Depletion of plasma vitamin C but not of vitamin E in response to cardiac operations

The whole-body inflammatory response produced by cardiopulmonary bypass is an important cause of perioperative morbidity after cardiac operations. This inflammatory response produces reactive oxygen species and other cytotoxic substances, such as the cytokines. The generation of reactive oxygen species might deplete principal antioxidant micronutrients, that is, vitamins C and E and the carotenoids. Therefore, we have investigated the time course of the plasma concentrations of vitamins C and E and the carotenoids in 18 patients undergoing coronary bypass operations after randomization for previous vitamin E supplementation (300 mg *dl*- α -acetyl-tocopherol 3 times daily for 4 weeks) or placebo. Supplementation with α -tocopherol doubled the lipid-standardized plasma vitamin E concentration to $63.7 \pm 14.5 \mu\text{mol/L}$ when compared with that of the control subjects ($31.2 \pm 9.0 \mu\text{mol/L}$) before the operation. The plasma concentrations of vitamin C ($36.0 \pm 19.0 \mu\text{mol/L}$ and $44.0 \pm 21.7 \mu\text{mol/L}$, respectively) and of the carotenoids were not statistically different between the two groups at baseline. The absolute plasma concentrations of both vitamin E and the carotenoids decreased during and after cardiopulmonary bypass, but after correction for hemodilution the plasma concentrations of vitamin E and the carotenoids showed no decrease. The vitamin E concentrations in the erythrocytes did not change either. In contrast, the plasma concentration of vitamin C decreased in all subjects within 24 hours after the operation by roughly 70 %. Correction for hemodilution still revealed a significant decrease in plasma vitamin C that persisted in most patients up to 2 weeks. In conclusion, the vitamin E and the carotenoid plasma concentrations are of no major concern during and after cardiac operations. In contrast, the serious depletion of vitamin C may deteriorate the defense against reactive oxygen species-induced injury during cardiac operations. (J THORAC CARDIOVASC SURG 1994;108:311-20)

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Cardiopulmonary bypass (CPB) for cardiac operations has been refined in recent years by pharmacologic means¹ and by the introduction of membrane oxygenators,² with a reduction in perioperative morbidity and mortality.¹⁻³ However, substantial morbidity after CPB remains. The whole-body inflammatory reaction produced by CPB⁴⁻⁶ is a major pathogenetic cause for post-

operative complications after cardiac operations. The inflammatory response has been shown to produce a large number of reactive oxygen species,⁷⁻¹⁰ cytokines,^{6, 11} and other cytotoxic materials.^{4, 5} Among all these harmful factors, the generation of reactive oxygen species has gained a major interest in recent years. The reexposure of the tissues to oxygen at the end of CPB produces a so-called "reperfusion injury," which is caused by reactive oxygen species.^{7, 9, 12-14} Aggressive oxygen species can damage various cell constituents, and the damage may continue by the chain reaction of peroxidation of polyunsaturated fatty acids. However, a multidefense system against injury induced by reactive oxygen species, including enzymes (e.g., superoxid dismutase), endogenous antioxidants (e.g., glutathione and the serum proteins), and exogenous dietary antioxidants (e.g., vitamins C and E and the carotenoids), protects the body against radical-

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induced injuries. Depletion of these vitamins might, therefore, be a prerequisite for harmful effects of reactive oxygen species in organ dysfunction after CPB. In recent studies,^{15,16} an acute decrease in plasma vitamin E concentration of 50% in patients undergoing cardiac operations was reported. Unfortunately, those workers measured only absolute plasma vitamin E concentrations. The common hemodilution during CPB was not accounted for, and therefore the fall in plasma vitamin concentrations is likely to be a reflection of hemodilution. Coghlan and associates,¹⁷ who corrected for the hemodilution during CPB, found only a transient fall of vitamin E, when expressed as the difference between coronary sinus and systemic arterial concentrations, up to 10 minutes after crossclamp removal. Mickle and coworkers¹⁸ found no decrease of myocardial vitamin E concentration after reperfusion, whereas Barsacchi's group¹⁹ reported an insignificant decrease of vitamin E under conditions of ischemia and reoxygenation. Similarly, the human data on plasma vitamin C concentrations are conflicting. Cavarocchi and coworkers¹⁵ reported an increase in vitamin C concentration by roughly 50% without correction for hemodilution immediately after CPB and a decrease 1 day after CPB when compared with preoperative values, but this difference was statistically not significant. In contrast, another group found a continuous hemodilution-adjusted decrease in plasma vitamin C concentration from the beginning of CPB up to the third postoperative day.²⁰

In the present study, we have, therefore, studied the time course of the principal antioxidant micronutrients, that is, the vitamins C and E, β -carotene, and lycopene, corrected for hemodilution. We hypothesized that the generation of reactive oxygen species in patients supported by CPB might deplete antioxidant micronutrients. As an indicator of myocardial damage, heart enzymes such as creatinine kinase were measured and some characteristics of erythrocytes, another potential target of radical injury, were investigated.

