# The effect of vitamin A, vitamin C, and vitamin E levels on reperfusion injury during coronary bypass surgery

**Ali SARIGÜL, Selim İSPİR, Şükrü MERCAN, Bora FARSAK, Rıza DOĞAN, Metin DEMİRCİN, İlhan PAŞAOĞLU**

Dept. of Thoracic and Cardiovascular Surgery, Medical School of Hacettepe University, Ankara, TURKEY

*During open heart operations, by the effect of cardiopulmonary bypass, a large number of reactive oxygen species, cytokines, and cytotoxic materials are released. Reactive oxygen species are extremely important for reperfusion injury in open heart operations. Reperfusion injury is the tissue necrosis after reexposition of tissue to oxygen at the end of cardiopulmonary bypass. There is multi-defence system against reperfusion injury in the body including enzymes, endogeneous anti oxidants, and exogenius dietary anti-oxidants. In the present study we searched for blood levels of vitamin A, vitamin E, and vitamin C during coronary bypass operations. Ten patients undergoing coronary artery bypass grafting (CABG) are selected and serum levels of the three parameters before, during and after the operative compared. Results are corrected for the hemodilution during CPB and the leveis of the vitamin A are more or less the same before, during and after the operation whereas that of the vitamin E is slightly decreased. Vitamin C levels, unlike the other two parameters, decreased significantly (P<0.025) during and after the operation. It is concluded that, Vitamin E prophylaxis might be helpful for the low risk patients, therefore especially for those who require emergency CABG, Vit. C prophylaxis, and its addition to cardioplegic solutions is very important for reperfusion injury.*
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**Key Words:** Reperfusion injury, Open heart operations, Antioxidants, Vitamin, Coronary bypass surgery

In cardiovascular surgery, the effects of cardiopulmonary bypass (CPB) on heart is very critical. The whole-body inflammatory reaction produced by CPB 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, cytokines, and other cytotoxic materials. Among these harmful factors, the generation of reactive oxygen species has gained a major interest (1-5). Reexposure of the tissue to oxygen at the end of CPB produces a so-called "reperfusion injury", which is caused by reactive oxygen species. A multidefense system against injury induced by reactive oxygen species including enzymes (superoxide dismutase) (6,7), endogenous antioxidants (glutathione and serum proteins) (8,9), exogenious dietary antioxidants (e.g. vitamin C, E and carotenoids) (10) protects the body against radical induced injuries.

The purpose of this study was to determine whether there is a depletion of vitamin C, vitamin E and vitamin A which could account for the harmful effects of reactive oxygen species in organ dysfunction after CPB.

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**Correspondence:** Ali SARIGÜL
Dept. of Thoracic and
Cardiovascular Surgery,
Medical School of Hacettepe University,
06100 Ankara, TURKEY

## MATERIALS AND METHODS

Ten patients undergoing coronary artery bypass operations were studied. Written informed consent was obtained. The patients age were between 41 and 71 (mean 55.50) 7 were men and 3 were women. Characteristics of the patients, are given in Table 1.

CPB was established with a single two-stage right atrial venous cannula and an arterial cannula in the ascending aorta. During bypass, moderate hemodilution (hematocrit 22% to 24%) and moderate hypothermia (nasopharyngeal temperature of 26°C) were used. A membrane oxygenator (polystan) and as arterial filter between the pump and the patient were used in all subjects. A roller pump (Shiley-Stockert, Munich Germany) provided a flow rate of 2.4 L/min/$m^2$ at normothermia and of 1.2 L/min/$m^2$ at hypothermia.

Blood samples were drawn at intervals of;

51) on admission
52) after induction of anesthesia
53) 30 min after the start of operation
54) after 60 min of CPB
55) Immediately after CPB
56) 24 hours after CPB
57) at dismissal

**Table 1.** Characteristics of the patients.

| | Mean | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|
| Patient's age | 55.50 | 10.61 | 41 | 71 |
| Aortic clamp time | 20.70 min | 7.69 min | 9 min | 35 min |
| CPB time | 28.40 min | 10.05 min | 12 min | 47 min |
| Number of the bypassed grafts | 2.90 | 1.37 | 1 | 5 |

