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Free Radical Reaction Products and Antioxidant Capacity in Beating Heart Coronary Artery Surgery Compared to Conventional Bypass

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Abstract—Oxygen-derived free radicals are important agents of tissue injury during ischemia and reperfusion. The aim of this study was to investigate changes in protein and lipid oxidation and antioxidant status in beating heart coronary artery surgery and conventional bypass and to compare oxidative stress parameters between the two bypass methods. Serum lipid hydroperoxide, nitric oxide, protein carbonyl, nitrotyrosine, vitamin E, and β-carotene levels and total antioxidant capacity were measured in blood of 30 patients undergoing beating heart coronary artery surgery (OPCAB, off-pump coronary artery bypass grafting) and 12 patients undergoing conventional bypass (CABG, on-pump coronary artery bypass grafting). In the OPCAB group, nitric oxide and nitrotyrosine levels decreased after reperfusion. Similarly, β-carotene level and total antioxidant capacity also decreased after anesthesia and reperfusion. In the CABG group, nitric oxide and nitrotyrosine levels decreased after ischemia and reperfusion. However, protein carbonyl levels elevated after ischemia and reperfusion. Vitamin E, β-carotene, and total antioxidant capacity decreased after ischemia and reperfusion. Significantly decreased nitration and impaired antioxidant status were seen after reperfusion in both groups. Moreover, elevated protein carbonyls were found in the CABG group. The off-pump procedure is associated with lower degree of oxidative stress than on-pump coronary surgery.

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Key words: oxidative stress, coronary artery bypass, antioxidant, protein carbonyls, nitrotyrosine

Reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, and hydrogen peroxide are produced during normal cellular function, and their high chemical reactivity leads to the oxidation of proteins, lipids, and DNA. ROS generated reperfusion injury has been reported in heart, kidney, liver, lung, and intestine. Several studies have proposed the significant role of ROS in the pathogenesis of myocardial ischemia–reperfusion injury [1-8]. An increase in the formation of reactive oxygen species during ischemia–reperfusion and the adverse effects of oxy-radicals on myocardium have now been well established by both direct and indirect measurements.

The first biological molecules for oxidative damage in cells are proteins, and their side chains can be carbonylated by reactive carbonyl compounds. Oxidative modification of proteins by oxygen radicals can cause loss of catalytic activity and subsequent proteolytic degradation of the proteins [9, 10]. Oxygen radicals are capable of reacting with unsaturated lipids and initiating chain reactions of lipid peroxidation in the membranes. Measures of lipid peroxidation include expired pentane, malondialdehyde (MDA), lipid hydroperoxides, isoprostanes, and conjugated dienes. Lipid hydroperoxides are formed earlier in the pathway leading to MDA.

Another important molecule, nitric oxide, is a free radical that has recently been found to have a key role in both normal physiological processes and disease states. The presence of nitric oxide in biological systems leads to the formation of reactive nitrogen species such as peroxynitrite, which reacts with tyrosine residues in proteins to

Abbreviations: CABG, on-pump coronary artery bypass grafting; LPx, lipid hydroperoxide; NOx, nitric oxide; NT, nitrotyrosine; OPCAB, off-pump coronary artery bypass grafting; ROS, reactive oxygen species; TAC, total antioxidant capacity.

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form nitrotyrosine. Thus, nitrotyrosine formation is an indicator of peroxynitrite production in various clinical situations. It has been demonstrated that peroxynitrite is produced in human myocardium after ischemia–reperfusion by the measurement of plasma nitrotyrosine [11, 12].

The effect of reactive species is balanced by the antioxidant action of antioxidant enzymes as well as by non-enzymatic antioxidants. α-Tocopherol is the major lipid-soluble antioxidant; it is present in the blood, mainly in the VLDL and LDL fractions, where it prevents free radical oxidation of these lipoproteins [13]. β-Carotene, another important lipid-soluble membrane-associated antioxidant, is able to quench singlet oxygen, thus interrupting the generation of ROS at a very early stage [14, 15]. Oxidative stress is the result of imbalance between antioxidant defenses and the formation of ROS. There is increasing evidence for the existence of an antioxidant network in human ischemia–reperfusion injury. For instance, many studies have demonstrated oxidative modification and release of myocardial antioxidants in patients undergoing coronary bypass surgery [16-18].

