Regional Anesthesia and Pain

Epidural anesthesia may attenuate lipid peroxidation during aorto-femoral surgery

[L'anesthésie péridurale peut diminuer la peroxydation lipidique pendant une intervention chirurgicale aorto-fémorale]

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Purpose: To determine the effect of epidural anesthesia (EP) on oxygenation of the chronically ischemic limb in patients undergoing aorto-femoral bypass grafting and to assess whether it produces an alteration of lipid peroxidation and antioxidant status following revascularization.

Methods: In this prospective, randomized, single-blinded study 40 ASA II or III patients undergoing elective aorto-femoral bypass grafting were allocated to receive general anesthesia (group GA, $n = 20$), or epidural + GA (group EP, $n = 20$) during surgery. Femoral venous blood-gas status, activities of the protecting antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-px), glutathione reductase (GSH-rd), glutathione (GSH) and thiobarbituric acid-reactive substances (TBARS) as a marker of lipid peroxidation were determined in blood samples taken from the femoral vein at different intervals before and after revascularization.

Results: Before the induction of anesthesia in group EP, femoral venous PO_2 [mean (standard deviation), 95% confidence interval] increased after achieving an adequate level of blockade by EP extending to the dermatomal level of T6–8 [29.32 (4.6), 26.34–32.30 to 36.29 (4.6), 33.37–39.22 mmHg, $P < 0.05$]. Femoral venous PO_2 was similar in both groups thereafter. In the GA group a significant increase in erythrocyte TBARS was observed immediately after restoration of blood flow when compared with baseline values [221.32 (102), 148.35–294–29 to 337.26 (123) 248.99–425.53 $nmol \cdot g^{-1}$ hemoglobin, $P < 0.01$] but not at any other moment. In the EP group TBARS did not increase throughout the study. Within group comparisons revealed no significant differences in GSH, GSH-px, GSH-rd and SOD.

Conclusion: In patients with atherosclerotic aorto-iliac occlusive disease EP may possibly attenuate lipid peroxidation following revascularization but has no effect on antioxidant enzyme activities.

Objectif : *Déterminer l'effet de l'anesthésie péridurale (AP) sur l'oxygénation du membre soumis à l'ischémie chez les patients qui subissent un pontage aorto-fémoral et évaluer si elle produit une altération de la peroxydation lipidique et de l'état antioxydant à la suite de la revascularisation.*

Méthode : *Une étude prospective, randomisée et à simple insu a été menée auprès de 40 patients d'état physique ASA II ou III qui devaient subir un pontage aorto-fémoral sous anesthésie générale (groupe AG, $n = 20$) ou anesthésie péridurale + AG (groupe AP, $n = 20$). La gazométrie du sang veineux fémoral, les activités des enzymes protecteurs antioxydants superoxyde dismutase (SOD), glutathion peroxydase (GSH-px), glutathion réductase (GSH-rd), glutathion (GSH) et les substances réactives à l'acide thiobarbiturique (SRATB), comme marqueur de la peroxydation lipidique, ont été mesurés dans les échantillons sanguins prélevés de la veine fémorale à différents intervalles avant et après la revascularisation.*

Résultats : *Avant l'induction de l'AP, la PO_2 veineuse fémorale [moyenne (écart type), intervalle de confiance de 95 %] s'est élevée après le blocage péridural adéquat s'étendant au niveau du dermatome T6–8 [29,32 (4,6) 26,34–32,30 à 36,29 (4,6) 33,37–39,22 mmHg, $P < 0,05$]. La PO_2 veineuse fémorale a été similaire dans les deux groupes par la suite. Dans le groupe AG, une hausse significative d'érythrocyte SRATB, en comparaison avec les valeurs de base, a été notée immédiatement et seulement après la restauration du débit sanguin [221,32 (102) 148,35–294–29 à 337,26 (123) 248,99–425,53 $nmol \cdot g^{-1}$ hémoglobine, $P < 0,01$]. Dans le groupe AP, les SRATB n'ont pas augmenté pendant l'étude. Aucune différence intragroupe significative de GSH, GSH-px, GSH-rd et SOD n'a été notée.*

Conclusion : *Chez les patients atteints d'occlusion aorto-iliaque, l'AP peut atténuer la peroxydation lipidique à la suite de la revascularisation, mais n'a pas d'effet sur les activités antioxydantes des enzymes.*

