Effect of Vitamin C on Forearm Blood Flow and Glucose Metabolism in Essential Hypertension

Andrea Natali, Anna Maria Sironi, Elena Toschi, Stefania Camastra, Giovanna Sanna, Armando Perissinotto, Stefano Taddei, Ele Ferrannini

Abstract—In 9 patients with essential hypertension, we tested whether a high-dose (12 mg · min⁻¹) vitamin C infusion into the brachial artery, by improving endothelium-dependent vasodilatation, would also attenuate the insulin resistance of deep forearm tissues. We measured the effect of vitamin C on acetylcholine (Ach)-induced vasodilatation and on forearm glucose uptake during systemic hyperinsulinemia; in all studies, the contralateral forearm served as the control. Intrabrachial Ach infusion produced a stable increase in forearm blood flow, from 2.6±0.3 to 10.6±2.1 mL · min⁻¹ · dL⁻¹; when vitamin C was added, a further rise in forearm blood flow (to 13.4 mL · min⁻¹ · dL⁻¹; P<0.03 vs Ach alone) was observed. In response to insulin, blood flow in both the infused and control forearms did not significantly change from baseline values (+10±16% and +2±11%, respectively). In contrast, when vitamin C was added, blood flow in the infused forearm increased significantly (to 3.7±0.7 mL · min⁻¹ · dL⁻¹; P<0.02 vs 2.8±0.6 mL · min⁻¹ · dL⁻¹ in the control forearm). Insulin stimulated whole-body glucose disposal to 20±2 μmol · min⁻¹ · kg⁻¹, compatible with the presence of marked insulin resistance. Forearm glucose uptake was similarly stimulated after 80 minutes of insulin infusion (to 2.11±0.42 and 2.06±0.43 μmol · min⁻¹ · dL⁻¹, infused and control, respectively). When intrabrachial vitamin C was added, no difference in glucose uptake was observed between the 2 forearms (infused, 2.37±0.44 μmol · min⁻¹ · dL⁻¹ and control, 2.36±0.53 μmol · min⁻¹ · dL⁻¹). Forearm O₂ uptake at baseline was also similar in the 2 forearms (infused, 9.7±0.7 μmol · min⁻¹ · dL⁻¹ and control, 9.6±1.1 μmol · min⁻¹ · dL⁻¹) and was not changed by either insulin or vitamin C. We conclude that in the deep forearm tissues of patients with essential hypertension and insulin resistance, an acute improvement in endothelial function, obtained with pharmacological doses of vitamin C, restores insulin-mediated vasodilatation but does not improve insulin-mediated glucose uptake. Thus, the endothelial dysfunction of essential hypertension is unlikely to be responsible for their metabolic insulin resistance. (Arterioscler Thromb Vasc Biol. 2000;20:2401-2406.)

Key Words: essential hypertension ■ insulin resistance ■ vitamin C ■ forearm

Recent studies have hypothesized that normal endothelial function (namely, normal endothelium-dependent vasodilatation of the resistance vessels) is necessary for insulin to fully exert its metabolic effects on skeletal muscle tissue.¹² In previous studies in a large group of patients with essential hypertension, we could not find any association between insulin sensitivity and endothelial function.³ Nevertheless, a direct test of this hypothesis requires verifying whether the insulin resistance observed in conditions such as obesity, hypertension, and diabetes (which are all characterized by endothelial dysfunction⁴⁻⁵) can be corrected by maneuvers that improve endothelial function. Physical exercise and good metabolic control, for example, have been shown to be associated not only with more efficient endothelium-mediated vasodilatation⁶⁻⁷ but also with improved insulin sensitivity.⁸ Because a large number of factors have been shown to be associated with both insulin resistance and endothelial dysfunction (eg, smoking, hypertension, obesity, aging, hyperglycemia, oxidative stress, and elevated free fatty acid concentrations), it seems possible that the endothelium plays a direct role in modulating the metabolic response to insulin. In healthy humans, Baron et al⁹ showed that both insulin-stimulated leg glucose uptake and vasodilatation were reduced when nitric oxide (NO) synthesis was inhibited by the infusion of N⁵-nitro-L-arginine into the femoral artery. From their data, these authors estimated that local NO generation was responsible for 30% of the overall insulin effect on leg glucose extraction. However, attempts at improving the metabolic effect of insulin through an enhancement of skeletal muscle perfusion in patients with insulin resistance have repeatedly failed. In patients with essential hypertension, neither adenosine¹⁰ nor sodium nitroprusside¹¹ could enhance forearm glucose uptake; in obese patients, bradykinin had no effect on leg glucose uptake.¹² In addition

