

# Decrease of Total Antioxidant Capacity during Coronary Artery Bypass Surgery

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## Abstract

**OBJECTIVE:** Cardiac surgery induces an oxidative stress, which may lead to impairment of cardiac function. In this study, we aimed to measure the changes of oxidative and antioxidative status of patients undergoing coronary artery bypass surgery (CABG).

**MATERIALS AND METHODS:** We studied 79 patients who underwent CABG with and without cardiopulmonary bypass (CPB). Of the 79 patients, 39 had CPB and 40 did not. Blood samples were drawn before, during, and after the surgery. Antioxidant status was evaluated by measuring total antioxidant capacity (TAC), and oxidative status was evaluated by measuring total peroxide (TP) levels and oxidative stress index (OSI).

**RESULTS:** TP and OSI levels increased, while TAC decreased progressively after the beginning of surgery, for all patients. There were negative correlations between TAC levels and aortic cross-clamping period and anastomosis time ( $r = -0.553$ ,  $p < 0.001$  and  $r = -0.500$ ,  $p < 0.001$ , respectively). In addition, there was a positive correlation between TAC and ejection fraction ( $r = 0.647$ ,  $p < 0.001$ ).

**CONCLUSIONS:** During CABG, oxidant and OSI levels significantly increase and TAC significantly decreases. This situation is influenced by long CPB and anastomosis time, and also by low ventricular ejection fraction. We concluded that the patients who undergo CABG are exposed to potent oxidative stress that impairs their TAC. We speculate that supplementation with antioxidant vitamins such as vitamins C and E may be beneficial for patients undergoing CABG.

**Key Words:** Antioxidant capacity, cardiac surgery, coronary artery bypass grafting, oxidative stress.

## Introduction

ISCHEMIA-REPERFUSION INJURY may cause damage to the myocardium following blood flow restoration after a critical period of coronary occlusion (1). In fact, ischemia-reperfusion is a clinical problem associated with procedures such as thrombolysis, angioplasty, and coronary bypass surgery, which are commonly used to reestablish the blood flow and minimize damage to the heart due to severe myocardial ischemia. Two main hypotheses, namely oxidative stress and  $Ca^{2+}$ -overload, have been proposed to explain the pathogenesis of ischemia-reperfusion injury (2, 3).

Oxidative stress, which is usually associated with increased formation of reactive oxygen species (ROS), modifies phospholipids and proteins, leading to lipid peroxidation and oxidation of thiol groups (4, 5). These lipids and thiol groups

are closely linked to inflammatory responses, including complement activation, release of cytokines, and leukocyte activation, along with expression of adhesion molecules (6). Many studies have described the nature of these ROS and the time course of their formation during cardiopulmonary bypass (CPB) (7). The nature of these oxidative events leads to depletion of plasma antioxidants, increased lipid peroxidation, and formation of other damaging metabolites (1, 8, 9). To learn how to counterbalance this sequence of events and diminish oxidative injury, several studies have investigated the use of antioxidant supplements during extracorporeal circulation (ECC) (10, 11).

Antioxidant molecules prevent and/or inhibit these harmful reactions (12). Serum (or plasma) concentrations of various antioxidants can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive, and costly, and they require complicated techniques. Because measuring different antioxidant molecules separately is not practical, and their antioxidant effects are additive, total antioxidant capacity (TAC) of a sample is measured (13).

In the last few years, off-pump coronary artery bypass surgery (CABG) has gained widespread acceptance as an alternative technique to conven-

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tional on-pump CABG. It has moreover been postulated that off-pump surgery of CPB avoids myocardial ischemia-reperfusion and significantly reduces the postoperative systemic inflammatory response and other biological derangements and, possibly, may improve the clinical outcomes (14). There are only a few studies comparing the degree of oxidative stress of patients with on-pump vs. patients with off-pump technique, and the results of these studies are debatable (6, 15–17).

In this study, we aimed to measure and compare oxidative and antioxidative status in patients undergoing off-pump and on-pump CABG.

