

Juha Koskenkari

MYOCARDIAL ISCHEMIA-
REPERFUSION INJURY AND
SYSTEMIC INFLAMMATORY
RESPONSE IN HIGH-RISK
CARDIAC SURGERY

*A CLINICAL STUDY OF THE EFFECTS OF HIGH-DOSE
GLUCOSE-INSULIN TREATMENT AND THE USE OF
LEUKOCYTE-DEPLETING FILTER*

FACULTY OF MEDICINE,
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NATIONAL PUBLIC HEALTH INSTITUTE, OULU

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JUHA KOSKENKARI

**MYOCARDIAL ISCHEMIA-REPERFUSION
INJURY AND SYSTEMIC
INFLAMMATORY RESPONSE IN HIGH-
RISK CARDIAC SURGERY**

A clinical study of the effects of high-dose glucose-insulin treatment and the use of leukocyte-depleting filter

Academic dissertation to be presented, with the assent of the Faculty of Medicine of the University of Oulu, for public defence in Auditorium I of Oulu University Hospital, on October 13th, 2006, at 12 noon

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Abstract

Cardiac surgery with cardiopulmonary bypass induces the activation of systemic inflammatory response syndrome (SIRS) and results in at least some degree of global myocardial ischemia. Although these responses are usually short-lived, they may lead to serious complications and organ system failures.

The present study evaluated the effects of high-dose glucose-insulin (1IU/kg/h) treatment (GIK) administered with the hyperinsulinemic normoglycemic clamp technique and a leukocyte-depleting filter on markers of systemic inflammatory response and myocardial ischemia-reperfusion injury in certain cardiac surgical risk groups.

The study involved four prospective randomized controlled clinical trials and 119 patients. Cardioprotective effects were measured as myocardial enzyme release, recovery of contractile function and incidence of arrhythmias in all studies. The hemodynamic and metabolic effects of high-dose glucose-insulin treatment were evaluated in patients admitted for combined aortic valve (AS) and coronary surgery (40) and for urgent coronary surgery (39), and the latter study also involved proinflammatory cytokine and C-reactive protein analyses. The impacts of leukocyte filter on the expression of neutrophil adhesion molecules along with proinflammatory cytokines were evaluated in patients admitted for combined aortic valve (AS) and coronary surgery (20) and for solitary coronary surgery (20).

The high-dose glucose-insulin treatment was associated with better preserved myocardial contractile function and less need for inotropic support after combined aortic valve and coronary surgery (I) and attenuation of postoperative CRP release after urgent coronary surgery (II). No effects on postoperative myocardial enzyme release (I, II) or on proinflammatory cytokine responses (II) were detected. The number of hypoglycemic events was low. The use of a leukocyte filter throughout the cardiopulmonary bypass period increased the neutrophil adhesion molecule CD11b expression in patients with both normal and prolonged CPB times and was associated with an enhanced proinflammatory cytokine response (III, IV).

In conclusion, high-dose glucose-insulin treatment is safe, but requires strict control of blood glucose level. It reduces the need for inotropic support in patients with compromised cardiac status. The use of leukocyte filter leads to increased leukocyte activation and proinflammatory reaction.

Keywords: cardiopulmonary bypass, glucose, insulin, leukocyte reduction procedures, myocardial reperfusion injury, systemic inflammatory response, thoracic surgery

To Päivi, Ella and Eetu

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Juha Koskenkari

Abbreviations

AC	Aortic cross-clamp
AS	Aortic stenosis
AUC	Area under curve
CABG	Coronary artery bypass grafting
CPB	Cardiopulmonary bypass
CRP	C-reactive protein
ECG	Electrocardiogram
FFA	Free fatty acid
GIK	Glucose-insulin-potassium
IL-8	Interleukin-8
IL-6	Interleukin-6
NO	Nitric oxide
SD	Standard deviation
SIRS	Systemic inflammatory response
SVRI	Systemic vascular resistance index
TnI	Cardiac troponin-I

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

- I Koskenkari JK, Kaukoranta PK, Kiviluoma KT, Raatikainen MJ, Ohtonen PP & Ala-Kokko TI (2005) Metabolic and hemodynamic effects of high-dose insulin treatment in aortic valve and coronary surgery. *Ann Thorac Surg* 80: 511-517.
- II Koskenkari JK, Kaukoranta PK, Rimpiläinen J, Vainionpää V, Ohtonen PP, Surcel H-M, Juvonen T & Ala-Kokko TI (2006) Anti-inflammatory effect of high-dose insulin treatment after urgent coronary revascularization operation. *Acta Anaest Scand* 50: 962-969.
- III Koskenkari J, Rimpiläinen J, Biancari F, Surcel H-M, Kaukoranta P, Kiviluoma K, Ala-Kokko T & Juvonen T (2005) Leukocyte depleting filter attenuates myocardial injury during elective coronary artery bypass surgery. *Scand Cardiovasc J* 39: 358-368.
- IV Koskenkari JK, Rimpiläinen J, Öhman H, Surcel H-M, Vainionpää V, Biancari F, Ala-Kokko TI & Juvonen T (2006) Leukocyte filter enhances neutrophil activation in combined aortic valve and coronary surgery. *Heart Surg Forum* 9: 693-699.

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1 Introduction

Although mortality associated with cardiac surgery is rare, different postoperative organ system failures are common and often lead to complicated recovery (Estafanous *et al.* 1998). Cardiac surgery with cardiopulmonary bypass (CPB) induces activation of systemic inflammatory response syndrome (SIRS), which was first described by Kirklin *et al.* as “postperfusion syndrome” (Kirklin *et al.* 1983). This complex series of events consists of humoral, cellular and hemostatic factors and has several similarities to sepsis. Complement and cytokine cascade, coagulation and fibrinolysis, neutrophil and platelet activation along with the expression of adhesion molecules and widespread endothelial activation have been established as central mechanisms mediating the effects of SIRS.

Furthermore, despite cardioplegic protection, aortic cross-clamping inevitably results in to some degree of myocardial ischemia. Restoration of normal circulation may lead to reperfusion injury, which manifests clinically as contractile dysfunction (stunning), reperfusion arrhythmias or, ultimately, myocardial cell death (Heyndricks *et al.* 1975, Braunwlad & Kloner 1982, Moens *et al.* 2005). Ischemia-reperfusion injury results from several interdependent mechanisms, namely oxidative stress, intracellular calcium overload and hypercontracture, endothelial cell activation with microvascular dysfunction and altered myocardial metabolism (Verma *et al.* 2002, Anselmi *et al.* 2004). Although these responses are usually short-lived, they may lead to serious complications and organ system failures, particularly in certain subgroups of patients at risk.

Nowadays, cardiac surgical teams are challenged with an increasing number of patients with compromised left ventricular function, unstable angina pectoris or advanced age and patients admitted for complex cardiac surgery (Harz *et al.* 2000, Gaudino *et al.* 2004). Preceding ongoing ischemia makes the myocardium particularly vulnerable to the consequences of cardioplegic arrest (Bonacchi *et al.* 2001), and a prolonged CPB time is assumed to be one of the central determinants of the intensity of SIRS (Roth-Isigkeit *et al.* 2000). Although the links between perioperative inflammation and myocardial reperfusion injury induced organ dysfunctions are largely unclear, various pharmacological and mechanical therapeutic strategies to attenuate these effects have been evaluated, ranging from complete avoidance of CPB (Penttilä *et al.* 2001) to the use of mechanical techniques such as biocompatible circuits (De Somer *et al.* 2000) and pharmacologic agents (Gott *et al.* 1998, Schmartz *et al.* 2003).

Insulin with glucose and potassium (GIK) has usually been used as an adjuvant therapy in cardiac surgery, mainly because of its effects on myocardial metabolic disorder and oxygen consumption during and after ischemia (Cave *et al.* 2000). In clinical and experimental studies, insulin has also been demonstrated to have inotropic (Doenst *et al.* 1999), vasodilatory (Sundell *et al.* 2002), antiapoptotic (Jonassen *et al.* 2000) and anti-inflammatory properties (Jeschke *et al.* 2004). However, the exact mechanisms of action and the optimal dosage of both glucose and insulin in cardiac surgical patients are largely unclear, and the methods of application have varied. High-dose GIK administered with the hyperinsulinemic normoglycemic clamp technique (short-acting insulin 1 IU/kg/h and glucose infusion administered separately) is intended to minimize surgical stress response induced insulin resistance, to optimize the hemodynamic effects of insulin and to avoid variation in blood glucose levels (Svedjeholm *et al.* 1995).

Cardiac surgery induced neutrophil activation, adhesion to the endothelium, transmigration into the extravascular space and subsequent release of proteolytic enzymes have been shown to be the central mechanisms mediating myocardial ischemia-reperfusion injury (Romson *et al.* 1983). This chain of events may be potentially attenuated by a leukocyte filter, which is intended to remove activated leukocytes from the circulation before they reach their target, i.e. the activated endothelium (Byrne *et al.* 1992). Since its introduction, the leukocyte filter has been tested in several trials with different filtration strategies and patient groups. Until recently, however, the results have been controversial in trials with anti-inflammatory and clinical end-points (Chen *et al.* 2002, Patel *et al.* 2003, Ilmakunnas *et al.* 2005, Whitaker *et al.* 2006).

The aims of the present research were to evaluate the effects of high-dose glucose-insulin treatment and a leukocyte-depleting filter as adjunctive strategies on markers of myocardial reperfusion injury and SIRS in elevated risk cardiac surgery.

2 Review of the literature

2.1 Systemic inflammatory response syndrome

Inflammation is a host response to infection, antigen challenge or tissue injury, which serves to eradicate the cause and to facilitate tissue repair and healing. Although acute inflammation is fundamentally beneficial, failure of local control of the inflammatory process and imbalance between pro- and anti-inflammatory responses can precipitate systemic inflammatory response syndrome (SIRS) (Bone *et al.* 1992). This generalized inflammatory process allows the inflammatory stimulus to spill over and to damage organs remote from the primary site of injury, and it typically occurs after different types of major insult such as severe infection, major trauma, pancreatitis and burns. After cardiac surgery, SIRS usually manifests merely as subclinical organ dysfunctions, but may also generate an excessive inflammatory response, particularly in patients with limited physiological reserves (Butler *et al.* 1993, Cremer *et al.* 1996, Wan *et al.* 1997, Holmes *et al.* 2002, Levy & Tanaka 2003). Organ damages after cardiac surgery with cardiopulmonary bypass (CPB) are largely caused by SIRS and another closely linked but distinct pathophysiological process, myocardial ischemia-reperfusion injury. A schematic representation of the mechanisms and consequences initiated by cardiac surgery and CPB is given in figure 1. The term “neuroendocrine stress response“, is here used to refer to a wider concept of metabolic changes induced by cardiac surgery and CPB, characterized by hypermetabolism, insulin resistance and synthesis of acute phase proteins.

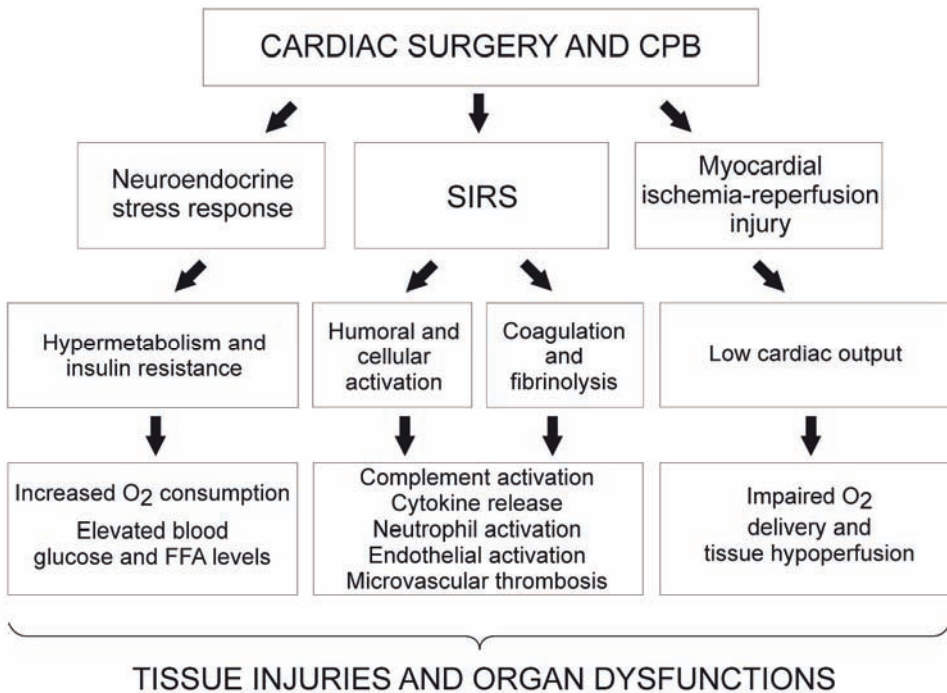


Fig. 1. Schematic representation of the pathophysiological mechanisms and consequences initiated by cardiac surgery and CPB.

2.1.1 Initiating factors

Based on current knowledge, SIRS induced by cardiac surgery is considered to be of multifactorial origin, and it is unclear which of the initiating mechanisms are clinically the most important. CPB-induced physiological changes such as interaction between blood and the non-endothelial artificial surfaces of extracorporeal circulation (El Habbal *et al.* 1997, Fromes *et al.* 2002), conversion of pulsatile to laminar blood flow (Dekker *et al.* 2002), blood-air interface (Gong *et al.* 1996), retransfusion of cardiotomy suction blood and mediastinal shed blood (Westerberg *et al.* 2004, Westerberg *et al.* 2006), myocardial ischemia-reperfusion injury following cardioplegic arrest (Wan *et al.* 1996) and splanchnic region hypoperfusion and endotoxemia (Fiddian-Green *et al.* 1987, Kharazmi *et al.* 1989, Wan *et al.* 1999) have been suggested as the main triggering factors in this process. In addition, the surgical trauma itself contributes markedly to the inflammatory response that occurs after cardiac procedures, although the studies on its importance in this respect have been inconclusive (Ascione *et al.* 2000, Czerny *et al.* 2000, Prondzinsky *et al.* 2005).

