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Original article

Ascorbic acid prevents vascular dysfunction induced by oral glucose load in healthy subjects

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ABSTRACT

Objectives: To examine the effects of oral glucose load on forearm circulatory regulation before and after ascorbic acid administration in healthy subjects.

Design: Microcirculation study with laser Doppler was performed at the hand in basal conditions, after ischemia and after acetylcholine and nitroprusside; strain gauge plethysmography was performed at basal and after ischemia. The tests were repeated in the same sequence 2 hour after oral administration of glucose (75 g). The subjects were randomised for administration of ascorbic acid (1 g bid) or placebo (sodium bicarbonate 1 g bid) for 10 days. After that, the tests were repeated before and after a new oral glucose load. Blood pressure and heart rate were monitored.

Results: Macrocirculatory flux, pressure values and heart rate were unvaried throughout the study. The glucose load caused a reduction in the hyperemic peak flow with laser Doppler and plethysmography; it reduced flux recovery time and hyperemic curve area after ischemia; acetylcholine elicited a minor increase in flux with laser Doppler. The response to nitroprusside was unvaried after glucose load as compared to basal conditions.

Treatment with ascorbic acid prevented the decrease in hyperemia after glucose, detected with laser Doppler and plethysmography. Ascorbic acid prevented the decreased response to acetylcholine after glucose, the response to nitroprusside was unaffected by ascorbic acid. Results after placebo were unvaried.

Conclusions: Oral glucose load impairs endothelium dependent dilation and hyperaemia at microcirculation, probably via oxidative stress; ascorbic acid can prevent it.

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1. Introduction

Several studies document the impairment of vasoregulation and in particular of endothelial function in diabetic patients [1]. Endothelial dysfunction in diabetes is attributed to multiple factors and in particular a pivotal role is attributed to increased reactive oxygen species with consequent nitric oxide (NO) degradation [2–4] and production of peroxynitrite, which is a toxic molecule [5]. In fact, in hyperglycaemic conditions an augmented production of reactive oxygen species has been demonstrated to take place [6]. Some authors have observed that an oral glucose load reduces endothelial dependent vasodilation in healthy individuals at humeral artery [2,7,8] and in microcirculation [9]. Oxidative stress is evoked for such an impairment [6]. Furthermore hyperglycaemia induces mitogen activated protein kinase, protein kinase C, transcription factor nuclear factor (NF)- κ B that consequently activates the expression of adhesion molecules [10]. Ascorbic acid is one of the most efficacious natural scavengers of reactive oxygen species and acts

enhancing NO function [11]. Several authors have demonstrated that ascorbic acid can recover endothelial dysfunction in different clinical conditions such as ischemic heart disease [12], hyperhomocysteinaemia [13], hypertension [14] and smoking [15]. Other authors have demonstrated that endothelial dysfunction, induced by infusion of mannitol, can be prevented by ascorbic acid infusion [16]. It has, furthermore, been recently described how slight hyperinsulinaemia impairs endothelium dependent vasodilation in large arteries; these data highlight the links between hyperinsulinemia/insulin resistance and atherosclerosis [17]. It is not still clear how endothelial vasoregulation in micro vessels and reactive hyperaemia are affected during a physiological metabolic stress such as an oral glucose load, which leads to a slight increase in glucose and in insulin plasma levels; secondly how ascorbic acid modulates these modifications. Further information can be obtained by studying the ipoxic-reducing conditions determined by ischemia with the analysis of the recovery phase during post ischemic hyperaemia.

2. Aim of the study

To evaluate the effects of an oral glucose load on macro- and microcirculatory haemodynamic parameters in healthy subjects and if ascorbic acid administration can modify these parameters.

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Table 1
Study population.

	Baseline	After treatment
AGE (years)	29–36	–
BMI	19 ± 3	–
TOTAL CHOL. (mg/dl)	165 ± 27	162 ± 31
TRIGLYCERIDES (mg/dl)	136 ± 12	133 ± 15
HDL CHOL. (mg/dl)	58 ± 8	59 ± 9
TOTAL CHOL./HDL (ratio)	5.4 ± 1.1	5.3 ± 1.3
GLUCOSE (mg/dl)	91 ± 10	89 ± 12
INSULIN (mU/l)	5.8 ± 1.1	5.6 ± 1.2

3. Patients and methods

3.1. Study population

Thirty-four healthy subjects, non smokers, aged 29–36 (17 females and 17 males). Lipid profile (total cholesterol, HDL, LDL, triglycerides), fasting blood glucose and glucose/insulin curves after oral glucose load (75 g) were within range. They all had BMI < 21 (Table 1).

