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Changes in α-tocopherol and retinol levels during cardiopulmonary bypass correlate with maximal arterial partial pressure of oxygen

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Abstract
Cardiopulmonary bypass (CPB) is associated with oxidative stress. This study examined antioxidant levels in adults undergoing CPB surgery and their correlation with clinical variables. Arterial blood samples were obtained from 27 patients undergoing CPB. The time-course variation of vitamin C (spectrofluorimetry), α-tocopherol and retinol (HPLC) levels were determined. Plasma vitamin C rose initially but gradually decayed during reperfusion until 60% reduction of baseline values post-surgery. α-Tocopherol and retinol were reduced along CPB with post-operative values ~ 25% lower than baseline. No significant changes were found for selenium and glutathione peroxidase. PaO_2 values rose steadily throughout CPB. A correlation existed for α-tocopherol and retinol depletion vs maximal PaO_2 throughout CPB but no correlation was found for antioxidant consumption vs duration of ischaemia and reperfusion and hypothermia level. In conclusion, consumption of arterial blood antioxidant vitamins occurs with CPB in relation with PaO_2 levels but not for other clinical variables measured in this study.

Keywords: *Cardiopulmonary bypass, oxidative stress, antioxidants, vitamin C, α-tocopherol, retinol*

Introduction

An increasing number of patients are undergoing cardiac surgery and in most cases cardiopulmonary bypass (CPB) is required [1]. This procedure involves diverting blood from the heart and then returning it after oxygenation to a high-capacity artery, thus creating a bypass which functions in series with the circulation to the rest of the body. CPB is an aggressive technique frequently associated with post-surgical complications including myocardial, pulmonary, renal, liver and neurological dysfunction, coagulation disorders and, ultimately, multiple organ failure [2] in which perioperative oxidative stress appears implicated [3–6]. Reactive oxygen species originate from various cellular and enzymatic sources such as myocardial cells, activated neutrophils and endothelial xanthine oxidase [3]. Many studies have described the nature of these oxidant species and the time course of their formation during CPB [3–7]. The oxidative stress is usually the result of an increased formation of reactive oxygen species which is not appropriately balanced by the wide spectrum of endogenous antioxidant defences [8]. Thus, in order

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to counterbalance this sequence of events and to diminish oxidative damage, several studies have investigated the use of exogenous antioxidant supplements during CPB [9–11]. However, although the systemic increase in oxidative stress during CPB and ischemia-reperfusion is well-documented [4–6, 12–14], the relative contribution of the various components of the endogenous antioxidant system and their correlation with clinical variables remains unsettled and continues being the object of further investigation.

In the present study, we have selected ascorbic acid, α-tocopherol, retinol, selenium and glutathione peroxidase activity as relevant markers of endogenous antioxidant defences. These markers were examined in arterial blood samples obtained at different times during adult CPB to study the temporal sequence of oxidative events that occur during this surgical procedure and if any correlation exists with some clinical variables.

Materials and methods

Patients and study design

The study protocol conforms the principles outlined in the Declaration of Helsinki (Edinburgh, 2000) and was approved by the Ethical Committee of the General University Hospital Consortium (Valencia, Spain) and written informed consent was obtained prior to enrolment. A total of 27 patients with elective, first-time cardiac surgery with CPB were included in this study. Cardiovascular and lipid-lowering medication taken by these patients was the conventional therapy for their associated pathologies and not reported to affect antioxidant levels. The patients enrolled in this study showed no indication of impaired nutritional status according to physical examination, blood cells and chemistry and absence of significant previous weight loss. All patients reported standard dietary habits. Patients had received no dietary supplements including vitamins or antioxidant drugs and had no evidence of a systemic inflammatory response (C-reactive protein ≤ 10 mg/l) [15]. Patients receiving blood transfusions before or during surgery were excluded. Patients were given antibiotic prophylaxis before surgery.

After standard intravenous anaesthesia, CPB was established by cannulation of the ascending aorta and right atrium, with moderate hypothermia and haemodilution as previously outlined [16]. Myocardium was preserved with cold anterograde-retrograde crystalloid multidose cardioplegia using a standard potassium-based solution (Cardibraun®, Braun, Melsugen, Germany; osmolarity 657 mOsm/l) mixed with blood at a ratio of 1:4. Surgery was performed using the intermittent cross-clamp technique. After decannulation, heparin was neutralized with protamine chloride (1:1 ratio).

