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Antioxidant Supplementation Attenuates Oxidative Stress in Patients Undergoing Coronary Artery Bypass Graft Surgery

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Ischemia-reperfusion has been reported to be associated with augmented oxidative stress in the course of surgery, which might be causally involved in the onset of atrial fibrillation (AF), the most common arrhythmia after cardiac surgery. We hypothesized that supplementation of antioxidants and n-3 polyunsaturated fatty acids (n-3 PUFAs) might lower the incidence of AF following coronary artery bypass graft (CABG) surgery. In the present study, by monitoring oxidative stress in the course of CABG surgery, we analyzed the efficacy of vitamins (ascorbic acid and α-tocopherol) and/or n-3 PUFAs (eicosapentaenoic acid and docosahexaenoic acid). Subjects (n = 75) were divided into 4 subgroups: control, vitamins, n-3 PUFAs, and a combination of vitamins and n-3 PUFAs. Fluorescent techniques were used to measure the antioxidative capacity, i.e. ability to inhibit oxidation. Total peroxides, endogenous peroxidase activity, and antibodies against oxidized LDL (oLAb) were used as serum oxidative stress biomarkers. Post-operative increase in oxidative stress was associated with the consumption of antioxidants and a simultaneous onset of AF. This was confirmed through an increased peroxide level and a decreased oLAb titer in control and n-3 PUFAs groups, indicating the binding of antibodies to oxidative modified epitopes. In both subgroups that were supplemented with vitamins, total peroxides decreased, and the maintenance of a constant IgG antibody titer was facilitated. However, treatment with vitamins or n-3 PUFAs was inefficient with respect to AF onset and its duration. We conclude that the administration of vitamins attenuates post-operative oxidative stress in the course of CABG surgery.

Keywords: cardio-vascular diseases; free radicals; ischemia/reperfusion; parenteral nutrition; vitamin C
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Introduction

Atrial fibrillation (AF) is a frequent self-perpetuating complication of cardiac surgery due to electrophysiological and structural remodeling (Van Wagoner and Nerbonne 2000). The incidence of AF after coronary artery bypass graft (CABG) surgery is a function of age, severity of cardiac disease, case complexity and co-morbidities. It is associated with greater in-hospital mortality, more strokes, longer length of stay in the ICU and hospital, increased complications, an increase in co-medication and a reduction in long-term survival after surgery (Villareal et al. 2004). Key events leading to AF were shown to be myocyte calcium overload in initiating atrial electrophysiological remodeling (Van Wagoner et al. 1999) and inflammation initiating a second messenger system and creating oxidative stress (Mihm et al. 2001). Several pharmacologic strategies beyond beta-blockers in the prevention of AF have been reported; among them ascorbic acid and n-3 polyunsaturated fatty acids (n-3 PUFAs = eicosapentaenoic acid and docosahexaenoic acid) have been suggested as effective therapy (Davis et al. 2010).

Ascorbic acid was shown to reduce post-operative AF, decrease time in the ICU and hospital, and reduce the time interval needed for rhythm restoration (Papoulidis et al. 2011). Female patients suffering from coronary artery dis-

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ease have also been reported to have significantly decreased α-tocopherol levels (Cavalca et al. 2009). The administration of a vitamin cocktail in the favorable proportion of about 10:1 (ascorbic acid vs. α-tocopherol) as in the human circulation (Stocker and Frei 1991) was shown to provide constant anti-oxidative protection for lipids and proteins and thus prevent a drastic peroxide excess in patients suffering from peripheral arterial disease (PAD) undergoing vascular surgery (Wonisch et al. 2005). Another study showed that supplementation with α-tocopherol was associated with a decrease in lipid peroxidation, i.e. plasma 8-isoprostanes and plasma total peroxides, as well as a decrease in urinary 8-hydroxydeoxyguanosine, a marker for oxidative DNA modification (Winklhofer-Roob et al. 2004). n-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), confer their cardio-protective action through anti-thrombotic and anti-arrhythmic action (Metcalf et al. 2007). The relationship between EPA and arachidonic acid and AF is inconsistent and still unclear (Gronroos and Alonso 2010).

In the past decade several oxidative stress biomarkers have been developed for use with body fluids to measure lipid peroxidation products and lipid peroxidation-induced tissue damage. The estimation of oxidative stress involves measurement of a complicated network of interlocking anti-oxidative systems, immunological reactions against reactive oxygen species, and the determination of breakdown products. Otherwise, the most important way to study oxidative stress is a combination of complementary methods. We decided upon fluorescent techniques to specifically measure water- and lipid-soluble antioxidants (Hofer et al. 1995; Mayer et al. 2001). Peroxidase activity was used to measure the endogenous anti-oxidative capacity (Tatzber et al. 2003). Antibodies against oxidized LDL (oLAb) (Tatzber and Esterbauer 1995) and total peroxides (Tatzber et al. 2003) were determined as oxidative stress biomarkers. In this prospective pilot study we focused on monitoring of oxidative stress in the course of CABG surgery with special attention to anti-oxidative effects of ascorbic acid and α-tocopherol as well as the anti-inflammatory effects of EPA and DHA (Mori and Beilin 2004). AF and its duration were used as a clinical target and oxidative stress as a biochemical objective. We hypothesized that in the course of CABG surgery free radical-induced oxidative stress consumes anti-oxidants in a time-dependent manner and might provoke AF. Supplementation of water- and lipid-soluble antioxidants might attenuate the decreased anti-oxidative capacity and the n-3 PUFAs could also guard against AF.