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to differences in methods (plethysmography versus thermic dilution versus tracer) and/or tissue composition (forearm versus leg), a possible explanation for these discrepancies is that the vasodilation obtained with the intra-arterial infusion of vasoactive drugs may not reproduce the dilatation induced by insulin. In the perfused rat hindlimb model, it has been consistently demonstrated that not all vasoactive substances exert favorable effects on metabolism. On these grounds, the hypothesis that skeletal muscle blood flow can follow different pathways (nutritive versus nonnutritive) has been proposed.13 If insulin-induced NO synthesis were to occur mainly in the vessels carrying nutritive flow, then the intra-arterial infusion of vasodilators would not necessarily reproduce the quality of tissue perfusion achieved by insulin.

As reported by numerous though not all (see Reference 14 for a thorough review) studies in patients with essential hypertension, the response to endothelium-dependent vasodilators (eg, acetylcholine [Ach]) is reduced; this defect has been attributed to an impaired NO synthesis.4 However, more recent experiments in humans15 have provided evidence that NO action can be reduced by an increased generation of O2 radicals in response to muscarinic receptor stimulation. Accordingly, pharmacological doses of vitamin C can restore the forearm response to Ach in patients with essential hypertension, and this effect, which is not seen in normotensive subjects, is prevented by the inhibition of NO synthase with N \textsuperscript{G}-monomethyl-L-arginine. Similarly, in patients with type 2 diabetes, intra-arterial vitamin C almost completely restores the endothelial response to Ach.16 In addition, when given systemically to diabetic patients, vitamin C has also been shown to improve whole-body insulin sensitivity.17 In the latter study, however, limb blood flow was not measured, and the effect of vitamin C on glucose metabolism was attributed to changes in plasma cell membrane fluidity.

In the present study in insulin-resistant patients with essential hypertension, we tested whether vitamin C, by countering O2 radical production, would facilitate insulin-stimulated NO action at the physiological sites and through this mechanism, also improve the metabolic response to the hormone.

Methods

Subjects
Nine middle-aged (52 ± 3 years, mean ± SEM), overweight (body mass index = 28.3 ± 1.6 kg · m \textsuperscript{-2}), male patients with essential hypertension (ambulatory blood pressure = 166 ± 2/96 ± 2 mm Hg) were recruited from the outpatient clinic. Each patient had a complete clinical work-up to exclude secondary forms of hypertension and hepatic, renal, or endocrine diseases. Diabetes was excluded on the basis of the following criteria: unawareness of the disease, absence of hypoglycemic treatment, and fasting plasma glucose values < 6 mmol/L on at least 2 recent occasions. All subjects were on a weight-maintaining diet, and they discontinued their antihypertensive treatment 2 weeks before the study. The purpose and the potential risks of the study were explained to all patients before their informed consent was obtained. The study protocol was approved by the Institutional Review Board.

Experimental Protocol
The study began at 8:30 AM after an overnight fast, with the subject lying supine in a quiet room at a constant temperature of 21°C to 24°C. A Teflon catheter (20G, 2 in.) was inserted retrogradely into an antecubital vein of each forearm and was considered to be correctly placed when its tip could not be palpated. These 2 catheters were used to collect blood samples from the deep tissues of the forearm. Another Teflon cannula (20G) was inserted, also retrogradely, into the brachial artery of the nondominant arm under local anesthesia (2% Xylocaine). This arterial access served for both blood sampling and local infusion. The forearm with the arterial catheter was designated the infused forearm and the contralateral, the control forearm. Another catheter (20G) was inserted antegradely into a superficial vein of the control forearm for the systemic infusion of insulin and glucose.