The patients were randomly given high doses of vitamin E before CPB, with the aim of equilibrating any fall in vitamin E plasma concentrations and to reduce myocardial damage induced by reactive oxygen species.

Methods

Patients. Eighteen patients undergoing bypass operations for coronary heart disease were studied. They were contacted 6 weeks before the operation and were informed about the purpose of the study. Written informed consent was obtained. Thereafter, the subjects were randomized into two groups: half of them received *dl*- α -acetyl-tocopherol (Ephynal, Hoffmann-La Roche Ltd., Basel, Switzerland) 300 mg 3 times daily for 4 weeks, and the other half had placebo. Patient compliance was ascertained

by capsule counting on admission. The study protocol was approved by the local ethics committee.

Extracorporeal circulation and bypass grafting. Midazolam, fentanyl, enflurane (Ethrane), and pancuronium bromide (Pavulon) were used for general anesthesia. Mannitol (10 gm), a hydroxyl radical scavenger, and heparin (200 U/kg body weight) were given routinely during CPB. A membrane oxygenator (Maxima, Medtronic Inc., Minneapolis, Minn., or CML2, Cobe Laboratories, Lakewood, Colo.) and an arterial filter (Bentley AF 1040 C, 40 mm, Baxter Healthcare Corp., Irvine, Calif.) between the pump and the patient were used in all subjects. A roller pump (Shiley-Stöckert, Munich, Germany) provided a flow rate of 2.4 L/min per square meter at normothermia and of 1.2 L/min per square meter at hypothermia (26° C). The priming solution consisted of 1.25 L 0.9% saline and 1.25 L Ringer's lactate containing sodium bicarbonate 48 μ mol/L, potassium 10 μ mol/L, magnesium 4 μ mol/L, and 3 mg calcium gluconolactobionate. Arterial oxygen pressure was maintained at about 200 mm Hg by an oxygen blender (Cobe Laboratories). The patients received on average 3.8 units of blood (310 ± 50 gm per unit, hematocrit value $60\% \pm 1\%$, stored <6 days) within the first 24 hours after the operation.

Methods. Blood samples were drawn at intervals: on admission, after induction of anesthesia, 30 minutes after the start of the operation, after 60 minutes of CPB, immediately after transition from CPB to the patient's own circulation, 24 hours after CPB, and at dismissal. Plasma concentrations of vitamins C and E were determined at all time points and erythrocyte parameters before the operation, after 60 minutes of CPB, and 24 hours after CPB.

For analysis of plasma antioxidant concentrations, 10 ml of heparinized blood was centrifuged immediately at 2000 g for 10 minutes at room temperature. An aliquot of 0.5 ml plasma was mixed with 4.5 ml metaphosphoric acid for the fluorimetric determination of vitamin C as described previously.²¹

Vitamin E (α -tocopherol), β -carotene, lycopene, and vitamin A (retinol) were extracted into *n*-hexane and analyzed by high-performance liquid chromatography²² with a fluorimetric detector (extinction 290 nm, emission 330 nm). Plasma cholesterol and triglycerides were determined enzymatically by an automated analyzer. Plasma vitamin E concentrations were expressed as lipid-standardized values,²³ because they are dependent on the concentration of carriers, that is, cholesterol and triglycerides. The adjustment of plasma vitamin E was based on a linear regression coefficient elaborated in large epidemiologic studies²³ with standard values of 2.2 gm/L for cholesterol and 1.1 gm/L for triglycerides. Vitamin E in erythrocytes was expressed as the vitamin E concentration (in micromoles) per unit hemoglobin (in nanomoles).

Hemodilution of the vitamins C and E and β -carotene was also controlled for by taking into account the hematocrit value. Corrected values of the measured plasma concentrations of all three vitamins were calculated by multiplying the vitamin concentration by $100 - \text{baseline hematocrit} / 100 - 100 \text{ actual hematocrit}$.

Red blood cell (RBC) shape and deformability were determined from blood samples containing ethylenediaminetetraacetic acid as anticoagulant. Drops of blood were fixed in cacodylate-buffered 1% glutaraldehyde. The shape of the RBC was analyzed under the light microscope and classified according to the nomenclature of Bessis.²⁴ Cell deformability was assessed by RBC filtration as described previously.^{24,25} In brief, RBCs were

washed three times and resuspended in phosphate-buffered saline to a hematocrit value of 10%. The RBC suspension was pumped at a flow rate of 0.5 ml/min through a polycarbonate filter (Nuclepore Corp., Pleasanton, Calif.) with mean pore diameters of $2.8 \pm 0.2 \mu\text{m}$, and the filtration pressure was measured on the upstream side of the filter. The relative resistance of an RBC (β) was calculated as follows:

$$\beta = 1 + ([P_i/P_o - 1]) V/h$$

where P_i is the initial pressure rise, P_o the filtration pressure for suspending medium alone, V the fraction of the pore volume occupied by the RBC, and h the fractional volume of RBCs in suspension.²⁵ The dimensionless parameter β is the ratio of resistance in a filter pore containing an RBC in passage compared with that in a filter pore with suspending medium.

