Reversal of Postprandial Endothelial Dysfunction by Cyclo-oxygenase Inhibition in Healthy Volunteers

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Abstract: The aim of this study was to evaluate the role of cyclo-oxygenase (COX) in venous vascular reactivity changes after an oral lipid overload (OLO). Venous endothelial function (dorsal hand vein technique) was evaluated in fasting, 30 minutes after COX inhibition (aspirin-fasting), 2 to 4 hours after an OLO (1000 kcal, 58% fat), and again after COX inhibition (aspirin-OLO, 600 mg/200 mL water) in 10 healthy adults (age, 28.1 ± 1.3 years; body mass index, 22.3 ± 0.6 kg/m^2). Fasting, 2- to 4-hour post-OLO, and 60-minute post-aspirin plasma glucose, insulin, and lipids were also evaluated. The OLO increased triglycerides and insulin, reduced low-density lipoprotein and high-density lipoprotein, but glycemia and total cholesterol remained unchanged. There were no metabolic differences between OLO and aspirin-OLO. In fasting, aspirin reduced acetylcholine-induced venodilation (107.0% ± 14% versus 57.3% ± 11%; $P < 0.001$). Vascular reactivity was blunted after the OLO (phenylephrine dose: 0.3 ± 0.2 fasting versus 1.9 ± 0.8 nmol/min after OLO; $P < 0.001$) and was partially corrected by aspirin (0.4 ± 0.2; $P < 0.001$). Similar changes were observed in maximum venodilation after acetylcholine (107.0% ± 14% fasting versus 60.4% ± 9% after OLO, $P < 0.001$; aspirin-OLO: 95.9% ± 6%; $P < 0.001$). The responses to sodium nitroprusside remained unchanged during the study. We conclude that the OLO reduction in the endothelium-dependent venoconstriction and venodilation is partially the result of the action of COX.

Key Words: cyclo-oxygenase inhibitors, postprandial period, endothelium, aspirin, lipid metabolism

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INTRODUCTION

Sustained high levels of triglyceride-rich lipoprotein (chylomicrons, very-low-density lipoproteins, and their remnants) can cause arterial endothelial dysfunction in the postprandial state.[1] The postprandial period is characterized by enhanced oxidative stress,[2] transient increase in proinflammatory cytokines, soluble adhesion molecules,[3] and platelet aggregation,[4] all important changes in the development of atherosclerosis. Recent studies showed that in healthy subjects, the magnitude of postprandial lipemia after a single oral lipid overload (OLO) is negatively related to vascular function.[3,5–7]

We recently described that an OLO determines, in healthy individuals, increased triglycerides and plasma insulin that are associated with reduced total antioxidant capacity (TRAP), a lower α_1-adrenergic response, and diminished endothelium-dependent venodilation.[8] The reported changes in vascular reactivity are probably mediated by postprandial changes in insulin and/or triglyceride levels.[3,9] Also, stimulation of the vascular wall to produce reactive oxygen species, especially when the superoxide anion ($O_2^{\bullet -}$) production goes beyond the antioxidant systems' capacity, may result in endothelial cell function impairment and tissue damage by inhibiting the action of nitric oxide (NO) in producing peroxynitrite ($ONOO^-$).[10]

Peroxynitrite production and/or lipid peroxidation both stimulate the production of eicosanoids; thus, this mechanism links the production of the eicosanoids to the NO redox signaling pathways.[10,11] Because eicosanoid production is regulated by the arachidonic acid availability and by the activity of prostaglandin H synthase (or cyclo-oxygenase [COX]),[12] we hypothesized that post-OLO venous endothelial dysfunction could be mediated by NO and also by COX. Therefore, the purpose of this study was to evaluate the role of COX on venous vascular reactivity in fasting and after an OLO.

METHODS

Ten nonsmoking healthy subjects, six men, 24 to 36 years old without chronic diseases and dyslipidemia and who were not on drugs known to interfere in lipid metabolism, were recruited. All of them signed written informed consent approved by the ethics committee of our institution. The individuals were instructed not to perform physical activity within the last 72 hours before evaluation and not to use alcoholic beverages and caffeine on the days of the examinations. Each subject performed two evaluations on 2 different

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days: 1) in fasting and 2 to 4 hours after the OLO (1000 kcal, 27% carbohydrates, 15% proteins, and 58% lipids or 64 g total fat; 20 g saturated fat, 22 g monounsaturated, 20 g polyunsaturated, and 300 mg cholesterol)[8] and again 30 to 90 minutes after COX inhibition (aspirin-OLO); and 2) in fasting and after aspirin (aspirin-fasting). Blood pressure and anthropometric variables were measured and blood samples were collected in the fasting state (12 hours), 2 to 4 hours after the OLO, and 60 minutes after COX inhibition with acetylsalicylic acid (aspirin, 600 mg in 200 mL of water orally), which corresponds approximately to the fifth hour after the OLO. Samples were evaluated for plasma glucose, cholesterol, triglycerides (automated enzymatic commercial kits; Roche, Mannheim, Germany) and insulinemia (enzyme immunoassay commercial kits; Abbott-Murex, Abbott Park, IL).