The study consisted of 3 periods: basal, clamp, and vitamin C supplemented (Figure 1). During the basal period, 2 sets of blood samples were drawn (at times −60 and −40 minutes) from the artery and deep vein of both forearms for the determination of blood gases and plasma glucose. One minute before each blood sampling, blood flow to the hands was interrupted by means of a pediatric cuff placed around the wrists and inflated to a suprasystolic pressure. Total forearm blood flow (FBF) was measured in both forearms by strain-gauge plethysmography (EC4, Hokansom) immediately after each blood sampling, with the circulation to the hand still excluded. Each FBF determination was the mean of at least 3 consecutive measurements. Intra-arterial blood pressure was continuously measured through a transducer (critical care system monitoring kit, Abbott) placed on the arterial line and connected to a bedside intensive care monitor (Dynascope 5100E, Fukuda Denshi); similarly, heart rate was monitored by means of a 3-lead ECG recording throughout the study. After these 2 basal determinations, FBF was measured 5 minutes after a constant (4.5 pg · min \textsuperscript{-1} · dL \textsuperscript{-1} in the forearm) intra-arterial infusion of Ach (Miovisin, Farmiga) and again after the coinfusion of vitamin C (Vitamina C, Bracco) at the rate of 12 mg · min \textsuperscript{-1}, or 68 μmol · min \textsuperscript{-1}. To allow FBF to return to baseline and to verify that forearm metabolism was not altered by these infusions, 20 minutes were allowed before a third baseline set of blood samples was collected. Subsequently, a primed (163 pmol · kg \textsuperscript{-1} · over 7 minutes), continuous (7 pmol · min \textsuperscript{-1} · dL \textsuperscript{-1} in the forearm) intra-arterial infusion of regular insulin was started through the superficial antecubital vein while plasma glucose was maintained constant at basal values by means of a variable 20% glucose infusion (euglycemic insulin clamp). Sixty minutes into the clamp period, another 3 sets of blood samples were collected, and FBF was measured at 10-minute intervals. When FBF, exogenous glucose infusion rate, or arterial plasma glucose concentrations were not stable (ie, within 10% of 1 another), further measurements were postponed by 5 to 10 minutes. After the last clamp FBF measurement, vitamin C was infused into the infused forearm at the rate of 12 mg · min \textsuperscript{-1} for 30 minutes; during this time, another 3 sets of blood samples were collected, and FBF was measured at 10-minute intervals.

Blood and Plasma Determinations
Each blood sample was divided into 2 aliquots: 1 mL was collected into heparinized microtubes and immediately centrifuged, and plasma glucose concentration was measured in the supernatant; 1.5 mL was collected into heparinized syringes for immediate blood gas determination and oximetry (Instrumentation Laboratory systems...
1302 and 282 CO-oximeter). Another 2 mL was also collected from the arterial line only for the determination of insulin (Insik 5, Sorin Biomedica).

Calculations
The metabolic response of deep forearm tissues was estimated from glucose and O2 fluxes across the limb. Two different indices of substrate disposal were calculated: the extraction ratio (ie, [arterial minus venous]/arterial) and the net balance (FBF times the arterial-venous concentration difference). The former provides an index of the efficiency with which a substrate is handled by the amount of tissue actually perfused. The net balance calculation yields the net rate of substrate exchange across the limb and is the standard way of expressing forearm metabolism. Because forearm O2 uptake can be considered proportional to the amount of metabolically active tissue, we also calculated the ratio of net glucose to O2 balance to correct for recruitment as well as any differences in forearm muscularity or deep vein drainage.

Whole-body glucose disposal was estimated by averaging the glucose infusion rates every 20 minutes and then adjusting for changes in the body glucose pool (assuming a distribution volume of 0.25 L · kg⁻¹).

Statistical Analysis
Each triple set of measurements within each study period (basal, clamp, and vitamin C supplemented) was averaged, and a paired t test analysis was then used to compare the 2 forearms (control and infused) within the same study period or different study periods within the same forearm. This choice was based on the consideration that all of the comparisons were actually made within the same subject. ANOVA for doubly repeated measures (over the 3 study periods and the 2 forearms) was also carried out on the mean values.

Results
At baseline, blood flow was similar in the 2 forearms (2.6±0.3 versus 2.7±0.4 mL · min⁻¹ · dL⁻¹, infused versus control). The intra-arterial infusion of Ach induced a prompt and stable vasodilatation (+302±70%, P<0.001) in the infused forearm, whereas blood flow in the control forearm did not change (Figure 2). The addition of vitamin C resulted in a further increase in blood flow (from 10.6±2.1 to 13.4±2.6 mL · min⁻¹ · dL⁻¹, P<0.03). Neither intra-arterial blood pressure (160±5/87±3 mm Hg) nor blood flow to the control forearm changed during infusion of the 2 compounds.

As shown in Figure 3, systemic insulin infusion (7 pmol · min⁻¹ · kg⁻¹) produced a stable rise in plasma insulin concentrations and significantly stimulated whole-body glucose uptake, which, during the last 40 minutes of the clamp, averaged 19.9±1.9 μmol · min⁻¹ · kg⁻¹. Plasma glucose remained stable throughout the study.