Erythrocyte membrane skeleton proteins were analyzed in RBC ghosts, obtained by lysing RBC in hypotonic phosphate buffer as described previously.²⁵ The harmful effect of proteolytic enzymes was eliminated by addition of the protease inhibitors pepstatin A (2 mg/ml) and phenylmethylsulfonyl fluoride (20 mg/ml). Sodium dodecyl sulfate polyacryl gel electrophoresis (SDS-PAGE) was performed with approximately 20 mg protein, and the gels were stained with Coomassie brilliant blue and scanned at 560 nm.

Creatinine kinase (CK) and its heart-specific isoenzyme (CK-MB) were determined before the operation and 6, 12, 18, 24, 36, 48, 60, and 72 hours after the operation with a Hitachi 705 AutoAnalyzer (Hitachi Instruments, Inc., San Jose, Calif.). Creatinine, urea, and uric acid were also analyzed in hospital routine. The results are expressed as mean values with standard deviation, if not stated otherwise. Statistical analyses were used to compare two treatment groups measured at different time points. The methods used were analysis of variance with repeated measurements and two-group comparisons with summary measures.

Results

The characteristics of the subjects are summarized in Table I. The only difference between the two groups was the preoperative ventricular ejection fraction, which was significantly lower in the vitamin E-supplemented group. This might be a reflection of the fact that eight of nine patients had had a myocardial infarction, whereas in the control group only five of nine patients had had a myocardial infarction. On the average 2.5 vessels were diseased in the control subjects versus 2.8 in the patients receiving supplemental vitamin E. Triple drug regimens consisted of β -blockers, calcium-channel blockers, and nitrates. Patients being treated with two drugs received nitrates and β -blockers or calcium-channel blockers, and single-drug treatment consisted of β -blockers/nitrates or an angiotensin-converting enzyme inhibitor. Three patients in the control group and four in the vitamin-supplemented group were receiving additional treatment with salicylates or dicumarol.

The initial plasma antioxidant status on hospital admission and the effects of CPB are shown in Figs. 1 to

Table I. Characteristics of the patients ($n = 18$; mean $\pm$ standard deviation)

	Control group	Vitamin E group
No. of patients	9	9
Age (yr)	56.1 ± 12.1	62.6 ± 7.4
History of myocardial infarction	5	8
No. of diseased coronary vessels (mean)	2.5	2.8
Ventricular ejection fraction (%)	70 ± 14	$52 \pm 13^*$
Preoperative treatment		
Three drugs (nitrate, β -blocker, calcium-antagonist)	4	2
Two drugs (nitrate, β -blocker, or calcium antagonist)	5	4
One drug (nitrate, or β -blocker, or ACE inhibitor)	0	3
CPB		
CPB time (min)	101 ± 45	103 ± 21
Aorta clamping time (min)	56 ± 34	57 ± 15
Surgical procedures		
Arteria mammaria interna graft	9	9
Aorta-coronary bypasses	14	21
Transfusions (units blood)	3.9	3.7
Stay in hospital (days)	13.7 ± 2.2	13.3 ± 3.7

ACE, Angiotensin-converting enzyme; CPB, cardiopulmonary bypass.

* $p < 0.02$ compared with control group by unpaired t test.

4. Initial plasma vitamin C concentrations were on average about $40 \mu\text{mol/L}$ ($36.0 \pm 19.0 \mu\text{mol/L}$ in the control group versus $44.0 \pm 21.7 \mu\text{mol/L}$ in the vitamin E-supplemented group). However, on admission one third of the patients already had "critical" vitamin C concentrations at the borderline of marginal deficiency ($\leq 0.4 \text{ mg/dl}$ or $\leq 23 \mu\text{mol/L}$; three patients) or even at "scorbutic levels," that is, plasma concentrations of accepted vitamin C deficiency ($\leq 0.2 \text{ mg/dl}$ or $\leq 11 \mu\text{mol/L}$; three patients) (Fig. 1). Twelve patients were ex-smokers, who had probably not given up their smoking-related dietary habits (typically low in vitamin C-providing fruits and vegetables). During and especially after CPB, vitamin C plasma concentration decreased strongly, on average by 71% at 24 hours. In contrast, the mean values of various water-soluble parameters showed smaller decreases at this time point: creatinine by less than 10%, urea by 20%, uric acid by 40%, and hematocrit by 22% to 42%. The quotient of vitamin C/creatinine changed highly significantly over the time period ($p < 0.00001$, analysis of

