor and pressoric response. The divergence with our results (higher dose of phenylephrine needed after the OLO) was probably due to the methodology used: our study reproduces the daily physiological conditions, in which fasting individuals present high levels of free fatty acids that diminish after feeding [8], whereas in the studies that used free fatty acid infusions, an increase in their plasma levels occurs already during the fasting period.

In young adults, postprandial epinephrine levels follow a biphasic pattern that is inversely related to that of glucose and insulin [34]. We speculate that the higher phenylephrine doses that were necessary and that correlated positively with triglyceride levels were associated with the insulin rise provoked by the OLO and reduced epinephrine effects.

Endothelial-dependent venodilation after the OLO was reduced for the percentage of E_{max} and for the mean of E_{max} in each acetylcholine dose. These data agree with studies found in similar populations in the arterial bed where an OLO caused transient damage in endothelial-dependent vasodilation without altering muscle function [5,6,11,25]. These endothelial alterations are probably mediated by

changes in the triglyceride, insulin, and/or resistin levels, which were also observed in our study and supported by the negative correlations between endothelial function (E_{max}) and triglycerides and insulin and by the positive correlation between triglyceride levels and phenylephrine dose needed to reach $ED_{70\%}$ with resistin levels.

A couple of possible methodological limitations of the present study should be highlighted. First, distension of the stomach induced by different types of meals causes cardiac output, left ventricular volumes, and forearm blood flow increase [35]. This gastrovascular reflex induced by food ingestion is counterbalanced by baroreflex changes, which prevent significant blood pressure changes [36], as we observed (no subject showed any blood pressure change during the experiments). Furthermore, hemodynamic changes were observed to be induced by gastric distension if pain and discomfort occur [37], which was not the case in our study. Second, ideally, the results obtained after the ingestion of a control isocaloric meal should be evaluated. However, the ingestion of isocaloric, isovolumetric high-protein, high-carbohydrate, or high-fat meals induces postprandial cardiovascular changes that are not substantially different from each other [35].

Previous studies in healthy subjects showed the influence of intravenous lipid overload acutely upon the cardiovascular system, changing peripheral arterial resistance and the central venous system. We showed here, for the first time, that an acute OLO in healthy individuals leads to increased resistin levels, reduced TRAP, α_1-adrenergic response, and endothelium-dependent venodilation. The subacute and chronic effects of usually eaten fatty meals have not yet been described and should be the focus of future research to explain possible cardiovascular end points.

We conclude that the acute effect of an OLO induces an expected triglyceride and insulin rise, accompanied by lower antioxidant capacity, resistin rise, and venous endothelial dysfunction. This is characterized by changes in α_1-adrenergic reactivity and lower endothelium-dependent venodilation. The mechanisms involved in signal transduction and their interaction with other pathways, such as cyclooxygenase and hyperpolarizing factor derived from the endothelium, have not yet been studied in the venous endothelium after an oral lipid overload.

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