Because blood flow, glucose, and O2 gradients returned to baseline values after the Ach plus vitamin C infusion and did not change significantly within each study period (by ANOVA for repeated measures), the 3 determinations of each period were averaged. Systemic insulin infusion alone was associated with limb blood flow changes ranging from −39% to +124%; on average, the change in FBF was small and not statistically significant (infused forearm=10±16%, control forearm=2±11%; Figure 4). Local vitamin C coinfusion was associated with a further 13% increase in FBF, which made the increment with respect to baseline values (+28±12%) close to statistical significance (P=0.06), whereas flow to the control forearm remained stable, and the difference between the 2 arms (3.7±0.7 versus 2.8±0.6 mL · min⁻¹ · dL⁻¹) was statistically significant (P<0.02). During the clamp, intra-arterial blood pressure (158±4/86±3 mm Hg) did not change with respect to baseline values (160±5/87±3 mm Hg), whereas heart rate showed a small but significant increment (from 65±3 to 69±4 bpm; P<0.03). As depicted in Figure 4, systemic insulin infusion produced a 10-fold rise in forearm glucose extraction in both forearms; adding vitamin C locally did not alter glucose extraction. Forearm glucose uptake was similar in the 2 forearms and tended to increase in both over time (Figure 4).

At baseline, venous hemoglobin O2 saturation in the infused forearm was 7% higher than in the control forearm (59±2% versus 55±3%; P<0.05); this resulted in a slightly lower O2 extraction (40±4% versus 44±3%, P<0.04) that, when coupled with the higher FBF, yielded similar rates of O2 consumption in the 2 forearms (the Table). During the clamp, O2 consumption rose slightly in both the infused and control forearms, with no significant difference between the 2. The

Figure 2. FBF response to Ach and ACh plus vitamin C in infused (continuous line) and control (dotted line) forearms. *P<0.03 vs Ach alone.

Figure 3. Plasma glucose (top), plasma insulin (middle), and glucose infusion rates (bottom) during basal and clamp periods. The arrows indicate times when the blood samples were drawn.
insulin resistance would benefit from such an intervention. Because the degree of endothelial dysfunction is extremely variable among patients with essential hypertension,\(^1\) assuming its presence a priori may be misleading. In the current study, endothelial function was directly assessed in the patients through the vascular response to Ach and by measuring the effect of vitamin C on this vasodilatation. The finding that vitamin C improved Ach vasodilatation (Figure 2) documents the presence of endothelial dysfunction and supports the contention that it is due to an excessive production of \(\text{O}_2\) radicals. \(^15\) The presence of insulin resistance of glucose metabolism in our study subjects is documented by the value of whole-body, insulin-stimulated glucose uptake (19.9±1.9 \(\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}\), or 3.6±0.3 \(\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}\)) that falls within the bottom quintile (with individual values ranging from the third to the 30th percentile) of the frequency distribution of whole body glucose uptake values in nondiabetic, normotensive, lean male subjects (\(n=373\)) observed in a large series of clamp studies that used the same insulin infusion rate.\(^19\) Incidentally, in the whole group, insulin-stimulated glucose uptake was correlated to the vascular response to our single dose of Ach (\(r=0.83, P<0.01\)) but not to the effect of vitamin C. In a larger group of more extensively characterized patients,\(^3\) we could not observe any correlation between the indices of insulin sensitivity and endothelial function. With small patient numbers, the possibility that 2 variables are spuriously associated should be considered, and caution should be taken in the interpretation of such data.

The mechanism by which vitamin C potentiates NO action on the vasculature in humans is uncertain, but animal and in vitro studies support the view that its antioxidant properties are involved. These studies have clearly shown that NO is rapidly inactivated by \(\text{O}_2\)-reactive species\(^20\) and that NO synthesis (at least when induced by Ach) is associated with \(\text{O}_2\) radical generation.\(^21\) Thus, it has been proposed that high doses of vitamin C make more NO available by “quenching” the local generation of \(\text{O}_2\) radicals.\(^22,23\) Whether NO synthesis is coupled to \(\text{O}_2\) radical generation when it is also stimulated by insulin is not known. Our finding during the clamp that blood flow increased only in the forearm infused with vitamin C (Figure 3) suggests that this might be the case. In animal studies,\(^24\) insulin has been shown to increase the intracellular production of \(\text{O}_2\) radicals. In our laboratory, we have demonstrated that euglycemic hyperinsulinemia lowers plasma vitamin E levels.\(^25\) Whether this pro-oxidant action of insulin is coupled to NO synthesis cannot be directly ascertained from the current experiments. Nevertheless, because vitamin C selectively potentiates NO-mediated vasodilatation (because it does not alter basal blood flow\(^15,16,26\)), its effect on insulin-stimulated blood flow suggests that in patients with essential hypertension also, insulin-stimulated NO synthesis is coupled with the production of \(\text{O}_2\) radicals that limit the vascular effect of the gas.

