DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
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ISCHAEMIA-REPERFUSION INJURY OF THE HEART DURING CORONARY SURGERY: A CLINICAL STUDY INVESTIGATING THE EFFECT OF HYPEROXIA

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IV **Karu I**, Zilmer K, Starkopf J, Zilmer M. Changes of plasma asymmetric dimethylarginine levels after coronary artery bypass grafting. *Scand Cardiovasc J* 2006; 40(6): 363–367.

ABBREVIATIONS

ADMA	asymmetric dimethylarginine
CABG	coronary artery bypass grafting
CK-MB	creatine kinase MB-isoenzyme
CPB	cardiopulmonary bypass
cTn I	troponin I
FiO_2	inspired fraction of oxygen
GPx	glutathione peroxidase
GSH	reduced glutathione
GSSG	oxidised glutathione
H_2O_2	hydrogen peroxide
IL-6	interleukin-6
IPC	ischaemic preconditioning
IR	ischaemia-reperfusion
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
NO	nitric oxide
NFκB	nuclear factor κB
OPCAB	off-pump coronary artery bypass grafting
OxS	oxidative stress
PaO_2	arterial partial pressure of oxygen
ROS	reactive oxygen species

1. INTRODUCTION

Ischaemic heart disease is a major cause of morbidity and mortality worldwide. Each year 3.8 million men and 3.4 million women die from the coronary heart disease (WHO).

Atherosclerosis leads to narrowing and occlusion of coronary arteries, resulting in inadequate oxygen supply for maintenance of normal oxidative metabolism. To avoid profound ischaemia and subsequent cell death, blood flow has to be restored by means of thrombolysis, percutaneous coronary intervention, or surgical revascularisation. But the magic bullet of reperfusion turned out to be a double-edge sword (Braunwald and Kloner 1985). Besides restoring the oxygen supply to the cells, introduction of molecular oxygen to the ischaemic tissue results in a spectrum of unfavourable events, termed altogether as reperfusion injury. Despite of vast amount of experimental research, translation of experimentally effective cardioprotective interventions and therapies against IR injury to the clinical practice has had several drawbacks. One of the strategies that has been proven effective in reduction of myocardial IR injury in experimental animals is pre-treatment by hyperoxia. Although contrary to many others, this is easily applicable, but so far has not been evaluated in a clinical setting.

2. REVIEW OF THE LITERATURE

2.1. Coronary surgery

Since the first coronary artery bypass grafts performed in 1950s, the number of procedures has been increasing constantly, and by now it is among the most common major operations performed in the world, accounting for more resources expended in the cardiovascular medicine than any other single procedure. Achievability of open-heart surgery has been drawn by two important developments – mechanical circulatory support and myocardial protection. The latter refers to all strategies that increase the ability of the myocardium to withstand an ischaemic insult. Hypothermia (Bigelow *et al.* 1950), crystalloid (Melrose *et al.* 1955; Baker *et al.* 1957) and blood (Follett *et al.* 1980) cardioplegia have been standard cardioprotective strategies for several decades.

CABG was until recently almost exclusively performed with cardioplegia and CPB, which provide motionless and bloodless surgical field for construction of anastomoses. The 1990s have witnessed the renaissance of bypass procedures on the working heart, the technique first used by the Russian surgeon Vassili Kolessov already in 1964 (Konstantinov 2004). The conditions for grafting on the working heart are less optimal, however, two major concerns of conventional CABG are eliminated. First, contact of blood to the foreign surfaces of the CPB circuit that causes inflammatory response (Butler *et al.* 1993) is avoided and, second, aortic cross clamping, leading to global ischaemia and subsequent IR injury to the heart, is not mandatory. Regardless of these clear benefits, the outcome favouring OPCAB is not as straightforward. There appear to be trends in OPCAB surgery towards less myocardial enzyme release up to 24 postoperative hours, less early neurocognitive dysfunction, less renal insufficiency, less blood loss, and need for transfusion when compared to the conventional CABG (Sellke *et al.* 2005). Mid-term results do not favour either of the methods clearly (Puskas *et al.* 2004; Racz *et al.* 2004; Sabik *et al.* 2004; Cheng *et al.* 2005). Some data suggest that the subgroups of patients to gain most benefit from the off-pump approach are the patients with atheromatous aorta (Sharony *et al.* 2004; Mishra *et al.* 2006) and the elderly (Al-Ruzzeh *et al.* 2003; Kilo *et al.* 2003). As global ischaemia and following reperfusion are avoided, OPCAB surgery has shown to result in significantly less OxS both in the heart (Matata *et al.* 2000; Akila *et al.* 2007) and in the whole organism (Cavalca *et al.* 2006).

Despite of aforenamed advantages, OPCAB surgery *per se* does not eliminate the risk of IR injury completely, as even short ischaemic periods during the grafting procedure may cause vascular endothelial injury, myocardial stunning and necrosis (Bufkin *et al.* 1998; Laurikka *et al.* 2002; Selvanayagam *et al.* 2004). To further reduce the risk of myocardial damage, the use of temporary intracoronary shunts has been advocated (Franzone *et al.* 1977; Yeatman *et al.* 2002).

2.2. Ischaemia-reperfusion injury of the heart

Brief periods of coronary occlusion result in prolonged depression of myocardial function in the ischemic zone
(Heyndrickx *et al.* 1975).

2.2.1. Mechanisms of IR injury

There are two interrelated hypotheses, oxidative stress and intracellular Ca^{2+}-overload, which have been proposed to explain the pathogenesis of IR injury. Oxidative damage caused by the interaction of ROS with proteins, lipids, and nucleic acids has been documented in a number of experimental studies from subcellular and cellular to *in vitro* and *in vivo* models (Marczin *et al.* 2003). Occurrence of oxidative stress has also been demonstrated during the reperfusion of the human heart (Ferrari *et al.* 1990).

In case oxygen-deprived cardiomyocytes become energy depleted, they accumulate Na^+ and Ca^{2+} in the cytosol. When oxidative phosphorylation is then resumed with the resupply of oxygen, activation of the myofibrils at (still) increased cytosolic Ca^{2+} concentrations provokes a sustained maximal force development and consecutive mechanical cell injury due to hypercontraction.

In addition to the direct injury caused, oxidants may also provoke increased production of inflammatory cytokines, inducible NO synthase, and proteinases via intracellular signal transduction pathways.

Antioxidant systems of the (human) heart
Myocardium is equipped with a variety of endogenous enzymatic and nonenzymatic antioxidant systems that metabolise ROS, generated during normal cellular activity. This inhibits or delays oxidative damage to the cellular proteins, carbohydrates, lipids, and DNA. In particular, dismutation of superoxide anion by cytosolic copper/zinc- and mitochondrial manganese-containing superoxide dismutase (CuZnSOD and MnSOD, respectively), and the degradation of H_2O_2 by glutathione peroxidase and catalase limit the cytotoxic effects of reactive oxygen metabolites (Fridovich 1978; McCord and Fridovich 1978; Hess and Manson 1985). The other extensively studied antioxidants include ubiquinone, α-tocopherol, carotenoids and ascorbic acid. Of primary importance is GSH, which is a preferential substrate for many oxidising agents, thus sparing protein thiol-groups from oxidation (Kosower, 1976).

Ischaemia and reperfusion have shown to induce a significant fall in tissue reduced glutathione content concomitant with an increase of its oxidised form (Arduini *et al.* 1988). Transgenic mice overexpressing glutathione peroxidase have been shown to exhibit markedly depressed contractile function, increased

release of creatine kinase, and size of infarction in comparison with non-transgenic mice (Yoshida *et al.* 1996). Glutathione peroxidase is the key enzyme protecting against lipid peroxidation. It also catalyses the peroxidation of H_2O_2 in the presence of GSH, to form H_2O and GSSG. GSSG recycles back to give GSH by glutathione reductase, which requires NADPH from the pentose monophosphate shunt (Fig. 1). Thus, glutathione peroxidase plays a significant role as a H_2O_2 scavenger in the heart, since its activity is much higher than that of catalase. During pathophysiological conditions, such as IR, the balance between ROS and antioxidants may shift in favour of a relative increase in pro-oxidants, resulting in depletion of endogenous antioxidants in the ischaemic heart upon reperfusion (Ferrari *et al.* 1985; Haramaki *et al.* 1998). The glutathione redox ratio (called also as redox buffer) has an impact on cell signalling system influencing the translocation of the transcription factor NFkB, which regulates the synthesis of cytokines and adhesion molecules. Activation of NFκB and AP-1 with concomitant increase in myocardial antioxidant enzyme gene expression (Das *et al.* 1993; Chandrasekar *et al.* 1997a), which occurs during ischaemic as well as hyperoxic preconditioning, can be considered as a defence mechanism of the heart in an attempt to counteract with excessive OxS.

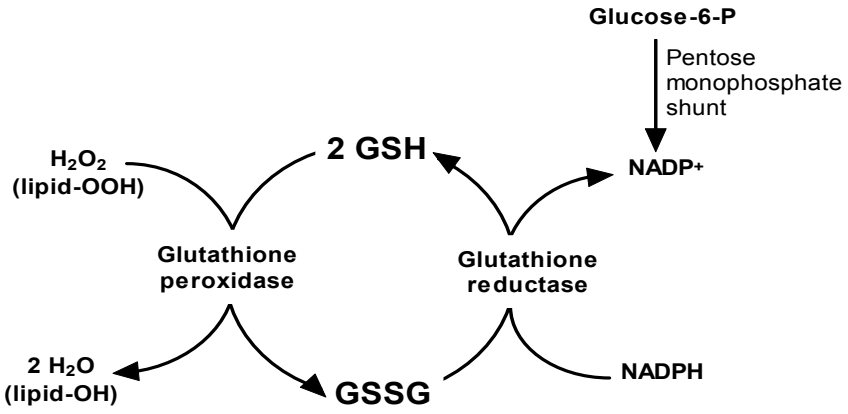

Figure 1. Overview of the glutathione system.

2.2.2. Clinical manifestations of IR injury

Early reperfusion is an absolute prerequisite for survival of the ischaemic myocardium. Even if this is achieved, an adverse complex of events, instead of expectedly good myocardial performance, can develop. Since the first descriptions of reperfusion accelerated necrosis in experimental (Jennings *et al.* 1960) and clinical setting (Bulkley and Hutchins 1977), reversible postischaemic

myocardial dysfunction (myocardial stunning), reperfusion arrhythmias, necrosis of cardiomyocytes, endothelial and microvascular dysfunction including the no-reflow phenomenon have been described as manifestations of the IR injury of the heart.

Reperfusion arrhythmias

Reperfusion arrhythmias are observed shortly after restoration of blood flow to the ischaemic heart. While commonly seen in experimental animals, the phenomenon is not so often observed in humans, for example, in course of thrombolysis or cardiac surgery. The incidence of ventricular fibrillation is higher, if reperfusion is achieved sooner after onset of ischaemia (The European Myocardial Infarction Project Group 1993). Residual coronary stenosis of 80% or less is a predisposing factor in the occurrence of ventricular fibrillation, ventricular tachycardia or accelerated idioventricular rhythm at reperfusion (Della Grazia *et al.* 1986). Frequency of ventricular premature complexes and ventricular tachycardia correlates with peak creatine kinase (Gressin *et al.* 1992) concentrations, indicating that ischaemia or incomplete myocardial protection are among major causes of the immediate postoperative rhythm disturbances (Pehkonen *et al.* 1995).

It has been suggested, that the cause of reperfusion arrhythmias is the formation of ROS acting together with the intracellular calcium overload (Hearse and Tosaki 1988). The incidence of reperfusion arrhythmias decreases after extended duration of ischaemia, presumably as the ATP stores are depleted and calcium ions cannot recycle (Opie 1989).

Myocardial stunning

Myocardial stunning is defined as a prolonged postischaemic mechanical dysfunction, that persists after reperfusion of previously ischaemic tissue in the absence of irreversible damage (Braunwald and Kloner 1982). Myocardial stunning is mediated by the effects of reperfusion, including ROS and calcium loading on myocytes, that retain viability and ultimately recover contractile function (Buja 1998). This form of IR injury appears to be a rule, rather than exception after CABG (Roberts *et al.* 1981; Breisblatt *et al.* 1990; Kloner *et al.* 1994; Mangano 1985). It affects both right and left ventricles, is independent of changes in preload, afterload and type of cardioplegia used, has its nadir during the first 6 postoperative hours, and resolves gradually during the 24–48 hours (Gray *et al.* 1979; Bolli *et al.* 1990; Breisblatt *et al.* 1990).

Stunned myocardium remains responsive to both endogenous and intravenously administered catecholamines, yet not at the expense of worsening or delaying the functional recovery, which is often the case in other ischaemic conditions (Ito *et al.* 1987). In the clinical setting, many of these patients are treated with inotropes during the early postoperative period. By now, there is some evidence that may change this practice, as an inotropic support to the

postischaemic heart increases intracellular calcium and cell death due to apoptosis (Stamm *et al.* 2002), which is probably accentuated in the segments of the heart that have not been adequately revascularised during CABG (Levitsky 2006). Levosimendan may offer an alternative in this setting (Sonntag *et al.* 2004), and it is also exerting a preconditioning-like effect (Tritapepe *et al.* 2006).

Death of cardiomyocytes

Different factors such as trauma caused by sewing needles or surgical manipulation of the heart, ischaemia from the inadequate myocardial protection or some complication of the procedure, and the events occurring upon reperfusion can all cause myocardial cell death during cardiac surgery. Ischaemia followed by reperfusion can trigger both basic patterns of cell death: oncosis and apoptosis, depending on the rate and magnitude of ATP depletion, as apoptosis is an ATP dependant process (Buja 1998; Buja and Entman 1998).

Once the damage has occurred, no biomarker is not yet capable to distinguish between procedure-associated, ischaemic, or reperfusion injury. In a clinical setting, quantitative measurement of markers of myocardial necrosis such as troponin T or I and CK-MB, is widely applied to differentiate acute infarction from the usually small quantity of myocardial cell damage associated with the procedure itself. The amount of released CK-MB or cTn I depends on the type of surgery (OPCAB < CABG+CPB < valve+CPB) (Swaanenburg *et al.* 2001) and correlates well with the aortic cross-clamping time (Benoit *et al.* 2001; Swaanenburg *et al.* 2001) or duration of CPB (Jain *et al.* 1997).

The cut-off limits for diagnosis of perioperative myocardial infarction after cardiac surgery vary significantly in different studies. The reported cTn I levels indicative for perioperative myocardial infarction 24 hours after CABG are higher than 3.9 μg/L (Carrier *et al.* 2000), 15 μg/L (Alyanakian *et al.* 1998) or 36 μg/L (Benoit *et al.* 2001). The suggested cut-off limit for OPCAB procedures is 8.35 μg/L (Peivandi *et al.* 2004).

CK-MB thresholds for perioperative myocardial infarction are greater or equal to 30 μg/L (Alyanakian *et al.* 1998; Jacquet *et al.* 1998); 100 μg/L in any postoperative sample, greater or equal to 70 μg/L more than 12 h after release of aortic occlusion, or greater or equal to 12 μg/L more than 24 h after release of aortic occlusion (Multicenter Study of Perioperative Ischemia Research Group 1995). The other criterion can be defined as a rise above upper limit of normal, whereby the infarction may be suspected with more than 5 to 10 times rise. According to the last consensus document redefining the myocardial infarction (Myocardial infarction redefined 2000), an increased value for cardiac troponin should be defined as a measurement exceeding the 99th percentile of a reference control group, and reference values must be determined in each laboratory.

Endothelial dysfunction

The consequences of IR are well established on the level of the myocardium, but less known what concerns the vasculature of the heart. Endothelial cells appear to be more sensitive to IR than myocytes, and during ischaemia endothelial dysfunction temporally precedes (and contributes to) the appearance of IR-induced myocardial necrosis (Mankad *et al.* 1997; Kharbanda *et al.* 2001).

Endothelial dysfunction is defined as an impaired endothelium-dependant vasodilation, whereas the responses to endothelium-dependant vasoconstrictors are exaggerated. The major early event leading to the endothelial dysfunction is loss of endothelial release of NO, occurring already within 2.5 minutes and progressing to near maximum values at about 20 minutes after initiation of reperfusion (Tsao *et al.* 1990).

