Biochemical and Molecular Actions of Nutrients

Vitamin C Deficiency Exerts Paradoxical Cardiovascular Effects in Osteogenic Disorder Shionogi (ODS) Rats

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ABSTRACT Vitamin C is considered to be a very efficient water-soluble antioxidant, for which several new cardiovascular properties were recently described. The aim of this study was to determine in vivo the effects of a severe depletion of vitamin C on cardiac and vascular variables and reperfusion arrhythmias. For this purpose, we used a mutant strain of Wistar rats, osteogenic disorder Shionogi (ODS). After 15 d of consuming a vitamin C–deficient diet, ODS rats had a 90% decrease in plasma and tissue levels of ascorbate compared with ODS vitamin C–supplemented rats and normal Wistar rats. However, plasma antioxidant capacity, proteins, α-tocopherol, urate, catecholamines, lipids, and nitrate were not influenced by the vitamin C deficiency in ODS rats. Moreover, there was no difference between ODS vitamin C–deficient and –supplemented rats in heart rate and arterial pressure. After 5 min of an in vivo regional myocardial ischemia, various severe arrhythmias were observed, but their intensities were not modified by vitamin C in vitamin C–deficient ODS rats. The vascular reactivity, measured in vitro on thoracic arteries, was not altered by ascorbate deficiency in ODS rats. These unexpected results suggest that unidentified compensatory mechanisms play a role in maintaining normal cardiac function and vascular reactivity in vitamin C–deficient rats.

KEY WORDS: • ascorbic acid • myocardial ischemia-reperfusion • arrhythmia • oxidative stress • ODS rats

Ascorbic acid (vitamin C) is considered to be a pleiotropic “old” molecule; a deficiency of this vitamin is associated with the development of scurvy. Ascorbate, its major form at physiological pH, is the essential cofactor of several hydroxylases such as proline-hydroxylation and dopamine β-hydroxylase (1). Moreover, due to its remarkable redox properties, ascorbate is generally considered to be one of the most efficient antioxidants in biological fluids (2). In the past decade, knowledge about the role of ascorbate in the cardiovascular domain has expanded dramatically, and new properties of this molecule have been discovered, opening the way for a wide range of potential therapeutic uses. For instance, vitamin C was proposed to augment cardiac function (3), enhance cardioprotection during myocardial reperfusion (4), inhibit endothelial cell apoptosis in congestive heart failure (5), lower diastolic blood pressure (6), and enhance endothelium-dependent vasorelaxation (7–13).

Recently, using a mutant strain of Wistar rats, osteogenic disorder Shionogi (ODS)3 (14,15), which lack the key enzyme for ascorbate synthesis, we investigated in vitro the effects of lowering ascorbate cardiac tissue levels on the ability of the heart to recover from an ischemia-reperfusion injury (16). In this pathological situation, in which free radicals are produced and exert detrimental effects, some antioxidant therapies were shown to lessen the extent of the injury (17). Paradoxically, we observed that ascorbate-deficient hearts recovered better from the ischemic insult during reperfusion, and released the same amount of radical species as hearts from control Wistar rats (16). Therefore, because some antioxidant proteins were upregulated in hearts of ascorbate-deficient rats, we propose that the absence of only one antioxidant, even as essential as vitamin C, was compensated by other protective mechanisms that act together to protect the heart from the oxidative stress subsequent to ischemia reperfusion. However, some unanswered questions were raised by this study (16), especially the behavior of the heart during this oxidative stress–generating situation in vivo.

Therefore, we designed this study to evaluate in vivo the incidence of lowering plasma and tissue levels of vitamin C on cardiovascular variables in rats, before, during and after an ischemia-reperfusion sequence, realized through the ligation of the left anterior descending (LAD) coronary artery. Moreover, we assessed in vitro the vascular reactivity of ascorbate-deficient cardiac blood pressure; SNAP, S-nitroso-N-acetyl-penicillamine; VPB, ventricular premature beats; VT, ventricular tachycardia; VF, ventricular fibrillation.
cient arteries. The conditions of the ascorbate deficiency were arranged in such a way that only vitamin C, and no other component that could influence cardiovascular variables, was affected by the diet in ODS rats. Therefore, ODS vitamin C–deficient or –supplemented rats were used and compared with Wistar rats because the last-mentioned is generally a reference strain in such studies.