Fig. 1. Individual plasma vitamin C concentrations before, during, and after CPB. **A.** Control subjects. **B.** Subjects receiving supplemental vitamin E. Each *symbol* represents one subject. The *dashed lines* represent thresholds for marginal deficiency (<0.4 mg/dl) and "scorbutic" levels (<0.2 mg/dl), respectively.

variance with repeated measurements. The *p* values have been corrected for lack of sphericity by means of the greenhouse Geisser factor). The same was true for vitamin C/urea ($p < 0.00001$) and for vitamin C/uric acid ($p = 0.0001$), indicating a significant decrease of plasma vitamin C concentration. One day after CPB the absolute vitamin C values had further decreased to critically low concentrations, regardless of the initial state. At this stage other nonlipid plasma parameters had already started to return toward normal. When hemodilution was accounted for by inferring the hematocrit value, similar statisti-

Table II. Vitamin E (μmol vitamin E per nmol hemoglobin) concentration in erythrocytes

	Control group	Vitamin E group
Admission	44.2 ± 12.5	83.9 ± 18.1
Anesthesia	40.3 ± 13.2	82.4 ± 10.8
Operation	39.9 ± 15.3	87.3 ± 9.7
60 min on CPB	40.7 ± 11.8	85.6 ± 12.0
Immediately after CPB	41.4 ± 8.2	89.0 ± 6.0
24 hr after CPB	39.1 ± 14.9	72.0 ± 11.3
Dismissal	45.8 ± 9.9	77.5 ± 28.0

CPB, Cardiopulmonary bypass.

cal significances resulted ($p = 0.007$ for the control group versus $p = 0.009$ for the vitamin E-supplemented group). At dismissal the mean vitamin C concentration was still reduced by 47% (Fig. 2), that is, in the range of marginal deficiency, corresponding to 11 of 18 patients remaining in the critical range (Fig. 1). Return to higher levels before dismissal occurred in only six of 12 subjects, who initially had fair levels above 0.6 mg/dl, whereas the vitamin C concentrations in the other 12 (of 18) patients did not exceed the threshold of marginal vitamin C deficiency at dismissal (Fig. 1). In contrast to vitamin C, all other nonlipid plasma parameters were back to pre-CPB concentrations or above at dismissal.

At baseline, the mean value of lipid-standardized plasma vitamin E concentration was "fair" in patients not receiving supplements²⁶ and increased about twofold in the supplemented group, roughly 30 and 60 $\mu\text{mol/L}$, respectively (Fig. 3). There was no significant time effect in lipid-standardized plasma vitamin E concentrations at any time point during and after CPB (Fig. 3). The same was true when the hemodilution of vitamin E was corrected by inferring the hematocrit value. In contrast, uncorrected plasma vitamin E concentrations dropped by about 40% or more but completely paralleled the dilution of cholesterol-rich lipoprotein carriers of vitamin E during CPB (Figs. 3 and 4). The vitamin E concentration in erythrocytes was also doubled by prior vitamin E supplementation, but there was no significant time effect by CPB either (Table II).

The plasma β -carotene concentrations on admission showed marginal β -carotene levels below the desirable 0.4 $\mu\text{mol/L}$ ²⁶ in five patients. Plasma β -carotene and lycopene (initially also at rather low levels) decreased during CPB, similar to vitamin E and cholesterol. Unlike the latter, the carotenoids had not fully regained the preoperative level at dismissal, and their return to normal might have been slightly slower than that of cholesterol (Fig. 4). The mean absolute vitamin A concentrations were initially in a fair range of 2.3 $\mu\text{mol/L}$ ($2.4 \pm 0.6 \mu\text{mol/L}$ in control subjects, $2.3 \pm 0.4 \mu\text{mol/L}$ in patients receiving

Fig. 2. Decrease of mean values of plasma vitamin C, creatinine, urea, and uric acid during and after CPB. Values are relative, in comparison with the baseline values on the right (means $\pm$ standard deviation of absolute baseline values on the right = 100%). **A**, Control subjects. **B**, Subjects receiving supplemental vitamin E.

supplemental vitamin E) and paralleled cholesterol concentrations during CPB, similar to the other lipid-soluble antioxidants (data not shown).

The results on RBC shape and deformability are summarized in Table III. Echinocytic shape transformation occurred after 60 minutes of CPB; it was due to a plasmatic factor because washed RBCs did not show any shape change. Echinocytosis was reversible within 24 hours after CPB. No time effect was found for vitamin E supplementation. RBC deformability was not affected. The analysis of the RBC membrane proteins revealed no high molecular complexes indicative of an oxidative damage. The individual membrane skeleton proteins (spectrin, ankyrin, protein band 3, 4.1, 4.2, 4.9, and actin) were normal with respect to molecular size and relative amount (data not shown).

