Ascorbate improves circulation in postural tachycardia syndrome

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Submitted 4 January 2011; accepted in final form 20 May 2011

Stewart JM, Ocon AJ, Medow MS. Ascorbate improves circulation in postural tachycardia syndrome. Am J Physiol Heart Circ Physiol 301: H1033–H1042, 2011. First published May 27, 2011; doi:10.1152/ajpheart.00018.2011.—Low flow postural tachycardia syndrome (LFP) is associated with vasoconstriction, reduced cardiac output, increased plasma angiotensin II, reduced bioavailable nitric oxide (NO), and oxidative stress. We tested whether ascorbate would improve cutaneous NO and reduce vasoconstriction when delivered systemically. We used local cutaneous heating to 42°C and laser Doppler flowmetry to assess NO-dependent conductance (%CVCmax) to sodium ascorbate and the systemic hemodynamic response to ascorbic acid in 11 LFP patients and in 8 control subjects (aged 23 ± 2 yr). We perfused intradermal microdialysis catheters with sodium ascorbate (10 mM) or Ringer solution. Predrug heat response was decreased in LFP, particularly the NO-dependent plateau phase (56 ± 6 vs. 88 ± 7% CVCmax). Ascorbate increased baseline skin flow in LFP and control subjects and increased the LFP plateau response (82 ± 6 vs. 92 ± 6 control). Systemic infusion experiments used Finometer and ModelFlow to estimate relative cardiac index (CI) and forearm and calf venous occlusion plethysmography to estimate blood flows, peripheral arterial and venous resistances, and capacitance before and after infusing ascorbic acid. CI increased 40% after ascorbate as did peripheral flows. Peripheral resistances were increased (nearly double control) and decreased by nearly 50% after ascorbate. Calf capacitance and venous resistance were decreased compared with control but normalized with ascorbate. These data provide experimental support for the concept that oxidative stress and reduced NO possibly contribute to vasoconstriction and venoconstriction of LFP.

METHODS

POSTURAL TACHYCARDIA SYNDROME (POTS) accounts for most cases of chronic orthostatic intolerance (21, 28, 39, 47, 48). POTS is defined by excessive increase in upright heart rate and symptoms of orthostatic intolerance (39) improving with recumbence. Our laboratory (42) has described a subset of POTS designated “low flow POTS” (LFP) in which there is marked upright tachycardia and circulatory insufficiency even while supine including resting tachycardia, decreased cardiac output (CO), and decreased regional blood flows with peripheral and cutaneous vasoconstriction, findings often ascribed to a hyperadrenergic state (1, 6). A consistent observation has been increased plasma angiotensin II (ANG II; Refs. 40, 43, 45), which can act locally to decrease bioavailable nitric oxide (NO) by superoxide scavenging to create peroxynitrite (36). ANG II also acts through reactive oxygen species (ROS; Ref. 35) to increase central and peripheral sympathetic activity (9, 52). Both increased ANG II due to deficient ACE2 and decreased bioavailable NO have been demonstrated in LFP during prior experiments using intradermal microdialysis techniques. Experiments made extensive use of the local vasodila-

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Protocol 1

Cutaneous microdialysis of sodium ascorbate improves the local heating response in LFP? Two microdialysis catheters were employed. One was used to perfuse 10 mM sodium ascorbate dissolved in lactated Ringer solution, and the other catheter contained Ringer solution and served as a control. Before microdialysis catheter insertion, laser Doppler flow (LDF) was measured over each insertion site to estimate precatheterization laser Doppler blood flows. Laser probes were removed and two microdialysis catheters were inserted and laser probes positioned over the dialysis membranes. After recovery (see below), LDF was measured for 10 min while the catheters were perfused with lactated Ringer solution to obtain baseline blood flows. Without moving the laser probes LDF was then recorded during local heating of both catheters. A recovery period followed requiring 30–60 min until LDF reached baseline levels. We then perfused one catheter with sodium ascorbate dissolved in Ringer and, after a 40-min loading period, verified LDF and repeated local heating while continuing ascorbate perfusion. The control catheter received Ringer solution only and underwent repeat local heating. After recovery from this second local heating, and as a final perfusion, 28 mM sodium nitroprusside was delivered through each microdialysis catheter to produce maximum blood flow.

