Cardiopulmonary bypass and the kidney

Studies on patients undergoing cardiac surgery

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Gothenburg 2018

Cover illustration: Rangitoto (Maori for “Bloody Sky”), the volcano dominating the harbor of Auckland, New Zealand, where most of this thesis was written. Photo by Emma Törnroth.

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To Emma, Amos and Jona

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ABSTRACT

Acute kidney injury is a common complication after cardiac surgery with cardiopulmonary bypass (CPB), and has a major impact on morbidity, mortality and costs. The mechanism of CPB-related renal impairment is not fully understood. The aim of this thesis was to describe how CPB affects the kidneys, and whether increased CPB flow might improve renal oxygenation. In addition, we compared the systemic and renal effects of two inotropes in patients with impaired cardiac and renal function.

Methods: In patients undergoing cardiac surgery we used urine measurement of N-acetyl-β-D-glucosaminidase (NAG) to assess tubular cell injury (n=61). Renal vein catheterization was used to study renal blood flow, oxygenation, and filtration during normothermic CPB at 2.5 L/min/m^2 (n=18), and at different CPB flow levels (2.4, 2.7 and 3.0 L/min/m^2) applied in a randomized order (n=17). In 32 patients with cardiac and renal impairment, pulmonary artery and renal vein catheters were used to study the differential effects of levosimendan and dobutamine in a randomized blinded trial.

Results: NAG was elevated already after 30 minutes of CPB, and increased to a six-fold peak early after discontinuation of CPB. In a multivariate analysis, the duration of CPB and the degree of rewarming were independent predictors of peak NAG excretion. Renal oxygenation was impaired during CPB, mainly through reduced oxygen delivery due to hemodilution and renal vasoconstriction. After CPB, renal oxygenation was further impaired due to increased oxygen consumption and inefficient sodium transport. At higher than

normal CPB flow rates, renal oxygen extraction was reduced by 12 – 23 % at an unchanged filtration fraction, indicating that renal oxygenation was improved. In contrast to dobutamine, levosimendan did not only increase cardiac output and renal blood flow, but also increased the glomerular filtration rate by 22%.

Conclusions: Cardiopulmonary bypass impairs renal oxygenation due to renal vasoconstriction and hemodilution during and after cardiopulmonary bypass, accompanied by increased release of a tubular injury marker. The postoperative tubular injury is increased after longer CPB times and higher degree of rewarming. Increasing the CPB flow rate may ameliorate the impaired oxygenation seen during CPB. In patients with heart failure and renal impairment, levosimendan may be the inotrope of choice.

Keywords: cardiac surgery, cardiopulmonary bypass, glomerular filtration rate, renal blood flow, renal oxygenation, tubular injury, N-acetyl-ß-D-glucosaminidase

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SAMMANFATTNING PÅ SVENSKA

Sedan 1950-talet har användning av hjärtlungmaskin där maskinen tar över hjärtats pumpmekanism möjliggjort operation på stillastående hjärta. Tyvärr drabbas upp till 30 % av patienterna av njursvikt efter operationen, vilket leder till ökad kostnad, vårdtid, lidande och dödlighet. Risken för njurskador vid hjärtlungmaskinanvändning har varit känd i decennier, men fortfarande är orsakssambandet inte klarlagt, vilket gör det svårt att ta fram effektiva förebyggande åtgärder. Samtidig hjärt- och njursvikt, ett tillstånd med hög dödlighet, behandlas ibland med hjärtstärkande läkemedel. Syftet med den här avhandlingen var att studera hur hjärtkirurgi med hjärtlungmaskin påverkar njurarna, och att undersöka om ökat blodflöde i hjärtlungmaskinen kan förbättra njurens syresättning. Därtill studerades skillnaderna i effekt på hjärta och njure mellan två hjärtstärkande läkemedel hos patienter med samtidig hjärt- och njursvikt.

I de tre första artiklarna visades bland annat att man redan efter 30 minuter på hjärtlungmaskin ser en njurcellskada, och skadan är som störst ca en timme efter avslutad hjärtlungmaskin. Skadans blir större ju längre tid patienten tillbringar i hjärtlungmaskin och ju större återuppvärmning av kroppen som behövs. Under användning av hjärtlungmaskin sker en spädning av blodet och en omfördelning av blodflödet bort från njurarna, vilket försämrar njurarnas syresättning. Efter hjärtlungmaskin blir njurarnas förmåga att koncentrera urin mer ineffektiv, vilket ökar syrgasbehovet och förvärrar syrebristen i vävnaden. Om man ökar blodflödet i hjärtlungmaskinen förbättras njurarnas syresättning, sannolikt på grund av ökat njurblodflöde och syrgastillförsel. I det fjärde delarbetet visades att båda de hjärtstärkande läkemedlen levosimendan och dobutamin ökar hjärtats pumpförmåga och blodflödet till njurarna hos patienter med hjärt- och njursvikt. Vid behandling med levosimendan sågs dessutom en förbättrad njurfunktion.

Vår slutsats är att användning av hjärtlungmaskin försämrar njurarnas syretillförsel, vilket leder till en syrebristskada i njurarna, som ytterligare förvärras efter avslutad behandling. Denna kunskap kan bidra till förståelsen av njursvikt efter hjärtkirurgi och strategier för att förhindra eller behandla detta allvarliga tillstånd. Ökat blodflöde i hjärtlungmaskin kan förbättra syresättningen, och vi planerar nu en uppföljande jämförande studie av olika blodflödens effekt på postoperativ njurskada och njursvikt. Vid behov av hjärtstärkande behandling hos patienter med hjärt- och njursvikt kan levosimendan förbättra både syresättning och funktion hos njurarna, och bör därför övervägas som förstahandsval.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Lannemyr L, Lundin E, Reinsfelt B, Bragadottir G, Redfors B, Oras J, Ricksten SE.

Renal tubular injury during cardiopulmonary bypass as assessed by urinary release of N-acetyl-ß-D-glucosaminidase.

Acta Anaesthesiologica Scandinavica. 2017;61(9):1075-1083.

II. Lannemyr L, Bragadottir G, Krumbholz V, Redfors B, Sellgren J, Ricksten SE.

Effects of Cardiopulmonary Bypass on Renal Perfusion, Filtration, and Oxygenation in Patients Undergoing Cardiac Surgery.

Anesthesiology, 2017;126(2):205-213.

III. Lannemyr L, Bragadottir G, Hjärpe A, Redfors B, Ricksten SE.

Impact of cardiopulmonary bypass flow on renal oxygenation in patients undergoing cardiac surgery.

Accepted for publication, Annals of Thoracic Surgery,

August 2018

IV. Lannemyr L, Ricksten SE, Rundqvist B, Andersson B, Bartfay SE, Ljungman C, Dahlberg P, Bergh N, Hjalmarsson C, Gilljam T, Bollano E, Karason K.

Differential effects of levosimendan and dobutamine on glomerular filtration rate in patients with heart failure and renal impairment: A randomized double-blind controlled trial.

Journal of the American Heart Association. 2018;7: e008455.

CONTENT

ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACT	Activated clotting time
AKI	Acute kidney injury
ANOVA	Analysis of variance
ARB	Angiotensin receptor blockade
ASA	Acetyl salicylic acid
BMI	Body mass index
BSA	Body surface area
CaO_2	Arterial oxygen content
CABG	Coronary artery bypass grafting
CI	Cardiac index
CKD	Chronic kidney disease
CO	Cardiac output
CPB	Cardiopulmonary bypass
^{51}Cr-EDTA	Chromium ethylenediamine tetraaceticacid
CRTD	Cardiac resynchronization therapy defibrillator
CVP	Central venous pressure
DCM	Dilated cardiomyopathy
DO_2I	Systemic oxygen delivery index
eGFR	Estimated glomerular filtration rate

FF	Renal filtration fraction
GFR	Glomerular filtration rate
HF	Heart failure
HR	Heart rate
ICD	Implantable cardioverter defibrillator
KDIGO	Kidney Disease: Improving Global Outcomes
LMM	Linear mixed model
LVEF	Left ventricular ejection fraction
MAP	Mean arterial pressure
MDRD	Modification of diet in renal disease
mGFR	Measured glomerular filtration rate
MPAP	Mean pulmonary artery pressure
NAG	N-acetyl-β-D-glucosaminidase
NGAL	Neutrophil gelatinase-associated lipocalin
NT-pro-BNP	N-terminal probrain natriuretic peptide
NYHA	New York Heart association
PAH	Para-aminohippuric acid
PCI	Percutaneous coronary intervention
PCWP	Pulmonary capillary wedge pressure
PVRI	Pullmonary vascular resistance

RBF	Renal blood flow
RO_2Ex	Renal oxygen extraction
RPF	Renal plasma flow
RPP	Renal perfusion pressure
RRT	Renal replacement therapy
RVR	Renal vascular resistance
SaO_2	Arterial oxygen saturation
SCr	Serum creatinine
SD	Standard deviation
SEM	Standard error of the mean
$SrvO_2$	Renal vein oxygen saturation
SVI	Stroke volume index
SvO_2	Mixed venous oxygen saturation
SVRI	Systemic vascular resistance index
VO_2I	Systemic oxygen consumption index

1 INTRODUCTION

1.1 CARDIOPULMONARY BYPASS

Since its first use in 1953, cardiopulmonary bypass (CPB) has made open cardiac surgery possible.[1] CPB allows the surgeon to operate on a non-beating heart, under fairly blood-less conditions and good visibility while the function of the heart and lungs are replaced by the CPB system. The technique has undergone extensive development, but retains some key features, which are briefly described below.

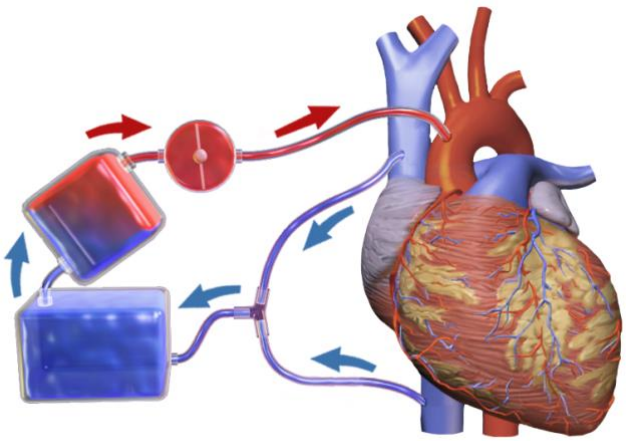


Figure 1. Schematic drawing of the cardiopulmonary bypass circuit. CC Creative Commons License.

The basic bypass circuit consists of a pump, an oxygenator, a heater/cooler system and tubing. These parts allow for the clinical perfusionist, a highly specialized nurse/technician, to control the systemic blood flow, gas exchange and temperature of the patient. The CPB circuit, usually constituting a volume of 1–1.5 L, is filled with fluid, *primed*, before the patient is connected to the system. This priming solution can be blood, but is often a cell-free electrolyte solution, which means that the patient undergoing cardiac surgery with CPB is to some extent hemodiluted. After systemic heparinization, the ascending aorta (or other sites) and caval vein(s) are cannulated, and the patient is connected to the CPB circuit. The venous return is drained from the vein cannulas, flows passively into a reservoir and is then pumped through the oxygenator and back

into the patient via the aortic cannula. An aortic clamp placed proximal to the aortic cannula isolates the heart from the body, and a potassium-rich solution, cardioplegia, is then injected into the aortic root. A competent aortic valve prevents back-flow into the left ventricle, and the aortic clamp prevents systemic flow, thus forcing the cardioplegic solution to perfuse the coronary arteries and cause cardiac arrest. Additional cardioplegia may be administered as needed during the CPB period. Upon completion of the surgery, the aortic clamp is removed, the returning coronary blood flow washes out the cardioplegia and the cardiac contraction resumes.

Historically, a pump flow of 2.2–2.5 L/min/m^2, mimicking the cardiac output of an unsedated adult person, has been considered adequate for normothermic CPB.[2,3] When hypothermia is used, the CPB flow is reduced due to the lower whole-body oxygen consumption. Mean arterial pressure is usually kept within 50 – 80 mmHg by use of vasoactive substances.

Although the vast majority of patients undergoing cardiac surgery with CPB emerge unscathed, some complications remain a concern. These include coagulopathy and bleeding, and cerebral dysfunction, both short- and long-term. Renal impairment, the main focus of this thesis, is a well-known complication after cardiac surgery with CPB, and will be discussed in depth in the following section.

1.2 ACUTE KIDNEY INJURY AND CHRONIC KIDNEY DISEASE

Acute kidney injury (AKI), previously called acute renal failure, is a clinical syndrome of rapidly deteriorating renal excretory function. The current definition of AKI by Kidney Disease: Improving Global Outcomes (KDIGO) is based on elevated levels of serum creatinine (SCr) and urine output, see table.[4] Both criteria reflect the ability of the kidney to uphold its functional capacity, i.e. glomerular filtration. Creatinine is formed upon breakdown of creatine in muscle cells, and produced at a fairly constant rate depending on the muscle mass. Creatinine is then eliminated in the kidneys through glomerular filtration and secretion by the proximal tubulus cells, with little or no reabsorption. Thus, when glomerular filtration is reduced, SCr concentration increases. The cut-off value was based on the findings by Lassnig and colleagues that patients with SCr increase ≥26.5 μmol/l after cardiac surgery suffered a four-fold mortality increase.[5]

Table 1. KDIGO criteria for the definition of AKI.[4]

Stage	*Serum creatinine*	*Urine output*
1	*1.5 – 1.9 times baseline OR ≥ 26.5 µmol/l increase (within 48 hours)*	*< 0.5 ml/kg/h for 6 – 12 hours*
2	*2.0-2.9 times baseline*	*< 0.5 ml/kg/h for ≥ 12 hours*
3	*3.0 times baseline OR increase to ≥ 354 µmol/l OR initiation of RRT*	*< 0.3 ml/kg/h for ≥ 24 hours OR anuria for ≥ 12 hours*

RRT; renal replacement therapy.

CKD is defined as abnormalities of kidney structure or function persisting for more than 3 months with implication for health. GFR is considered the best overall indicator of kidney function, and a decreased GFR is defined as <60 ml/min/1.73 m^2, which means a roughly halved filtration compared to healthy young men and women.[6] Common causes of CKD include diabetes mellitus, parenchymal kidney disease and cardiac or hepatic failure.

1.3 SCOPE OF THE PROBLEM

After cardiac surgery, up to one third of the patients develop AKI of any grade, as assessed by increased serum creatinine or urinary output (KDIGO).[7] The incidence is around 10 % in patients undergoing isolated CABG[8], and is higher after valvular surgery and combined procedures.[9] The importance of AKI is underlined by the close correlation between AKI, mortality and morbidity. Indeed, even minor increases in serum creatinine after cardiac surgery are associated with impaired prognosis[5,8] and the mortality increases with the grade of renal impairment.[10] Thus, the in-hospital risk of death is quadrupled in patients with milder AKI[11], and the mortality rates in patients who require dialysis as a result of AKI are above 35 %.[10] The economic impact of AKI is monumental. In a recent study, patients with AKI had a longer stay (median 3 days), and when renal replacement therapy was needed, the length of stay was 11 days longer than for comparable patients without renal impairment.[12] AKI is associated with higher costs than myocardial infarction or gastrointestinal bleeding, and comparable with the cost of stroke. Another recent US study found that the mean hospitalization cost was doubled in patients with post-cardiac surgery AKI, and that the annual cost of AKI was more than 1 billion USD.[9]

Furthermore, patients who suffer an episode of AKI are at increased risk of chronic kidney disease and end-stage renal failure requiring dialysis or transplantation.[8] This emphasizes that an AKI episode might be more serious

than previously believed. It has also been argued that AKI and CKD may be viewed as a continuum.[13] Thus, an initial renal insult may cause AKI, either transient or persisting, with a further progress to CKD.

1.4 RENAL ANATOMY AND PHYSIOLOGY

The kidneys are paired organs with retroperitoneal location at each side of the vertebral column at the level of the twelfth thoracic vertebra. In a cross section, the outer cortex region and the inner medulla are clearly visible. Each kidney is normally perfused via a single renal artery, which divides into interlobar arteries, with further subdivisions down to the afferent arterioles and the glomerular capillaries, where the filtration takes place. The blood flow then enters the efferent arterioles, perfusing each, single nephron.