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THE choice of anesthesia for revascularization of the ischemic limb due to atherosclerotic occlusive disease has been debated for many years and many clinicians have developed strong convictions that certain anesthetic techniques are preferable for these patients.[1,2] Objective measures of outcome in patients undergoing lower extremity revascularization are of great significance because of the high incidence of morbidity after such procedures, as well as the relationship of that morbidity to pathophysiologic changes in the perioperative period.[3,4] Progressive occlusion of the aorto-iliac/femoral arterial tree results in vascular insufficiency and ischemia of the lower extremities. Restoration of blood flow to the ischemic limb may initiate a cascade of cellular and biochemical events culminating in muscle edema, necrosis and impaired muscle function. A significant fraction of this damage is caused by oxygen-derived free radicals (OFR) generated at reperfusion, rather than the ischemia itself. The most damaging effect of free radicals is lipid peroxidation of the cell membranes, which occurs when the oxygen flux exceeds the capability of the multiple endogenous antioxidant mechanisms.[5,6]

Perioperative morbidity including cardiac, pulmonary and vascular graft complications in patients receiving general anesthesia (GA) alone or GA in combination with epidural anesthesia (EP) for aortic and lower extremity vascular surgery has been compared in some studies.[7] However there has been no study inquiring if EP-induced vasodilation ameloriates oxygenation of the ischemic limb before reperfusion begins and thus improves oxidative stress by acting as a preconditioner.

In the present study we examined the effect of EP supplemented with GA on ameliorating the oxygenation of the ischemic limb in patients with atherosclerotic aorto-iliac occlusive disease (AOD). We also assessed if EP could improve lipid peroxidation and antioxidant enzyme activities after revascularization in comparison with patients receiving GA alone.

Methods

After local Ethics Committee approval and written informed consent, 40 patients scheduled for elective aorto-femoral bypass grafting because of atherosclerotic AOD were enrolled in the study. The patients were randomly allocated into two groups using computer-generated random numbers to receive either GA (GA group, n = 20), or EP supplemented with GA (EP group, n = 20). Patients with coagulation disorders, preexisting neurological deficit or patients who refused insertion of an epidural catheter were excluded. Peri-aortic collateral vessels seen on preoperative aortograms were quantitated by a vascular radiologist blinded to group assignment, according to the method described by Johnston *et al.*[8] All patients were premedicated with midazolam, 5 mg intramuscularly one hour before admission to the operating room. In the operating room patients were monitored with a radial artery cannula and a central venous catheter in addition to routine monitoring. Before induction of GA, epidural puncture was performed with an 18-gauge Tuohy needle in the lumbar region using the loss of resistance technique. A 20-gauge catheter was inserted cephaled 4 to 6 cm into the epidural space and proper placement was tested with 3 mL lidocaine 2%. Following fluid replacement with isotonic saline 7 $mL \cdot kg^{-1}$, the patients in the EP group received 14 to 18 mL of bupivacaine, 5 $mg \cdot mL^{-1}$ to achieve an analgesic T_{6-8} dermatomal level. The effect of blockage was tested bilaterally at the midclavicular line after 30 to 40 min by pinprick. The block was maintained with the infusion of bupivacaine 2.5 $mg \cdot mL^{-1}$, 6 to 8 $mL \cdot hr^{-1}$, until the end of surgery. In the GA group no epidural medication was injected until the end of the study period and the epidural route was used only for postoperative analgesia. Induction of anesthesia was similar in both groups and was accomplished with *iv* fentanyl 2 to 5 $\mu g \cdot kg^{-1}$ followed by etomidate 0.3 $mg \cdot kg^{-1}$. Vecuronium was used to facilitate tracheal intubation and surgical relaxation. After intubation of the trachea, anesthesia was maintained with oxygen in air and sevoflurane. Supplemental doses of fentanyl were administrated as needed. Sodium nitroprusside was used to treat systemic hypertension when necessary. In all patients, a median laparotomy was carried out and aorto-femoral prosthetic grafts were implanted.

Radial arterial and femoral venous blood-gas status, erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GSH-px), glutathione reductase (GSH-rd) activities, glutathione (GSH) and thiobarbituric acid-reactive substance (TBARS) levels in femoral venous blood were determined.