Instrumentation. Testing was at ~25°C ≥2 h after a light breakfast. Experiments were performed with the subject supine. The two catheters were placed ≥5 cm apart and inserted in the dermal space of the left lateral calf after hair was gently removed from the insertion site. Each site was cooled with an ice-pack before catheter insertion to reduce discomfort. Each probe (MD-2000 Linear Microdialysis Probes; Bioanalytical Systems, West Lafayette, IN) has a 10-mm microdialysis membrane section that is placed in the intradermal space using a 25-gauge needle as an introducer. The molecular mass cutoff of the membrane is nominally 30,000 Da.

Following placement, both catheters were perfused with Ringer solution at 2 μl/min. An integrating LDF probe (Probe 413; Perimed, Stockholm, Sweden) containing seven individual probe tips was placed directly over each microdialysis catheter to measure LDF. LDF was observed until flow recovered from the trauma of catheter insertion. To estimate recovery, we used precatheterization laser Doppler blood flows as a rough guide. Baseline LDF data were thereafter measured.

Once baseline LDF values were obtained and local heating response measured under untreated conditions, subjects received perfusate containing 10 mM sodium ascorbate dissolved in Ringer at a rate of 2 μl/min. The concentration of ascorbate was based on the work of Holowatz and Kenney (17).

Local heating. Once baseline LDF values were obtained, the areas under each laser were heated at 1°C/10 s to 42°C for ≥30 min until a plateau was reached. Heat was then turned off to allow for recovery to baseline LDF.

Monitoring. Heart rate was monitored by electrocardiography and blood pressures were measured by finger plethysmography (Finometer; TNO, Amsterdam, The Netherlands) of the right index or middle finger intermittently recalibrated against oscillography. Mean arterial pressure (MAP) was obtained by averaging the signal over 5 min. The Finometer MAP was calibrated by oscillography [using the formula MAP = (systolic pressure + 2 * diastolic pressure)/3].

Protocol 2

Does systemic administration of ascorbic acid reduce peripheral vasoconstriction and increase CO in LFP? INSTRUMENTATION AND HEMODYNAMIC CALCULATIONS. On another day, tests began at 10 AM after 4 h of fasting. Subjects refrained from caffeine for ≥72 h. Experiments were performed with the subjects supine. An intravenous catheter was placed in the left antecubital vein.

1) Blood pressure, heart rate, and CO; we used the ModelFlow feature of the Finometer to estimate relative change in CO. Relative total peripheral resistance (TPR) in Woods units (mmHg·1−1·min) was calculated as MAP/CO. Single lead electrocardiogram was obtained for heart rate and rhythm.

2) Impedance plethysmography: impedance plethysmography (IPG) can detect internal fluid volume shifts (32), during orthostatic stress (31). We used a four-channel digital impedance plethysmograph (THRM; UFI, Morro Bay, CA) to measure volume shifts in four anatomic segments designated the thoracic segment, the splanchnic segment, the pelvic segment incorporating the lower pelvis to upper leg, and the leg segment. Ag/AgCl electrocardiographic electrodes were attached to the left foot and hand, which served as current injectors. Additional electrodes were placed in pairs representing anatomic segments as follows: the ankle-upper calf just below the knee (leg), the knee-iliac crest (pelvic), the iliac crest-midline xyphoid process (splanchnic), and the xyphoid process to supraventricular area (thoracic). We estimated the change in segmental fluid volume during the ascorbic acid infusion from the following formula:

\[ \Delta \text{Segmental blood volume} = p \times \left( \frac{L}{R_0} \right)^2 \times \Delta R \]

where \( p \) is the electrical conductivity of blood estimated as 33.2 × exp(hematocrit × 0.022) (10) and is assumed to be constant over the course of the experiment, \( R_0 \) is the baseline resistance of a segment, and \( \Delta R \) is the change in resistance of that segment. Volume changes used for intergroup comparisons were calculated from maximum changes in \( \Delta R \) during the infusion using the average baseline value of \( R_0 \) before ascorbate infusion and averaging over the entire change in resistance.

3) Forearm and calf hemodynamics: forearm and calf blood flow, venous pressure, and volume-pressure capacitance relationship were measured by venous occlusion plethysmography (VOP) using mercury in silastic, Whitney-type strain gauges (Hokanson, Bellevue, WA). Arm and thigh occlusion cuffs were placed around the upper and lower limbs 10 cm above the strain gauge. Forearm and calf blood flows were obtained by rapidly inflating occlusion cuffs to a pressure just below diastolic pressure to prevent venous egress. Inflating a smaller secondary cuff to above systolic blood pressure briefly prevented wrist and ankle flow. Arterial inflow in units of ml/(100 ml tissue)/min was estimated as the rate of change of limb cross-sectional area.