The first evidence of postischaemic coronary endothelial dysfunction was demonstrated in a canine model of ischaemia and reperfusion (Ku 1982). In humans, the studies of endothelial dysfunction are often conducted in a forearm model, which is shown to correlate with coronary endothelial dysfunction in patients with cardiovascular disease (Wilkinson and Webb 2001).

A clinically relevant period of IR causes early, profound and sustained endothelial dysfunction and systemic neutrophil activation in humans (Lefer and Lefer 1996; Kharbanda *et al.* 2001), which does not correlate with microscopically assessed structural damage (Mankad *et al.* 1997).

Microvascular dysfunction and no-reflow

After approximately 20 minutes of reperfusion, adhesiveness of leucocytes increases and they migrate across the endothelium into the reperfused tissue. The infiltration of neutrophils leads to the reperfusion injury (i.e. necrosis), which is significant at 3 hours, but becomes profound at 4.5 hours following reperfusion (Lefer and Lefer 1996).

Activated neutrophiles release a whole complex of cytotoxic and chemotactic substances, further exacerbating the injury. A combination of endothelial dysfunction, clogging of the microvasculature with neutrophils and platelets, ultrastructural changes of the vasculature, oedema and OxS can lead to flow-limiting dysfunction, or eventually no reflow phenomenon which, in turn, may further jeopardize the survival of the myocytes. Whereas no reflow may be of little importance if it occurs in the already necrotic tissue, total inability of the affected tissue to be reperfused in a potentially salvable myocardium could be of considerable significance (Hearse *et al.* 1993). In humans, the no-reflow phenomenon has been detected in about 30% of cases after thrombolysis or percutaneous coronary interventions (Schofer *et al.* 1985; Ito *et al.* 1992; Porter *et al.* 1998; Wu *et al.* 1998), and is associated with incomplete ST-segment recovery (Claeys *et al.* 1999), increased incidence of acute myocardial infarction, myocardial rupture and death (Abbo *et al.* 1995; Morishima *et al.* 1995).

2.2.3. Assessment of IR injury

Long list of methods exists for assessment of IR injury in experimental animals (Ytrehus 2000). In clinical studies, the choices are far more limited. Assessment of the function of the heart, usually by measurement of the cardiac output with a (thermo)dilution technique or echocardiography, and dynamics of the markers of myocardial infarction, for example, arterial CK-MB and/or troponins, are typically applied in the early postoperative period after coronary surgery.

Although the severity of injury depends on the ischaemic time (Jennings and Reimer 1983), there is a lack of evidence to show that the extent of necrosis determines early postischaemic function of the heart. In experimental conditions, poor correlation between necrosis and contractile function has been demonstrated (Bugge and Ytrehus 1996; Starkopf *et al.* 1998), and in clinical studies the results remain controversial as well (Koh *et al.* 1998; Chang *et al.* 2002; Kwinecki *et al.* 2003).

Measurements of cTn I and CK-MB are well-accepted standards for assessment of myocardial cell necrosis. The rate of appearance of CK in plasma early after reperfusion reflects the extent of irreversible myocardial injury (Devries *et al.* 1989). To detect minor damage, blood sampling form coronary sinus instead of systemic arterial or venous blood has been advocated. The coronary sinus predominantly drains the left ventricle and receives approximately 85 % of the coronary venous blood. Thereby, the difference in concentrations between systemic arterial and coronary sinus blood reflects the release of the markers exclusively from the heart. Early myocardial release and later venous concentrations of Tn T have been correlated with the recovery of myocardial aerobic metabolism during OPCAB and CABG procedures (Koh *et al.* 1999). Additionally, assessment of arterio-coronary venous difference in the concentrations of lactate can be successfully used for perioperative assessment of the cardiac energy metabolism (Wollert *et al.* 1990). Myocardial lactate production is considered to be a good indicator of myocardial ischaemia (Hall *et al.* 1995).

In the CABG patients, direct measurement by electron spin-trapped spectroscopy (Wu *et al.* 2001), or release of GSSG (Ferrari *et al.* 1990; De Vecchi *et al.* 1998; Volk *et al.* 2003) has provided the evidence of ROS generation after cardioplegia. In some studies, the severity of OxS has been related to the postoperative recovery of the cardiac function (Ferrari *et al.* 1990; De Vecchi *et al.* 1998; Wu *et al.* 2001), whereas others have not found such correlation (Biagioli *et al.* 1997). Ferrari *et al*. were the first who nicely demonstrated the evidence of oxidative stress upon reperfusion of the human heart (Ferrari *et al.* 1990). They showed that the release of GSSG from the heart after cardioplegia correlated well with the impaired postoperative function of the heart. No other investigator, however, has reported similar results in the setting of cardiac surgery.

Taking together, it is important to realise that suppressed function of the heart upon reperfusion may result from stunning or necrosis, or be the mixture of both. Especially in clinical studies investigating whatever cardiprotective strategies, simultaneous assessment of the function of the heart, oxidative stress indices, and markers of myocardial necrosis is warranted in order to distinguish between these two types of injury. On that background we investigated whether early post-cardioplegic performance of the heart is related to the extent of myocardial necrosis, lactate and GSSG release in patients undergoing coronary surgery. By complex assessment of biochemical parameters and the function of the heart, we also wanted to establish a set of measurements required for the comprehensive description of IR injury of the human heart.

2.3. Myocardial preconditioning

Ischaemic preconditioning

In 1986 a landmark study demonstrating a new concept of myocardial protection – ischaemic preconditioning – was published by Murry *et al*. (Murry *et al.* 1986). IPC refers to the phenomenon of inducing tolerance against the IR injury by controlled brief periods of ischaemia and reperfusion prior to a sustained ischaemic insult. It is characterised by an early phase of protection manifesting from the first minutes and lasting for few hours after the preconditioning stimulus (classic preconditioning), and a longer delayed phase evincing 24–72 hours after the preconditioning stimulus (second window of protection).

The first clinical study during CABG was published in 1993, where a significant 76% increase in the ATP concentration in myocardial biopsies from the IPC group following the ischaemic insult was found (Yellon *et al.* 1993). Followed by years of extensive research, it is recognised by today, that IPC is one of the most powerful manifestations of endogenous adaptation against ischaemic injury in all species and tissues tested. In numerous clinical studies it has shown to reduce size of myocardial infarction and improve cardiac function (Illes and Swoyer 1998; Lu *et al.* 1998; Wu *et al.* 2000), attenuate the release of markers of myocardial necrosis (Jenkins *et al.* 1997; Illes and Swoyer 1998; Szmagala *et al.* 1998) and IL-6 (Wei *et al.* 2001), reduce the incidence of postoperative ventricular arrhythmias (Wu *et al.* 2002), and attenuate endothelial dysfunction and systemic neutrophil activation (Kharbanda *et al.* 2001).

During the OPCAB procedure, temporary occlusion of target vessel has shown to suppress the heart rate elevation, decrease the episodes of supraventricular and ventricular tachycardia after surgery, enhance the recovery of stroke volume index, and tended to decrease the immediate postoperative myocardial enzyme release (Laurikka *et al.* 2002; Wu *et al.* 2003).

In the clinical practice IPC can be achieved by intermittent aortic cross-clamping. The necessity to repeatedly cross-clamp the aorta, and resulting risk of atheroembolism limits its clinical applicability (Vaage *et al.* 2000). Besides, in patients with coronary artery disease, an extra ischaemic load as a preconditioning stimulus may further endanger the already diseased myocardium. To overcome these problems, a large body of studies has tried to identify triggers, mediators and end-effectors to develop pharmacological agents to mimic this powerful phenomenon.

To summarise, so far none of the numerous agents proven to mimic preconditioning in experimental studies have been implemented for the routine clinical use. The only exceptions may be the volatile anaesthetics (De Hert *et al.* 2002; Bein *et al.* 2005; Yu and Beattie 2006), and opioids (Bell *et al.* 2000; Murphy *et al.* 2006), although their indication as "preconditioning drugs" not as "anaesthetic drugs" is still very limited in the everyday clinical practice.

2.4. Hyperoxia

Oxygen is routinely administered during the perioperative period, with the inspired concentrations varying from 30% to 100%. From one side, this is essential to ensure adequate tissue oxygenation. On the other side, introduction of molecular oxygen may cause cellular injury due to formation of ROS. This concept has been termed the oxygen paradox (Hearse *et al.* 1973; Davies 1995).

Preconditioning by hyperoxia

Besides other strategies aimed to mimic preconditioning, the effect of hyperoxia has been explored in experimental animals. In brief, the theory is based upon the moderate oxidative stress mediated effect, which causes upregulation of the antioxidant enzymes and promotes tolerance to ischaemia of the myocardial cells (Lai *et al.* 1996; Zhou *et al.* 1996). Intermittent exposure to 100% oxygen allows time for protective enzyme concentrations to increase before severe toxic effects occur (Hendricks *et al.* 1977; Paegle *et al.* 1977).

The studies on rats and mice conducted so far, have revealed that exposure to >96% inspired oxygen for a short period before sustained ischaemia protects the heart against IR injury. It induces a low-grade systemic OxS, improves recovery of postischaemic function, and reduces the infarct size both in normal and atherosclerotic hearts (Li *et al.* 2001; Tähepõld *et al.* 2001; Tähepõld *et al.* 2002a). The minimal concentration to evoke protective effect in the rat heart is 80% (Tähepõld *et al.* 2002b). Similar to the ischaemic preconditioning, hyperoxia evokes immediate and delayed phases of protection (Tähepõld *et al.* 2002a; Tähepõld *et al.* 2002b). The protective effect of hyperoxia is mediated through a NFκB- (Tähepõld *et al.* 2003), tumour necrosis factor receptor I (Labruto *et al.* 2005), NO- (Ruusalepp *et al.* 2007) and NOS-3 (Cabigas *et al.* 2006) –

dependant mechanisms. After exposition to the hyperbaric hyperoxia, myocardial infarction is prevented by expressing Bcl-2 (Choi *et al.* 2006).

The oxygen therapy possesses also other potentially beneficial side-effects, as briefly discussed below.

Other cardiovascular effects of hyperoxia

Breathing of hyperoxic gas mixture has several well established effects upon the cardiovascular system, namely reduction of cardiac index, stroke index, heart rate, and left ventricular diastolic relaxation with concomitant rise in systemic vascular resistant index and left ventricular filling pressures. The effects are reversed when FiO_2 is reduced back to normal, and they occur similarly in healthy volunteers (Frobert *et al.* 2004), anaesthetised or awake patients (Anderson *et al.* 2005), after CABG (Anderson *et al.* 2005; Harten *et al.* 2005), or in patients with congestive heart failure (Mak *et al.* 2001). In the coronary circulation, breathing 100% oxygen reduces coronary blood flow velocity and increases coronary resistance without significantly changing the diameter of capacitance arteries (McNulty *et al.* 2007).

In experimental animals, increasing of PaO_2 during an acute low-flow myocardial ischaemia, in contrast, improved both function and flow distribution to the ischaemic myocardium, and decreased glycolytic metabolism in the ischaemic zone (Cason *et al.* 1992). Besides, hyperoxic ventilation increased tolerance to acute normovolemic anaemia by creating readily usable plasmatic oxygen reserve (Meier *et al.* 2005), and reduced 6 h mortality after haemorrhagic shock (Meier *et al.* 2004).

Systemic effects of hyperoxia

The lung serves as a primary target organ for hyperoxia. Among clinicians hyperoxic lung injury is one of the best known side-effects of long-term oxygen administration. Studies in humans have described the first signs of toxicity after 12–16 hours of oxygen administration (Stogner and Payne 1992), while intermittent exposure to hyperoxia increases the tolerance against such injury (Hendricks *et al.* 1977). However, the growing body of experimental and clinical evidence shows that mechanical ventilation, especially usage of high tidal volumes, appears to be more deleterious to the lung than hyperoxia *per se* (Carvalho *et al.* 1998; Sinclair *et al.* 2004; Li *et al.* 2007). One of the effects, that clinicians have to be aware of while exploiting 100% oxygen, is the formation of absorption atelectases (Rothen *et al.* 1995; Benoit *et al.* 2002). Still, reducing the inspired concentration of oxygen to 80% does not cause more atelectases than breathing of 30% oxygen (Akca *et al.* 1999b).

In the clinical studies, other beneficial systemic effects of hyperoxia have been described. Administration of 80% oxygen reduces the wound infection rate in patients undergoing colorectal resection (Greif *et al.* 2000), but poorly treated postoperative pain abolishes this effect (Akca *et al.* 1999a). Breathing 100%

oxygen preserves antimicrobial function of alveolar macrophages after surgery (Kotani *et al.* 2000). Initial reports describing the effect of hyperoxia on postoperative nausea and vomiting showed very promising results (Greif *et al.* 1999; Goll *et al.* 2001), but later reports could not find such benefits (Turan *et al.*; Treschan *et al.* 2005).

The other favourable effects of normobaric hyperoxia include extension of the reperfusion window in focal cerebral ischaemia and reduction of neurological deficit after stroke (Kim 2005; Singhal *et al.* 2005), and also improvement of liver transplant function and survival (Corradini *et al.* 2005). In experimental animals, pre-treatment with hyperoxic gas mixture has shown to reduce tissue injury after spinal cord injury (Dong *et al.* 2002).

Taking together, these experimental and clinical data lead us to design a clinical study investigating the effect of hyperoxia upon IR injury of the human heart. We choose the setting of elective coronary surgery, where the ischaemia and reperfusion are well standardised, and the myocardial damage could be closely monitored. The model of injury, established in the first part of the present thesis (Paper I), was taken as a basis for studies on hyperoxia. If proven effective, the hyperoxic pre-treatment can easily be applied in a wide variety of clinical conditions associated with a possible threat of myocardial ischaemia.

2.5. Cardiac surgery and IL-6

Persisting, chronic inflammation plays an important role in the pathogenesis of atherosclerosis (Ross 1993) and coronary plaque disruption (Buja and Willerson 1994).

IL-6 levels are shown to be associated with subclinical atherosclerotic lesions independently of traditional risk factors (Amar *et al.* 2006). Raised levels of IL-6 are common in unstable angina (Biasucci *et al.* 1996), as well as in patients having atrial fibrillation (Gedikli *et al.* 2007). These elevated levels may have also beneficial long term effects, as upregulation of the proinflammatory cytokines in plasma by unstable angina induces late preconditioning effects, and shifts the myocardium to a preconditioned phenotype upon exposure to impending stress (Wang *et al.* 2007).

During acute inflammatory response, IL-6 is involved in the induction of acute phase reactions, and controlling the level of acute inflammatory responses by downregulating the expression of proinflammatory and upregulating anti-inflammatory molecules (Xing *et al.* 1998). Cardiac surgery with CPB provokes an acute phase inflammatory reaction, the causes being IR injury, contact of blood components with the artificial surfaces of the CPB circuit, endotoxaemia, and direct trauma. The CPB *per se* is not the most important contributor to the development of inflammatory response, but myocardial ischaemia during aortic-cross clamping, or surgical trauma (Liebold *et al.* 1999; Wan *et al.* 2004;

Prondzinsky *et al.* 2005). Proinflammatory cytokines, such as TNFα, IL-1ß, and IL-6, are not constitutively expressed in the normal heart (Kapadia *et al.* 1995; Kapadia *et al.* 1997). mRNA for proinflammatory cytokines is only expressed early in the reperfusion (Chandrasekar *et al.* 1997b; Kukielka *et al.* 1995). Ischaemic and reperfused myocardium has shown to be a major source of IL-6 during CPB (Wan *et al.* 1996a; Gasz *et al.* 2006), and usage of OPCAB attenuates this proinflammatory response (Wan *et al.* 1999; Czerny *et al.* 2000; Yamaguchi *et al.* 2005). Upregulation and production of proinflammatory cytokines represent an intrinsic or an innate stress response against myocardial injury (Wilson *et al.* 2004).

The degree of release of proinflammatory cytokines is directly related to the duration of ischaemia (Hennein *et al.* 1994; Wan *et al.* 1996b). Due to its procoagulant properties, raised preoperative levels of IL-6 are predictors of both early graft occlusion and late cardiovascular events after CABG (Hedman *et al. 2006*).

Most authors believe, that increased local inflammatory response is linked to the postischaemic endothelial and contractile dysfunction of the heart (Prabhu 2004). On the other hand, there is a recent evidence from experimental studies that IL-6 induces protection of cardiomyocytes, and is involved in mediating effects of late preconditioning (Dawn *et al.* 2004; Smart *et al.* 2006).