MATERIALS AND METHODS

Chemicals. All drugs and chemicals used were purchased from Sigma-Aldrich Chimie: indomethacin, L-N^G-nitro arginine methyl ester (L-NAME), acetylcholine chloride, papaverine, S-nitroso-N-acetyl-penicillamine (SNAP), and phenylephrine.

Animals and diets. The investigators complied with authorization 21CAE024 from the French government, ensuring respect of the agreement with the Guidelines for the Protection of Experimental Animals issued by European Convention.

Male ODS rats (10 wk old) were purchased from CLEA Laboratories and 10-wk-old Wistar rats were purchased from INRA-CREDO. All rats were fed a purified diet (INRA-UPAE) containing 65% of total energy as carbohydrate, 23.4% as casein, and 11.6% as vegetable fat (Table 1), to which a known amount of L-ascorbic acid had been added. Two diets were used, a vitamin C–deficient diet (0 mg/kg) and a vitamin C–supplemented diet (800 mg/kg). Two weeks before the beginning of the experiment, the usual diet was changed to the vitamin C–deficient or –supplemented diet. Wistar rats were fed the vitamin C–deficient diet (n = 15), and ODS rats were fed either the vitamin C–supplemented (n = 15) or the vitamin C–deficient (n = 15) diet. Normal Wistar rats do not require a dietary source of ascorbic acid.

Surgeal preparation. The rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and cannulated for carrying out the in vivo ischemia-reperfusion protocol, as described before (18). Functional cardiovascular variables were measured: diastolic and systolic arterial blood pressure (DAP and SAP), pulse pressure (SAP–DAP); mean arterial pressure (MAP); and heart rate (HR). The electrocardiogram (D derivation) was taken via electrodes placed on the anterior and left posterior paws and recorded on the polygraph. Rhythm disturbances such as ventricular premature beats (VPB), ventricular tachycardia (VT), and fibrillation (VF) were quantified. Briefly, a ligature was placed around the LAD coronary artery to cause a regional ischemia for 5 min. The occluding ligature was then released and the ischemic area was reperfused for 15 min.

Criteria of arrhythmias and death. Classification of arrhythmias was carried out according to the Lambeth Conventions (19) with slight modifications: VPB were defined as discrete and identifiable premature QRS complexes, VT as a run of ≥5 consecutive VPB, and VF as a signal for which individual QRS deflections can no longer be distinguished from one another and for which a rate can no longer be measured. When VP-induced hypotension (<20 mmHg) lasted for >2 min, the rat was considered to be dead. Irreversible ventricular fibrillation was the unique cause of death.

At the end of the stabilization phase, certain criteria led to the exclusion of the rat from the protocol: MAP < 70 mmHg or HR < 300 beats/min. The absence of any reduction in MAP and of ST segment elevation during ischemia was also a specific criterion for exclusion. The final number of rats in each group is given in the tables and figures.

Plasma and tissue processing. After anesthesia, 1 mL of arterial blood was collected from the carotid artery and replaced with an equal volume of 9 g/L NaCl to evaluate the antioxidant capacity and the catecholamine and ascorbic acid concentrations in fresh plasma. At the end of the experimental procedure, total blood was withdrawn from the aorta, and the plasma was divided into aliquots and stored at −70°C until analysis. For vitamin C measurements, 2 volumes of 5% metaphosphoric acid solution were added to plasma samples before freezing and storage.

The thoracic aortas were rapidly removed and maintained in cold (4°C) Krebs-Henseleit solution (concentration mmol/L: NaCl, 116.3; KCl, 5.4; CaCl2, 1.8; Na2HPO4, 1.04; MgSO4, 0.83; NaHCO3, 19; and glucose, 5.5; pH 7.4) to carry out contractility experiments. The heart was rapidly removed and rinsed with saline solution. The atria were excised and the remainder of the heart was separated into septum left and right ventricles, weighed and instantaneously frozen, crushed in liquid nitrogen, and kept at −70°C until use. After thawing, the hearts were homogenized in 5 volumes of 50 mmol/L phosphate buffer (pH 7.4, 0.1 mmol/L EDTA) and used for vitamin C, vitamin E, and protein determination. For vitamin C, 2 volumes of a 5% metaphosphoric acid solution were added to samples before freezing and storage. The atrial glands were excised, weighed, and frozen in liquid nitrogen to measure the catecholamine content.