2.1.2 Pathophysiological mechanisms

SIRS is a result of synergistic immunologic reactions consisting of both humoral and cellular components, and it is closely linked with the activation of the coagulation-fibrinolytic system (Chandler *et al.* 1995, Hunt *et al.* 1998). Soluble mediators and plasma proteins, such as complement and cytokines, initiate the complex series of events leading to the expression of specific adhesion molecules in immune and endothelial cells to facilitate their contact (Chenoweth *et al.* 1981, Kirklin *et al.* 1983). The production of various substances, including oxygen free radicals, arachidonic acid metabolites, platelet-activating factor, nitric oxide and endothelins, mediates the process ultimately leading to increased vascular permeability, neutrophil transmigration across the endothelium and consequent tissue injury. However, the interactions between different pro- and anti-inflammatory reactions, cells and substances involved in this process are not completely clear.

2.1.2.1 Cytokine release

Cytokines are soluble polypeptides produced by various cell types including activated monocytes, tissue macrophages, lymphocytes, and endothelial cells in response to a different types of physiologic stimuli. Cytokines are key mediators of acute phase response modulating the inflammatory cell functions and production of acute phase proteins. (Marshall & Cohen 1999). Cytokines may exert either proinflammatory or anti-inflammatory properties or both and the balance between these effects, modulate the extension of inflammatory response after cardiac surgery (Franke *et al.* 2002). Plasma levels of inflammatory cytokines, interleukin-6 (IL-6) and interleukin-8 (IL-8) are usually demonstrated to increase after the CPB, reaching their top values 4-6 hours after the end of operation (Franke *et al.* 2005). Although the elevated proinflammatory cytokine levels have been considered to reflect the degree of inflammatory response and to predict adverse outcome by several studies, a direct cause-and-effect relation has not been demonstrated (Grunenfelder *et al.* 2000). However, the elevated pro-inflammatory cytokines may alter myocardial contractility and cause haemodynamic instability and hence contribute to the development of organ system failures after cardiac surgery (Finkel *et al.* 1992, Hennein *et al.* 1994). Individual studies, however, vary greatly in the degree of cytokine response measured during and after CPB (Tassani *et al.* 2000, Schmarzt *et al.* 2003).

2.1.2.2 Neutrophil activation

Numerous experimental and clinical studies have demonstrated that activated neutrophils are key mediators of the systemic inflammatory response, and neutrophil-endothelial interaction has been shown to be a central mechanism in the development of organ system failures both after cardiac (Litt *et al.* 1989, Gillinov *et al.* 1994, Cameron 1996) and transplantation surgery (Turunen *et al.* 2004). Neutrophil activation occurs as a

response to surgical trauma and contact of blood with foreign materials induced by several inflammatory mediators such as activation of factor XII (el Habbal *et al.* 1997). As leukocytes are activated, they go through a rapid conformational change with expression of surface adhesion molecules. Activated cells are recruited to localized areas of injury or inflammation by chemokines, complement, cytokines and adhesion molecules (Paugam *et al.* 1997, Baggiolini *et al.* 1998). Thereafter, the freely moving neutrophil slows down and starts to roll along the endothelial surface in a postcapillary venule (Engler *et al.* 1986a). After the formation of a tight adhesion between the neutrophil and an endothelial cell, the neutrophil transmigrates across the microvascular endothelial barrier via intercellular junctions and invades into the underlying tissue (Vestweber 2000). This is followed by neutrophil degranulation with release of free oxygen radicals (Wachtfogel *et al.* 1987), cytotoxic proteases and elastases (Romson *et al.* 1983, Faymonville *et al.* 1991), which contribute to the formation of tissue injury and capillary leak (Wedmore *et al.* 1981).

2.1.2.3 Endothelial activation

Vascular endothelium has a central role in the control of vascular tone and permeability, the regulation of coagulation and fibrinolytic systems and inflammation-induced neutrophil-endothelial cell interaction. Cardiac surgery with CPB leads to widespread endothelial activation and diffuse endothelial dysfunction, and endothelial cells start to release inflammatory mediators such as cytokines, cellular adhesion molecules and chemotactic substances that regulate leukocyte extravasation. (Boyle *et al.* 1996a). Furthermore, widespread vascular injury, cellular events and fibrin deposition in the microvasculature all contribute to altered microvascular flow and end-organ failure (Boyle *et al.* 1996b, McGilvray & Rotstein 1998).

2.1.2.4 Adhesion molecules

Specific adhesion molecules, which are expressed on leukocytes, platelets and endothelial cells, are shown to mediate the contact between neutrophils and endothelial cells. Neutrophil rolling is mediated by the expression of a family of adhesion molecules known as selectins, which are divided into three classes: L-selectins on leukocytes, E-selectins on activated endothelial cells and P-selectins on endothelial cells and platelets. The tight adhesion between the neutrophil and the endothelial cells is mediated by CD11/CD18 neutrophil adhesion molecules (β 2-integrins) on neutrophils and intercellular adhesion molecules (ICAM-1 and ICAM-2) on endothelial cells (immunoglobulin superfamily). One subfamily of integrins, which share the same β -chain (CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), is expressed only on leukocytes. (Albelda *et al.* 1994, Asimakopoulos & Taylor 1998). The expression of neutrophil surface and soluble endothelial cell adhesion molecules is suggested to reflect the degree of neutrophil and endothelial activation during and after cardiac surgery (Gillinov *et al.*

1993, Eikemo *et al.* 2004). Particularly, the expression of neutrophil CD11b is shown to be a valuable marker in this respect (Paugam *et al.* 1997, Ilton *et al.* 1999)

2.2 Neuroendocrine stress response

The cardiac surgery induced neuroendocrine stress response induces several profound metabolic effects, which are initially intended to enhance survival, but may be partly more harmful than beneficial in the context of modern critical care (Rumelin *et al.* 1999). These metabolic changes are characterized by hypermetabolism (Tulla *et al.* 1991), changes in plasma protein synthesis (Marshall & Cohen 1999) and alterations in glucose metabolism leading to elevated blood glucose levels even in patients with normal glucose tolerance (Chaney *et al.* 1999). The acute phase response is a cytokine-induced physiological reaction to tissue injury and infection characterized by hepatic production of acute phase proteins, including C-reactive protein (CRP) (Marshall & Cohen 1999). CRP is a sensitive but non-specific marker of inflammation, tissue damage and infection, since measurements of CRP concentrations are widely used to monitor the extent of inflammatory response (Aouifi *et al.* 1999).

The transient loss of the anabolic effects of insulin i.e. insulin resistance, is the central factor contributing to these adverse changes in glucose metabolism (Greisen *et al.* 2001) and they may be overcome by exogenous insulin administration, although large doses are often needed (Brandi *et al.* 1990). The mechanisms of insulin resistance are complex and not fully understood, but stress hormones and proinflammatory cytokines are suggested to be closely involved in this process (Ljungqvist *et al.* 2000). In addition, increased lipolysis leads to elevation of serum free fatty acid levels (FFA), which have been suggested to play a role in inflammatory processes, although this connection between the hormonal stress response and immunological changes is largely unclear (Brix-Christensen *et al.* 2004).

2.3 Myocardial ischemia-reperfusion injury

During cardiac surgery with CPB, some degree of global myocardial ischemia and repetitive periods of reperfusion are almost inevitable, although measures have been taken to protect the myocardium by cardioplegia, hypothermia and electromechanical arrest (Braunwald & Maroko 1974, Braimbridge *et al.* 1977, Buckberg *et al.* 1977). Reperfusion after a short episode of ischemia is usually accompanied by rapid restoration of cellular metabolism and functional recovery without any structural or biochemical evidence of tissue injury. However, an ischemic event of sufficient duration and severity may lead to significant changes in myocardial cell metabolism, function and structural integrity and promote even more widespread and permanent cell injury (Braunwald & Kloner 1985, Vinten-Johansen *et al.* 1988).

2.3.1 Pathophysiological mechanisms

The underlying pathophysiological mechanisms of ischemia-reperfusion injury are not completely clear, and several interdependent mechanisms are suggested to contribute this phenomenon.

2.3.1.1 Oxidant stress

Oxygen-derived free radicals are generated in small amounts during normal cellular metabolism and are normally inactivated by endogenous scavenging systems. Reintroduction of oxygen into a previously ischemic myocardium leads to the formation of large quantities of oxygen-derived free radicals within minutes via several different mechanism, i.e. by activated neutrophils (Zweier *et al.* 1987, Bolli *et. al.* 1988). Oxidative stress results in peroxidation of lipid cell membranes and impairment of the membrane-bound enzyme system, leading to a loss of cell integrity and cell death (Lazzarino *et al.*1994). Free oxygen radicals are also connected to postischemic contractile dysfunction (Bolli *et. al.* 1988) and neutrophil recruitment and adherence to endothelium (Patel *et al.* 1991).

2.3.1.2 Calcium

Intracellular calcium accumulation is considered to play an important role in the development of myocardial ischemia reperfusion injury (Kusuoka *et al.* 1987, Meissner & Morgan 1995). A calcium overload has been suggested to lead to excessive and uncontrollable contraction of myocytes (hypercontracture) and to a result in severe cell injury or cell death (Piper 1989). In addition, decreased responsiveness of contractile filaments to calcium after ischemia-reperfusion has been observed and contributes to the contractile dysfunction that appears after ischemia (stunning) (Kusuoka *et al.* 1987, Bolli & Marban 1999).

2.3.1.3 Altered metabolism

Persistent myocardial lactate release after reperfusion of ischemic myocardium, as measured by coronary sinus blood samples, has been shown to predict postoperative contractile dysfunction in CABG patients (Rao *et al.* 2001). This suggests delayed recovery of normal aerobic metabolism, which may, in turn, contribute to the prolonged recovery of contractile function postoperatively. The improvement of myocardial aerobic metabolism after ischemia has been demonstrated to be enhanced by the stimulation of myocardial pyruvate dehydrogenase enzyme activity with insulin in experimental models (Rao *et al.* 1998). In heart failure, myocardial cells have also been suggested to have impaired capacity to use their normal predominant fuel, free fatty acids, without a sufficiently high compensatory increase in glucose oxidation (Sack *et al.* 1996).

Furthermore, the cardiac surgery induced stress response leads to elevated levels of circulating FFAs, which have been shown to have deleterious effects on the postischemic myocardium (Liu Q *et al.* 2002) and to increase the occurrence of arrhythmias (Oliver & Opie 1994).

2.3.1.4 Endothelial dysfunction

Coronary vasodilatation is regulated by several endothelial derived substances such as prostacyclin, adenosine and nitric oxide (NO). Reperfusion of ischemic vasculature after cardioplegia, results in marked endothelial cell dysfunction with impaired vasodilatation and increased response to vasoconstrictors potentially limiting the coronary blood flow (Metais *et al.* 1999, Metais *et al.* 2001). This is potentially due to the enhancement of myocardial expression of inducible form of cyklo-oxygenase (COX-2) and production of vasoconstrictive prostaglandins such as tromboxane A2 along with other potent endothelial vasoconstrictors, like endothelin and angiotensin II (Metais *et al.* 1999). On the other hand, simultaneous decrease in constitutive nitric oxide synthase (eNOS) activity lead to decreased concentration of vasodilative nitric oxide (NO) (Engelman *et al.* 1995).

2.3.1.5 Neutrophils

Several studies have shown that neutrophil accumulation into the ischemic myocardium (Engler *et al.* 1986b) after reperfusion with a subsequent burst of oxygen free radicals (Zweier *et al.* 1987), degranulation and release of proteases and elastases (Romson *et al.* 1983) is the crucial step in the formation of myocardial cell injury. In addition, neutrophil activation increases its size and cytoskeletal rigidity, which prevents neutrophil conformation to capillary dimensions and thereby contributes to the obstruction of the capillaries and the “no reflow” phenomenon (Engler *et al.* 1983a).

2.4 Cardiovascular manifestations and organ dysfunction

2.4.1 Perioperative risk factors

Despite the improvements in surgical outcomes, there is conclusive evidence that the condition of cardiac surgical patients has become progressively more complicated with a higher incidence of comorbidities and a larger proportion of elderly patients with reduced physiological reserves (Hartz *et al.* 2000, Gaudino *et al.* 2004). The predictors for adverse events are often complex but essential for optimal resource use, and different scoring systems have hence been developed to facilitate risk stratification (Kurki & Kataja 1996, Kurki *et al.* 2001). However, there are certain particular risk factors such as left ventricular hypertrophy, long duration of aortic cross-clamp and perfusion and preceding

myocardial ischemia, which may complicate the perioperative course and challenge the attending physicians from the surgical theatre to the intensive care unit (Roques *et al.* 1999).

The hypertrophied left ventricle, often due to aortic stenosis, is shown to be particularly vulnerable to the effects of ischemia and reperfusion (Warner *et al.* 1987), which may manifest as contractile dysfunction or myocardial infarction after surgery (Menasche *et al.* 1985). This is suggested to result from alterations in myocardial cell calcium processing (Allard *et al.* 1994), disturbed energy metabolism (Allard *et al.* 1994, Friehs & del Nido 2003) and high energy demand (Ascione *et al.* 2001). The long aortic cross-clamp time and the inadequate delivery of cardioplegia may aggravate the situation, as in combined aortic valve and CABG surgery.

The duration of aortic cross-clamp and CPB times has been shown to be the main determinants of postoperative outcome in most of studies that have attempted to identify the determinants of the outcome of cardiac surgery (Bjessmo *et al.* 2000). Prolonged CPB time is suggested to affect adversely splanchnic perfusion (Kumle *et al.* 2003), to strengthen the effects of SIRS (Gott *et al.* 1998, Roth-Isigkeit *et al.* 2000) and to contribute to the incidence of postoperative organ failures (Grayson *et al.* 2003). Long aortic cross-clamp time probably worsens the ischemia-reperfusion injury and has been shown to predict the need for inotropic medication both for separation from CPB (McKinlay *et al.* 2004) and postoperatively (Royster 1993).

Unstable angina pectoris is a common cause for urgent CABG operation (Disch *et al.* 1994) and it is associated with a higher incidence of perioperative myocardial infarction, contractile dysfunction and for the need of postoperative inotropic support than patients with stable angina pectoris. (Bonacchi *et al.* 2001). The state is characterized by ongoing myocardial ischemia with depleted energy reserves (Cave *et al.* 2000) and by a current knowledge, also by local and systemic low-grade inflammation (Buffon *et al.* 2002), which may both reduce myocardial tolerance to cardioplegic arrest and reperfusion. Unstable angina pectoris is shown to be associated with increased risk for new perioperative myocardial infarction and death from cardiac causes according several studies particularly if combined with other risk factors, such as advanced age and prolonged aortic cross-clamp time (Naunheim *et al.* 1989, Luoagie *et al.* 1995, Bjessmo *et al.* 2000).