Blood samples were collected from the radial artery or from the arterial side of pump during the extracorporeal circulation as follows: (i) baseline, immediately after cannulation of the arterial system before cardiac surgery (T_0); (ii) 3 min after initiating CPB but before aortic clamp (T_1); (iii) during the ischemic period of CPB, at 3 and 30 min after aortic cross-clamping (T_2, T_3); (iii) during reperfusion, with samples obtained 10 min before (T_4) and 15 min after (T_5) switching pump off; and (iv) 3 h after cessation of CPB with the patient within the intensive care unit (T_6). Blood was drawn into plastic syringes, immediately transferred to tubes with or without EDTA (Vacutainer, Becton Dickinson, Inc., Oxnard, CA) as appropriate, placed on ice and protected from daylight. Samples with hemolysis were excluded from analysis. Blood samples were processed within 10 min after sampling to avoid auto-oxidation of endogenous antioxidants [3]. After centrifugation at 3000 g for 10 min at 4°C, the supernatant was distributed into aliquots and tubes were immediately frozen and stored at -20°C until analysis. Measurements were performed within 4 h after blood sampling for vitamin C, 48 h for selenium and within 14 days for the rest of the analytes.

In separate experiments, we demonstrated that analyte concentrations remained stable during this time of storage (not shown). A separate aliquot of EDTA-blood was centrifuged in microcapillaries (10 000 g for 3 min, 22°C) for the determination of the hematocrit (HCT, ratio of erythrocytes to total blood volume). Correction to account for the haemodilution secondary to the pump-priming solution used for the extracorporeal CPB circuit was necessary. Thus, the concentrations of analytes at a particular time point were multiplied by the ratio of the initial hematocrit to the hematocrit at the specified time.

Analytical methods

Plasma vitamin C (ascorbate and dehydroascorbate) was analysed in *meta*-phosphoric acid-stabilized plasma by spectrofluorimetry [17]. Serum retinol and α-tocopherol were quantified by ultraviolet detection at 292 nm after extraction into hexane and separation by reversed-phase high performance liquid chromatography [18]. Plasma selenium was determined by flameless atomic absorption spectrophotometry (Perkin Elmer series 4100ZL, with Zeeman background correction and stabilized temperature platform furnace STPF) [19]. Plasma glutathione peroxidase (GPx) activity was estimated using a spectrophotometric assay (340 nm) according to the FLAIR protocol [20].

In additional experiments we assured that (i) concentrations of the different analytes in the samples were above the limit of detection of the corresponding

techniques, (ii) intra- and inter-assay coefficients of variation of the assays were below 5 and 10%, respectively, and (iii) determinations repeated at intervals along a 12 h period of time after samples were unfrozen yielded reproducible values with variation below 5% (not shown).

Statistical analysis

Values are expressed as mean ± SD. Normality of variable distribution was assessed by the Shapiro-Wilk test. One-way ANOVA was used to test the time-dependent change in oxidative stress markers. *Post-hoc* comparisons were performed with Bonferroni adjustment for multiple comparisons. Correlations between variables were assessed by linear regression analysis. Statistical analysis was carried out using the software SPSS 9.0 for Windows (SPSS Inc., IL). $p < 0.05$ was considered significant.

Results

Patient characteristics

The main clinical characteristics of patients and a summary of their operative data are shown in Table I.

Table I. Clinical and operative data of 27 adult patients undergoing cardiac surgery with cardiopulmonary bypass (CPB).

Clinical data	
Age (years; mean ± SD)	64.7 ± 9.36
Sex, male:female (%)	19 (70.4):8 (29.6)
Body weight (kg)	77.2 ± 14.9
Height (cm)	164 ± 10
Body surface (m^2)	1.83 ± 0.23
Smoking status, no:yes:ex-smoker:lost (%)	14 (51.9):4 (14.8): 8 (29.6):1 (3.7)
Alcohol consumption, no:yes:lost (%)	25 (92.6):1 (3.7):1 (3.7)
NYHA class[a], II:III:IV:lost (%)	2 (7.4):19 (70.4): 1 (3.7):5 (18.5)
ASA class[b]	21 (77.8):1 (3.7):5 (18.5)
Associated pathologies[c,d]	4:5:9:5:3:4
Operative data	
Surgical procedure[e], CABG:VR	20:7
CPB time (min)	116 ± 36
Aortic cross-clamp time (min)	77 ± 26
Reperfusion time (min)	39 ± 19
Temperature during hypothermia	29.8 ± 1.9
Maximal PaO_2 (mmHg)[f]	387 ± 76
Administered volume[g] (ml)	3341 ± 474