## Methods

### Patients

Seventy-nine patients elected CABG. Preoperative and postoperative patient data were reviewed using registry databases, medical notes, and charts. Thirty-nine patients (group A) underwent CABG using the CPB technique while 40 patients (group B) were operated on using the off-pump technique. Selection of either technique was made by the individual surgeon, based on his experience and preference.

All patients were on standard anti-anginal treatment, and they all stopped taking aspirin 7 days before the operation. The study was approved by the local ethics committee, and informed consent was obtained from all patients. Patients' data are summarized in Table 1.

**TABLE 1**

*Perioperative and Operative Characteristics of Patients Undergoing Coronary Artery Bypass (CPB) Grafting*

Characteristic	Group A (n=39)	Group B (n=40)	p
Age (years)	59.66 ±13.80	63.57±12.72	0.45
Female sex (%)	43.6	37.5	0.58
Current smoker (%)	51.2	67.5	0.14
Diabetes mellitus (%)	17.9	20.0	0.81
Hypertension (%)	38.5	45.0	0.55
Chronic obstructive pulmonary disease (%)	53.8	60.0	0.58
Anastomosis/patient (mean ± SD)	2.03 ± 0.7	1.93 ± 0.7	0.91
Distal anastomosis time (min ± SD)	25.5 ± 4.5	17.5 ± 5.7	0.16
CPB time (min ± SD)	74.3 ± 6.2		
Length of stay in intensive care unit (h ± SD)	15.55±1.58	14.73±1.35	0.46
Length of stay in hospital (d ± SD)	7.33 ± 1.11	7.44 ± 1.14	0.91

### Anesthesia and Operative Technique

All routine cardiac medications were continued up to the morning of surgery. After premedication of 5 mg midazolam intramuscularly and 0.18 mg/kg of morphine diluted in 4 mL were given intrathecally for postoperative analgesia, anesthesia was induced using 0.3 mg/kg of etomidate and 1 µg/kg of remifentanyl and 0.6 mg/kg of rocuronium intravenously. After endotracheal intubation, desflurane (3–10%) and remifentanyl 0.25–1.0 µg/kg/min in air/oxygen and rocuronium were given to maintain the anesthesia.

After the standard median sternotomy, aorta-right atrial cannulation and cardiopulmonary bypass were performed in on-pump patients. During CPB, hematocrit, mean arterial pressure, and pump flow were kept between 20 and 30%, 50–80 mm Hg, and 2.2–2.5 L/m<sup>2</sup>, respectively. Adequacy of tissue perfusion was monitored by arterio-venous partial carbon dioxide difference (P<sub>v-a</sub> CO<sub>2</sub>), urine output, and base deficit. Patients were cooled to 32°C with moderate hypothermia. Desflurane-remifentanyl anesthesia was administered during CPB. Revascularization procedures were performed with aortic cross-clamping. During myocardial ischemia antegrade cold hyperkalemic crystalloid cardioplegia was used (Plegisol®, Abbot Laboratories, IL). After completion of distal anastomosis, the proximal anastomosis was performed to the ascending aorta by using a side-biting clamp.

Left internal mammary artery and saphenous vein grafts were harvested for off-pump patients. To provide better access to lateral and posterior target vessels, the pericardium was retracted by two or three deep sutures and two sponges were placed under the heart. Neither a heart stabilizer nor intraluminal shunts were used. Silicone snare sutures were placed proximal and distal to the anastomosis, in order to provide a bloodless field. Remifentanyl infusion and desflurane were discontinued at skin closure, and all patients were extubated in the operating room.

### Samples

Blood samples were drawn from the central venous catheter immediately before, midway through and at the end of the surgery, as well as 24 h and 48 h after cessation of CABG. The obtained heparinized blood was immediately stored on ice at 4°C. The plasma was then separated from the cells by centrifugation at 3,000 rpm for 10 min, and the plasma samples were stored at –80°C until analysis.