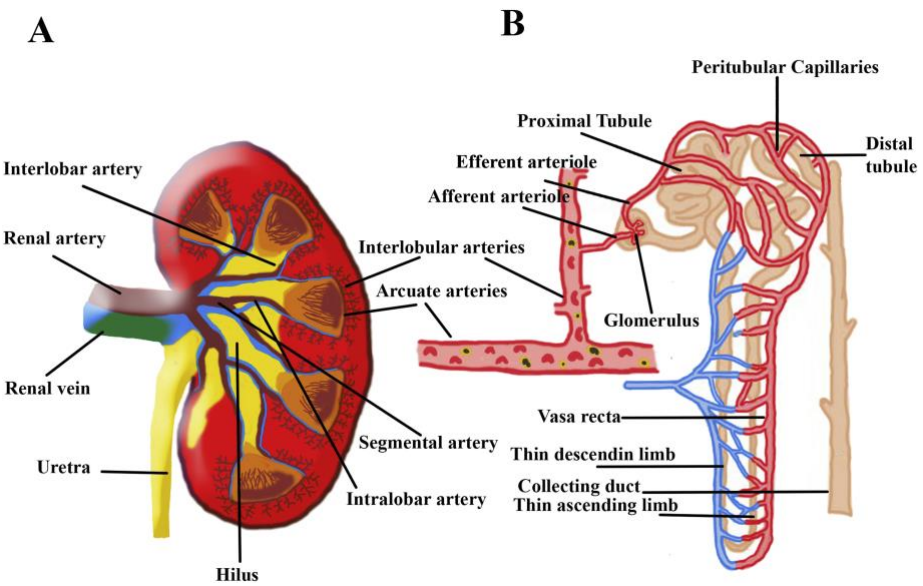


Figure 2. Representative image of the renal preglomerular vessels (Panel A), glomerular vessels, and tubules (Panel B). From Guercy 2017[14], with permission.

Nephrons are the basic functional unit of the kidneys, and around one million nephrons are found in each kidney. The nephrons consist of the Bowman's capsule, the proximal tubule, the thin loop, the distal tubule and the collecting duct. The afferent arteriole feeds into the glomerulus, where primary urine is formed by filtration of plasma across the basal membrane into Bowman's

capsule. The filtration is influenced by several factors; the permeability of the basal membrane (ultrafiltration coefficient, K_{UF}) and the differences in hydrostatic and colloid osmotic pressure across membrane. The glomerular filtration rate (GFR) relationship can be expressed as:

$$GFR = K_{UF} \times [(P_{glom} + \pi_{Bow}) - (P_{Bow} + \pi_{glom})]$$

where P_{glom} and P_{Bow} are the hydrostatic pressures, and π_{glom} and π_{Bow} are the colloid osmotic pressures in the glomerulus and Bowman's capsule, respectively. Blood flow may affect GFR by changes the colloid oncotic pressure. When plasma flow through the glomerulus is reduced, the increased transit time allows for more filtration and higher π_{glom}, which acts to reduce GFR. The opposite is true for increased RBF. Thus, the GFR is to some extent flow dependent.

The primary urine is concentrated by a 100-fold along its way through the tubular system. Thus, the primary urine volume filtered through the glomeruli, approximately 180 L/day, is reduced to an excreted volume of 1–2 L/day. This is a highly energy demanding process, which is discussed in further detail below.

Cortical nephrons constitute the majority (85 %), and have glomeruli close to the surface of the kidney and shorter loops of Henle. The remaining 15 % are the juxta-medullary nephrons, with have long loops of Henle that penetrate deep into the renal medulla. The urine concentration capacity of the nephron is proportional to the length of the loop of Henle.

1.4.1 RENAL PERFUSION AND BLOOD FLOW CONTROL

Although the kidneys' combined weight is less than 350 g, they receive about 20 % of the cardiac output, or 1 L per minute in healthy adults. The renal blood flow is controlled by several mechanisms, which affect the vascular tone of the afferent renal arterioles. The so-called autoregulation of renal blood flow maintains the blood flow and GFR over a wide range of mean arterial pressures (MAP). Thus, in the MAP range of 80 – 180 mmHg, the renal blood flow is held fairly constant.[15] The renal autoregulation appears to be effective in controlling cortical blood flow, but less so in the medulla.[15] The two primary mechanisms of renal autoregulation are the myogenic response and the tubulo-glomerular feedback mechanism (TGF).

The **myogenic response** is a rapid mechanism by which elevated systemic blood pressures induce vasoconstriction of the afferent arterioles. Stretch of

the vascular smooth muscle activates ion channels, which allows cellular influx of calcium and cellular contraction. Several vascular beds, including muscle, brain and kidneys, are autoregulated through this mechanism.[15]

The **TGF**, a somewhat slower mechanism, is sensitive to renal metabolic changes. It is dictated by signaling from the macula densa cells, which reside adjacent to the distal tubule and the afferent arterioles. Chemoreceptors in the macula densa respond to the sodium concentration in the filtrate by release of adenosine triphosphate and/or adenosine, which affect the tone of the afferent arterioles. Increased blood pressure leading to increased GFR and sodium concentration induces afferent arteriolar constriction and subsequent reduction in blood flow and GFR. Decreased blood pressure or GFR leads to increased blood flow by vascular relaxation. TGF has been suggested to have a role in the prevention of tubular ischemia by reducing the sodium load when metabolic demand exceeds DO_2.[16]

These two mechanisms operate through changes in the afferent arteriolar tone, and thus in theory has no effect on the filtration fraction, i.e. GFR/RBF. However, GFR and RBF may change independently if the tone of the efferent arterioles is altered.

The **renin-angiotensin system** exerts control of the renal circulation through renin release from granular cells in the afferent arterioles. Renin may be released in response to reduced blood pressure (vascular wall tension), by sympathetic stimulation or by reduced sodium concentration at the macula densa cells. Renin then reacts with angiotensin to produce angiotensin I, which is converted to angiotensin II by angiotensin converting enzyme. Angiotensin II causes vasoconstriction of the efferent arterioles, which reduces RBF but maintains or increases GFR. High levels of angiotensin II leads to contraction of mesangial cells in the glomerulus (which reduces GFR) and causes systemic vasoconstriction with further reduction of RBF. Increased renin-angiotensin activity is common in heart failure patients[17], and might be one mechanism by which reduced cardiac function may negatively affect renal function, e.g. the cardiorenal syndrome.

Several agents may cause vasodilatation or vasoconstriction in the renal circulation and balances or counteracts the above-mentioned mechanisms. Prostaglandins and natriuretic peptides induce vasodilatation, and may have important roles in the control of intrarenal flow distribution. Vasopressin modulates the medullary blood flow, and the effect appears to be dependent on the hydration status of the studied animals.[18]

Nitric oxide (NO) is an important regulator of oxygen supply and oxygen consumption. NO induces vasodilation of both afferent and efferent arterioli, increases GFR and also act on mitochondria to reduce oxidative metabolism.[14] Reducing NO production by blocking of the NO-synthase, has been shown to increase renal oxygen consumption and reduce renal plasma flow.[19,20]

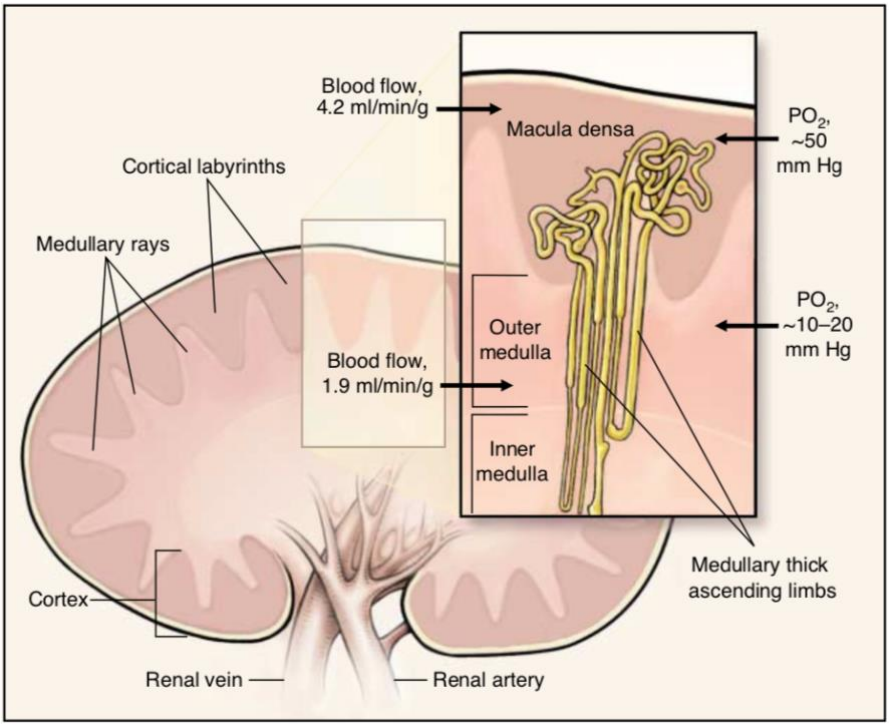


Figure 3. Intrarenal blood flow and oxygen tension. Reproduced with permission from Brezis 1995, Copyright Massachusetts Medical Society.[21]

The intrarenal blood flow distribution is highly uneven. The medullary circulation is arranged in parallel to the cortical circulation, which in turn is parallel to the total body circulation.[22] The renal medulla is perfused by cortical efferent arterioles, and receives less than 50 % of the cortical blood flow.

Medullary oxygen tension is low, around 10–15 mmHg, compared to 50 mmHg in the cortex.[21] While the osmolality is plasma-like in the cortex, the low blood flow in combination with counter-current arrangement of the vasa recta, supplying the loops of Henle, permits the medulla to maintain a high osmolality. This facilitates efficient electrolyte and water reabsorption.

1.4.2 RENAL OXYGEN CONSUMPTION

The oxygen consumption of the kidneys (RVO_2) can be regarded as the sum of the contributions from the basal metabolism and the cost of active electrolyte transport. The basal metabolism, i.e. cellular processes apart from sodium reabsorption, constitutes approximately 15–25 % of the total RVO_2.[23,24]

The bulk of the renal oxygen consumption is linked to tubular sodium reabsorption, which is a key process in the urine concentration mechanism. Sodium ions passively diffuse from the tubular lumen through the apical membrane of the tubular cells. The Na-K-ATPase actively transports sodium into the intersititum, from where it is absorbed into the tubular capillaries along a gradient formed by the intravasal colloid osmotic pressure. Tight junctions between the cells prevent the return of sodium into the tubular lumen.

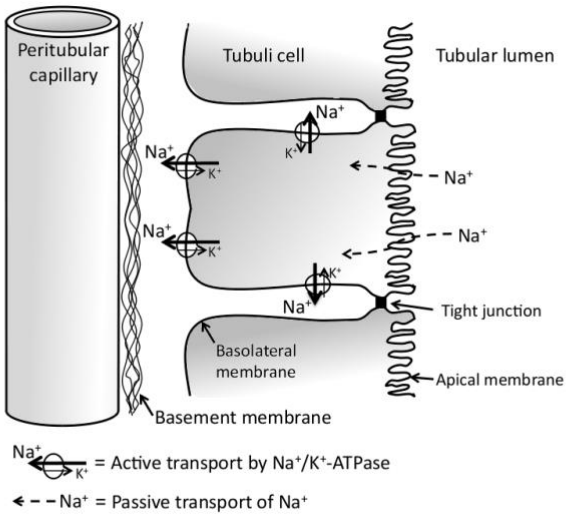


Figure 4. Tubulus cells and sodium transport. Sodium diffuses passively from the tubular lumen through the apical membrane of the tubulus cell, and is then pumped by the Na-K-ATPase through the basolateral membrane to the interstitium. Low intravascular colloid osmotic pressure draws the sodium into the peritubular capillaries. Tight junctions prevent the sodium from leaking back into the tubular lumen. From Redfors 2010, with permission.

While the basal metabolic energy requirements are fairly constant at a given temperature, the cost of sodium transport varies with the load of filtered

sodium and the cost of reabsorption. Several studies have shown a close relationship between GFR, tubular sodium load and RVO_2 in different settings.[25-27]

The energetic cost of sodium reabsorption may vary between different sites along the nephron.[28] It may also be affected by nitric oxide availability and neuro-hormonal milieu[29], which may be significantly altered in kidney injury or disease.

The renal medulla, more specifically the medullary thick ascending limbs (mTAL), harbors the highest concentration of Na-K-ATPase, and consequently has the highest oxygen consumption.[30] Thus, the renal medulla is on the verge of hypoxia even in normal conditions due to the high medullary oxygen consumption, in combination with low blood flow and oxygen delivery.[21] Therefore, the renal medulla is particularly susceptible to ischemia.

1.5 CARDIOPULMONARY BYPASS AND KIDNEY INJURY

The link between CPB and AKI has been debated and studied for decades. The pathophysiology is complex, and still poorly understood, which makes targeted interventions to ameliorate injury difficult. Some established risk factors for AKI after cardiac surgery are summarized in the table below.

Table 2. Risk factors of AKI after cardiac surgery.

Preoperative	***Intraoperative***	***Postoperative***
Advanced age	*Complex surgery*	*Hypovolemia*
Female gender	*CPB duration*	*Hypotension*
Hypertension	*Hemodilution*	*Anemia*
Chronic kidney disease	*Hypoperfusion*	*Venous congestion*
Emergency surgery	*Transfusion*	*Cardiogenic shock*
COPD	*Hypothermia*	*Sepsis*
Diabetes Mellitus	*Emboli*	
Anemia	*Inflammation*	
Peripheral vascular disease	*Hemolysis*	

Adapted from O'Neal, Crit Care, 2016,[7], and Nadim, JAHA 2018.[31]

In cardiac surgery, possible mechanisms of renal injury include inflammation, altered perfusion pressure and blood flow, micro-embolization and reperfusion injury.[32] There is mounting evidence that renal oxygenation is of central

importance in the development of cardiac surgery-associated AKI (CS-AKI). During CPB, both the oxygen carrying capacity of the blood and systemic oxygen delivery seems to affect renal oxygen delivery. It has been shown that the degree of hemodilution[33] and a decreased systemic oxygen delivery[34,35] are independent risk factors for the development of postoperative AKI. More specifically, studies have found that nadir systemic oxygen delivery index (DO_2I) below the range of 225–272 ml/min/m^2 is a strong predictor of postoperative AKI.[34,36,37] Temperature management may affect the oxygen consumption, and intraoperative hypothermia, elevated postoperative temperatures and rapid rewarming have been associated with AKI.[38,39] The mechanisms of CS-AKI will be discussed further in detail in the discussion chapter.

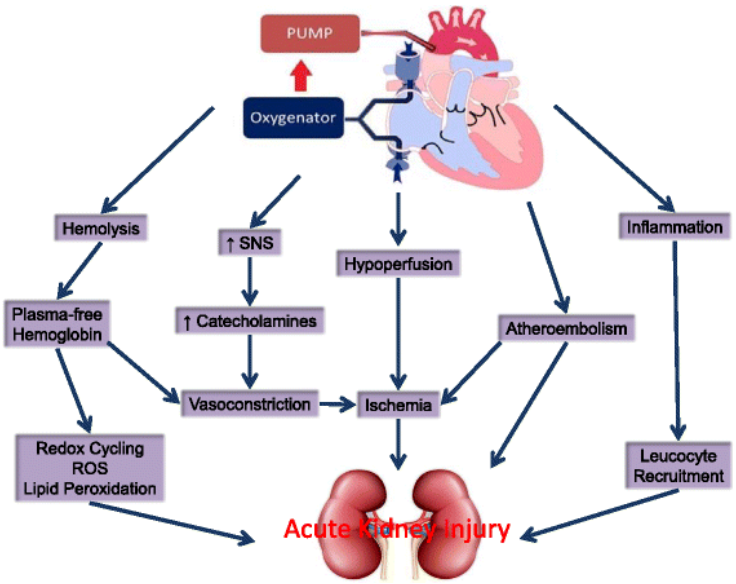


Figure 5. Pathophysiology of acute kidney injury following cardiac surgery. SNS; sympathetic nervous system, ROS; reactive oxygen species. From O'Neal 2016,[7] CC License.