The study was carried out in our vascular laboratory at 9.00 a.m. in a comfortable environment and at a constant temperature (22 ± 1 °C). After a 20-min period of stabilisation in supine position subjects underwent microcirculatory evaluation with jontophoretic tests for endothelium dependent and endothelium independent vasodilation on the right hand. Ten minutes later a post ischemic test was performed; on the left arm we studied brachial flow with plethysmography at rest and during reactive hyperaemia; blood pressure and heart rate monitoring was performed during the tests. Afterwards subjects were asked to drink a solution containing 75 g of glucose; 2 hours later the same sequence of tests was performed. The subjects were randomised to assume ascorbic acid (1 g bid) or placebo (sodium bicarbonate 1 g bid) for 10 days, then the tests were repeated before and after oral glucose load.

3.2. Haemodynamic measurements

The cutaneous microcirculatory system was studied by means of laser Doppler (LD) (Periflux PF 3, Perimed, Stockholm, Sweden). This device has a helio-neon laser that emits light at a wave-length of 632.8 nm, transmitted by optic fibre to a probe. The device can measure the dermal microcirculatory flow through the analysis of the back-scattered light and it expresses the flow in perfusion units (PU—arbitrary units). With this method we can analyse dermal rest flow (thermoregulatory and nutritive flow), hyperflow after ischemia, endothelial-dependent dilation (with acetylcholine administration) and endothelial-independent dilation (with nitroprusside administration). Laser Doppler probe was placed on the dorsum of the third finger of the right hand and cutaneous microcirculatory flux was evaluated at rest, after jontophoretic administration of acetylcholine (dose–response curve of 2% acetylcholine, 0.1 mA; doses administered for 10, 20, 40 s) and nitroprusside (dose–response curve of 1% nitroprusside, 0.1 mA; doses administered for 10, 20, 40 s) [18–20] and after 3 min ischemia induced with a cuff placed 2 cm above the elbow and inflated 10 mm Hg over systolic pressure. The reactive hyperaemia was studied calculating the peak flow

(maximum flux value reached after the release of the cuff), the area under the curve (describing the increase and decrease of hyperaemic flow), and the time to recovery of the flux (return to rest value). These parameters were automatically calculated by the dedicated software of laser-Doppler. We also calculated microcirculatory resistance as the ratio between mean arterial pressure (diastolic pressure plus 1/3 of the pulse pressure) and laser-Doppler rest flow for each step of the protocol (Table 2). Traces of laser-Doppler were evaluated by operator unaware of the subject identity and study phase.

Evaluation of the flow at the arm was performed by means of strain gauge plethysmography (Periquant 3800, Gutmann Medizintechnik, Eurasburg, Germany) at rest and during reactive hyperaemia. We also calculated forearm resistance as the ratio of mean pressure and forearm blood flow, at each step of the protocol. Plethysmographic traces were evaluated by an operator unaware of the subject identity and study phase [27].

Pressure and heart rate monitoring was done with an oscillometric device (Dinamap 845 XT, Critikon, Johnson and Johnson, Tampa, FL, USA).

Blood glucose values were determined in the capillary blood with a stick (Glucocard Memory 2, Arkray Inc Shia Japan), insulin values were determined with Insulin DPC, Immulite.

3.3. Statistical analysis

The data is expressed as mean ± SD and the statistical analysis was carried out using two tailed analysis of variance (ANOVA; SPSS, SPSS Italia srl, Italy) followed by post-hoc Student's *t* test for paired and unpaired data, Bonferroni's correction was applied. Values of *p* < 0.05 were considered to be significant.

All patients gave their informed consent to the participation in the study.

4. Results

Blood pressure, heart rate values never changed throughout the study.

Blood glucose 2 hours after the load was 94 ± 15 mg/dl, insulin 34.3 ± 7.4 pmol/l.