2.4.2 Hemodynamic consequences and tissue injuries

Myocardial ischemia-reperfusion injury is commonly considered to manifest clinically as contractile dysfunction with a need for inotropic support, reperfusion arrhythmias and, ultimately, lethal cell injury or myocardial infarction (Bolli *et al.* 2004, Moens *et al.* 2005). The mediators for SIRS, in addition to other deleterious effects, may interfere with the distribution of systemic blood flow leading to organ hypoperfusion (Uusaro *et al.* 1996, Pölönen *et al.* 2000). Although these effects are usually short-lived, they may lead to different organ system failures and adverse outcomes, particularly in certain risk groups.

2.4.2.1 Myocardial contractile dysfunction

Myocardial stunning is the best-known manifestation of reperfusion injury, and it was first described by Heyndrickx *et al.* (1975) and named by Braunwald & Kloner (1982). It is defined as prolonged but transient postischemic contractile dysfunction without evidence of permanent myocardial injury or marked cell necrosis (Bolli 1998). The time course of post-bypass myocardial contractile function has been shown to be remarkably consistent in many studies and to decline after the period immediately following CPB, reaching its peak 4-6 hours after CPB and usually returning to near normal 1-2 days after surgery (Breisblatt *et al.* 1990, Royster 1993), but it may also persist for a prolonged period and lead to adverse consequences and prolonged ICU stay (Christakis *et al.* 1996). Reduced cardiac output may lead to inadequate oxygen delivery during the increased metabolic needs in a postoperative situation. However, the use of inotropic drugs may potentially increase myocardial oxygen consumption in ischemic areas (Schwenzer *et al.* 1990, Rao *et al.* 2001) and the occurrence of arrhythmias (Salaria *et al.* 2005)

2.4.2.2 Myocardial cell injury

The diagnosis of perioperative myocardial infarction after cardiac surgery is difficult, and all diagnostic methods have their pitfalls. Electrocardiography (ECG) fails to detect small areas of myocardial infarction, and ST segment alterations or conduction abnormalities are often hard to interpret. Furthermore, myocardial cell injury and enzyme release may be caused by several mechanisms in addition to global ischemia induced by inadequate myocardial protection (Alpert *et al.* 2000). Some degree of myocardial enzyme release usually occurs as a result of direct surgical trauma and intracardiac manipulation, particularly in valve operations (Alpert *et al.* 2000). However, it is evident that the higher the value for the cardiac biomarker, the greater the amount of damage to the myocardium (Costa *et al.* 2001), and both abundant myocardial enzyme release (Costa *et al.* 2001, Klatte *et al.* 2001, Ramsay *et al.* 2005) and new Q waves on the postoperative ECG are associated with adverse outcome (Yokoyama *et al.* 2000).

2.4.2.3 Arrhythmias

Although the arrhythmias in the setting of acute myocardial infarction and reperfusion strategy are usually considered as ischemia-reperfusion arrhythmias, the supraventricular arrhythmias often seen after cardiac surgery are obviously multifactorial, even though possibly related to the inflammatory response and ischemia-reperfusion injury (Lamm *et al.* 2006). Atrial fibrillation is one of the most often seen complications after cardiac surgery, and although usually well tolerated, may compromise hemodynamic recovery and prolong the ICU stay. Atrial fibrillation occurs most commonly on the second postoperative day and affects over one third of patients undergoing CABG and up to 60 percent of those who undergo combined CABG and valve replacement. (Mathew *et al.*

2004, Maisel, *et al.* 2001). Many perioperative strategies have been tested to reduce its incidence, but the results have been inconclusive (Creswell *et al.* 2005).

2.4.2.4 Vasomotor dysfunction

Vasomotor dysfunction during and after cardiac surgery may clinically manifest as decreased peripheral vascular resistance in the skeletal muscle bed and, conversely, as an increased trend towards vasoconstriction in the coronary, pulmonary, mesenteric and cerebral circulation (Ruel *et al.* 2004). The exact mechanisms mediating both maldistribution of microvascular blood flow and hypotension due to vasodilatation are largely unclear, but they are connected to cascades of activated systemic inflammatory response syndrome (Kristof & Magder 1999, Cugno *et al.* 2001) and endothelially mediated mechanisms, leading to alterations in the local nitric oxide concentration (Ruel *et al.* 2004). However, the redistribution of blood flow into organs and microcirculatory dysfunction alter tissue perfusion along with oedema formation and microthrombosis, and the situation is potentially influenced by vasoactive medication (O'Dwyer *et al.* 1997, Brander *et al.* 2006).

2.4.2.5 Tissue hypoperfusion and organ system failures

The exact mechanisms of SIRS and reperfusion injury influencing the incidence and severity of organ system failures are not well understood and are obviously multifactorial. It is evident that different occult organ dysfunctions are common, despite the low incidence of manifest organ failures (Tang *et al.* 2002, Brudney *et al.* 2005). There is powerful clinical and experimental evidence to link perioperatively occurring splanchnic hypoperfusion and commonly occurring systemic endotoxin release as a significant factor strengthening the effects of SIRS and contributing to the development of postoperative organ system failures (Uusaro *et al.* 1996, Neuhof *et al.* 2001, Aydin *et al.* 2003). Perioperative hemodynamic instability has been shown to predict morbidity and mortality following CPB, and the low output syndrome has been considered the crucial initial insult leading to multiorgan failure and death, probably due to organ hypoperfusion (Nugent 1999, Reich *et al.* 1999). Furthermore, optimization of myocardial function and maintenance of adequate tissue perfusion during the immediate postoperative period has benefits for the long-term outcome (Routsis *et al.* 1993, Pölönen *et al.* 2000).

2.5 Glucose-insulin-potassium (GIK) treatment

Glucose-insulin-potassium (GIK) administration was first introduced as treatment of myocardial infarction by Sodi-Pallares *et al.* (1962) and applied in cardiac surgery by Brainbridge *et al.* (1969). The term 'GIK' covers a wide variety of different glucose, insulin and potassium treatment protocols and applications, and it is therefore no surprise that, despite the long history of usage, controversy still exists over its use for myocardial

protection both in the treatment of acute myocardial infarction (Fath-Ordoubadi *et al.* 1997, Mehta *et al.* 2005) and as an adjunctive therapy in cardiac surgery (Quinn *et al.* 2006, Shipke *et al.* 2006).

2.5.1 Potential effects on myocardial metabolism

Glucose-insulin-potassium solution (GIK) was initially intended to increase myocardial electrical stability by stimulating potassium/hydrogen ATPase and restoring intracellular potassium reuptake. The mechanism was suggested to stabilize cell membranes and to reduce the incidence arrhythmias (Zierler 1966).

Thereafter preischemic administration of glucose and insulin was proposed to improve myocardial ischemia tolerance by preserving and restoring the myocardial glycogen stores, which was suggested to increase glycolytic ATP production and thereby to result in enhanced myocardial protection (Haider *et al.* 1984). Enhanced myocardial recovery was also demonstrated in clinical trials, but later experimental studies have questioned the relevance of this mechanism (Cross *et al.* 1996).

According to recent experimental studies, both aerobic and anaerobic myocardial metabolism appears to persist during low-flow (myocardial infarction) and repetitive periods of ischemia (CPB) (Cave *et al.* 2000). The beneficial effects of glucose and insulin administration in this situation are thought to be due to both increased glucose uptake and oxidative phosphorylation at the expense of reduced free fatty acid metabolism and in addition the enhanced anaerobic glycolysis (Rao *et al.* 1998, Cave *et al.* 2000). This is supported by the increased myocardial glucose uptake and oxidation after hypothermic cardioplegic arrest demonstrated by Pietersen *et al.* (1999). On the other hand, although ATP production by anaerobic glycolysis is minimal, it is suggested to maintain vital cell functions, such as membrane stabilization, during ischemia (Xu *et al.* 1995). However, anaerobic energy production prerequisites some degree of remaining nutritive flow or reperfusion to wash out toxic metabolites, such as lactate and hydrogen ion, or otherwise deleterious intracellular acidosis will occur and interfere with cell function (Liu *et al.* 2002).

Although FFAs are normally the predominant substrates for myocardial energy production, their metabolism is impaired during and after ischemia, which may lead to accumulation of toxic intermediate metabolites, e.g. acyl-carnitine, which has been shown to interfere with the ionic pumps maintaining myocardial cell integrity and to lead to intracellular calcium ion accumulation (Lopashuck *et al.* 1993, Hendrickson *et al.* 1997). Furthermore, aerobic glucose oxidation consumes less oxygen than FFAs, which makes it a potentially better substrate during ischemia-reperfusion (Lopashuk 2001). Insulin administration has been shown to bring down systemic FFA levels, to reduce myocardial FFA uptake and to increase glucose oxidation both in normal and chronically dysfunctional but viable myocardium (Oliver & Opie 1994, Mäki *et al.* 1996).

Administration of insulin has also been shown to stimulate pyruvate dehydrogenase enzyme resulting in improved aerobic metabolism after ischemia (Rao *et al.* 1998) and the replenishment of Krebs cycle intermediates after ischemia (Taegtmeyer 1997), to proper potential anti-apoptotic properties (Gao *et al.* 2002) and to reduce ischemia-

induced myocardial cell injury independently of its metabolic effects in experimental studies (Sack & Yellon. 2003).

2.5.2 Hemodynamic effects

Insulin itself, has been shown to improve myocardial contractile function in experimental trials (Doenst *et al.* 1999) without any evident effect on myocardial oxygen consumption (Tune *et al.* 1998). The improved myocardial function following administration of glucose and insulin has also been demonstrated in clinical studies on patients with chronic ischemic cardiomyopathy (Cottin *et al.* 2002), acute myocardial infarction (Rackley *et al.* 1981) or chronic ischemic left ventricular dysfunction (Khojry *et al.* 2003), but controversial results also exist (Bergstra *et al.* 2006).

In healthy subjects, insulin has been shown to have dose-dependent vasodilatory effects on both skeletal muscle (Baron *et al.* 1993) and coronary vasculature (Laine *et al.* 2000, Sundel *et al.* 2002), which are considered to be mediated through the sympathetic nervous system and the endothelium-dependent L-arginine nitric oxide pathway (Sobrevia *et al.* 1996). Furthermore, enhanced coronary blood flow has also been demonstrated in patients with coronary artery disease (McNulty *et al.* 2000). Improvement of regional myocardial perfusion in areas next to a recently infarcted region by GIK has been demonstrated in single photon emission tomography (Marano *et al.* 2000). However, the connections between these coronary and systemic vasodilatory effects of insulin and the potentially beneficial hemodynamic effects of GIK are unclear (Parsonage *et al.* 2001).

2.5.3 Systemic metabolism and inflammation

Episodes of hyperglycemia during the perioperative period, has several deleterious effects. In clinical studies on cardiac surgical patients hyperglycemia has been associated with increased incidence of infections (Furnary *et al.* 1999), resource utilization (Estrada *et al.* 2003) and mortality both in patients with and ones without diabetes (Doenst *et al.* 2005). A decrease in ICU mortality following maintenance of strict normoglycemia with intensive insulin therapy, was also demonstrated by Van den Berghe *et al.* in their landmark study including mainly critically ill cardiac surgical patients (Van den Berghe *et al.* 2001) and in a subgroup of medical patients with longer ICU stay (Van den Berghe *et al.* 2006).

The biochemical pathways mediating improved survival during the ICU stay with insulin treatment and maintenance of normoglycemia have not been defined (Van den Berghe *et al.* 2001). The mechanisms have been suggested to be related to either an avoidance of the proinflammatory effects of hyperglycemia (Esposito *et al.* 2002, Turina *et al.* 2005) or the direct protective and potentially anti-inflammatory effects of insulin, as demonstrated in recent experimental (Brix-Christensen *et al.* 2004), and clinical studies (Jeschke *et al.* 2004). Interestingly, Hansen *et al.* (2003) demonstrated a pronounced decrease in C-reactive protein levels following intensive insulin therapy in a subgroup of

patients from the study of Van den Berghe *et al.* (2001), and Chaudhuri *et al.* (2004) showed low-dose insulin infusion to attenuate high-sensitivity C-reactive protein and myocardial enzyme release in patients with thrombolitically treated acute myocardial infarction.

2.5.4 GIK in cardiac surgery

The effects of different glucose-insulin treatment regimens during cardiac surgery have been mainly studied in small trials with varying adjuvant strategies and techniques of myocardial protection (Wistbacka *et al.* 1995, Jeppsson *et al.* 1997). Furthermore, the large variation in the dosage and application of both glucose and particularly insulin interferes with the interpretation of the results (Schipke *et al.* 2006).

2.5.4.1 Insulin dosage

The optimal dosage of both glucose and insulin during cardiac surgery are largely unclear and seem to depend on the targeted endpoint of the therapy. However, with regard to myocardial glucose uptake and hemodynamic effects, several authors have suggested that high doses of insulin, i.e. up to 1 IU/kg/h are needed to overcome the effects of insulin resistance (Svensson *et al.* 1990) and to achieve the optimal inodilatory effects on myocardial contractile function (Svedjeholm *et al.* 1995) and tissue perfusion (Lindholm *et al.* 2001), although controversial results also exist with in respect (Lazar *et al.* 2000, Quinn *et al.* 2006). On the other hand, it seems that lower levels of insulin can suppress FFAs (Nilsson *et al.* 1987), reduce the deleterious effects of hyperglycemia (Lazar *et al.* 2004) and exert potential anti-inflammatory effects during cardiac surgery (Visser *et al.* 2005).

2.5.4.2 Strategy of administration

In most of studies insulin and glucose have been administered blindly in a combined fashion at a fixed rate without observing the effects of the individually varying degree of insulin resistance, which have often led to a mismatch between the glucose and insulin doses with uncontrollable variation of blood glucose levels (Bruemmer-Smith *et al.* 2002, Doenst *et al.* 2003). In the hyperinsulinemic normoglycemic clamp technique used by some investigators, plasma insulin concentration is raised to a predetermined level by continuous short-acting insulin infusion and the blood glucose level is kept within an optimal range by adjusting separate glucose infusion. This method prerequires systematic monitoring of blood glucose levels, but has been shown to results in better maintenance of normoglycemia in some studies (Szabo *et al.* 2003, Visser *et al.* 2005).

Controversy also exists with regard to the timing and duration of glucose-insulin treatment, and enhanced myocardial recovery after cardiac surgery has been

demonstrated with preoperative (Besogul *et al.* 1999), perioperative (Quinn *et al.* 2006) and postoperative treatment protocols (Svedjeholm *et al.* 1995).