No perioperative pharmacologic intervention has consistently shown effect in reducing CS-AKI[40], and the efforts to find preventive measures has been hampered by the lack of understanding of the mechanisms behind of the CPB-related renal impairment. Strategies aimed at minimizing the risk of CS-AKI are largely centered on systemic oxygen delivery, such as maintaining

perfusion pressure and hematocrit above critical levels, and provide adequate systemic blood flow.[41] One approach to prevent renal hypoxia during CPB could be to optimize renal hemodynamics during CPB by e.g. increasing CPB pump flow rate with the aim to improve renal perfusion. Remarkably enough, this approach has, to our knowledge, not been studied in patients undergoing cardiac surgery with CPB.

1.6 BIOMARKERS OF RENAL INJURY

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[42] A number of biomarkers reflecting renal dysfunction or tubular injury have been developed. These are heterogeneous substances that originate from different cells or processes involved in renal injury. The biomarkers reflect changes in renal function (i.e. glomerular filtration) or structure, such as the integrity of renal tubulus cells. Their use may include prediction or early diagnosis of AKI and prediction of outcome. Structural or subclinical AKI is an emerging concept where cellular injury (assessed by biomarkers) occurs after an insult, but serum creatinine remains stable.[43]

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme with high molecular weight (130 kDa) found in the renal proximal tubular cells. The large size of the molecule precludes glomerular filtration, and the low concentrations normally found in urine are mainly the result of exocytosis.[44] Thus, increased urine NAG-levels are considered to be an indication of tubular cell injury. This has been demonstrated in several settings, such as ischemic reperfusion injury after renal transplantation[45], administration of nephrotoxic agents or radio contrast.[44] NAG-excretion may also increase as a result of local infection or inflammation, such as glomerulonephritis[46]. In a review of biomarkers, postoperative urinary NAG had a modest predictive value for CS-AKI[47], comparable to most other studied compounds. However, the review found no studies where biomarkers were assessed, intraoperatively, during CPB.

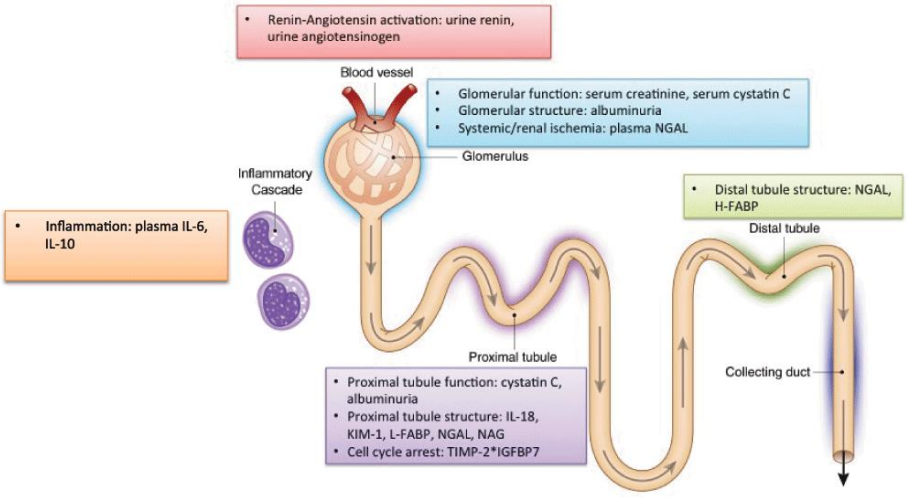


Figure 6. Physiology of biomarkers of AKI (acute kidney injury). H-FABP; heart fatty acid binding protein, IGFBP-7; insulin-like growth factor binding protein 7, IL-6; interleukin-6, IL-10; interleukin-10, IL-18; interleukin-18, KIM-1; kidney injury molecule-1, L-FABP; liver fatty acid binding protein, NAG; N-acetyl-β-D-glucosaminidase, NGAL; neutrophil gelatinase-associated lipocalin, TIMP-2; tissue inhibitor metalloproteinase-2. From Schaub 2016[48], CC License.

1.7 INOTROPES AND RENAL FUNCTION

In patients with heart failure (HF), renal impairment is common, and is an even stronger predictor of mortality than low left ventricular ejection fraction or New York Heart Association (NYHA) class.[49] The use of inotropes in decompensated HF is considered an option for patients with severe reduction of cardiac output and compromised perfusion of vital organs, such as the kidneys.[50] The drugs most commonly used are dopamine, dobutamine, milrinone and levosimendan. All of these agents increase cardiac output, but it is uncertain whether there are differences in their effects on renal function.

Levosimendan is a calcium sensitizer and an opener of ATP-dependent potassium channels that has inotropic and arterial and venous dilating properties.[51] Several studies have suggested that levosimendan may have beneficial effects on renal function, both in cardiac surgery[52], HF [53] and heart

transplantation.[54] Bragadottir et al found that levosimendan, when compared with placebo, increased both renal blood flow and glomerular filtration rate in post-cardiac surgery patients with normal preoperative serum creatinine.[55]

Dobutamine is a catecholamine with beta-1 and beta-2-adrenergic effects, which causes increased cardiomyocyte contractility and reduced afterload.[56] Animal studies are conflicting regarding how dobutamine may influence renal vascular resistance and renal blood flow.

Selected patients with HF may undergo cardiac transplantation. In these patients, preoperative renal impairment has been associated with higher early and late post-transplant mortality.[57] It is unclear if strategies to optimize renal function may translate into improved survival. Currently, the guidelines from the American Heart Association and European Society of Cardiology give no suggestion on the choice of inotrope in the treatment of patients with HF and reduced renal function.[58,59]

2 AIM

1. To study the effect of cardiac surgery with cardiopulmonary bypass (CPB) on a renal tubular cell injury marker, and to identify independent predictors of intraoperative tubular injury
2. To study the impact of CPB on renal blood flow, glomerular filtration rate and renal oxygenation during cardiac surgery
3. To study the effects of various CPB flow rates on renal oxygenation and renal filtration fraction during cardiac surgery
4. To study the differential renal effects of the two inotropic agents, dobutamine and levosimendan, in patients with heart failure and reduced renal function

3 PATIENTS AND METHODS

3.1 ETHICS AND TRIAL REGISTRATION

All studies were performed in accordance with the recommendations guiding physicians in biomedical research involving human patients adopted by the 18th World Medical Assembly, 1964 and later additions (Declaration of Helsinki). In addition, study IV was performed in accordance with the principles of ICH Harmonized Tripartite Guideline for Good Clinical Practice. The study protocols were approved by the Gothenburg Regional Ethics Committee. All studies were registered in ClinicalTrials.gov, with identifier for Paper I; NCT02410642, Paper II; NCT02405195, Paper III; NCT02549066, Paper IV; NCT02133105.

3.2 PATIENTS

3.2.1 Paper I, II and III – studies during cardiopulmonary bypass

Studies I-III were undertaken at the Department of Cardiothoracic Anesthesia and Intensive Care, at Sahlgrenska University Hospital. All patients were informed at the pre-operative evaluation, and written informed consent was obtained from all patients before enrollment in the studies.

In paper I, 70 adult patients scheduled for cardiac surgery with CPB and with a normal preoperative serum creatinine, were enrolled. Exclusion criteria were: CPB duration <60 minutes, lowest bladder temperature during CPB ≤30 °C and on-going treatment with nephrotoxic agents. In seven patients, surgery was cancelled or delayed, and 2 patients were excluded (one due to CPB duration <60 minutes, and one due to bladder temperature ≤30 °C). Thus, 61 patients completed the study protocol. Patient characteristics are summarized in table 3.

In paper II, 28 adult patients scheduled for cardiac surgery with CPB and with a normal preoperative serum creatinine and a left ventricular ejection fraction (LVEF) ≥50 % were enrolled. The exclusion criteria were: CPB duration <60 min, unsuccessful catheterization of the renal vein, contraindication to radio-contrast, cardiac transplantation and thoracic aortic surgery. In four patients, surgery was cancelled or delayed, two patients were considered non-eligible by the surgeon, and four patients were excluded (three due to unsuccessful

catheterization of the renal vein and on due to CPB duration <60 minutes). The characteristics of the 18 patients who completed the protocol are summarized in table 3. NAG-excretion data obtained from these patients were also used in study I.

In Paper III, 28 adult patients scheduled for cardiac surgery with CPB and with a normal preoperative serum creatinine and a LVEF ≥50 % were enrolled. The exclusion criteria were: CPB time <60 min, unsuccessful catheterization of the renal vein, a body mass index ≥32 kg/m^2, previous cerebrovascular lesion, and radiographic contrast allergy. In 10 patients, surgery was cancelled or postponed. Eighteen patients were randomized; one patient was excluded due to CPB duration <60 minutes. Thus, 17 patients completed the protocol, and their characteristics are summarized in the table 3.

Table 3. Patient characteristics in papers I, II and III.

Variable	*Paper I*	*Paper II*	*Paper III*
No of patients	*61*	*18*	*17*
Male gender	*43 (70)*	*16 (89)*	*14 (82)*
Age (years)	*71±8*	*70±7*	*69±10*
Body Surface Area (m^2)	*1.93±0.08*	*1.95±0.20*	*1.95±0.22*
Left Ventricular Ejection Fraction (%)	*57±8*	*58±5*	*58±4*
Preoperative S-creatinine (µmol/L)	*87±13*	*87±11*	*86±12*
CPB time (minutes)	*133±44*	*132±31*	*123±41*
Aortic cross clamp time (minutes)	*100±32*	*103±24*	*93±39*
Comorbidities			
- Hypertension	*34 (58)*	*10 (56)*	*13 (76)*
- COPD	*NA*	*NA*	*3 (18)*
- Atrial fibrillation	*NA*	*7 (39)*	*8 (47)*
- Diabetes Mellitus	*5 (8)*	*1 (6)*	*1 (6)*
Type of surgery			
- Isolated valve surgery	*19 (31)*	*9 (50)*	*7 (41)*
- Valve surgery and CABG	*32 (53)*	*9 (50)*	*6 (35)*
- Other	*10 (16)*	*0*	*4 (24)*

Values are n (%) or mean±SD. CPB; cardiopulmonary bypass, CABG; coronary artery bypass grafting, COPD; Chronic obstructive pulmonary disease. Other surgical procedure includes combinations of Maze surgery and valve surgery. NA; data not available.

3.2.2 Paper IV – levosimendan vs. dobutamine

Individuals with chronic heart failure (HF), scheduled for a right-sided cardiac catheterization as a part of an elective heart transplant evaluation, were screened for study participation. The inclusion criteria were: 1) signed informed consent, 2) age ≥18 years, 3) chronic congestive heart failure 4) left ventricular ejection fraction (LVEF) ≤40 %, 5) serum-N-terminal pro-brain natriuretic peptide (NT-pro-BNP) ≥500 ng/L and 6) estimated (MDRD) or a measured GFR between 30–80 ml/min (clearance of chromium ethylene diamine tetra acetic acid [^{51}Cr-EDTA]). The exclusion criteria were: 1) untreated acute HF, 2) systolic blood pressure <100 mmHg, 3) heart rate >100 beats per minute, 4) a Canadian Cardiovascular Society class III angina pectoris or higher, 5) aortic stenosis, 6) hypertrophic cardiomyopathy, 7) restrictive cardiomyopathy, 8) presence of kidney disease diagnosed before HF, 9) recent administration of radiographic contrast, 10) radiographic contrast allergy, and 11) in the opinion of the investigator, a clinically significant disease that could be adversely affected by study participation.

In total, 33 patients were enrolled. One patient, randomized to levosimendan, developed atrial fibrillation with circulatory instability prior to the study drug administration and was excluded. Thus, 32 persons completed the study. Patient characteristics are summarized in table 4.

Table 4. Patient characteristics from paper IV.

Variable		*Levosimendan (n=16)*	*Dobutamine (n=16)*
Gender	*Male*	*14 (88)*	*14 (88)*
Age	*Years*	*58.1±11.6*	*58.6±10.0*
BMI	*kg/m²*	*29.1±4.2*	*28.6±5.5*
NYHA class	*II*	*1 (6)*	*1 (6)*
	III	*14 (88)*	*12 (75)*
	IV	*1(6)*	*3 (19)*
DCM		*8 (50)*	*9 (56)*
Ischemic heart disease		*8 (50)*	*6 (38)*
Other cause of HF		*0*	*1 (6)*
Hypertension		*4 (25)*	*3 (19)*
Diabetes mellitus		*6 (38)*	*5 (31)*
Atrial fibrillation		*8 (50)*	*7 (44)*
Pulmonary disease		*1 (6)*	*3 (19)*
LVEF	*%*	*27.2±8.0*	*26.0±8.1*
Heart rate	*beats/min*	*72±7*	*76±15*
Hemoglobin	*g/L*	*127±18*	*136±16*
S-creatinine	*µg/L*	*143±37*	*122±31*
NT-proBNP	*ng/L*	*2290 [1500–4650]*	*1760 [1057–5995]*
eGFR	*ml/min*	*49.4±16.3*	*55.3±18.7*
mGFR	*ml/min*	*42.8±15.4*	*53.4±15.2*

Values are numbers (%), mean±SD, or median [interquartile range]. BMI; body mass index, DCM; dilated cardiomyopathy, eGFR; estimated glomerular filtration rate according to the Modification of Diet in Renal Disease formula, HF; heart failure, LVEF; left ventricular ejection fraction, mGFR; measured glomerular filtration rate, NT-proBNP; N-terminal pro-brain natriuretic peptide, NYHA, New York Heart Association.

3.3 METHODS

3.3.1 Anesthesia and CPB - Papers I–III

Anesthesia and CPB were conducted in accordance with our department's clinical standard, unless specified below. Premedication consisted of oxazepam (5–10 mg) and oxycodone (10 mg). Anesthesia was induced by administration of fentanyl (5–10 µg/kg), propofol (1–1.5 mg/kg) and intubation facilitated by rocuronium (0.6 mg/kg). Before and after CPB, anesthesia was maintained with sevoflurane (0.5–2.5%) in a 50% O2/air mixture. During CPB, anesthesia was maintained with an intravenous infusion of propofol (2.5–4 mg/kg/hour).

CPB circuit and fluid management

The CPB circuit consisted of a Primox® or Inspire 8® oxygenator (Sorin Group, Italy), an HVR Hard-shell reservoir (Sorin Group), a Sorin Adult® tubing system, a Stöckert S5® heart-lung machine, and a Stöckert Heater Cooler System 3T® (Stöckert Instrumente, Germany). The priming solution consisted of 1,200 ml acetated Ringer's solution and 10,000 IU heparin. Hydroxyethyl starch was not used in the pump prime, nor during or after CPB. Furthermore, loop-diuretics or albumin was not used before, during, or after CPB.

In Paper I, mannitol (200 ml, 150 mg/ml) was given in the priming solution in some patients at the discretion of the surgical team. No mannitol was administered to patients in Paper II or III.

After heparinization with 400 IU/kg, the patients were cannulated in the aortic root followed by venous mono- or bicaval cannulation depending on the surgical procedure. Activated clotting time was kept at more than 480 s during CPB. During CPB, the target hematocrit was 25–35%. The target body temperature (bladder) was 33–36°C in Paper I, and 35–36°C in Papers II and III. Before weaning from CPB, the patients were rewarmed to a target body temperature of 36.0–36.5°C. Cold, hyperkalemic blood cardioplegia was given at an induction dose of 800–1000 ml followed by subsequent doses when deemed necessary by the surgeon. Alpha-stat pH management was used during CPB. After weaning from CPB, the heparin was antagonized by protamine sulphate (4 mg/kg).

CPB flow and pressure

In studies I and II, non-pulsatile CPB was conducted with a target flow of 2.5 L/min/m^2. In study III, non-pulsatile CPB was initiated at a flow of 2.4 L/min/m^2 and the flow was later changed according to the study protocol.

In Paper I, mean arterial pressure (MAP) was allowed to vary between at 50 – 80 mmHg as deemed appropriate by the attending anesthetists considering co-morbidities and preoperative blood pressure. In studies II and III, mean arterial pressure was maintained at 60 to 80 mmHg. Vasopressor (norepinephrine) or vasodilator (nitroprusside) therapy was used when necessary.