Blood samples from the radial artery and femoral vein and hemodynamic measurements including heart rate, systolic, diastolic and mean arterial pressure and central venous pressure were obtained at baseline before the epidural puncture (T0), just before the induction of GA, after achieving the analgesic dermatomal level of T_{6-8} only in the EP group (T1), just before aortic cross-clamping (T2), immediately after the completion of revascularization and restoration of blood flow to the ischemic limb (T3), 20, 40, 60 min after revascularization (T4, T5, T6 respectively). Radial arterial and femoral venous blood-gas status were measured using a

Ciba Corning 860 blood gas analyzer (Ciba Corning, Albertville, MI, USA) immediately after sampling. Femoral vein blood samples collected for the measurement of SOD, GSH, GSH-px, GSH-rd and TBARS were sent immediately for biochemical analyses.

Biochemical analyses

Venous blood samples were drawn into EDTA containing tubes. The tubes were centrifuged for ten minutes at 1500 g, the plasma was separated, the buffy coat discarded and the packed erythrocytes were washed with sterile 0.9% sodium chloride (w/u) making one to ten dilutions three times. A complete blood count by an automated counter was performed on washed cell samples to check the contamination by leukocytes. Preparation of erythrocytes and biochemical analysis was performed immediately after blood collection by a biochemist who was blinded to the study design.

Erythrocyte TBARS levels were measured according to the method of Buege and Aust.[9] Results are expressed as nmol·g^{-1} hemoglobin (Hb). The intra and inter-assay coefficient of variation for TBARS were 4.5% and 4.8%, respectively.

Erythrocyte Cu-Zn SOD activity was determined by the method of Sun *et al.*[10] The assay involves inhibition of nitroblue tetrazolium (NBT) reduction with xanthine-xanthine oxidase used as a superoxide generator. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%. Results are expressed as units·g^{-1} Hb. The intra and inter-assay coefficients of variations were 9.5% and 10.5%, respectively.

Erythrocyte GSH levels were measured according to the procedure of Beutler,[11] using metaphosphoric acid for protein precipitation and 5,5' dithio-bis-2-nitrobenzoic acid for colour development at 412 nm. Results are expressed as µmol·g^{-1} Hb. The intra and inter-assay coefficients of variation for GSH were 4.7% and 4.9%, respectively.

Erythrocyte GSH-px activity, comprising both selenium-dependent and non-selenium-dependent peroxidase was measured using the method of Paglia and Valentine.[12] One unit of enzyme activity was defined 1 µmoL NADPH oxidized per minute. Activity is expressed as units·g^{-1} Hb. The intra and inter-assay coefficients of variation were 4.8% and 4.5%, respectively.

GSH-rd activity of erythrocyte was measured in the presence of the oxidized form of GSH by following the oxidation of NADPH spectrophotometrically.[13] Results are expressed as units·g^{-1} Hb. The intra and inter-assay coefficients of variations were 4.5% and 4.7%, respectively.

Statistical analysis

Sample size was estimated using data from a preliminary study (n = 10). To detect a difference of 1 standard deviation (SD) between baseline and post revascularization (SD for TBARS = 99), it was calculated that 16 patients per group were required for the study to have a power of 80% and Type I error of 0.05. Data are presented as mean, SD and 95% confidence interval (CI). Differences in the time course of the various variables within the groups were tested by repeated measures analysis of variance with a post hoc Tukey test when appropriate. Between group comparisons were assessed with the unpaired Student's t test considering $P < 0.05$ as significant.

Results

There were no differences between the groups with regard to preoperative demographic information. Ankle-brachial pressure indexes of the groups were similar. Aortic cross-clamp time and the time from the beginning of the surgery until the completion of the revascularization were comparable between the groups (Table I). The degree of peri-aortic collateral vascularization seen on the preoperative aortograms was also similar. The aorta was clamped below the renal arteries in all patients. Hemodynamic variables remained within a clinically acceptable range and were similar in both groups. None of the patients received inotropic agents. Three patients in the GA group and one patient in the EP group received sodium nitroprusside during the surgery. Changes in arterial blood gases and acid-base status were similar in both groups (data not shown).

