Protective effects of ascorbic acid on arterial hemodynamics during acute hyperglycemia

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Mullan, Brian A., Ciaran N. Ennis, Howard J. P. Fee, Ian S. Young, and David R. McCance. Protective effects of ascorbic acid on arterial hemodynamics during acute hyperglycemia. Am J Physiol Heart Circ Physiol 287: H1262–H1268, 2004.—Mortality increases when acute coronary syndromes are complicated by stress-induced hyperglycemia. Early pulse wave reflection can augment central aortic systolic blood pressure and increase left ventricular strain. Altered pulse wave reflection may contribute to the increase in cardiac risk during acute hyperglycemia. Chronic ascorbic acid (AA) supplementation has recently been shown to reduce pulse wave reflection in diabetes. We investigated the in vivo effects of acute hyperglycemia, with and without AA pretreatment, on pulse wave reflection and arterial hemodynamics. Healthy male volunteers were studied. Peripheral blood pressure (BP) was measured at the brachial artery, and the Sphygmocor pulse wave analysis system was used to derive central BP, the aortic augmentation index (AIx; measure of systemic arterial stiffness), and the time to pulse wave reflection (T r; measure of aortic distensibility) from noninvasively obtained radial artery pulse pressure (PP) waveforms. Hemodynamics were recorded at baseline and then every 30 min during a 120-min systemic hyperglycemic clamp (14 mmol/l). The subjects, studied on two separate occasions, were randomized in a double-blind, crossover manner to placebo or 2 g intravenous AA before the initiation of hyperglycemia. During hyperglycemia, AIx increased and T r decreased. Hyperglycemia did not change peripheral PP but did magnify central aortic PP and diminished the normal physiological amplification of PP from the aorta to the periphery. Pulse wave reflection, as assessed from peripheral pulse wave analysis, is enhanced during acute hyperglycemia. Pretreatment with AA prevented the hyperglycemia-induced hemodynamic changes. By protecting hemodynamics during acute hyperglycemia, AA may have therapeutic use.

HYPERGLYCEMIA is common in critically ill patients, even if they have not previously had diabetes (24, 27). The hyperglycemia is a manifestation of the neuroendocrine stress response and induced insulin resistance. Stress-induced hyperglycemia has been associated with increased mortality after myocardial infarction (5). Several in vivo experiments have reported impaired nitric oxide (NO)-mediated endothelial function during acute hyperglycemia (1, 3, 18, 40, 54). Endothelium-derived NO may play an important role in the functional regulation of arterial stiffness (9, 10, 25, 48, 53). Stiffening of the arterial tree increases the velocity and amplitude of pulse waves reflected from the periphery back to the heart (31). This results in larger reflected pressure waves reaching the ascending aorta earlier and augmenting the central aortic systolic blood pressure. Augmentation of central systolic pressure increases left ventricular workload and myocardial oxygen demand. Although the peripheral arterial hemodynamic effects of acute systemic hyperglycemia have been described (15, 21–23), the effects on pulse wave reflection and central aortic hemodynamics are unknown.

Acute hyperglycemia can generate oxidative stress (6). Hyperglycemia may also promote ascorbic acid deficiency in endothelial cells (35). Ascorbate is a potent free radical scavenger and regulator of intracellular redox state. Intra-arterial administration of ascorbic acid has been observed to improve NO-mediated endothelium-dependent vasodilatation during a forearm hyperglycemic clamp, in which 50% dextrose was infused locally into the brachial artery (3). The results from this study may not be entirely relevant to clinical practice. The locally induced hyperglycemia caused vasodilatation, which is not the normal physiological response to systemic hyperglycemia (15, 21–23). In addition, the study was not randomized, double-blind, or placebo-controlled, and the ascorbate levels attained locally in the brachial artery would be difficult to achieve with systemic administration. We have recently shown that chronic ascorbic acid supplementation can reduce blood pressure and arterial stiffness in Type 2 diabetes (28). However, individuals with Type 2 diabetes display dyslipidemia as well as hyperglycemia. The hemodynamic effects of ascorbic acid in Type 2 diabetes may therefore not be related to hyperglycemia. If acute hyperglycemia adversely affects pulse wave reflection, the induced change to central aortic hemodynamics may contribute to the increase in cardiac risk observed during stress-induced hyperglycemia. Using pulse wave analysis, based on a previously validated transfer function, we investigated the in vivo effects of acute systemic hyperglycemia on pressure wave reflection and arterial hemodynamics. In addition, we also investigated the potential for pretreatment with intravenous ascorbic acid to modify the vascular response to acute hyperglycemia.