Forearm blood flow increased after ascorbic acid treatment—after glucose load; while cutaneous microcirculation was unaffected by this treatment (Table 4).

Forearm resistance decreased in the group treated with ascorbic acid after oral glucose load (Table 4); microcirculatory resistance did not change throughout the protocol (Table 4).

Oral glucose load determined a significant reduction in post-ischemic microcirculatory hyperaemia, at the beginning of the study and after 10 days of treatment with ascorbic acid: hyperaemia was studied at the peak flow (Table 2) and calculated as area under the curve (Fig. 1). The analysis of recovery time at laser Doppler during reactive hyperaemia demonstrated a reduction after glucose load and a recovery after treatment with ascorbic acid (Fig. 2). No differences were relieved after placebo.

Glucose load reduced the response to acetylcholine administration; after 10 days of ascorbic acid and post-glucose load, the dose–

Table 2
Blood pressure, heart rate, peripheral resistance (**p* < 0.005 vs rest and correspondent baseline).

	Baseline		After vit C		After placebo	
	Rest	Glucose	Rest	Glucose	Rest	Glucose
Systolic pressure (mmHg)	119 ± 4	109 ± 6	120 ± 8	110 ± 7	120 ± 5	109 ± 5
Diastolic pressure (mmHg)	79 ± 9	74 ± 6	81 ± 7	74 ± 6	79 ± 6	81 ± 8
Heart rate (b/min)	74 ± 3	71 ± 8	73 ± 6	66 ± 5	74 ± 8	72 ± 9
Forearm resistance (mmHg/ ml/100 ml min ⁻¹)	34.1 ± 2.1	39.3 ± 1.5	32.3 ± 2.1	23.2 ± 1.9*	33.1 ± 1.4	33.4 ± 1.9
Microcirculatory resistance (mm Hg/PU)	1.7 ± 0.4	1.7 ± 0.8	1.8 ± 0.5	1.7 ± 0.6	1.6 ± 0.8	1.7 ± 0.8

Table 3
% increase in flux with laser Doppler and plethysmography after ischemia and absolute values at rest (mean ± SD).

% increase during reactive hyperemia	Baseline		Ascorbic		Placebo	
	Rest	Glucose	Rest	Glucose	Rest	Glucose
Laser Doppler	117.3 ± 9	35.9 ± 8*	131.5 ± 12	65.5 ± 8 #	120.1 ± 10	33.8 ± 9*
SG Pletysmography	285.7 ± 23	182.6 ± 13*	391.4 ± 19	275.8 ± 14 #	279.8 ± 26	189.7 ± 16*
* <i>p</i> <0.05 vs rest; # <i>p</i> <0.05 vs after-glucose/baseline.						
Absolute values at rest	Baseline		Ascorbic		Placebo	
	Rest	Glucose	Rest	Glucose	Rest	Glucose
Laser Doppler (PU)	52.6 ± 9.2	49.9 ± 8.3	48.5 ± 12.1	50.5 ± 8.4	54.8 ± 10.1	51.7 ± 11.4
SG pletysmography (ml/100 ml min ⁻¹)	2.7 ± 0.3	2.6 ± 0.3	3.1 ± 0.3	3.7 ± 0.6*	2.8 ± 0.2	2.7 ± 0.4

**p*<0.05 vs correspondent baseline.

response curve with acetylcholine showed a recovery of the perfusion values with the higher stimulus (Table 4). Nitroprusside response curve was unvaried throughout the study (Table 4).

A reduction in peak flow after ischemia was also detected with plethysmography after glucose, a recovery was observed after ascorbic acid treatment, rest flow was unchanged throughout the study (Table 3). Placebo did not cause any change.

5. Discussion

The results show that oral glucose load in healthy subjects impairs hyperaemic response at forearm and reduces endothelium dependent vasodilation at cutaneous microcirculation; oral ascorbic acid can recover these modifications. Forearm resistance decreased after ascorbic acid treatment and after glucose load.