The present study was designed to investigate changes in protein and lipid oxidation and antioxidant status during bypass operation in blood of patients with off-pump and on-pump coronary artery bypass surgery and to compare oxidative stress parameters between the two bypass methods. Hence, all patients in the study were operated under identical conditions, and the only variable was the use of different bypass operation methods.

MATERIALS AND METHODS

Chemicals. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium persulfate, ABTS (2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid) diammonium salt), sodium chloride, potassium chloride, ferrous ammonium sulfate, methanol, ethanol, acetonitrile, butanol, *n*-hexane, butylated hydroxytoluene, ethyl acetate, sulfuric acid, 3-nitrotyrosine, α-tocopherol, β-carotene, sodium sulfate, and sodium carbonate were purchased from Sigma-Aldrich (USA); sodium dihydrogen orthophosphate dihydrate, disodium hydrogen orthophosphate 12-hydrate, hydrogen peroxide, and xylenol orange tetrasodium were purchased from Merck (Germany).

Patients. Forty-two patients (33 male, 9 female) were studied. We compared 30 patients who underwent off-pump coronary artery bypass grafting (OPCAB) versus 12 patients who underwent on-pump coronary artery bypass grafting (CABG). Thirty patients (24 male, 6 female) were operated by the OPCAB technique. Twelve patients (9 male, 3 female) were operated using the CABG technique. Patients who used antioxidants such as captopril and allopurinol were excluded from the study. Patients who received blood transfusion or blood products during the operation were also excluded, since the antioxidant properties of such products are not yet established. None of the patients were taking vitamins or dietary supplements with established antioxidant properties before the study. The study was approved by Gazi University Medical Ethics Committee, and written informed consent was obtained from all participants.

Anesthetic technique. Anesthetic technique was standardized for all patients and consisted of balanced anesthesia. All patients were premedicated with diazepam (5 mg) the night before surgery and with midazolam (0.07 mg/kg) intramuscularly 30 min before the surgery. Patients underwent initiation of anesthesia with midazolam (0.15 mg/kg), fentanyl (4 μg/kg), and propofol (1-2 mg/kg). Pancuronium or vecuronium was used as a muscle relaxant (0.1-0.15 mg/kg). After tracheal intubation, anesthesia was maintained with a 50% air/oxygen mixture with isoflurane at a concentration of 0.5-1%. Additional fentanyl and propofol were applied when necessary.

Surgical procedure. *Coronary artery surgery without CABG (OPCAB).* The operation was performed through a median sternotomy. After harvesting the bypass conduits, heparin was given at a dose of 100 IU/kg to achieve a target activated clotting time of 250 to 300 sec. Beta-blocker drugs were used to decrease heart rate during the anastomosis. No myocardial stabilization devices were used except cotton sutures. During the anastomosis coronary flow was occluded by bulldog clamps. The proximal anastomosis was performed using a site clamp on the aorta or on the internal mammary artery. All grafts were arterial (IMA or radial artery).

Coronary artery surgery with CABG. The operation was performed through median sternotomy. After harvesting the bypass conduits, heparin was given at a dose of 400 IU/kg to achieve a target activated clotting time of 450 sec or above. Cardiopulmonary bypass was instituted using ascending aortic cannulation and two-stage venous cannulation in the right atrium. The extracorporeal circuit consisted of a membrane oxygenator and a roller pump primed with crystalloid solution. CABG was managed according to the alpha-stat principle, moderate hypothermia, and the CABG flow rate was maintained at 2.4 liters/min per m^2. Myocardial protection was achieved with cold potassium cardioplegia (Plegisol; Abbot Inc., USA), and warm blood cardioplegia before removing the aortic cross-clamp. The cardioplegia was given retrogradely, except the first two-thirds of crystalloid cold cardioplegia was given anterogradely. All distal and proximal anastomosis were completed before the aortic cross-clamp was removed. At the end of CABG, heparin was neutralized by protamine chloride until the activated clotting time was less then 180 sec. In the CABG group hematocrit was kept more than 20% during CABG, whereas in the OPCAB group hematocrit was kept more than 25%.