After recovery to baseline, we increased occlusion pressure gradually until a change in limb volume was detected. This represents resting venous pressure (Pv) (7). We calculated the forearm and calf peripheral arterial resistance in units of ml-100 ml tissue/min-1-mmHg-1 from

\[ \text{mmHg} / (\text{ml} / 100 \text{ml tissue}) / \text{min} \times \frac{\text{from MAP-Pv}}{\text{flow}} \]

We also used VOP to estimate the pressure dependent forearm and calf capacitance (volume-pressure) relation for comparison between groups, before and after ascorbic acid infusion. VOP measures volume changes in normalized units of ml/100 ml of tissue (7, 8).

Resting venous volume (volume at Pv) was measured. With cuffs deflated and the subject supine, we elevated the forearm and calf until there was no further decrease in limb size. Heart rate and blood pressure remained unchanged suggesting that autonomic status was unperturbed.

After a return to baseline, we used 10-mmHg pressure steps to a maximum of 60 mmHg to produce progressive limb enlargement. Pressure steps started at the first multiple of 10 exceeding Pv. By fixing pressure with the cuff, the ascending volume-pressure relation was obtained as shown in Fig. 1.

Separating filtration from vascular filling was performed. At lower occlusion pressures, as shown in Fig. 1, the limb size reached a plateau. With higher pressures, an initial curvilinear change represent-
ing venous filling occurred, and thereafter limb volume increased linearly due to microvascular filtration. This is shown in Fig. 1, bottom right. With the use of least squares analysis (37), venous filling was separated from filtration by “curve stripping” the later linear portion leaving only the plateau-reaching curvilinear portion representing capacitance vessel filling (left lower panel of Fig. 1). Occlusion pressure was maintained constant for ≥4 min to accomplish this.

The volume-pressure relation was computed. The volume-pressure relation was constructed once the volume response was partitioned into contributions from filling of capacitance vessels and from filtration.

The venous resistance was as computed. After stepwise increases in pressure were completed, the occlusion cuff was rapidly deflated. The instantaneous pressure difference between limb and central veins was estimated by the final occlusion pressure (60 mmHg), and the central PV (assumed to be close to 0 mmHg). Venous resistance ($R_v$) was estimated from the initial slope shown in Fig. 1, bottom right, using the formula $R_v = \frac{P(60 \text{ mmHg})}{\text{efflux}}$ (34).

Protocol for Ascorbic Acid Infusion

Baseline data were collected for 10 min. Forearm and calf blood flow was measured by VOP. Thereafter, we generated limb volume-pressure relationships. The cuffs were then rapidly deflated to calculate venous resistance.

After initial tests were completed and following a 30 min rest, all subjects received 60 mg/(kg fat-free mass) infusion of ascorbic acid dissolved in 100 ml of saline over 20 min followed by a maintenance infusion of 20 mg/(kg fat-free mass) of ascorbic acid dissolved in 30 ml saline administered over an hour. During the infusion blood pressure, heart rate, ModelFlow, and IPG were measured continuously while VOP blood flows were performed intermittently. After infusions were complete, we repeated measurements of arterial pressure, ModelFlow, segmental blood volume changes by IPG, and VOP measurements of forearm and calf blood flow, PV, capacitance measurements, and estimated venous resistance.

On another day, time and fluid volume control experiments were performed on three POTS patients and two control subjects. Baseline measurements were performed, and 130 ml of saline were infused following the same time and fluid volume schedule that was used for ascorbic acid infusions. In both groups, hemodynamic parameters were unchanged throughout the saline infusion.

Data and Statistical Analysis

Cutaneous microdialysis perfusion of sodium ascorbate. LDF was measured in perfusion units (pfu) to inform on cutaneous vascular conductance (CVC). CVC measurements were then converted to a percent maximum conductance (%CVC$_{max}$) by dividing CVC by the maximum CVC achieved after administration of 28 mM sodium nitroprusside at the end of experiments.

Changes in baseline LDF before and after sodium ascorbate were compared by paired $t$-test. Comparisons were made examining differences between each phase of the local heating response pre- and postascorbate infusion. Group comparisons of the heat response were also averaged over POTS and controls subjects and graphically depicted. Data were obtained from individual heating curves before and after drugs.
Table 1. Local heat response to sodium ascorbate (%CVC\textsubscript{max})