METHODS

Subjects. Twelve healthy men (mean age 25.2 years, range 20–34 years) were recruited from the staff of the Royal Victoria Hospital, Belfast, Northern Ireland. The study was approved by the Research Ethics Committee of the Queen’s University Belfast, and all individuals gave written informed consent. Exclusion criteria included smoking, heart disease, hypertension, diabetes mellitus, dyslipidemia, and renal disease. None of the subjects were receiving medication or vitamin supplements.

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Protocol. The vascular effects of acute systemic hyperglycemia, with and without intravenous ascorbic acid pretreatment, were studied using a randomized, double-blind, placebo-controlled, crossover trial design. Subjects were studied on two occasions, 2–3 wk apart. All the experiments were performed in the morning in an undisturbed, temperature-controlled environment (22–24°C, within ±0.5°C during each study). Each individual fasted from 22.00 hours the night before and abstained from ingestion of alcohol or caffeine-containing beverages. Subjects were placed in a comfortable supine position during the studies. Intravenous cannulae were placed in the left arm for the collection of blood samples, for the infusion of octreotide, and for the infusion of 20% dextrose during the hyperglycemic clamp. Octreotide (Sandoz Pharmaceuticals; Surrey, UK) was given at the start of each study, as a 25-mcg intravenous bolus, followed by a 0.5-mcg/min intravenous infusion, to maintain endogenous insulin at basal levels throughout and to simulate the insulin resistance associated with stress-induced hyperglycemia (3, 21–23, 54).

Blood pressure and heart rate were measured with an Omron HEM-705CP oscillometric sphygmomanometer. The SphygmoCor (model SCOR-Px, PWV Medical; Sydney, Australia) was used for pulse wave analysis. All measurements were made in triplicate and averaged. Hemodynamics and pulse wave analysis were recorded after 60 min of supine rest (baseline, euglycemia). Individuals were then randomized, in a double-blind fashion, to 2 g iv ascorbic acid or matching placebo. The alternative treatment was given on the next study day. After the subjects received ascorbic acid/placebo, blood glucose was acutely raised to 14 mmol/l using a 0.3-g/kg intravenous bolus of 20% dextrose. Venous blood samples were obtained every 5 min, and a variable dextrose infusion maintained the blood glucose at the desired level. Hemodynamic measurements and pulse wave analysis were repeated every 30 min for 120 min of induced hyperglycemia. A subgroup of eight volunteers participated in a control study in which an equal volume of saline was infused instead of glucose.