In the present study, we investigated whether hyperoxia modifies myocardial or systemic IL-6 release during coronary surgery.

2.6. Cardiovascular disease and ADMA

ADMA – asymmetric dimethylarginine – is a naturally occurring inhibitor of all three isoforms of NO synthase (Tsikas *et al.* 2000). It accumulates in plasma in a variety of diseases associated with endothelial dysfunction and enhanced atherosclerosis. By affecting NO generation in vascular endothelial cells, ADMA significantly raises systolic blood pressure and causes vasoconstriction, increases renovascular resistance, and reduces heart rate and cardiac output (Achan *et al.* 2003; Kielstein *et al.* 2004). Thus, increased ADMA levels have been reported in patients with hypertension (Surdacki *et al.* 1999), hypercholesterolemia (Boger *et al.* 1998), type 2 diabetes mellitus (Stuhlinger *et al.* 2002), hyperhomocysteinemia (Sydow *et al.* 2003), and end-stage renal failure (MacAllister *et al.* 1996). Importantly, ADMA has been found to be an independent risk factor for the coronary artery disease (Schulze *et al.* 2006), and cardiovascular events and death in patients with the coronary artery disease (Schnabel *et al.* 2005). Its values are related to the extent of coronary lesions (Sahinarslan *et al.* 2006).

In the heart, most of NO is produced by the endothelial NO synthase, present in the endothelium of coronary vessels and myocardium (Ursell and Mayes

1993; Balligand *et al.* 1995). NO, although decreased during reperfusion (Amrani *et al.* 1995), has been shown to afford cardioprotection, as described by the reduced infarct size and neutrophil accumulation in the reperfused heart (Jones *et al.* 1999). Furthermore, overexpression of ADMA metabolising enzyme DDAH-I, has shown to attenuate OxS, release of inflammatory cytokines, and graft coronary artery disease in the experimental animals (Tanaka *et al.* 2005).

In patients with unstable angina, reduction in the levels of ADMA has been reported after two weeks of standard medical therapy (Bae *et al.* 2005) and six weeks after percutaneous coronary intervention (Krempl *et al.* 2005). How ischaemia due to cardioplegia, and the following reperfusion influence the levels of ADMA in the perioperative period, or does hyperoxia induce any changes in ADMA concentrations, is not known. There is only one relevant experimental study describing the decrease of endothelial NOS expression following chronic hyperoxia in the model of IR injury (Felaco *et al.* 2000).

3. AIMS OF THE STUDY

The general aim of the present work was to answer the question, whether pre-treatment with hyperoxia will protect the human heart from ischaemia-reperfusion injury, similar to already proven on rat and mice.

Based on this, the present study was aimed specifically:

1. to establish a clinically exploitable model for evaluation of myocardial damage during the cardiac surgery;
2. according to the established model, to evaluate possible myocardial protection caused by pre-treatment by hyperoxia in patients undergoing coronary artery by-pass grafting with cardioplegia and cardiopulmonary bypass;
3. according to the established model, to evaluate possible myocardial protection caused by pre-treatment by hyperoxia in patients undergoing off-pump coronary artery by-pass grafting;
4. to evaluate changes in levels of ADMA in connection with cardiac surgery and pre-treatment by hyperoxia.

4. MATERIALS AND METHODS

4.1. Patients

Adult patients scheduled for CABG at the Centre for Cardiothoracic Surgery in North Estonia Regional Hospital, Tallinn were allocated for selection into the study. The study design was approved by the Ethics Review Committee on Human Research of the University of Tartu, and written informed consent was obtained from all patients the day before surgery.

To have prolonged ischaemic times during surgery, and thus more pronounced OxS during the reperfusion of the ischaemic heart (Ferrari *et al.* 1990), only patients with multivessel coronary artery disease (at least 3 distal anastomoses planned by the surgeon) were included. To eliminate as much variability as possible, patients with isolated primary CABG, and without concomitant diseases (diabetes mellitus treated either with insulin or oral medications, hepatic, renal (serum creatinine >150 μmol/l), or pulmonary pathology) were incorporated. All of the patients had transthoracic echocardiography done before surgery, and in case of ejection fraction below 40%, the patient was also not included into the study. This also served as an attempt to unify the study group, as in case of preoperative low ejection fraction OxS is more pronounced during reperfusion (De Vecchi *et al.* 1998). As the primary endpoint of the study was to evaluate the amount of cTn I and CK-MB in the 1st postoperative morning as the response to the pretreatment by hyperoxia, patients with the confounding situations like recent myocardial infarction (less than two weeks prior surgery), unstable angina pectoris, or already elevated cTn T/ cTn I or CK-MB were also excluded. All medications were allowed until the morning of surgery, except salicylates which were interrupted a week before operation.

4.2. Anaesthesia and operative procedure

In the morning of the operation day, all patients were premedicated with intramuscular Morphine 7.5 or 10 mg depending on the body-built of the patient. Standardised intravenous anaesthesia (midazolam, fentanyl and pancuronium) was used in all cases. For induction of anaesthesia 5 mg Midazolam and 0.5–0.75 mg Fentanyl was used, followed by 8–10 mg Pancuronium as a muscle relaxant. For maintenance Midazolam, Fentanyl and Pancuronium were exploited as well. In order to avoid the confounding effect of a preconditioning-like state caused by volatile anaesthetics (Weber and Schlack 2005), none of these were used. To avoid spasm of arterial conduits, nitroglycerine infusion (0.5–1 mg/min) was started after induction of anaesthesia in all cases. After

surgery patients were transferred to the postoperative care unit and extubated in the same evening.

The anastomoses were performed either with conventional CABG technique with cardioplegia and CPB (Papers I, II, IV), or on the working heart without CPB – OPCAB (Paper III).

4.4.1. CABG with cardioplegia and cardiopulmonary bypass

After midline sternotomy, left internal mammary artery, radial artery and saphenous veins were harvested for grafting. Thereafter pericardium was opened and secured with sutures. After cannulation of the aorta (curved tip cannula, Medtronic Inc., Minneapolis, USA) and the right atrium (two stage venous cannula, Medtronic Inc., Minneapolis, USA), the cannulas for cardioplegia were inserted into the aortic root and transatrially to the coronary sinus (15 Fr, manually inflatable, Medtronic Inc., Minneapolis, USA). CPB was performed with a roller pump (Stöckert Instrumente GmbH, Munich, Germany) and membrane oxygenator (Dideco, Mirandola, Italy) under mild hypothermia (nasopharyngeal temperature 33–35°C). To accomplish cardioplegia, St. Thomas' solution II was given into the aortic root followed by infusion into the coronary sinus. Infusion technique of cardioplegia was standardised in all cases. Coronary sinus pressure was carefully monitored and kept between 20 and 40 mmHg, not to cause coronary venous injury. Infusion was repeated after completion of each anastomosis, or at least once every 20 minutes. Both distal and proximal anastomoses were performed under a single cross-clamping period.

4.4.2. Off-pump coronary artery bypass grafting

After conventional midline sternotomy, left internal mammary artery, radial artery and saphenous veins were harvested for grafting. Thereafter pericardium was opened and a manually inflatable 15 Fr coronary sinus cannula (Medtronic Inc., Minneapolis, USA) was introduced through the right atrial wall into the coronary sinus for blood sampling. Pericardial traction sutures and elevating gauze pads were used to facilitate visibility and access to the left and right sides of the heart during the grafting procedure. The Octopus heart stabiliser system (Medtronic, Minneapolis, Minnesota, USA) was used for myocardial stabilisation. In all cases intracoronary shunt (Medtronic Inc., Minneapolis, USA) was inserted immediately after opening the coronary artery. To improve visibility, a carbon dioxide blower was used. The left anterior descending artery to the left mammary artery was always grafted first. Additional bypasses were performed using a radial artery and saphenous veins in a sequence as decided by the

surgeon. In case haemodynamic instability occurred, the right pericardium was opened to the pleural cavity, and intravenous boluses of phenylephrine together with infusion of crystalloid and colloid solutions were used. Proximal anastomoses were performed to the ascending aorta with partial aortic cross-clamping.

Total grafting time was counted from the opening the first coronary artery (left anterior descending artery in all cases) for grafting until releasing the partial aortic cross-clamp after completion of the last proximal anastomosis.

4.3. Pretreatment by hyperoxia

After induction of anaesthesia and intubation of the trachea, patients were randomly allocated to receive either 40% or >96% oxygen until the beginning of the CPB (Papers II, IV), or grafting of the first coronary artery (Paper III). Fraction of oxygen in inspired gas mixture was continuously monitored by the gas analyser of the patient monitor Datex-Ohmeda S5 (Datex – Ohmeda Division, Instrumentarium Corp, Helsinki, Finland). Arterial blood gases were analysed (Radiometer ABL 700 series, Ratiometer Medical A/S, Copenhagen, Denmark) 15 minutes after randomisation and verified again just before end of the pre-treatment phase. In the further course of the operation mixture of oxygen and air was adjusted to obtain arterial PaO_2 in the range of 110–150 mmHg.

4.4. Blood sampling and assay of biochemical indices

Blood samples were simultaneously collected from the coronary sinus and radial artery cannulae at time points described subsequently in detail.

During sampling of the coronary sinus blood, the balloon of the cannula was manually inflated to get blood exclusively from the coronary sinus. Besides simultaneous arterial/coronary sampling, in all studies additional arterial samples were drawn 60 minutes after restoration of blood flow to the heart, and in the morning of the 1st postoperative day. Blood was centrifuged immediately after sampling and serum stored at –80°C until analysing, except for glutathione measurements, which is described in the subsection regarding glutathione measurements.

During *CABG*, blood was sampled immediately after inserting the coronary sinus cannula and before starting CPB (baseline values). The following blood samples were drawn after declamping the aorta – in the 1st, 5th, 10th, and 20th minutes of reperfusion.

During *OPCAB*, the coronary sinus cannula was inserted and blood was sampled before turning the heart for inspection and putting pericardial traction

sutures (baseline values). The following blood samples were drawn 1 minute after restoration of blood flow to each grafted coronary artery, and in the 5th, 10th and 20th minutes of reperfusion.

Troponin I and *creatine kinase MB-isoenzyme* mass were measured using commercially available chemiluminescent immunoassay on Bayer ACS:180 analyser (Bayer Corp., Tarrytown, NY, USA) and l*actate* photometrically on Konelab 60i (Thermo Electron Corp., Vantaa, Finland).

To remove protein for *glutathione* measurements, one aliquot of plasma was mixed in equal portions with 10 % solution of metaphosphoric acid in water immediately after initial centrifugation and stored at +4°C for 15 minutes, and thereafter centrifuged again. Protein free supernatant was collected and stored at -80°C for further analysis.

Glutathione was measured by using an enzymatic method of Tietze (Tietze 1969), modified by Griffith (Griffith 1980), and by us (Muda *et al.* 2003). The concentration of GSH was calculated as the difference between total amount of glutathione and GSSG. The glutathione redox ratio was expressed as μmol GSSG/ μmol GSH.

Coronary sinus and arterial values for *interleukin-6* were sampled at baseline, and in the 5th and 20th reperfusion minutes, followed by the arterial samples in the 60th reperfusion minute and in the 1st postoperative morning. The concentrations were determined using the commercially available kit and the quantitative sandwich enzyme immunoassay technique (Human IL-6 Immunoassay kit, R&D Systems, Inc., Minneapolis, USA).

ADMA was determined using the competitive ADMA-ELISA (DLD Gesellschaft für Diagnostika und Medizinische Geräte mbH; Hamburg, Germany).

4.5. Haemodynamic measurements

Thermodilution pulmonary artery (Swan-Ganz) catheter was inserted through the right jugular vein after induction of anaesthesia and intubation of the trachea. Heart rate, mean arterial, pulmonary artery and pulmonary capillary wedge pressures, and cardiac output were recorded as the mean value of three sequential measurements differing less than 10%. The baseline values were recorded before sternotomy, i.e. 15–20 minutes after randomisation, followed by measurements 15 minutes, 1, 2, 4, 6, 9, and 12 hours after restoration of blood flow to the myocardium. Cardiac index, right and left ventricular stroke work indices, and pulmonary vascular resistance index were calculated using standard formulae.

4.6. Power analysis

Based on data provided in the literature about cTn I release after CABG (Alyanakian *et al.* 1998; Benoit *et al.* 2001), we used 8 ng/mL as a standard deviation of cTn I release in calculations of the sample size for the study (Paper II). According to that, the number of patients to be studied was 18 in each group. Assuming the possibility of non-parametric distribution of the data, an extra 10% was added to the sample size, and in total 40 patients were included.

4.7. Statistical methods

The method of permuted blocks was used for randomisation to the hyperoxia and control groups.

Patient, oxygenation and surgical data were analysed using Student's t-test or Fischer's exact test as appropriate. Depending on the data distribution (Shapiro-Wilk test), the differences in biochemical data between groups were analysed using either non-parametric ANOVA and Mann-Whitney U-test, and the results represented as median (interquartile range); or parametric tests (ANOVA for repeated measures and Student's t-test) and represented as mean (standard deviation). In Study I Wilcoxon sign test was used to evaluate differences between time points. In Study IV Friedman's ANOVA followed by Dunnet´s test was applied to locate significant differences over time. In Study III not all patients needed to have 4 grafts, therefore the time-point after 4th distal anastomosis was excluded from the statistical analysis. As haemodynamic data showed normal distribution in all cases, these were analysed with ANOVA for repeated measures and represented as mean (standard deviation).

In case of significant p-values were calculated, multiple comparisons were performed with Duncan's test or Mann-Whitney U-test with Bonferroni correction, depending on the distribution of the data.

With normal distribution of the data, correlation was expressed as Pearson correlation coefficient, otherwise Spearman's rank order correlation was used.

Value of $p<0.05$ was always considered as significant.

5. RESULTS

5.1. Patient characteristics

This research has been divided into four parts, each published as a separate paper. Altogether data sets form 63 patients were analysed. 12 patients from Study I served also as controls for Study II. To evaluate release of ADMA (Study IV), refrigerated sera of 22 lastly included patients to study II (11 from both groups) were taken and ADMA concentrations were measured.

Study groups were similar regarding age of the patients, severity of coronary artery disease, preoperative cardiac function and medications, and number of grafted arteries. Number of male patients exceeded that of female in all groups. This is most probably due to the higher prevalence of coronary artery disease among men in general population. In Study I, the patients had 3–6 coronary arteries grafted, and in Studies II to IV, the number of grafts was 3–5. In the control group of Study III, five patients (out of 11), and in the hyperoxia group seven patients (out of 12) had four distal anastomoses, in all other cases three coronary arteries were bypassed.

Demographic data of the patients are presented in Table 1.

Table 1. Characteristics of patients, oxygenation and surgical data.

	Study I	Study II		Study III		Study IV	
	(n=12)	Controls (n=20)	Hyperoxia pre-treated (n=20)	Controls (n=11)	Hyperoxia pre-treated (n=12)	Controls (n=11)	Hyperoxia pre-treated (n=11)
Age (years)	65 (7)	64 (9)	60 (7)	67 (6)	63 (7)	64 (10)	60 (6)
No. of patients (male/female)	12 (8/4)	20 (12/8)	20 (16/4)	11 (10/1)	12 (12/0)	11 (6/5)	11 (9/2)
Preoperative EF (%)	64 (8)	60 (11)	53 (8)	57 (8)	57 (10)	58 (11)	53 (7)
p_aO_2 15 min after intubation (mmHg)		127 (39)	330 (74)	106 (33)	301 (94)	133 (39)	357 (65)
p_aO_2 before CPB / grafting (mmHg)		121 (36)	302 (96)	115 (17)	314 (68)	124 (36)	301 (119)
Exposition time to oxygen (40 % vs >96%, min)		130 (24)	144 (22)	128 (31)	126 (25)	135 (20)	148 (24)
Cross-clamping / grafting time (min)	85 (21)	86 (20)	85 (19)	135 (36)	152 (53)	85 (18)	89 (13)

Values are represented as mean (SD).

5.2. Myocardial damage during coronary surgery

All patients had cTnI and CK-MB mass within reference values before cardioplegia.

During *CABG* (Papers I, II), myocardial release of both CK-MB and cTnI into the coronary sinus blood was apparent already 1 minute after declamping the aorta, and peaked in the 20th minute of reperfusion (Fig. 2). The arterial values showed constant increase up to the 1st postoperative morning (Fig. 3, control group; Table 1, Paper I).