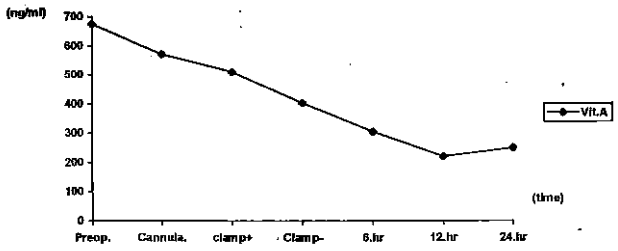


**Figure** 1. Individual plasma vitamin A concentration before, during and after CABG.

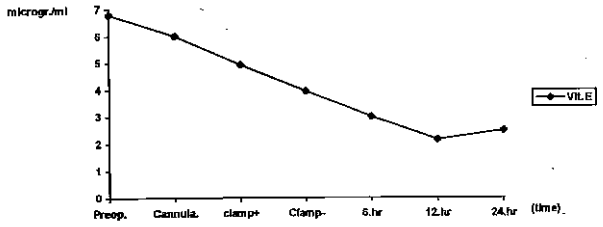


**Figure** 2. Individual plasma vitamin E concentration before, during and after CABG.

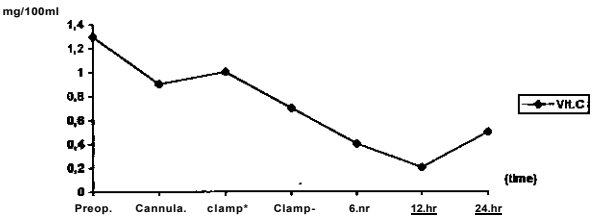


**Figure 3.** Individual plasma vitamin C concentration before, during and after CABG.

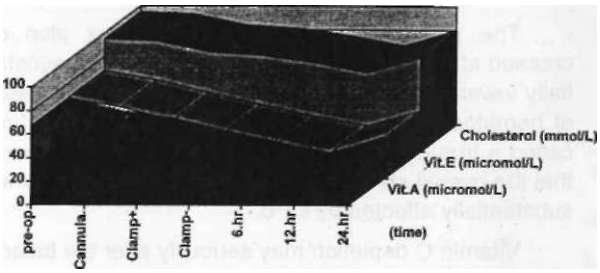


**Figure** 4. CPB related time course of plasma concentration of lipid soluble essential antioxidants and of cholesterol.

For analysis of plasma antioxidant concentration, 10 ml of heparinized blood was centrifuged 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 by high performance liquid chromotography (HPLC) as described by Brubacher (11). Vitamin E and vitamin A (retinol) were extracted into n-hexane and analyzed by HPLC with a fluorimetric detector (extinction 290 nm, emission 330 nm) (12). Plasma cholesterol and triglycerides were determined enzymatically by an automated analyzer. Plasma vitamin E determined enzymatically by an automated analyzer. Plasma vitamin E concentrations were expressed as lipid standardized values because they are dependent on the concentrations of cholesterol and triglycerides that are carriers. Hemodilution of vitamin E, vitamin A and vitamin C were controlled for by taking into account the hematocrit, creatinine, urea and uric acid values. The MB isoenzyme of creatinekinase (CK-MB) was measured by an antibody inhibition technique (CK-MB Boehringer Mannheim, Montreal).

## RESULTS

The characteristics of the subjects are summarized in Table 1.

Plasma antioxidant status on hospital admission and the effects of CPB are shown in Fig. 1 to 4.

Initial vitamin A concentrations on hospital admission were on avarage about 0.5 urnol/L. Plasma vitamin A concentrations decreased during CPB and had not fully regained the preoperative level at dismissal. There was no statistical difference between S2, S3, S4 and S5. But for the rest values the difference was significant ($P<0.025$). The plasma vitamin C concentration were in average about 40 umol/L (36.0±19.0 umol/L). During and after CPB vitamin C concentrations decreased strongly.

The plasma vitamin E concentration dropped by about 40% or more during CPB when the hemodilution of vitamin E is not accounted for. But there was no significant time effect in lipid standardized plasma vitamin E concentrations at any time during and after CPB.

## DISCUSSION

The generation of reactive oxygen species is a major pathogenetic factor for tissue damage resulting from CPB. In the myocardium, however there are series of de-

fense mechanisms able to protect the cell against the cytotoxic oxygen metabolites. These include the enzyme superoxide dismutase (SOD) (6,7) and glutathione peroxidase (GPD) (5), plus other endogenous antioxidants like -tocopherol, vitamin C- carotene and cysteine (10). In the present study, therefore we have investigated the effects of CPB on plasma concentrations of the vitamin C, E and carotene in patients with coronary artery disease undergoing cardiac operations.