## Measurement of TAC

The TAC of the plasma was measured using a novel, automated, colorimetric measurement method developed by Erel (12). In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution, which is present in Reagent 1, is mixed with hydrogen peroxide, which is present in Reagent 2. The sequential produced radicals, such as brown-colored dianisidine radical cation, produced by the hydroxyl radical, are also potent radicals. In this assay, the antioxidative effect of the sample against the potent free radicals' reactions, which is initiated by the produced hydroxyl radical, is measured. The assay results are expressed as mmol Trolox equivalent/L; the precision of this assay is excellent. Accurate measurements of TAC can be obtained in as little as 10 min, making this assay eminently suitable for the clinical biochemistry laboratory (18).

## Measurement of Total Peroxide Concentration

Total peroxide (TP) concentrations were determined using the FOX2 method with minor modifications (18, 19). The FOX2 test system is based on oxidation of ferrous ion to ferric ion by various types of peroxides contained within the plasma samples, to produce a colored ferric-xylenol orange complex whose absorbency can be measured. The FOX2 reagent was prepared by dissolving ammonium ferrous sulfate (9.8 mg) in 250 mM H<sub>2</sub>SO<sub>4</sub> (10 mL), to give a final concentration of 250 μM ferrous ion in acid. This solution was then added to 90 ml of HPLC-grade methanol containing 79.2 mg butylated hydroxytoluene (BHT). Finally, 7.6 mg xylenol orange was added with stirring to make the final working reagent (250 μM ammonium ferrous sulfate, 100 μM xylenol orange, 25 mM H<sub>2</sub>SO<sub>4</sub> and 4 mM BHT in 90% vol/vol methanol in a final volume of 100 mL). The blank reagent contained all the components of the solutions except ferrous sulfate. Aliquots (200 μL) of plasma were mixed with 1800 μL FOX2 reagent. After incubation at room temperature for 30 min the vials were centrifuged at 12,000 g for 10 min. Absorbency of the supernatant was then determined as a function of the absorbency difference between test and blank tubes, using a solution of H<sub>2</sub>O<sub>2</sub> as standard. The coefficient of variation for individual plasma samples was less than 5%.

## Oxidative Stress Index

The percent ratio of the TP to the TAC gave the oxidative stress index (OSI), an indicator of the de-

gree of oxidative stress (18–20). To perform the calculation, the result unit of TAC, mmol Trolox equivalent/L, was converted to μmol equivalent/L and the OSI value was calculated by the formula: OSI = [(TP, μmol/L)/(TAC, μmol Trolox equivalent/L)×100].

## Statistical Analysis

Results were expressed as mean ± standard deviation. Differences were considered significant at a probability level of  $p < 0.05$ . Obtained results were evaluated by repeated measurement variance analysis, student's t test correlation analysis, and chi-square tests. SPSS, Systat, and SigmaPlot computer programs were used.

## Results

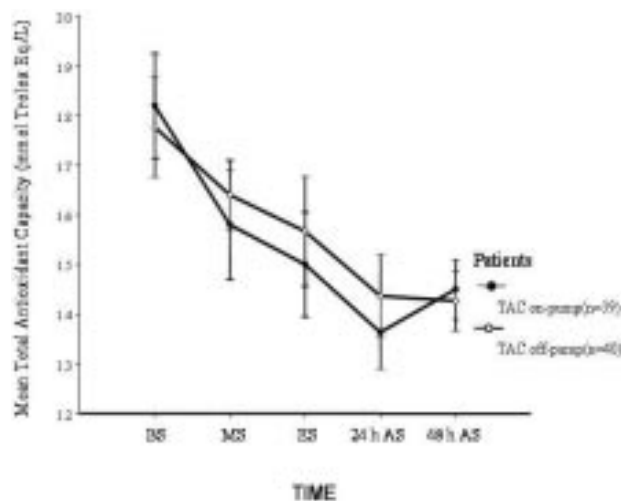
The preoperative characteristics of patients are listed in Table 1. As seen there, no statistically significant demographic differences were observed between the on-pump and off-pump groups. Problems included chronic obstructive airway disease (COPD), hypertension, and diabetes mellitus requiring active treatment at the time of the operation.