Sample collection and analyses. Blood samples were collected at different time points. In all patients the first samples were obtained from the venous line, while other times blood samples were obtained from the arterial line. The blood was drawn into plastic syringes, and plasma was separated by centrifugation and kept in plastic tubes at −70°C until analysis. Samples were obtained before anesthesia (t1), before operation (t2), 10 min after start of CABG operation (ischemic period) (t3), and 10 min after cessation of CPB (reperfusion time) (t4) in the CABG group. In the OPCAB group blood collections were similar to the CABG group except ischemic period (t3).

Determination of lipid hydroperoxides. Serum lipid hydroperoxide (LPx) levels were determined with the FOX2 (ferrous oxidation in xylenol orange, version 2) method described by Nourooz-Zadeh et al. [19]. Xylenol orange and ammonium ferrous sulfate were dissolved in 250 mM H_2SO_4 to final concentrations of 1 and 2.5 mM, respectively. FOX2 reagent consists of one volume of this mixture and nine volumes of methanol containing 4.4 mM butylated hydroxytoluene (BHT). Mixture of 200 μl serum, 300 μl water, and 250 μl methanol was vortexed for 20 sec. Ethyl acetate (500 μl) was then added and the mixture was vortexed. The suspension was centrifuged at 3000*g* for 5 min. The upper layer was then transferred to a test tube, 500 μl ethyl acetate was added to the residual aqueous phase, and the mixture was vortexed and centrifuged as above. The organic layers were pooled and concentrated by evaporation to a final volume of 100 μl under a stream of nitrogen. Samples (100 μl) and 900 μl FOX2 reagent were mixed and incubated at room temperature for 30 min. The mixture was centrifuged at 12,000*g* for 5 min, and the absorbance of the supernatant was measured at 560 nm.

Determination of nitric oxide. Plasma levels of nitrate + nitrite (NOx; as a marker of NO production) were used as the measurement of NO synthase activity and NO biosynthesis. Nitrate was measured as nitrite after enzymatic conversion by nitrate reductase (Assay Designs, USA). The Griess reaction was then used for nitrite determination.

Determination of carbonyl. Modified from Carty et al. [20], a carbonyl ELISA was used to determine the extent of LDL protein oxidation from samples oxidized *in vitro* and from those isolated from the patient groups. Five carbonyl standards (0-9.9 nmol protein carbonyl/mg protein) were generated from a combination of borohydride reduction or iron-catalyzed oxidation of bovine serum albumin and calibrated as previously described [20] to construct a standard curve. All standards and samples were diluted to 20 μg/ml in coating buffer (50 mM sodium carbonate buffer, pH 9.2; BDH Chemicals) and incubated in a NUNC MaxiSorp plate in triplicate (50 μl). Protein was allowed to bind for 1 h at 37°C before washing three times with PBS-Tween (0.5%). 2,4-Dinitrophenylhydrazine (DNPH) was added in 2 M HCl (1 mM) and allowed to react for 1 h at room temperature before washing as before. Nonspecific binding sites were blocked overnight at 4°C with PBS-Tween (1%). After washing, rabbit anti-DNPH primary antibody (1 : 1000) was applied and incubated for 1 h at 37°C and, following washing with PBS/Tween, anti-rabbit IgG conjugated to peroxidase (1 : 5000) was also incubated at 37°C for 1 h. The reaction was visualized by *o*-phenylenediamine tablets and stopped by the addition of sulfuric acid (2 N). Absorbance was read at 490 nm, and a standard curve, from which the carbonyl content of the lipoprotein samples was calculated, was constructed.

Determination of nitrotyrosine. Nitrated tyrosine was determined using reversed-phase HPLC with electrochemical detection (+995 mV) [21]. Lyophilized samples were reconstituted in aqueous mobile phase (50 μl) and were loaded onto a Luna C18 column (Phenomenex, UK) in phosphate (10 mM) and perchlorate buffer (100 mM), pH 2.5. Amino acids were eluted with a linear increasing gradient of methanol to 40% over 40 min at 0.7 ml/min. Standard tyrosine and 3-nitrotyrosine were used to calibrate unknowns. Samples were hydrolyzed for 24 h using Pronase (10 μg/ml; 37°C), as acid hydrolysis frequently introduces nitration from contaminating glassware. An inter-batch coefficient of variation of 11% was observed.