Pulse wave analysis. The SphygmoCor pulse wave analysis system relies on the noninvasive technique of application tonometry to record arterial pressure waveforms. The waveforms obtained by this method have previously been validated by comparing them with waveforms obtained by a high-fidelity transducer within an artery (19). During the study, the radial artery of the right arm was used for assessment. Data were collected directly into a portable computer, and an averaged peripheral pressure waveform was automatically generated from 20 sequential waveforms using the integral software provided (SCOR-2000, version 6.2). The radial waveform was calibrated according to sphygmanometric pressure measured in the brachial artery under the valid assumptions that mean pressure is similar throughout the arterial tree and that diastolic pressure is similar between brachial and radial artery (31). A validated transfer function (8, 17, 33, 39) enabled the software to generate the corresponding central (ascending aortic) pressure waveform. From this waveform central pressure, augmentation, augmentation index (AIx), and the timing of the reflected pressure wave could be determined. The features of the central aortic pressure waveform are illustrated in Fig. 1. Augmentation was defined as the difference between the second systolic peak (caused by wave reflection) and the first systolic peak (caused by left ventricular ejection). The AIx was this difference expressed as a percentage of the central pulse pressure. The index was used as a measure of overall systemic arterial stiffness (4, 38, 45, 46, 50). Pilot data from our unit allowed us to estimate reproducibility. Because AIx varies around zero, the data were analyzed using Bland-Altman plots rather than coefficients of variation (CVs). In 54 subjects, aged 21–66 yr, diabetic and nondiabetic, the mean ± SD of the difference between repeated measurements was 0.2 ± 1.7%. This compares favorably with other published studies (13, 37, 47). The time to wave reflection (T_r) was the time between the foot of the pressure wave and the inflection point on the central pressure waveform. T_r represented the time taken for the pulse pressure wave to reach the first main reflectance site (the aortic bifurcation) and then return to the ascending aorta. T_r was used to indirectly assess aortic pulse wave velocity and, hence, aortic stiffness (20, 29, 49).

Laboratory analyses. Whole blood glucose was measured at the bedside by means of a reflectance glucometer. Blood was also sent to the hospital biochemistry laboratory for determination of plasma glucose. Plasma insulin was determined by radioimmunoassay, and plasma ascorbic acid was measured from samples of EDTA-anticoagulated blood using the technique described by Vuillemin and Keck (43). At a mean concentration of 4.2 μmol/l, the interassay CV was 6.80%, and at 50.6 μmol/l, the interassay CV was 0.72%, whereas at 151.0 μmol/l, the interassay CV was 1.50%.

Statistical analysis. Results were analyzed with the SPSS statistical package (SPSS; Chicago, IL). Data are reported as means ± SD unless otherwise stated. Changes in brachial and central aortic hemodynamics during hyperglycemia were assessed within groups by one-way repeated-measures ANOVA. Two-way repeated-measures ANOVA compared the effects of ascorbic acid with placebo. If differences reached statistical significance, post hoc analyses with a two-tailed paired t-test was used to assess differences at individual time periods in the study with a Bonferroni correction for multiple comparisons. Two-sided P values <0.05 were considered to indicate statistical significance. The study had 90% power at the 5% level of significance to detect a between treatment difference of ≥6.7 mmHg for mean arterial pressure, ≥8.9% for AIx, and ≥17.9 ms for T_r.

RESULTS

The baseline clinical characteristics of the 12 study subjects are shown in Table 1. All subjects completed both parts of the study. Plasma glucose stabilized at 14 mmol/l during the systemic hyperglycemic clamp, and octreotide successfully maintained plasma insulin at basal concentrations throughout (Fig. 2). When subjects were pretreated with placebo, 120 min of hyperglycemia caused the plasma ascorbate concentration to decrease from 44.0 ± 15.2 to 37.3 ± 13.5 μmol/l (P < 0.0001). As expected, pretreatment with intravenous ascorbic acid resulted in a higher plasma ascorbate concentration at 120 min (134.2 ± 27.0 μmol/l, P < 0.0001). Ascorbic acid had no effect on the volume of dextrose infused to maintain plasma glucose at the desired concentration. Equal volumes of intravenous fluid were infused during the placebo and ascorbic acid limbs of the study.

Within 30 min of induced hyperglycemia, brachial systolic blood pressure, brachial diastolic blood pressure, and mean arterial blood pressure increased significantly from their baseline values (Table 2). The magnitude of these changes was greatest at 120 min. Ascorbic acid significantly attenuated the
peripheral arterial hemodynamic responses to hyperglycemia (Table 2). When subjects were pretreated with ascorbic acid, the only statistically significant change in brachial blood pressure occurred at 120 min. Acute hyperglycemia had no effect on heart rate or peripheral pulse pressure.