Before the induction of GA in group EP a significant increase in partial pressure of oxygen (PO_2) was noted

TABLE I Demographic and operative characteristics of patients undergoing GA and EP + GA

	GA (n = 20)	*EP + GA (n = 20)*
Age; yr	63.0 (6.7)	60.1 (10.6)
Sex; M:F	18:2	19:1
Weight; kg	66.1 (12.3)	62.5 (9.8)
Preexisting medical conditions		
Hypertension	8	9
Coronary artery disease	2	2
Diabetes mellitus	6	4
Ankle-brachial pressure index	0.32 (0.19)	0.28 (0.07)
Intraoperative characteristics		
Aortic cross-clamp time; min	18.1 (6.19)	17.4 (3.62)
*Revascularization time; min	117.3 (9.45)	120.5 (10.59)

GA = general anesthesia; EP = epidural anesthesia. Data are expressed as mean (SD). *Time from the beginning of surgery to the completion of revascularization of the studied limb.

TABLE II Antioxidant status of the groups [mean (SD)]

					Reperfusion			
		Preoperative	*After EP*	*Before X clamp*	*Immediately after*	*20 min*	*40 min*	*60 min*
GSH	GA	10.69 (1.4)		10.72 (1.2)	9.96 (1.9)	10.65 (1.6)	9.94 (1.5)	10.44 (8.8)
($\mu mol \cdot g^{-1}$ Hb)	EP	10.26 (1.3)	9.67 (1.2)	9.88 (1)	10.08 (1.5)	10.42 (1.8)	9.5 (1.4)	8.85 (1.5)
GSH-pX	GA	32.87 (12.9)		30.86 (11.6)	30.8 (12.4)	26.8 (5)	27.78 (5.3)	28.39 (6.8)
($U \cdot g^{-1}$ Hb)	EP	29 (5.7)	30.36 (4)	28.57 (4.3)	28.5 (4.1)	26.27 (4)	27.91 (2.8)	27.2 (2.9)
GSH-rd	GA	15.55 (4.1)		16.05 (6.4)	14.85 (5.9)	16 (4.5)	17.89 (6.4)	16.55 (4.9)
($U \cdot g^{-1}$ Hb	EP	20.07 (2.9)	18.15 (5.1)	17.6 (4)	17.97 (5.6)	17.63 (3)	16.91 (3.6)	17.82 (7.1)
SOD	GA	1362.8 (391)		1621.9 (437)	1841.2 (806)	1875.5 (725)	1825.8 (493)	2051.7 (923)
($U \cdot g^{-1}$ Hb)	EP	1652.3 (447)*	1749.9 (426)	1740.9 (507)	1694.9 (704)	1447.8 (486)	1337.5 (504)†	1424.6 (652)*

EP = epidural anesthesia; GA = general anesthesia; X-clamp = aortic cross-clamp; GSH = glutathione; Hb = hemoglobin; GSH-pX = glutathione peroxidase; GSH-rd = glutathione reductase; SOD = superoxide dismutase. *$P < 0.05$; †$P < 0.01$ *vs* group GA.

in femoral venous blood after achieving an adequate level of block by EP when compared to baseline values [mean (SD), 95% CI; 36.29 (4.6), 33.37–39.22 *vs* 29.32 (4.6), 26.34–32.3 mmHg, $P < 0.05$]. After revascularization the PO_2 of femoral venous blood ($P_{fv}O_2$) increased in both groups. When compared with baseline values, in group EP, $P_{fv}O_2$ was found to be significantly higher at the 20th [48.53 (11.1), 41.51–55.55 mmHg], 40th [53.81 (15.09), 44.22–63.4 mmHg], and 60th min [55.26 (20.43), 42.28–68.24 mmHg] of revascularization ($P < 0.001$, $P < 0.001$, $P < 0.001$ respectively), while it was significantly increased in group GA at the 40th [44.98 (12.66), 36.93–53.07 mmHg] and 60th min [48.44 (10.66), 41.66–55.21 mmHg], ($P < 0.01$, $P < 0.001$ respectively). No statistical difference in $P_{fv}O_2$ was found between the groups (Figure 1a).

In the GA group a significant increase in erythrocyte TBARS was observed immediately after restoring the blood flow when compared to baseline values [337.26 (123.4), 248.99–425.59 $nmol \cdot g^{-1}$ Hb *vs* 221.32 (102.02), 148.35–294.29 $nmol \cdot g^{-1}$ Hb, $P < 0.01$]. In the EP group TBARS values were stable throughout the study (Figure 1b).