The increase of forearm flow after vitamin C treatment and after glucose may be attributed to a synergic action by insulin vasodilatory properties and antioxidant improvement in these subjects. We can hypothesise that insulin dependent vasodilation at baseline or after placebo can be counteracted by increased oxidative stress due to increased glucose concentration, with balance in circulatory regulation. The administration of ascorbic acid can contrast the oxidative stress, with enhanced effects of insulin on middle and small calibre arteries.

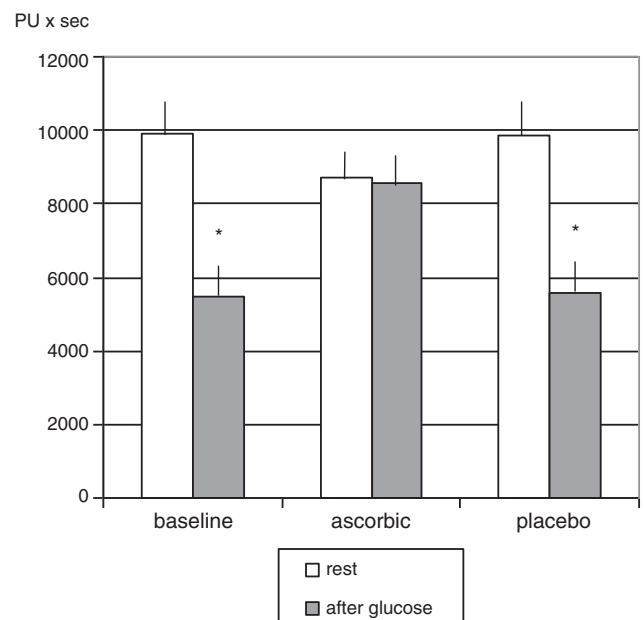
No effects are detected in cutaneous microcirculation at rest, vasoregulation in this district may be affected also by autonomic balance; hyperinsulinemia may cause vagal withdrawal that can mask the vasodilation effects of metabolic activation induced by hyperglycemia and glucose storage.

Some authors observed impairment of endothelial dependent vasodilation at brachial artery after glucose administration [21,22],

other authors have studied microcirculation focusing mainly on acetylcholine stimuli during hyperglycaemia [9]. Some other authors, on the contrary, have shown a lack of effect of oral glucose on endothelial function at brachial artery [23], this fact was highlighted in young subjects with prevalence of female. In our experimental setting, ischemia is generated by cuff inflation above the site of vessel measurements in contrast to studies of vasodilation of the brachial artery. Hence, ischemic metabolites could theoretically influence our results and contribute to differences between our results and results of studies performed on the brachial artery. We documented a reduction in endothelium dependent vasodilation in microcirculation after administering of glucose, these findings show that the response to glucose can impair endothelial function in microcirculation, this phenomenon can be attributed to oxidative stress induced by glucose metabolism [8] and, probably, to the increase in insulin output, as documented in studies on large arteries [17]. Beckman observed an impairment in endothelium dependent vasodilation during hyperglycaemia, prevented by ascorbate administration; the study was performed with infusive technique so through an invasive approach [16]. Prostaglandins, catabolites, nerves activity and nitric oxide determine vasodilation during and after ischemic stimulus [18–20]; another mechanism of increased flow in the ischemic area is the dilation of middle calibre arteries (e.g. humeral artery), through an endothelial dependent dilation. Glucose impairs hyperemia in our work, both in peak flow and in duration-amplitude of the phenomenon. A lack in the release or action of prostaglandins [24] and

Table 4
% Increase in laser Doppler flux with administration of acetylcholine and nitroprusside (mean ± SD).

Acetylcholine 0.10 mA		10 s	20 s	40 s
Baseline	Rest	65.4 ± 14	194.2 ± 18	378.2 ± 25
	After glucose	35.4 ± 11*	123.4 ± 14*	260.8 ± 21*
Ascorbic	Rest	79.4 ± 20	215.7 ± 21	445.7 ± 35
	after glucose	31.8 ± 10*	101.9 ± 19*	345.3 ± 21 #
Placebo	Rest	63.4 ± 13	200.2 ± 18	381.2 ± 24
	After glucose	36.8 ± 15*	128.4 ± 13*	245.7 ± 20*
* <i>p</i> <0.05 vs rest; # <i>p</i> <0.05 vs after-glucose/ascorbic.				
Nitroprusside–0.10 mA		10 s	20 s	40 s
Baseline	rest	69.5 ± 12	211.2 ± 19	399.2 ± 24
	after glucose	67.8 ± 11	209.3 ± 22	388.8 ± 28
Ascorbic	rest	70.1 ± 18	215.7 ± 25	397.7 ± 35
	after glucose	69.8 ± 15	208.9 ± 28	397.9 ± 29
Placebo	rest	66.5 ± 13	215.1 ± 21	388.4 ± 25
	after glucose	68.6 ± 14	211.5 ± 25	376.9 ± 27