The effects of hyperglycemia, with and without ascorbic acid, on the central aortic hemodynamics derived from peripheral pulse wave analysis are shown in Table 3. After 30 min of acute systemic hyperglycemia, aortic augmentation, aortic systolic pressure, aortic diastolic pressure, and central pulse pressure all increased and continued to increase by 120 min. Hyperglycemia diminished the normal physiological amplification of pulse pressure from the central aorta to the brachial artery. Ascorbic acid prevented the changes to aortic augmentation, central pulse pressure, and pulse pressure amplification. Only aortic systolic and diastolic pressures showed slight increases at 120 min. The additional adverse effects of hyperglycemia on pulse wave reflection caused aortic systolic pressure to increase more than brachial systolic pressure (Fig. 3). Ascorbic acid was therefore most protective of central aortic hemodynamics.

The aortic AIx (measure of systemic arterial stiffness) rose significantly during hyperglycemia (Fig. 4). This did not occur when subjects were pretreated with ascorbic acid. $T_r$ (measure of aortic distensibility) shortened with hyperglycemia, a response also prevented by ascorbic acid pretreatment (Fig. 5). The change in plasma ascorbate concentration after 120 min of hyperglycemia correlated with the changes in mean arterial pressure ($r = -0.578, P = 0.004$), AIx ($r = -0.734, P < 0.001$), and $T_r$ ($r = 0.466, P = 0.025$).

Peripheral arterial hemodynamics, central aortic hemodynamics, AIx, and $T_r$ did not change with time during the control study, in which an equal volume of saline was infused instead of dextrose (data not shown).

**DISCUSSION**

This study confirms previous evidence that acute systemic hyperglycemia can increase peripheral arterial blood pressure (15, 21–23). However, to our knowledge, this is the first report on the in vivo effects of hyperglycemia on pulse wave morphology and central aortic hemodynamics using peripheral pulse wave analysis based on a previously validated transfer function. We have shown through pulse wave analysis that hyperglycemia-induced increases in brachial systolic pressure are exaggerated in the central ascending aorta (Fig. 3). This is because hyperglycemia also affects pulse wave reflection (Figs. 4 and 5). As it is aortic pressure, and not brachial pressure, that determines left ventricular workload, one can see that conventional techniques, which measure blood pressure at peripheral sites, underestimate the adverse effects of hyperglycemia on the heart. Although mean arterial pressure remains nearly constant along the arterial tree, pulse pressure increases markedly from central to peripheral arteries (31). When the arterial tree becomes stiffer, as occurs during ageing or in cardiovascular disease, pulse wave velocity increases. Reflected pressure waves then reach the ascending aorta earlier and augment central systolic blood pressure. This increases left ventricular workload and myocardial oxygen demand. The amplification of pulse pressure along the arterial tree is also reduced. We have shown that acute hyperglycemia, in young healthy individuals, can magnify central pulse pressure and diminish the normal physiological amplification of pulse pressure from the aorta to the periphery (Fig. 5). Increased central pulse pressure and reduced pulse pressure amplification have recently been reported to be strong independent predictors of cardiovascular mortality (36).