Interestingly, insulin-enhanced cardioplegia failed to attenuate the effects myocardial ischemia-reperfusion injury as measured by the incidence of postoperative atrial fibrillation (Hynninen *et al.* 2001), myocardial enzyme release and the incidence of low output syndrome (Rao *et al.* 2002) in two large-scale studies on patients admitted for urgent CABG surgery due to unstable angina pectoris. However, the effects of insulin-enhanced cardioplegia are mainly limited to the myocardium and the time of cardioplegic arrest, since these studies do not support this route of administration.

2.5.4.3 *Effects on myocardial ischemia-reperfusion injury*

Increased cardiac output and a decreased need for inotropic medication after cardiac surgery in patients treated with GIK, have been reported by several investigators (Hiesmayr *et al.* 1995, Jeppsson *et al.* 1997, Lazar *et al.* 1997, Quinn *et al.* 2006), but controversial results also exist (Bruemmer-Smith *et al.* 2002). However, administration of insulin to merely maintain normoglycemia during cardiac surgery failed to reduce the need for inotropic or anti arrhythmic medication (Groban *et al.* 2002).

In many studies with low-risk patients and a low degree of inflammatory stimulus, glucose insulin treatment has failed to show any benefit, whereas several trials with higher risk patients, such as diabetics (Lazar *et al.* 2004) and patients with impaired myocardial contractile function (Wistbacka *et al.* 1995) or unstable angina pectoris (Lazar *et al.* 1997), have shown promising results. Interestingly, two trials with off-pump techniques, failed to show any clinical benefit of GIK (Lell *et al.* 2002, Smith *et al.* 2002). This may be due to a shorter period of myocardial ischemia and a lower degree of proinflammatory response in off-pump operations, which may limit the potential benefit of the therapy.

In some studies GIK has also been shown to reduce the incidence of reperfusion arrhythmias such as postoperative atrial fibrillation (Lazar *et al.* 1997, Lazar *et al.* 2000), although controversy exists (Smith *et al.* 2002).

2.5.4.4 *Effects on inflammatory response*

Studies evaluating the effects of insulin on cardiac surgery and CPB-initiated systemic inflammatory response are rare. However, an insulin dose of 0.1 IU/kg/h administered with the hyperinsulinemic normoglycemic clamp technique has been shown to bring down CRP levels after elective CABG surgery without marked effect on proinflammatory cytokines (IL-6 and IL-8), but with better maintained normoglycemia in the GIK group (Visser *et al.* 2005). On the other hand, strict glycemic control with intensive insulin therapy after elective CABG surgery failed to show any effect on proinflammatory cytokine levels in the study of Hoedemaekers *et al.* (2005).

2.5.4.5 Potential side effects

There are certain potential risk factors associated with the administration of GIK, particularly when high doses of glucose and insulin are used. Surgery-induced insulin resistance and alterations in glucose metabolism make the balance between optimal glucose and insulin delivery complex, leading easily to variation in blood glucose levels and episodes of both hyperglycemia (Brummer-Smith *et al.* 2002, Lell *et al.* 2002) and hypoglycaemia (Wistbacka *et al.* 1994) have been reported during such treatment.

Although GIK is primarily intended to optimize myocardial glucose uptake, it also has major effects on systemic metabolism. Cardiac surgery has been shown to induce hypermetabolism during the postoperative period. (Tulla *et al.* 1991). Thus, insulin administration may increase the whole-body metabolic drive and increase oxygen consumption and excessive carbohydrate loading further increases the systemic carbon dioxide production and places a burden on the cardiovascular system during this sensitive postoperative period (Kiiski & Takala 1994). Furthermore, the glucose load needed to maintain normoglycemia, particularly when high insulin doses are used, may exceed the physiological needs and current recommendations and be associated with adverse consequences (Krishnan *et al.* 2003).

Insulin treatment often also leads to shifts in plasma electrolyte concentrations, particularly potassium levels. Insulin infusion increases potassium transfer into the intracellular space. Hence, most high-dose glucose-insulin regimens include some degree of simultaneous potassium supplementation during the therapy (Szabo *et al.* 2003). Hypokalemia may increase the incidence of reperfusion arrhythmias after the operation and prolong the patient's ICU stay (Wahr *et al.* 1999).

Insulin-induced vasodilatation may also interfere with postoperative hemodynamic treatment. The altered systemic vascular resistance may increase the need for vasopressor medication (Quinn *et al.* 2006) and also affect the organ blood flow. The effects of insulin on the distribution of splanchnic blood flow are, however, largely unclear (Lindholm *et al.* 2001).

2.6 Leukocyte-depleting filter

The leukocyte filter was primarily developed to remove the leukocytes from packed red cells and platelets, i.e. to eliminate their adverse effects on the host immune system (Van De Watering *et al.* 1998), and the first leukocyte filter designed to be connected to a CPB circuit's arterial return line was introduced in 1992 (Pall *LeukoGuard* LG6). The filter consists of a polyester fiber screen intended to trap enlarged activated leukocytes by their physical size ($>40 \mu\text{m}$) and electrical charge in addition to microaggregates, debris and emboli, even though there is some proof that leukocyte adhesion to the filter material could also play a part in this process (Gourlay *et al.* 1992). During the last decade, a number of different leukocyte filters and filter combinations have been used in different parts of the CPB circuit in experimental and clinical studies, but conclusive evidence of their utility is still lacking (Salamonsen *et al.* 2005).

2.6.1 Experimental studies with arterial line leukocyte filter

In experimental models, an arterial line leukocyte filter has been shown to improve brain protection after a prolonged period of hypothermic circulatory arrest in a chronic porcine model (Rimpiläinen *et al.* 2000) and to attenuate ischemia-reperfusion-induced lung injury in isolated ventilated rabbit lung model leading to decreased microvascular permeability disorder and improved lung function (Ross *et al.* 1999). Interestingly, Lazar *et al.* (1995) demonstrated a significant reduction in ischemic myocardial damage with arterial line leukofiltration compared to solitary cardioplegia line leukofiltration, in a pig model simulating ischemic conditions before urgent surgical revascularization.

2.6.2 Clinical studies with arterial line leukocyte filter

Different types of arterial line filters are an optional accessory commonly connected to the CPB circuit, and they are intended to remove bubbles and microparticles from the circulation (Whitaker *et al.* 2001). In addition to this, a leukocyte filter should remove most of the activated leukocytes and restrict their contact with the activated endothelium of different target organs (Gourlay *et al.* 1992). In the majority of the clinical studies, the Pall LG6 type arterial line leukocyte filter has been used in a continuous fashion throughout the whole CPB period and compared to the ordinary arterial line filter used in the control group. The endpoints in leukocyte filter trials have varied and focused primarily on inflammatory mediators, markers of organ injury or functional outcome, usually in low-risk CABG patients. However, the heterogeneity of study settings, the CPB techniques and the adjunctive therapies make any comparison and interpretation of results rather difficult.

2.6.2.1 Filtration strategy

Firstly, controversy exists with regard to the filtration strategy, which is, according to current knowledge, a crucial factor influencing the effects of the leukocyte filter (Scholz *et al.* 2002). Since leukocyte activation is considered to begin at the very start of surgery (Faymonville *et al.* 1991), the aim of continuous use of the leukocytefilter throughout the CPB period is to remove a maximal amount of activated cells before the reperfusion phase (Sutton *et al.* 2005). In the strategic filtration method, the filter is usually not activated until 0-15 minutes before aortic declamping and reperfusion, and filtration is continued until the end of CPB (Matheis *et al.* 2001). The rationale for this is based on concerns that the efficacy of the filter to capture activated cells may decrease over time before the critical moment of reperfusion (Baksaas *et al.* 1998). Furthermore, increased arterial line pressures following prolonged use have also been reported by some investigators, which is probably due to cells and debris plugging the filter and obstructing blood flow (Smit *et al.* 1999, Salamonsen *et al.* 2005). This has also been demonstrated by scanning electron microscopic data of the filter after the use (de Vries *et al.* 2003). In

recent trials, some investigators have also combined the use of an arterial line leukocyte filter with i.e. blood cardioplegia line leukocyte filter, to attain more efficient leukodepletion but with varying success (Olivencia-Yurvati *et al.* 2003, Salamonsen *et al.* 2005, Sutton *et al.* 2005).

2.6.2.2 *Leukocyte counts*

During CPB, total leukocyte and neutrophil counts usually increase due to the rapid mobilization of neutrophils from bone marrow, even though a transient drop is usually seen after the initiation of CPB, probably associated with the hemodilution, leukocyte margination and adhesion (Wachtfogel *et al.* 1987, Hiesmayr *et al.* 1999). In experimental trials, the arterial line leukocyte filter has been shown to remove around 70% of leukocytes (Gourlay *et al.* 1992), but not surprisingly, clinical studies have been rather controversial in this respect, showing decreased leukocyte counts (Chen *et al.* 2002, Alexiou *et al.* 2004, Ilmakunnas *et al.* 2005) and no effect (Mihaljevic *et al.* 1995, Hurst *et al.* 1997, Baksaas *et al.* 1998). The filter has been suggested to remove more efficiently morphologically changed activated cell forms, but even the results concerning this have been conflicting (Thurlow *et al.* 1996, Hurst *et al.* 1997).

2.6.2.3 *Leukocyte activation*

Chen *et al.* (2002) demonstrated decreased expression of neutrophil CD11b and L-selectin by the leukocyte filter and reduction in endothelial activation and neutrophil transmigration as measured by soluble intercellular adhesion molecule-1 (ICAM-1) and platelet-endothelial cell adhesion molecule-1 (PECAM-1) levels (Chen *et al.* 2004). Controversial findings were presented by Stefanou *et al.* (2001), with no differences between the study groups, and Ilmakunnas *et al.* (2005), who showed that a leukocyte filter actually increased CD11b expression on neutrophils across the filter line, resulting in elevated plasma lactoferrin levels and hydrogen peroxide production, as a marker of increased intravascular neutrophil activation. Increased neutrophil activity by the filter as measured by the plasma elastase (Mihaljevic *et al.* 1995, Mair *et al.* 1999, de Vries *et al.* 2003, Whitaker *et al.* 2006) and myeloperoxidase levels (Scholz *et al.* 2002) have also been reported by other investigators. However, decreased levels or no effect on leukocyte degranulation have also been reported, but in a minority of the studies (Baksaas *et al.* 1999).

2.6.2.4 *Inflammatory mediators*

Most studies with a leukocyte filter have failed to report any measurable effect on inflammatory mediators such as cytokines IL-6 and IL-8, regardless of whether they have used the continuous (Johnson *et al.* 1995, Hurst *et al.* 1997) or the strategic filtration method (Matheis *et al.* 2001). Baksaas *et al.* performed two studies with 40 elective

CABG patients, each involving both filtration methods and comparable study protocols, but without evident attenuation in these markers and complement C3 activation products (Baksaas *et al.* 1998, Baksaas *et al.* 1999). Interestingly, Hamada *et al.* (2001) demonstrated reduction in both IL-6 and IL-8 when they compared patients with a leukocytefilter on a heparin-coated CPB circuit to controls with a standard arterial line filter on a conventional CPB circuit.

2.6.2.5 Myocardial protection and organ dysfunction

Some of the early studies focusing on myocardial injury and contractile function have shown a leukocyte filter to attenuate myocardial enzyme release and to decrease the need for inotropic medication after cardiac surgery (Hachida *et al.* 1995, Di Salvo *et al.* 1996), while most of the more recent studies have failed to show any cardioprotective effect of the filter (Mair *et al.* 1999, Sahlman *et al.* 2001, Whitaker *et al.* 2006). Recently, Leal-Noval *et al.* (2005) could not demonstrate any effect on myocardial enzyme release and contractile function in patients admitted for mixed cardiac operations, and similar results were also reported by Salamonsen *et al.* (2005) with their combined leukocyte depletion strategy, which included a cardioplegia line, homologous blood and residual oxygenator content leukofiltration with leukodepleted blood products (Salamonsen *et al.* 2005). One study with strategic timing of leukofiltration showed positive results (Matheis *et al.* 2001), and another revealed a reduced incidence of postoperative atrial fibrillation (Olivencia-Yurvati *et al.* 2002).

Improved oxygenation and pulmonary function after CABG surgery have been reported following both continuous (Alexiou *et al.* 2004, Sheppard *et al.* 2004) and strategic (Hachida *et al.* 1995, Karaiskos *et al.* 2004) use of leukocyte filter. On the other hand, several studies have failed to show any effect on pulmonary function (Fabbri *et al.* 2001, Leal-Noval *et al.* 2005, Salamonsen *et al.* 2005). Interestingly, Olivencia-Yurvati *et al.* (2003) combined strategic leukofiltration (started 30 minutes prior to aortic cross-clamp release) with cardioplegia line leukofiltration, leukoreduced allogenic blood products and leukoreduced reinfused salvaged blood in their prospective study of 225 elective CABG patients, and demonstrated a decreased pulmonary shunt fraction with markedly reduced hospital stay and costs. Shorter hospital stay (Patel *et al.* 2003, Sutton *et al.* 2005) and lower cost have also been reported by some other authors (Gott *et al.* 1998).

3 Aims of the present research

The aims of the present research were to evaluate the effects of high-dose glucose-insulin treatment and leukocyte-depleting filter as adjunctive strategies combined with our current perioperative methods on myocardial ischemia-reperfusion injury and systemic inflammatory response syndrome in patients at elevated perioperative risk.

More specifically the aims of the present study were to:

1. Evaluate the safety issues and metabolic effects of high-dose glucose-insulin treatment administered with the normoglycemic hyperinsulinemic clamp technique (I, II).
2. Evaluate the effects of high-dose glucose-insulin treatment on myocardial ischemia-reperfusion injury in patients with left ventricular hypertrophy and prolonged CPB time and in patients with unstable angina pectoris and normal CPB time (I, II).
3. Evaluate the effect of high dose glucose- insulin treatment on systemic inflammatory response syndrome in patients with unstable angina pectoris admitted for urgent coronary artery revascularization (II).
4. Evaluate the effect of leukocyte-depleting filter with the continuous filtration method on neutrophil activation after both normal coronary artery revascularization in elderly patients (III) and prolonged CPB time surgery in patients with left ventricular hypertrophy (IV).
5. Evaluate the effect of leukocyte-depleting filter on markers of systemic inflammatory response after both normal coronary artery revascularization in elderly patients (III) and prolonged CPB time surgery in patients with left ventricular hypertrophy (IV).