The postoperative CK and CK-MB values are summarized in Table IV. A significant increase of both CK and CK-MB values occurred in both groups after bypass grafting ($p < 0.001$), regardless of the previous vitamin E supplements. Both the CK and CK-MB values tended to be lower in the vitamin E-supplemented group (CK: range 14% to 31%; CK-MB: range 9% to 33%), without the difference reaching statistical significance, however. This was also true for areas under the curves.

Discussion

The generation of reactive oxygen species is a major pathogenetic factor for tissue damage resulting from CPB. In particular, at the time of reperfusion the body is exposed to a harmful burst of reactive oxygen species. Antioxidant micronutrients, that is, the vitamins C and E

Fig. 3. Plasma vitamin E concentration before, during, and after CPB. *Open symbols* are unadjusted values (means $\pm$ standard error of the mean) and *closed symbols* are lipid-standardized concentrations. *Squares* indicate patients receiving supplemental vitamin E, and *circles* indicate control subjects.

and the carotenoids form (together with the endogenous glutathione) the major nonenzymatic defense system against injury induced by reactive oxygen species. In the present study, therefore, we have investigated the effects of CPB on plasma concentrations of the vitamins C and E and β -carotene in patients with coronary heart disease undergoing cardiac operations.

The present data confirm a substantial decrease of absolute vitamin E plasma concentrations during and 24 hours after CPB, when hemodilution of vitamin E is not accounted for, as has been shown by other authors.^{15, 16} However, when the vitamin E concentrations were expressed in lipid-standardized values, taking into

account plasma cholesterol and triglycerides, the natural carriers of lipid-soluble vitamins, or when the hemodilution of vitamin E was corrected by means of the hematocrit, we have not found any relevant decrease in vitamin E concentrations during and after CPB. Similarly, Coghlan and associates¹⁷ have also not found a decrease in coronary sinus blood vitamin E concentrations 10 minutes after crossclamp removal. Because not the absolute plasma vitamin E but only the lipid- or dilution-adjusted vitamin E value is indicative for the biologic vitamin E status, our results suggest that CPB does not affect substantially the overall vitamin E status. The present interpretation of vitamin E plasma concentrations is also in line with that of Mickle and coworkers,¹⁸ who found no decrease in myocardial tocopherol concentrations 20 minutes after reperfusion. Our conclusion that the overall status of liposoluble antioxidants of the whole body is not substantially affected is corroborated by the fact that the plasma status of other liposoluble antioxidants, that is, β -carotene, lycopene, and vitamin A, remained unchanged too.

The absolute vitamin C plasma concentrations decreased also at the beginning of CPB. However, the vitamin C concentration was also substantially lower when hemodilution was corrected by means of the hematocrit level and various other water-soluble parameters such as creatinine, urea, and uric acid, indicating a true depletion of this vitamin. Twenty-four hours after the operation, the plasma vitamin C concentrations further declined below the commonly accepted threshold of marginal vitamin C deficiency, that is, below 0.4 mg/dl ($=22.7 \mu\text{mol/L}$), whereas the other water-soluble parameters started to return to normal limits. At dismissal, the average vitamin C concentration was still reduced by roughly 50%, in the range of marginal deficiency. Thus our results confirm two previous preliminary reports on a critically low vitamin C status after CPB.^{15, 20} This persistence of low vitamin C concentrations in the majority of the patients suggests a substantial exhaustion of vitamin C stores during CPB. Vitamin C depletion may seriously alter the balance of the entire antioxidant defense system, by a subsequent decrease in blood glutathione concentration, and render the organism to increased oxidative stress with the sequels of tissue and cell damage, a potential source of CPB-related morbidity. Moreover, vitamin C depression after CPB might also be an important factor for postoperative cardiac complications, because a poor vitamin C status is associated with rhythm disturbances and cardiac failure.²⁷⁻³³ The present findings of substantial vitamin C depletion are an extension of in vitro studies on plasma vitamin C, where various kinds of aggressive oxygen species and other radicals, for

Fig. 4. CPB-related time course of plasma concentrations (means $\pm$ standard deviation) of lipid-soluble essential antioxidants and of cholesterol. The values are not lipid-standardized. Values are relative, in comparison with the baseline values on the right (=100%). **A**, Control subjects. **B**, Subjects receiving supplemental vitamin E.

example, from cigarette smoke, caused primarily a fast depletion of vitamin C, and of vitamin E only after exhaustion of vitamin C.³⁴⁻³⁸ This specific vitamin C depletion is evidence for a unique function of vitamin C as a first-line "free radical sink."³⁸ Moreover, our data on vitamin C link with the recent observational data on cardiovascular disease, in which a poor vitamin C status was shown to be a potential risk factor for angina pectoris,³⁹ as well as for deaths from coronary and cerebrovascular disease.^{26, 40}