Preparation of the aortic rings and functional procedure. The thoracic aortas were cleaned of connective tissues, cut into 3-mm rings and suspended in organ chambers as described earlier (20).

In the first series of experiments, once the precontraction to phenylephrine had reached a plateau value, the aortic rings were relaxed by cumulative additions of acetylcholine (0.003–30 μmol/L) or by the NO donor SNAP (0.003–30 μmol/L). In the second series of experiments, the involvement of nitric oxide in the vasodilator response to acetylcholine was determined by incubating arterial rings for 30 min with the NO synthase inhibitor L-NAME (100 μmol/L) before contraction.

The effects of agonists were expressed as a percentage of maximal relaxation induced with papaverine (100 μmol/L) added to the bath at the end of the experiment, as previously described (20). pD3 values correspond to the negative logarithm of the concentration required to produce a half-maximum relaxing effect, and they reflect the sensitivity of arterial segments to the various compounds. This value was derived from log-logit transformation of individual concentration-response curves. L-NAME, acetylcholine, phenylephrine, KCl and papaverine were prepared in distilled water. Drug concentrations are expressed as final bath concentrations.

Biochemical assays. Ascorbic and dehydroascorbic acid concentrations in the plasma and heart homogenates were measured by HPLC using fluorimetric detection at 360 nm excitation and 440 nm emission, as previously described (21). The level of vitamin E (α-tocopherol) in the plasma was determined by a method derived from Lehman and Martin (22). Uric acid concentration in the plasma was determined using the SIGMA enzymatic procedure (kit 686 A, Sigma Diagnostics, France) using uricase and peroxidase. Cardiac protein levels were determined according to the method of Lowry et al. (23). Cholesterol and triglycerides were measured using a colorimetric assay (Dimensions X-Pand). Total nitrites were determined in plasma after deproteinization (Milipore Ultrafree UFC4 BTK25) with a Griess reaction, using the kit provided by Boehringer-Mannheim (kit BM 905 958). Levels of catecholamines [epinephrine (E) and norepinephrine (NE)] in the

<table>
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<tr>
<th>TABLE 1</th>
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<td>Composition of the experimental diets</td>
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<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg</th>
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<tbody>
<tr>
<td>Casein</td>
<td>23</td>
</tr>
<tr>
<td>α-Methionine</td>
<td>1</td>
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<tr>
<td>Cornstarch</td>
<td>379</td>
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<tr>
<td>Sucrose</td>
<td>260</td>
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<tr>
<td>Corn oil</td>
<td>50</td>
</tr>
<tr>
<td>Cellulose</td>
<td>25</td>
</tr>
<tr>
<td>L-Ascorbic acid</td>
<td>0 or 0.8</td>
</tr>
<tr>
<td>Vitamin mixture¹</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mixture²</td>
<td>45</td>
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</tbody>
</table>

¹ Vitamin mixture (g/kg diet): thiamin-HCl, 1 g; riboflavin, 1 g; nicotinic acid 4.5 g; calcium p-pantothenate, 3 g; pyridoxine-HCl 1 g; folic acid, 200 mg; cyanocobalamin, 1.35 mg; retinyl palmitate, 500,000 IU; α-tocopherol, 5000 IU; cholecalciferol, 250,000 IU; menadione, 100 mg; biotin, 5 g; inositol 5 g; α-paminobenzoic acid, 5 g; choline, 75 g.

² Mineral mixture (g/kg diet): Ca, 7.1 g; K, 4.7 g; Na, 1.2 g; Mg, 0.95 g; Zn, 82 mg; Fe, 78 mg; Mn, 67 mg; Cu, 11 mg; Cr, 2 mg; Co, 0.5 mg; P, 4.9 g; Cl, 1.8 g; S, 0.65 g; F, 15 mg; I, 1.5 mg; Se, 0.4 mg; Mo, 0.5 mg.
plasma and adrenal gland were determined by HPLC with amperometric detection as described (24).