[a] New York Heart Association; [b] American Society of Anesthesiology; [c] insulin-dependent diabetes:non-insulin-dependent diabetes: hypercholesterolemia:hypertension:chronic obstructive pulmonary disease; [d] the total number of cases is greater than 27 because some patients have more than one associated pathology; [e] coronary artery bypass grafting:valve replacement; [f] Maximal PaO_2 values significantly increased after oxygenation from baseline values (T_0) of 85.4 ± 12 up to 280 ± 63 (T_1), 325 ± 60 (T_2), 370 ± 72 (T_3), 387 ± 76 (T_4) and 373 ± 78 (T_5) and decreased post-surgery to 122 ± 35 (T_6); [g] cardioplegic solution and priming of the pump.

CPB is associated with changes in endogenous antioxidant levels

Pre-operative levels of vitamin C in the population studied were mostly normal except for 3 patients (11%) with marginal deficiency status (values close to 20 μM) [4,21]. Vitamin C levels tended to increase during the initial period of CPB and ischemia followed by a gradual decrease during reperfusion (Figure 1A). Post-surgery haemodilution-corrected ascorbate levels were ~60% lower than baseline levels.

All patients in our study showed serum α-tocopherol and retinol pre-operative values within normal limits [22]. Serum α-tocopherol was reduced from early stages of CPB and during ischemia and reperfusion with the lowest valued reached after surgery (Figure 1B). Post-surgery haemodilution-corrected α-tocopherol levels were ~24% lower than baseline levels. Similar changes were observed for serum retinol with ~27% post-surgical decrease compared to pre-operative values (Figure 1B).

Plasma selenium and GPx activity did not significantly change during and after CPB (Figure 2).

Correlations between changes in plasma levels of antioxidants

The observed changes in plasma vitamin C concentrations were correlated with the corresponding changes in plasma α-tocopherol ($p = 0.043$) but the r value obtained ($r = 0.146$) indicates a weak correlation (Figure 3A). Correlation between vitamin C and retinol did not reach statistical significance ($p = 0.058$, $r = 0.138$) (Figure 3B). A significant correlation was found between plasma changes of α-tocopherol and retinol ($p = 0.0001$, $r = 0.566$) (Figure 3C).

There was a direct correlation between pre-operative vitamin C concentrations and the extent of its decrease from baseline in absolute values after CPB ($p = 0.002$, $r = 0.633$); i.e. the higher the pre-operative value, the greater the corresponding post-operative fall in plasma vitamin C. By contrast, no significant correlation was found between pre-operative values and their corresponding amounts of loss at post-operative time points for α-tocopherol ($p = 0.325$, $r = 0.196$) and retinol ($p = 0.669$, $r = 0.092$).

Correlations between post-operative loss in plasma antioxidants and clinical parameters

The amount of loss of vitamin C after cardiac surgery did not correlate with CPB time ($p = 0.372$, $r = 0.189$), ischemic time ($p = 0.257$, $r = 0.236$), reperfusion time ($p = 0.896$, $r = 0.030$), hypothermia ($p = 0.553$, $r = 0.128$) or PaO_2max ($p = 0.125$, $r = 0.315$; Figure 4A). The amount of loss of α-tocopherol and retinol was significantly correlated with PaO_2max