Venous–endothelial function was evaluated by the dorsal hand vein technique.[8] Briefly, a 23-gauge butterfly needle was inserted into a vein of the hand, the arm positioned at an upward angle of 30° to allow for complete emptying of the veins, and a continuous infusion of physiological saline solution (0.3 mL/min) was started and kept constant during the experimental session. A tripod holding a linear variable differential transformer (LVDT; 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, gives a measurement of the vein diameter. Readings are taken under a congestive pressure of 40 mmHg by inflation of a blood pressure cuff placed on the upper portion of the arm studied. Results are presented as normalized dose–response curves in which the diameter of the vein during saline infusion with the cuff inflated was defined as 100% relaxation. The vein was preconstricted by infusing increasing doses of the α_1-adrenergic selective agonist phenylephrine (0.06–8 nmol/min) until the dose that produced 65% to 75% vein constriction ($ED_{70\%}$ dose) was obtained. This $ED_{70\%}$ dose was defined as 0% dilation and subsequently used as the initial reference for the after OLO and aspirin evaluations. The vasodilation produced by six doses (0.02–24 nmol/min) of acetylcholine (endothelium-dependent) and three doses (1–6 nmol/min) of sodium nitroprusside (endothelium-independent) before and after the OLO was calculated as a percentage of the range between 100% and 0% vasodilation. After aspirin, $ED_{70\%}$ was defined again and also the endothelium-dependent and -independent maximum effects (E_{max}). Drug(s) were infused with a Harvard infusion pump (Harvard Apparatus Inc., South Natick, MA).

Data are presented as the mean ± standard error of mean. For parametrically distributed data, comparisons were made using the repeated-measure analysis of variance (ANOVA) and for nonparametrically distributed data Friedman ANOVA, both followed by the Student-Newman-Keuls post hoc test. $P < 0.05$ was considered statistically significant.

RESULTS

The studied subjects were 28.1 ± 1.3 years old, body mass index was 22.3 ± 0.6 kg/m^2, systolic blood pressure 114.7 ± 2 mmHg, diastolic blood pressure 73.5 ± 1 mmHg, cholesterol 150.8 ± 6 mg/dL, low-density lipoprotein (LDL) cholesterol 85.6 ± 5 mg/dL, high-density lipoprotein (HDL) cholesterol 51.9 ± 3 mg/dL, triglycerides 65.7 ± 9 mg/dL, and plasma glucose 81.7 ± 3 mg/dL, all inside the expected normal ranges.

Table 1 shows the data obtained for metabolic responses and endothelial venous function. Triglycerides peaked 2 to 4 hours after the OLO, reaching levels that were approximately 2.5 times the fasting levels, not returning to the basal values during the aspirin-OLO ($P < 0.001$). The LDL-cholesterol ($P < 0.001$) and HDL-cholesterol ($P = 0.002$) levels were significantly lower after the OLO. Insulin levels were elevated

TABLE 1. Metabolic Variables and Venous Endothelial Function at Fasting, Aspirin-fasting, After an Oral Lipid Overload (OLO), and Aspirin-OLO

Variables	Fasting	Aspirin-fasting	OLO	Aspirin-OLO	*P* value
Cholesterol (mg/dL)	150.8 ± 6	149.8 ± 4	147.7 ± 7	146.6 ± 6	0.701
High-density lipoprotein cholesterol (mg/dL)	51.9 ± 4	53.5 ± 4	47.3 ± 4†	47.2 ± 4†	<0.001
Triglycerides (mg/dL)	65.7 ± 9	68.7 ± 9	168.6 ± 30†	136.9 ± 26†	<0.001
Low-density lipoprotein cholesterol (mg/dL)	85.6 ± 5	82.7 ± 4	66.6 ± 5†	71.9 ± 5†	0.002
Plasma glucose (mg/dL)	81.7 ± 3	82.4 ± 2	82.5 ± 2	83.1 ± 2	0.968
Insulin (μU/mL)	7.2 ± 1	7.5 ± 1	12.0 ± 2†	10.8 ± 2†	0.001
Phenylephrine $ED_{70\%}$	73.1 ± 3	73.0 ± 3	72.5 ± 3	72.4 ± 2	0.974
Acetylcholine $\%E_{max}$	107.0 ± 14	57.3 ± 11*	60.4 ± 9*	95.9 ± 6‡	<0.001
Nitroprusside $\%E_{max}$	146.4 ± 16	153.9 ± 16	124.5 ± 11	129.8 ± 9	0.445
Phenylephrine $ED_{70\%}$ (nmol/min)	0.3 ± 0.2	0.4 ± 0.1	1.9 ± 0.8†	0.4 ± 0.2§	<0.001
Acetylcholine E_{max} (nmol/min)	9.7 ± 3	7.1 ± 3	10.6 ± 4	13.2 ± 3	0.748
Nitroprusside E_{max} (nmol/min)	1.9 ± 0.1	2.1 ± 0.2	1.9 ± 0.1	1.9 ± 0.1	0.420

Mean ± standard error of mean.
*$P < 0.05$ versus fasting.
†$P < 0.05$ versus fasting and aspirin-fasting.
‡$P < 0.05$ versus aspirin-fasting and OLO.
§$P < 0.05$ versus OLO.
$ED_{70\%}$, percentage of venoconstriction; E_{max}, maximum effect. Repeated-measure analysis of variance or Friedman variance followed by Student-Newman-Keuls test.