**Fig. 1.** Area under the hyperemic curve at laser Doppler. **p*<0.05 vs rest-baseline.

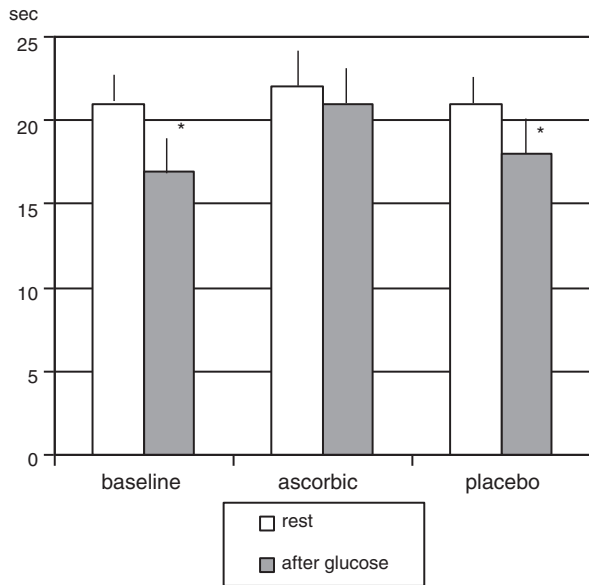


Fig. 2. Recovery time of hyperemic curve. * $p < 0.05$ vs rest-baseline.

neurogenic mechanisms may be considered for the reduction in peak-flow. Nitric oxide system impairment can be evoked in the reduction of duration-amplitude of hyperaemic curve, since nitric oxide plays a role in determining the last part of hyperaemic curve, in prolonging the increased flow supply through a flux mediated dilation [24,25]. Ascorbic acid can recover these alterations, so we can infer that oxidative stress is involved and that it acts not only at endothelium level but also in the complexity of circulatory regulation. In fact, hyperaemia is a crucial test, showing how vasoregulation systems can respond to maximal request of oxygen and metabolites from peripheral tissues.

Furthermore, ischemic conditions create a reductive stress [26], this is followed by reperfusion which produces a condition of oxidative stress (ischemia–reperfusion damage); this last aspect is probably amplified by oxidative stress due to hyperglycemia and glucose metabolism. Ascorbic acid counteracts these phenomena, by improving the redox state. As a consequence hyperaemic curve is restored after vitamin C. The curve is, in fact, recovered in peak flow (mainly prostaglandins mediation), in amplitude (neurogenic and myogenic response), and in total time (nitric oxide contribution for flow mediated dilation). Antioxidant acts on multiple stages in vasoregulation, and preserves hyperaemic response; some consequences, speculative and therapeutical, may be traced in clinical conditions that determine an oxidative–reductive stress, in particular for diabetic patients affected with atherosclerosis. Large studies on antioxidant supplementation in this setting have been deluding, but at least on speculative basis we can imagine a positive role in particular dynamic conditions with higher oxidative stress, such as strenuous exercise in patients with peripheral atherosclerosis and in anaerobic phases of training in rehabilitation programmes; in fact these conditions may elicit an ischemia–reperfusion phenomenon with increasing oxidation.

Our study demonstrates that oral glucose load can cause endothelial microcirculatory impairment as well as a reduction of hyperaemia in macro a microcirculatory circulation. This is due to oxidative–reductive stress as demonstrated by the recovery of these phenomena obtained by administering ascorbic acid.

Conflict of interest statement

The authors state that they have no conflicts of interest.

Learning points

- Oral glucose load can cause endothelial microcirculatory impairment an macro a microcirculatory reduction of hyperemia.
- This is due to oxidative–reductive stress.

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