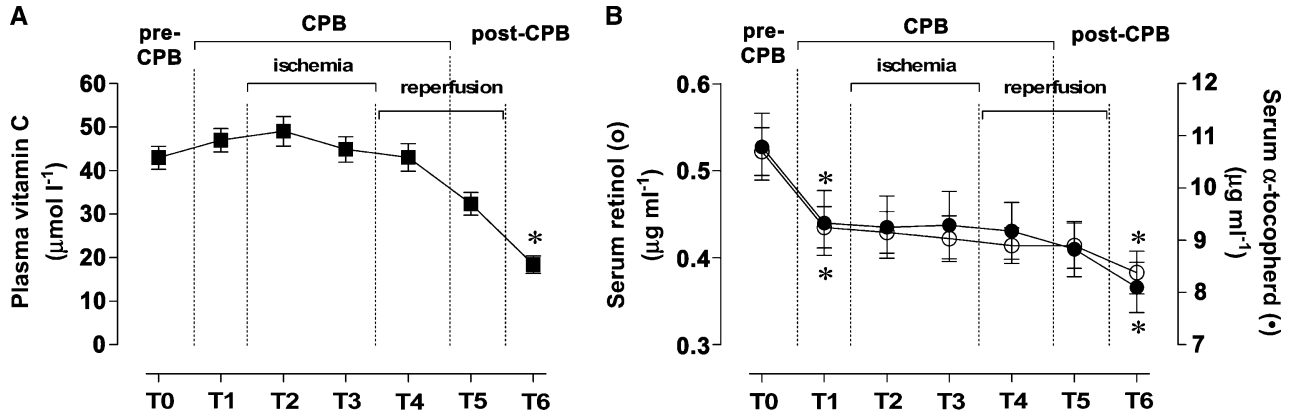

Figure 1. Time-course of changes in concentration of vitamin C (A) and α-tocopherol and retinol (B) in arterial blood from adult patients subjected to cardiac surgery with bypass (CPB). Samples were obtained pre-operatively (T_0), after commencing the CPB (T_1), during ischemia (T_2, T_3) and reperfusion (T_4, T_5) and after surgery (T_6), as indicated in Methods. Data are mean ± SD of 27 patients. $^{\star}p < 0.05$ from baseline values.

($p = 0.047$, $r = 0.395$ and $p = 0.014$, $r = 0.467$, respectively) but did not correlate with duration of CPB, ischemia and reperfusion nor with hypothermia (Figure 4B and C).

Discussion

In the present study, we examined the temporal profile of arterial blood antioxidant vitamins in a cohort of 27 adult patients undergoing elective heart surgery with CPB and compared these changes to some clinical variables. The intermittent clamp technique used during CPB imposes an initial period of hypoxic-ischemic insult followed by reperfusion-reoxygenation damage, both likely to involve the early generation and release of oxidant species followed at a later stage by an inflammatory reaction [3,4]. In addition, extracorporeal circulation involves contact of blood with foreign surfaces which also leads to free radical generation and inflammation. Increased oxidant burden throughout CPB may overwhelm endogenous antioxidant defences and cause damage [3].

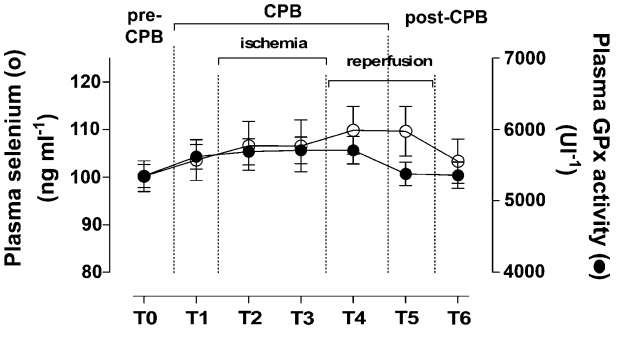

Figure 2. Time-course of changes in concentration of selenium and glutathione peroxidase (GPx) in arterial blood from adult patients subjected to cardiac surgery with cardiopulmonary bypass (CPB). Samples were obtained pre-operatively (T_0), after commencing the CPB (T_1), during ischemia (T_2, T_3) and reperfusion (T_4, T_5) and after surgery (T_6), as indicated in Methods. Data are mean ± SD of 27 patients.

We have selected vitamins like ascorbate, α-tocopherol and retinol, the trace element selenium and the selenoprotein GPx as relevants in defence against oxidant stress, although other key antioxidants have been recently studied in cardiac surgery [4]. Vitamin C appears as a particularly sensitive target of CPB-mediated oxidation in patients. Thus, plasma ascorbate levels were found markedly diminished in adult [23] and paediatric [4] patients after CPB. Others found that ascorbate concentrations, corrected for haemodilution, rose initially from those prior to surgery, remained elevated throughout CPB and returned to pre-operative levels by 24 h post-surgery [21]. We found an initial rise in plasma vitamin C followed by progressive decrease during reperfusion and a marked loss (~60% decrease from baseline values) after surgery. The transient increase in vitamin C levels at the start of CPB may be due to its release from body stores as an early response to oxidant stress [24]. Alternatively, this augmentation may simply reflect the release to plasma of vitamin C from blood cells damaged by the extracorporeal circulation since blood cells have high intracellular levels of this antioxidant [25]. A direct correlation was found between pre-operative vitamin C concentrations and its decrease after surgery; although this relation has not been described by others, a similar finding was reported for total plasma antioxidant status [26].