3.3.2 Measurements of systemic hemodynamics

In all papers, mean arterial pressure (MAP) was measured with a radial or femoral artery catheter, and central venous pressure (CVP) was measured with a central venous catheter with the tip in the upper caval vein. In papers II–IV, a pulmonary artery thermodilution catheter (Baxter Healthcare Corporation, USA) was inserted through either the left subclavian vein or the right jugular internal vein and placed in the pulmonary artery. Measurements of thermodilution cardiac output (CO) were performed in triplicate and indexed to the body surface area (BSA) for cardiac index (CI). The pulmonary capillary wedge pressure (PCWP) was measured intermittently. Other variables were calculated according to standard formulas, see table below.

Table 5. Formulas for calculation of systemic variables.

Variable	***Formula***
Arterial oxygen content (CaO_2)	*1.39 x Hb x SaO_2 x 0.01 + 0.0023 x PaO_2*
Venous oxygen content (CvO_2)	*1.39 x Hb x SvO2 x 0.01 + 0.0023 x SvO_2*
Systemic oxygen delivery index (DO_2I)	*CI x CaO_2*
Systemic oxygen consumption index (VO_2I)	*CI x (CaO_2 - CvO_2)*
Stroke volume index (SVI)	*CI / HR*
Systemic vascular resistance index (SVRI)	*80 x (MAP - CVP) / CI*
Pulmonary vascular resistance index (PVRI)	*80 x (MAP - PCWP) / CI*

CI; cardiac index (L/min/m^2), CVP; central venous pressure (mmHg), Hb; hemoglobin level (g/L), HR; heart rate (beats/minute), MAP; mean arterial pressure (mmHg), PaO_2; arterial oxygen tension (kPa), PCWP; pulmonary capillary wedge pressure (mmHg), SaO_2; arterial oxygen saturation (%), SvO_2; mixed venous oxygen saturation.

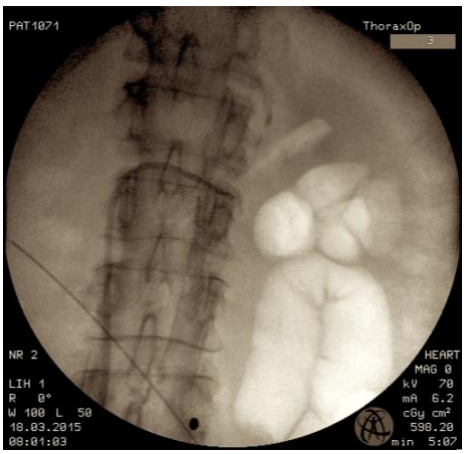
3.3.3 Measurements of renal variables

All renal data were normalized to a body surface area of 1.73 m^2.

Renal vein catheterization (Papers II–IV)

In Papers II–IV renal vein catheterization was used for invasive measurement of renal variables. In Paper II and III, a 7.5-Fr CCO Pulmonary Artery Catheter® (Edwards Lifesciences Corporation, USA) or an 8-Fr catheter (Webster laboratories, USA) was inserted in the left or right renal vein via the left or right femoral vein under fluoroscopic guidance. In Paper IV, an 8-Fr catheter (Webster laboratories, USA) was inserted in the left renal vein *via* the right internal jugular vein under fluoroscopic guidance. The catheter was placed in the central portion of the renal vein, and its position was verified by venography using ultralow doses of iohexol (Omnipaque® 300 mg I/ml; GE Healthcare, Sweden). Since the cross-sectional area of the renal vein is approximately 25 times the cross-sectional area of the renal vein catheter, the risk of the catheter to partially occlude the vein is minimal.

Figure 7. Radiograph showing a renal vein catheter placed in the left renal vein.



Renal blood flow by infusion clearance of para-aminohippuric acid (Papers II & IV)

Renal blood flow (RBF) was measured by infusion clearance of para-aminohippuric acid (PAH), corrected for the PAH-extraction by the renal vein catheter. After renal vein catheterization, blood and urine blanks were taken. An intravenous priming dose of PAH (Merck, NJ, USA, or Bachem AG, Bubendorf, Switzerland) was given, followed by infusion at a constant rate,

individualized to body surface area and preoperative serum creatinine. The equilibrium time before start of the study was 60–90 minutes, and plasma concentrations of PAH activity was measured with a spectrophotometer (Beckman DU 530; Life Science UV/Vis, USA). Renal plasma flow was calculated as the amount of infused PAH divided by the difference in arterial-renal vein PAH concentrations.

Table 6. Formulas for calculation of renal variables.

Variable	*Formula*
Renal plasma flow (RPF)	*Amount of PAH infused/(PAH_{art}-PAH_{rv})*
Renal blood flow (RBF)	*RPF/(1-hematocrit)*
Filtration fraction	*[RPF x ^{51}Cr-$EDTA_{art}$–(RPF-UF) x ^{51}Cr-$EDTA_{rv}$] /RPF x ^{51}Cr-$EDTA_{art}$*
Glomerular filtration rate (GFR)	*FF x RPF*
Renal vascular resistance (RVR)	*(MAP – CVP)/RBF*
Renal oxygen consumption (RVO_2)	*RBF x (CaO_2 – $CrvO_2$)*
Renal oxygen delivery (RDO_2)	*RBF x CaO_2*
Renal oxygen extraction (RO_2Ex)	*(CaO_2 – $CrvO_2$)/CaO_2*
Renal sodium filtration	*GFR x serum sodium concentration*
Renal sodium excretion	*UF x urine sodium concentration*
Renal sodium reabsorption	*(GFR x serum sodium concentration – (UF x urine sodium concentration)*

^{51}Cr-EDTA = ^{51}chromium-ethylenediaminetetraacetic acid; CVP = central venous pressure; CaO_2 and $CrvO_2$ = arterial and renal vein oxygen contents; FF = filtration fraction; GFR = glomerular filtration rate; MAP = mean arterial pressure; PAH = para-aminohippuric acid; PAH_{art} = arterial PAH concentration, PAH_{rv} = renal vein PAH concentration, RBF = renal blood flow; RPF = renal plasma flow.

Renal filtration fraction (Papers II, III and IV)

Renal filtration fraction (FF) was defined as the renal extraction of chromium ethylenediaminetetraacetic acid (^{51}Cr-EDTA). After the collection of blood and urine blanks, an intravenous priming dose of ^{51}Cr-EDTA was given, followed by infusion at a constant rate, individualized to BSA and preoperative serum creatinine. Serum activity of ^{51}Cr-EDTA in arterial and renal vein blood were measured with a well counter (Wizard 3” 1480, Automatic Gamma Counter; Perkin Elmer LAS, Finland). The filtration fraction was corrected taking the urine flow into account, in order to eliminate errors due to variations in RBF and urine flow.

Urine analysis (Papers I, II and III)

All patients had a Foley catheter for measurements of urine flow and urine concentration of sodium and creatinine.

In Papers I and II, urine samples were assayed for N-acetyl-β-D-glucosaminidase (NAG) by a spectrophotometric method (ABX Pentra 400, Horiba Medical, CA, USA) using a commercially available kit (Reference no. 10 875 406 001, Roche Diagnostics GmbH, Mannheim, Germany) with an intra-assay coefficient of variation of 4.6–10.4% and a lower limit of detection of 0.30 U/L. The urinary NAG levels were corrected for urinary creatinine levels and expressed as units/mmol creatinine.

Analysis of oxygen, sodium and hemoglobin

Arterial, mixed venous and renal vein blood was analyzed for the content of oxygen, hemoglobin and sodium using an automated blood gas analyzer (Radiometer ABL 700 series, Copenhagen, Denmark).

3.3.4 Experimental procedures

Paper I

In a prospective observational study, 61 patients with normal preoperative serum creatinine undergoing cardiac surgery with CPB were studied. Urine NAG (corrected for urine creatinine) was measured before, during and after CPB. Urine samples were collected at ten occasions: after induction of anesthesia but before surgery and CPB (baseline), at 30, 60, 90 and 120 minutes after start of CPB, 30 minutes after end of CPB, upon arrival in the ICU (60–90 minutes after weaning from CPB) and at 4, 8 and 18 hours after arrival in the ICU. Peak NAG excretion was defined as the difference between baseline NAG and the postoperative peak NAG. Hemodynamic data, blood gases and CPB-related data were recorded. MAP was allowed to vary between 50–80 mmHg as deemed appropriate by the attending anesthesiologist considering co-morbidities and preoperative blood pressure. Vasopressor (norepinephrine) or vasodilator (nitroprusside) therapy was used when necessary.

Post-operative AKI was defined according to the KDIGO criteria based on changes in serum creatinine at postoperative day 1–2.

Paper II

In a prospective observational study, 18 patients with a normal preoperative serum creatinine undergoing cardiac surgery procedures with normothermic cardiopulmonary bypass (2.5 L/min/m^2) were included. After induction of anesthesia, pulmonary artery and renal vein catheters were inserted. Systemic and renal hemodynamic variables, and urine NAG were measured before, during, and after cardiopulmonary bypass. Arterial, mixed venous and renal venous blood samples were taken for measurements of systemic and renal oxygen delivery and consumption. Measurements were made before CPB (baseline), after 30 and 60 minutes of CPB and at 30 and 60 minutes after weaning from CPB. Renal blood flow and filtration fraction were measured by the infusion clearance technique of PAH and ^{51}Cr-EDTA, respectively. Mean arterial pressure was allowed to vary between 60–80 mmHg, and infusions of norepinephrine or nitroprusside was used as needed to keep blood pressure within these limits.

Paper III

In a randomized crossover study, 17 patients with normal preoperative serum creatinine and LVEF ≥50 % undergoing cardiac surgery with normothermic CPB were included. After induction of anesthesia, pulmonary artery and renal vein catheters were inserted, and baseline systemic hemodynamic and renal measurements were obtained (Pre-CPB). CPB was initiated at 2.4 L/min/m^2. The study commenced after aortic cross-clamp and cardioplegia administration under stable hemodynamic conditions. In a randomly determined order (sealed envelopes), the cardiopulmonary bypass flow was set to 2.4, 2.7 and 3.0 L/min/m^2. Each pump flow level was maintained for 10 minutes, followed by blood samples and recording of hemodynamic data. Filtration fraction was measured by the infusion clearance technique of ^{51}Cr-EDTA. Mean arterial pressure was allowed to vary between 60–80 mmHg, and infusions of norepinephrine or nitroprusside was used as needed to keep blood pressure within these limits. The venous reservoir volume was held above 10 % of the CPB flow rate (L/min), and crystalloid solution (Ringers acetate, Baxter, Sweden) was administered into the reservoir to reach or maintain this safety limit.

Paper IV

In a randomized double-blind study, 32 patients with chronic heart failure (LVEF <40 %) and impaired renal function (GFR <80 ml/min/1.73m^2) were included. The patients were randomized (1:1) to receive levosimendan or

dobutamine. Their order was stratified according to the level of the right ventricular end-diastolic pressure (above or below 12 mmHg at baseline). A study nurse, not otherwise involved in study procedures, performed the randomization and administration of the study drug. The infusion pump containing the study drug was concealed behind a curtain and equipped with an opaque infusion line to ensure blinding. Levosimendan administration was initiated with a loading dose of 12 μg/kg given over 10 minutes followed by a continuous infusion of 0.1 μg/kg/min for 65 min. Dobutamine was given as a continuous infusion started at 5.0 μg /kg/min for 10 minutes, and thereafter increased to 7.5 μg/kg/min for 65 minutes.

A pulmonary artery catheter was used for hemodynamic measurements, and a renal vein catheter was used to determine renal plasma flow (RPF) using the infusion clearance technique for PAH, and FF was measured by renal extraction of ^{51}Cr-EDTA.

Duplicate baseline measurements (B1 and B2) of systemic hemodynamics and renal variables (arterial and renal vein blood samples) were performed before initiation of the drug infusion. The study drug was then administered as described above. Duplicate measurements were repeated after 60 and 75 minutes of treatment (T1 and T2).

To prevent drug-induced hypotension (i.e. MAP falling below 60 mmHg for 3 minutes or more), a crystalloid fluid (Ringer-Acetate®, Baxter Viaflo, Lund, Sweden) was administered (50–100 ml/hour) from the start of the study drug administration in patients without clinical signs of hypervolemia (e.g. jugular vein distension and/or central venous pressure (CVP) ≥12). Response to hypotension was standardized; administration of Ringers-Acetate with the aim of keeping CVP 5–10 mmHg, or secondary, norepinephrine infusion with the aim of keeping MAP at 70±5 mmHg.

3.4. Statistical analyses and sample size

Quantile regression in Paper I was made using Stata version 14 (StataCorp LCC, Texas, USA). All other statistical analyses were made using Predictive Software Statistics version 18–25 (SPSS Inc., USA). A probability level (p-value) of less than 0.05 was considered statistically significant.

Paper I

The primary outcome variable was the longitudinal NAG-excretion. The sample size was chosen to allow for the detection of three independent predictors of NAG-excretion**.** In a quantile regression, 20 observations are deemed a necessary sample size for each predictor. Thus, 60 patients (3 x 20) were needed for the analysis, and 70 patients were enrolled to allow for exclusions.

The longitudinal NAG-excretion was analyzed statistically with a linear mixed model (LMM) followed by a Fisher's least significant difference *post hoc* test.

The intra-operative tubular injury was defined as the difference between baseline NAG and the post-operative peak NAG, i.e. peak increase in NAG. For evaluation of variables associated with intra-operative tubular injury, a quantile regression model of the median was used. This regression method is valid also in circumstances where the dependent variable is not normally distributed, as was the case for peak increase in NAG. The predictors were chosen based on previously shown association with AKI.

Univariable correlates for intra-operative renal injury among baseline characteristics and comorbidities and intra-operative variables (see below) were tested. Variables with a p-value <0.10 in the univariable analysis were included in a multivariate analysis, and variables with a p-value <0.05 in the multivariate analysis were considered significant independent predictors of tubular injury.

The baseline characteristics and comorbidities that were explored were: Gender, Age, BMI, Hypertension, Diabetes Mellitus, LVEF, pre-operative serum-creatinine, pre-operative estimated GFR (using the Modification of Diet in Renal Disease [MDRD] formula), and pre-operative hemoglobin. The explored intra-operative variables were: (1) CPB duration (min), (2) the degree of rewarming, defined as the temperature (°C) difference between the lowest bladder temperature during CPB and the bladder temperature 30 min after

discontinuation of CPB, (3) fluid delivery during CPB, defined as the total amount of crystalloid fluid given during CPB divided by patient weight and CPB duration (ml/kg/min), (4) change in renal perfusion pressure (RPP), defined as the difference in renal perfusion pressure before and the mean RPP during CPB, where the RPP is the mean arterial pressure minus central venous pressure (mmHg), (5) the use of mannitol in the prime solution, (6) the intra-operative use of low-dose (2 μg/kg/min) dopamine, (7) pump flow index (L/min/m^2), defined as the mean CPB pump flow during CPB, (8) lowest DO_2I (ml/min/m^2) defined as the lowest oxygen delivery during CPB, indexed to body surface area [i.e. CPB pump flow index · hemoglobin · 1.39 · arterial oxygen saturation] and (9) the intra-operative change in serum hemoglobin, defined as the difference between pre-operative hemoglobin and the mean hemoglobin during CPB. The ability of NAG-excretion to predict post-operative AKI was tested using binary logistic regression.

Paper II

The primary outcome variable was renal oxygen extraction (RO_2Ex). In previous studies, RO_2Ex has had a SD of 4 % in repeated measures.[60] Thus, to detect a relative change of 30% in RO_2Ex during CPB at a power of 80 % and a two-sided significance level of 0.05, 15 patients were needed. We aimed to compile approximately 18 to 20 patients who could be analyzed, and to include 30–50 % more to allow for dropouts.

Data were analyzed by repeated measures ANOVA. A significant ANOVA was followed by a Bonferroni-Holm *post hoc* test for comparison of baseline (pre-CPB) values versus data from subsequent measuring points. Data obtained after CPB (30 and 60 min) were pooled. A within-subject correlation was performed to correlate NAG/creatinine ratio to RO_2Ex.

Paper III

The primary outcome variable was RO_2Ex. In Paper II, renal oxygen extraction had a SD of 4 % in repeated measures during CPB. Thus, 15 patients were needed to detect a relative change in renal oxygen extraction of 30 % with a power of 80 % and a two-sided significance level of 0.05. We planned to include 50 % more patients to allow for dropouts.