Within group comparisons revealed no significant differences in GSH, GSH-px, GSH-rd and SOD. There are also no significant differences in GSH, GSH-px and GSH-rd between the groups, however SOD activity in group EP was found to be significantly higher than that of group GA preoperatively ($P < 0.05$) and lower at the 40th and 60th min of revascularization ($P < 0.01$, $P < 0.05$ respectively; Table II).

Discussion

The present study showed that before revascularization oxygenation of the ischemic limb in the patients with AOD can be improved by EP, which then may possibly attenuate the generation of by products of lipid peroxidation after restoration of blood flow to the ischemic limb.

Sudden restoration of arterial flow after prolonged ischemia may contribute additional damage to the energy-depleted tissue with consequent impairment of blood flow and accelerated tissue necrosis.[14] One mechanism of this injury is explained by OFR damage to cellular membranes, which leads to the generation of by products of lipid peroxidation such as malondialdehyde (MDA), the mean component of TBARS, and widely accepted marker of OFR-mediated lipid peroxidation.[15] Although a number of mechanisms exist by which aerobic organisms normally prevent or limit the damage caused by toxic oxygen metabolites, the defense mechanisms that first evolved seem to have been the SOD, catalases and GSH-px which are in the highest concentration within erythrocytes.[6]

In the present study femoral venous PO_2 was measured in order to assess the perfusion and oxygenation of the ischemic limb and TBARS to assess OFR induced lipid peroxidation. Erythrocyte GSH levels, Cu-Zn-SOD, GSH-px, GSH-rd activities were also analyzed to show the endogenous antioxidant defense mechanisms. We demonstrated a significant increase in femoral venous PO_2 in patients receiving EP preoperatively. EP produces sympathetic blockade-induced vasodilatation which might probably ameloriate blood flow to the ischemic limb before revascularization via collaterals originating above the occlusion of the aorto-iliac tree. Depending on the rate of progression of the occlusion, the extent of chronic collateral circulation is expected to be more in the patients with atherosclerotic AOD, as in the patients involved in this study. This was also shown in a study in which the influence of peri-aortic collateral vessels in patients with AOD were compared with those of patients with

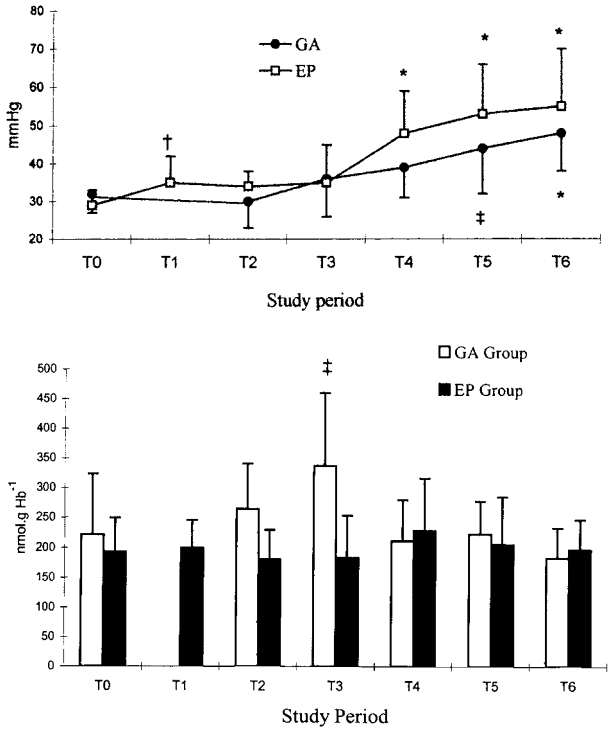

FIGURE Femoral venous partial pressure of oxygen (a), and mean erythrocyte thiobarbituric acid-reactive substances (TBARS) levels (b) in patients receiving GA and GA + EP. T0 = baseline; T1 = before the induction of GA, after achieving the T_{6-8} dermatomal level; T2 = just before aortic cross-clamping; T3 = immediately after the completion of revascularization and restoration of blood flow to the ischemic limb; T4, T5, T6, 20, 40, 60 min after revascularization respectively. †$P < 0.05$, ‡$P < 0.01$ and *$P < 0.001$ compared to baseline. GA = general anesthesia; EP = epidural anesthesia.