Determination of vitamin E and β-carotene. α-Tocopherol and β-carotene levels in serum were determined according to the method described by Lee et al. [22]. A 200 μl aliquot of serum in a polypropylene microfuge tube wrapped in aluminium foil was extracted with 200 μl butanol–ethyl acetate (1 : 1 v/v) and further mixed for 1 min. Approximately 10 mg of sodium sulfate was added. After vortex mixing for another 1 min, the sample was allowed to stand at −20°C for 20 min before centrifugation at 15,000*g* for 2 min (Eppendorf microcentrifuge Jouan MR 1822). The organic upper layer was transferred into a sealed amber sample vial and stored at −70°C until HPLC analysis. HPLC analysis was performed by isocratic elution. A Hewlett Packard (HP) Series 1050 liquid chromatograph was set at flow-rate 1 ml/min. The mobile phase consisting of methanol–butanol–water (75 : 20 : 5 v/v) was premixed and vacuum filtered through a 0.45 μm polypropylene membrane filter (Whatman, USA) and degassed (Bandelin-Sonorex RK 100 H) before use. A 10-μl aliquot of organic extract for α-tocopherol and 4-μl aliquot of organic extract for β-carotene were taken using a Hamilton syringe. The analytical column used was a LiChrospher RP 18-e (HP) (5 μm, 250-4) for α-tocopherol and HP-MOS Hypersil (5 μm, 100 × 4.6 mm) for β-carotene and maintained at 45°C. Vitamin E and β-carotene were detected by a UV detector (Model 1050; Hewlett Packard, USA) at different wavelengths programmed for analysis as follows: 9.8 min/290 nm and 4 min/450 nm, respectively. Analytes were identified by retention time and quanti-

fied by peak height with a HP Model 3396 integrator (USA).

Determination of serum total antioxidant capacity (TAC). Serum TAC levels were determined according to the ABTS radical cation ($ABTS^{\bullet+}$) decolorization assay described by Re et al. [23]. Using this method, when an aliquot of serum is added to the $ABTS^{\bullet+}$ solution, decolorization as a result of the presence of serum antioxidants that reverse the formation of $ABTS^{\bullet+}$ is observed. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate (final concentration) in the ratio of 1 : 0.5 and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. $ABTS^{\bullet+}$ solution was diluted with PBS, pH 7.4, to give absorbance of 0.700 ± 0.020 at 734 nm and 30°C. After addition of 1 ml of diluted $ABTS^{\bullet+}$ solution (A_{734} = 0.700 ± 0.020) to 10 μl serum or Trolox standard in PBS, the absorbance reading was taken at 30°C exactly 6 min after initial mixing. Percentage inhibition values of samples and standards were calculated, and TAC levels were calculated from a calibration curve.

Determination of other blood parameters. Serum total cholesterol, HDL-cholesterol, triglycerides, fasting blood glucose, ALT, AST, albumin, and total bilirubin were measured spectrophotometrically on a Hitachi 912 autoanalyzer using a commercial kit (Roche, Germany). Concentration of serum LDL-cholesterol was calculated by the standard Friedewald formula.

Statistical analyses. All results are expressed as means ± SE. Statistical analyses were performed using SPSS software (version 10; SPSS Inc., USA). Student's *t*-test was used to compare the results between the patient groups. One-way analysis of variance was performed in repeated measures. Pearson correlation coefficients were calculated for the relationship between measured parameters. *P* values of less than 0.05 were regarded as significant.

RESULTS

No patient received inotropic support during the operation and none developed any significant complication over 24 h of observation, i.e. stroke, arrhythmia, renal failure, pulmonary insufficiency, infection, or excessive bleeding. During the study period there was no mortality of any patient.

The clinical characteristics and laboratory data from the OPCAB and CABG groups are summarized in Table 1. The mean age and Quetelet index at the time of blood sampling did not differ significantly among the groups. There were no significant differences in lipid profile, fasting glucose, serum ALT, AST, LDH, albumin, bilirubin, protein, creatinine, and uric acid levels between the groups.