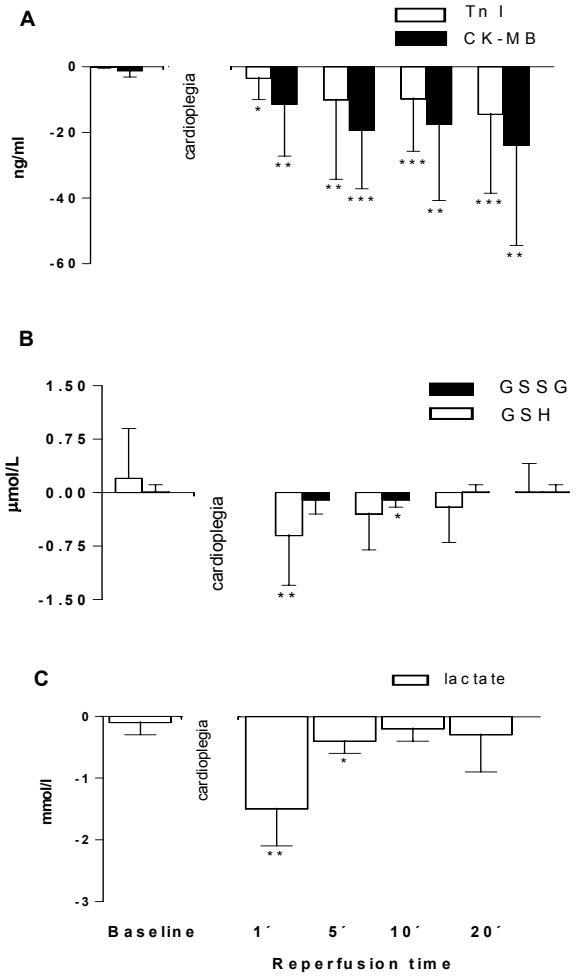

Figure 2. Myocardial release (arterial-coronary sinus difference) of cTn I and CK-MB (panel A), oxidised and reduced glutathione (panel B), and lactate (panel C). Data are represented as mean (SD). * $p<0.05$, ** $p<0.01$, *** $p=0.001$ in comparison with preclamping values.

Reperfusion after cardioplegia resulted in a significant release of both *oxidised and reduced glutathione* from the heart (Fig. 2). Significant release of GSSG occurred in the 5th minute of reperfusion, with concomitant increase in the glutathione redox ratio (Results section, Paper I). After cardioplegia, temporary release of *lactate* from the heart was observed (Fig. 2). Despite of biochemical signs of myocardial damage, the function of the heart showed no significant depression as evaluated by the right and left stroke work indices (Table 2, Paper I).

Relation between the release of biochemical markers and postoperative myocardial function. Amount of released cTn I and CK-MB had poor influence on the early postoperative myocardial function. Neither the absolute nor relative values (change from baseline) of myocardial function parameters after cardioplegia, at any time-point when assessed, were correlated with the release of CK-MB or cTn I.

Also, no significant correlations were evident between the *duration of cardioplegia* and early postoperative cardiac function. Furthermore, the postoperative function of the heart was not determined by the amount of GSSG and GSH released during the immediate reperfusion.

During *OPCAB,* both cTnI and CK-MB appeared into the coronary sinus already after the 1st distal anastomosis, and increased gradually over the whole study period (Fig. 6, control group). The extent of markers released, however, was of a magnitude smaller in comparison with the *CABG* patients.

5.3. Effect of pretreatment by hyperoxia during CABG

Hyperoxia did not have any significant effects upon myocardial necrosis (Fig. 3) and postoperative cardiac function (Fig. 4), although some favourable tendencies, as better myocardial function and reduced release of *cTn I* and *CK-MB* in the 1st postoperative morning occurred in the hyperoxia group.

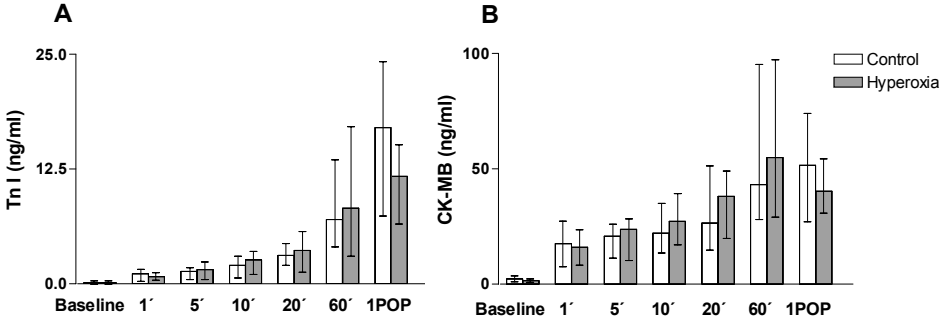

Figure 3. Arterial values of cTn I (panel A) and CK-MB (panel B) during immediate reperfusion, and in the 1st postoperative morning (1POP) after CABG. Data are represented as median (interquartile range).

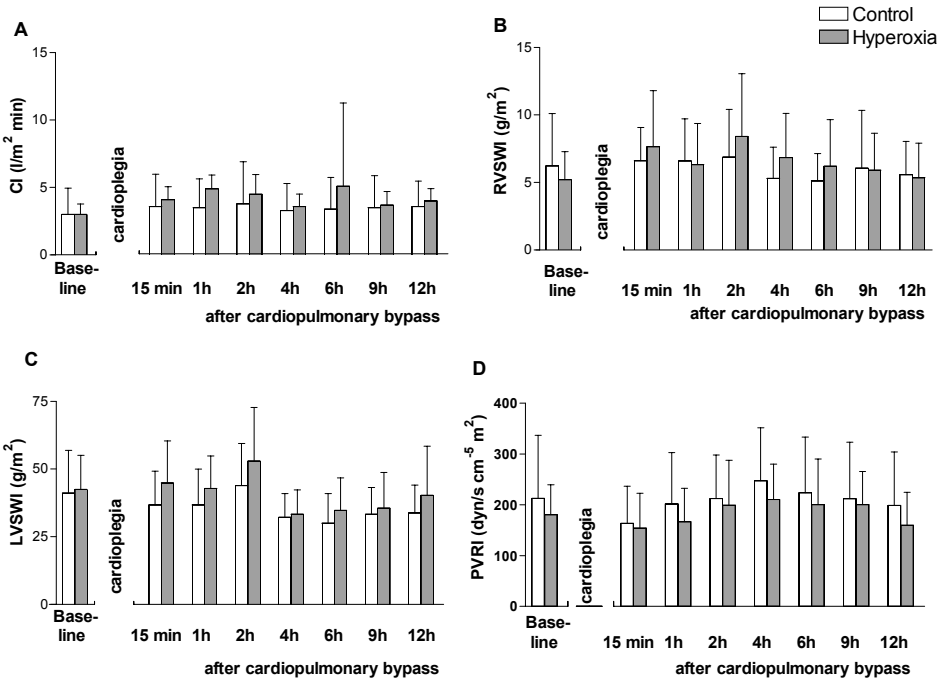

Figure 4. Haemodynamic parameters – cardiac index (CI, panel A), right ventricular stroke work index (RVSWI, panel B), left ventricular stroke work index (LVSWI, panel C), and pulmonary vascular resistance index (panel D) after intubation (baseline) and at timed intervals after cardiopulmonary bypass. Data are represented as mean (SD).

No significant differences were found between groups with respect to the coronary sinus blood levels of cTnI and CK-MB (Paper II, Results section).

At the end of the hyperoxic pre-treatment, no difference in the levels of *GSH* and *GSSG* between control and hyperoxia groups was detected. Also, transmyocardial release of *GSH* did not differ between groups. There was no release of GSSG in the 1st min of reperfusion in the control group, but in the hyperoxia group the arterio-coronary sinus difference was –0.1 (–0.13, –0.01) µmol/L (p=0.015 between groups). In the further course of reperfusion, the values did not differ between groups.

The *glutathione redox ratio* in the coronary sinus blood did not differ between groups during the whole study period.

5.4. Effect of pretreatment by hyperoxia during OPCAB

The baseline levels of *cTn I* and *CK-MB* mass were within reference limits and did not differ between groups. The arterial values of both cTn I and CK-MB increased postoperatively (Fig. 5). Sixty minutes after completion of grafts, the level of cTn I was higher in the hyperoxia group (p=0.031 in comparison with controls). By the 1st postoperative morning this difference was not evident any more (2.8 (2.4, 6.4) ng/ml in the hyperoxia and 2.6 (1.7, 3.8) ng/ml in the control group, p=n.s). Arterial values for CK-MB did not differ between groups at these measurement points. The values in the 1st postoperative morning were 18.2 (13.8, 43.3) in the control and 20.8 (12.9, 25.2) in the hyperoxia group.

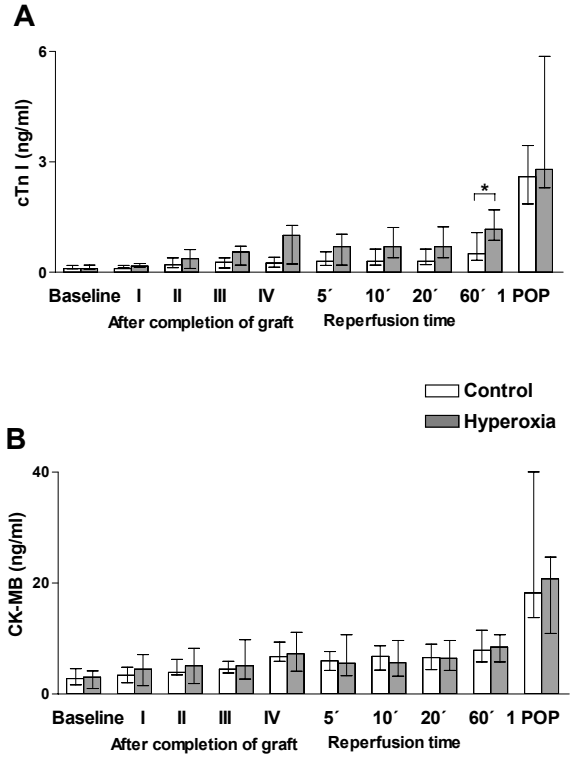

Figure 5. Arterial values of cTn I (panel A) and CK-MB (panel B) during OPCAB, early reperfusion and in the 1st postoperative morning (1POP). Data are represented as median (interquartile range). * p=0.03 between groups.

Already after the 1st distal anastomosis both markers appeared into the coronary sinus blood, and were gradually increased over the whole study period (Fig. 6; Results section, Paper III). In the control group maximal values in the coronary

sinus blood were observed in the 10th min of reperfusion (8.0 (5.6, 8.6) ng/ml for CK-MB and 0.5 (0.3, 0.94) ng/ml for cTnI, resp). In the hyperoxia group, both CK-MB and cTn I peaked in the coronary sinus blood in the 20th minute of reperfusion (7.3 (4.2, 10.6) and 1.2 (0.75, 1.9) ng/ml, resp.). Either during the procedure or in the immediate reperfusion period no difference between groups could be demonstrated.

Arterial and coronary sinus values of *GSSG* and *GSH* did not differ between groups neither immediately after the hyperoxic pre-treatment nor during the whole study period. The glutathione redox ratio did not differ between groups (Results section, Paper III).

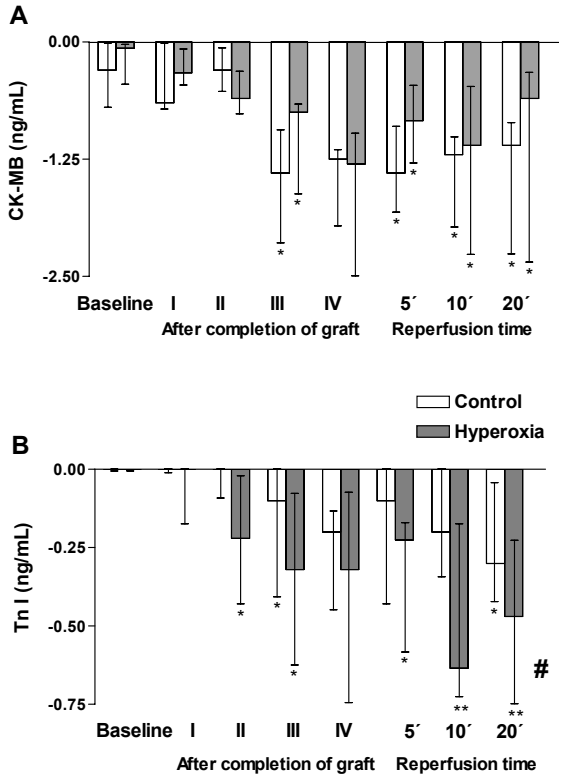

Figure 6. Myocardial release (expressed as difference between arterial and coronary sinus concentrations) of CK-MB (panel A) and cTn I (panel B) during OPCAB and early reperfusion. Values are given as median (interquartile range). * $p<0.05$, **<0.001 in comparison with preclamping values; # $p<0.01$ in comparison with the control group.

Haemodynamic measurements

Pre-treatment by hyperoxia had no significant effect upon the function of the heart during the procedure (Fig. 7).

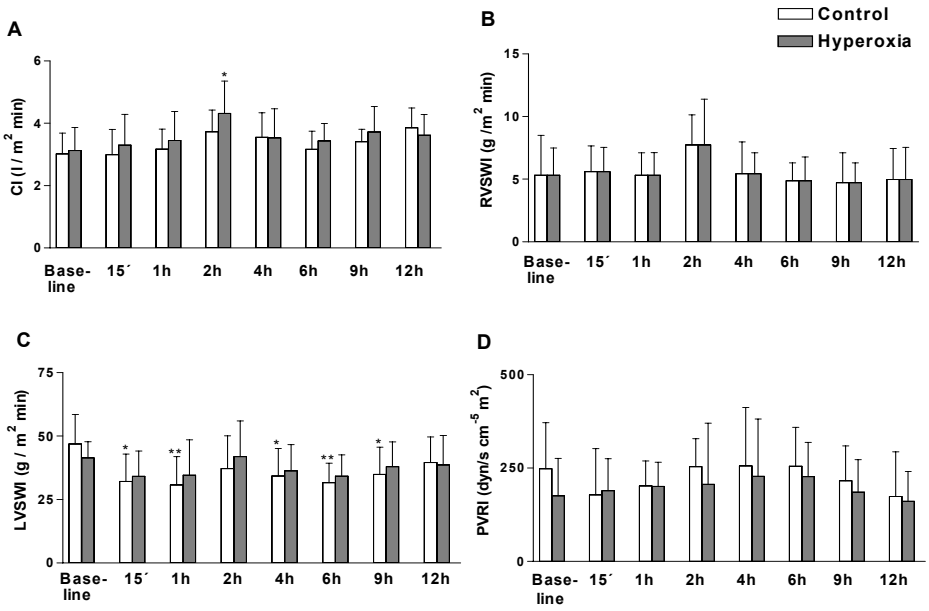

Figure 7. Haemodynamic parameters – cardiac index (CI, panel A), right ventricular stroke work index (RVSWI, panel B), left ventricular stroke work index (LVSWI, panel C) after intubation (baseline) and at timed intervals after OPCAB. Data are represented as mean (SD). * $p<0.05$, ** $p<0.01$ in comparison with the preclamping values.

5.5. Alterations of the IL-6 levels during coronary surgery

In both studies (Papers II, III), the concentrations of IL-6 in the arterial and coronary sinus blood were almost negligible immediately after termination of the hyperoxic pre-treatment, and difference between hyperoxia and control groups was not detected.

CABG. During the reperfusion phase, the levels of IL-6 were significantly increased in both arterial and coronary sinus blood in comparison with the pre-CPB values, whereas higher myocardial release was detected in the control group (Fig. 8). To the contrary, in the 1st postoperative morning the arterial IL-6 concentrations were higher in the hyperoxia group ($p=0.016$ between groups).

In the control group, the arterial concentrations of IL-6 were related to the ischaemic time in the 5th minute ($r=0.61$, $p=0.04$) and in the 60th minute ($r=0.62$, $p=0.04$) of reperfusion, but this relationship was not followed in the hyperoxia group.