α-Tocopherol has been reported not to change significantly during CPB [4] or to decrease after reperfusion or after surgery [3]. By contrast, a transient increase of α-tocopherol at the end of CPB has been also reported [13]. We found a decrease of serum α-tocopherol throughout CPB and after surgery. Changes in retinol levels during CPB have been scarcely studied. No change of plasma retinol during CPB with decrease at 4 h post-surgery [3] or decrease during CPB [27] have been reported. We found a decrease in serum retinol

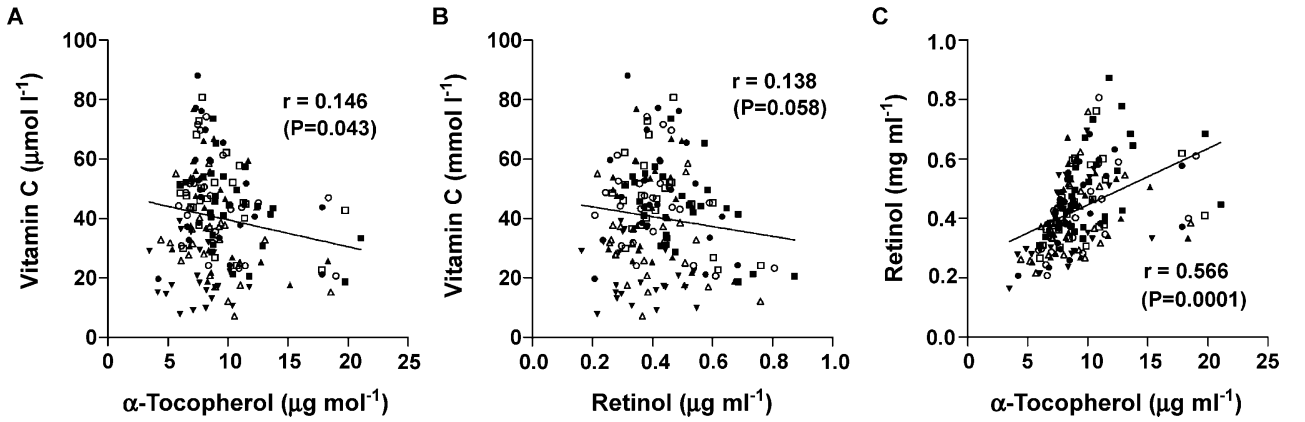

Figure 3. Correlations between the plasma levels of vitamin C, α-tocopherol and retinol at different stages of cardiopulmonary bypass surgery as shown in A, B and C. Points represent the absolute values at T_0 (■),T_1 (□),T_2 (●),T_3 (○), T_4 (▲),T_5 (△) and T_6 (▼) as indicated in the legend of Figure 1 and Methods ($n=189$ points in each graph).

which parallels that of α-tocopherol. Since we have not studied oxidant damage, it is not clear whether decreases of α-tocopherol and retinol indicate consumption of these lipid soluble vitamins or changes in their distribution pattern [28,29].

Plasma selenium was reported to decrease at 3 h after reperfusion but values were uncorrected for haemodilution [30]. Plasma GPx activity was found increased [3] or not changed [30,31] during CPB. We found no significant change for corrected values of plasma selenium and GPx during CPB and after surgery compared to pre-operative values.

The correlations between changes in endogenous antioxidant defences and clinical variables of CPB have been scarcely reported. In the present study, a significant correlation was found between maximal levels of PaO_2 during CPB and the extent of α-tocopherol and retinol loss after surgery. A similar tendency existed for vitamin C consumption but this correlation failed to reach significance. There is concern about whether excessive oxygenation in cardiac surgery induces oxygen-derived free radicals [32]. In fact, hyperoxic CPB in adults results in oxidative myocardial damage [33]. The basis for the relationship between maximal PaO_2 values and lipid soluble antioxidant vitamin levels found in this study remain uncertain since we did not investigate oxidant damage in this study. By contrast, we did not find any significant correlation between the amounts of loss in plasma antioxidant concentrations of vitamin C, α-tocopherol and retinol vs the duration of CPB, the duration of the ischemia and reperfusion periods and the degree of hypothermia. Although the duration of CPB has been shown to be an independent factor of CPB-associated post-operative complications [34], others have also failed in finding a correlation between total plasma antioxidant status and duration of CPB