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n = 8)</th>
<th>POTS Patients (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before ascorbate</td>
<td>9 ± 1</td>
<td>6 ± 1(^*)</td>
</tr>
<tr>
<td>After ascorbate</td>
<td>15 ± 2(^†)</td>
<td>13 ± 3(^‡)</td>
</tr>
<tr>
<td>First thermal peak</td>
<td>62 ± 8</td>
<td>42 ± 6(^*)</td>
</tr>
<tr>
<td>After ascorbate</td>
<td>72 ± 6</td>
<td>52 ± 6(^*)</td>
</tr>
<tr>
<td>Nadir</td>
<td>37 ± 5</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>After ascorbate</td>
<td>44 ± 4</td>
<td>43 ± 3(^‡)</td>
</tr>
<tr>
<td>Plateau</td>
<td>88 ± 7</td>
<td>56 ± 6(^*)</td>
</tr>
<tr>
<td>After ascorbate</td>
<td>92 ± 6</td>
<td>82 ± 6(^‡)</td>
</tr>
</tbody>
</table>

Values are means ± SE. %CVC\textsubscript{max}, maximum percent cutaneous vascular conductance; POTS, postural tachycardia syndrome. \(^*\) P < 0.05, smaller than control subjects. \(^†\) P < 0.01, larger than preascorbate %CVC\textsubscript{max}.

Systemic administration of ascorbic acid. Unpaired \(t\)-tests with Bonferroni correction were used to assess group differences between control and POTS hemodynamics at baseline. Limb capacitance data were compared between POTS and controls by two-way ANOVA for repeated measures. Pre- and postascorbate infusion data were compared by one-way ANOVA corrected for multiple comparisons. Since percent changes are shown, paired data were used. Capacitance curves were compared before and after ascorbate between POTS and controls using multiple ANOVA for repeated measures. Results are graphically depicted as means ± SE. The Statistical Package for the Social Sciences software version 11.0 was used, and statistically significant differences are reported for P < 0.05.

RESULTS

Cutaneous Microdialysis Perfusion of Sodium Ascorbate

Effects of sodium ascorbate on the local heating response. Table 1 and Fig. 2 show averaged %CVC\textsubscript{max} measured along the heating curves. Data are reported for baseline, first thermal peak, nadir, and plateau. Error bars have been omitted from Fig. 2 for clarity, but SE values are shown in the Table 1. Maximum conductance during Ringer perfusion was 2.16 ± 0.13 pfu/mmHg for LDF compared with 2.25 ± 0.13 pfu/mmHg for control where pfu refers to perfusion units of flow measured by LDF. There was no effect of ascorbate on the maximum CVC achieved with sodium nitroprusside: maximum conductance during Ringer perfusion was 2.31 ± 0.16 for POTS compared with 2.22 ± 0.14 for control.

**Baseline.** Baseline %CVC\textsubscript{max} in POTS was decreased compared with control subjects before ascorbate (P < 0.05). Ascorbate similarly increased baseline %CVC in POTS and control subjects.

**First Thermal Peak.** Before sodium ascorbate, the first thermal peak %CVC\textsubscript{max} was reduced in POTS compared with control subjects (P < 0.01). The first thermal peak remained reduced in POTS compared with control subjects after ascorbate administration.

**Nadir.** Before ascorbate, the nadir %CVC\textsubscript{max} was similar in POTS and control and increased (P < 0.05) in POTS after ascorbate. POTS remained similar to control after ascorbate.

**Plateau.** Before ascorbate, the plateau %CVC\textsubscript{max} was reduced in POTS compared with control (P < 0.001). After ascorbate the plateau was unchanged in control subjects but increased in POTS (P < 0.001) to approximate the control subjects’ plateau. There was no change in the plateau of control subjects with ascorbate.

Systemic Administration of Ascorbic Acid

**Dimensions and preascorbic acid hemodynamic data.** Preascorbic acid data are shown in Table 2. POTS patients weighed less than control subjects (P < 0.05) despite similar heights and had reduced body mass indexes (P < 0.025). The supine heart rate was increased in POTS compared with control (P < 0.001). Arterial pulse pressure was increased in POTS (P < 0.05). Calf blood flow in LFP was decreased by definition. Calf arterial resistance was increased in POTS patients (P < 0.001) while calf venous resistance was also increased in POTS (P < 0.01). Both forearm and calf venous pressures were increased in POTS compared with control (P < 0.05).

**Capacitance (volume-pressure) relation.** The capacitance relation before ascorbic acid infusion is shown in Fig. 3. While individual forearm volumes at given pressures did not differ individually, there was a small overall decrease in calf capacitance in POTS compared with control (P < 0.05). There were highly significant differences in calf capacitance (P < 0.001) and reductions in volume at each pressure in POTS compared with control.