Materials and Methods

Subjects

In this randomized double-blinded clinical trial we investigated 75 subjects (68 males and 7 females, aged 66 ± 8 (mean ± S.D.) years). These subjects were divided into 4 subgroups, i.e. controls (C; n = 20), vitamins (Vit; n = 19), n-3 PUFAs (n = 19), and vitamins plus n-3 PUFAs (Vit-n-3 PUFAs; n = 17). The rationale for this nutritional regimen was the investigation of anti-oxidative and anti-inflammatory effects of Vit and n-3 PUFAs both singly and in combination in the course of CABG surgery. Patients were enrolled at the Cardiosurgical Department, Paracelsus Medical University Hospital Salzburg, Austria. Patients were in good pharmacological control, in particular for lipid profile, blood pressure values and glycemic control as recommended by current guidelines. This trial was conducted according to the Helsinki Declaration of the World Medical Association (2000). Ethical approval of the study was obtained from the Ethical Clinical Board of the hospital. Informed written consent was obtained from all participants before the trial.

Blood samples

Blood samples were taken pre-operatively (T1) and intra-operatively: 5 minutes after the start of extra-corporal circulation (ECC) (T2), 2 minutes before opening the aorta clamp (T3), 2 minutes after opening the aorta clamp (T4), and 10 minutes after opening the aorta clamp (T5); thereafter post-operatively: at 6 hours (T6), 24 hours (T7), 48 hours (T8) and 72 hours (T9) (Fig. 1). Samples were centrifuged 30 minutes after collection at 1,500 × g for 10 minutes. Serum was stored at −20°C until use, i.e. for a maximum of 2 weeks.

Treatment with n-3 PUFAs

Subgroups n-3 PUFAs and Vit-n-3 PUFAs received two preoperative infusions (42 hours and 18 hours before surgery) of Omegaven® (n-3 PUFAs - EPA and DHA ~ 0.15 g fish oil/kg body weight) (Fresenius/Kabi, Graz, Austria) with a velocity of max. 0.5 ml/kg body weight for at least 3 hours. The third Omegaven® infusion was given as a single dose of 50 ml (= 5 g fish oil) 42 hours after surgery (Fig. 1).

Treatment with Vitamins

Subgroups Vit and Vit-n-3 PUFAs received a cocktail of ascorbic acid contained in 1 ampoule Mel-C® (= 500 mg ascorbic acid) and α-tocopherol delivered as Tocovenös® (3 ampoules = 45IE Vitamin E, all-rac-α-tocopherol-acetate) dissolved in 250 ml physiological sodium chloride solution. All ingredients were purchased from Fresenius-Kabi, Graz, Austria. This vitamin cocktail infusion was administered intra-operatively 30 minutes before reperfusion and then post-operatively, 120 minutes after reperfusion (Fig. 1).

Serum total peroxides

Serum total peroxide concentrations were determined by a rapid enzymatic in vitro diagnostic assay (TOC®) supplied by LDN (Labor Diagnostik Nord, Nordhorn, Germany) as previously described (Tatzber et al. 2003). The test system was based on a peroxide/peroxidase reaction using 3,5,3´,5´-tetramethylbenzidine (TMB) as substrate. Reference values were reported to be less than 200 μM (Lindschinger et al. 2004). Peroxide levels were evaluated as arbitrary units with a CV of 3.73% (intra-assay variance) and 5.51% (inter-assay variance), and expressed as percentage of baseline.

Endogenous Peroxidase-Activity

Serum peroxidase activity (EPA®) was determined by the reaction of endogenous peroxidases with hydrogen peroxide, using 3,5,3´,5´-tetrametayhlbenzidine (TMB) as the chromogenic substrate supplied by LDN (Labor Diagnostik Nord, Nordhorn, Germany) as previously described (Tatzber et al. 2003). Reference values are in the range of 5 ± 3 mU/mL. Results were evaluated as mU/mL with a