The present data confirm that vitamin E concentration were not decreased during and after CPB. Several other investigators found that when hemodilution and lipid-standardized values were not expressed they show that vitamin E concentrations decreased significantly. However, when the vitamin E concentrations were expressed in lipid-standardized values, taking into account plasma cholesterol and triglycerids, that are the carriers of lipid-soluble vitamins and hemodilution and was corrected by means of the hematocrit, they found no relevant decrease in vitamin E concentrations during and after CPB. We believe that only the lipid or dilution adjusted vitamin E value is indicative for the biologic vitamin E status and our results suggest that CPB does not affect the vitamin E status.

The plasma vitamin A concentrations also decreased during CPB. Beside vitamin E, we also searched for vitamin A concentrations in lipid standardized values and found some relevant decrease in vitamin A concentrations during and after CPB. The present interpretations of vitamin A and vitamin E plasma concentrations are accordance with that of Ballmer and coworkers who found no decrease in plasma vitamin A and E concentrations during and after CPB (8).

The plasma vitamin C concentrations also decreased at the begining of CPB and it was also substantially lower when hemodilution was corrected by means of hematocrit level, urea, uric acid and creatinine, indicating a true depletion of this vitamin. Our conclusion is that the overall status of vitamin C in the whole body was substantially affected by CPB.

Vitamin C depletion may seriously alter the balance of the entire antioxidant defence system and render the organism to increased oxidative stress during CPB. Because poor vitamin C concentration is associated with rhythm problems and cardiac failure, vitamin C depletion after CPB might play an important factor in the induction of postoperative cardiac complications.

Yau and cowokers found that pretreatment with alpha-tocopherol had a small but significant effect on tissue protection compared with placebo and suggested that oral vitamin E would not improve myocardial function for low-risk patients but it might be beneficial for the high risk patients such as those requiring urgent coronary revascularisation (13).

The present findings of substantial vitamin C depletion are an extension of studies on plasma vitamin C concentrations.

Critically low vitamin C concentrations after CPB suggested that in most patients it was utilised in large quantities and that even deeper body stores including the myocardium were likely to have been defected. This consumption might have occurred by direct reaction with reactive oxygen species or by regeneration of other antioxidants like vitamin E. When taking into account that the overall status of liposoluble antioxidants they have no major concern during CPB.

As it was reported by Eddy, Hurvitz and Horchstein intravenous injection at vitamin C before CPB and addition to cardioplegic solutions might be beneficial (14). Our conclusion according to the present data is, vitamin E and vitamin C together are promising candidates for prophylactic and therapeutic interventions in clinical conditions during CPB related reperfusion injury.

**Vitamin A, vitamin C ve vitamin E seviyelerinin koroner bypass ameliyatı sırasındaki reperfüzyon hasarı üzerindeki etkisi**

*Açık kalp ameliyatları sırasında; kardiopulmoner bypass'ın etkisiyle çok sayıda reaktif oksijen türevleri, sitokinler ve sitotoksik materyaller salınır. Reaktif oksijen türleri açık kalp ameliyatındaki reperfüzyon hasarı için son derece önemlidir. Reperfüzyon hasarı kardiopulmoner bypass sonunda, dokunun oksijenle karşılaşması sonrası doku nekrozudur. Vücutta reperfüzyon hasarına karşı enzimler; endojen antioksidanlar ve ekzojen diyet antioksidanlarmı içeren çoklu savunma sistemi vardır. Mevcut çalışmada koroner bypass ameliyatları sırasında vitamin A, vitamin E ve vitamin C'nin kan seviyelerini araştırdık. Koroner arter bypass greftleme (KABG) yapılan 10 hasta seçildi ve operasyon sırasında ve sonrasında 3 parametrenin serum seviyeleri karşılaştırıldı. KABG sırasında sonuçlar hemodilüsyon açısından kontrol edildi ve vitamin E seviyeleri hafifçe düşmesine rağmen; vitamin A seviyeleri az veya çok öncesiyle aynıydı. Vitamin C seviyeleri operasyon esnasında ve sonrasında diğer iki parametreden farklı olarak belirgin olarak düştü (p<0.025). Sonuç olarak, vitamin E profilaxisi düşük riskli hastalar için faydalı olabilir. O halde özellikle acil KABG'e ihtiyaç duyanlar için vitamin C profilaxisi ve kardioplejik solüsyonlara eklenmesi reperfüzyon hasarı için çok önemlidir.*
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