Effects of ascorbic acid on hemodynamic data. Hemodynamic data for a representative POTS patient during the infusion of ascorbic acid are shown in Fig. 4: CO and MAP
increased and TPR and heart rate decreased during the infusion. An increase in CO and decrease in TPR were observed in every POTS patient, but decreases in heart rate and increases in MAP were not observed in every POTS patient. Individual POTS patients with findings similar to those shown in Fig. 4 exhibited a visible color change from pasty white to pink, with warming of the peripheral extremities and a sensation of overall warmth. Averaged percent changes in hemodynamic data are shown in Table 3. Ascorbate did not result in significant changes in hemodynamics in control subjects. However, in POTS patients pulse pressure increased ($P < 0.05$), CI increased by 40% ($P < 0.01$), while TPR decreased ($P < 0.05$). Forearm and calf blood flows increased markedly ($P < 0.001$) with a reciprocal decrease in arterial resistances ($P < 0.005$). Forearm and calf venous resistances were also reduced ($P < 0.01$) in association with reduced venous pressures.

Regional blood volume changes in POTS are shown in Fig. 5. While small, they were significant with a shift of blood from the splanchnic ($-3 \pm 1\%$), pelvic ($-2 \pm 0\%$), and leg ($-2 \pm 1\%$) regional vasculature towards the thorax ($7 \pm 1\%$), which may help to enhance CO.

Vascular capacitance was markedly affected by ascorbic acid infusion in POTS patients as depicted in Fig. 6, which compares pre- and postascorbate capacitance for all subjects. Ascorbate had minimal effect on control capacitances, but produces a statistically significant overall increase of forearm capacitance ($P < 0.05$) and a larger increase of calf capacitance in POTS.

**DISCUSSION**

**Key Findings**

The current data show that at baseline LFP is associated with decreased bioavailability of cutaneous NO, increased calf peripheral resistance, and reduced volume-pressure relationship under resting conditions in POTS compared with control. Past work from our laboratory (42) using indicator dye techniques demonstrated reduced CO and increased total peripheral resistance in similar LFP patients (42). These findings have been consistent across experiments and different from results found in other POTS subgroups. Our results show improvement in CO and peripheral blood flow by the reduction of arterial and total peripheral resistance in LFP patients following ascorbic acid infusion. Afterload reduction results in increased CO. Relatively small changes in regional blood volumes were observed with increased

**Table 2. Dimensions and hemodynamics**

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects ($n = 8$)</th>
<th>POTS Patients ($n = 11$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>22 ± 1.3</td>
<td>22.5 ± 0.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>67 ± 3</td>
<td>59.2 ± 2*</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168 ± 2</td>
<td>172 ± 3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24 ± 0.9</td>
<td>20.1 ± 5*</td>
</tr>
<tr>
<td>Supine HR, beats/min</td>
<td>64 ± 2</td>
<td>74 ± 3*</td>
</tr>
<tr>
<td>Upright HR during HUT screen</td>
<td>86 ± 4</td>
<td>124 ± 5*</td>
</tr>
<tr>
<td>Change in HR during HUT</td>
<td>23 ± 2</td>
<td>49 ± 3*</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>120 ± 2</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>62 ± 2</td>
<td>66 ± 3</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>57 ± 2</td>
<td>48 ± 3*</td>
</tr>
<tr>
<td>Venous occlusion forearm blood flow, ml·100 ml⁻¹·min⁻¹</td>
<td>4.0 ± 0.6</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>Forearm arterial resistance, ml·100 ml⁻¹·min⁻¹·mmHg⁻¹</td>
<td>26 ± 4</td>
<td>36 ± 5</td>
</tr>
<tr>
<td>Forearm venous pressure, Pv, mmHg</td>
<td>6 ± 1</td>
<td>11 ± 2*</td>
</tr>
<tr>
<td>Forearm venous resistance, ml·100 ml⁻¹·min⁻¹·mmHg⁻¹</td>
<td>0.62±0.16</td>
<td>0.82±0.12</td>
</tr>
<tr>
<td>Venous occlusion calf blood flow, ml·100 ml⁻¹·min⁻¹</td>
<td>3.0 ± 0.4</td>
<td>1.0 ± 0.2*</td>
</tr>
<tr>
<td>Calf arterial resistance, ml·100 ml⁻¹·min⁻¹·mmHg⁻¹</td>
<td>32 ± 4</td>
<td>66 ± 8*</td>
</tr>
<tr>
<td>Calf venous pressure, Pv, mmHg</td>
<td>11 ± 1</td>
<td>16 ± 2*</td>
</tr>
<tr>
<td>Calf venous resistance, ml·100 ml⁻¹·min⁻¹·mmHg⁻¹</td>
<td>0.71±0.11</td>
<td>1.8 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. HR, heart rate; HUT, head-up tilt; BP, blood pressure. *$P < 0.05$, smaller than control.

