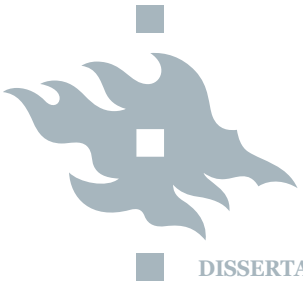

DISSERTATIONES SCHOLAE DOCTORALIS AD SANITATEM INVESTIGANDAM
UNIVERSITATIS HELSINKIENSIS

9/2014

JUHA LEMPIÄINEN

Ischaemia-Reperfusion-Induced Kidney Injury

Experimental studies on the effects of caloric restriction, AMPK activator AICAR and α2-adrenoceptor agonists in the rat

INSTITUTE OF BIOMEDICINE
PHARMACOLOGY
FACULTY OF MEDICINE AND
DOCTORAL PROGRAMME IN DRUG RESEARCH
UNIVERSITY OF HELSINKI

Ischaemia-Reperfusion-Induced Kidney Injury

Experimental studies on the effects of caloric restriction, AMPK activator AICAR and α_2-adrenoceptor agonists in the rat

Juha Lempiäinen
Institute of Biomedicine, Pharmacology
University of Helsinki

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine, University of Helsinki, for public examination in Biomedicum Lecture hall 2 on 29th August, 2014, at 12 noon

Supervisors :
Professor Eero Mervaala, MD, PhD
Institute of Biomedicine, Pharmacology
University of Helsinki
Helsinki, Finland

Docent Piet Finckenberg, PhD
Institute of Biomedicine, Pharmacology
University of Helsinki
Helsinki, Finland

Reviewers :
Docent Petteri Piepponen, PhD
Faculty of Pharmacy, Division of Pharmacology and Toxicology
University of Helsinki
Helsinki, Finland

Docent Sanna Lehtonen, PhD
Haartman Institute, Department of Pathology
University of Helsinki
Helsinki, Finland

Opponent:
Professor Markku Koulu, MD, PhD
Institute of Biomedicine, Phamacology, Drug Development and Therapeutics
University of Turku
Turku, Finland

ISBN 978-952-10-9998-4 (paperback)
ISBN 978-952-10-9999-1 (PDF)
ISSN 2342-3161 (Print)
ISSN 2342-317X (Online)
http://ethesis.helsinki.fi

Helsinki Hansaprint 2014
Helsinki 2014

Table of Contents

List of original publications

This thesis is based on the following original publications, referred to in the text by Roman numerals (I-III). Some unpublished data related to the original articles are also provided.

I Lempiäinen J, Finckenberg P, Mervaala EE, Sankari S, Levijoki J, Mervaala EM. Caloric restriction ameliorates kidney ischemia reperfusion injury through PGC-1alpha-eNOS-pathway and enhanced autophagy. *Acta Physiol (Oxf).* 2013; 208: 410-21.

II. Lempiäinen J, Finckenberg P, Levijoki J, Mervaala E. AMPK activator AICAR ameliorates ischemia reperfusion injury in the rat kidney. *Br J Pharmacol.* 2012; 166: 1905-15.

III Lempiäinen J, Finckenberg P, Mervaala EE, Storvik M, Kaivola J, Levijoki J, Mervaala EM. Dexmedetomidine preconditioning ameliorates kidney ischemia reperfusion injury. *Pharmacology Research & Perspectives*. Accepted for publication 13.3.2014.