4 Patients and methods

4.1 Patients and study designs

These four prospective, randomized, open-label trials were carried out at the Departments of Anesthesiology and Surgery at Oulu University Hospital during the years 2000-2002 (I), 2003-2004 (II) and 2004-2005 (III, IV). The study involved a total of 119 patients (male gender 75 %, mean age 69.6 years (SD 7.3)), of whom 60 were admitted for combined aortic valve (AS) and coronary artery bypass surgery, 39 for a urgent coronary artery bypass surgery and 20 for elective coronary artery bypass surgery performed with the aid of CPB. The study protocols were approved by the Oulu University Ethics Committee and informed written consent was obtained from all patients. Randomization was carried out by using numbered sealed envelopes. The principal exclusion criteria were altered renal function (serum creatinine >150 mg/l), significant end-target organ failure or other severe chronic disease, emergency or re-operation, need for inotropic medication, corticosteroid medication (I-IV), oral anti-diabetic medication or insulin-dependent diabetes mellitus (I, II) and altered immune function or evident infection (II, III, IV). One patient was excluded because of technical problems in cardiac protection during the operation (I) and one patient due to protocol violation (II). The study designs are summarized in table 1.

Table 1. Patients and study designs.

Study	Patients and groups (n)	Design	Focus	Main variables
I	Combined CABG and AVR operation (40)	High-dose insulin vs. placebo	reperfusion injury, regimen safety	CI, TnI, metabolic effects
II	CABG, unstable angina pectoris (39)	High-dose insulin vs. placebo	SIRS, glycemic control vs. insulin effect	CRP, cytokines, TnI
III	CABG, elderly patients (20)	CPB with or without leukocyte filter	reperfusion injury, neutrophil activation	adhesion molecules, TnI
IV	Combined CABG and AVR operation (20)	CPB with or without leukocyte filter	neutrophil activation, SIRS	adhesion molecules, cytokines

Table 2. CPB technique modifications in different studies

Study	CPB circuits	Cardioplegia type	Cardioplegia temperature	Systemic temperature	Aprotinin	Tranexamic acid	Corticosteroid administration
I	uncoated	aspartate/glutamate enriched	25°C	31°C	+	-	+
II	coated	Ringer acetate	15°C	34°C	+	-	-
III	uncoated	Ringer acetate	15°C	34°C	-	+	-
IV	coated	Ringer acetate	15°C	34°C	-	+	-

Aprotinin = 2 million IU i.v. after the induction of anesthesia and 2 million IU in the priming solution (Trasylol®, Bayer, Leverkusen, Germany), Aspartate/glutamate enriched = Aspartate/Glutamate solution 500 ml (Monosodium-L-Aspartate-monohydrate and Monosodium-L-Glutamate-monohydrate 65 mmol in Aqua ster 500 ml) (Medipolar, Oulu, Finland), KCl 40 mmol, Tribonate 150 ml, CPD 125 ml and 5% Glucose 250 ml, Corticosteroid administration = Dexamethason (Decadron, MSD, USA) 60-80 mg i.v. after induction of anesthesia, Coated = phosphorylcholine coated CPB circuits (Ph.i.s.i.o., Dideco, Mirandola, Italy) (III, IV), Ringer acetate = Ringer acetate 1000 ml with KCL 80mmol/L, Tranexamic acid = 2 g of tranexamic acid (Caprilon®, Leiras, Finland) after induction of anesthesia and 2 g during protamine administration.

4.2 Anesthesia and cardiopulmonary bypass

The anesthesia and cardiopulmonary bypass techniques were primarily based on the normal standards and practices of the hospital during the years 2000-2005. The patients were premedicated with oral lorazepam (1-2 mg) and zopiclone (7.5-15mg) the night before the operation and with oral diazepam (0.1-0.15mg/kg) and intramuscular morphine (0.10-0.15 mg/kg) one hour before the induction of anesthesia. Antianginal medication and β -blockers at a reduced dose were continued, but calcium channel blockers and ACE inhibitors were discontinued before the operation. Before the induction of anesthesia, pulmonary and radial artery catheters were introduced under local anesthesia. Anesthesia was induced with a fentanyl bolus (5-7.5ug/kg) and propofol (5-8mg/kg/h) and alfentanil (0.05mg/kg/h) infusions until intubation. Muscle relaxation was achieved with pancuronium 8mg i.v. Anesthesia was maintained with propofol (1-2 mg/kg/h) and alfentanil (0.05mg/kg/h.) infusions, and the patients were ventilated with isoflurane (0.8-1.5 MAC) in 40% O₂ with air. Before aortic cannulation, heparin (3mg/kg) was given

and additional heparin was administered if necessary to maintain an activated clotting time of more than 400 seconds during the bypass.

The differences in CPB and cardioplegia techniques between the studies are shown summarized in table 2. Due to the manufacturer's decision to discontinue the production of aspartate/glutamate solution, which was used in the first study (I), and due to the changed clinical practice in our institution, the targeted cardioplegia and systemic temperatures in addition to cardioplegia type differ between the studies I and II-IV. Uncoated CPB circuits were used for the same reason in the studies I and III, which were carried out before the studies II and IV. Aprotinin was administered in the studies I-II because of its anti-fibrinolytic properties, but not in the studies III-IV due to its potential effects on neutrophil adhesion molecules.

Cardiopulmonary bypass was maintained using a non-pulsatile flow roller pump (Stöckert, Munich, Germany) and a membrane oxygenator (Compactflo or D 903 AVANT, Dideco, Mirandola, Italy). The CPB circuit was primed with Ringer acetate (1500ml), 15% mannitol (250ml) and heparin 75mg. Pump flow was maintained at 2.4 l/min/m² and perfusion pressure was kept at 50 to 70 mmHg. The hematocrit value was maintained over 0.25 during and after CPB. Blood retrieved by cardiotomy suction was returned into the cardiopulmonary bypass reservoir in all patients.

Blood cardioplegia means a mixture of clear hyperkalemic solution (table 2.) and oxygenated blood from the oxygenator in volume ratio of 1:9 to 1:30. After aortic cross-clamping, blood cardioplegia was initially delivered into the aortic root, and induction of asystole was facilitated by 5 to 15 ml of potassium-magnesium cardioplegia concentrate solution (KCL and MgCl₂ 0.8 mmol/ml) (Kardioplegia-K™, Medipolar, Oulu, Finland) (I) or 10 to 20 mmol KCL (II-IV). After cardiac arrest, blood cardioplegia was delivered continuously in a retrograde fashion via the coronary sinus, and whenever possible, antegrade delivery via the venous grafts (CABG) and/or coronary ostiums (AVR) was also used. The cardioplegic temperature was raised to 36°C at 5 minutes ("hot shot") before the removal of the aortic cross-clamp.

4.3 Hemodynamic support and postoperative care

All patients were primarily weaned off CPB without inotropic or vasopressor agents, but dobutamine and noradrenaline were used primarily as needed. The goals of hemodynamic support were to maintain the cardiac index (CI) above 2.2 l/min/m² and mixed venous oxygen saturation above 58% after perfusion and during the ICU stay. These goals were achieved with dobutamine following an adequate preload (pulmonary capillary wedge pressure 8-14 mmHg), afterload and heart rate optimization. Hypotension (mean arterial pressure below 65 mmHg) was treated with noradrenaline. Bradycardia (heart rate below 65) was treated with temporary ventricular pacing if the cardiac index was not sufficient. Propofol (1mg/kg/h) and oxycodone (0.03 mg/kg/h) infusions were continued in the intensive care unit until the patient was considered ready for extubation. The criteria for extubation were central temperature above 36.0°C, adequate spontaneous ventilation, co-operation, stable hemodynamics and absence of significant bleeding. The patients were

transferred into a surgical ward when their vital signs were stabilized and they no longer needed ventilatory or hemodynamic support.

4.4 Experimental protocols

The experimental protocols and their relation to laboratory sample collection are presented in the figure 2.

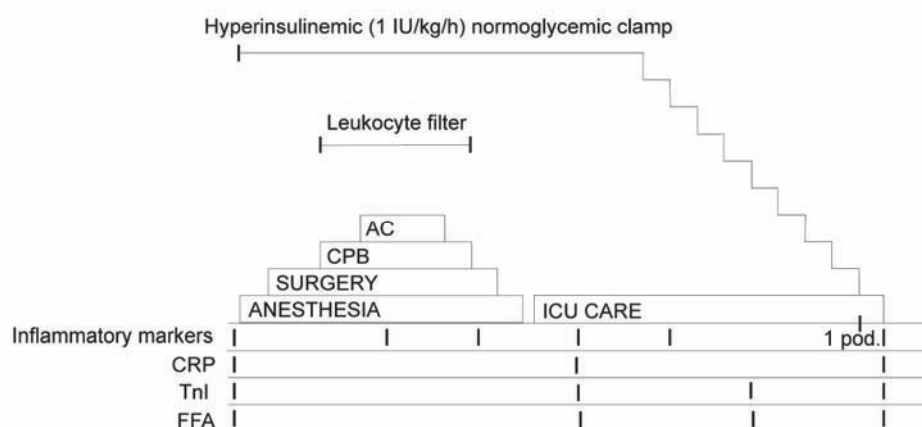


Fig. 2. Experimental protocols. AC = Aortic cross-clamp, CPB = Cardiopulmonary bypass, FFA = Free fatty acids, ICU = Intensive care unit, Inflammatory markers = Total leukocyte and neutrophil count, neutrophil adhesion molecules, proinflammatory cytokines, 1 pod. = First postoperative day, TnI = Troponin-I.

4.4.1 Normoglycemic hyperinsulinemic clamp technique (I, II)

The modified normoglycemic hyperinsulinemic clamp technique was primarily adopted from the Department of Cardiothoracic Surgery, Linköping Heart Center, University Hospital, Sweden in the mid-1990s (Svedjeholm *et al.* 1995), to be used as an adjunctive therapy in high-risk cardiac surgery. The high-dose GIK therapy consisted of separate glucose and insulin infusions. After the induction of anesthesia, 30% glucose solution (1000 ml with 20 mmol KCL and 20 mmol Mg) at a rate of 1.5 ml/kg/h (0.45 g/kg/h) and fast-acting insulin (Insulin Actrapid®, Novo Nordisk A/S, Denmark) infusion (5 IU/ml) at a dose of 1 IU /kg/h were started via the central venous line. The blood glucose levels were targeted to 6-10 (I) and 6-8 mmol/l (II) by adjusting the glucose infusion rate. Withdrawal of insulin infusion was begun six hours (I) or right after (II) the admission into the ICU at a rate of 5 IU per hour, and the glucose infusion rate was decreased gradually according to the blood glucose levels. The control group was started on 0.9% saline (1.5 ml/kg/h, 1000 ml with 20mmol KCL and Mg 20 mmol), and 4-8 IU of short-acting insulin was administered intravenously whenever the blood glucose levels

exceeded 10 (I) or 8 (II) mmol/L. After the termination of cardiopulmonary bypass, heparin was antagonized with protamine sulfate (3mg/kg).

4.4.2 Blood glucose and plasma potassium control (I, II)

Arterial blood glucose levels were measured with the ACCU-CHEK® Sensor (Roche Diagnostics Corporation, 9115 Hague Road, Indianapolis, USA) whole-blood bedside strip test. After the start of high-dose insulin treatment, blood glucose levels were checked at every 15 minutes during the first hour and after that at every half hour until the end of the operation. In the control group, blood glucose levels were checked once per hour during the operation. After the admission into the ICU, arterial blood glucose levels were checked at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, and 28 hours in both groups.

Plasma potassium concentrations were maintained within a range from 3.0 to 5.0 mmol/l, and supplemental potassium boluses were administered as necessary.

4.4.3 Leukocyte-depleting filter (III, IV)

The leukocyte-depleting filter (LeukoGuard LG6, Pall Biomedical, Portsmouth, England) used in the studies III and IV was incorporated into the extracorporeal circulation arterial line and primed according to manufacturer's instructions. Cardiopulmonary bypass was performed with a leukocyte filter and the continuous filtration method (filter group) or without any filter (control group).

4.5 Laboratory analyses

4.5.1 Blood sample collection

The design of laboratory sample collection is presented in table 3.

Table 3. Laboratory sampling design.

Study	Laboratory marker	Time of measurement
I	FFA, TnI,	0 and 8 hours after ICU admission and 1 st postoperative morning
II	CRP	before anesthesia induction, after ICU admission, 1 st and 3 rd postoperative morning
	IL-6, IL-8	before anesthesia induction, 45 minutes after the start of CPB, 5 minutes, 2 and 18 hours after the end of CPB
	FFA, TnI	before anesthesia induction, 0 and 8 hours after ICU admission and 1 st postoperative morning
III, IV	CRP	before anesthesia induction, after ICU admission, 1 st and 3 rd postoperative morning
	IL-6, IL-8	before anesthesia induction, 45 minutes after start of perfusion, 5 minutes, 2, 4 and 18 hours after the end of perfusion
	neutrophil count	
	neutrophil CD11a,CD11b,CD62L	
	TnI	before anesthesia induction, 0 and 8 hours after ICU admission and 1 st postoperative morning

4.5.2 Neutrophil adhesion molecules and cytokine analysis

Blood samples for cytokine analysis and neutrophil adhesion molecules (III, IV) were drawn from the radial artery line and collected into tubes containing acid-citrate-dextrose (Baxter Healthcare, Thetford, England) as an anticoagulant. Each tube was immediately cooled in an ice-cold water bath to prevent *ex vivo* neutrophil activation and subsequently kept at 0°C until staining of neutrophils for flow cytometry within 24 hours. Plasma was separated from each specimen within 4 hours of sampling and stored at –70°C for cytokine analysis.

Neutrophil surface antigens were stained with the whole blood technique and the direct immunofluorescence method using a fluorescence flow cytometer analyser (FACS). Monoclonal antibodies (BD Biosciences, San Jose, USA) were used as follows: anti-CD16b (neutrophils), anti-CD11a (integrin α_L chain), anti-CD11b (integrin α_M chain), anti-CD62L (L-selectin) and appropriate isotype control antibodies. Cells were measured on a dual-laser flow cytometer (FACSCalibur, BD Biosciences), and data were analysed by the CellQuest software package. CD16b-positive neutrophils were gated and analysed for the expression of CD11a, CD11b and CD62L antigen. The results are expressed as geometric mean of the fluorescence intensity (channel number) of the FITC- or PE-positive histograms.

Interleukin-6 (IL-6) and interleukin-8 (IL-8) concentrations (pg/ml) were determined by the enzyme immunoassay method according to the manufacturer's instructions using commercially available ELISA kits (DuoSet, R&D Systems, Minneapolis, USA).