The operative characteristics of patients are also presented in Table 1. There was no significant difference in the number of grafts between the off-pump and on-pump patients. CPB patients received  $2.03 \pm 0.7$  grafts per patient, while off-pump patients received  $1.93 \pm 0.7$  grafts per patient ( $p = 0.91$ ). For the CPB patients, distal anastomosis time was  $25.5 \pm 4.5$  min.; for the off-pump patients,  $17.5 \pm 5.7$  ( $p = 0.16$ ).

No patient required exploration for postoperative bleeding, and there were no operative deaths or important adverse complications; all patients were discharged from the intensive care unit on the first postoperative day. There was no significant difference in the length of stay in intensive care unit and length of stay in hospital between the off-pump and on-pump patients ( $p = 0.46$ ;  $p = 0.91$ ).

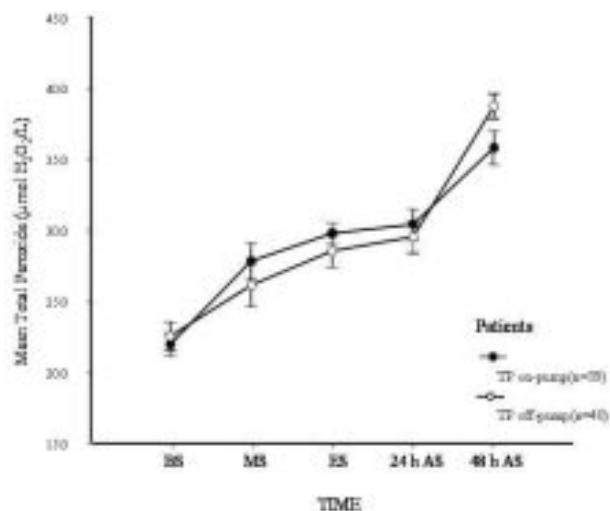
TAC, TP, and OSI changes are presented in Figs. 1, 2 and 3, respectively. As seen in Fig. 1, in group A (on-pump) patients, TAC decreased during surgery and also for 48 hours postoperatively ( $p < 0.005$ ). OSI level increased progressively after the beginning of surgery ( $p < 0.001$ ). There was a negative correlation between TAC levels and aortic cross-clamping period ( $r = -0.553$ ,  $p < 0.001$ ). There was also a positive correlation between TAC and ejection fraction ( $r = 0.647$ ,  $p < 0.001$ ).

As seen in Fig. 1, in group B (off-pump) patients TAC decreased during the operation and it



**Fig. 1.** Time changes of total antioxidant capacity (TAC) during and following on-pump and off-pump coronary artery surgery. All the preoperative, operative, and postoperative values showed statistically significant differences from the baseline value ( $p < 0.005$ ).

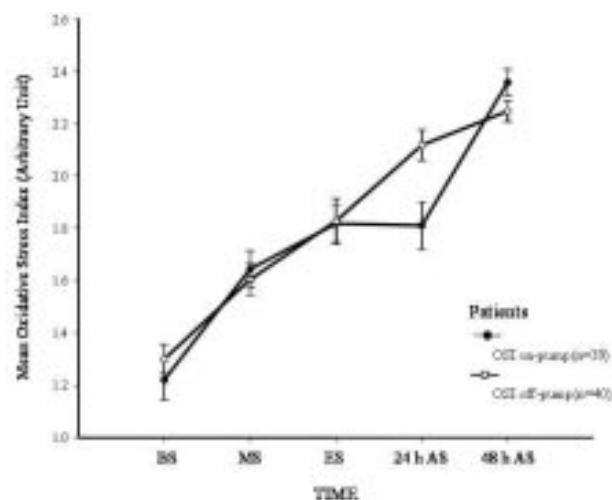
BS = before surgery; MS = midpoint of surgery; ES = end of surgery; 24 h AS = 24 hours after surgery; 48 h AS = 48 hours after surgery.



**Fig. 2.** Time changes of total peroxide (TP) concentration during and following on-pump and off-pump coronary artery surgery. All the preoperative, operative, and postoperative values showed statistically significant differences from the baseline value ( $p < 0.005$ ).