In our study, in the OPCAB group there was no difference among before anesthesia, after anesthesia, and after reperfusion in terms of LPx and carbonyls ($p > 0.05$) (Table 2). Preoperative NOx levels were not changed after initiation of anesthesia ($p > 0.05$). The mean nitrotyrosine (NT) level was diminished in the OPCAB group from 21.39 ± 5.73 nmol/mg preoperatively to 20.70 ±

Table 1. Clinical and laboratory data of the study groups

	OPCAB group (N = 30)	CABG group (N = 12)
Sex (M/F)	23/7	10/2
Age, years	66.80 ± 1.36	59.00 ± 1.38
Quetelet index, kg/m^2	28.32 ± 0.71	27.76 ± 1.08
Total cholesterol, mg/dl	196.07 ± 11.12	186.50 ± 7.50
HDL-cholesterol, mg/dl	44.83 ± 2.16	37.00 ± 2.24
LDL-cholesterol, mg/dl	110.43 ± 7.61	110.08 ± 6.50
VLDL-cholesterol, mg/dl	42.20 ± 4.80	36.22 ± 4.03
Triglycerides, mg/dl	233.53 ± 33.74	248.00 ± 40.26
Fasting blood glucose, mg/dl	141.97 ± 11.82	118.83 ± 21.79
ALT, U/liter	22.91 ± 1.74	24.25 ± 4.09
AST, U/liter	18.33 ± 0.90	19.99 ± 1.96
LDH, U/liter	289.33 ± 8.14	289.92 ± 8.06
Albumin, mg/dl	4.22 ± 6.74 × 10^{-2}	4.20 ± 9.92 × 10^{-2}
Total bilirubin, mg/dl	1.02 ± 0.23	0.91 ± 0.10
Total protein, g/dl	7.03 ± 0.23	7.32 ± 0.14
Creatinine, mg/dl	1.05 ± 3.17 × 10^{-2}	1.02 ± 5.62 × 10^{-2}
Uric acid, mg/dl	6.00 ± 0.31	5.50 ± 0.35

Table 2. Comparison of LPx, NOx, carbonyl, and NT levels according to two different bypass methods

	LPx, nmol/liter	NOx, μmol/liter	Carbonyl, nmol/mg	NT, nmol/mg
OPCAB ($N = 30$)				
t1	41.13 ± 8.27	65.39 ± 3.10	3.83 ± 0.19	21.39 ± 1.05
t2	38.20 ± 7.09	68.27 ± 3.69	3.85 ± 0.19	20.70 ± 0.89
t4	28.37 ± 5.56	61.11 ± 3.04^{b}*	4.00 ± 0.21	18.36 ± 1.11^{a}*,^{b}*
CABG ($N = 12$)				
t1	40.33 ± 11.73	60.02 ± 4.36	4.00 ± 0.25	25.48 ± 2.47
t2	22.25 ± 4.33	59.65 ± 2.68	4.13 ± 0.31	26.29 ± 1.61
t3	47.58 ± 15.63	44.71 ± 3.27^{a}**,^{b}**	4.85 ± 0.35^{a}*,^{b}**	13.54 ± 1.90^{a}**,^{b}**
t4	24.42 ± 6.14	48.24 ± 3.02^{a}**,^{b}**	4.71 ± 0.33^{b}**	13.44 ± 1.08^{a}**,^{b}**

Notes: [a] Compared with t1; [b] compared with t2; * $p < 0.05$; ** $p < 0.01$.

4.89 nmol/mg after initiation of anesthesia ($p < 0.05$). NT levels after initiation of anesthesia were decreased after reperfusion time (18.35 ± 6.07 nmol/mg) ($p < 0.05$).

In the CABG group, LPx levels were not different at the blood collection times ($p > 0.05$) (Table 2). Preoperative NOx levels were not changed after initiation of anesthesia. The NOx levels decreased during the ischemic period compared with preoperative and initiation of anesthesia values ($p < 0.01$). Similarly, the level at reperfusion time was lower than preoperative and initiation of anesthesia ($p < 0.01$). Carbonyl levels after initiation of anesthesia were similar to preoperative levels ($p > 0.05$). The mean levels of carbonyl increased significantly after ischemia compared with initiation of anesthesia values. This increase was from 4.13 ± 0.31 to 4.85 ± 0.35 nmol/mg ($p < 0.01$). The mean carbonyl levels in the ischemic period diminished to 4.71 ± 0.33 nmol/mg. The mean NT levels decreased from 25.48 ± 2.47 nmol/mg preoperatively to 13.54 ± 1.90 nmol/mg in the ischemic period ($p < 0.01$). The decrease in the ischemic period remained during the reperfusion time ($p < 0.01$).