Pretreatment with a 2-g intravenous bolus of ascorbic acid attenuated, or completely prevented, the adverse effects of acute systemic hyperglycemia on peripheral arterial and central aortic hemodynamics (Tables 2 and 3). Ascorbic acid’s beneficial cardiovascular effects were most evident centrally. Ascorbic acid has been shown to reverse impaired endothelium-dependent NO-mediated vasodilatation in a number of conditions, including acute hyperglycemia induced locally in the brachial artery (3). Endothelium-derived NO is thought to have an important role in the functional regulation of arterial stiffness (9, 10, 25, 48, 53). The preservation of endothelial NO bioactivity, by ascorbic acid, may explain our observed findings. Proposed mechanisms include reduced NO degradation by free radicals (16), increased endothelial NO synthase activity (2), or augmented vascular smooth muscle sensitivity to NO.
Acute treatment with ascorbic acid may reduce cardiac high-dose ascorbic acid can attenuate these changes (Tables 2
namic changes can occur within 30 min of hyperglycemia, and experiment has shown that peripheral and central hemody-
24 h to achieve normoglycemia in such patients (41). This
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creased mortality after myocardial infarction (5). Adverse
plasma ascorbate.
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may promote ascorbic acid de
(30). A recent review article (35) suggested that hyperglycemia
on central aortic hemodynamics
Table 2. Effects of acute systemic hyperglycemia, with or without ascorbic acid pretreatment, on peripheral arterial hemodynamics

<table>
<thead>
<tr>
<th>Hemodynamic Variable</th>
<th>Time</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial systolic pressure, mmHg</td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>107.0±9.5</td>
<td>113.4±9.4*</td>
</tr>
<tr>
<td>Hyperglycemia + ascorbic acid</td>
<td>108.1±10.5</td>
<td>109.6±10.1</td>
</tr>
<tr>
<td>Brachial diastolic pressure, mmHg</td>
<td>59.4±5.9</td>
<td>62.8±7.5*</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>58.5±6.7</td>
<td>59.9±6.0</td>
</tr>
<tr>
<td>Hyperglycemia + ascorbic acid</td>
<td>49.5±9.9</td>
<td>49.7±9.2</td>
</tr>
<tr>
<td>Peripheral pulse pressure, mmHg</td>
<td>47.6±7.6</td>
<td>50.6±7.1</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>74.0±6.7</td>
<td>79.5±8.3*</td>
</tr>
<tr>
<td>Hyperglycemia + ascorbic acid</td>
<td>74.1±7.7</td>
<td>75.0±6.8</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>57.0±5.6</td>
<td>54.8±7.0</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>57.0±6.1</td>
<td>58.2±6.5</td>
</tr>
<tr>
<td>Hyperglycemia + ascorbic acid</td>
<td>57.0±6.1</td>
<td>58.2±6.5</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. baseline values (time = 0 min).

Stress-induced hyperglycemia has been associated with increased mortality after myocardial infarction (5). Adverse effects on central aortic hemodynamics may partly explain this phenomenon. Strict glycemic control is difficult during acute illness. Even with intensive insulin therapy, it may take up to 24 h to achieve normoglycemia in such patients (41). This experiment has shown that peripheral and central hemodynamic changes can occur within 30 min of hyperglycemia, and high-dose ascorbic acid can attenuate these changes (Tables 2 and 3). Acute treatment with ascorbic acid may reduce cardiac workload and potentially improve outcome in acute coronary syndromes complicated by stress-induced hyperglycemia. Further clinical investigation is warranted to test this hypothesis. Study limitations. Radial artery pulse wave analysis to determine central aortic hemodynamics has been described in numerous publications. The accuracy of the radial-to-aortic transfer function has, however, been questioned (12, 14). Most of the reported discrepancies have been for the derivation of the aortic AIx. The transfer function shows greater between subject variability at higher frequencies and is therefore less likely to provide an accurate estimate of aortic AIx than of aortic blood pressure. If anything, the aortic AIx is underestimated by using a radial-to-aortic transfer function (8, 26). Close agreement between transfer function-derived and invasively measured central blood pressures has been observed in many validation studies (8, 17, 26, 33). Unfortunately, a recent study by Davies et al. (11) reported a significant bias between derived and measured aortic pressures. The discrepancy in this study may have been due to the fact that the authors calibrated

Table 3. Effects of acute systemic hyperglycemia, with or without ascorbic acid pretreatment, on central aortic hemodynamics