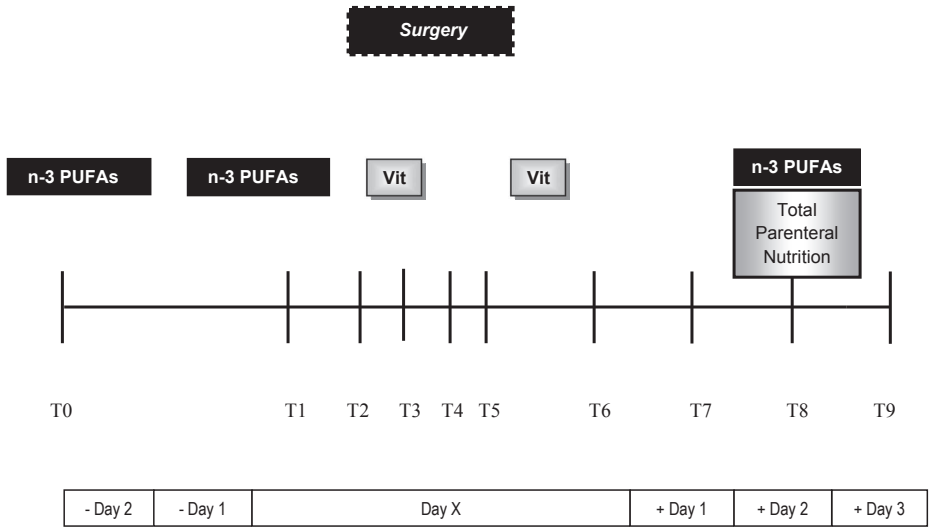

Fig. 1. Study design.
Time schedule for blood sampling (T0-T9) and supplementation with n-3 PUFAs and vitamins, including total parenteral nutrition with StructoKabiven®, relevant to respective subgroups.
Pre-operatively (T1); intra-operatively: 5 minutes after the start of extra-corporal circulation (ECC) (T2); two minutes before opening the aorta clamp (T3); two minutes after opening the aorta clamp (T4); 10 minutes after opening the aorta clamp (T5); post-operatively: six hours (T6), 24 hours (T7), 48 hours (T8) and 72 hours (T9).

CV of 3.1% (intra-assay variance) and 5.5% (inter-assay variance) and expressed as percentage of baseline.

Determination of oLAb

oLAb titres were measured in serum with a commercial enzyme immunoassay (oLAb®) supplied by Biomedica (Vienna, Austria) according to the method of (Tatzber and Esterbauer 1995). The assay is based on the binding reaction of the 1:50 diluted samples to the previously oxidized LDL bound (by cupric ions) to the microtiter wells. Detection was accomplished by binding a secondary, peroxidase-coupled anti-IgG antibody, which permitted colorimetric detection of this enzyme, with tetramethylbenzidine as substrate. Reference values were found between 200-600 mU/mL (Pincemail et al. 2000). Results were evaluated as mU/mL with a CV of 4.3% (intra-assay variance) and 8.2% (inter-assay variance) and expressed as percentage of baseline.

Determination of water- and lipid-soluble antioxidants

Lag-time of ex vivo degradation of the fluorophores 1,6-diphenylhexatriene propionic acid (DPHPA) and 1-palmitoyl-2-((2-(4-(6-phenyl-trans-1,3,5-hexatrienyl)phenyl)ethyl)-carbonyl)-*sn*-glycero-3-phosphocholine (DPHPC) by ROS in serum was determined by fluorescence techniques according to Mayer et al. (2001) and Hofer et al. (1995), with modifications to the oxidation part (Protein-Ox® and Lipid-Ox®, Omnignostica GmbH., Höflein/Klosterneuburg, Austria). Briefly, 100 μL sera were incubated with the fluorophore DPHPA at 37°C for 1 hour and kept under argon with DPHPC at 37°C for 12 hours. Oxidation was started by peroxide (0.004%)/ peroxidase (10 U/ml) and the time-dependent decrease in fluorescence intensity was monitored at 430 nm (excitation at 360 nm) on a BMG Optima fluorescence well-plate reader (Offenburg, Germany) at 37°C. The radical-induced degradation of the fluorescent dye resembles the destruction of natural substances, i.e. proteins and lipids. Results were evaluated as "lag time" in minutes with the respective coefficients of variation (CV) for DPHPA and DPHPC (DPHPA: Intra-assay variance 5.2%; Inter-assay variance 4.5%; DPHPC: Intra-assay variance 3.9%; Inter-assay variance 3.9%). Raw data were corrected for hematocrit and expressed as percentage of baseline i.e. "pre-operative" (T1) values were determined as 100%. This procedure was applied for all biomarkers.

Statistics

For the statistical analysis both a per-protocol and an intention-to-treat approach were used. By definition, the per-protocol analysis would take precedence in the evaluation of efficacy. Missing values were not replaced.

Group comparisons of metric variables were performed by a non-parametric analysis of variance (Kruskal-Wallis test followed by Nemenyi's multiple comparisons). For group comparison of nominal variables, the exact chi-square test was used.

Differences between 2 cohorts (AF, yes/no) were analyzed by the Mann-Whitney-*U* test for metric variables and by the exact chi-square test or the Fisher's exact test for nominal variables. Pre-post comparisons of metric variables were performed by the Wilcoxon test. All tests were two-tailed with a confidence level of 95% ($p < 0.05$). As no adjustment was made for type-I-error, the respective *p*-values are only descriptive. For all calculations PASW Statistics 18 (SPSS Inc., IBM Company Headquarters, 233 S. Wacker Drive, Chicago, Illinois 6060) was used. Unless otherwise mentioned descriptive data in the text reflect medians and quartiles (in brackets).

Results

Baseline characteristics of patients

All data were corrected for hematocrit. Demographic data are listed in Table 1.

Table 1. Baseline characteristics of patients subjected to CABG surgery.