Vitamin E, β-carotene, and TAC values of study groups at blood collection times are presented in Table 3. There was no difference in vitamin E levels of the OPCAB group among before anesthesia, initiation of anesthesia, and after reperfusion. β-Carotene levels were significantly decreased from 0.45 ± 0.46 μg/ml preoperatively to 0.15 ± 0.17 μg/ml in the ischemic period ($p < 0.01$). The decrease in the ischemic period remained during the reperfusion time ($p < 0.01$). Similarly, total antioxidant capacity decreased with initiation of anesthesia (1.97 ± 0.14 mmol/liter) ($p < 0.05$). The TAC level during the ischemic period diminished to 1.87 ± 0.13 mmol/liter after reperfusion ($p < 0.01$).

In this study, in the CABG group vitamin E levels after initiation of anesthesia were similar to the preoperative levels. The mean vitamin E level decreased after ischemia ($p < 0.01$). This decrease was from 3.02 ± 1.68 to 1.45 ± 1.08 μg/ml. The mean level of vitamin E decrease in the ischemic period remained during the reperfusion time ($p < 0.01$). β-Carotene levels diminished significantly after ischemia compared with preoperative and initiation of anesthesia ($p < 0.01$ and $p < 0.05$, respectively). The mean β-carotene level during the ischemic period diminished to 0.09 ± 0.05 μg/ml after reperfusion. Preoperative TAC levels were unaltered after initiation of anesthesia. However, TAC levels decreased in the ischemic and reperfusion times compared with those of others ($p < 0.01$). These values are 1.59 ± 0.12 and 1.67 ± 0.10 μg/ml, respectively.

Overall data taken from the OPCAB group (see Table 4) showed that there was a significant negative correlations between NOx and TAC in the preoperative period ($r = -0.448$, $p = 0.013$), NOx and TAC after initiation of anesthesia ($r = -0.402$, $p = 0.028$), and LPx and TAC after initiation of anesthesia ($r = -0.486$, $p = 0.006$).

Table 3. Comparison of vitamin E, β-carotene, and TAC levels for the two bypass methods

	Vitamin E, μg/ml	β-Carotene, μg/ml	TAC, mmol/liter
OPCAB ($N = 30$)			
t1	3.37 ± 0.48	0.45 ± 0.08	2.02 ± 0.03
t2	3.16 ± 0.56	0.15 ± 0.03[a]**	1.97 ± 0.03[a]*
t4	3.36 ± 0.49	0.13 ± 0.03[a]**	1.87 ± 0.02[a]**,[b]**
CABG ($N = 12$)			
t1	3.77 ± 0.51	0.41 ± 0.13	2.05 ± 0.03
t2	3.02 ± 0.48	0.14 ± 0.03[c]*	1.99 ± 0.03
t3	1.45 ± 0.31[a]**,[b]**	0.08 ± 0.01[a]**,[b]*	1.59 ± 0.04[a]**,[b]**
t4	1.47 ± 0.27[a]**,[b]**	0.09 ± 0.02[a]**	1.67 ± 0.03[a]**,[b]**

Notes: [a] Compared with t1; [b] compared with t2; [c] compared with t4; * $p < 0.05$; ** $p < 0.01$.

Table 4. Pearson correlation coefficients between measured parameters in patient groups

			TAC	Vitamin E	NOx
OPCAB patients ($N = 30$)	t1	NOx	–0.448*	–	–
	t2	NOx	–0.402*	–	–
		β-carotene	–	0.494**	–
	t4	LPx	–0.471**	–	–
CABG patients ($N = 12$)	t1	β-carotene	0.622**	–	–
	t3	carbonyls	–	–	0.727**
		NT	–	–	0.585*

* Correlation is significant for $p < 0.05$.
** Correlation is significant for $p < 0.01$.