BS = before surgery; MS = midpoint of surgery; ES = end of surgery; 24 h AS = 24 hours after surgery; 48 h AS = 48 hours after surgery.

remained so until 48 hours postoperatively ( $p < 0.001$ ). In addition, as seen in Fig 3, OSI increased progressively on serial blood samples taken from the time of coronary anastomosis ( $p < 0.001$ ). A negative correlation was also obtained between anastomosis time and TAC ( $r = -0.500$ ,  $p < 0.001$ ).



**Fig. 3.** Time changes of oxidative stress index (OSI) during and following coronary artery surgery for both groups ( $p < 0.001$ ). BS = before surgery; MS = midpoint of surgery; ES = end of surgery; 24 h AS = 24 hours after surgery; 48 h AS = 48 hours after surgery.

TAC, TP and OSI mean and standard deviation (SD) values are presented in Table 2.

## Discussion

The systemic increase in oxidative stress during CABG is well-documented (1, 5), but the various components of the oxidant-antioxidant balance and the contribution of the various mechanisms involved have not yet been fully evaluated. This situation is closely connected with overproduction of ROS. ROS is produced during the ischemia/reperfusion process and systemic inflammatory response, both of which are associated with cardiac surgery performed with CPB (6, 15). In the present study, we found that patients undergoing on-pump or off-pump CABG are exposed to potent oxidative stress.

There are only a few studies comparing the degree of oxidative stress for patients undergoing on-pump vs. those undergoing off-pump techniques. However, it has been demonstrated that the on-pump procedure gives rise to a more pronounced systemic inflammation and oxidative stress than the off-pump procedure (14). The mechanisms explaining these observations may be related to several deleterious events occurring during CPB (21), which are either material-dependent (caused by exposure of blood to nonphysiologic surfaces and conditions during the ECC) or material-independent (caused by surgical trauma, ischemia-reperfusion, and changes in body temperature). These events are closely linked to inflammatory responses, including complement activation, release

**TABLE 2**  
*TP, TAC, and OSI Mean and Standard Deviation (SD) Values*

	BS	MS	ES	24 h	48 h	<i>p</i>
<b>Total Peroxide</b>						
TP on-pump (Mean ± SD)	22.43 ± 1.0	26.00 ± 1.5	28.40 ± 1.2	29.40 ± 1.1	38.54 ± 0.9	0.001
TP off-pump (Mean ± SD)	21.86 ± 0.8	27.70 ± 1.3	29.63 ± 0.8	30.26 ± 1.1	35.63 ± 1.2	0.001
<b>Total Antioxidant Capacity</b>						
TP on-pump (Mean ± SD)	1.82 ± 0.10	1.58 ± 0.11	1.50 ± 0.10	1.36 ± 0.07	1.45 ± 0.06	0.001
TP off-pump (Mean ± SD)	1.78 ± 0.10	1.64 ± 0.07	1.56 ± 0.11	1.44 ± 0.08	1.43 ± 0.60	0.001
<b>Oxidative Stress Index</b>						
TP on-pump (Mean ± SD)	1.22 ± 0.08	1.66 ± 0.07	1.82 ± 0.07	1.81 ± 0.09	2.36 ± 0.05	0.001
TP off-pump (Mean ± SD)	1.30 ± 0.05	1.62 ± 0.06	1.82 ± 0.09	2.12 ± 0.06	2.25 ± 0.04	0.001

BS = before surgery; MS = midpoint of surgery; ES = end of surgery.

of cytokines, and leukocyte activation, along with expression of adhesion molecules (6). Many studies have described the nature of these ROS and the time course of their formation during CPB (7). These oxidative events in turn lead to depletion of plasma antioxidants, increased lipid peroxidation, and formation of other damaging metabolites (1–11). In our present study, we demonstrated that off-pump patients experience decreased oxidative stress compared with patients undergoing CPB, but this difference is not significant statistically (Fig. 1–3 and Table 2).