The differential effects of the cardiopulmonary bypass pump flow levels were studied using a linear mixed model with a compound symmetry matrix, using CPB flow as a fixed factor. *Post-hoc* paired T-tests were used to assess differences between the 2.4 and 3.0 L/min/m^2 flow rates.

Paper IV

The primary outcome variables were GFR and RBF. Based on previous studies, the standard deviation for the difference between two GFR measurements estimated by infusion clearance is approximately 10 ml/min. Thus, to detect an estimated 20% difference in GFR between groups, with a power of 80% and an alpha of 0.05, a sample size of 26 (13 patients in each group) was required. In total, we planned to include 32 patients to allow for a 20% dropout.

Normal distribution of continuous data was checked using histograms. Continuous normal distributed data are presented as mean±SD, and non-normal distributed continuous data are presented as median and interquartile range. Data on renal and systemic hemodynamic variables from the two baseline measurements (B1 and B2) as well as during study drug administration (T1 and T2) were pooled. The differential effects of levosimendan and dobutamine were studied using a linear mixed model with a compound symmetry matrix, with "time" (baseline and treatment) and "group" (levosimendan or dobutamine) as fixed factors. Changes within-groups were studied with paired t-tests. Differences between the groups at baseline were studied with independent samples t-tests.

4 RESULTS

4.1 PAPER I

To evaluate the effects of CPB on the renal tubular injury marker, NAG, 61 patients with a normal preoperative serum creatinine undergoing open cardiac surgery with CPB were studied. Urinary NAG (U-NAG) release was measured before, during and after CPB, and factors influencing peak U-NAG (defined as the postoperative peak value minus preoperative baseline concentration of U-NAG) were studied in a regression model.

Urinary excretion of NAG

Urine samples were obtained for all patients at 30 and 60 min after the start of CPB. Forty-two patients (69%) were sampled at 90 min, and 20 (33%) at 120 min of CPB. In five patients (8%) data on U-NAG after ICU arrival was missing due to logistic reasons.

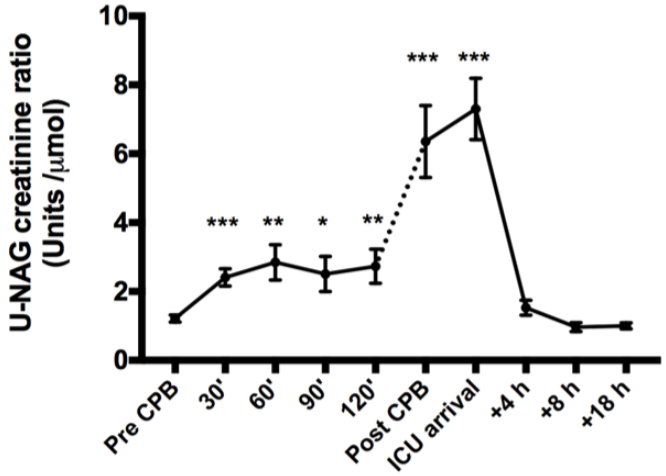


*Figure 8. Excretion of N-acetyl-b-D-glucosaminidase (NAG) before, during and after cardiopulmonary bypass (CPB). The levels of urinary NAG in patients undergoing cardiac surgery with CPB were measured; before (Pre-CPB), at 30 min intervals during CPB, 30 min after CPB, at arrival in the intensive care unit (ICU arrival), and at 4, 8 and 18 h after ICU arrival. Data are presented as mean± SEM. Asterisks indicate significant difference vs. Pre CPB at *P<0.05, **P<0.01, ***P<0.001.*

U-NAG increased during and after surgery ($p<0.001$). The level of U-NAG was significantly increased, doubled, compared to baseline, already at 30 min

after the start of CPB, and remained significantly higher throughout the CPB period. After discontinuation of CPB, the NAG excretion peaked at a mean of 7.3±7 units/µmol creatinine upon ICU arrival. At 18 h after arrival in the ICU, NAG had returned to the preoperative baseline level in all patients.

Determinants of intra-operative tubular injury

CPB duration and the degree of rewarming were the only significant predictors of the peak increase in NAG-excretion in the univariable regression model, and both remained significant in the multivariate model (p=0.022 and p=0.032, respectively).

Table 7. Variables associated with peak increase in NAG excretion.

Variable	*Univariable regression*			*Multivariable regression*		
	B	***95 % CI***	***p***	***B***	***95 % CI***	***p***
CPB time	*0.066*	*0.014–0.117*	*0.013*	*0.063*	*0.009 –0.117*	*0.022*
Degree of rewarming	*2.421*	*0.44–4.43*	*0.019*	*2.12*	*0.185–4.05*	*0.032*
Fluid delivery	*-7.29*	*0.57–18.2*	*0.569*			
Change in RPP	*-0.018*	*-0.16–0.13*	*0.806*			
Use of Mannitol	*1.07*	*-3.9–6.1*	*0.670*			
Use of Dopamine	*1.76*	*-4.9–8.5*	*0.602*			
Pump flow index	*-14.2*	*-38.1–9.81*	*0.242*			
Lowest DO_2I	*0.011*	*-0.04 –0.063*	*0.665*			
Change in Hb	*-0.029*	*-0.27–0.22*	*0.811*			
Male Gender	*-0.411*	*-6.6–5.8*	*0.895*			
Age	*-0.116*	*-0.48 –0.24*	*0.519*			
Body mass index	*-0.030*	*-0.65–0.59*	*0.924*			
Hypertension	*-1.60*	*-6.6–3.4*	*0.524*			
Diabetes	*-1.36*	*-11–8.0*	*0.771*			
LVEF	*-0.014*	*-0.34–0.31*	*0.930*			
Preoperative SCr	*0.031*	*-0.19–0.25*	*0.778*			
Preoperative eGFR	*-0.036*	*-0.20–0.13*	*0.658*			
Preoperative Hb	*-0.009*	*-0.18–0.17*	*0.922*			

CPB; Cardiopulmonary Bypass, DO_2I; systemic oxygen delivery, eGFR; estimated glomerular filtration rate, LVEF; left ventricular ejection fraction, RPP; renal perfusion pressure, SCr; serum creatinine.

After surgery, 18 patients (30%) developed AKI within 48 h (grade 1, n=15; grade 2–3, n=3). No patient required hemodialysis. In the logistic regression, peak NAG-excretion did not predict the development of AKI.

In a post-hoc analysis, a receiver operating characteristic (ROC) curve was created using the NAG-excretion at 4 hours after surgery to predict AKI. The area under the ROC-curve was 0.651. A urinary NAG level of 1.35 U/mmol creatinine at 4 hours postoperatively had a sensitivity of 64 % and a specificity of 74 % to predict the development of AKI.

4.2 PAPER II

To evaluate the effects of CPB on renal perfusion, filtration and oxygenation, 18 patients with a normal preoperative serum creatinine and LVEF ≥50 % undergoing elective cardiac surgery with normothermic CPB at 2.5 L/min/m^2 were studied.

Effects of CPB on systemic variables

Cardiac index (CI) before CPB was 1.87±0.39 L/min/m^2. Mean systemic perfusion flow rate during CPB was 2.47±0.08 at 30 min and 2.49±0.08 L/min/m^2 at 60 min. Systemic perfusion flow thus increased by 32 to 33% ($p<0.05$ and $p<0.001$), and SVRI decreased by 15 to 17% ($p<0.05$ and $p<0.01$) during CPB, compared with pre-CPB values, while mean arterial pressure (MAP) was not significantly changed. Hematocrit, serum hemoglobin, and CaO_2 decreased by 16 to 20% ($p<0.001$) during CPB. In spite of this, systemic oxygen delivery index (DO_2I), if anything, increased (8%), due to the increase in systemic perfusion flow rate during CPB. Body temperature and VO_2I were not significantly affected during CPB.

After CPB, CI was higher (18%; $p<0.01$), SVRI was lower (−21%; $p<0.01$), while MAP was not different from the pre-CPB values. After CPB, hematocrit, serum hemoglobin, and CaO_2 were lower (16 to 19%; $p<0.001$) when compared with the pre-CPB values. After CPB, body temperature and VO_2I (20%, $p<0.05$) were significantly higher when compared with the pre-CPB values. Two patients received nitroprusside during the trial to maintain MAP less than 80 mmHg. Twelve patients required norepinephrine to maintain a target MAP between 60 and 80 mmHg. The dose of norepinephrine was not changed during CPB. After CPB, the dose of norepinephrine was significantly higher when compared with the pre-CPB dose. No other inotropic or vasoactive agents were used during the study.

Table 8. Effects of CPB on systemic hemodynamics.

	Time				
Variable	***Pre-CPB***	***CPB 30 min***	***CPB 60 min***	***Post-CPB***	***p***
MAP (mmHg)	*76±12*	*75±10*	*73±10*	*70±7*	*0.16*
CI or perfusion flow (L/min/m²)	*1.87±0.39*	*2.47±0.08 #*	*2.49±0.08 #*	*2.21±0.52 **	*< 0.001*
CVP (mmHg)	*11±4*	*-1±4 #*	*-1±4 #*	*9±3*	*< 0.001*
Hb (g/L)	*125±14*	*102±13 #*	*100±12 #*	*103±12 #*	*< 0.001*
DO_2I (ml/min/m²)	*319±59*	*346±45*	*343±38*	*305±78*	*0.038*
VO_2I (ml/min/m²)	*74±17*	*81±11*	*81±11*	*89±16 **	*0.007*
SvO_2 (%)	*75±6*	*76±4*	*76±3*	*67±5*	*<0.001*
Temperature (℃)	*35.7±0.41*	*35.6±0.32*	*35.7±0.43*	*36.4±0.39 #*	*< 0.001*
Norepinephrine (µg/kg/min)	*0.020±0.011*	*0.017±0.007*	*0.020±0.009*	*0.076±0.030**	*0.046*

*Values are mean±SD. CPB; cardiopulmonary bypass, CI; cardiac index, CVP; central venous pressure, DO_2I; systemic oxygen delivery index (ml/min/m²), MAP; mean arterial pressure, SvO_2: mixed venous oxygen saturation, VO_2I; systemic oxygen consumption (ml/min/m²). * p<0.05, # p<0.001 vs. Baseline (Pre CPB).*

Effects of CPB on renal variables

During CPB, renal vascular resistance (RVR) increased by 15–23 % ($p<0.005$) with no change in RBF. Thus, as systemic perfusion flow increased, the relationship between RBF and perfusion flow, the RBF/CI ratio, decreased by 25 to 29% ($p<0.01$ and 0.001), suggesting a redistribution of blood flow away from the kidneys during CPB. Hemodilution, in combination with a maintained RBF, caused an 18 to 23% decrease in RDO_2 ($p<0.05$ and $p<0.001$). GFR, filtration fraction, sodium filtration, sodium reabsorption, and urine flow were not affected by CPB. RVO_2 was not affected, while RO_2Ex increased by 33 to 44% ($p<0.05$) during CPB. Neither arterial PAH concentration nor renal PAH extraction was changed during CPB.

After CPB, RDO_2 was still lower (-17%; $p<0.05$), while RBF and RVR were not different from the pre-bypass values. After CPB, GFR, filtration fraction, sodium filtration, sodium reabsorption, and urine flow did not differ from baseline. After CPB, RVO_2 was higher (50%; $p<0.05$) compared with baseline, and RO_2Ex increased further and was 78% higher ($p<0.001$) than the baseline value. After CPB, arterial PAH concentration and renal PAH extraction did not differ from baseline.

Table 9. Effects of CPB on renal variables.

	Time				
Variable	***Pre-CPB***	***CPB 30 min***	***CPB 60 min***	***Post-CPB***	***p***
RPP (mmHg)	*66±11*	*76±12**	*75±12**	*61±7*	*<0.001*
RBF (ml/min/1.73m^2)	*554±126*	*524±116*	*564±162*	*558±130*	*0.638*
RVR (mmHg/ml/min)	*0.13±0.04*	*0.16±0.04 **	*0.15±0.06*	*0.12±0.04*	*0.005*
RBF/CI	*0.30±0.06*	*0.21±0.05#*	*0.23±0.06**	*0.26±0.09*	*< 0.001*
RDO_2 (ml/min)	*96±22*	*74±17#*	*79±24 **	*80±17 **	*0.001*
GFR (ml/min/1.73/m^2)	*67±23*	*68±23*	*70±19*	*67±18*	*0.972*
Filtration fraction	*0.20±0.07*	*0.20±0.06*	*0.19±0.07*	*0.18±0.06*	*0.794*
Sodium filtration (mmol/min)	*8.6±3.8*	*9.5±3.2*	*8.9±3.2*	*9.1±3.0*	*0.674*
Sodium reabsorption (mmol/min)	*8.4±3.7*	*9.3±3.1*	*8.7±3.1*	*8.9±2.8*	*0.889*
Urine flow (ml/min)	*2±0.4*	*2±0.5*	*1±0.2*	*3±0.7*	*0.177*
RVO_2 (ml/min)	*8.0±2.8*	*8.7±3.0*	*8.6±3.1*	*11.6±3.8 **	*0.017*
RO_2Ex	*0.09±0.03*	*0.12±0.05 **	*0.13±0.06 **	*0.16±0.05#*	*< 0.001*
PAH_{art}	*0.28±0.07*	*0.27±0.07*	*0.26±0.07*	*0.27±0.07*	*0.248*
PAH extraction	*0.76±0.1*	*0.75±0.2*	*0.74±0.1*	*0.71±0.1*	*0.692*

*Values are mean±SD. CPB; cardiopulmonary bypass, GFR; glomerular filtration rate, PAH; para-aminohippuric acid, PAH_{art}; arterial PAH concentration, RBF; renal blood flow, RBF/CI; renal blood flow divided by cardiac index, RDO_2; renal oxygen delivery, RO_2Ex; renal oxygen extraction, RPP; renal perfusion pressure, RVO_2; renal oxygen consumption, RVR; renal vascular resistance. * p <0.05, # p <0.001 vs. baseline.*

The oxygen cost per millimole reabsorbed sodium (RVO_2/mM sodium) was 0.9±0.3 ml/mM before CPB and increased by 55% to 1.4±0.4 ml/mM after CPB (p<0.01).

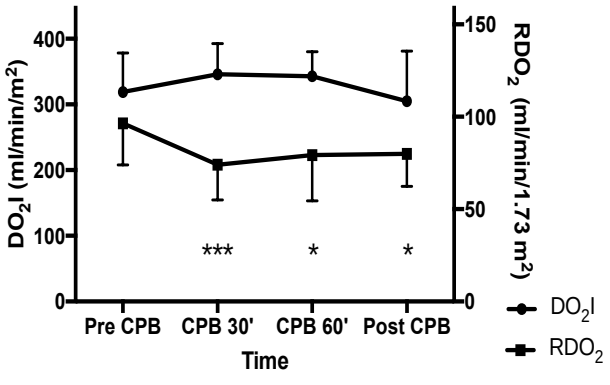


*Figure 9. The effects of cardiopulmonary bypass (CPB) on systemic (DO_2I) and renal(RDO_2) oxygen delivery before (Pre-CPB), 30min (CPB 30'), and 60 min (CPB 60') after initiation of CPB, and after end of CPB (Post-CPB). * p < 0.05, *** p < 0.001 versus baseline (Pre-CPB).*

4.3 PAPER III

To evaluate the effects of different CPB flow rates on renal oxygenation, 17 patients with a normal serum creatinine and a LVEF ≥50 % undergoing cardiac surgery with normothermic CPB were studied.

Effect of different CPB flow rates on systemic variables and the CPB circuit

At CPB flow rates of 2.7 and 3.0 L/min/m^2, MAP (6–9%, p=0.003), renal perfusion pressure (7–9%, p=0.007), SvO_2 (4–7%, p<0.001) and DO_2I (16–28%, p<0.001) were higher, SVRI (-4 to -12%, p<0.001) was lower, while CVP, arterial hemoglobin, VO_2I, body temperature and the norepinephrine dose were unchanged, when compared to a CPB flow rate of 2.4 L/min/m^2.

Table 10. Systemic variables before CPB and at different CPB flow levels.