	C	Vit	n-3 PUFAs	Vit-n-3 PUFAs	*p*-value
Age (years)	65 (10)	67 (6)	65 (6)	66 (10)	n.s.
Sex (male/female)	17/3	17/2	19/0	15/2	n.s.
Weight (kg)	78.58 (9.51)	78.86 (9.70)	75.76 (6.75)	77.45 (12.72)	n.s.
Height (cm)	168.55 (7.52)	173.26 (6.24)	171.32 (5.51)	172.24 (7.33)	n.s.
BMI (kg/m^2)	27.76 (3.85)	26.21 (2.23)	25.85 (2.50)	26.04 (3.32)	n.s.
Comorbidities (%)					
Systemic hypertension	85	83	80	71	n.s.
Diabetes mellitus II	15	22	32	25	n.s.
COPD	12	17	0	26	n.s.
Smoker	65	52	54	65	n.s.
Myocardial infarction	46	26	29	44	n.s.
Stent	15	13	13	22	n.s.
Medication (%)					
Betablockers	85	87	72	92	n.s.
Aceteylic salicylic acid (ASS)	89	91	100	96	n.s.
Statins	85	87	92	92	n.s.
ACE Inhibitors	69	65	72	38	n.s.
Nitrates	12	9	20	13	n.s.
Perioperative features					
OP time (min)	229	208	229	243	0.007[1]
Cross-clamp time (min)	43	42	50	54	0.014[2]
Extracorporeal circulation (min)	83	82	90	102	0.010[3]
Valve replacement (n)	4	4	3	4	n.s.

C, control; Vit, vitamin supplemented; n-3 PUFAs, n-3-polyunsaturated fatty acids supplemented; BMI, body-mass index; COPD, chronic obstructive pulmonary disease; ACE, angiotensin converting enzyme; OP time (min), operation time in minutes; 1-3: $p < 0.05$: Vit versus Vit-n-3 PUFAs.

AF

Post-operative AF was observed between the first and third post-operative days in 6 C subjects, 5 Vit subjects, 4 n-3 PUFAs subjects and 7 Vit-n-3 PUFAs-subjects (Table 2). The frequency of AF was proportional to the time after CABG surgery: first post-operative day (POD) (C = 1; Vit = 1; n-3 PUFAs = 1; Vit-n-3 PUFAs = 1), first to second POD (C= 2; Vit = 2; n-3 PUFAs = 2; Vit-n-3 PUFAs = 3), and second to third POD (C = 5; Vit = 5; n-3 PUFAs = 3; Vit-n-3 PUFAs = 5). The frequency of AF was not significantly different between the groups (first POD: $p > 0.999$; first to second POD: $p = 0.895$; second to third POD: $p = 0.742$; end of surgery until end of observation: $p = 0.821$). Although there were indications for the shortest time for rhythm restoration in the Vit-n-3 PUFAs subjects (Table 2), this was not statistically significant.

Total peroxides

Vitamin supplementation was associated with a significant decrease in total peroxides both in Vit as well as in Vit-n-3 PUFAs, compared to C and n-3 PUFAs in the time-slot from 2 minutes before opening the aorta clamp to 6 hours after surgery (Fig. 2), as calculated in the multiple comparisons analysis according to Nemenyi (Table 3). Furthermore, we observed significantly decreased total peroxides in Vit ($p < 0.001$); n-3 PUFAs ($p < 0.006$) and Vit-n-3 PUFAs ($p < 0.004$) at the first POD, compared to C, where peroxides remained at baseline levels ($p = 0.179$; Wilcoxon test) (Table 4). The anti-oxidative effect of the vitamin supplementation was no longer active on the second and third POD.

Peroxidase activity

Peroxidase activity was significantly reduced at the first POD in C ($p = 0.015$), Vit ($p = 0.011$) and Vit-n-3 PUFAs ($p < 0.01$), while in n-3 PUFAs ($p = 0.21$) there was no statistically significant difference in comparison to baseline values. Significant decreases in peroxidase activities were observed in vitamin-supplemented groups Vit ($p = 0.013$) and Vit-n-3 PUFAs ($p = 0.013$) at the second POD, while C ($p = 0.064$) and n-3 PUFAs ($p = 0.084$) did not significantly differ from baseline levels (Fig. 3).

oLAb

Antibodies against oxidized LDL of the IgG type were significantly decreased only in C ($p = 0.002$) at the first POD while all the other subgroups [Vit ($p = 0.573$), n-3 PUFAs ($p = 0.246$) and Vit-n-3 PUFAs ($p = 0.381$)] did not

Table 2. Duration of atrial fibrillation occurred after CABG surgery.

			Min	Percentile 25%	Median	Mean	Percentile 75%	Max	STD
EoS- T7	C	(n = 1)	427.00	427.00	427.00	427.00	427.00	427.00	–
	Vit	(n = 1)	1140.00	1140.00	1140.00	1140.00	1140.00	1140.00	–
	n-3 PUFAs	(n = 1)	14.00	14.00	14.00	14.00	14.00	14.00	–
	Vit-n-3 PUFAs	(n = 1)	0.50	0.50	0.50	0.50	0.50	0.50	–
T7-T8	C	(n = 2)	45.00	45.00	220.00	220.00	–	395.00	247.50
	Vit	(n = 2)	84.00	84.00	312.50	312.50	–	541.00	323.00
	n-3 PUFAs	(n = 2)	406.00	406.00	553.00	553.00	–	700.00	207.00
	Vit-n-3 PUFAs	(n = 3)	20.00	20.00	104.00	159.50	–	355.00	174.50
T8-T9	C	(n = 5)	71.00	80.00	130.00	281.00	557.00	930.00	365.50
	Vit	(n = 5)	66.00	69.00	202.00	502.00	1085.50	1473.00	601.50
	n-3 PuFAs	(n = 3)	46.00	46.00	240.00	313.50	–	654.00	310.50
	Vit-n-3 PUFAs	(n = 5)	1.50	23.50	58.00	93.00	180.00	240.00	92.50
EoS-T9	C	(n = 6)	71.00	118.50	305.50	378.50	626.50	930.00	322.50
	Vit	(n = 5)	72.00	111.00	202.00	855.00	1926.00	3154.00	1308.50
	n-3 PUFAs	(n = 4)	46.00	94.50	330.00	515.00	1120.50	1354.00	580.00
	Vit-n-3 PUFAs	(n = 7)	0.50	1.50	120.00	135.00	240.00	355.00	128.50