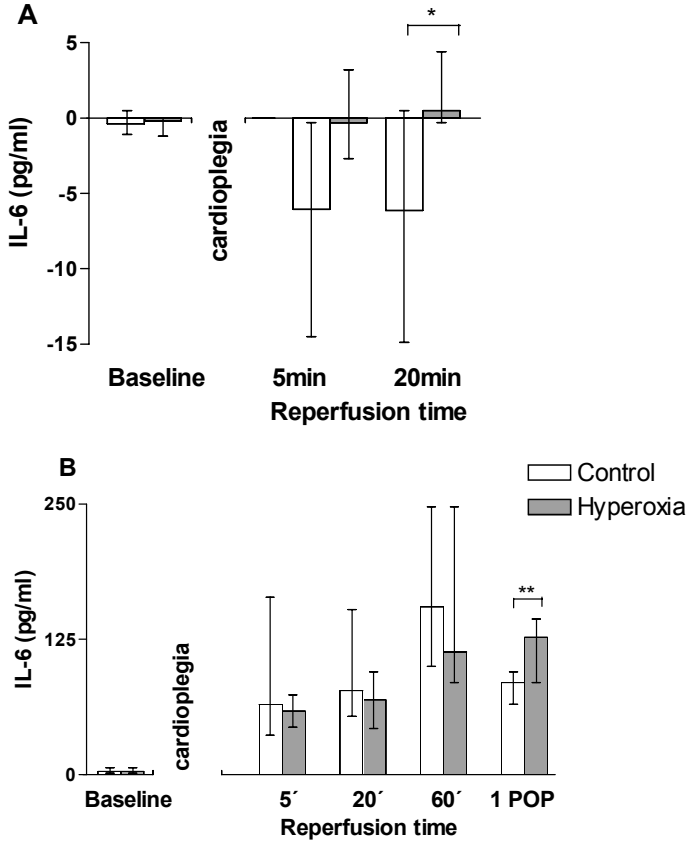

Figure 8. Arterio-coronary sinus differences (panel A) and arterial concentrations (panel B) of IL-6 during reperfusion and in the 1st postoperative morning (1 POP) after CABG. Data are represented as median (interquartile range). * p=0.04, ** p=0.02 between groups.

OPCAB. In both groups increased transmyocardial release of IL-6 was evident in the 5th and 20th minutes of reperfusion (Fig. 9). In the arterial blood, IL-6 started to increase from the 5th post-grafting minute, reaching its maximum by the 1st postoperative morning (20 h after completion of the grafts). At this time maximal difference between groups was observed, the concentrations being 202.0 (188.0, 246.0) pg/mL in the control and 162.0 (138.0, 223.0) pg/mL in the hyperoxia group. The duration of the grafting procedure influenced both arterial and coronary sinus blood concentrations of IL-6. Significant correlations were observed during the whole reperfusion period, but not in the 1st postoperative morning any more. The relationship was most clear-cut in the coronary sinus blood in the 5th (r=0.59, p=0.006) and 20th minutes (r=0.58, p=0.01) of reperfusion.

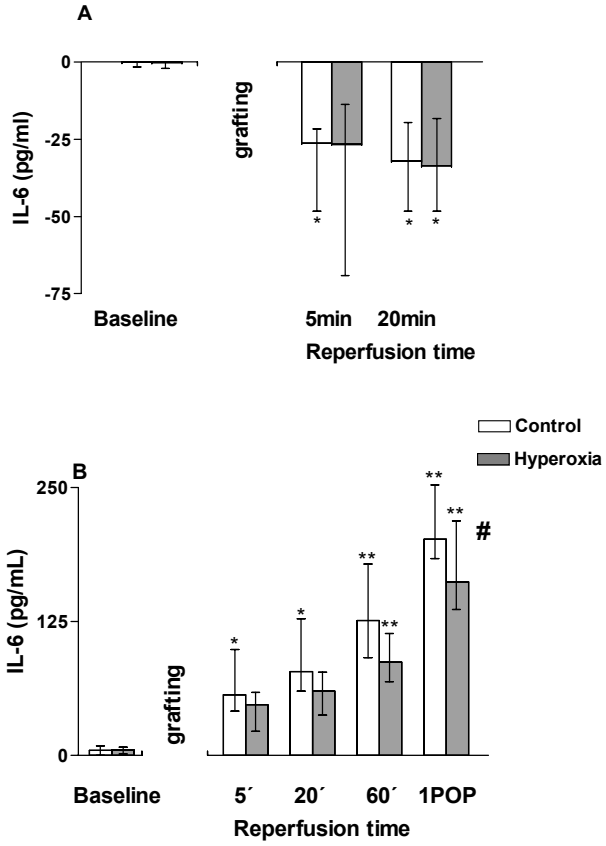

Figure 9. Difference between arterial and coronary sinus concentrations (panel A) and arterial values (panel B) of of IL-6 during reperfusion and in the 1st postoperative morning (1 POP) after OPCAB. Data are represented as median (interquartile range). * $p<0.05$, ** $p<0.01$ in comparison with preclamping values; # $p=0.046$ between groups over time.

5.6. Alterations in the ADMA levels during coronary surgery

The baseline arterial levels of ADMA, measured before CPB, were similar in both groups. During the 1st hour of reperfusion no alterations in arterial concentrations were seen, but a significant decrease ($p<0.01$) was observed by the 1st postoperative morning both in the hyperoxia and control groups (Fig.10).

In the control group, reperfusion of cardioplegic heart did not result in washout of ADMA. Some extent of ADMA was released from the myocardium of the hyperoxia pretreated patients during the 20 minutes of reperfusion. In

comparison with the control group, the values, however, did not reach significant difference. In the hyperoxia pre-treated, but not in the control patients, the concentration in the coronary sinus blood in the 5th and 20th minutes of reperfusion correlated with the duration of the cardioplegic arrest (Fig.3, Paper IV).

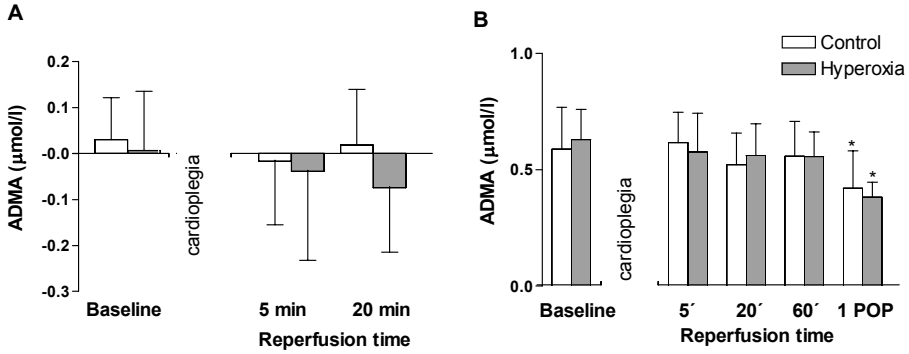

Figure 10. Difference between arterial and coronary sinus concentrations (panel A) and arterial values (panel B) of ADMA before cardioplegia, during reperfusion and in the1st postoperative morning (1 POP). Data are represented as mean (SD). * $p<0.01$ in comparison with the preclamping values.

6. DISCUSSION

In the present thesis we demonstrate that elective CABG with cold crystalloid cardioplegia was associated with significant release of CK-MB, cTn I, lactate, GSSG, and impaired glutathione status. Early postoperative function of the heart was not determined by the extent of myocardial necrosis and abnormal pro-oxidant status. Despite of biochemical signs of myocardial injury, contractile function of the heart was not significantly altered in the immediate reperfusion period. The release of markers of myocardial necrosis and OxS from the heart did not correlate with the indices of myocardial function. In comparison with CABG, OPCAB was associated with diminutive release of markers of myocardial necrosis, although their release started already after completion of the first graft, even before the heart was extensively handled for the grafting procedure.

The proposed cardioprotective effect of hyperoxia could not be demonstrated in the setting of coronary surgery. Ventilating the lungs with hyperoxic gas mixture before cardioplegia did not affect the myocardial release of cTn I and CK-MB after CABG. Clinically significant changes were detected neither in the release of markers of OxS nor in the postoperative haemodynamic indices, measured with the thermodilution pulmonary artery catheter. To the best of our knowledge, this is the first clinical study reporting the effects of pre-treatment with hyperoxia on the myocardial necrosis, function and oxidative stress.

This study provides the first evidence of changes of ADMA concentrations in association with coronary surgery. We found a significant decrease of arterial ADMA concentrations by the first postoperative morning, and demonstrated that a cardioplegic arrest did not cause the release of ADMA from the human heart. Ventilation with hyperoxic gas mixture had no influence on either arterial or coronary sinus levels of ADMA.

6.1. Methodological considerations

Sample size

One of the major pitfalls of the study is the underestimation of variance for cTn I release in calculating the sample size (Paper II). *A posteriori* analysis, taking into account the variance of our data, revealed power of 75 %, indicating the study being underpowered.

Patients

Despite of strict patient selection criteria and standardising the techniques for anaesthesia and cardioplegia as much as possible, influence of clinical variability regarding age, sex, and degree of coronary atherosclerosis cannot still be excluded. The between-patient differences regarding the quality of cardioplegia

and surgical handling may also be responsible for the marked variability of the data. One of the confounding factors could be usage of nitroglycerin to avoid spasm of the arterial grafts. Nitroglycerin as a NO donor has shown to affect not only the late (Leesar *et al.* 2001), but also an early phase of preconditioning (Cohen *et al*. 2006).

Oxidative stress
Huge variety of methods exists for evaluation of OxS. Some of these are impossible to exploit in humans, others are not suitable for clinical investigations. Our measurements of GSSG and GSH release were much inspired from Ferrari *et al.* (Ferrari *et al.* 1990), who was the first to demonstrate the occurrence of oxidative stress during the reperfusion of the human heart. In the future, isofurans, products of free radical-induced peroxidation of arachidonic acid that exhibit favored formation with increasing oxygen concentrations (Fessel and Jackson Roberts 2005), may be used as a sensitive index of free-radical induced lipid peroxidation assessment *in vivo.*

Interleukin-6
Although some concerns exist about the exact role of IL-6 during ischaemia and reperfusion, it is still one of the most widely used to evaluate the inflammatory response. Besides IL-6, a variety of proinflammatory cytokines (TNF, IL-1β etc) are evaluated in conjunction with IR injury of the heart. However, IL-6 and IL-10 have showed the least interindividual variations, suggesting that these cytokines may give reliable information regarding modulation of the immune response following CPB and its consequences for the patients' outcome (Misoph and BabinEbell 1997).

6.2. Myocardial damage during coronary surgery

It is clear, that some amount of myocardial damage is associated with every intervention, during which the myocardium is manipulated. Be it due to the procedure *per se*, its complications or reperfusion-associated events. Consequently, eliminating any of the abovementioned should result in reduced myocardial damage. And there is extensive number of published data showing that conventional CABG, where CPB and cardioplegia are exploited, is associated with increased myocardial necrosis in comparison with OPCAB, where global myocardial ischaemia and following reperfusion are not applicable.

In both of our studies evaluating myocardial damage (Papers II, III), similar inclusion criteria were applied. The only difference was the technique used for coronary artery bypass grafting. As a result, similar to others, we see concentrations of cTn I and CK-MB order of a magnitude smaller in the OPCAB study patients (Fig. 3 and 5). The small elevation of cTn I and CK-MB during

OPCAB is most probably procedure-associated, which in addition to direct trauma while sewing the grafts, causes unfavorable blood flow during repeated turning the heart for better visualisation. The diminished lactate release immediately after discontinuation of the handling of the heart (Paper III) provides further support for this. The cumulative effect of brief periods of ischaemia, which when single do not cause necrosis, has been previously demonstrated in dogs (Geft *et al.* 1982).

As described earlier (section 2.2.2), the cut-off limits for detection of myocardial necrosis vary to a great extent. Despite of presence of noticeable irreversible myocardial injury in our studies (Papers I, II), the function of the heart was not depressed in the immediate postoperative period. Electrocardiography and clinical course showed no signs of perioperative myocardial infarction as well. It is in line with the data of Holmvang *et al*., as in 25% of post-CABG patients a CK-MB mass value > 35.8 μg/L can be detected with otherwise no indications of ischaemic complications (Holmvang *et al.* 2002).

The duration of ischaemic time is without doubt one of the causative factors for changes in myocardial structure and metabolism. Both close relationship between cardiac enzyme release, recovery of oxidative metabolism and ischaemic time (Koh *et al.* 1998; Raman *et al.* 2001), and not such a clear correlation (Biagioli *et al.* 1997; Alyanakian *et al.* 1998) have been shown not only during CABG, but also in association with OPCAB surgery (Laurikka *et al.* 2002). It supports the hypothesis that duration of cardiac arrest is not the dominating determinant of perioperative myocardial damage. Most likely complex of factors like severity of atherosclerosis, quality and route of delivery of cardioplegia, temperature, surgical technique, etc. rather than the ischaemic time only, is responsible for the ultimate injury.

How extensive leakage of biomarkers is of importance?

The most recently described and preferred biomarker for myocardial damage is cardiac troponin (I or T), which has nearly absolute myocardial tissue specificity, as well as high sensitivity. cTn I is a regulatory protein of the muscular thin filament and part of the troponin-tropomyosin complex in the striated muscle. Creatin kinase MB-isoenzyme is a predominantly cytosolic protein, which after myocardial damage should be released somewhat more rapidly than structurally bound regulatory proteins. Remmpis *et al*. have showed that protein release from nonstructurally bound cytosolic pools accounts for up to 6% of the total cTnI content of the cardiomyocyte. Washout of this cytosolic component (reflecting surgery-induced increased cell permeability without cellular death) could result in one seeing troponin blips in the first few hours after surgery without corresponding irreversible myocardial injury on imaging (Remppis *et al.* 1995). Others confirm, that cTn I and CK-MB can be released only after irreversible, but not during reversible myocardial cell injury (Klein *et al.* 1977; Fishbein *et al.* 2003). According to the latest consensus

document about redefinition of myocardial infarction, any amount of myocardial necrosis caused by ischaemia should be labeled as myocardial infarction, as there is a continuous relation between minimal myocardial damage, characterised by elevation of cardiac troponin without elevation of other cardiac biomarkers (e.g., CK-MB) and large infarcts, characterised by complications such as heart failure or shock (Myocardial infarction redefined, 2000). Release of troponins (Dehoux *et al.* 2001; Fellahi *et al.* 2003) and CK-MB (Costa *et al.* 2001; Steuer *et al.* 2002) after CABG is reported to predict both short and long term adverse outcome after CABG. Fellahi *et al*. suggested, that the best cut-off value to predict death over a 2-year period after surgery was between 12.1 and 13.4 μg/mL (Fellahi *et al.* 2003).Our results on postoperative release of both CK-MB and cTn I strongly support the superiority of OPCAB technique over the conventional CABG in this context.

Do pro-oxidative processes exceed protective mechanisms in the postoperative dysfunction of the heart?

Our measurements of GSSG and GSH release were much inspired from Ferrari *et al.*, who was the first to demonstrate the occurrence of OxS during the reperfusion of the human heart (Ferrari *et al.* 1990). They further showed existing correlations between postcardioplegic OxS and myocardial dysfunction. Other investigators (De Vecchi *et al.* 1998; Wu *et al.* 2001) have later supported these findings. In our patients, subtle, but still significant release of GSSG occurred during the first minutes of reperfusion. The glutathione redox ratio was impaired (which means more oxidised) immediately after release of the aortic cross clamp, and increased in time. After 20 minutes of reperfusion the values of the glutathione redox ratio in the coronary sinus blood exceeded these in the arterial blood almost fivefold. The presence of OxS, however, was not associated with the depressed left ventricular function. Taking together, our results are only partly in accordance with Ferrari *et al.*, as we did not find any correlation between the GSSG efflux and the function of the heart. Biagioli *et al*. and Ohsawa *et al.* did also not report of a such association (Biagioli *et al.* 1997; Ohsawa *et al.* 2004).

In our study (Paper I) mean ischaemic time, patient preoperative status and cardioplegia regimen were very similar as from Ferrari *et al.* (Ferrari *et al.* 1990). The only difference was that in our study both distal and proximal anastomoses were performed with a single aortic cross clamping period, which provides good immediate reperfusion upon declamping. Also, we used manually inflatable coronary sinus cannula, which probably ensures more correct sampling of the coronary sinus blood. Thus, our data suggest that pro-oxidant mechanisms do not affect the postoperative recovery of myocardial function in a carefully selected group of CABG patients with good preoperative left ventricular function. Thus, it can be speculated, that the underlying cellular mechanisms of myocardial stunning are far more complex than originally thought.