		CPB flow (L/min/m^2)			
Variable	***Baseline (Pre-CPB)***	***2.4***	***2.7***	***3.0***	***p***
CI (L/min/m^2)	*1.8±0.4*				
MAP (mmHg)	*73±8*	*70±10*	*74±9 **	*76±8 **	*0.003*
CVP (mmHg)	*11±5*	*2±4*	*2±4*	*3±3*	*0.470*
SvO_2 (%)	*72±9*	*76±2*	*79±2#*	*81±2#*	*<0.001*
Hemoglobin (g/L)	*120±11*	*102±10*	*102±9*	*101±9*	*0.423*
DO_2I (ml/min)	*278±42*	*322±45*	*374±32#*	*412±36#*	*<0.001*
SVRI (dyn/s/cm^5/m^2)	*3587±768*	*2810±443*	*2704±339*	*2485±301#*	*<0.001*
Norepniephrine (µg/kg/min)	*0.06±0.05*	*0.08±0.07*	*0.07±0.06*	*0.06±0.06*	*0.064*

*Values are mean±SD. CI; cardiac index, CPB; cardiopulmonary bypass, CVP; central venous pressure, DO2I; systemic oxygen delivery index, MAP; mean arterial pressure, SVRI; systemic vascular resistance index, VO2I; systemic oxygen consumption index. Difference vs. 2.4 L/min/m2 pump flow rate is indicated * p <0.05, # p <0.001.*

Effects of different CPB flow rates on renal variables

At CPB flow rates of 2.7 and 3.0 L/min/m^2, renal vein oxygen saturation was higher (2–4%, p=0.001) and renal oxygen extraction was lower (-12% to -23%, p=0.001) when compared to a CPB flow rate of 2.4 L/min/m^2. This corresponds to an increase in the renal oxygen supply/demand ratio (DO_2/VO_2 ratio, i.e. the reciprocal of RO_2Ex) by 14 and 30%, respectively, at the two higher flow rates. There was a trend for an increase in renal sodium excretion, while renal FF was not affected by increasing CPB flow rates. During CPB,

the changes in RO_2Ex were negatively correlated with SvO2 (R^2=-0.853, p=0.003), but not with MAP (p=0.671).

Table 11. ***Renal variables before CPB and at different CPB flow levels.***

		CPB flow (L/min/m²)			
Variable	***Baseline (Pre-CPB)***	***2.4***	***2.7***	***3.0***	***p***
$SrvO_2$ (%)	*86±4*	*83±6*	*85±6 **	*86±4 ***	*0.001*
RO_2Ex (%)	*12.1±4.0*	*15.7±6.2*	*13.8±5.8 **	*12.1±4.4 ***	*0.001*
Filtration fraction	*0.26±0.8*	*0.20±0.9*	*0.21±0.9*	*0.22±0.9*	*0.476*
Urine flow (ml/min)	*1.3±1.6*	*1.7±1.3*	*2.1±1.6*	*2.8±1.8 ***	*0.012*
Sodium excretion (mmol/min)	*0.09±0.10*	*0.10±0.12*	*0.15±0.17*	*0.21±0.24*	*0.069*

*Values are mean±SD. CPB; cardiopulmonary bypass, RO_2Ex; renal oxygen extraction, $SrvO_2$; renal vein oxygen saturation. Asterisks indicate difference vs. 2.4 L/min/m2 pump flow rate at * p<0.05, ** p<0.01.*

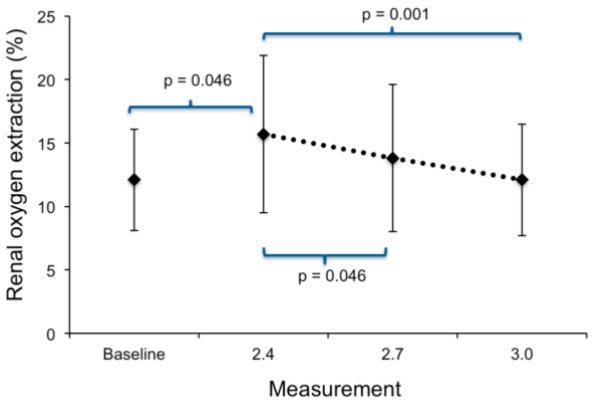


Figure 10. Renal oxygen extraction before CPB (baseline) and during CPB at 2.4, 2.7 and 3.0 L/min/m² flow rates. Values are mean±SD.

Effects on reservoir volume and trans-oxygenator pressure

Increasing CPB flow from 2.4 to 3.0 L/min/m², increased the pressure head over the oxygenator by 34% and decreased the blood volume of the reservoir by 18%. To ensure the safety minimum reservoir level, one patient received a 200 ml crystalloid bolus at the CPB flow rate of 2.4 L/min/m², and two patients were given 200 and 400 ml, respectively, at the CPB flow of 3.0 L/min/m².

4.4 PAPER IV

To evaluate the differential effects of levosimendan and dobutamine, 32 patients with heart failure and impaired renal function (GFR<80 ml/min/1.73m^2) were studied. Patients were randomized (1:1) to receive either levosimendan (loading dose 12 µg/kg + 0.1 µg/kg/min) or dobutamine (7.5 µg/kg/min) for 75 minutes. The duplicate measurements made at baseline and after treatment were pooled before analysis.

In three patients (all in the levosimendan group) renal data were incomplete: due to missing data (n=1) and/or displacement of the renal vein catheter during the experimental procedure (n=2). In the two latter patients, exceptionally high PAH concentrations confirmed that the blood samples were, to a great extent, sampled from the inferior vena cava and not exclusively from the renal vein.

Systemic variables

Systemic variables did not differ between study groups at baseline, nor did they display between group differences with respect to treatment effects. Levosimendan and dobutamine increased stroke volume index (14 % and 13 %, respectively), cardiac index (17 % and 28 %, respectively), systemic oxygen delivery (18% and 29 %, respectively) and SvO_2 (4.7 % and 7.8 % units, respectively). CVP and PCWP decreased in both groups. Both drugs caused an increase in heart rate, which tended to be more pronounced in patients receiving dobutamine. There was a trend for a larger fall in SVRI in the dobutamine group (-21%) compared to the levosimendan group (-8%).

In the dobutamine group, 3 patients received both norepinephrine and crystalloid, and 1 patient received only norepinephrine. One patient in the levosimendan group received norepinephrine for hypotension. Neither the mean infusion rate of the crystalloid nor the mean dose of norepinephrine differed between the groups. No serious adverse events occurred during the trial.

Table 12. Systemic variables before and after study drug administration.

	Levosimendan, n=16		*Dobutamine, n=16*		
Variable	***Baseline***	***Treatment***	***Baseline***	***Treatment***	***p***
CI (L/min/m^2)	*2.30±0.36*	*2.70±0.59 **	*2.41±0.58*	*3.08±0.53 #*	*0.162*
HR (min^{-1})	*71±5*	*73±5 **	*78±19*	*88±20 **	*0.057*
MAP (mmHg)	*69±10*	*71±9*	*70±9*	*70±9*	*0.349*
MPAP (mmHg)	*31±9*	*29±9*	*25±10*	*24±11*	*0.864*
CVP (mmHg)	*9±5*	*7±4 **	*8±9*	*6±8 **	*0.728*
PCWP (mmHg)	*19±7*	*17±6*	*14±8*	*12±9 **	*0.795*
DO_2I (ml/min/m)	*348±73*	*409±89 **	*391±110*	*504±102 #*	*0.116*
VO_2I (ml/min/m^2)	*129±18*	*136±24*	*130±29*	*129±16*	*0.367*
SaO_2 (%)	*93.4±3.6*	*93.8±2.5*	*95.1±2.2*	*96.1±1.9 **	*0.298*
SvO_2 (%)	*57.4±10.3*	*62.1±6.3 **	*62. ±7.9*	*70.5±7.8 #*	*0.369*
SVRI (dyn/s/cm^5/m^2)	*2141±494*	*1961±491*	*2162±574*	*1704±338 **	*0.092*
PVRI (dyn/s/cm^5/m^2)	*440±300*	*394±205*	*386±245*	*349±219*	*0.506*

*CI; cardiac index, CO; cardiac output, CVP; central venous pressure, DO_2I; systemic oxygen delivery index; HR; heart rate, MAP; mean arterial pressure, MPAP; mean pulmonary artery pressure, PCWP; pulmonary capillary wedge pressure, PVRI; pulmonary vascular resistance index, SaO_2; arterial oxygen saturation, SVI; stroke volume index, SvO_2; mixed venous oxygen saturation, SVRI; systemic vascular resistance index, VO_2I; systemic oxygen consumption index. An asterisk indicates difference vs. baseline at: * p<0.05, # p<0.001.*

Renal variables

Baseline measurements (B1 and B2) of arterial PAH concentration were 0.29±0.12 and 0.29±0.11 in the levosimendan group (p=0.98), respectively and 0.31±0.06 and 0.31±0.07 in the dobutamine group (p=0.51), respectively; suggesting that a steady state was reached in both groups. There were no significant differences between the groups at baseline.

After treatment, RBF increased by 22 % in the levosimendan group and 26 % in the dobutamine group, with corresponding increases in renal oxygen delivery and no significant differences between groups. The renal vascular resistance decreased in both groups (-9 % in the levosimendan group, and -16 % in the dobutamine group, p=0.25). GFR increased by 22 % in the levosimendan group, but remained unchanged in the dobutamine group

(p=0.012). Filtration fraction was not affected by levosimendan and decreased by 17 % with dobutamine (p=0.045). Renal oxygen extraction decreased in both groups with no differences between groups. The ratio between RBF and CI was not affected by either of the two agents.

Table 13. Renal variables before and after study drug administration.

	Levosimendan, n=13		***Dobutamine, n=16***		
Variable	***Baseline***	***Treatment***	***Baseline***	***Treatment***	***p***
RBF (ml/min/1.73 m^2)	*426±197*	*518 ± 276 **	*397 ± 121*	*499 ± 154 #*	*0.732*
GFR (ml/min/1.73 m^2)	*36.5±18*	*44.5±19 **	*47.1±15*	*47.3±6.9*	*0.012*
FF	*0.146±0.08*	*0.143±0.07*	*0.19±0.07*	*0.16±0.08 **	*0.045*
PAH_{ext}	*0.70±0.21*	*0.65±0.22 **	*0.793±0.15*	*0.754±0.19 **	*0.614*
PAH_{art}	*0.29±0.11*	*0.26±0.10 #*	*0.31±0.06*	*0.27±0.07 #*	*0.194*
RVO_2 (ml/min)	*9.2±6.3*	*10.1±6.2*	*8.3±2.6*	*8.9±4.3*	*0.801*
RDO_2 (ml/min)	*67.0±36.5*	*82.4±50.3 **	*65.0±23.8*	*82.2±29.3 #*	*0.728*
RO_2Ex (%)	*15.5±6.7*	*13.8±5.0*	*14.5±7.0*	*12.0±6.5 **	*0.487*
$SrvO_2$ (%)	*78.6±8.6*	*80.5±5.9*	*81.3±7.7*	*84.6±7.1 **	*0.117*
RBF/CI (%)	*18.5±7.7*	*19.5±9.8*	*16.9±4.9*	*16.8±6.3*	*0.474*
RVR (mmHg/ml/min)	*0.161±0.05*	*0.147±0.05 **	*0.171±0.07*	*0.144±0.06 **	*0.249*

*GFR; glomerular filtration rate, FF; filtration fraction, PAH_{art}; arterial para-aminohippurate concentration, PAH_{ext}; renal extraction of PAH, RBF; renal blood flow, RDO_2; renal oxygen delivery, RO_2Ex; renal oxygen extraction, RVO_2; renal oxygen consumption, RVR; renal vascular resistance, $SrvO_2$; renal vein oxygen saturation. An asterisk indicates difference vs. baseline at: * $p<0.05$, # $p<0.001$.*

5 DISCUSSION

5.1 STUDY POPULATION AND ETHICAL ISSUES

In papers I–III, the study populations were elective cardiac surgery patients. The main inclusion criterion in these studies was an expected CPB time exceeding 60 minutes, which would allow for repeated measurements during the CPB period. This, and logistical reasons excluded many faster CABG cases. In papers II and III, the included patients had a LVEF ≥50 %, but the indication for surgery in a majority of these patients was valvular disorders, which might explain the relatively low levels of cardiac index in these patients when anesthetized, before CPB (1.9 and 1.8 L/min/m^2 for paper II and III, respectively)

The second main inclusion criterion in these studies, a preoperative serum creatinine within the normal range, was chosen since renal oxygenation and possibly also renal autoregulation might be affected in patients with renal impairment. However, the normal creatinine range is wide, and in an elderly population a normal creatinine might veil a dwindling kidney function. The mean preoperative estimated GFR (using the MDRD formula)[61] was 75±15, 79±11, and 78±11 ml/min/1.73m^2 in studies I–III, respectively.

Thus, the patients in these studies were possibly more marginal in their cardiac and renal function than the average CABG patient, but they are, nonetheless, representative of patients undergoing more complex cardiac surgery.

One limitation in papers I and II is that there were no control groups subjected to cardiac surgery without the use of CPB. Ideally, controls undergoing off-pump cardiac surgery or other major surgery could have been studied and compared to the study group in papers I–II. On the other hand, off-pump cardiac surgery is no longer performed at our institution.

In paper III, the choice of flow rates was based on clinical practice and safety considerations. We chose to exclude patients with a BMI >32 kg/m^2, as their body surface area would lead to high flow with the possible associated risks. In addition, patients with a history of cerebral lesions were excluded.

In paper IV, we studied patients with chronic heart failure and reduced renal function. The patients were scheduled for right heart catheterization either as a

work-up for possible heart transplantation or as a comprehensive investigation to optimize heart failure therapy. Since patients with acute decompensated heart failure were excluded, the study participants were not in immediate need of inotropic therapy. One could argue that this reduces the clinical applicability of the study. However, our method of choice, with invasive measurement of renal variables, by renal vein catheterization, requires the patient to be cooperative and able to lie supine for the duration of the entire study procedure, often 2–3 hours. As patients with acute decompensated heart failure might, for obvious reasons, have difficulties to comply with such a protocol, we chose (after ethical and practical considerations) to include only patients with stable chronic heart failure and impaired renal function. To perform a similar invasive pharmacological investigation on patients suffering from acute heart failure would be very difficult, if not impossible.

The study's definition of a renal impairment, GFR <80 ml/min/1.73 m^2, is above the definition of cardiorenal syndrome, i.e. a GFR <60 ml/min/1.73 m^2.[6] This cutoff was chosen due to problems of recruiting suitable patients with a more severe renal dysfunction. However, the measured GFR in both the levosimendan and dobutamine groups were well below 60, at 43±15 and 53±15 ml/min/1.73 m^2, respectively.

In all four studies, a majority of the participants were men. The study populations partly reflect the population of patients undergoing cardiac surgery, where women are underrepresented.[62] In addition, catheterization of the renal vein is often significantly harder in smaller persons, and most of the patients who were excluded due to failed catheterization were women.

5.2 METHODOLOGICAL CONSIDERATIONS

5.2.1 RBF by infusion clearance of PAH

We used the so-called constant infusion clearance technique, in which renal clearance of PAH is calculated from the arterial serum level of PAH and the infusion rate of PAH, a technique that makes the collection of urine redundant. This technique for estimation of RBF, corrected for renal extraction of PAH, has been validated in cardiac surgery patients against standard urinary clearance for PAH.[63] It was found to have a high reproducibility and a high level of agreement with the urinary clearance reference method. The requirements for an infusion clearance technique are that the test substance (e.g., PAH) is rapidly equilibrated after the start of infusion, not metabolized and only excreted by the kidney. Furthermore, the rate of infusion and the rate

of excretion should be at equilibrium, as indicated by stable serum concentrations of the test substance.

In paper II, this technique was used to measure renal blood flow in patients during CPB. Both at the initiation and discontinuation of CPB, there are major shifts of fluid that could affect the volume of distribution. Thus, a reasonable time must be allowed for a steady state, i.e. equilibrium in the infusion and excretion of PAH. In paper II, arterial concentration of PAH was unchanged throughout the measurements before, during, and after CPB, suggesting that a steady state was reached. In paper III, the periods of each CPB flow level (10 minutes) were deemed too short to allow for reliable measurements of RBF with the infusion clearance technique.