**Fig. 3.** Volume-pressure data points from supine POTS (gray) and control (black) subjects obtained from the forearm (top) and calf (bottom). Data were obtained before ascorbate infusion. Data for the forearm were similar for POTS and control while there was a significantly reduced calf volume at given pressure for POTS and thus decreased vascular capacitance. *$P < 0.05$, compared with control.
thoracic blood volume that could increase preload. Venous return and CO were clearly augmented by ascorbate and may enhance peripheral blood delivery. Reduction of venous resistance is also observed in LFP following ascorbate and facilitates peripheral venous return. Reduced venous resistance was associated with increased vascular capacitance. While small compared with arterial resistance, venous resistance can critically regulate pooling in the venous vasculature. Similar changes in capacitance and resistance properties were not observed in healthy volunteers receiving ascorbic acid nor during time and volume control experiments, which ensured that ascorbate effects did not depend on exogenous volume loading. Cutaneous data showing increased NO-dependent local heating response indicate that part of the overall effect of ascorbate possibly involves improved bioavailability of NO in LFP. The results are consistent with the hypothesis that ascorbate provides an antioxidant pronitricergic effect that improves circulatory insufficiency observed in LFP patients.

**Dilator Properties of Ascorbate**

Vasodilator and venodilator properties of ascorbic acid have been previously demonstrated. Thus, for example, high dose ascorbic acid infusion abolishes the vasoconstriction of age in men (19) and in estrogen-deficient postmenopausal women (33). Ascorbate can also modulate venous tone in humans (12). This is largely attributed to its antioxidant properties, which can exert its effects on an array of reactive oxidative species including superoxide and peroxides (3).

**Table 3. Percent changes in hemodynamics with ascorbic acid**

<table>
<thead>
<tr>
<th>Percent Change</th>
<th>Control Subjects</th>
<th>POTS Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>$-2 \pm 4$</td>
<td>$-10 \pm 5$</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>$-4 \pm 6$</td>
<td>$11 \pm 5$</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>$0 \pm 5$</td>
<td>$1 \pm 5$</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>$-1 \pm 4$</td>
<td>$10 \pm 3^*$</td>
</tr>
<tr>
<td>Cardiac index, l·min$^{-1}$·m$^{-2}$</td>
<td>$4 \pm 3$</td>
<td>$40 \pm 14^*$</td>
</tr>
<tr>
<td>Total peripheral resistance Woods units, mmHg·l·min$^{-1}$·min$^{-1}$</td>
<td>$1 \pm 3$</td>
<td>$-23 \pm 11^*$</td>
</tr>
<tr>
<td>Forearm occlusion forearm blood flow, ml·100 ml$^{-1}$·min$^{-1}$</td>
<td>$2 \pm 3$</td>
<td>$30 \pm 5$</td>
</tr>
<tr>
<td>Forearm arterial resistance, ml·100 ml$^{-1}$·min$^{-1}$·mmHg$^{-1}$</td>
<td>$-2 \pm 1$</td>
<td>$-25 \pm 10$</td>
</tr>
<tr>
<td>Forearm venous pressure, P$v$, mmHg</td>
<td>$0 \pm 3$</td>
<td>$-21 \pm 7^*$</td>
</tr>
<tr>
<td>Forearm venous resistance, ml·100 ml$^{-1}$·min$^{-1}$·mmHg$^{-1}$</td>
<td>$10 \pm 8$</td>
<td>$-37 \pm 9$</td>
</tr>
<tr>
<td>Venous occlusion calf blood flow, ml·100 ml$^{-1}$·min$^{-1}$·mmHg$^{-1}$</td>
<td>$10 \pm 11$</td>
<td>$80 \pm 24^*$</td>
</tr>
<tr>
<td>Calf arterial resistance, ml·100 ml$^{-1}$·min$^{-1}$·mmHg$^{-1}$</td>
<td>$-15 \pm 13$</td>
<td>$-60 \pm 18^*$</td>
</tr>
<tr>
<td>Calf venous pressure, P$v$, mmHg</td>
<td>$-12 \pm 8$</td>
<td>$-20 \pm 4^*$</td>
</tr>
<tr>
<td>Calf venous resistance, ml·100 ml$^{-1}$·min$^{-1}$·mmHg$^{-1}$</td>
<td>$-10 \pm 13$</td>
<td>$-44 \pm 12^*$</td>
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</tbody>
</table>