Min, minimum; Max, maximum; STD, standard deviation; EoS, end of surgery; C, control; Vit, vitamin supplemented; n-3 PUFAs, n-3-polyunsaturated fatty acids supplemented; Vit-n-3 PUFAs, vitamins and n-3-polyunsaturated fatty acids supplemented.

Shown are the duration of atrial fibrillation (in minutes) from the end of surgery until the first post operative day POD (EoS-T7), from the first to the second POD (T7-T8), from the second to the third POD (T8-T9), and from the end of surgery until the third POD (Eos-T9) in each subgroup.

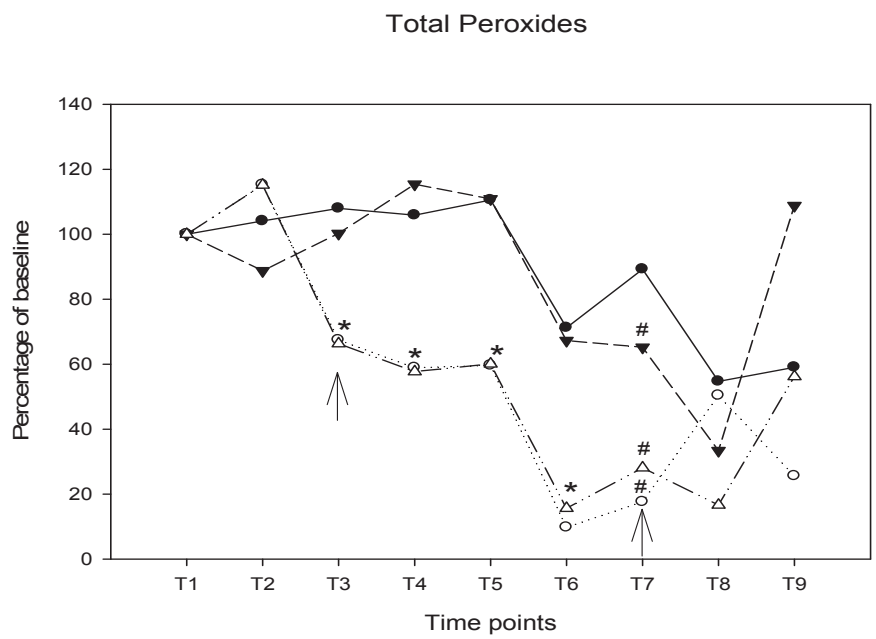

Fig. 2. Breakdown products of ROS.

Total peroxides in the course of CABG surgery and in the follow-up in the subgroups C (n = 20; ●), Vit (n = 19; ○), n-3 PUFAs (n = 19; ▼), and Vit-n-3 PUFAs (n = 17; Δ). The arrow at T3 comprising asterisks from T3-T6 indicate significant decreased peroxide levels in vitamin-supplemented groups. The arrow at T7 and hash-markers indicate significant decreased peroxide levels in n-3 PUFAs, Vit-n-3 PUFAs and Vit compared to baseline. All data were corrected for hematocrit and values were expressed as percentage of baseline.

Table 3. Impact of vitamins in respect of total peroxides and water-soluble antioxidants - multiple comparison analysis according to Nemenyi.

Biomarker	Time	C: Vit	C: n-3 PUFAs	C: Vit-n-3 PUFAs	Vit: n-3 PUFAs	Vit: Vit-n-3 PUFAs	n-3 PUFAs: Vit-n-3 PUFAs
TOC	(AUC: T4 to T6-T1)	0.067	0.963	0.141	0.018*	0.995	0.046*
TOC	(T3)	0.059	0.840	0.032*	0.005**	0.993	0.003**
TOC	(T4)	0.029*	0.924	0.016*	0.004**	0.994	0.002**
TOC	(T5)	0.042*	0.928	0.038*	0.007**	> 0.999	0.006**
TOC	(T6)	0.018*	0.997	0.016*	0.010*	0.999	0.009**
Proteinox	(T3)	0.941	0.197	0.907	0.047*	0.999	0.038*

TOC, total peroxides; Proteinox, water soluble antioxidants; Nemenyi (post hoc test); C, control; Vit, vitamin supplemented; n-3 PUFAs, n-3-polyunsaturated fatty acids supplemented; Vit-n-3 PUFAs, vitamins and n-3-polyunsaturated fatty acids supplemented; *$p < 0.05$; **$p < 0.01$.