**Determination of oxygen radical absorbance capacity.** Oxygen radical absorbance capacity (ORAC) of the plasma was determined according to a modified method of Cao et al. (25), which was described in detail elsewhere (26). Briefly, the reaction mixture contained a final concentration of 37.5 nmol/L β-allylphycocyanin in 75 mmol/L phosphate buffer, pH 7.0, at 37°C in the presence or the absence of Trolox or diluted plasma (dilution 1:500). The reaction was initiated by the introduction of 3 mmol/L of 2,2′-azobis(2-amidinopropane) 4-hydrochloride, and followed spectrophotometrically by the decrease in fluorescence at 598 nm excitation and 615 nm emission. Trolox was used as a reference antioxidant for calculating the ORAC values, with 1 ORAC unit defined as the net protection area provided by 1 μmol/L final concentration of Trolox.

**Statistical analysis.** All data are presented as means ± SEM, and tests were performed with α = 0.050. For biochemical measurements, data were evaluated by 1-way ANOVA followed by intergroup pairwise multiple comparisons using Tukey’s test (plasma vitamin C, vitamin E, lipids and uric acid) or Dunn’s method (heart vitamin C and adrenal gland catecholamines), comparing only Wistar rats with ODS vitamin C-supplemented rats, and ODS vitamin C-supplemented with ODS vitamin C–deficient rats. For the rhythm disturbances, data were analyzed with a Mann-Whitney Rank Sum Test. For the evolution of heart rate and mean arterial blood pressure, a 1-way ANOVA for repeated measures followed by Dunn’s test intergroup comparisons was used. Relaxation was expressed as a percentage of maximum relaxation induced by 0.1 mmol/L papaverine and presented as means ± SEM. Differences between groups were determined by ANOVA for repeated measures followed by the Bonferroni corrected t test.

**RESULTS**

**Biochemical variables.** The body weight of the ODS rats was less than that of Wistar rats (Fig. 1), but was not modified by the 15-d vitamin C–deficient diet.

In blood samples taken from anesthetized rats before ischemia and reperfusion, ODS rats fed the 800 mg/kg vitamin C–supplemented diet had plasma and tissue levels of vitamin C that did not differ from those in Wistar rats fed the vitamin C–deficient diet (Table 2). However, after 15 d of consuming the vitamin C–deficient diet, the plasma vitamin C levels of ODS rats dropped dramatically, falling to 10% of the levels in the two other groups (P < 0.001). Wistar rats and ODS vitamin C–supplemented rats had heart vitamin C concentrations that did not differ, whereas ODS vitamin C–deficient rats had a 90% lower level (P < 0.001).

The plasma total catecholamine levels (E + NE) did not differ among the three diet groups (Fig. 1). However, NE in blood samples increased significantly in the ODS group compared to Wistar rats (P < 0.001). The plasma uric acid levels in ODS rats fed the vitamin C–deficient diet were significantly lower than those in Wistar rats (P < 0.001). For plasma triglyceride, no significant differences were noted among the groups (P = 0.058).

![Graph](https://example.com/graph.png)

**FIGURE 1** Body weights of Wistar rats fed a vitamin C–deficient diet and ODS rats fed a vitamin C–supplemented diet or a vitamin C–deficient diet for 2 wk. Values are means ± SEM. Means at a time without a common letter differ, P < 0.001.