4.5.3 Other laboratory analyses

CRP levels were measured by ADVIA® 2400 Chemistry Systems (Bayer HealthCare LLC, Benedict Avenue, NY, USA). Serum cardiac troponin-I (TnI) concentrations were measured by AxSYM® (Abbot laboratories, USA) (I, II) or Innotrac Aio!™ (Innotrac Diagnostics OY, Turku, Finland) (III, IV) systems and free fatty acids (FFA) by JC-2401 PC (Shimazu, Kyoto, Japan). Arterial blood lactate (Rapidlab 865, CIBA CORNING Diagnostics Corp.) and arterial and mixed venous blood gases were analyzed before the induction of anesthesia (II -IV) and at 0, 4 and 8 hours postoperatively and on the first postoperative morning (I-IV).

4.6 Other measurements

Cardiac index was measured using a thermodilution method and systemic vascular resistance (SVRI) and pulmonary vascular resistance indices (PVRI) were calculated using the standard formulae (I- IV). The mean and total doses and the length of administration of inotropic and vasopressor agents and the use of an intra-aortic balloon pump during the operation and the ICU stay were recorded (I, II). Atrial fibrillation and severe arrhythmias were recorded. Electrocardiograms were obtained for evaluation before the operation, immediately after the operation, on the first postoperative day and in the surgical ward before discharge, and they were assessed by an experienced cardiologist blinded to the patient's treatment group. Myocardial infarction was defined as new Q-wave, depression of the R-wave combined with the rise of serum TnI above 60 (µg/l) (I, III) and 5.0 (µg/l) (II, IV).

4.7 Statistical analysis

Statistical analysis was performed using the SPSS statistical software (SPSS 12.0.1, SPSS Inc., Chicago, IL, U.S.A) and SAS (version 9.1, SAS Institute Inc., Gary, NC., USA) (II, IV). Summary statistics for continuous and ordinal variables were expressed as median with the 25th and 75th percentiles. The groups were compared by t-test or Mann-Whitney U-test when the t-test assumption (approximate normality) was not met. Fisher's exact test was utilized for categorical data. Area under the curve (AUC) was calculated for the average administration rate (µg/kg/min) of dobutamine and noradrenaline for each patient from the start of the operation to the 2nd postoperative morning (I). For repeatedly measured data the analysis of variance for repeated measurements (I, III) or the linear mixed model was utilized (II, IV) (Brown & Prescott 2006). For the latter method, complete independence was assumed across subjects. If the measurement distribution was not normal, log-transformation was used ($\log(x)$ or $\log(x+1)$), using the latter if zero values occurred (II). If the measurements were done at uneven time intervals, the spatial covariance structure was defined for the R matrix, with the even time intervals first-order autoregressive covariance matrix defined. Reported p-values are as follows: p_g , indicates

the level of difference between groups, p_{t*g} , indicates the interaction between groups and time. The correlation between continuous variables was assessed by Spearman's correlation coefficients (III). Linear regression was used to assess the impact of different parameters on the postoperative levels of troponin I (III). Two-sided p-values are reported.

5 Results

5.1 Perioperative characteristics

In all of the papers, the study and control groups were comparable with regard to the main intraoperative characteristics (Table 4.), and no marked differences in the duration of postoperative mechanical ventilation or the length of ICU or surgical ward stay between the groups were detected during the postoperative period. There was one death in the control group in study I on the 21st postoperative day due to mesenterial thrombosis and subsequent multi-organ failure.

Table 4. Main intraoperative patient characteristics.

Study	Group	Ischemic time (min)	Cross-clamp time (min)	CPB time (min)
I	GIK	18 (11)	135 (33)	176 (40)
	Control	26 (16)	152 (29)	186 (32)
II	GIK	26 (11)	93 (22)	121 (28)
	Control	22 (19)	84 (13)	109 (14)
III	Filter	18 (7)	81 (21)	106 (23)
	Control	24 (12)	87 (8)	102 (11)
IV	Filter	18 (14)	146 (17)	183 (21)
	Control	16 (9)	134 (31)	175 (37)

CPB = cardiopulmonary bypass, min = minutes. Data are presented as mean and standard deviation (SD)

Table 5. Postoperative data (n = 119)

Study	Group	n	Death	MI	AF	Pulmonary infecton	Renal failure	Cerebral infarction	ICU stay > 3 days	Inotropic medication	Vasopressor medication
I	GIK	20	0	1	12	2	1	0	4	14	18
	Control	20	1	2	14	1	1	0	2	17	16
II	GIK	19	0	1	5	1	0	1	2	2	13
	Control	20	0	1	6	2	2	0	2	6	16
III	Filter	10	0	0	5	0	0	0	2	3	7
	Control	10	0	1	3	0	0	0	0	2	5
IV	Filter	10	0	1	6	1	1	0	2	3	4
	Control	10	0	1	5	2	1	0	2	8	7

AF = atrial fibrillation, ICU = intensive care unit, MI = myocardial infarction.

5.2 Metabolic effects of high-dose glucose-insulin treatment (I, II)

The main metabolic effects of high-dose insulin treatment in the studies I and II are presented in table.

Table 6. Metabolic effects of high-dose insulin treatment during the ICU stay.

Variable	Study I (n = 20)	Study II (n = 19)
Targeted blood glucose level	6-10 mmol/L	6-8 mmol/L
Mean blood glucose during ICU stay	8.2 (0.6)	7.1 (0.5)
Number of hypoglycaemic events recorded	3	1
Average glucose infusion rate during 24 hours	4.9 mg •kg-1•min-1	4.2 mg •kg-1•min-1
Mean value for daily glucose uptake	7.1 g •kg-1	6.1 g •kg-1
Mean value for daily caloric intake	28 kcal •kg-1	24.4 kcal •kg-1
VO ₂ (ml •min-1) *	215 (43)	210 (50)
VCO ₂ (ml •min-1) *	197 (48)	207 (46)
RQ*	0.89 (0.13)	0.99 (0.06)

RQ = mean respiratory quotient, VCO₂ = mean total-body carbon dioxide production, VO₂ = mean total-body oxygen consumption, * = during mechanical ventilation. Data are presented as mean and standard deviation (SD) unless otherwise stated.

Blood glucose levels in the studies I and II are presented in the figure. In study I, high-dose glucose-insulin treatment led to lower blood glucose levels ($p_g < 0.001$) and better maintenance of normoglycemia during the ICU stay as compared to the standard insulin treatment protocol in the controls. In study II, glucose levels were comparable during the ICU stay ($p_g = 0.5$), although some interaction between group and time was detected ($p_{t*g} < 0.001$). In study II, elevated blood glucose values above the targeted range (6-8 mmol/L) were recorded in both groups, but no clear trend towards marked hyperglycemia

was recorded. The number of hypoglycaemic effects was low and the lowest blood glucose value recorded was 2.8mmol/L. Both studies demonstrated a profound attenuation in free fatty acid ($p_g < 0.001$) levels during the ICU stay as compared to the control group.

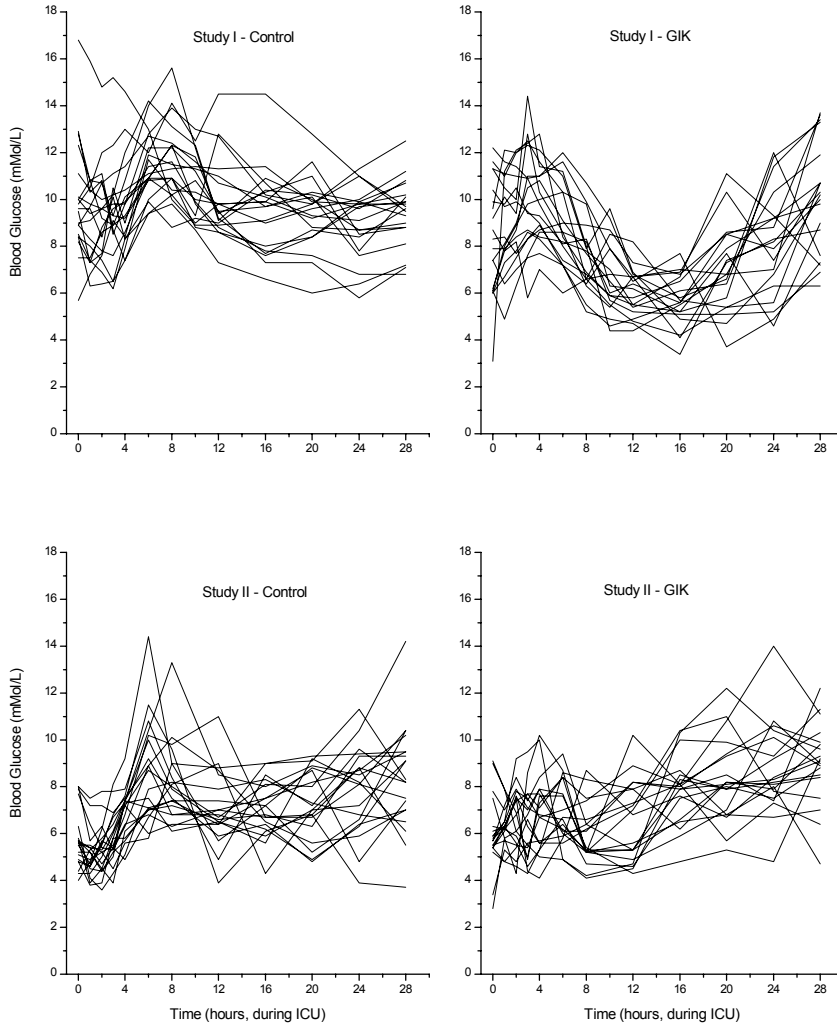


Fig. 3. Blood glucose levels for each patient in the ICU during the study period (I, II).

5.3 Markers of systemic inflammation

5.3.1 Cytokine analyses (II, III, IV)

In general, all of the studies showed a wide degree of interindividual variation even in the baseline systemic cytokine levels. In study II, high-dose glucose-insulin treatment did not have evident effects on IL-6 ($p_g=0.8$) and IL-8 ($p_g=0.2$) levels during the perioperative period.

Serum cytokine levels in the studies III and IV are presented in figure 4. In study III, higher IL-8 levels were detected 5 minutes after the termination of CPB ($p=0.03$), but the difference between the groups was not statistically significant over the whole study period ($p_g=0.08$). In study IV, one control patient had over tenfold baseline IL-6 and IL-8 levels compared to the other patients, and the p -values are hence presented without this outlier (see figure 4.). There was enhanced IL-6 elevation with time in the filter group 5 minutes and 2 hours after the termination of CPB ($p_g = 0.09$, $p_{I^*g} < 0.001$). The elevation in IL-8 levels was also faster at these same measuring points ($p_g = 0.16$, $p_{I^*g} = 0.007$).

5.3.2 Leukocyte counts and neutrophil adhesion molecules (III, IV)

The levels of neutrophil adhesion molecules are presented in figure 5. The use of a leukocyte filter failed to reduce the total leukocyte and neutrophil counts in both studies during the study period. Instead, a marked increase in neutrophil adhesion molecule CD11b expression was detected in the filter group after 45 minutes of CPB, lasting for up to 5 minutes after CPB in both study III ($p_g = 0.09$, $p_{I^*g} < 0.001$) and study IV ($p_g = 0.003$, $p_{I^*g} < 0.001$). Thereafter, the levels paralleled and returned to the baseline in both groups. The neutrophil adhesion molecule CD11a and L-selectin (CD62L) expression rates were not affected by the filter.

5.3.3 C-reactive protein (II, III, IV)

High-dose glucose-insulin treatment was associated with significantly lower average C-reactive protein release in study II (23.8 vs. 40.1 mg/L, $p=0.008$) (II). The difference in CRP levels vanished on the third postoperative day after the discontinuation of insulin treatment and strict control of glucose levels. None of the studies with leukocyte filter (III-IV) showed marked effects in this respect ($p_g=0.33$ and $p_g=0.12$, respectively).

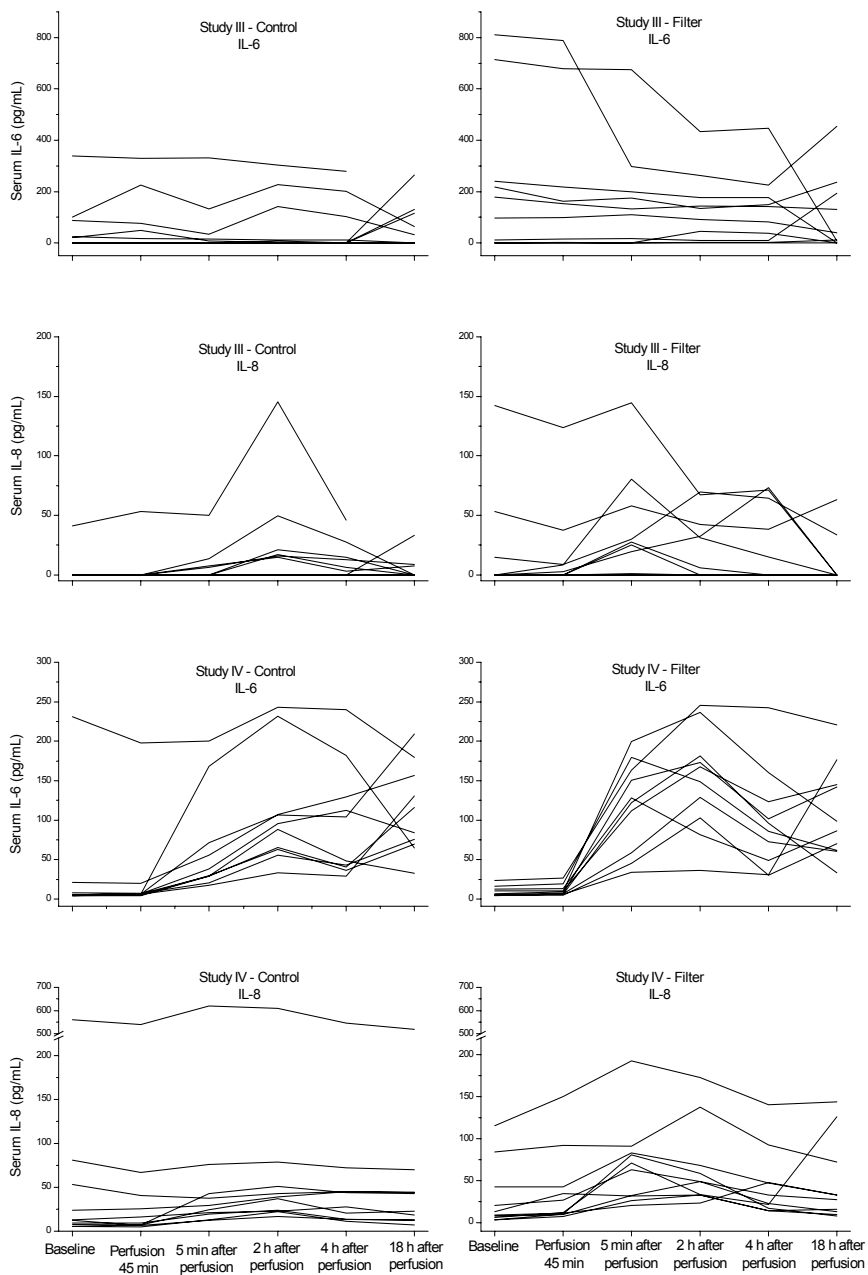


Fig. 4. Individual serum cytokine responses in the studies III and IV. IL-6 = interleukin-6, IL-8 = interleukin-8.