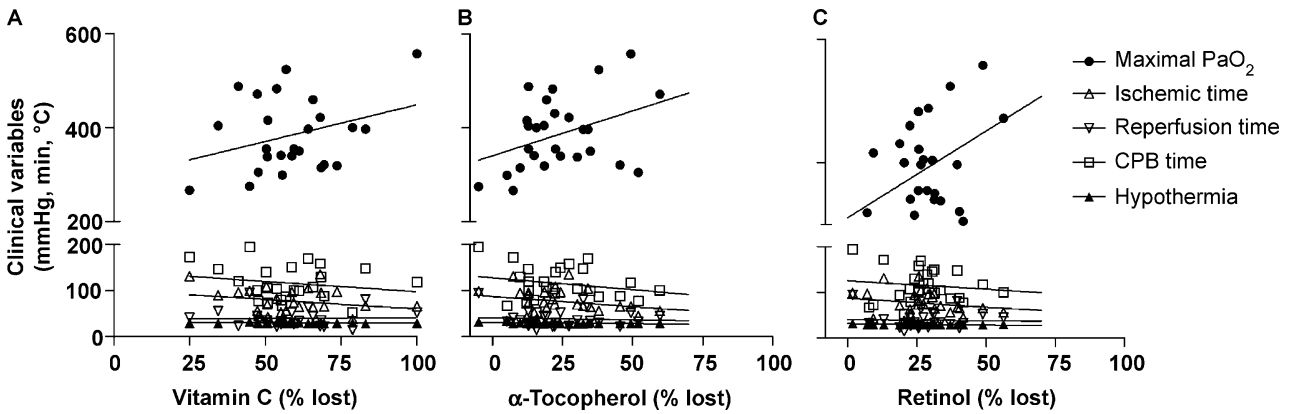

Figure 4. Correlations between the lost in plasma levels of vitamin C (A), α-tocopherol (B) and retinol (C) expressed as a percentage of the initial values and the maximal values of PaO_2 (mmHg; ●), ischemic time (min; △), reperfusion time (min; ▽), CPB time (min; □) and hypothermia (°C; ▲) during surgery for each patient ($n=27$ points for each clinical variable in each graph). (A) The loss of vitamin C did not correlate with PaO_2 ($p=0.125$, $r=0.315$), ischemic time ($p=0.257$, $r=0.236$), reperfusion time ($p=0.896$, $r=0.030$), CPB time ($p=0.372$, $r=0.189$) and hypothermia ($p=0.553$, $r=0.128$). (B) The loss of α-tocopherol correlates with PaO_2 ($p=0.047$, $r=0.395$) but not with ischemic time ($p=0.199$, $r=0.255$), reperfusion time ($p=0.704$, $r=0.078$), CPB time ($p=0.268$, $r=0.226$) and hypothermia ($p=0.064$, $r=0.382$). (C) The loss of retinol correlates with PaO_2 ($p=0.014$, $r=0.467$) but not with ischemic time ($p=0.348$, $r=0.188$), reperfusion time ($p=0.886$ $r=0.030$), CPB time ($p=0.537$, $r=0.127$) and hypothermia ($p=0.076$, $r=0.354$).

but differentiation among endogenous antioxidants was not reported [26].

A limitation of our study is that we have not searched for direct evidences of oxidative injury by measuring biomarkers of lipid peroxidation [5]. Nevertheless, the progressive decrease of plasma non-enzymatic antioxidant levels from baseline is likely to reflect their consumption in relation to oxidant stress elicited by free radicals generated during the hypoxia and reoxygenation of myocardium. In fact, several clinical studies have examined the potential benefits of pre-operative antioxidant supplementation with vitamins C and E [10,35,36] as well as with other antioxidants [9,11] in the setting of CPB.

In summary, the present study shows that a significant consumption of plasma antioxidant vitamins occurs during and after CPB in adult patients, which correlates with arterial partial pressure of oxygen but not with other clinical variables measured in the present study. Optimization of surgical procedures, nutritional status and therapeutic interventions with antioxidant vitamins should therefore be considered as strategies to lower the rate of post-operative complications in patients undergoing cardiac surgery with CPB.

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