Vitamin E positively correlated with β-carotene values in the anesthetic period ($r = 0.438$, $p = 0.015$). Positive correlations were seen between TAC and β-carotene in the preoperative period ($r = 0.622$, $p = 0.031$), protein carbonyls and NOx after ischemia ($r = 0.727$, $p = 0.007$), and protein nitration and NOx after ischemia ($r = 0.585$, $p = 0.044$) in the CABG group.

DISCUSSION

There is now convincing evidence that oxidative stress and reactive oxygen species (ROS) play an important role in the aetiology and/or progression of a number of human diseases [24]. Some previous studies have demonstrated oxidative stress during cardiopulmonary

bypass (CPB) as well as during ischemia/reperfusion of the human heart [25-28]. Alterations in the myocardium during ischemia–reperfusion were suggested to be in part due to oxidative stress. At the cellular level, the excessive production of oxygen free radicals causes lipid peroxidation, protein denaturation, enzyme inactivation, carbohydrate breakdown, and finally tissue injury [29]. Conflicting results regarding lipid peroxidation in ischemia–reperfusion injury have been reported [30-33]. Matata et al. reported that lipid hydroperoxide levels were not affected in anesthesia and surgical trauma, but were significantly elevated between 1 and 4 h after the initiation of CABG. Similarly, Starkopf et al. observed a significant elevation in lipid peroxidation after ischemia and after reperfusion. Hadjinikolaou et al. found a significant increase in LPx production only at 1 h after the operation. Carlucci et al. could not find any significant change in reactive oxygen metabolites during the ischemic phase or during reperfusion. Gerritsen et al. in a prospective study showed that OPCAB was associated with lower levels of urinary lipid peroxidation than those of CABG [34]. In this study, we found that concentrations of lipid hydroperoxide did not change during ischemia–reperfusion in either group. Contrary to many other studies [30-32], we found no evidence that lipid hydroperoxides are elevated in the plasma of patients with CABG compared with samples from matched patients with OPCAB.

Protein carbonyls are considered as markers of oxidative stress [35]. They arise from direct free radical attack on vulnerable amino acid side chains or the protein backbone or from the products of glycation, glycoxidation, and lipid peroxidation reactions with proteins [36-38]. Serdar et al. demonstrated increased protein oxidation products related to the severity of disease in coronary artery patients [39]. In another study, Fu et al. investigated the role of radicals in protein oxidation in advanced human atherosclerotic plaque, and they concluded that reactive products of protein oxidation have to be considered as possible contributors to atherogenesis [40]. In a prospective study comparing OPCAB with CABG, OPCAB patients displayed lower levels of protein carbonylation [30]. They were unaffected by anesthesia and surgical trauma, but were rapidly elevated upon the initiation of CABG. This increase was confined to the first hour after the operation, and after this time levels were similar to those seen before operation. In this study, elevated carbonyl levels were found during ischemia–reperfusion in the CABG group compared to the OPCAB group. The carbonyl concentrations did not change during ischemia–reperfusion in the OPCAB patients.

Nitric oxide is a polyfunctional signaling molecule controlling processes of vasodilatation, platelet aggregation, leukocyte adhesion, and smooth muscle cell proliferation [41-43]. However, under pathological conditions nitric oxide may be converted into potent nitrating oxidants that promote oxidative damage. Nitric oxide (NO) is readily oxidized to nitrite and nitrate in biological systems. The role of NO in myocardial ischemia/reperfusion is controversial. Myocardium subjected to ischemia is reported to enhance NO production in the early phase [44, 45]. However, endogenous NO production decreases on myocardial reperfusion, which is thought to accelerate intraoperative myocardial damage [46, 47]. Carlucci et al. observed a significant decrease in serum nitrate and nitrite levels during both ischemic and reperfusion phases in CABG patients [33]. Similarly, in the present study we demonstrate a significant decrease in plasma NOx levels in both groups during these times. However, the CABG patients displayed lower levels of NOx than those of the OPCAB patients. Free radical production and antioxidant system imbalance during ischemia–reperfusion could lead to alteration of NO bioactivity. Peroxynitrite is a highly reactive oxidant metabolite of nitric oxide. It nitrates free and protein bound tyrosine residues to produce nitrotyrosine. The measurement of nitrotyrosine provides an indirect estimation of plasma peroxynitrite concentrations. Plasma nitrotyrosine has been shown to be generated under oxidative stress conditions with a severe endothelial insult, such as in chronic renal failure with septic shock [48] and in myocardial ischemic reperfusion injury [12]. Increased nitrotyrosine levels in CABG and OPCAB patients were reported by Matata et al. [30]. Nitrotyrosine concentrations were significantly elevated after initiation of anesthesia and in the early ischemic phase. Values decreased by 2 h, a time when the operation was already complete. In the present study, decreased NT levels were found during ischemia–reperfusion in both the CABG and the OPCAB groups. These decreased NT levels are probably associated with falling of NOx levels.