6.3. Effects of hyperoxia during coronary surgery

In experimental studies, it has been repeatedly demonstrated that exposing animals to hyperoxia prior IR attenuates development of myocardial necrosis and improves post-ischaemic function of the heart. In the clinical setting we could not see such clear benefits, although some tendencies towards similar effects exist. These include less cTn I and CK-MB release in the 1st post-operative morning after CABG, and less depression of right and left ventricular stroke work indices during the first postoperative hours.

Why hyperoxia in a clinical setting did not induce myocardial protection, as demonstrated in the mouse and rat hearts (Tähepõld *et al.* 2002a), remains unclear. We tested only one inspiratory concentration of oxygen with delimited exposure-time. It cannot be excluded that an average exposition of two hours as in the present study would be on the borderline between induction of protection and tissue injury, especially, while keeping in mind the additional oxidative burst possibly caused by the institution of CPB. In the animal experiments exposure of one hour has been used, which repeatedly appeared protective against IR injury. Further studies should address exposure of this duration also in patients. There are also other factors, like the protective effect of cardioplegia and usage of opioid anaesthetics, exploitation of 40% but not 21% oxygen (due to anaesthesia safety) in controls, which would have very likely interfered with the results. Finally, the differences among species might also be the explanation for this discrepancy.

Regarding clinical studies of myocardial preconditioning, the consequences are not as unequivocally beneficial as in experimental setting. Both benefits (Jenkins *et al.* 1997; Illes and Swoyer 1998; Li *et al.* 1999) and no superiority (Cremer *et al.* 1997; Kaukoranta *et al.* 1997) over, or in addition to conventional myocardial protection in terms of cardiac function and metabolic or necrosis markers have been described. This is a common problem of clinical studies in the cardiac surgery, where modern cardioprotective techniques are applied also to control patients, and thereby the degree of injury is often insufficient to demonstrate the additive effect of a studied intervention.

The situation is similar during *OPCAB* procedure, where the control group has only minor myocardial damage, and thus there is nothing to protect from. Laurikka *et al*. found less cTn I release, but only marginal changes in haemodynamic parameters after ischaemic preconditioning, whereas Penttilä *et al*. detected no effect at all (Laurikka *et al.* 2002; Penttila *et al.* 2003). The reason for that could be lack of effect of early preconditioning on myocardial stunning (Malkowski *et al.* 1998; Jahania *et al.* 1999). These divergent results have led to the hypothesis that in the setting of coronary artery bypass surgery, the additional protection conferred by ischaemic preconditioning may only be demonstrable where a potential for suboptimal myocardial protection increases the risk of perioperative infarction (Perrault and Menasche 1999).

Increased OxS could be of concern in association with hyperoxic pre-treatment. Immediately after administration of hyperoxic gas mixture the glutathione redox ratio, a sensitive index of OxS, did not show presence of extensive OxS and the values did not differ between hyperoxia and control groups. Although increased release of GSSG was evident in the 1st minute of reperfusion, no aggravation of OxS was detected at later time-points.

Pre-treatment by hyperoxia affected both myocardial release and systemic levels of IL-6. As levels of IL-6 during reperfusion have been shown to correlate with the severity of injury – left ventricular wall motion abnormalities (Hennein *et al.* 1994) and negative inotropic effect (Finkel *et al.* 1992), a marked reduction of IL-6 concentrations in the coronary sinus blood after declamping the aorta could be considered as a sign of reduced injury. However, as we did not observe any protective effect of hyperoxia upon myocardial infarction or function, the exact importance of this finding remains to be elucidated.

The effects of pre-treatment by hyperoxia upon systemic levels of IL-6 in the 1st postoperative morning are more confusing. The elevated arterial IL-6 levels after CABG may be of concern, as it may suggest that hyperoxia increases systemic inflammatory reaction. To the contrary, after OPCAB we observed a significant reduction of IL-6 in the hyperoxia group by the first postoperative morning. Further studies should clarify whether pre-treatment with hyperoxia has a cause-effect relationship with systemic inflammatory response in the coronary surgery.

6.4. Alterations in the interleukin-6 and ADMA levels during coronary surgery

IL-6 is not constitutively expressed in the heart (Kapadia *et al.* 1997), but only after cardiac injury, regardless of its origin. This universal nature suggests that these molecules constitute part of an intrinsic or innate cardiac stress response system (Mann 2003).

Myocardial production of IL-6 has been described after cardiac surgery with CPB (Wan *et al.* 1996a) and coronary stenting for stable angina (Kefer *et al.* 2004). Similar production of IL-6 results in OPCAB surgery, indicating that even minor myocardial injury during the procedure is sufficient to activate the stress response system. Moreover, if we compare the control groups of CABG and OPCAB studies, the myocardial production of IL-6 during the 20-minute reperfusion period is of a magnitude smaller after CABG. This can be partly due to the time gap for the IR stimulus to take effect after declamping the aorta. The arterial values did not differ 60 minutes after declamping the aorta in CABG and OPCAB patients, but surprisingly, by the 1st postoperative morning the OPCAB group showed significantly higher IL-6 values (Fig. 8 and 9). This is

not in line with the previously reported reduced IL-6 release after OPCAB (Mei *et al.* 2007), or no difference between the two techniques (Wippermann *et al.* 2005). Our results suggest that not the contact of the blood with foreign surfaces (i.e. CPB circuit), but rather the general stress response to the surgery is a key factor for IL-6 production in the cardiac surgery patients.

In Paper IV of the present thesis we demonstrate that CABG surgery is associated with a significant decrease of arterial *ADMA* concentrations by the 1st postoperative morning. Cardioplegic arrest did not cause the production of ADMA in the human heart, and ventilation with hyperoxic gas mixture had no influence on either arterial or coronary sinus levels of ADMA.

The measured ADMA levels in the in the 1st postoperative morning after CABG were on the very low end, and in some cases even lower than proposed reference limits for ADMA in the healthy population (Schulze *et al.* 2005). The early decrease of ADMA concentration may reflect adaptive changes, aimed to enhance NO release through better functioning of endothelial NOS. Whether these are only temporary alterations in the immediate postoperative period, or reflect true improvement of endothelial functioning after surgery, remains to be elucidated. There is a number of different powerful stimuli interfering in the early postoperative period, and therefore the changes during this short period of time are difficult to interpret. Obviously, only persisting changes in ADMA could reflect the modification of cardiovascular risk in particular patients.

Oxidative stress may decrease the activity of dimethylarginine dimethyl-aminohydrolase with the concomitant increase in ADMA levels. In our study (Paper IV), exposure to >96 % of oxygen on the average for 135 minutes before CPB did not cause an increased production of ADMA in the heart (Fig 10). During the reperfusion period, there was a tendency, albeit non-significant, for increased ADMA production in the hyperoxia pre-treated, but not in the control hearts (Fig 10).

In the hyperoxia pre-treated patients we observed the correlation between ADMA levels in the coronary sinus and aortic cross-clamping time, which could be interpreted as a relation between the severity of endothelial reperfusion injury and duration of the ischaemic period. Why such correlation occurred only in the hyperoxia pre-treated but not in control patients, remains unclear. It cannot be excluded that additional pro-oxidativity induced by hyperoxia aggravated endothelial dysfunction.

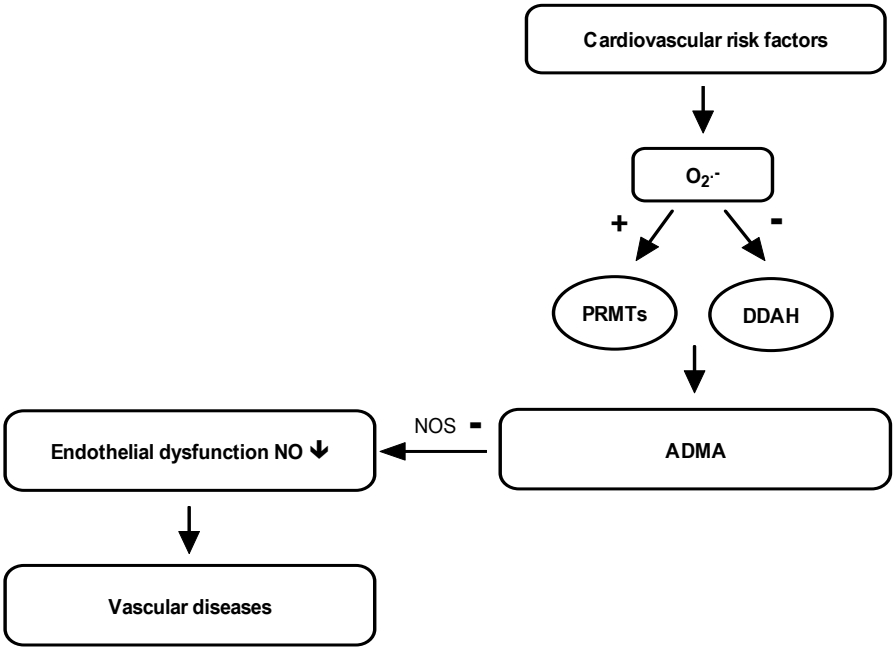

Figure 11. Overview of the ADMA related functions.
Elevated superoxide ($O2^{\cdot -}$ production caused by cardiovascular risk factors like diabetes mellitus, hypercholesterolaemia, hypertension, hyperhomocysteinaemia) may increase ADMA levels by upregulating the activity of protein arginine N-methyltransferases (PRMTs) or by inhibiting dimethylarginine dimethylaminohydrolase (DDAH) activity. Increased ADMA concentrations inhibit NO synthase (NOS)-mediated NO activity. Adapted from (Sydow and Munzel 2003).

7. CONCLUSIONS

1. We established a clinically exploitable model for evaluation the myocardial damage in patients undergoing conventional CABG as well as OPCAB. Blood samples for detection of the myocardial necrosis and OxS indices assay are taken simultaneously from the coronary sinus and radial artery cannulae, and the values of different biochemical markers are analysed together with the data of the cardiac function. Early postoperative function of the heart was not predicted by the extent of myocardial cTn I and CK-MB release or oxidative stress. Despite of biochemical signs of significant injury, contractile function of the heart was not altered in the immediate reperfusion period. The changes in the concentrations of biochemical indices did not correlate with the postoperative function of the heart.
2. Administration of >96% oxygen before CPB did not offer such clear protection of the heart from the IR injury in humans as seen in experimental animals, although it can offer some potentially beneficial effects such as decreased transmyocardial release of IL-6.
3. Off-pump coronary surgery is associated with a minor release of cardiac markers already from the very beginning of the grafting procedure. Cardioprotective effect of hyperoxia could not be demonstrated in this setting of patients. Pretreatment by hyperoxia had an effect upon whole-body inflammatory response, expressed as diminished systemic production of IL-6.
4. CABG surgery with cardioplegia resulted in changes of ADMA levels in the coronary sinus during early reperfusion, which in the hyperoxia pretreated patients was related to the aortic cross-clamping time. A significant decrease in the arterial concentrations of ADMA was detected in the first postoperative morning. Pretreatment with hyperoxia had no influence on either myocardial release or arterial levels of ADMA.

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9. SUMMARY IN ESTONIAN

Südame isheemia-reperfusiooni kahjustus koronaarkirurgias: kliiniline uurimus hüperoksia toimest

Verevoolu katkemine ja hapnikuvaegus tekitavad tõsiseid häireid aeroobsete organismide rakkude talitluses. Verevoolu taastamine isheemilisse koesse võib põhjustada hapniku reaktiivsete osakeste kontrollimatu produktsiooni, mis omakorda viib erinevate rakustruktuuride kahjustumiseni. Seda fenomeni tuntakse kui isheemia-reperfusiooni (IR) kahjustust. Igapäevses kliinilises praktikas avaldub see isheemilise müokardi verevarustuse taastamisel – nt trombolüüsil, perkutaansete koronaarinterventsioonide käigus või kardiokirurgias. IR kahjustuse kliinilisteks väljendusteks on reperfusioonil tekkivad südame rütmihäired, südamelihase pöörduv funktsioonihäire ehk oimetu müokard, südamelihase nekroos, erineva raskusastmega mikrovaskulaarne kahjustus ja endoteeli düsfunktsioon. Üheks võimsamaks endogeenseks kaitsemehhanismiks IR kahjustuse vastu on isheemiline eelkohastumus e enne potentsiaalselt letaalset isheemilist episoodi läbiviidavad lühiaegsed isheemia ja reperfusiooni episoodid.

Loomkatsetes on tõestatud, et isheemia-eelsel hüperoksilise gaasisegu hingamisel on eelkohastumusega sarnane südant kaitsev toime, mille tulemusena väheneb infarktikolde suurus ja paraneb müokardi kontraktiilse funktsiooni taastumine. Hüperoksilise ekspositsiooni käigus tekkivad hapniku reaktiivsed osakesed toimivad rakusiseseid antioksüdantsüsteeme käivitavate signaalmolekulidena. Sellest tulenevalt on rakkudel „valmisolek" toimetulekuks reperfusioonil vabaneva hapniku reaktiivsete osakeste tulvaga ning tekkiv koekahjustus on väiksem.

Erinevalt paljudest teistest eelkohastumise mudelitest oleks hüperoksilise gaasisegu hingamine kliinilises praktikas kergesti rakendatav. Siiani ei ole aga teada, kas see loomkatsetes kaitsvat toimet omav meetod aitab vähendada ka inimsüdame kardiopleegiajärgset IR kahjustust.

Uurimuse eesmärgid

Uurimistöö peamiseks eesmärgiks oli kindlaks teha, kas katseloomadel efektiivseks osutunud hüperoksiline eelkohastumus omab kaitsvat toimet ka inimorganismis.

Probleemi lahendamiseks püstitasime täpsemad ülesanded:

1. välja töötada kliinilises praktikas kasutatav mudel müokardi kahjustuse hindamiseks kardiokirurgias;

2. väljatöötatud mudeli alusel hinnata hüperoksilise eelkohastumise mõju IR kahjustusele kehavälise vereringega läbiviidavatel aortokoronaarse šunteerimise operatsioonidel;
3. väljatöötatud mudeli alusel hinnata hüperoksilise eelkohastumise mõju IR kahjustusele ilma kehavälise vereringeta (töötaval südamel) läbiviidavatel aortokoronaarse šunteerimise operatsioonidel;
4. hinnata asümmeetrilise dimetüülarginiini (ADMA) kontsetratsiooni muutusi aortokoronaarse šunteerimise ajal ning hüperoksia mõju ADMA kontsentratsiooni muutustele.

Patsiendid ja metoodika

Uuritavateks patsientideks olid Põhja-Eesti Regionaalhaigla kardiotorakaalkirurgia keskusesse plaaniliseks aorto-koronaarseks šunteerimiseks hospitaliseeritud täiskasvanud patsiendid. Uuringusse arvamise kriteeriumiteks olid: (1) esmane, isoleeritud aortokoraarse šunteerimise operatsioon; (2) kirurgi poolt planeeritud vähemalt kolm distaalset anastomoosi; (3) kaasuvate haiguste puudumine; (4) ehhokardiograafiliselt hinnatud südame väljutusfraktsioon > 40%. Uuringusse ei arvatud hiljutise (st alla kahe nädala vanuse) müokardi infarkti ja operatsioonieelselt tõusnud troponiin I/T või kreatiin kinaasi-MB fraktsiooni väärtustega patsiente.

Aortokoronaarne šunteerimine viidi läbi kas kehavälise vereringe kasutamisega (publikatsioonid I, II, IV) või ilma (publikatsioon III).

Peale anesteesia induktsiooni ja trahhea intubeerimist randomiseeriti patsiendid kas kontrollgruppi (40% O_2 sissehingatavas gaasisegus) või uuringugruppi (> 96% O_2 sissehingatavas gaasisegus). Vastavat gaasisegu kasutati juhitava hingamise läbiviimiseks kuni kehavälise vereringe (publikatsioonid II, IV) või esimese koronaararteri šunteerimise alustamiseni (publikatsioon III). Seejärel hoiti mõlema grupi patsientide arteriaalse vere hapniku osarõhk vahemikus 110–150 mmHg.