Redfors et al found that the mean renal extraction of PAH was 0.68 in post-cardiac surgery patients with AKI, as compared to 0.85 in uncomplicated postoperative patients[24], and that the difference might be attributable to tubular injury which might reduce the tubular secretion of PAH in the AKI group. In the patients of paper II, the mean renal extraction of PAH was 0.74–0.76 during CPB. The early postoperative NAG-peak suggests that a tubular injury has occurred during CPB, which could explain lower PAH extraction during CPB, when compared to an uncomplicated post-cardiac surgery group.[24] Another explanation could the slight hypothermia seen during CPB (35.5–36.0 °C), which could decrease the efficiency of the tubular secretory pumps due to the lower metabolic rate. In paper IV, the arterial PAH level was stable between the two baseline measurements, indicating that a steady state was reached before start of the intervention. Both levosimendan and dobutamine induced similar reductions in PAH extraction. These findings suggest that both agents redistribute blood flow from the outer cortex to the inner cortex and medulla, as the latter areas have the lowest extraction of PAH, as previously described for dopamine.[27,64,65]

5.2.2 Filtration fraction by renal extraction of ^{51}Cr-EDTA

^{51}Cr-EDTA is filtered freely into the primary urine, but neither reabsorbed nor secreted by the kidney. Thus, its rate of excretion in the urine is directly proportional to the rate of filtration of water and solutes across the glomerular membrane. It can easily be shown that the renal extraction of ^{51}Cr-EDTA, ([^{51}Cr-EDTA arterial] - [^{51}Cr-EDTA renal vein]) / [^{51}Cr-EDTA arterial] is a direct measurement of the renal filtration fraction (FF), i.e. GFR/RPF. Thus, by measuring RPF and FF, GFR can be calculated. The coefficients of variation for repeated measurements of FF by renal extraction of ^{51}Cr-EDTA has been shown to be 5%.[63] The formation of urine induces a

hemoconcentration of ^{51}Cr-EDTA, which will increase renal vein concentration of ^{51}Cr-EDTA and thereby underestimate FF and GFR. The formula for renal extraction of ^{51}Cr-EDTA should therefore be corrected for urine flow, particularly at high levels of urine flow, as was done in paper II. In paper IV, urine correction was not performed, as these patients had no urinary catheter for measurement of urine flow.

5.2.3 Tubular injury marker N-acetyl-β-D-glucosaminidase

Strong evidence points towards renal, and especially medullar, ischemia as a key factor in the development of post cardiac surgery AKI.[66] Thus, to assess renal ischemia in papers I and II, we chose to measure NAG in urine. Urine NAG is considered a sensitive marker of tubular injury.[44] Although NAG may increase in cases of elevated lysosomal activity[67], it has not, unlike NGAL, been shown to increase in response to systemic inflammation *per se*.[68] Furthermore, due to its high molecular weight, it is not subject to glomerular filtration, in contrast to NGAL, and increased levels of urinary NAG is associated with tubular necrosis in cardiac surgery patients.[69]

Urinary biomarkers and proteins are commonly reported as a ratio to urinary creatinine concentration in order to reduce the impact of urine flow. However, urinary creatinine levels are affected by renal function. Creatinine is filtered in proportion to GFR and the excreted creatinine is subject to leakage back into the blood stream through damaged cells in the tubular epithelium. This could bias the levels of urinary biomarkers expressed as a ratio to creatinine, probably mainly in the direction of increasing sensitivity at the cost of decreased specificity.[70] Since the urine flow might be highly variable during surgery, we chose to correct the NAG-values for creatinine to compensate for changes in diuresis.

5.2.4 Dose selection of levosimendan and dobutamine

The protocol used in paper IV was based on a previous study on the renal effects of levosimendan vs. placebo in post-cardiac surgery patients.[55] The recommendation of the manufacturer is to start levosimendan treatment with a bolus dose of 6–12 µg/kg, but this has been abandoned in many centers due to the risk of hypotension. The elimination half-time of levosimendan is approximately 1 hour, and without a bolus dose, a steady state is reached after >3 hours.[51] In the present study, in which blood pressure was monitored by an intra-arterial line, a bolus infusion was used to reach a steady state more quickly and, thereby, allow for measurements of clinical effects within a reasonable time frame.

Levosimendan is metabolized in the liver, and forms several active metabolites, e.g. OR-1896 and OR-1855.[51] OR-1896 has a prolonged effect, which may explain the long duration, up to a week, of levosimendan. The metabolites are formed slowly and are at peak concentration at two days after the end of a 24-hour infusion.[51] Thus, it is unlikely that the concentration of the metabolites would be high enough to have an impact on renal variables in study IV. To our knowledge, there is no information on the renal effects of OR-1896 or OR-1855.

Our aim was to use a dose of dobutamine that would increase CO to a similar degree, at least, as would levosimendan. The dose increase after 10 min from 5 to 7.5 µg/kg/min was made to mimic the change in levosimendan infusion rate and thereby facilitate and ensure blinding. The recommended starting dose of dobutamine is 5 µg/kg/min, which can be adjusted to 2–20 µg/kg/min according to the clinical response. In the present study, dobutamine and levosimendan exerted comparable effects on stroke volume, while there was an insignificant trend for a more pronounced heart rate increase in the dobutamine group.

5.3 RENAL TUBULAR INJURY DURING CPB (PAPER I)

The main findings of paper I were that the tubular injury marker NAG increased already after 30 minutes of CPB, remained elevated throughout the CPB period and peaked after weaning from CPB. This excretion pattern suggests that there is an ongoing renal insult during CPB, which is even more aggravated early after discontinuation. The nature of this insult is not entirely clear, but the NAG-levels correlate with the renal oxygen extraction ($r=0.57$; $p<0.001$, paper II), which points toward ischemia as an important factor in the development of CPB-related tubular injury.

The study's second findings were that the magnitude of postoperative tubular injury is dependent on the duration of CPB and the change (increase) in temperature, i.e. rewarming after CPB. This is in line with a study of biomarkers by Boldt and colleagues, where patients with a CPB time exceeding 90 minutes showed more pronounced kidney injury.[71] The negative effects of rewarming found in our study is also consistent with the predictions made in a computer model, where the medullary oxygen consumption is greatly increased during the rewarming phase, but the oxygen delivery is increased only moderately during the same period, causing a medullary oxygen supply demand mismatch.[72]

One could speculate that the hemodilution and reduced oxygen delivery during CPB renders the renal medulla ischemic, with loss of tubular cell integrity as a result. Thus, the injury sustained during CPB leaves the kidney in a vulnerable state. The second insult, after discontinuation of CPB, might represent a reperfusion injury of the previously hypo-perfused tissues. At this time point, the added rewarming increases oxygen consumption[72], which further offsets the medullary oxygen balance.

In addition to a possible direct ischemic insult to the kidneys, activation of the immune and complement systems during surgery and CPB might cause tubular injury through several pathways. The CPB-induced provocation of the systemic inflammatory response syndrome (SIRS) may explain the link between CPB duration and peak NAG. Systemic inflammation will activate the neutrophils, platelets and vascular endothelium with upregulation of adhesion molecules and release of proteases, free oxygen radicals, chemokines and cytokines, which will cause capillary leak, lipid peroxidation edema and tubular cell injury.[73-77] Some of the pro-inflammatory cytokines (TNF-alpha, IL-1, IL-8) are smaller molecules and undergo glomerular filtration, and it has been shown that there is a correlation between levels of urinary cytokines and tubular injury (NAG release) in cardiac surgery using CPB.[78]

In this material, the peak NAG excretion seen at 90 minutes after weaning from CPB was *not* associated with postoperative AKI, according to the KDIGO criteria.[4] Previous studies on the performance of NAG at predicting AKI have shown conflicting results. Liangos et al found no association between u-NAG at 2 hours after CPB and AKI.[79] Vermeulen-Windsant and colleagues found higher u-NAG levels at 15 minutes after CPB in patients who later developed AKI, but there was no difference between the groups at 2 hours after CPB.[80] Han and colleagues found a modest correlation (AUROC 0.61) between u-NAG collected immediately after CPB and postoperative AKI.[69] In a recent study of several urinary tubular injury markers, the biomarker levels at 3 hours after ICU admission could improve a clinical model of AKI detection, but the biomarker levels at ICU admission did not differ between patients who developed postoperative AKI and those who did not.[81] Thus, in addition to different timing of the urine sampling, differences in strategies regarding postoperative rewarming and circulatory goals might attribute to the disparity of data on NAG and other biomarkers for AKI prediction. Furthermore, the use of serum creatinine for assessment of AKI could be dubious, particularly in cardiac surgery patients, as serum concentration of creatinine may be heavily affected by hemodilution due to water overload in the early postoperative period.

Prediction of AKI was not the aim of the present study, nor was the study powered for it, but it is nonetheless an interesting finding. If anything, it underscores the considerable functional reserve capacity and regenerative potential of the kidneys. After milder tubular injury, a profound proliferation of the uninjured epithelial cells is seen, which leads to restoration of the normal epithelium.[82] However, if the tubular cells suffer repeated mild or more severe injury, it results in interstitial fibrosis, inflammation and glomerulosclerosis.[83] Thus, the ischemic tubular insult during and shortly after CPB may not be deleterious if additional injury can be prevented in the early postoperative period. On the other hand, it was recently shown that subclinical/structural AKI assessed by biomarkers of tubular injury was associated with reduced renal functional reserve capacity, even in the absence of clinical postoperative AKI.[84] This might represent a renal impairment, undetected by serum creatinine changes, that may have long-lasting impact for the patient.

Limitations

One major limitation of the present study was the relatively low number of included patients. Furthermore, no inflammatory markers were measured, so any correlation between inflammation and NAG-release could not be explored. Another limitation was that urine output criteria for AKI diagnosis were not used.

5.4 RENAL BLOOD FLOW, OXYGENATION AND FILTRATION DURING CPB (PAPER II)

The main finding was that the renal oxygenation is impaired during CPB. This was seen as an increase in RO_2Ex, mainly due to reduced renal oxygen delivery (RDO_2) at maintained oxygen consumption (RVO_2). The reduction in RDO_2 during CPB was caused by hemodilution and increased renal vascular resistance (RVR), which caused redistribution of blood flow away from the kidneys.

The mean systemic DO_2 during CPB was around 345 ml/min/m^2, well above the critical levels of systemic DO_2 of 225–272 ml/min/m^2, that have been shown to be predictive of postoperative AKI.[34,36,37] Hematocrit levels below 22–26 % during CPB are associated with cardiac surgery-associated AKI (CS-AKI).[36,85,86] The mean hematocrit in the present study was only reduced to around 30 % during CPB. We therefore believe that CPB was safely performed in the current study with respect to systemic oxygen delivery, as mixed venous oxygen saturation was well maintained. However, in spite of this, RDO_2 was

reduced by 20 % during CPB. The increased RO_2Ex and corresponding NAG-release suggest that at normothermia and a pump flow of 2.5 L/min/m^2, renal ischemia and tubular injury is ongoing throughout the CPB period.

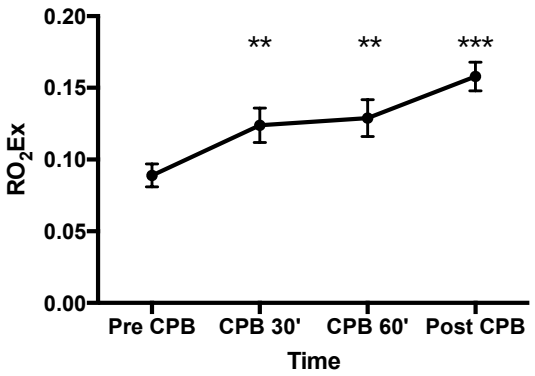


*Figure 11. Renal oxygen extraction before, during and after cardiopulmonary bypass (CPB). Values are mean±SEM. Asterisks indicate difference vs. Pre CPB at * $p<0.05$, ** $p<0.01$.*

The renal oxygen supply/demand mismatch starting already during CPB was further aggravated after CPB, as shown by a nearly 80% increase in RO_2Ex. The increase in RVO_2 after CPB could not be attributed to an increase in GFR and tubular reabsorption, since neither of these variables differed from baseline. One obvious explanation is the higher body temperature seen after CPB (36.4° vs. 35.7°C), which increased VO_2I. On the other hand, the increase in RVO_2 was considerably higher than the increase in VO_2I (45% vs. 20%). Another explanation could be the finding that after CPB, the oxygen consumption per millimole of reabsorbed sodium was 55% higher than before, indicating a shift in the relationship between sodium reabsorption and RVO_2. Such an increased oxygen utilization for tubular sodium transport has previously been described in patients with post–cardiac surgery AKI.[24] Efficient vectorized sodium reabsorption is dependent on polarized tubular cells and intact tight junctions. Ischemic tubular damage has been shown to depolarize tubular cells and disrupt tight junctions.[87-89] Thus, reabsorbed sodium ions may leak back to the tubular lumen to be reabsorbed again, which might explain the high oxygen utilization per millimole net sodium reabsorbed, as seen in the current study. This might, in turn, be caused by tubular injury/dysfunction, as also manifested by the release of renal injury marker NAG.

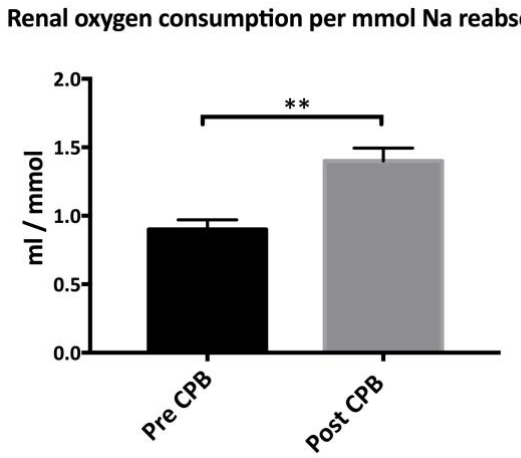


Figure 12. Renal oxygen consumption per millimole sodium reabsorbed before (Pre CPB and after (Post CPB) cardiopulmonary bypass. Values are mean±SEM. Asterisks indicate difference with a p-value <0.01 (paired T-test).

Limitations

The use of vasopressors may have influenced renal vascular tone and RDO_2. Twelve patients required norepinephrine during CPB to maintain a MAP between 60 and 80 mmHg. One could argue that the use of norepinephrine in the majority of the patients (67%) could have contributed to the increase in renal vascular tone and impaired oxygen delivery during and after CPB. We believe that this is less likely, as we have previously shown in post–cardiac surgery patients with AKI that restoration of MAP from 60 to 75 mmHg increased RDO_2 and GFR and improved renal oxygenation.[90] Furthermore, the switch from sevoflurane to propofol during CPB could have influenced renal vascular tone and RDO_2. However, experimental studies have shown that propofol does not affect RBF or RVR.[91,92]

5.5 IMPACT OF CPB FLOW ON RENAL OXYGENATION (PAPER III)

As a consequence of the finding of impaired renal oxygenation at normothermic CPB at 2.5 L/min/m^2, we studied the effect of increased pump flow on renal oxygenation. The main finding of the study was that increased CPB flow rates improved renal oxygenation, expressed as reduced renal oxygen extraction. Thus, increasing the pump flow from 2.4 to 3.0 L/min/m^2 improved the oxygen supply/demand relationship by 30 %, and RO_2Ex was reduced to pre-bypass levels.

In a retrospective study, Svenmarker and colleagues found that systemic blood flow control to target SvO_2 >75 % during CPB was associated with reduced risk of AKI, and this was more pronounced for procedures exceeding 90 minutes.[93] Our results are in line with these findings, and the CPB-induced changes in SvO_2 were closely correlated to RO_2Ex (R^2=0.853, p=0.003). This implies that the target SvO_2 of 75 % might be too low to avoid impaired renal oxygenation during CPB.

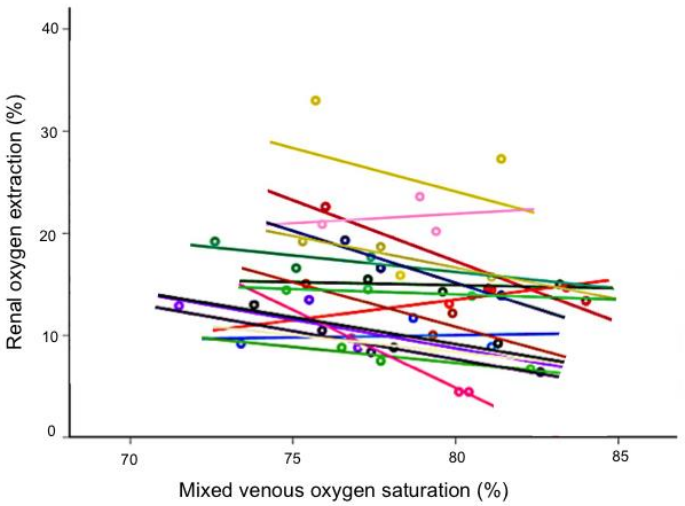


Figure 13. Individual change in renal oxygen extraction and mixed venous oxygen saturation at different CPB flow levels.