Values are means ± SE. *$P < 0.05$, different from preascorbate.
Vasoconstriction and Decreased NO Bioavailability in LFP

The vasoconstriction and potentially decreased NO bioavailability of LFP can be explained by effects of ANG II excess. While ANG II can directly produce vasoconstriction (49), potent vasoconstrictive actions occur through ANG II type 1 receptor (AT1R)-dependent interactions with NADPH oxidase and other oxidases; these enhance production of ROS (11, 13, 14), specifically superoxide, which scavenges NO to produce peroxynitrite. Thus, simultaneously, a potent oxidative/nitritative agent is produced while available NO is reduced (51).

Increased ROS and specifically increased peroxynitrite oxidize tetrahydrobiopterin (BH4; Ref. 27), a vital cofactor for constitutive NOS. Absence of reduced BH4 uncouples NOS, causing it to produce superoxide and diverse ROS (26). Once begun, the process results in positive feedback for oxidative stress and reduction in NO resulting in endothelial dysfunction and defective regulatory nitrergic pathways that adversely affect myogenic tone in mesenteric artery and veins (2).

How Ascorbate Improves LFP

Ascorbic acid can interfere with this cycle of oxidative stress. At first it was thought to exert its primary effects on superoxide, but subsequent studies (3) have confirmed its ability to block lipid peroxidation. Ascorbate is a potent, perhaps the most potent, antioxidant against peroxynitrite-induced oxidation reactions (24), effectively quenching the nitrosation reaction (18) and restoring intracellular BH4 (19).

Mechanistic Predictions Based on the ROS Paradigm in LFP Patients

As proposed, the hypothetical pathophysiology of LFP depends on ANG II-induced superoxide formation, peroxynitrite generation, and uncoupling of NOS through the removal of BH4. The effects of ascorbic acid infusion and the cutaneous effects of ascorbate support this hypothesis. Potential benefit might derive from AT1R inhibition. To date, our pilot results using single dose losartan have been disappointing although chronic testing is underway and may yield different outcomes. The hypothesis might also indicate benefit from BH4 supplementation, which is also under consideration. However, if the primary defect is reduced ACE2, one wonders how that could be addressed. Decreased ACE2 expression occurs in heart failure and is normalized by exercise, which also decreases AT1R and increases NO synthesis (22). There is evidence that a vigorous program of exercise may improve POTS through mechanisms that are not yet clear (4). While exercise training...
clearly increases blood volume and while volume loading offers some palliative benefits in POTS, many of the pleitropic effects of exercise remain to be determined.

Clinical Perspective

Patients with LFP present with a consistent phenotype of circulatory insufficiency even while supine. This includes decreased CO, increased peripheral vasoconstriction, cutaneous pallor, and tachycardia. Venous capacitance is reduced presumably to accommodate this smaller blood volume. Ascorbate improves these abnormalities in all patients. The pallid, cold skin grows warm and ruddy. Improvement of the venous volume-pressure capacitance relation suggests that alterations are not due to remodeling but rather to vasoconstriction. Thus both arterial vasoconstriction and peripheral venoconstriction are enhanced in LFP and are remediated by ascorbate therapy. The literature suggests that vasoconstriction is mediated by reactive oxygen and reactive nitrogen species. Ascorbate appears to rapidly reverse vascular constriction in acute patients and may be proposed as a form of remedial therapy for LFP patients. However, enterically administered ascorbic acid is excreted in the urine nearly as rapidly as it is administered presenting a problem in obtaining plasma concentrations high enough to produce a vascular response. A blinded placebo control study of orally administered ascorbic acid that includes ascorbate pharmacokinetics may therefore be clinically important. Further experiments comparing the effects of ascorbic acid across POTS subgroups are also indicated.

Limitations

There may be selection bias in comparing POTS patients who have low calf blood flow measured by VOP, with control subjects who have a range of calf blood flows. Ideally, the most informative study design would be to perform the current experiments on all POTS patients identifying baseline flow (or “low flow” designation) as an a priori covariate and then compare with a group of controls (with a similar covariate). It has been suggested that we selected POTS patients simply as those with the lowest flow values and that lower flow per se dictates the response to ascorbate. To address this possibility, we compared control subjects with lower than average calf blood flow to control subjects with greater than average calf blood flow. There were no significant differences in hemodynamic results between control subgroups while POTS results were different from controls. The smallest calf blood flow for controls was 1.2 ml·kg⁻¹·min⁻¹ compared with the largest calf blood flow in LFP, which was 1.2 ml·kg⁻¹·min⁻¹. There was no overlap. Also, although our original early studies described three subtypes using calf blood flow to distinguish different subjects, then relative changes are thought to reflect true alterations in CO and TPR were consistent with changes in arm and calf blood flows, suggesting that similar phenomena may occur system wide.