We have also measured CK and its heart-specific isoenzyme, CK-MB, as indicators for skeletal and heart muscle injury. However, no correlation was detected between any of the antioxidant plasma concentrations and myocardial damage, as indicated by the moderate increase of CK-MB. Because CK and CK-MB alone are

not very specific indicators of myocardial damage, our study might be unsuitable to test whether antioxidants are involved in CPB-related myocardial injury. The echinocytic shape transformation of RBCs during CPB, reported earlier,^{25, 41} was confirmed. However, the two-fold increase in plasma and erythrocyte vitamin E by supplements of high doses of vitamin E did not reverse the shape transformation. This suggests that vitamin E-related stress is not an important pathogenetic factor for the CPB-related echinocytosis.

In summary, the present data indicate that in patients with a "fair" (if not optimal²⁶) initial vitamin E plasma concentration, both the lipid-standardized plasma vitamin E and the vitamin E in red blood cells (and thus, most likely the intracardiac vitamin E status) and the carotenoids were maintained during and after cardiac opera-

Table III. Influence of vitamin E on occurrence of echinocytes and erythrocyte deformability

Time	Echinocytes (%)		Relative filtration resistance of RBC (β)	
	Control group	Vitamin E group	Control group	Vitamin E group
Before operation	3.7 $\pm$ 2.7	4.8 $\pm$ 2.7	79.8 $\pm$ 21.4	92.3 $\pm$ 17.5
60 min on CPB				
Unwashed	17.6 $\pm$ 8.5*	16.3 $\pm$ 9.1*		
Washed in buffer	2.7 $\pm$ 2.0	3.4 $\pm$ 1.7	82.0 $\pm$ 14.6	83.9 $\pm$ 26.2
24 hr after CPB	5.8 $\pm$ 2.6	3.7 $\pm$ 1.9	86.1 $\pm$ 24.7	87.6 $\pm$ 28.6

RBC, Red blood cell; CPB, cardiopulmonary bypass.

* $p < 0.001$ compared within group.**Table IV.** Time course of CK and CK-MB

Time	Control			Vitamin E			Difference: control-vitamin E	
	CK (U/L)	CK-MB (U/L)	% CK-MB	CK (U/L)	CK-MB (U/L)	% CK-MB	Δ CK (U/L)	Δ CK-MB (U/L)
Preop.	99 $\pm$ 36	—	—	112 $\pm$ 53	—	—	—	—
Postop.								
6 hr	667 $\pm$ 410	68 $\pm$ 48	10.2	508 $\pm$ 156	58 $\pm$ 11	11.4	159	10
12 hr	643 $\pm$ 454	56 $\pm$ 40	8.7	475 $\pm$ 187	38 $\pm$ 10	8.0	168	18
18 hr	821 $\pm$ 515	48 $\pm$ 27	5.8	564 $\pm$ 268	32 $\pm$ 11	5.7	257	16
24 hr	955 $\pm$ 648	48 $\pm$ 27	5.0	687 $\pm$ 344	39 $\pm$ 18	5.7	268	9
36 hr	788 $\pm$ 441	34 $\pm$ 11	4.3	562 $\pm$ 212	30 $\pm$ 12	5.3	226	4
48 hr	506 $\pm$ 299	23 $\pm$ 6	4.5	431 $\pm$ 181	21 $\pm$ 9	4.9	75	2
60 hr	376 $\pm$ 218	17 $\pm$ 4	4.5	325 $\pm$ 140	18 $\pm$ 8	5.5	51	-1
72 hr	309 $\pm$ 224	25 $\pm$ 14	8.1	266 $\pm$ 137	17 $\pm$ 4	6.4	43	8

CK, Creatinine kinase; CK-MB, heart-specific isoenzyme of CK.

tions. Maintenance of these substances suggests that the status of liposoluble antioxidants is of no major concern during CPB. In contrast, critically low plasma concentrations of the water-soluble vitamin C occurred within 24 hours after the operation regardless of the initial vitamin C status. The lack of return to preoperative concentrations at dismissal in most patients implies that vitamin C was consumed in large quantities and that even deeper body stores including the myocardium were likely to have been depleted. The consumption of vitamin C might have occurred by direct reaction with reactive oxygen species or by regeneration of other antioxidants, for example, vitamin E from the vitamin E radical.^{12, 42} The generation of aggressive oxygen species by myeloperoxidase released from activated neutrophils in the pulmonary capillary beds¹⁶ might be another important mechanism causing vitamin C depletion in the whole-body inflammatory response caused by CPB.^{12, 43}