Although renal blood flow (RBF) was not directly measured in the present study, it is not unreasonable to interpret the improved renal oxygenation at a CPB flow rate of 2.7 and 3.0 L/min/m^2, as shown in the present study, as a result of increased RBF and oxygen delivery. The dependency of RBF on CPB flow is supported by a study of Mackay and colleagues, who found that changes in pump flow and perfusion pressure could significantly alter RBF in a porcine model of normothermic CPB.[94] The only published clinical human study, using hypothermic (28 °C) CPB and low CPB flows (1.4–2.2 L/min/m^2), also revealed that RBF was primarily determined by the pump flow rate and less so by MAP.[95]

The urine flow in study III was increased by 24–65 % when CPB flow rate was increased to 2.7 and 3.0 L/min/m^2. This is in line with a retrospective study by Hori and colleagues, where urine flow rate correlated with CPB flow rate. In

addition, the authors also found that a urine flow of <1.5 ml/kg/h during CPB was independently associated with CS-AKI.[96]

It is not immediately evident that an increase in RBF, induced by e.g. an increase in CPB flow rate, would improve renal oxygenation. Renal tissue oxygenation is dependent on the balance between renal oxygen consumption and oxygen delivery. Experimental studies have shown that renal oxygen consumption is dependent on RBF and that changes in RBF and oxygen delivery will cause correspondent changes renal oxygen consumption. Thus, an increase in RBF will increase GFR and the filtered amount of sodium, which in turn will increase the tubular sodium load and consequently the renal oxygen consumption.[16,97] It has repeatedly been shown in patients undergoing cardiac surgery that both tubular sodium reabsorption and renal oxygen consumption are closely correlated to GFR, which is a major determinant of renal oxygen consumption.[25,26,98] In the present study, the renal filtration fraction, i.e. the renal extraction of ^{51}Cr-EDTA, did not change significantly with variations in CPB flow, indicating a maintained balance between GFR, renal oxygen consumption and RBF. One would therefore have expected no change in renal oxygenation at increasing levels of CPB flow rates. The fall in renal oxygen extraction with increasing CPB flow rates, at an unchanged filtration fraction, as demonstrated in paper III, thus implies that other mechanisms in general and during CPB in particular, may be of importance for renal oxygenation.[97] Thus, the improved renal oxygenation and concomitant increased urine flow at increased CPB flow suggest an uncoupling of renal oxygen consumption and GFR. A dissociation of sodium reabsorption from oxygen consumption has been observed in several experimental studies.[97] The mechanisms are not fully understood, but might include intrarenal redistribution of blood flow, or a shift of sodium reabsorption to sites of higher oxygen utilization efficiency.[99] Renal autoregulation is mainly effective in controlling cortical blood flow[15], and increases in MAP and RBF during CPB may thus redirect flow to the medulla. In our patients, higher CPB flows were accompanied by increased MAP and a trend for an increase in sodium excretion. This could reflect pressure natriuresis, which is mediated by an inhibition of sodium reabsorption,[100] and might be another mechanism by which renal oxygenation is improved at higher CPB flow rates.

Higher CPB flow may have negative consequences, such as increased tissue edema,[101] exaggerated inflammatory response, increased blood cell shear stress with hemolysis and activation of cellular blood components.[102-104] Other potential problems include increased venous blood return to the surgical field and low reservoir volume with need for volume replacement. As expected, we found that the trans-oxygenator pressure head increased with higher CPB flow

rates. Although the level of hemolysis could not be adequately measured due to the short time spent at each CPB flow rate, the available evidence does not support a linear correlation between trans-oxygenator pressure and hemolysis in contemporary oxygenators.[105,106] The venous reservoir volume decreased with increased flow, but the changes occurred within the first minute of altered CPB flow, and no ongoing fluid shifts could be detected throughout the measurement periods.

Limitations

The main limitation of the study is that renal blood flow was not measured. Consequently, GFR, renal oxygen delivery and consumption could not be determined. The PAH-method for renal plasma flow assessment requires a steady state, and is not suitable for rapid changes in RBF. The retrograde renal vein thermodilution technique for measurements of RBF was tested in some pilot cases, but yielded unstable measurements.

It is also unclear whether the beneficial effect of a higher CPB flow on renal oxygenation is sustained beyond the 10-minute intervention period. Due to the nature of the experimental procedure, we have no data on whether high CPB flow rates are beneficial in terms of renal outcome. Thus, whether the improved renal oxygenation seen at the higher than normal CPB flow rates, as shown in the present study, will translate into a lower incidence of postcardiac surgery AKI, remains to be shown in a randomized trial.

5.6 RENAL PHYSIOLOGY DURING AND IMMEDIATELY AFTER CPB

Our findings suggest that the renal medulla is in a state of continuous hypoxia/ischemia with ongoing tubular injury (increased NAG-excretion) during CPB. This might be caused by the mismatch in renal DO_2/VO_2, as evidenced by the increased RO_2Ex, which is correlated with the NAG-excretion ($r=0.57$; $p<0.001$). Hemodilution from cell-free priming solution in the CPB circuit reduces the oxygen carrying capacity of the blood, and renal vasoconstriction reduces the proportion of blood perfusing the kidneys in relation to the total systemic blood flow. Furthermore, the renal function, as assessed by GFR, and renal oxygen consumption, is not reduced during CPB, adding to the oxygenation imbalance.

After weaning from CPB, reperfusion of the injured tubular epithelium adds to the injury, with further increased NAG-excretion and RO_2Ex. In this phase, rewarming also increases oxygen consumption. The dramatic increase in NAG and RVO_2 suggests that the integrity of the tubular cells and the tight junctions are disrupted. This loss of polarization leads to inefficient sodium transport, where the Na-K-ATPase continues its oxygen-consuming pumping action but the sodium leaks freely back into the tubular lumen. Hence, the oxygen cost of sodium reabsorption is increased by 55 % compared to pre-CPB levels.

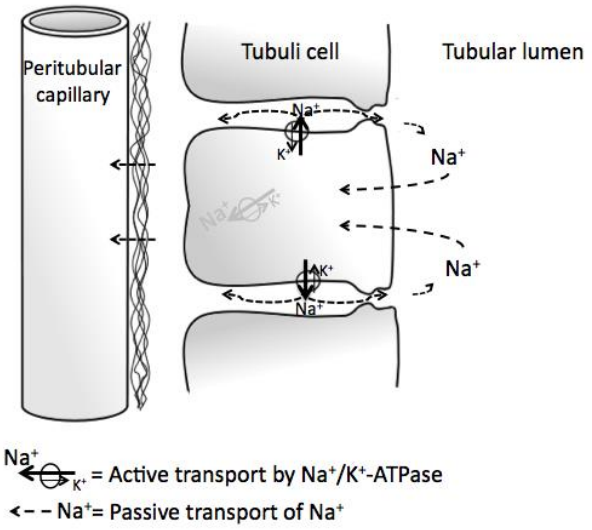


Figure 14. Tubulus cell injury and sodium transport. Ischemic injury leads to disruption of the cell membrane and tight junctions, which allows sodium to leak back into the tubular lumen. Thus, the net oxygen cost per mmol sodium that is transported into the peritubular capillaries is increased. From Redfors, 2010, with permission.

The intrarenal blood flow distribution may be altered during CPB. In a rat model, Darby and colleagues found that anemia during CPB increased medullary blood flow compared to cortical, but that tissue hypoxia was more severe and sustained in the medulla.[107] In addition to the macro-circulatory changes during CPB, the microcirculation may be altered. In patients undergoing CABG, Koning and colleagues found that CPB reduced sublingual capillary perfusion, but that pulsatile flow during CPB could preserve the postoperative microcirculation.[108] More recently, the same authors found systemic microvascular shunting in patients undergoing CABG with CPB, but

not in off-pump CABG controls.[109] Based on experimental studies, it has been suggested that diffusive shunting of oxygen from arteries to veins in the cortex and from the descending to ascending medullary vasa recta, may limit renal oxygen delivery.[97] One could speculate that this intrarenal oxygen shunting might be aggravated during CPB, inducing tissue hypoxia and tubular injury. These perfusion alterations and oxygen shunting at capillary levels may not be reflected in renal or systemic VO_2 or oxygen extraction.

Data on the physiological state of the kidneys beyond the first hour after cessation of CPB are limited. Redfors compared patients with AKI and uncomplicated controls after cardiac surgery.[24] Both groups were sedated and mechanically ventilated. The control group was studied 4–6 hours after weaning from CPB, while the AKI group was studied 2–6 days after surgery.

Table 14. ***Systemic and renal variables after cardiac surgery with CPB.***

Variable	***Early post CPB***	***Postoperative AKI***	***Postop controls***
	N=18	***N=12***	***N=37***
CI	*2.21±0.12*	*2.77±0.16*	*2.63±0.08*
RO_2Ex	*0.16±0.01*	*0.163±0.01*	*0.097±0.004*
RBF	*558±30*	*496±34*	*822±40*
GFR	*67±4.2*	*33.6±3.4*	*80.3±4.2*
FF	*0.18±0.01*	*0.11±0.01*	*0.148±0.005*
RVR	*0.116±0.01*	*0.131±0.095*	*0.086±0.004*
PAH_{ext}	*0.71±0.03*	*0.68±0.04*	*0.85±0.01*
RDO_2	*80±4.0*	*71±4.5*	*120±6.6*
RVO_2	*12±4*	*11.8±0.8*	*11.4±0.5*
O_2/Na^+	*1.4±0.94*	*1.94±0.36*	*0.82±0.06*

Values are mean±SEM. CI; cardiac index, FF; renal filtration fraction of ^{51}Chromium-EDTA, GFR; glomerular filtration rate, O_2/Na^+; oxygen consumption per millimole reabsorbed sodium, PAH_{ext}; renal extraction of para-aminohippurate, RBF; renal blood flow, RDO_2; renal oxygen delivery, RO_2Ex; renal oxygen extraction, RVO_2; renal oxygen consumption, RVR; renal vascular resistance. Data from Paper II and Redfors[24], with permission.

Although these groups are not entirely comparable, the state of the renal oxygenation shortly after CPB does have some similarities with that of AKI. In both groups, the renal oxygen extraction is high, mainly due to high RVR and reduced RDO_2, and the oxygen cost of sodium transport is increased, reflecting tubular dysfunction or injury.

5.7 DIFFERENTIAL EFFECTS OF LEVOSIMENDAN AND DOBUTAMINE (PAPER IV)

The main finding of the study was that levosimendan, in contrast to dobutamine, not only increased cardiac index and renal blood flow, but also GFR.

These findings are in line with a previous study of levosimendan in uncomplicated post-cardiac surgery patients with normal renal function, where levosimendan increased both RBF and GFR compared with placebo.[55]

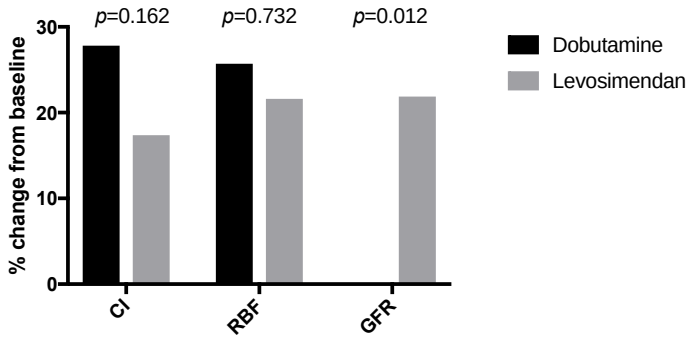


Figure 15. Relative (%) changes in cardiac index (CI), renal blood flow (RBF) and glomerular filtration rate (GFR) after administration of levosimendan and dobutamine.

Both agents induced a renal vasodilatory effect accompanied by an increase in RBF. The renal filtration fraction (GFR/renal plasma flow) remained unchanged in patients receiving levosimendan, and it decreased in those treated with dobutamine. These findings could mean that levosimendan preferentially causes vasodilation of the afferent arterioles, which, at a certain mean arterial pressure, induces a proportional increase in both RBF and GFR. The presence of ATP-dependent potassium channels on afferent arterioles and activation of these channels have previously been demonstrated in experimental studies.[110] Dobutamine, in contrast, seems to induce a balanced vasodilation of both afferent and efferent arterioles, thereby increasing RBF, while maintaining a constant glomerular filtration pressure, as indicated by no change in GFR. This pattern is similar to that previously described for low-dose dopamine in post-cardiac surgery patients, in whom it induced a pronounced increase in RBF with no effect on GFR.[27]

Experimental studies indicate that levosimendan may exert a beneficial effect on the glomerular capillary ultrafiltration coefficient.[111] Smooth muscle-like cells in the mesangium of the glomerulus, the mesangial cells, regulate the glomerular capillary surface area. They respond to vasoconstrictors such as angiotensin II and react by decreasing the available surface area for filtration. This angiotensin II-mediated mesangial cell contraction is reversed by levosimendan.[111] Heart failure patients have high circulatory levels of angiotensin II,[17] and the increase in GFR seen after levosimendan administration could to some extent be explained by an inhibition of the angiotensin II-mediated mesangial cell contraction and an increase of the available glomerular capillary surface area.

It has been assumed that any inotropic drug that displays a favorable effect on central and peripheral hemodynamics would, inevitably, also improve renal function.[112] Current guidelines on inotropic treatment in patients with heart failure provide no suggestions on the choice of agent.[58,59] The differential effects of levosimendan and dobutamine on GFR, as demonstrated in paper IV, are, therefore, of clinical interest and might imply that levosimendan could be the preferred inotropic agent for treatment of the cardiorenal syndrome.

Limitations

A major limitation is the relatively small sample size of the study population. Furthermore, our protocol was a pharmacological intervention of short duration, and only the acute effects of the administered inotropic agents were studied; therefore, the effect of a more prolonged (24–48 hours) period of levosimendan treatment on measured GFR is not known. Moreover, the participants of the study were not in need of inotropic support, in contrast to patients with acute HF, who are considered for such interventions. In addition, urine was not collected for analysis of, for example, sodium excretion.

6 CONCLUSION

- In cardiac surgery, a renal tubular cell injury is seen early after onset of CPB with a peak biomarker increase early after the discontinuation of CPB.
- The magnitude of this tubular injury is independently related to CPB duration and the degree of rewarming.
- Cardiopulmonary bypass impairs renal oxygenation due to renal vasoconstriction and hemodilution
- After CPB, the renal oxygenation is further deteriorated due to increased oxygen consumption.
- The renal oxygenation mismatch during and after CPB is correlated with tubular cell injury.
- The impaired renal oxygenation seen during CPB is ameliorated by an increase in CPB flow rate.
- The feasibility of higher CPB flow rates and the clinical relevance of these findings, in terms of renal outcome, should be explored in future studies.
- In patients with HF and renal impairment, the levosimendan-induced elevation of cardiac output not only increased RBF but also, and in contrast to dobutamine, enhanced GFR, suggesting a preferential dilation of preglomerular afferent arterioles.
- Levosimendan may be the preferred inotrope when treating patients with cardiac and renal failure

7 FUTURE PERSPECTIVES

The studies in this thesis have added to the current knowledge on the renal effects of cardiac surgery with cardiopulmonary bypass. Hopefully, this may lead to improvements in the patient care, and to the development of new strategies to reduce the risk of postoperative AKI. The dire state of the kidneys shortly after discontinuation of CPB suggests that this is a critical period, where care should be taken to optimize perfusion and oxygenation. Also, more studies of the "natural course" of renal oxygenation in the hours after CPB should be undertaken to see which interventions might be the most appropriate.

Increased CPB flow rates might be reno-protective, and could be one way to reduce postoperative AKI in selected patients. We plan to perform a randomized outcome study of different CPB pump flow levels, which may reveal the clinical applicability of our findings in paper III.

The findings of paper IV may have impact on the guidelines on the management of acute heart failure accompanied by AKI, where levosimendan might be the preferred agent. The renal and systemic effects of levosimendan in postoperative AKI remains to be studied.

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