<table>
<thead>
<tr>
<th>Hemodynamic Variable</th>
<th>Time</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic augmentation, mmHg</td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>3.2±2.6</td>
<td>6.6±3.1†</td>
</tr>
<tr>
<td>Hyperglycemia + ascorbic acid</td>
<td>2.9±3.9</td>
<td>2.6±4.4</td>
</tr>
<tr>
<td>Aortic systolic pressure, mmHg</td>
<td>92.1±6.3</td>
<td>99.8±7.4†</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>93.5±8.6</td>
<td>94.7±8.0</td>
</tr>
<tr>
<td>Hyperglycemia + ascorbic acid</td>
<td>60.0±6.3</td>
<td>63.7±7.9*</td>
</tr>
<tr>
<td>Aortic diastolic pressure, mmHg</td>
<td>59.2±6.9</td>
<td>60.1±6.5</td>
</tr>
<tr>
<td>Central pulse pressure, mmHg</td>
<td>32.1±4.5</td>
<td>36.2±3.5†</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>34.3±6.3</td>
<td>34.6±6.3</td>
</tr>
<tr>
<td>Hyperglycemia + ascorbic acid</td>
<td>148.7±15.5</td>
<td>139.9±13.5†</td>
</tr>
<tr>
<td>Peripheral/central pulse pressure, %</td>
<td>144.8±16.5</td>
<td>144.5±18.4</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. baseline values (time = 0 min).
Acute administration of ascorbic acid has previously been reported to lower AIx in healthy volunteers (51, 52). Thus it could be argued that the ascorbic acid given in this study simply masked the hemodynamic effects of hyperglycemia, by lowering AIx, rather than preventing the hyperglycemia-induced rise. However, we also showed that ascorbic acid could significantly attenuate the increase in blood pressure during hyperglycemia. Because ascorbic acid does not have hypotensive properties in healthy, disease-free individuals (7, 32, 34, 51, 52), it is therefore evident that ascorbic acid is not masking hyperglycemia-induced hemodynamic changes but is instead preventing the changes.

The octreotide was used to maintain plasma insulin at basal concentrations throughout the study. Extremely high insulin levels would have developed otherwise. Supraphysiological concentrations of insulin can directly stimulate the sympathetic system but may also cause NO-mediated vasodilatation (9, 45). Hyperinsulinemia would therefore have confounded the hemodynamic effects of hyperglycemia. Insulin resistance can occur during critical illness and may be a feature of stress-induced hyperglycemia (42, 44). By preventing an increase in plasma insulin concentration during induced hyperglycemia, octreotide artificially simulated the clinical effects of insulin resistance. The octreotide was always given at the start of each study. Baseline hemodynamics were only recorded after 60 min of supine rest with concomitant octreotide infusion. In addition to this, no octreotide-induced hemodynamic effects were observed in the control saline study.

In conclusion, we have shown that acute systemic hyperglycemia (14 mmol/l) can, within a short period of time (30 min), modify directly measured brachial artery hemodynamics and derived ascending aortic hemodynamics. The central hemodynamic variables were calculated from peripheral pulse wave analysis data and a previously validated transfer function. The hemodynamic changes observed during hyperglycemia included increased mean arterial blood pressure, increased aortic AIx (measure of systemic arterial stiffness), reduced $T_e$ (measure of aortic distensibility), increased central pulse pressure, and diminished amplification of pulse pressure from the aorta to the brachial artery. Hyperglycemia increased central aortic systolic pressure significantly more than peripheral arterial systolic pressure. Measurement of blood pressure at peripheral sites, such as the brachial artery, may therefore underestimate...
the adverse effects of hyperglycemia on the heart. Pretreatment with an intravenous bolus of ascorbic acid can protect the vasculature from these adverse effects. Acute treatment with ascorbic acid may help to reduce cardiac risk in acutely ill patients with stress-induced hyperglycemia. This has not been examined before and should be investigated further.

GRANTS

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REFERENCES