Table 4. Intra-group comparison for the first and second POD adjusted to baseline (T1) for oxidative stress biomarkers and antioxidant capacity according to Wilcoxon.

Biomarker	Time point	C P	Vit P	n-3 PUFAs P	Vit-n-3 PUFAs P
TOC (AU)	T7	0.179	0.001**	0.004**	0.006**
TOC (AU)	T8	0.879	0.334	0.266	0.102
Endogenous peroxidase-activity (mU/mL)	T7	0.015*	0.011*	0.210	0.010*
Endogenous peroxidase-activity (mU/mL)	T8	0.064	0.013*	0.084	0.013*
Anti-oxLDL antibody (mU/mL)	T7	0.002**	0.573	0.246	0.381
Anti-oxLDL antibody (mU/mL)	T8	0.043*	0.717	0.031*	0.227
Proteinox (min)	T7	0.003**	< 0.001***	< 0.001***	< 0.001***
Proteinox (min)	T8	0.020*	0.001**	< 0.001***	< 0.001***
Lipidox (min)	T7	0.002**	< 0.001***	< 0.001***	< 0.001***
Lipidox (min)	T8	0.027*	0.001**	< 0.001***	< 0.001***

POD, post operative day; C, control; Vit, vitamin supplemented; n-3 PUFAs, n-3-polyunsaturated fatty acids supplemented; Vit-n-3 PUFAs, vitamins and n-3-polyunsaturated fatty acids supplemented; AU, arbitrary units; TOC, total peroxides; oLAb, Antibodies against oxidized LDL; Proteinox, water-soluble antioxidants; Lipidox, lipid-soluble antioxidants; *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$.

differ from baseline (Table 4). Significantly decreased oLAb titers were further observed in C ($p = 0.043$) and n-3 PUFAs ($p = 0.031$) in comparison to the vitamin supplemented groups [Vit ($p = 0.717$) and Vit-n-3 PUFAs ($p = 0.227$)] at the second POD, which did not significantly differ from baseline (Fig. 4).

Water- and Lipid-soluble antioxidants

Water- and lipid-soluble antioxidants were significantly decreased at the first and second POD (Table 4) in all subgroups but recovered at the third POD (Fig. 5). Multiple comparison analysis according to Nemenyi revealed a significant difference between n-3 PUFAs compared to Vit ($p = 0.047$) and Vit-n-3 PUFAs ($p = 0.038$) two minutes before opening the aorta clamp in the case of water-soluble antioxidants, i.e. vitamin-supplemented subjects were better supplied with water-soluble antioxidants.

Discussion

We monitored oxidative stress in the course and follow-up of CABG surgery and investigated the impact of ascorbic acid, α-tocopherol and n-3 PUFAs in the follow-up for another 3 days.

Intra-operatively, we could not verify substantial quantities of emerging reactive oxygen species (ROS) in any of the subgroups, apart from decreased peroxidase activity 5 minutes after the start of ECC. We saw no changes in the other oxidative stress biomarkers in comparison to preoperative values. Nevertheless, 6 hours after surgery we discerned a distinct consumption of water- and lipid-soluble antioxidants, which became significantly different from baseline values between the first and second POD. This unequivocally reflects post-operative oxidative stress, which was associated with a decreased oLAb titer, a persistent peroxide level and a decrease in the endogenous antioxidant system, i.e. peroxidase activity in control subjects. The consumption of antioxidants confirms a recent report on patients undergoing coronary artery bypass grafting that also indicated a significant consumption of lipid-soluble antioxidants at the first POD, which was associated with a