**TABLE 2**

<table>
<thead>
<tr>
<th>Diet vitamin C</th>
<th>ODS 800 mg/kg</th>
<th>ODS 0 mg/kg</th>
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<tr>
<td></td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Total vitamin C (AH− + DHA)</td>
<td></td>
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<tr>
<td>Plasma, μmol/L</td>
<td>13.12 ± 0.82a</td>
<td>14.57 ± 0.59a</td>
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<tr>
<td>% AH−</td>
<td>63.6</td>
<td>52.0</td>
</tr>
<tr>
<td>Heart, nmol/g</td>
<td>283.9 ± 26.9a</td>
<td>275.2 ± 10.3a</td>
</tr>
<tr>
<td>Catecholamines</td>
<td></td>
<td></td>
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<tr>
<td>Plasma, ng/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>62.40 ± 10.85</td>
<td>94.80 ± 17.37</td>
</tr>
<tr>
<td>NE</td>
<td>292.8 ± 53.4</td>
<td>461.2 ± 70.4</td>
</tr>
<tr>
<td>Total</td>
<td>355.2 ± 56.7</td>
<td>548.0 ± 71.4</td>
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<td>Adrenal gland, pg/g</td>
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<tr>
<td>E</td>
<td>238.5 ± 17.8a</td>
<td>158.0 ± 5.2b</td>
</tr>
<tr>
<td>NE</td>
<td>12.32 ± 3.05a</td>
<td>4.67 ± 0.72b</td>
</tr>
<tr>
<td>Total</td>
<td>250.9 ± 19.4a</td>
<td>162.7 ± 5.4b</td>
</tr>
<tr>
<td>Vitamin E, μmol/L</td>
<td></td>
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<tr>
<td>Total</td>
<td>19.52 ± 1.05a</td>
<td>15.64 ± 0.65b</td>
</tr>
<tr>
<td>Cholesterol, μmol/L</td>
<td></td>
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<tr>
<td>Triglyceride, μmol/L</td>
<td></td>
<td></td>
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<tr>
<td>Uric acid, μmol/L</td>
<td>55 ± 8b</td>
<td>86 ± 10a</td>
</tr>
<tr>
<td>Protein, g/L</td>
<td>126.8 ± 15.8b</td>
<td>364.7 ± 38.8a</td>
</tr>
<tr>
<td>Nitrite + nitrate, μmol/L</td>
<td>5.70 ± 3.56</td>
<td>59.60 ± 1.58</td>
</tr>
<tr>
<td>Plasma ORAC value, AU</td>
<td>2.63 ± 0.21</td>
<td>2.16 ± 0.07</td>
</tr>
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</table>

1 Values are means ± SEM. Means in a row without a common letter differ; for total vitamin C and plasma uric acid, P < 0.001; for tissue catecholamines and plasma vitamin E, P < 0.01; for plasma triglyceride, P < 0.05.
2 Abbreviations: DHA, dehydroascorbate; AH−; ascorbate; AU; arbitrary units.
differ between Wistar and ODS rats (Table 2). In contrast, in the adrenal gland, E and NE levels were lower in ODS than in Wistar rats, but there was no difference between Vitamin C–supplemented or –deficient ODS rats.

Plasma α-tocopherol levels were lower in ODS rats compared with Wistar rats ($P < 0.01$), but were not modified in ODS rats by 15 d of vitamin C deficiency. ODS and Wistar rats did not differ in plasma cholesterol concentration, but triglyceride levels were higher in ODS rats than in Wistar rats. Two weeks of consuming a vitamin C–deficient diet did not modify the lipid levels in ODS rats.

The plasma uric acid concentration was 2–3 times higher in ODS rats than in Wistar rats, but was not modified by vitamin C deficiency in ODS rats. Plasma proteins, nitrates and nitrates, as well as antioxidant capacity evaluated as ORAC did not differ between Wistar and ODS rats and was not affected by dietary vitamin C in the latter.

Cardiovascular variables and arrhythmia during ischemia reperfusion. The heart rate of anesthetized Wistar rats was stable at ~400 beats/min before and during myocardial ischemia (Fig. 2). After reperfusion of the ischemic area, the heart rate gradually increased by ~50 beats/min at the end of the protocol. ODS rats had a lower heart rate than Wistar rats, with values staying at ~340 beats/min throughout the ischemia reperfusion protocol. Vitamin C deficiency did not influence the heart rate in ODS rats.

Mean arterial pressure (MAP) of anesthetized Wistar rats was stable at ~85 mmHg during the preischemic stabilization phase (Fig. 3). After ligation of the LAD coronary artery, MAP dropped rapidly to 40 mmHg and stayed at this low level during the 5 min of regional ischemia. With reperfusion, the MAP of Wistar rats initially remained at a low level for almost 5 min, but then increased progressively to its preischemic level at the end of the protocol. However, there were large individual variations due to the high occurrence of rhythm abnormalities. The pattern of variation of the MAP in the ODS rats did not differ from that in Wistar rats, before, during and after the LAD coronary artery ligation; the vitamin C–supplemented and –deficient rats also did not differ.