We did not measure plasma ascorbate levels; however, we followed the same intravenous ascorbic acid protocol employed successfully by Jablonski et al. (20).

We did not directly measure NO, ROS, or reactive nitrogen species. We do have past experience with attempted direct measurements of NO in skin and in blood using the chemiluminescence method. Skin NO was highly variable and interpretable only by retaining nitrite and discarding nitrate. Blood NO was even more variable because of hemoglobin scavenging of NO. Cutaneous microdialysis is a verified surrogate for systemic responses, and the plateau phase of the local heat response is a well-defined bioassay for NO (23, 30). The objective of performing cutaneous microdialysis was to demonstrate that ascorbate increases the NO-dependent heating plateau and that therefore NO bioavailability was increased by ascorbate in LFP. We also did not measure ROS. This is difficult to accomplish in humans although attempts are underway. Our past data indicate ANG II excess (40, 43) and decreased NO bioavailability (41) in LFP, consistent with literature (11, 14) in which ANG II induced superoxide overproduction scavenges NO producing peroxynitrite that in turn decreases BH4 further reducing NO formation via NOS uncoupling (15).

Investigators as well as subjects were not blinded although limited blinding of subjects occurred during saline placebo administration. Absent blinding weakens our conclusions.

We studied mostly females without regard to menstrual cycle. The phase of the menstrual cycle can exert important effects on NO-dependent mechanisms. There is also evidence indicating differences in renin activity and serum aldosterone but not in CO, stroke volume, blood pressure, heart rate, or total peripheral resistance in the midluteal phase compared with the early follicular phase of the menstrual cycle in POTS (5).

LDF measures relative changes in skin blood flow and conductance. For example, if the maximum absolute conductance during sodium nitroprusside dialysis is similar for different subjects, then relative changes are thought to reflect true vascular properties. However, changes in the numbers of microvessels or their properties, differences in skin color, and ambient temperature fluctuations can all exert effects on the measured “maximum flow” such that two patients with similar CVC_max may have different vascular properties.

Cooling the skin to help subject comfort before insertion of the catheter may affect blood flow and CVC measurements.

Our studies of hemodynamics were technically limited. While VOP afforded us the ability to accurately estimate changes in venous and arterial properties in the regional forearm and calf circulations, and while impedance methods offered additional information concerning thoracic, pelvic, and splanchnic blood volume shifts, equivalent volume-pressure relations could not be obtained. Also, impedance measurements lack anatomic specificity regarding liver, intestinal, renal, and other hemodynamically important circulations. Generalizing from the specific to the general is risky. However, changes in CO and TPR were consistent with changes in arm and leg blood flows, suggesting that similar phenomena may occur system wide.
However, there is no way to penetrate the skin without causing a response of some sort. Thus, for example, the act of catheterization changes blood flow response characteristics. Any and all attempts to reduce the pain of catheterization that is necessary for ethical reasons also changes the blood flow. This issue has been addressed in a study by Hodges et al. (16) in which the investigators found that maximum conductance in response to whole body heating was not affected by ice or local anesthesia but was decreased if no form of pain reduction was used.

Finally, the model proposed while consistent with the data has therapeutic limitations. Large doses of intravenous ascorbic acid were administered and can easily exceed the blood concentration range (not measured in these experiments) achievable by oral administration of ascorbic acid. Ascorbate is typically excreted in the urine more rapidly than it can be absorbed from large enteric doses.

ACKNOWLEDGMENTS

We thank members of the New York Medical College Department of Pediatrics, especially its Chairman, Dr. Leonard Newman, and the Division of Pediatric Cardiology, especially its Director, Dr. Michael H. Gewitz, for unflagging support. We also acknowledge our intellectual debt to our mentors Dr. Thomas H. Hintze, Dr. Gabor Kaley, Dr. David Robertson, and Dr. Phillip Low for constant inspiration and stimulation.

GRANTS

This work was supported by the National Heart, Lung, and Blood Institute Grants 1-RO1-HL-074873, 1-RO1-HL-087803, and 1-F30-HL-097380.

DISCLOSURES

We have nothing to disclose concerning any potential conflict of interest (e.g., consultancies, stock ownership, equity interests, patent-licensing arrangements, lack of access to data, or lack of control of the decision to publish).

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