The present finding of the substantial consumption of vitamin C suggests that a perioperative vitamin C supplementation, combined with other antioxidants, might attenuate the whole-body inflammatory reaction and thus reduce CPB-related morbidity. Eddy, Hurvitz, and Hochstein⁴⁴ reported that intravenous injection before CPB and addition to cardioplegic solutions of vitamin C markedly reduced the release of CK into the coronary

sinus. In analogy, benefits were shown in kidney transplants by infusing a multivitamin mixture with vitamin C as the major antioxidant vitamin.⁴⁵ This reduced lipid peroxidation, indicative of reperfusion injury, and substantially improved renal transplant performance when the multivitamin mixture was given immediately before the reperfusion of the kidney. In consequence, vitamin C is a promising "candidate" for prophylactic and therapeutic interventions in clinical conditions in which the balance between reactive oxygen species and antioxidants is likely to be disturbed.

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REFERENCES

1. Gardener TJ, Stewart JR, Casale AS, Downey JM, Chambers DE. Reduction of myocardial ischemic injury with oxygen-derived free radical scavengers. *Surgery* 1983; 94:423-7.
2. Van Oeveren W, Kazatchkine MD, Descamps-Latscha B, et al. Deleterious effects of cardiopulmonary bypass: a prospective study of bubble versus membrane oxygenation. *J THORAC CARDIOVASC SURG* 1985;89:888-9.

3. Kirklin JW, Kirklin JK, Lell WA. Cardiopulmonary bypass surgery. In: Sabiston DC, Spencer FC, eds. *Surgery of the chest*. 4th ed. Philadelphia: WB Saunders 1983:909-25.
4. Chenoweth DE, Cooper SW, Hugh TE, Stewart RW, Blackstone EH, Kirklin JW. Complement activation during cardiopulmonary bypass: evidence for generation of C3a and C5a anaphylatoxins. *N Engl J Med* 1981; 304:497-503.
5. Nilsson L, Brunnkvist S, Nilsson U, et al. Activation of inflammatory systems during cardiopulmonary bypass. *Scand J Thorac Cardiovasc Surg* 1988;22:51-3.
6. Butler I, Rocker GM, Westaby S. Inflammatory response to cardiopulmonary bypass. *Ann Thorac Surg* 1993; 55:552-9.
7. Ferreira RF, Llesuy S, Milei J, et al. Assessment of myocardial oxidative stress in patients after myocardial revascularization. *Am Heart J* 1988;115:307-12.
8. Bolli R, Jeroudi O, Patel BS, et al. Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion: evidence that myocardial "stunning" is a manifestation of reperfusion injury. *Circ Res* 1989;65:607-22.
9. Davies MJ. Direct detection of radical production in the ischaemic and reperfused myocardium: current status. *Free Radic Res Commun* 1989;7:275-84.
10. Weisel RD, Mickle DAG, Finkle CD, et al. Myocardial free-radical injury after cardioplegia. *Circulation* 1989; 80(Suppl):III14-18.
11. Butler J, Chong GL, Baigrie RJ, Pillai R, Westaby S, Rocker GM. Cytokine responses to cardiopulmonary bypass with membrane and bubble oxygenation. *Ann Thorac Surg* 1992;53:833-8.
12. Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. 2nd ed. Oxford: Clarendon Press, 1989:3-496.
13. Redl H, Gasser H, Schlag G, Marz I. Involvement of oxygen radicals in shock related cell injury. *Br Med Bull* 1993;49:556-65.
14. Rangan U, Bulkley GB. Prospects for the treatment of free-radical mediated tissue injury. *Br Med Bull* 1993; 49:700-18.
15. Cavarocchi NC, England MD, O'Brien JF, et al. Superoxide generation during cardiopulmonary bypass: Is there a role for vitamin E? *J Surg Res* 1986;40:519-27.
16. Pincemail J, Faymonville ML, Deby-Dupont G, Thirion A, Deby C, Lamy M. Neutrophil activation evidenced by plasmatic myeloperoxidase release during cardiopulmonary bypass in humans: consequence of vitamin E status. *Ann N Y Acad Sci* 1989;570:501-2.
17. Coghlan JG, Flitter WD, Clutton SM, Ilsley CD, Rees A, Slater TF. Lipid peroxidation and changes in vitamin E levels during coronary artery bypass grafting. *J THORAC CARDIOVASC SURG* 1993;106:268-74.
18. Mickle DAG, Weisel RD, Burton GW, Ingold KU. Effect of orally administered alpha-tocopheryl acetate on human myocardial alpha-tocopherol levels. *Cardiovasc Drugs Ther* 1991;5:309-12.
19. Barsacchi R, Pelosi G, Maffei S, et al. Myocardial vitamin E is consumed during cardiopulmonary bypass: indirect evidence of free radical generation in human ischemic heart. *Int J Cardiol* 1992;27:339-43.
20. Brodowski S, Jähnchen E, Hanck A, Roskamm H, Preiss DU. Intra- und postoperative Ascorbinsäure-Plasmakonzentrationen herzchirurgischer Patienten [Abstract]. *Anaesthesist* 1991;40:S101.
21. Brubacher G, Vuilleumier JP. Vitamin C. In: Curtius HC, Roth M, eds. *Clinical biochemistry: principles and methods*. 1st ed. Berlin: De Gruyter, 1974:989-97.
22. Hess D, Keller HE, Oberlin B, Bonfanti R, Schuep W. Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. *Int J Vitam Nutr Res* 1991;61:232-8.
23. Gey KF, Puska P, Jordan P, Moser UK. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am J Clin Nutr* 1991;53:326S-34S.
24. Bessis M. Red cell shapes: an illustrated classification and its rationale. *Nouv Rev Fr Hematol* 1972;12:721-46.
25. Reinhart WH, Ballmer PE, Rohner F, Ott P, Straub W. The influence of extracorporeal circulation on erythrocytes and flow properties of blood. *J THORAC CARDIOVASC SURG* 1990;100:538-45.
26. Gey KF, Moser UK, Jordan P, Stähelin HB, Eichholzer M, Lüdin E. Increased risk of cardiovascular disease at sub-optimal plasma levels of essential antioxidants: an epidemiological up-date with special attention to carotene and vitamin C. *Am J Clin Nutr* 1993;57(Suppl):787S-97S.
27. Follis RH. Sudden death in children with scurvy. *J Pediatr* 1942;20:347-57.
28. Shafar J. Rapid reversion of electrocardiographic abnormalities after treatment in two cases of scurvy. *Lancet* 1967;2:176.
29. Sament S. Cardiac disorder in scurvy. *N Engl J Med* 1970;282:282.
30. Singh D, Chan W. Cardiomegaly and generalized oedema due to vitamin C deficiency. *Singapore Med J* 1974;15: 60-3.
31. Clemetson CAB. Coronary thrombosis and myocardial infarction. In: Clemetson CAB, ed. *Vitamin C*. 1st ed. Boca Raton: CRC Press, 1989:247-56.
32. Bernier M, Manning AS, Hearse DJ. Reperfusion arrhythmias: dose related protection by anti-free radical interventions. *Am J Physiol* 1989;256:H1344-52.
33. Gey KF. Vitamin E and other essential antioxidants regarding coronary heart disease: risk assessment studies. In: Packer L, Fuchs J, eds. *Vitamin E: Biochemistry and clinical applications*. 1st ed. New York: Dekker, 1992:589-633.
34. Jialal I, Vega GL, Grundy SM. Physiological levels of ascorbate inhibit the oxidative modification of low density lipoprotein. *Atherosclerosis* 1990;82:185-91.
35. Frei B. Ascorbic acid protects lipids in human plasma and