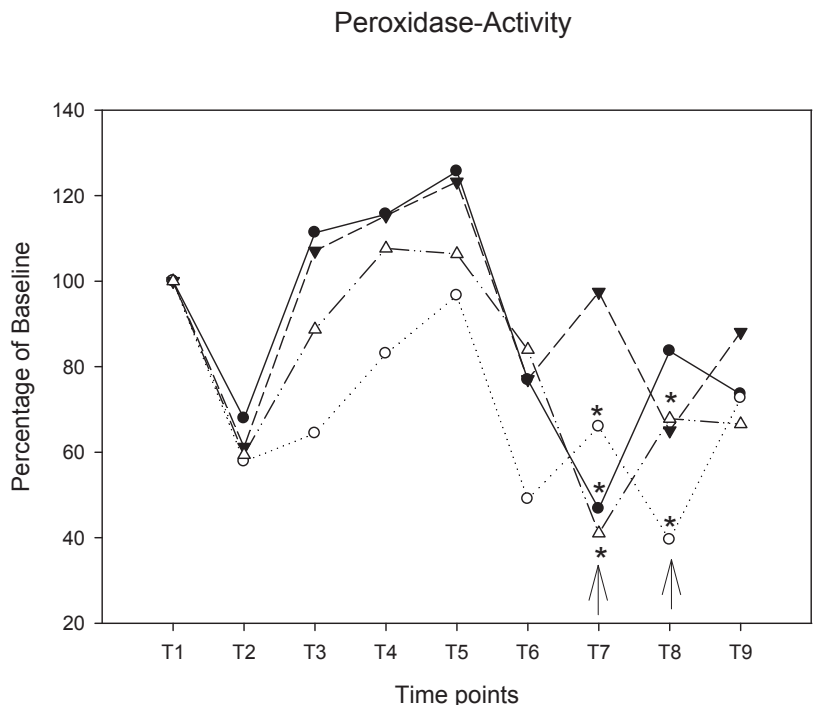

Fig. 3. Enzymatic protection.
Endogenous peroxidase activity in the course of CABG surgery and in the follow-up in the subgroups C ($n = 20$; ●), Vit ($n = 19$; ○), n-3 PUFAs ($n = 19$; ▼), and Vit-n-3 PUFAs ($n = 17$; Δ). The arrow at T7 indicates significant decreased peroxidase activities in control, Vit-n-3 PUFAs and Vit groups compared to baseline. The arrow at T8 indicates significant decreased peroxidase activities in Vit-n-3 PUFAs and Vit groups compared to baseline. All data were corrected for hematocrit and values were expressed as percentage of baseline.

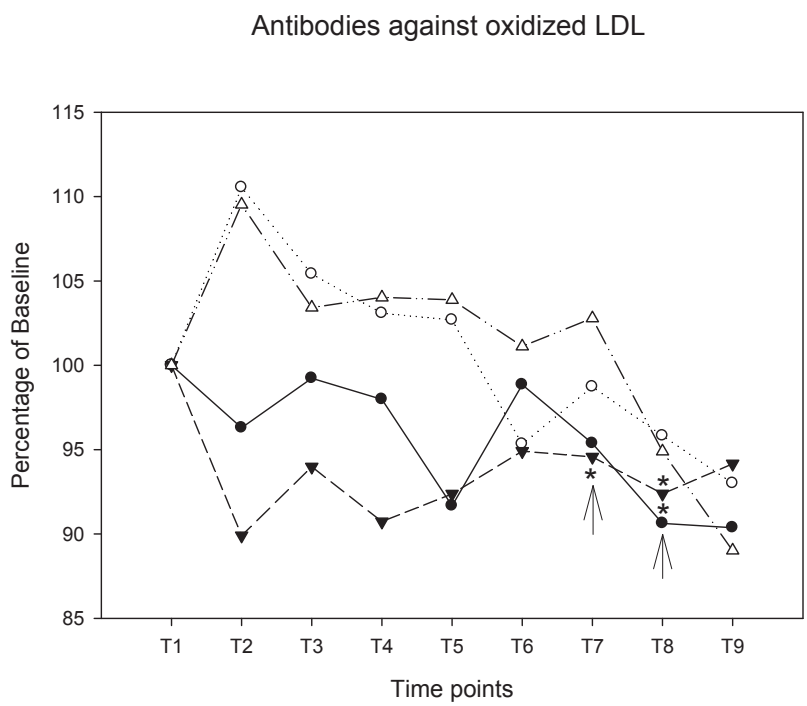

Fig. 4. Immunologic response.
Antibodies against oxidized LDL in the course of CABG surgery and in the follow-up in the subgroups C ($n = 20$; ●), Vit ($n = 19$; ○), n-3 PUFAs ($n = 19$; ▼), and Vit-n-3 PUFAs ($n = 17$; Δ). The arrow at T7 indicates a significant decreased oLAb titer in the control group compared to baseline. The arrow at T8 indicates significant decreased oLAb titers in control and n-3 PUFAs groups compared to baseline. All data were corrected for hematocrit and values were expressed as percentage of baseline.