The 5-min LAD coronary artery ligation protocol was chosen because this short period of ischemia is responsible for severe arrhythmias during reperfusion (Table 3). During the 15 min of reperfusion (equivalent to 900 s), the sinus rhythm was reduced to 353 ± 163 s in Wistar rats, with a large occurrence of VPB, VT, and life-threatening VF, leading to 50% mortality in these rats. ODS rats were not more or less susceptible to reperfusion arrhythmias. However, mortality was lower in this strain of rats, and the duration of the sinus rhythm was greater in ODS vitamin C–supplemented rats than in Wistar rats. In ODS rats, 2 wk of vitamin C–deficient diet did not affect the occurrence or duration of rhythm abnormalities due to reperfusion.

Aortic ring reactivity. In the 3 groups of rats, acetylcholine induced a concentration-dependent relaxation of aortic rings that were precontracted with phenylephrine (Fig. 4).
These effects were significantly higher in the ODS rats compared with the Wistar rats, and in the vitamin C–deficient ODS rats compared with the vitamin C–supplemented rats (P < 0.05). However, there was no difference in ascorbic acid potency (pD2) among the three groups of rats (8.86 ± 0.36; 8.99 ± 0.24; 8.94 ± 0.11 mol/L in Wistar, vitamin C–supplemented, and vitamin C–deficient ODS rats, respectively). The strain and diet did not influence the relaxation response of the aortic rings to the endothelial-independent NO-donor, SNAP (data not shown). Moreover, in the three groups of rats, the relaxing effects of acetylcholine were strongly inhibited by the perfusion of L-NAME (data not shown).

**DISCUSSION**

Several new aspects of the well-known antioxidant vitamin C were described in the literature in very recent years, in particular its ability to modify cardiac (3–6) or vascular variables. Many of these studies investigated the possibility of reinforcing the antioxidant plasma pool by the administration of massive doses of vitamin C. However, to our knowledge, none of these studies addressed the question of the effects of lowering the body levels of vitamin C. Ascorbate is an essential cofactor of dopamine β-hydroxylase, we investigated the plasma and adrenal pools of catecholamines in Wistar and ODS rats, for which there are no data available in the literature. Circulating catecholamines were not influenced by the strain of rats or the diet. However, in the adrenal gland, although E and NE concentrations were 35 and 60% lower, respectively, in ODS vitamin C–supplemented rats than in Wistar rats, they were not modified in ascorbate-deficient ODS rats. However, it is likely that a longer duration of ascorbate deficiency would decrease the synthesis of E and NE by the adrenal gland.

Ascorbate is implicated in lipid homeostasis as a cofactor for carnitine synthesis and for cytochrome P450. In ODS rats, ascorbate deficiency was shown by others to increase plasma total cholesterol and LDL cholesterol and to decrease HDL cholesterol (30,31), causing a higher susceptibility to atherosclerosis. Under our experimental conditions, plasma total cholesterol and triglyceride concentrations were not modified in ODS vitamin C–deficient rats, despite higher levels of triglycerides in ODS rats, compared with Wistar rats.

Nitrate synthesis was shown to be lower in ODS rats administered lipopolysaccharide by injection (32). However, under nonendotoxemic conditions, the plasma levels of nitrates were not affected by vitamin C deficiency in ODS rats. In fact, several studies suggested that ascorbate is able to reinforce nitric oxide synthesis (33), but it might not be readily measurable by the simple evaluation of nitrate levels;
in vivo, however, the very low levels of ascorbate might be sufficient to maintain a basal production of nitric oxide.

Thus, the differences in biochemical modifications (urate, tocopherol, catecholamine and lipids) between Wistar and ODS rats are related more to the difference in strain than to vitamin C deficiency. In the ODS strain of rats, the only modified biochemical factor was a dramatic drop in plasma and heart concentrations of this vitamin.

Therefore, the 2 wk of vitamin C deficiency did not affect other plasma components such as catecholamines and lipids that could influence cardiovascular variables. Under these conditions in which only vitamin C was modified, we investigated the effects of ascorbate deficiency on the cardiovascular variables of anesthetized rats under preischemic and postischemic conditions. As we observed earlier in vitro, the initial heart rate of anesthetized ODS rats was lower than that of Wistar rats (16), but was not modified by the vitamin C deficiency. Despite equivalent levels of plasma epinephrine and norepinephrine, measurements in the adrenal gland suggest that catecholamine synthesis might be lower in ODS rats than in Wistar rats, which could explain their lower heart rate. Under our experimental conditions, the blood pressures of Wistar and ODS rats did not differ during the preischemic period. Some clinical studies have shown that feeding a vitamin C–deficient diet for 1 mo could increase blood pressure (6). The reason generally advanced is that modifications of redox status might modify vascular resistance. However, these differences might also be attributable to other modifications related to long-term ascorbate deficiency such as the lowering of catecholamines, but also modifications of vessel collagen, a situation that is unlikely to have occurred in ODS rats after 2 wk of ascorbate deficiency.