- low-density lipoprotein against oxidative damage. *Am J Clin Nutr* 1991;54:S1113-8.
36. Mulholland CW, Strain JJ. Serum total free radical trapping ability in acute myocardial infarction. *Clin Biochem* 1991;24:437-41.
37. Wagner JR, Motchik PA, Stocker R, Sies H, Ames BN. The oxidation of blood plasma and low density lipoprotein components by chemically generated singlet oxygen. *J Biol Chem* 1993;268:18502-6.
38. Sharma MK, Buettner GR. Interaction of vitamin C and vitamin E during free radical stress in plasma: an ESR study. *Free Radic Biol Med* 1993;14:649-53.
39. Riemersma RA, Wood DA, Macintyre CCA, Elton RA, Gey FK, Oliver MF. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. *Lancet* 1991;337:1-5.
40. Gey KF, Stähelin HB, Eichholzer M. Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemia heart disease and stroke: Basel Prospective Study. *Clin Invest* 1993;71:3-6.
41. Kamada T, McMillan DE, Sternlieb JJ, Björk VO, Otsuji S. Erythrocyte crenation induced by free fatty acids in patients undergoing extracorporeal circulation. *Lancet* 1987;2:818-21.
42. Niki E. Interaction of ascorbate and α -tocopherol. *Ann N Y Acad Sci* 1987;498:186-99.
43. Hu ML, Louie S, Cross CE, Motchnik P, Halliwell B. Antioxidant protection against hypochlorous acid in human plasma. *J Lab Clin Med* 1993;121:257-62.
44. Eddy L, Hurvitz R, Hochstein P. A protective role for ascorbate in induced ischemic arrest associated with cardiopulmonary bypass. *J Appl Cardiol* 1990;5:409-14.
45. Rabl H, Khoschsorur G, Colombo T, et al. A multivitamin infusion prevents lipid peroxidation and improves transplantation performance. *Kidney Int* 1993;43:912-7.