Oxidative stress is the result of imbalance between antioxidant defenses and the formation of ROS. Oxygen-free radicals and their metabolites play an important role in the pathophysiology of ischemia-reperfusion injury. However, direct measurement of free radicals in humans is difficult because of the free radicals' transient nature and the complexity of the available techniques. Various measurement methods have been developed for total antioxidant status, but there is as yet no accepted reference method (12, 13). The most widely used methods for TAC measurement utilize colorimetry, fluorescence, and chemiluminescence (22–24). In these methods, a type of radical is generated in the assay and the antioxidant activity of the sample is measured against that of the assay radical. On the other hand, even when these technologies are available, their value is limited. In the present study, the total antioxidant status of the plasma was measured using a novel automated colorimetric measurement method developed by Erel for patients undergoing off-pump and on-pump CABG (12).

Reintroduction of oxygen to previously hypoxic myocardium can result in a sudden increase in the extent of irreversible tissue injury. There is

abundant evidence that the mechanism of ischemic-reperfusion injury involves increased generation of ROS, and that both endogenous and exogenous antioxidants and antioxidative enzymes ameliorate the extent of the damage (25–30). The recognition of these pathogenic mechanisms has made the understanding of the natural antioxidant defenses, intracellular and extracellular, of clinical importance.

Several studies have shown a direct connection between increased production of ROS and damage of tissues and organs, as well as deleterious effect of these substances on heart function (6, 14–17). In the present study, TAC decreased during surgery and for 48 hours postoperatively ( $p < 0.005$ ), while the total peroxide increased ( $p < 0.001$ ) operatively and postoperatively in all patients. However, this difference is not statistically significant between the groups.

Depending on the severity of the ischemic period, oxidative stress occurs during the reperfusion of patients with coronary artery disease who are subjected to on-pump cardiac surgery, and that stress may be linked to a delay in postoperative recovery of cardiac function. As previously proposed, not only is chronic ischemia responsible for a severe impairment of myocardial contractility, but it can also promote a loss of tissue glutathione and scavenging enzyme activity (by glutathione peroxide), thus resulting in a lack of defense against free radical toxicity (27). Prolonged ischemia is responsible for a decrease in myocardial non-protein sulphhydryl groups, mainly glutathione and high-energy phosphates, and this becomes a rate-limiting factor for detoxification of oxygen species (31). The final effect could be a vicious circle in which chronic ischemia impairs left ventricular ejection fraction and reduces natural scav-

enger availability, thus producing a low ability to counterbalance the damage of ischemia and worsening myocardial contractility (15). Patients with low left ventricular ejection fraction did not have lower preoperative TAC values or higher lipid peroxidation following CABG. However, they exhibited a more prominent plasma antioxidant depression immediately after surgery. This might be due to exacerbation of albumin loss into the extracellular space and through the kidneys, as well as decreased levels of vitamin C, observed in chronic heart failure (15). We found a positive correlation between TAC and ejection fraction ( $r = 0.647$ ,  $p < 0.001$ ). To the best of our knowledge, this is the first report showing the relationship between the TAC and ejection fraction.

We also found that there was a significant inverse correlation between TAC and cross-clamp time ( $r = -0.553$ ,  $p < 0.001$ ). To the best of our knowledge, this is the first report showing the inverse relationship between the TAC and cross-clamp time in the patients. Ferrari and coworkers, using a crystalloid cardioplegia, reported a positive correlation between cross-clamp time and oxidized glutathione release for clamping periods longer than 30 minutes, while the correlation was not evident for periods less than 30 minutes (27). On the other hand, we found positive correlations between cross clamp-time and TP and OSI.

In conclusion, during CABG, oxidants and OSI levels increase significantly and TAC decreases significantly. This situation is influenced by long CPB/anastomosis time and low ventricular ejection fraction. We conclude that patients who are undergoing CABG are exposed to potent oxidative stress and that their TAC is impaired. Therefore, prior to coronary bypass surgery, we suggest that supplementation with antioxidant vitamins such as vitamin C and E may be beneficial.

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