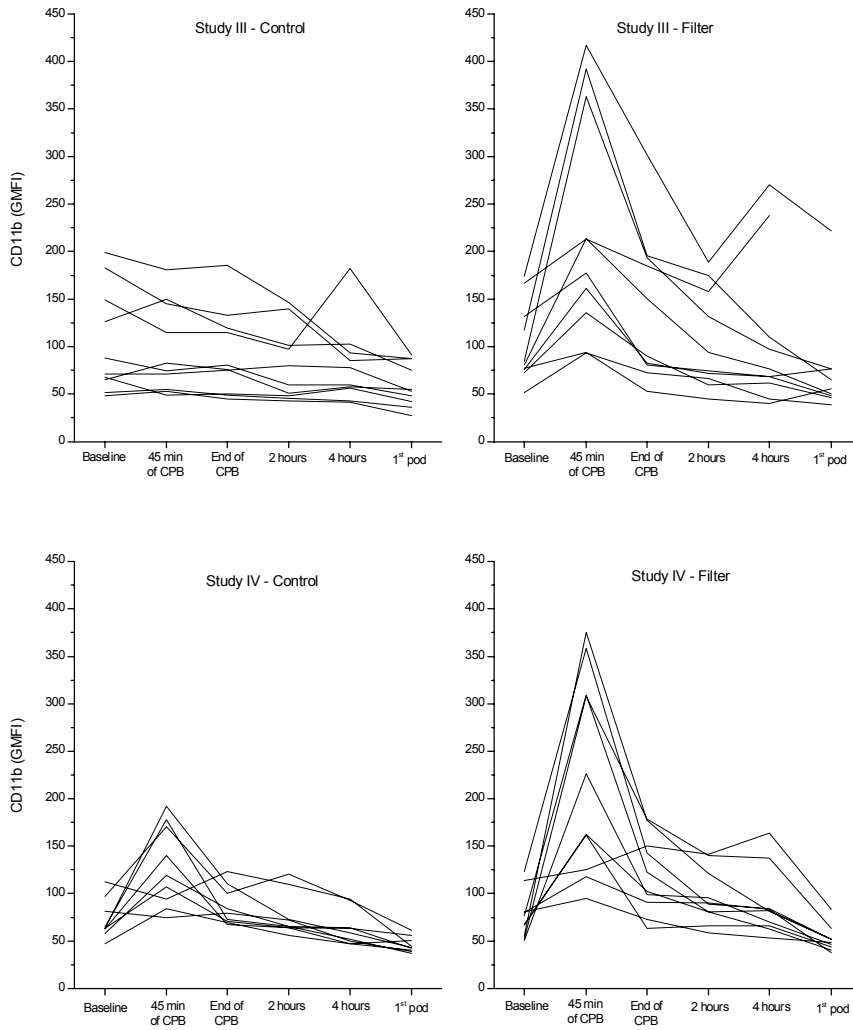


Fig. 5. Individual neutrophil CD11b expression in studies III and IV. GMFI = geometric mean of fluorescence intensity.

5.4 Markers of myocardial ischemia-reperfusion injury

5.4.1 Myocardial contractile function (I-IV)

High-dose insulin treatment did not have remarkable effects on cardiac indexes (I-II) but decreased the need for dobutamine medication (dobutamine) in study I (AUC 4.6 (SD 8.1) vs. 21.8 (SD 30.2), $p=0.014$). Similar decrease in the need for inotropic medication was not detected in study II. Both leukocyte filter studies failed to show a beneficial effect in this respect.

5.4.2 Myocardial infarction and enzyme release (I-IV)

The mean values for postoperative TnI release in the studies I-IV are presented in figure 6. None of the high-dose insulin studies showed a difference in the incidence of myocardial infarction between the study groups, and no marked postoperative effects on TnI release were detected (I, II) ($p_g=0.15$ and $p_g=0.5$, respectively). The use of leukocyte filter attenuated TnI release on the first postoperative day after normal coronary artery revascularization (III) ($p_g=0.024$), but failed to have a similar effect after combined aortic valve and coronary artery revascularization (IV) ($p_g=0.27$). No differences were seen in the incidence of postoperative myocardial infarctions (III, IV).

5.4.3 Rhythm disturbances (I-IV)

The incidence of atrial fibrillation was higher in patients admitted for combined surgery in the studies I (65%) and IV (55%) than in patients scheduled for only CABG in the studies II (40%) and III (28%). Neither high-dose insulin treatment nor the use of a leukocyte filter showed any evident effect on the incidence of atrial fibrillation. No other severe rhythm disturbances were detected during the ICU stay.

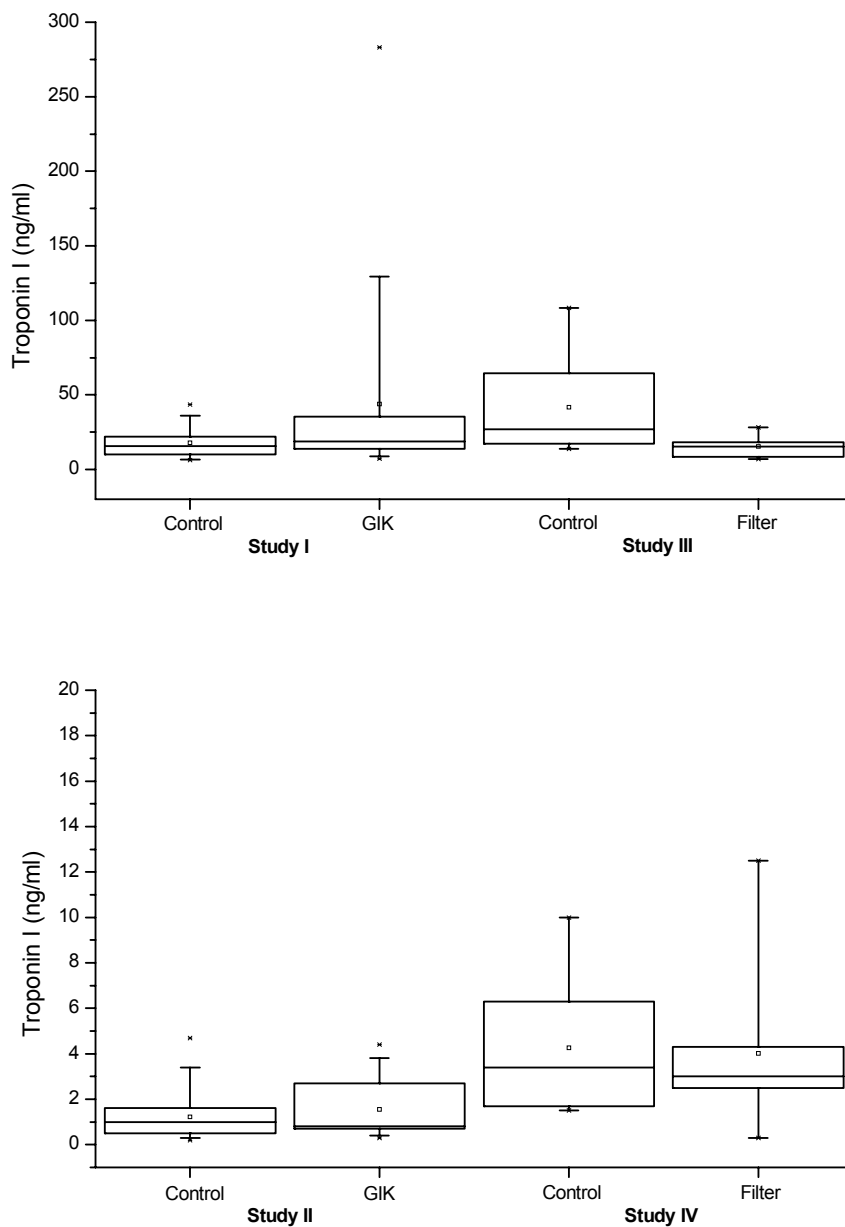


Fig. 6. Box-plots for postoperative TnI release in the studies I-IV. The values outside the whiskers are considered outliers. Due to a change in laboratory assays, the TnI values from the studies I and III are not comparable to those obtained in the studies II and IV. TnI = cardiac troponin-I.

6 Discussion

6.1 Strengths and weaknesses of the study

Numerous methods and pharmacological agents have been used in attempts to prevent cardiac surgery induced systemic inflammatory response and myocardial ischemia-reperfusion injury. The majority of studies have explored the effects on different laboratory markers and physiological scores. Demonstration of the effects on conventional clinical outcome variables such as mortality requires a large number of patients for results of clinical and statistical significance. For example, to detect a 2 percent decrease in mortality (from 5 to 3 percent, $\alpha=0.05$, $\beta=0.9$), approximately 2011 patients per each treatment group would be needed. In this study formal Power analysis was not done. Since most cardiac surgery nowadays involves uncomplicated perioperative course, it is evident that the potentially beneficial effects of different adjuvant strategies are only seen in complex operations with prolonged CPB times and in patients with elevated perioperative risk (Gott *et al.* 1998, Grover *et al.* 1999). However, the enrolment of a representative patient group with some particular risk factor for a single centre trial is difficult, due to different patient characteristics and variable comorbidities. This was also evident on the basis of the review of current papers with both treatment strategies for this study. The enrolment of patients into the first high-dose insulin study (I) took two years, and the other period of enrolment was one year per study, and even then the number of patients enrolled was relatively small. On the other hand, multi-centre trials may be potentially biased by differences in perioperative techniques and treatment protocols, while a single-centre protocol allows for more uniform patient treatment.

6.1.1 Methodological considerations

The methods of myocardial protection, the use of other adjunctive strategies and the postoperative treatment were standardized in each study but varied between different studies, depending on the practice in use in our institution at each time. All studies were

carried out as unblinded, which may introduce bias. In insulin studies, this was the inevitable practice due to safety issues and the high insulin dose used. In the leukocyte filter studies, leukocyte activation was the primary end point, and the measurements of leukocyte adhesion molecules and cytokines were performed blinded to the patient's treatment group after the end of the study period.

Cytokine analyses and leukocyte adhesion molecules were measured with established laboratory assays by professionals experienced in such laboratory work. Although serum cytokine levels are widely used to monitor the extent of inflammatory response during and after cardiac surgery, the reports have shown inconsistent results and several confounding factors. Perioperative measurements of cytokines has been shown to be influenced by several factors such as hemodilution (Roth-Isigkeit *et al.* 1999), hypothermia (Grunenfelder *et al.* 2000), cardiotomy suction (Westerberg *et al.* 2004) and adjuvant medication (Tassani *et al.* 2000) or to be dependent on the site and the methods of measurement (Visser *et al.* 2005). Furthermore, cytokine responses have shown wide interindividual variation (Misoph & Babin-Ebell 1997), which also became clear in the current study with wide variation of proinflammatory cytokine levels at baseline already (II-IV).

Due to the changed clinical practice in our institution, phosphorylcholine-coated CPB circuits were used in paper II and IV. The use of heparin- or phosphorylcholine-coated CPB circuits potentially affects inflammatory response and its markers (De Somer *et al.* 2000, Hamada *et al.* 2001). Similarly, aprotinin administration has been shown to attenuate the inflammatory mediators, which may have interfered with the cytokine analyses in study II (Tassani *et al.* 2000). However, aprotinin was not used in the studies III-IV, because of its potential effects on leukocyte surface antigens (Asimakopoulos *et al.* 2000). In this study, normal arterial line filters were not used and the blood retrieved by cardiotomy suction was returned into the cardiopulmonary bypass reservoir. Retransfusion of cardiotomy suction and mediastinal shed blood has been shown to contribute to the postoperative proinflammatory cytokine response (Westerberg *et al.* 2004) and to influence vascular resistance and hemodynamics (Westerberg *et al.* 2006).

Cardioprotective effects were measured as the prevention of clinical manifestations of ischemia-reperfusion injury, which includes myocardial cell injury, contractile dysfunction and arrhythmias (Bolli *et al.* 2004). Due to the manufacturer's decision to change the production of cardiac troponin-I assays, cardiac troponin-I levels were analyzed with two different laboratory methods; the AxSYM® system (Abbot laboratories, USA) was used in the studies I and III and Innotracs Aio!™ (Innotrac Diagnostics OY, Turku, Finland) in the studies II and IV. Hence, TnI levels are not directly comparable between the studies. Electrocardiograms were evaluated by an experienced cardiologist blinded to patient allocation in each study.

6.1.2 Safety issues

In our institution, high-dose insulin treatment has been used as occasional adjunctive therapy for years in the treatment of complicated cardiac surgical cases, and the members of the cardiac, anesthesia and ICU teams are therefore well trained to use it and to avoid hypoglycaemic events. This was also demonstrated in our studies, where the number of hypoglycaemic events was low. The most high-risk phase with regard to hypoglycaemia seems to be the first few hours after the admission into ICU, which has also been noted by other authors using low-dose insulin infusion (Chaney *et al.* 1999). This is obviously due to the decrease in insulin resistance after the termination of the surgical stimulus and the simultaneous increase in the need for glucose to maintain normoglycemia (Greisen *et al.* 2001). After the termination of insulin administration, the effects of the drug continue for several hours if large doses are used (Doenst *et al.* 2003). This requires gradual withdrawal of glucose infusion and blood glucose controls for safety reasons. In the first insulin study (I), withdrawal of insulin infusion was begun six hours after the admission into ICU, which delayed the transfer to the surgical ward by some hours in one fourth of the cases, due to the prolonged need for glucose infusion and blood glucose level controls, although no differences in the ICU stay time were detected. To avoid delayed withdrawal, the high-dose insulin protocol was changed in the second insulin study by starting the unloading of insulin after the admission into ICU (II), and no cases of delayed transfer occurred in this respect. In general, the normoglycemic hyperinsulinemic clamp technique seems to be a smooth way to adjust blood glucose levels, which has also been shown by other investigators (Szabo *et al.* 2003, Carvalho *et al.* 2004, Visser *et al.* 2005). The average glucose infusion rates in both studies were in concordance with previous studies on high-dose glucose-insulin treatment (Nilsson *et al.* 1985, Szabo *et al.* 2003). Despite the increased systemic carbon dioxide production and the elevated RQ levels, the time of mechanical ventilation did not differ between the study groups (I, II).