an abdominal aortic aneurysm. The degree of collateral vascularization was found to be significantly more in patients with AOD. Also, hemodynamic stress from aortic cross-clamping had been shown to be inversely proportional to the degree of peri-aortic collaterals.[16]

Placement of an aorto-femoral graft requires cross-clamping of the aorta and a period of lower extremity ischemia. In the present study, although the cross-clamp induced ischemia was thought to be partial on the basis of chronic ischemia and collateral vascularization, erythrocyte TBARS levels were found to be significantly elevated a few minutes after revascularization and near baseline values at the 20th min of reperfusion in patients having GA alone. Similarly, high levels of MDA have been reported after reperfusion of the lower limb following femoro-popliteal bypass grafting.[17]

Trewick *et al.*,[18] investigating patients undergoing femoro-popliteal bypass, also found a significant increase in xanthine oxidase (a major source of superoxide radical) activity in femoral vein blood peaking at two minutes of revascularization and then returning to near normal levels by 60 min. On the contrary, however, in their study free radical generation was not accompanied by lipid peroxidation as no increase was detected in MDA. The differences between studies regarding the occurrence of lipid peroxidation might be related to differences in the antioxidant enzyme activities of patients. In our study as the preoperative SOD activities of the groups were found to be different between group comparisons at any other time period might not reflect reality. Therefore only the changes in the time course within the groups were taken into consideration while assessing SOD activities. Neverthless GSH-px, GSH-rd activities and GSH levels appeared to be unaffected significantly by reperfusion or the anesthetic method and the changes in SOD within the groups did not reach statistical significance. A similar phenomenon was reported by Persson *et al.*[19] in a study in which the activities of Cu-Zn SOD, Mn SOD and GSH-px were found to be similar after reperfusion in comparison with baseline in chronically ischemic muscle although a significant increase was observed in TBARS ten minutes after reperfusion.

The increase in TBARS might have been more important in patients undergoing abdominal aortic aneurysm repair since acute ischemia would have occurred with aortic cross-clamping. However, we aimed to show the effect of EP which produces vasodilatation and an increased blood flow to the ischemic limb before revascularization, even during the cross-clamp period via collaterals originating above the cross-clamp. Similarly Neglen and co-workers[20] demonstrated high levels of hypoxanthine after revascularization of the leg in patients undergoing aortic reconstrictive surgery although aortic cross-clamp induced ischemia was reported to be partial due to pre-existing collateral circulation. In our study, TBARS did not increase throughout the study in patients receiving EP suggesting the lack of lipid peroxidation while a significant increase was detected a few minutes after restoration of blood flow in patients having no epidural medication during the study period.

EP, resulting in preoxygenation of the ischemic limb before reperfusion, may produce beneficial effects similar to ischemic preconditioning or intermittent reperfusion.[21] Several studies have attempted to minimize ischemia-reperfusion induced tissue damage in a similar manner. They include initial reperfusion with deoxygenated blood,[22,23] gradual

reintroduction of oxygen,[24,25] or controlled reperfusion[26] during the initial reperfusion. These approaches prevented the increase in permeability and vascular resistance and decreased postischemic muscle necrosis. Thus, tissues may need preconditioning or adaptation before reperfusion begins. In other words, oxygen may need to be reintroduced gradually into tissues after prolonged ischemia before sustained reperfusion begins. EP applied before revascularization may possibly lessen the burst of superoxide radical production, presumably by increasing oxygenated blood flow of the ischemic limb via collaterals before revascularization. It is also possible that EP spreads the production of free radicals over a longer period of time, avoiding the induction of lipid peroxidation. Similar to ischemic preconditioning, the mechanism of halogenated anesthetic-induced preconditioning involves the activation of adenosine triphosphate-sensitive potassium channels, stimulation of adenosine A(1) receptors and thus preserving energy substrate.[21,27] However further investigations with larger patient groups are needed to confirm that EP-induced preoxygenation potentiates preconditioning and then to explain possible biochemical mechanisms.

In our study, in patients with atherosclerotic AOD revascularization resulted in a transient increase in TBARS under GA which was not seen in patients receiving EP. While the limited number of patients and the isolated increase in TBARS do not allow us to conclude definitely, EP applied before revascularization may improve oxygenation of ischemic limbs in patients with AOD and, possibly, attenuate lipid peroxidation following the restoration of arterial blood flow.

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