Vereanalüüsid laktaadi, troponiin I, kreatiinkinaas-MB fraktsiooni, oksüdeeritud ja redutseeritud glutatiooni, interleukiin-6 ja ADMA määramiseks koguti nii koronaarsiinuse kui ka arteriaalsest verest enne operatsiooni põhietapi algust ning korduvalt reperfusiooni vältel. Biokeemiliste markerite määramiseks kasutati standardseid meetodeid. Sama ajavahemiku jooksul määrati ka südame funktsiooni iseloomustavad parameetrid kasutades kopsuarteri kateetrit ja termodilutsiooni tehnikat.

Andmetöötlusel kasutati programmi Statistica 6 (tootja: StatSoft Inc.)

Uurimuse peamised tulemused ja järeldused

1. Väljatöötatud mudeli alusel on võimalik hinnata müokardi kahjustuse ulatust nii kehavälise vereringega kui ka ilma selleta läbiviidavate aorto-koronaarse šunteerimise operatsioonide korral. Selleks analüüsitakse koronaarsiinuse ja arteriaalsest verest saadud müokardi nekroosi, oksüdatiivse stressi ja põletikureaktsiooni iseloomustavaid biomarkereid kompleksselt koos südame funktsiooni iseloomustavate andmetega. Selgus, et hoolimata märkimisväärsest südamelihase nekroosile vihjavast biomarkerite vabanemisest ei olnud südame jõudlus varases operatsioonijärgnes perioodis langenud. Biomarkerite vabanemise ja operatsioonijärgse südamefunktsiooni vahel puudus korrelatsioon.
2. Hüperoksilise gaasisegu (>96% hapnikku) hingamine enne kehavälist vereringet ei vähendanud kardiopleegiaga kaasnevat IR kahjustust. Samas võib hüperoksia omada potentsiaalselt kasulikke toimeid, näiteks vähenenud interleukiin-6 vabanemine müokardist.
3. Aorto-koronaarne šunteerimine töötaval südamel põhjustas vähese biomarkerite vabanemise südamelihasest koheselt protseduuri algfaasis. Selle võimalikeks põhjusteks on korduv südame asendi muutmine ning operatsiooni käigus tekkiv kudede trauma. Ebasoodsaid metaboolseid tingimusi kinnitab ka laktaadi vabanemine protseduuri ajal ning selle kohene vähenemine anastomooside valmimisel. Protseduurieelne hüperoksilise gaasisegu hingamine ei vähendanud ka selles patsientide rühmas müokardi kahjustust. Samas põhjustas hüperoksia olulist interleukiin-6 langust esimesel operatsioonijärgsel hommikul, mida võib käsitleda kui vähenenud organismi põletikureaktsiooni.
4. Aorto-koronaarne šunteerimine kehavälise vereringe tingimustes põhjustas ADMA kontsentratsiooni tõusu korornaarsiinuse veres juba varases reperfusioonifaasis, mis hüperoksia grupis oli seotud aordi klemmimise (st totaalse müokardi isheemia) ajaga. Selle põhjuseks võib olla pikemast isheemia ajast tulenev suurenenud endoteeli kahjustus reperfusioonil, mida võimendas veelgi hüperoksiast põhjustatud hapniku reaktiivsete osakeste vabanemine. Esimeseks operatsioonijärgseks hommikuks oli ADMA kontsentratsioon oluliselt langenud, mida võiks käsitleda suurenenud lämmastikoksiidi vabanemisele suunatud adaptatiivse mehhanismina.

Töö kokkuvõtteks järeldame, et ca 120 minutit kestev hüperoksia periood ei vähendanud aorto-koronaarse šunteerimisega kaasnevat müokardi isheemia-reperfusiooni kahjustust, kuid põhjustas muutusi interleukiin-6 kontsentratsioonis, mida võib käsitleda põletikureaktsiooni vähenemisena.

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11. PUBLICATIONS

I

Karu I, Loit R, Paapstel A, Kairane C, Zilmer M, Starkopf J.
Early postoperative function of the heart after coronary artery bypass grafting is not predicted by myocardial necrosis and glutathione-associated oxidative stress.
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II

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III

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IMPACT OF HYPEROXIA BEFORE OFF-PUMP CORONARY SURGERY ON MYOCARDIAL INJURY

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Abstract

Objectives: Off-pump coronary surgery does not eliminate the risks of ischemia-reperfusion injury. In the present study the hypothesis, that hyperoxia before grafting procedure protects human myocardium against necrosis and stunning as demonstrated in previous animal experiments, was tested.

Design: Twenty three patients were ventilated prior OPCAB with FiO_2 0.4 or >0.96. Blood for troponin I, creatine kinase MB, lactate, glutathione and interleukin-6 was sampled from arterial and coronary sinus cannulae.

Results*:* Release of creatinine kinase-MB, troponin I and lactate into coronary sinus was evident already after the completion of the 1st graft. By the 1st postoperative morning no differences in cTnI levels were observed (2.6 (1.7, 3.8) in the control vs. 2.7 (2.4, 5.1) in the hyperoxia group), but the patients pre-treated with hyperoxia had significantly lower arterial levels of IL-6 (162.0 (138.0, 223.0) vs. 202.0 (188.0, 246.0) pg/mL, p=0.046).

Conclusions: Cardioprotective effect of hyperoxic pre-treatment could not be demonstrated in setting of OPCAB surgery. Hyperoxia diminished systemic IL-6 levels. During OPCAB surgery minor myocardial necrosis was evident already after completion of the 1st graft.

Keywords
OPCAB, hyperoxia, preconditioning, troponin I, myocardial function, creatinine kinase-MB, glutathione

Introduction

Off- pump coronary artery bypass grafting (OPCAB) is gaining wide acceptance as it is associated with less myocardial enzyme release up to 24 postoperative hours, less early neurocognitive dysfunction, less renal insufficiency, less blood loss and need for transfusion when compared to conventional coronary artery bypass grafting (CABG) (1). Despite of these advantages, OPCAB surgery does not eliminate the risks completely, as even short ischaemic periods during the grafting procedure on the beating heart possess a risk of myocardial necrosis, stunning and vascular endothelial injury (2–4).

Due to cardiovascular compromise patients coming to the cardiac procedures often need inspiratory fractions of oxygen higher than usual. Although long-lasting hyperoxia is injurious, we and others have previously demonstrated in an experimental setting that a short exposure to hyperoxia induces a low-grade systemic oxidative stress and elicits myocardial protection analogous to ischaemic preconditioning (5–7). So far the effect of hyperoxia has been investigated on rat and mouse hearts, where minimal inspired fraction of oxygen needed to reduce the infarct size was 80% (8).

In cardiac surgery, the extent of myocardial injury is usually evaluated by arterial levels of cardiac markers, most often during early postoperative hours/ days. However, whether the release of cardiac biomarkers occurs already during the off-pump grafting procedure, is not so well described.

The main objective of this study was to evaluate the effect of pre-ischaemic hyperoxia upon the myocardial injury in patients undergoing OPCAB surgery. Arterial troponin I (cTn I) level in the first postoperative morning was set as the primary endpoint of the study. For detailed characterisation of the ischemia-reperfusion (IR) injury the coronary sinus levels of markers of necrosis, oxidative stress and inflammation were additionally assessed during the grafting procedure.

Material and methods

Patients

The study design was approved by the Ethics Review Committee on Human Research of the University of Tartu and written informed consent was signed by all patients. Twenty four adult patients scheduled for primary elective aorto-coronary bypass grafting on the working heart with at least 3 distal anastomoses were included, and randomised into the control (n=12) and hyperoxia (n=12) groups. Exclusion criteria included preoperative ejection fraction of the heart (evaluated by echocardiography) below 40%, unstable angina or elevated cTn I/cTn T or creatine kinase MB-isonezyme (CK-MB), type I or II diabetes mellitus, and hepatic, renal or pulmonary disease. All medications except salicylates were allowed up to the morning of the operation.

Study protocol

All patients were ventilated with 100% oxygen for a few minutes before and during induction of anaesthesia. After intubation of the trachea patients were allocated to ventilation with either 40% (control group) or >96% (hyperoxia group) oxygen according to the randomisation protocol based on the method of permuted blocks. From the beginning of the grafting until to the end of operation mixture of oxygen and air was adjusted to obtain PaO_2 levels between 110 and 140 mmHg in both groups. Arterial blood gases were analysed (Radiometer ABL 700 series, Ratiometer Medical A/S, Denmark) 15 minutes after randomisation, verified again before beginning of the grafting procedure and thereafter during the operation as decided by the anaesthesiologist.

Anaesthesia and operative procedure

Standardised anaesthetic technique with fentanyl, midazolam and pancuronium was used in all cases. Due to additional preconditioning effect (9) volatile anaesthetics were avoided. To avoid vasospasm of the radial artery graft (used in all patients) intravenous nitroglycerin (1–2 mg/min) was started in the beginning of the procedure. To correct the haemodynamic responses during the procedure, metoprolol was administered or dosage of nitrogycerin adjusted as needed. After midline sternotomy and opening the pericardium a manually inflatable 15 Fr coronary sinus cannula (Medtronic Inc., Minneapolis, USA) was introduced through the right atrial wall into the coronary sinus for blood sampling. Pericardial traction sutures and elevating gauze pads were used to facilitate visibility and access to the left and right sides of the heart during the grafting procedure. The Octopus heart stabiliser system (Medtronic, Minneapolis, Minnesota, USA) was used for myocardial stabilisation. In case of blood leakage occurred, a carbon dioxide blower was used. Intracoronary shunt (Medtronic Inc., Minneapolis, USA) was inserted immediately after opening the coronary artery in all cases. The left anterior descending artery was always grafted first with the left mammary artery. Additional bypasses were performed using a radial artery and saphenous vein grafts in the sequence as decided by the surgeon. In case of haemodynamic detoriation, the right pericardium was opened to the pleural cavity and intravenous boluses of phenylephrine together with infusions were given. Proximal anastomoses were performed to the ascending aorta. Two experienced surgeons performed all operations. No conversion to cardiopulmonary bypass was needed.

Total grafting time was counted from opening the first coronary artery (left anterior descending artery in all cases) for grafting until releasing the partial aortic cross-clamp after completion of the last proximal anastomosis.

In the postoperative period pulmonary capillary wedge pressure was kept over 8 mmHg with infusion of crystalloid and colloid solutions.

Biochemical markers

Blood samples for lactate cTn I, CK-MB mass and oxidised (GSSG) and reduced glutathione (GSH) were collected serially from the coronary sinus (CS) and radial artery immediately after insertion of the coronary sinus cannula (baseline), 1 minute after restoration of blood flow to each grafted artery, and at 5, 10 and 20 minutes after completion of the last anastomosis. Blood for interleukin-6 (IL-6) was sampled by the same methodology at baseline and 5th and 20th reperfusion minutes. The balloon of the CS cannula was manually inflated at these time points to get blood exclusively from the CS. Additional arterial samples for all markers were drawn 60 minutes after completion of the last anastomosis and in the morning of the 1st postoperative day. Blood was centrifuged immediately after sampling and serum stored at -80° C until analyses. To remove protein for glutathione measurements, one aliquot of plasma was mixed with metaphosphoric acid in equal proportions, stored at +4° C for 15 min and centrifuged again. Protein free supernatant was stored at -80° C until further analysis.

Lactate was measured photometrically on Konelab 60i (Thermo Electron Corp., Finland), CK-MB and cTn I by using chemiluminescent immunoassay on Bayer ACS:180 analyser (Bayer Corp., Tarrytown, NY, USA).

For measurement of total amount of glutathione and GSSG the sample was divided into two parts and previously described (10) enzymatic method was used. The concentration of GSH was calculated as the difference between total amount of glutathione and GSSG, and the glutathione redox ratio (GSSG/GSH) as μmol GSSG/ μmol GSH.

IL-6 was determined by the quantitative sandwich enzyme immunoassay technique (Human IL-6 Immunoassay kit, R&D Systems, Inc., Minneapolis, USA).

Myocardial release of biochemical markers is expressed as a difference between coronary sinus and arterial concentrations, thus positive values indicate myocardial release of these substances.

Haemodynamic measurements

Heart rate, mean arterial, pulmonary artery and pulmonary capillary wedge pressures and cardiac output were recorded with thermodilution pulmonary artery catheter at baseline (before sternotomy, 15–20 minutes after randomisation to either control or hyperoxia groups), and 15 minutes, 1, 2, 4, 6, 9 and 12 hours after completion of the grafting procedure. Cardiac index (CI), right and left ventricular stroke work indices (RVSWI, LVSWI) and pulmonary vascular resistance index (PVRI) were calculated using standard formulas.

Statistical analysis

For sample size determination, we assumed the standard deviation of arterial cTn I release in the 1st postoperative morning to be 2 ng/mL, difference between

groups 2.5 ng/mL, alpha error of 0.05 and and power of 80%. This results in a sample size of 11 per group to be sufficient to show the significant difference.

Patient data were analysed using Student's t-test or Fisher's exact test as appropriate. According to Shapiro-Wilk test the biochemical data were not distributed normally. The data from baseline up to the 20th reperfusion minute were analysed with non-parametric ANOVA, followed by multiple comparisons with Mann-Whitney U-test corrected according to the Bonferroni procedure and presented as median with upper and lower quartiles. As not all patients needed to have 4 grafts, this time point was left out from the statistical analysis. Tn I and CK-MB values at the 60th reperfusion minute and in the 1st postoperative morning were compared with Mann-Whitney U-test. As the haemodynamic data showed normal distribution, these were analysed with ANOVA for repeated measures and represented as mean±SD. In case of significant p-values were calculated, multiple comparisons were performed with Tukey test for unequal N (difference between hyperoxia and control groups) or Dunnet test (difference between baseline and the following time-points). Correlation is expressed as Spearman rank order correlation. Significance was assumed with $p<0.05$.

Results

Demographic data

Patients' demographic data are presented in the Table 1. One patient, who was assigned to the control group, refused to participate in the study just before induction of anaesthesia. Thus data of 11 patients in the control group and 12 patients in the hyperoxia group were analysed. The groups had similar characteristics regarding age of the patients, preoperative cardiac function and operative data. The average time of exposure to hyperoxia was 126±25 min (time from intubation up to the grafting of left anterior descending artery, Table 1), which does not differ from the 40% oxygen exposure (128±31 min) in the control group. The time gap between unclamping the aorta and taking arterial blood samples in the 1st postoperative morning was 20 h and 12 min (37 min) in the control group and 19 h 57 min (34 min) in the hyperoxia group ($p=0.59$).

In the control group 5 patients (out of 11), and in the hyperoxia group 7 patients (out of 12) had 4 distal anastomoses, in all other cases 3 coronary arteries were bypassed. One patient in the control group needed inotropic support with Dobutamine 5 μg/kg min and Norepinephrine 5 μg/min during the first postoperative night, but no myocardial infarction-specific changes were found on the ECG.

Table 1. Patient characteristics, oxygenation and surgical data. Data are given as mean±SD.

Variable	Control (n=11)	Hyperoxia-pretreated (n=12)	p-value
Age (years)	67±6	63±7	0.12
Gender (male / female)	10 / 1	12 / 0	
Triple vessel disease (n)	10	10	
Left main stem stenosis > 50% (n)	1	2	
Preoperative ejection fraction (%)	57±8	57±10	0.86
Preoperative medications			
Ca-channel blockers, n (%)	4 (36)	5 (42)	1
Nitrates, n (%)	8 (73)	8 (67)	0.73
β-blockers, n (%)	9 (82)	8 (67)	1
ACE-inhibitors, n (%)	2 (18)	5 (50)	0.42
Statins, n (%)	7 (64)	3 (25)	0.28
p_aO_2 15 min after intubation (mmHg)	106.6±32.9	300.9±93.6	<0.001
p_aO_2 before 1st distal (LAD) anastomosis (mmHg)	115.1±17.4	314.0±68.4	<0.001
Time from intubation until LAD grafting (min)	128±31	126±25	0.85
Total time of the grafting procedure (min)	135±36	152±53	0.38
Vessels bypassed	4±1	4±1	0.80
Need for inotropic support (n)	1	0	

LAD – left anterior descending artery

cTnI and CK-MB mass

The baseline levels of cTn I and CK-MB mass were within reference limits and did not differ between groups. Both markers appeared into the coronary sinus already after the 1st distal anastomosis and were gradually increased over the whole study period (Fig 1, A B). During the grafting procedure and the 20 minute reperfusion period more cTn I was released in the hyperoxia group ($p<0.01$, Fig. 1, B), whereas release of CK-MB showed no difference between groups.