No technical problems, such as increased CPB arterial line pressures, reported to be associated with the leukocyte filter use were detected (Smit *et al.* 1999).

6.2 Markers of systemic inflammatory response

6.2.1 Cytokine and C-reactive protein levels (II)

Several studies on GIK in both the treatment of myocardial infarction (Mehta *et al.* 2005) and cardiac surgery (Lazar *et al.* 1997) have failed to neutralize the proinflammatory effect of hyperglycemia induced by the treatment protocol, which might also have revealed the potentially beneficial direct effects of insulin. Thus, the second insulin study in patients with unstable angina pectoris aimed to avoid the interfering effect of hyperglycemia in either study group. Although the targeted blood glucose levels were set above the level recommended by van Den Berghe *et al.* (2001) and were reached only with moderate success, they were mainly comparable during the study period.

In paper II, high-dose insulin treatment failed to affect the systemic proinflammatory cytokine response, but showed a marked reduction on the first postoperative day CRP levels. Similar results were recently presented by Visser *et al.* (2005) with an insulin dose tenfold lower than ours. Our results and those presented by Visser *et al.* (2005) do not support the hypothesis of the ability of insulin to inhibit the proinflammatory cytokine response triggered by CPB and cardiac surgery. Interestingly, Hoedemaekers *et al.* (2005) also showed no effect on proinflammatory cytokine levels with strict glycemic control and insulin therapy after elective CABG surgery, suggesting that the beneficial effects of strict glycemic control are not due to changes in cytokine balance. However, these findings are in conflict with several experimental studies showing attenuation of the proinflammatory cytokine response by insulin (Brix-Christensen *et al.* 2004, Jeschke *et al.* 2004).

Elevated CRP levels have been shown to be associated with poor prognosis in patients with unstable angina pectoris (Liuzzo *et al.* 1994), acute myocardial infarction with primary coronary intervention (Yip *et al.* 2005) and CABG operation (Biancari *et al.* 2003). The significance of CRP attenuation with insulin treatment in the current study and that reported by Visser *et al.* (2005) remains unclear. However, the attenuation of CRP levels vanished after the termination of insulin treatment. In the setting of acute myocardial infarction, low-dose insulin treatment decreased CRP release, which was accompanied by attenuation of TnI levels. This is in contrast to our results and those of Visser *et al.* (2005), which showed no beneficial effects on the markers of myocardial ischemia-reperfusion injury and systemic inflammatory response. It is not known whether CRP serves only as a marker of the severity of the inflammatory process or whether it really plays an active role as a mediator, serving as a target to potential therapies, as it has been shown in recent experimental studies (Bisoendial *et al.* 2005).

6.2.2 Neutrophil adhesion molecules (III, IV)

Both of our trials with leukocyte filter demonstrated similarly significant elevation of leukocyte adhesion molecule CD11b expression in the leukocyte filter group after 45 minutes of CPB. Thereafter, the differences in CD11b levels slowly levelled off, probably due to margination and extravasation of activated cell forms. Expression of CD11b is considered a reliable indicator of leukocyte activation, and it has been shown to play an important role in leukocyte adherence and transmigration (Paugam *et al.* 1997). The increase in CD11b is associated with the occurrence of organ system failures by some authors (Rinder *et al.* 2003) and presented as a therapeutic target to diminish inflammation after cardiac surgery (Ilton *et al.* 1999), which has also been achieved in some trials by the administration of aprotinin and corticosteroids (Hill *et al.* 1994, Asimakopoulos *et al.* 2000). Similarly, increased CD11b expression by the filter was shown by Ilmakunnas *et al.* (Ilmakunnas *et al.* 2005), whereas opposite findings were presented by Chen *et al.* (Chen *et al.* 2001). One potential explanation for the conflicting results is that Chen *et al.* used a normal arterial line filter in the control group. The opinions and practices to use a normal arterial line filter vary between the centres across the Atlantic, and most studies have compared the leukocyte filter to such a filter, to

evaluate the effect of leukodepletion separately from the filtration effect (Whitaker *et al.* 2001). The effects of a normal arterial line filter on leukocytes are largely unclear, but the use of heparin-coated circuits has been shown to reduce the adhesion of cellular material to such a filter (Borowiec *et al.* 1993). In our institution, normal arterial line CPB filters are not routinely used during CPB, and no filters were thus used in the control group. However, the increased leukocyte CD11b expression combined with the markedly enhanced proinflammatory cytokine response in both of our studies are clear proof of enhanced leukocyte activation and a proinflammatory effect of the filter when used throughout the CPB period. Furthermore, Ilmakunnas *et al.* (2005) demonstrated increased CD11b expression across the filter line five minutes after the initiation of CPB already. Our findings are also supported by several studies which have demonstrated an increase of leukocyte degranulation products when a filter is used (Mihaljevic *et al.* 1995, Mair *et al.* 1999, Scholz *et al.* 2002, de Vries *et al.* 2003, Whitaker *et al.* 2006). Although these substances may also be released by captured leukocytes in the filter, they can hardly be considered beneficial. The clinical importance of elevated CD11b cannot be concluded based on these small studies, but these findings do not support the anti-inflammatory effect of the leukocyte filter.

6.3 Markers of myocardial ischemia-reperfusion injury

6.3.1 High-dose insulin studies (I, II)

Despite the metabolic (Cave *et al.* 2000), anti-apoptotic (Jonassen *et al.* 2000, Gao *et al.* 2002) and direct cardioprotective effects (Sack *et al.* 2003) demonstrated in experimental trials, our high-dose insulin treatment had no beneficial effects on myocardial enzyme release, as used in our current strategies of myocardial protection. The myocardial enzyme release after cardiac surgery reflects the amount of damage to the myocardium, irrespective of the mechanism of injury, and TnI is commonly considered a reliable marker of this (Alpert *et al.* 2000). Considering the fact that GIK is usually primarily intended to attenuate myocardial ischemia-reperfusion injury, there is a surprisingly small number of studies reporting the levels of myocardial injury markers or showing any benefit of treatment in this respect. Recent studies with low-dose GIK have also yielded controversial results (Bruemmer-Smith *et al.* 2002, Quinn *et al.* 2006). However, insulin administration has been shown to increase coronary blood flow during ischemia, and the possible effects of insulin treatment and potential coronary vasodilatation on cardioplegic protection and the washout of myocardial enzymes are unclear (Eberli *et al.* 1991, Groeneveld *et al.* 1992, McNulty *et al.* 2000).

Paper I demonstrated a decreased need for inotropic support due to the better preserved cardiac indexes of patients admitted for combined valve and CABG surgery. No such phenomenon was seen in patients with unstable angina, probably due to the overall smaller need for inotropic support. It seems obvious that the better preserved myocardial function occurred independently of metabolic or cardioprotective actions, since no effects on myocardial enzyme release were detected. Our results are in line with

several experimental (Tune *et al.* 1998, Doenst *et al.* 1999) and clinical studies showing improved myocardial performance following glucose-insulin treatment independent of dosage (Svedjeholm *et al.* 1995, Wistbacka *et al.* 1995, Lazar *et al.* 1997, Lazar *et al.* 2000, Quinn *et al.* 2006). The mechanism underlying the insulin-mediated increase in myocardial function has not been clearly defined. It remains unclear whether this was due to a direct inotropic effect or an afterload reduction, since calculation of afterload is not possible with the methods used in this study (Aittomäki & Salmenperä 1997). However, no differences in cardiac filling pressures were detected. Both studies demonstrated marked reduction in SVRI levels, although they reached statistical significance only in paper II. The number of patients treated with vasopressor medication did not differ between the groups.

Increased FFA levels have been shown to reduce myocardial contractility and to increase the incidence of arrhythmias (Oliver & Opie 1994). Although there was a profound suppression of FFAs in both studies, no differences were detected in the incidence of atrial fibrillation. The recent GIK studies have also shown conflicting results in this respect (Lazar *et al.* 2000, Bruemmer-Smith *et al.* 2002)

6.3.2 Leukocyte filter studies (III, IV)

Previous studies with a leukocyte filter and a continuous filtration strategy have shown controversial results about the markers of myocardial ischemia-reperfusion injury with attenuation in myocardial enzyme levels and need for inotropic medication (Chiba *et al.* 1993, Hachida *et al.* 1995) or with no effect on these parameters (Whitaker *et al.* 2006). The mechanism of filter-induced attenuation of TnI release in elderly patients with normal CABG operation (III) remains unclear, but no similarly beneficial effect was seen in patients with combined surgery and prolonged CPB times (IV). However, the myocardial enzyme release after combined valve and CABG surgery is also affected by surgical trauma, which may decrease the specificity of TnI as a marker in this respect. It seems clear that these potentially beneficial effects on myocardial ischemia-reperfusion injury were due to the filter effect and not associated with the leukocyte capture, similarly to the findings of Rimpiläinen *et al.* (2000) with an experimental chronic porcine model. They may even be chance errors due to the small number of patients or attributable to statistical error.

6.4 Clinical implications and further investigations

According to this study, our high-dose insulin protocol is safe, but provides strict control of the blood glucose level. In the first paper, the main finding was the decreased need for inotropic support after combined aortic valve and CABG surgery, which supports our hypothesis that the best benefit of high-dose glucose-insulin treatment will be achieved in patients with predictable postoperative contractile dysfunction. This is in line with the current literature on various GIK regimens and our own clinical experiences (Wistbacka *et al.* 1995, Besogul *et al.* 1999, Quinn *et al.* 2006). However, if improved contractility

occurs only via some inotropic effect without any other more profound influences on myocardial recovery or systemic circulation, transient postoperative contractile dysfunction is more easily and safely achieved with the use of conventional inotropic drugs. Their effects are better documented and the treatment is more easily and safely adjustable (Ruokonen *et al.* 1993, Parviainen *et al.* 1995). But in contrast to dobutamine, insulin does not appear to increase the myocardial or systemic oxygen demand (Schwenzer *et al.* 1990, Hiemayr *et al.* 1995), and it is thus unlikely that improved contractility occurs at the expense of the myocardial energy demand and ATP reserves (Tune *et al.* 1998). This may be a central factor in patients with a prolonged need for inotropic medication, including patients with compromised cardiac status and reduced ejection fraction. Thus, according to this study, the use of high-dose insulin treatment is indicated in this patient group, but does not offer evident clinical benefits in patients at lower risk.

In addition to postoperative myocardial dysfunction, the systemic inflammatory response induced changes in vasoregulation of the gut region are potentially causative of the emergence of different organ system failures (Uusaro *et al.* 1996, Hessel 2004). The effects of glucose-insulin treatment on splanchnic circulation are largely unclear, although improvement in central mixed and hepatic venous oxygenation without evident effects on the proinflammatory mediators C3a and IL-6 have been demonstrated in patients undergoing heart surgery. (Lindholm *et al.* 2001). In this study, no differences in global markers of tissue perfusion were detected, with the exception of the faster lactate clearance reported in paper I, obviously associated with the better glycemic control in the GIK group. However, this should be evaluated with adequate methods of measurement in further studies, because of the crucial importance of this issue for patients with postoperative cardiac failure.

The insulin effect on CRP is new and interesting but, like other parameters of this kind, should not be interpreted without reference to clinically relevant parameters. However, if the potential ability of insulin treatment to attenuate the inflammatory response is real and of clinical importance, should the treatment possibly be continued for a longer period after the surgery with a lower dose of insulin.

The leukocyte filter is a mechanical device primarily aimed to attenuate the effects of ischemia-reperfusion injury after cardiac surgery in different organ systems. Although the filter is a commercially produced device with a history of over a decade, it is surprising that no large-scale outcome trial have been carried out until recently (Leal-Noval *et al.* 2005, Salamonsen *et al.* 2005, Sutton *et al.* 2005). According to this study, the filter does not perform its primary task and attenuate leukocyte counts but, contrariwise, enhances leukocyte activation, and its use is hence potentially harmful. The recent conflicting findings with regard to leukocyte CD11b expression, clearly point out the importance of precise definitions for the filtration methods used and the study settings in general.

7 Conclusions

Based on this study, the following conclusions can be made

1. The normoglycemic hyperinsulinemic clamp technique offers a reliable method to administer glucose-insulin-potassium (GIK) treatment and to maintain normoglycemia. High-dose glucose-insulin treatment is a safe but laborious method and requires frequent checking of blood glucose levels. Due to the potential danger of hypoglycemia, it cannot be used without intensive care unit or operating theatre level of monitoring. The treatment leads postoperatively to an elevated respiratory quotient without an increase in systemic oxygen consumption but is well tolerated by patients with normal pulmonary capacity. (I, II)
2. High-dose glucose-insulin treatment reduces the need for inotropic support after combined valve and CABG surgery in patients with left ventricular hypertrophy and prolonged CPB time (I). No similar beneficial effect was detected in patients with unstable angina pectoris and well preserved myocardial function and normal CPB time (II). The treatment leads to profound suppression of free fatty acid levels, but does not reduce the postoperative myocardial enzyme release or the incidence of postoperative atrial fibrillation after the cardiac surgery (I, II).
3. High-dose insulin treatment attenuates postoperative CRP release without an evident effect on perioperative proinflammatory cytokine responses (II).
4. The use of a leukocyte filter throughout the cardiopulmonary bypass period does not lead to a marked reduction of leukocyte and neutrophil counts but increases leukocyte activation, as shown by the increased neutrophil CD11b expression in patients with both normal and prolonged CPB times (III, IV).
5. The use of a leukocyte filter does not attenuate, but probably enhances the systemic inflammatory response and the proinflammatory cytokine response, particularly in patients with a prolonged CPB time (IV).

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- I Koskenkari JK, Kaukoranta PK, Kiviluoma KT, Raatikainen MJ, Ohtonen PP & Ala-Kokko TI (2005) Metabolic and hemodynamic effects of high-dose insulin treatment in aortic valve and coronary surgery. *Ann Thorac Surg* 80: 511-517.
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