Protection against free radical attack is offered by a wide spectrum of antioxidants and scavengers that are distributed in all tissues. While antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase are responsible for intracellular protection, nonenzymatic molecules, especially α-tocopherol and carotenoids, are involved in extracellular defense. Myocardial antioxidants are defined as substances that inhibit or delay oxidative damage to subcellular proteins, carbohydrates, lipids, and DNA. The data reported in the literature concerning antioxidant vitamins in heart ischemia–reperfusion are controversial. Various studies have shown the beneficial effects of antioxidants as these agents render resistance of the heart against ischemic–reperfusion injury [49-51]. However, other investigators failed to observe such results [52, 53]. A recent study reported a decrease in endogenous antioxidants in the ischemic heart upon reperfusion [54]. Hydrophilic antioxidants were decreased during the reperfusion time, but not the ischemic period. However, vitamin E did not change during these periods; however, increasing the severity of ischemia–reperfusion by adding

oxygen radicals resulted in vitamin E depletion. Barsacchi et al. [55] evaluated changes in vitamin E content and observed a great variability in basal vitamin E content in the atria and a reproducible and substantial decrease in atrial vitamin E content after cardiopulmonary bypass. This was directly related to the aorta cross-clamping duration. In this study, it was demonstrated that vitamin E and β-carotene levels were significantly decreased in CABG patients during ischemia and reperfusion, but only β-carotene levels were reduced in the OPCAB group. Human plasma contains a wide spectrum of antioxidants with synergic action, so that individual measurements of antioxidant concentrations in blood do not always reflect the level of antioxidant status. Measurement of TAC can provide accurate and more valuable results. Previous studies have shown that there is a significant decrease in TAC after operation in patients with CABG. Hadjinikolaou et al. [32] found that TAC was suppressed for 72 h after the operation. It presented a sharp initial decrease followed by a tendency for partial recovery after 6 h, not reaching the preoperative levels. A second decrease between 6 and 24 h was followed by slow progressive recovery, which also did not reach the preoperative levels within the 72 h of observation. Another study also reported a significant decrease in TAC levels after ischemia–reperfusion compared with preoperative values [31]. In this study, we showed that serum total antioxidant capacity was decreased in sera of the CABG and OPCAB groups during ischemia–reperfusion, which demonstrated an inadequate capacity of plasma to protect its environment from free radical attack. In addition, we found significant inverse correlations between TAC and NOx values before and after anesthesia and between TAC and LPx after anesthesia in patients undergoing OPCAB. We also found positive correlations between NOx and carbonyls and between NOx and protein nitration after ischemia in patients undergoing CABG. CABG patients displayed lower levels of TAC than those of the OPCAB patients. The on-pump procedure was associated with higher degrees of oxidative stress than the off-pump coronary surgery. Activated neutrophils, xanthine oxidase of endothelial cells, and damaged heart mitochondria could be potential sources of free radical production during CABG operation. Extracorporeal circulation in the CABG operation, by increasing contact of blood with foreign substances, may also induce systemic inflammatory responses. These findings suggest that there are alterations in the non-enzymatic antioxidant defenses that can interfere in the direct removal of free radicals and in protection for biological sites.

Our results indicate an imbalance in total antioxidant capacity and free radical reaction products containing serum nitrates/nitrites and concentrations of nitrotyrosine during the ischemic phase and reperfusion, which is indirect evidence of free radical production in both the CABG and OPCAB groups. The patients undergoing CABG have more severe oxidative stress compared to the OPCAB patients. We conclude that CABG surgery elevates impairment of oxidant–antioxidant balance.

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