60 min after completion of grafts the arterial values of cTn I were still higher in the hyperoxia group ($p=0.031$), but by the 1st postoperative morning this difference was not evident any more. Arterial values for CK-MB did not differ between groups during the study period (Fig 1, C D).

Lactate

Myocardial release of lactate increased in time during during the grafting procedure, showed maximal values after the 3rd anastomosis and diminished quickly when all the anastomoses were ready and the heart was not handled any more (Fig 2).

Oxidised and reduced glutathione
Arterial and coronary sinus values of GSSG and GSH did not differ between groups neither immediately after the hyperoxic pre-treatment nor during the whole study period. The glutathione redox ratio in the coronary sinus blood, a sensitive index of oxidative stress, did not differ between groups (maximal value in the control group was in the 5^{th} min of reperfusion (0.11 (0.016, 0.15) and in the hyperoxia group in the 10^{th} min of reperfusion (0.59 (0.023, 0.23)).

Interleukin-6
Coronary sinus and arterial values did not differ between groups before grafting. In both groups the CS blood levels of IL-6 exceeded the arterial values in 5^{th} and 20^{th} reperfusion minutes (Fig 3, A).In the arterial blood IL-6 started to increase from the 5^{th} postgrafting minute, reaching its maximal value by the 1^{st} postoperative morning (Fig 3, B). By this time maximal difference between groups was observed, with the level of 202.0 (188.0, 246.0) pg/mL in the control and 162.0 (138.0, 223.0) pg/mL in the hyperoxia group ($p<0.046$ between groups).

Haemodynamic measurements
All patients had CI more than 2.2 L m^2 min as a baseline value. CI did not change significantly after the grafting procedure except at the 2 h time point ($p=0.018$ in comparison with the initial value in the hyperoxia group; Fig 4, A). LVSWI decreased in the immediate postgrafting period in both groups, but only in the control group the decrease was statistically significant as compared to the baseline (Fig 4, B). RVSWI (Fig. 4, C) and PVRI were not significantly altered during the study period.

Heart rate did not differ between groups during the whole study period.

Effects of duration of the grafting procedure
The duration of the grafting procedure influenced both arterial and coronary sinus blood concentrations of IL-6. The significant relationships were observed during the whole reperfusion period, but not in the 1^{st} postoperative morning any more. The relationship was most clear-cut in the CS blood in 5^{th} ($r=0.59$, $p=0.006$) and 20^{th} minutes ($r=0.58$, $p=0.01$) of reperfusion.

Duration of the total grafting time had also an impact on the immediate release of CK-MB (10^{th} reperfusion minute $r=-0.74$, $p=0.04$; 20^{th} reperfusion minute $r=-0.73$, $p=0.04$).

Discussion

The present study demonstrates that release of cardiac markers into the coronary sinus starts already after completion of the first graft, even before the heart is extensively handled for the grafting procedure. The proposed cardioprotective effect of hyperoxia could not be demonstrated in this study setting. OPCAB is associated with the release of IL-6 from the heart and pre-grafting exposure to hyperoxia decreases systemic levels IL-6 in the early postoperative period.

The preconditioning-like effect of hyperoxia has been repeatedly demonstrated in the animal experiments (6–7,11–12). In the current, to the best of our knowledge, first clinical study, the effect of hyperoxia appeared controversial. In one hand, somewhat higher release of cTnI to coronary sinus was observed, but at the same time, better postoperative heart function, although unimportant from the clinical point of view, was seen in the hyperoxia pre-treated patients. Some of the reasons, why protective effect of hyperoxia could not be demonstrated, might be first, the difference among species, and second, the inappropriate exposure time. Studies on time-response curve of hyperoxic treatment are lacking in humans. Finally, and most importantly, to demonstrate the efficacy of an intervention aimed to protect the heart from the ischaemia-reperfusion, certain degree of injury in the control arm is required. From that point of view it is hard to believe that any myocardial protection could have been verified in the present study as the control group had only a minor myocardial damage. Operations on the working heart have been believed to offer an alternative model for studies of preconditioning in humans, although so far conflicting results have been reported (3,13). These divergent results have led to the hypothesis that in the setting of coronary artery bypass surgery, the additional protection conferred by preconditioning may only be demonstrable where a potential for suboptimal myocardial protection increases the risk of perioperative infarction (14).

One of the pitfalls of the present study was underestimation of the variance of the cTn I release in the 1st postoperative morning. Only control group showed the assumed standard deviation of 2 ng/mL, whereas in the hyperoxia group data were much more scattered and therefore more patients needs to be included in further studies.

Working heart has a limited tolerance to ischaemia during normothermic conditions, and that possesses a risk for myocardial damage in case of even short ischaemic times. Lifting and turning the heart for optimal operating conditions may further lead to temporary and unfavourable blood flow redistribution. Although single episodes of disturbed coronary flow are as a rule of a very short duration, the total ischaemic burden may have a significant effect. It has been reported that prolonged target vessel occlusion time during the OPCAB procedure is associated with increased arterial cTn I and CK-MB levels and impaired contractile function (3). To limit coronary occlusion time

and to reduce the incidence of myocardial infarction, intracoronary shunts have been suggested (15,16). Despite of using shunts in all cases, we observed unfavourable metabolic conditions in the myocardium as shown by the release of lactate after completion of each graft. Although, glutathione-associated oxidative stress was not evident as shown by the values of GSSG and GSH in the coronary sinus blood. Some degree of myocardial cell injury still occurred, as evidenced by the release of cTn I and CK-MB. However, the extent of markers released was negligible and remained well below the cut-off limit for perioperative myocardial infarction (17).

In response to surgery, unspecific inflammatory response occurs and increased production of cytokines, including IL-6, is evident. Studies during conventional CABG have described a good correlation between the length of cardiopulmonary bypass and the degree of myocardial damage and serum IL levels (18). Myocardium as a source of IL-6 has been described after cardiac surgery with cardiopulmonary bypass (19) and coronary stenting for stable angina (20). OPCAB surgery can now be added to this line of research, indicating that even minor myocardial injury during the procedure is sufficient to activate the stress response system. The correlation between CK-MB, and particularly IL-6 values and the total grafting time further supports this hypothesis. Despite of limited number of patients, in our study hyperoxia somewhat modified the inflammatory response. Although it did not modify the levels of IL-6 observed in the coronary sinus blood, reduction of systemic IL-6 may suggest some possibly beneficial effects of hyperoxia. The importance and underlying mechanisms of reduced systemic IL-6 production remain to be elucidated in the further studies.

In conclusion, in this study we demonstrated that off-pump coronary surgery was associated with a minor release of cardiac markers already after completion of the 1st graft. OPCAB was associated with production of IL-6 in the heart and pre-treatment by hyperoxia diminished the systemic IL-6 production. Cardio-protective effect of hyperoxia could not be demonstrated in this setting of patients.

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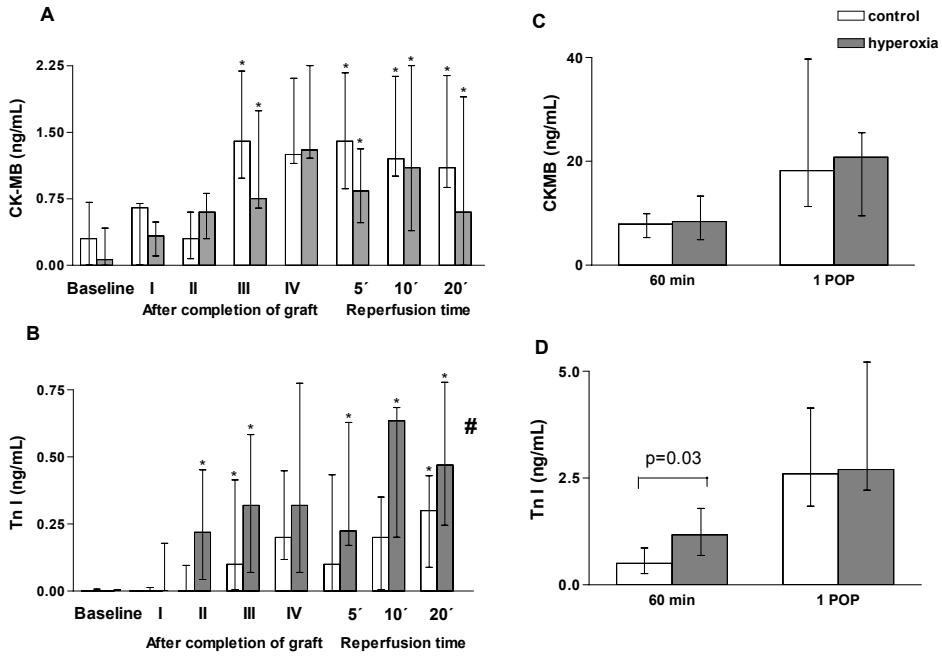

Figure 1. Myocardial release (expressed as difference between coronary sinus and arterial concentrations) of creatine kinase MB isoenzyme (panel A) and troponin I (panel B) during the grafting procedure and early reperfusion. Arterial values of CK-MB (panel C) and Tn I (panel D) 60 min after finishing the grafting procedure and in the 1st postoperative morning. Values are given as median with upper and lower quartiles. * $p<0.01$ in comparison with baseline, # $p<0.01$ between hyperoxia and control groups.

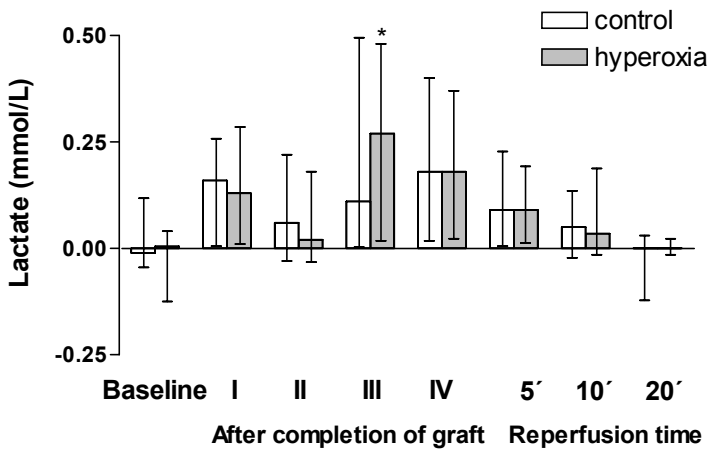

Figure 2. Myocardial release (expressed as difference between coronary sinus and arterial concentrations) of lactate. Values are given as median with upper and lower quartiles. * $p<0.01$ in comparison with baseline.

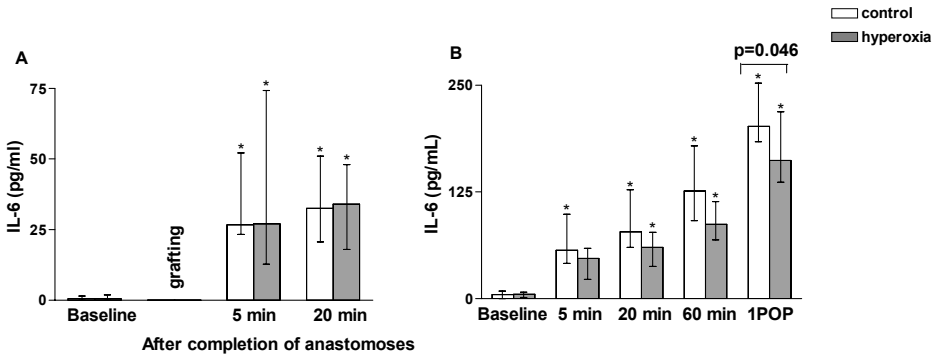

Figure 3. Difference between coronary sinus and arterial concentrations (panel A) and arterial values (panel B) of IL-6. Values are given as median with upper and lower quartiles. *p=0.01 in comparison with baseline.

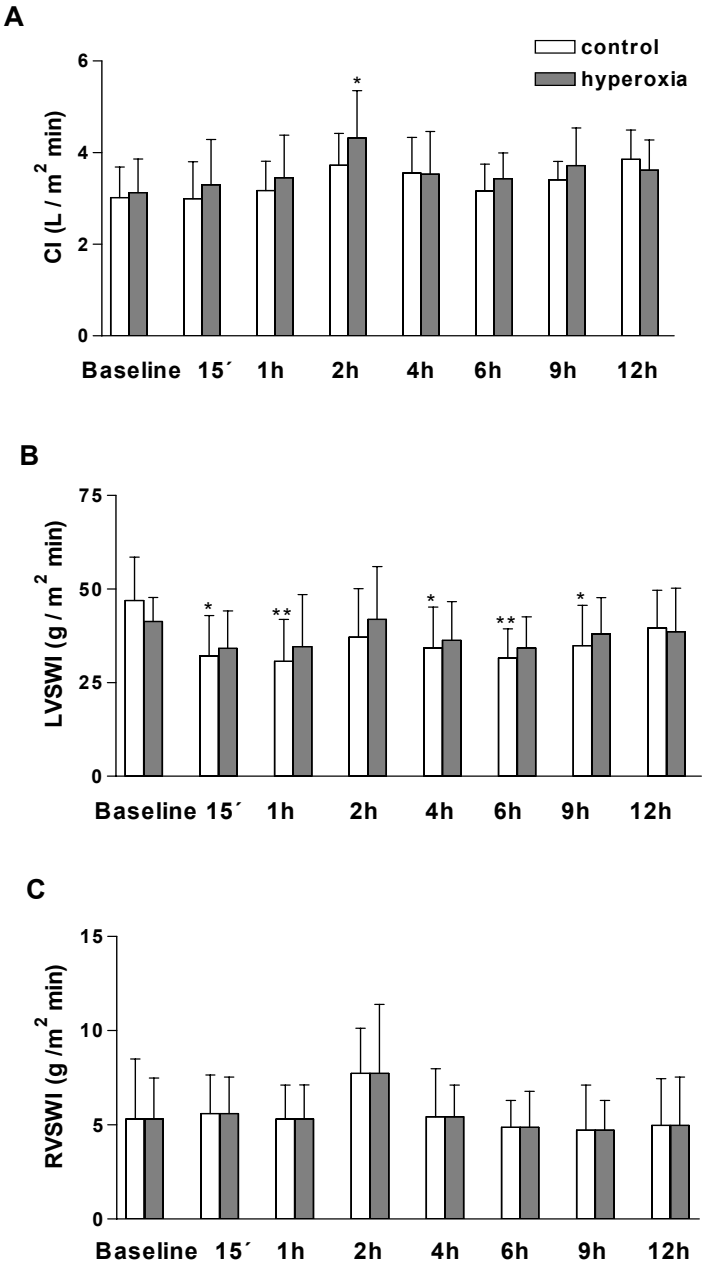

Figure 4. Changes in myocardial function before grafting and in the early postoperative period. Values are given as mean (SD). * $p<0.05$, ** $p<0.01$ in comparison with baseline. CI – cardiac index; RVSWI, LVSWI – right and left ventricular stroke work indices.

IV

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1995–1997	Anestesioloogia- ja intensiivravi internatuur, Tartu
2002–2007	Tartu Ülikool, doktoriõppe üliõpilane

Erialane enesetäiendus

1997– 1998	Lundi Ülikooli Haigla, Rootsi Külalisteadur Lundi Ülikooli Lasermeditsiini keskuses
Nov. 1999	Salzburgi keskhaigla, Austria
Märts 2001	Viini Ülikooli keskhaigla, Austria
Aprill 2002	Hartfordi Haigla, Hartford CT, USA

Erialane teenistuskäik

1996–1997	Tartu Ülikooli Kopsukliiniku intensiivravi osakond, arst
1997–	Põhja-Eesti Regionalhaigla, Anestesioloogia Kliinik, anestesioloog

Teadustegevus

Põhiliseks uurimisvaldakonnaks on südamelihase isheemia-reperfusiooni kahjustus ning selle vähendamise võimalused hüperoksilise eelkohastumise abil.

6 teaduslikku publikatsiooni, 8 ettekannet rahvusvahelistel konverentsidel.

Eesti Anestesioloogide Seltsi ja Euroopa Kardiotorakaalanestesioloogide Seltsi liige.

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