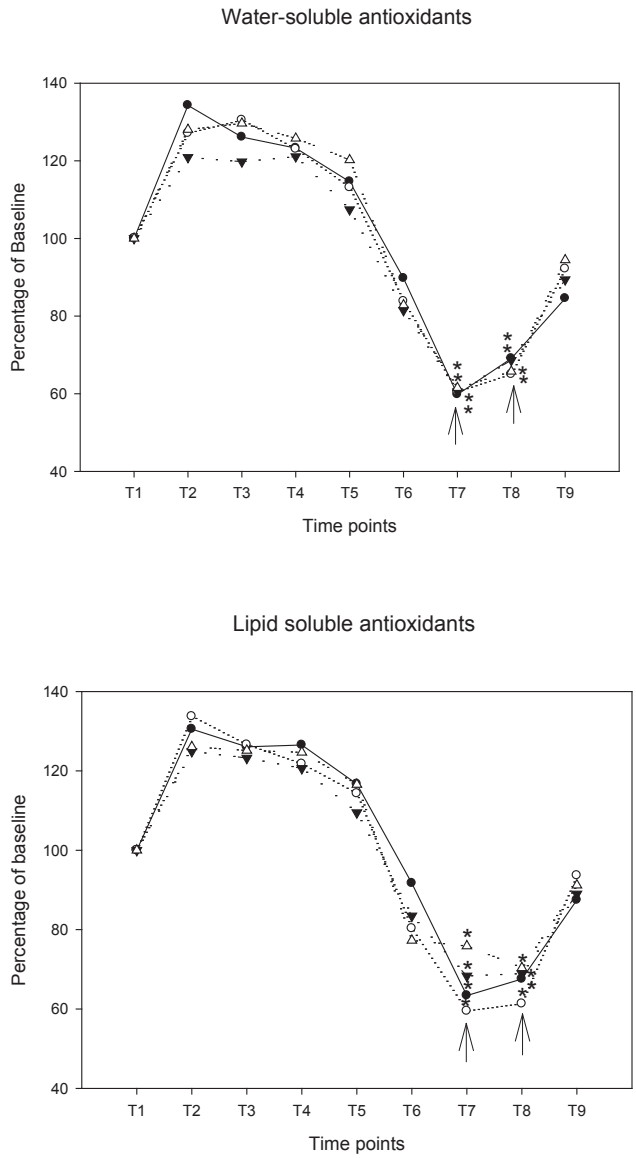

Fig. 5. Antioxidative capacity.
Water-soluble antioxidants (upper panel) and lipid-soluble antioxidants (lower panel) in the course of CABG surgery and in the follow-up in the subgroups C (n = 20; ●), Vit (n = 19; ○), n-3 PUFAs (n = 19; ▼), and Vit-n-3 PUFAs (n = 17; Δ). The arrows at T7 and T8 indicate significant decreased water- and lipid-soluble antioxidants in all sub-groups compared to baseline. All data were corrected for hematocrit and values were expressed as percentage of baseline.

significant increase in Interleukin-6 (Stoppe et al. 2013).

The vitamin cocktail was able to attenuate oxidative stress, i.e. decrease of total peroxides, for a period of six hours but was consumed thereafter and this is why we could not observe a protective effect with AF, which first occurred on the first POD. The anti-oxidative capacity of vitamins did take the load off the endogenous peroxidase activity, which was decreased even on the first and second POD compared to baseline levels and facilitated the maintenance of a constant IgG titer against oxidized LDL in the vitamin-supplemented groups. This indicates a lack of emerging epitopes that might decrease the free antibody titer. The anti-oxidative effect of ascorbic acid is consistent with previous reports indicating protection against ischemia/reperfusion damage with the aid of a vitamin cocktail in, among others, PAD patients to prevent oxidative stress (Wonisch et al. 2005) and AF (Carnes et al. 2001; Eslami et al. 2007) after vascular surgery.

It is worth mentioning that the consumption of antioxidants and the onset of AF emerged simultaneously. The highest incidence of AF occurred between the second and third POD, which is consistent with a previous report (Eslami et al. 2007). Moreover, peak incidence of AF coincides with peak levels of inflammation markers (Bruins et al. 1997), thus underscoring the connection between inflammation and oxidative stress (Mori et al. 2003; Korantzopoulos et al. 2006).

The anti-inflammatory and anti-oxidative features of

EPA and DHA might contribute to the favorable effect of n-3 PUFAs on post-operative AF (Mori et al. 2003). Considering the action of n-3 PUFAs, it is noteworthy that they are incorporated into phospholipids in a dose- and time-dependent manner at the expense of arachidonic acid and other n-6 PUFAs (Versleijen et al. 2012). Thus, they inhibit the pro-inflammatory action of arachidonic acid derived eicosanoids. In addition, these fatty acids can also influence cytokines and have effects on transcription factors that regulate inflammatory gene expression. This seems to be important in attenuating inflammatory processes. The anti-inflammatory and anti-oxidative effects were confirmed in patients subjected to cardiac surgery with extracorporal circulation, although the authors neither achieved a significant difference for post-operative AF nor in the duration of AF between n-3 PUFAs plus vitamin supplemented subjects compared to placebo (Castillo et al. 2011). This is consistent with results of our study, although not significant: in both studies the mean duration of AF was reduced. This finding contradicts other studies, which reported significant reductions of post-operative AF by administration of n-3 PUFAs (Mori et al. 2003; Sorice et al. 2011). The discrepancy might be explained by the fact that patients with low baseline n-3 PUFAs levels may benefit disproportionately from DHA and EPA substitutions (Skuladottir et al. 2011). Such divergent results could, however, be attributed to limited study populations. Indeed, the population of the present pilot-study comprised only 20% compared to (Sorice et al. 2011). Last but not least we observed a prolonged operation time, cross-clamp time and extracorporeal circulation for Vit-n-3 PUFAs patients compared to Vit subjects that might have adversely affected the therapeutic effect in this subgroup.

The weakness of the present study was the small number of cases making up the 4 subgroups. In addition, the time window for vitamin substitutions was too narrow. Thus, the tendency of n-3 PUFAs and Vit to improve AF should be investigated with a larger number of cases and continuous treatment until discharge from intensive care.

In conclusion, this study presents the monitoring of oxidative stress in CABG patients starting two days preoperatively until the third POD. Substantial quantities of ROS emerged 6 hours after surgery until the end of observation, i.e. the third POD. Furthermore, we observed a significant attenuation of oxidative stress in vitamin-supplemented CABG patients, as indicated by a significant decrease in total peroxides. The peroxidase activity was decreased even at the first and second POD compared to baseline levels, and IgG antibody titers against oxidized LDL were maintained in the Vit-supplemented groups. In contrast, treatment with vitamins and/or n-3 PUFAs did not significantly affect the frequency of AF and the duration of events. Nevertheless, the diminished occurrence and decreased duration of AF may become significant in a future study with a larger patient collective.

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Conflict of Interest

The authors declare no conflict of interest.

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