2 to 4 hours after the OLO and aspirin-OLO ($P = 0.001$). Plasma glucose levels did not change significantly ($P = 0.968$). No differences were observed between fasting and aspirin-fasting and between post-OLO and aspirin-OLO concerning the metabolic variables studied. In fasting, aspirin reduced the percentage of the venodilation induced by acetylcholine by approximately 50% (107% ± 14% versus 57.3% ± 11%, $P < 0.001$) with no changes in the other variables that were used to evaluate endothelial function. The OLO provoked an approximate 40% reduction of endothelium-dependent venodilation by acetylcholine response maximum effect (E_{max}) and the return to fasting values after COX inhibition as compared with the fasting state on the same day. The phenylephrine dose needed to reach $ED_{70\%}$ was approximately five times higher after the OLO, an alteration that was reverted by aspirin. The endothelium-dependent and -independent percentual venoconstriction after phenylephrine ($ED_{70\%}$) remained unchanged throughout the experiments. The drug doses needed to reach the E_{max} with acetylcholine (endothelium-dependent) and sodium nitroprusside (endothelium-independent) did not change during the experiments.

DISCUSSION

The present study shows that COX inhibition induced by aspirin decreased endothelium-dependent venodilation induced by acetylcholine in fasting. However, after an OLO, COX inhibition reverted the deleterious venous bed vascular changes characteristic of this period.

The endothelium maintains vascular tone by basal continuous formation of NO and PGI_2 through the action of endothelial NO synthase and activity of prostaglandin H synthase (PGHS), respectively.[9,10] The venous infusion of free fatty acids (NEFAs) increases venoconstrictive α_1-adrenergic (phenylephrine) and venodilating (acetylcholine) endothelium-dependent responses, changes that are not the result of NO synthesis reduction.[11] Previous data indicate that NEFAs (mainly the oleic and linoleic acids) increase the stable metabolites of prostacyclin (6-keto-$PGF_{1\alpha}$) without altering thromboxane (T_xB_2).[12] In physiological conditions, however, NEFAs are constantly maintained elevated only in the fasting state,[13] increasing the venodilation by PGI_2. Our results indicate that PGI_2 plays a key role in endothelium-dependent venodilation in fasting.

The OLO increases triglyceride-rich lipoprotein and insulin reduces α_1-adrenergic response and endothelium-dependent venodilation in healthy individuals as we recently described.[8] These changes are associated with reduced TRAP.[4] The increase in $O_2^{\bullet -}$ associated with the rise of triglycerides induced by meals[5,8] may impair endothelial function by inhibiting NO action,[1,3] reducing the NO bioavailability production of $ONOO^-$[9]. After a meal, PGI_2 is reduced as the result of lipid peroxidation and/or a rise in ONOO, causing reduced venodilation.[10] Using the urinary excretion of the 8-$PGF_{2\alpha}$ as a surrogate marker of lipid peroxidation, Tsai et al[7] showed its rise after an OLO in healthy individuals. Besides these changes, the postprandial period in healthy humans also promotes stomach distension, increases sympathetic activity,[14] lowers plasma epinephrine, and increases norepinephrine levels (α_1-adrenergic selective agonist).[15] These changes certainly do not occur in fasting, possibly explaining the unaltered α_1-adrenergic response after COX inhibition with aspirin found in our study.

The nonselective COX inhibitor aspirin also has an antioxidant capacity, inhibiting the formation of lipid peroxidation products (8-$PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, stable metabolites of the PGI_2),[16] an effect that can be achieved by single 500-mg oral doses.[17] In similar doses as used in our study (600 mg), the endothelium-dependent cutaneous vasodilatation by acetylcholine is limited by constrictor products of the COX pathway, including PGH_2, T_XA_2, and/or $O_2^{\bullet -}$.[18] We believe that there is a redox imbalance leading to the reduction of PGI_2 synthesis after an OLO, favoring the conversion of PGH_2 to T_xA_2, resulting in less endothelium-dependent venodilation. A limitation of the present study was that we did not evaluate markers of oxidative modification of arachidonic acid to effectively prove this hypothesis.

CONCLUSION

Our data indicate that aspirin reduces endothelium-dependent venodilation in fasting, but in the postprandial period, it reverts the endothelial changes (α_1-adrenergic and muscarinic receptors) provoked by the OLO, suggesting that COX is at least partially involved in postprandial endothelial dysfunction.

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