Although during hyperinsulinemia the effect of vitamin C on FBF was small, the difference between the infused and control forearms was statistically significant and quantitatively similar to that induced by insulin alone in healthy subjects (ie, +30%).\(^27\) In addition, our aim was to restore NO action with the idea that this would selectively improve
Blood Gas Arterial (A) Concentration, Fractional Exchange (FE), Net Balance (NB), Glucose-to-Oxygen Uptake Ratio (G/O), and Energy Expenditure (EE) in Control and Infused Forearms

<table>
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<th>Basal</th>
<th>Clamp</th>
<th>Vitamin C Supplemented</th>
<th>ANOVA P*</th>
<th>Time</th>
<th>Forearm</th>
<th>Time × Forearm</th>
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<td>O₂, A, mmol/L</td>
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<tr>
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<tr>
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*Two-way ANOVA for doubly repeated measures. Shown are the significances of the 2 factors (time and forearm) and their interaction.

nutritive tissue perfusion, which can also take place with only minor changes in total tissue blood flow.¹³

While potentiating insulin-induced vasodilatation, vitamin C was without effect on forearm glucose metabolism, because vasodilatation in the infused forearm was associated with a proportional dilution of the arteriovenous glucose gradient, such that net glucose uptake remained similar to that in the control forearm (Figure 4). Similarly, neither O₂ uptake nor CO₂ release was affected by vitamin C (the Table), indicating the absence of significant muscle fiber recruitment or changes in the pattern of substrate oxidation. Net hydrogen output was significantly increased by vitamin C, probably as a result of the low pH of the vitamin C solution (pH = 6.91). Because venous blood pH was similar in the infused and control forearms, the small acid load was likely diluted by forearm blood and did not result in significant tissue acidosis.

The study by Baron and coworkers⁹ is the only one to have reported an improvement in insulin-mediated glucose uptake after experimental vasodilatation.²⁸ That study was carried out in healthy young subjects and used an infrafemoral infusion of methacholine after 180 minutes of hyperinsulinemia. The discrepancy between Baron’s results and those of other studies (including ours) may relate to the use of different vasoactive agents or to the different experimental setup. In the current experiments, special care was taken to fulfill the requisites for the measurement of forearm metabolism from arterial-venous balances.²⁹ Strain-gauge plethysmography has been criticized because of its low accuracy. In our laboratory, the technique has been validated in experiments in which saline was infused into the brachial artery at rates designed to increase FBF by 1 or 2 mL · min⁻¹ · dL⁻¹; the measured FBF increments were 0.8±0.2 and 1.1±0.3 mL · min⁻¹ · dL⁻¹, respectively. Utirainen et al²⁷ estimated the absolute accuracy of the strain-gauge plethysmograph by comparing it with a direct method; they reported a correlation coefficient of 0.92, with a systematic overestimation of
plethysmography of 19±4%. In our experience, reproducibility, which includes spontaneous flow rate fluctuations, is 6±2%. Finally, the good correlation between forearm and whole-body glucose uptake in both forearms in the present as well as other studies from our laboratory1,2 indicates that the plethysmographic method yields rather accurate and precise estimates of FBF. Plethysmography together with dye dilution are, in fact, the only 2 methods used for metabolic balance studies and are used as a reference for new techniques.3,4 A possible alternative explanation of the discrepancy between our negative finding and Baron’s data could be that blood flow modulates insulin-mediated glucose metabolism in normal subjects only. To be clinically relevant, however, any positive effect of perfusion on insulin action should be demonstrated in insulin-resistant subjects.

A possible limitation of this study is the relatively small number of study subjects. However, given the number of paired observations (n=9) and the observed standard deviation of the differences in glucose uptake between the infused and the control forearms (0.429 μmol · min⁻¹ · dl⁻¹), we could calculate that the study had a 90% probability of detecting a difference >20% between the 2 forearms while accepting a 10% probability of making a type I error.

In conclusion, in the skeletal muscle of patients with essential hypertension and insulin resistance, an acute increase in local endothelium-derived NO availability obtained by administration of pharmacological doses of vitamin C allows insulin-mediated vasodilation to occur but has no effect on insulin-mediated glucose uptake. Thus, the vascular insulin resistance of essential hypertension is unlikely to be responsible for the metabolic insulin resistance observed in this condition.

References

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