During myocardial ischemia and reperfusion, the calcium overload and the production of radical species likely contribute to the occurrence of reperfusion injury. In fact, several studies demonstrated that the antioxidant pool was lowered by ischemia (34) and that antioxidant therapy was able to reduce the extent of the injury (35). In this context, clinical studies suggest that ascorbate can be beneficial during myocardial infarction (0.6–1 g/d) (36,37) and could reduce apoptosis associated with heart failure (2 g/d, i.v.) (5). Moreover, in isolated perfused rat hearts, vitamin C (0.5–1 mmol/L) was able to reduce reperfusion arrhythmias (35) and to enhance the cardioprotection of glutathione (4). However, as suggested by the data already obtained in vitro (16), lowering plasma and heart tissue vitamin C status by ~90% was not associated with a lower recovery in cardiovascular variables or a higher incidence of reperfusion arrhythmias. Therefore, the cardiac effects of vitamin C deficiency are distinct from those obtained with the administration of large doses of this vitamin, suggesting the existence of compensatory mechanisms, but also pharmacologic effects of ascorbate administration that are not necessarily related to its antioxidant properties.

Several authors investigated in vivo (7–11) or in isolated perfused vessels (12,13) the effects of vitamin C on vascular reactivity, and they concluded that vitamin C is effective in restoring impaired endothelium-dependent vasorelaxation, an effect that is generally attributed to its antioxidant properties (38,39). In fact, it has been suggested that ascorbate enhances nitric oxide production through the activation of tetrahydrobiopterin (33,40). Its protective role against nitric oxide degradation via its interaction with the superoxide anion is more controversial because its rate constant for the reaction with the superoxide anion is lower than that of NO with superoxide. Therefore, such a protective effect seems to occur solely in the presence of large doses of vitamin C, reaching concentra-

tions that are very unlikely to occur in vivo (12,13). To our knowledge, none of these studies focused on the much more relevant effect of vitamin C deficiency. Surprisingly, our experimental data suggest that endothelium-dependent relaxation was slightly better in ODS than in Wistar rats, and even more so in ODS vitamin C–deficient rats. These differences are probably attributable to endothelial function because the relaxation response to an NO donor was equivalent in all groups. These results agree with very recent studies suggesting that ascorbate can impair endothelium-derived vasodilation in rat mesenteric bed (20) or rabbit aortic rings (41). However, the endothelium-dependent NO pathway seems to remain the main way of relaxation, whatever the strain or regimen because the acetylcholine-induced relaxation in the 3 groups of rats was strongly inhibited by the NO synthase inhibitor. Mc Neish et al. (40) suggested that physiologic concentrations of ascorbate block endothelium-derived hyperpolarizing factor–mediated vasodilator responses. This hypothesis was not confirmed in our study because the NOS inhibitor completely blocked the relaxing effects of acetylcholine in the 3 groups of rats. Finally, the possibility that the rat strain explains the discrepancies in endothelium-dependent vasoreactivity cannot be excluded.

In conclusion, although mega-doses of vitamin C are able to augment vasorelaxation, or to exert some cardioprotective effects in the context of myocardial ischemia-reperfusion, the physiologic effects of acute ascorbate deficiency cannot be extrapolated from these results. An antioxidant is a compound that at very low doses is able to lessen the oxidative processes occurring in a biologic environment. However, at millimolar concentrations, the dose at which ascorbate is usually added in many experiments, any physiologic or pharmacologic molecule, e.g., mannitol, can act as an antioxidant. Because ascorbic acid is also a weak acid, when large amounts of this vitamin are added to the extracellular compartment, it is able to modify the local pH and to change ionic transport through the cellular membrane, an effect that is independent of its antioxidant properties. Therefore, it would be reasonable to investigate the biological effects of vitamin C considering its physiologic concentrations.

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LITERATURE CITED