Abbreviations

ACh	acetylcholine
AFOS	alkaline phosphatase
AGE	advanced glycation end product
AICAR	5-amino-4-imidazolecarboxamide riboside-1-β-D-ribofuranoside
ALAT	alanine aminotransferase
AMPK	adenosine monophosphate kinase
AKI	acute kidney injury
ATN	acute tubular necrosis
AUC	area under curve
α_2-AR	α_2-adrenoceptor
Ang II	angiotensin II
ASAT	aspartate aminotransferase
ATP	adenosine triphosphate
BP	blood pressure
CKD	chronic kidney disease
CO	cardiac output
CR	caloric restriction
Dex	dexmedetomidine
eNOS, NOS III	endothelial nitric oxide synthase
ER	endoplasmic reticulum
ET_A	endothelin receptor A
EPO	erythropoietin
Fado	fadolmidine
GFR	glomerular filtration rate
HMGB1	high-mobility-group-protein B1
HR	heart rate
H_2O_2	hydrogen peroxide
ICU	intensive care unit
IGF-1	insulin-like growth factor 1
iNOS, NOS II	inducible nitric oxide synthase
IPC	ischaemic preconditioning
I/R	ischaemia-reperfusion
KEGG	*Kyoto Encyclopedia of Genes and Genomes*
MAP	*mean arterial pressure*
MAPK	*mitogen activated protein kinase*
MAP1	microtubule-associated protein 1
MCP-1	monocyte chemotactic protein 1
MnSOD	mitochondrial superoxide dismutase
MOF	multi organ failure
mTOR	mammalian target of rapamycin
NA	noradrenaline
NAD+	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide hydrogen

NADPH	nicotinamide adenine dinucleotide phosphate
NAMPT	nicotinamide phosphoribosyltransferase
NF-κB	nuclear factor kappa B
nNOS, NOS I	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
NSAID	non-steroidal anti-inflammatory drug
O_2^-	superoxide
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator 1α
RAS	*renin*-angiotensin system
RIFLE	risk, injury and failure; and loss; and end-stage kidney disease
RIPK1	death receptor-interacting protein kinase 1
RIR	renal ischaemia reperfusion
RSV	resveratrol
ROS	reactive oxygen species
SGLDH	serum glutamate dehydrogenase
SGGT	serum gamma-glutamyl transferase
SIRT	silent information regulator
SOD	superoxide dismutase
XO	xanthine oxidase
3-MA	3-methyladenine

Abstract

Ischaemia-reperfusion (I/R) injury of the kidneys is a common cause of acute kidney injury (AKI). The incidence of AKI among hospitalized patients varies between 2% and 10%, and the mortality rate among patients undergoing renal replacement therapy in intensive care units may be over 50%, since the ischaemic kidney injury often occurs in the context of multi organ failure (MOF) and sepsis. Furthermore, patients suffering from AKI have increased risk of developing chronic kidney disease. Currently, there is no curative therapy for I/R injury-induced AKI. Accumulating evidence indicates a major role for haemodynamic alterations, oxidative stress and inflammatory response, as well as endothelial and tubular cell injury in the pathophysiology of I/R-induced AKI. The aim of the present study was to increase our understanding of the cellular pathophysiological processes underlying kidney injury and repair and to identify novel drug targets for AKI. We therefore investigated whether caloric restriction (CR), adenosine monophosphate-activated protein kinase (AMPK) activation by 5-amino-4-imidazolecarboxamide-1-β-D-ribofuranoside (AICAR), or α_2-adrenoceptor (α_2–AR) activation by α_2–AR agonists could ameliorate kidney I/R injury. These three treatment strategies have provided protection against tissue I/R injury, at least in part, through their anti-inflammatory properties. An established and widely used murine model of kidney I/R injury was used. The drugs were given intravenously at different dosages, and the salutary effects of the various treatment strategies were assessed by combining biochemical measuments of kidney function, kidney histology and immunohistochemistry. The present study revealed that CR improved renal function, protected against the development of acute tubular necrosis and attenuated nitrosative stress as well as inflammatory responses. These effects were mediated at least partially through induction of endothelial nitric oxide synthase (eNOS) and peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α). The present study provided evidence that AICAR also ameliorated I/R-induced renal dysfunction, acute tubular necrosis, as well as monocyte/macrophage infiltration and oxidative stress. The effects of AICAR were dose-dependent and mediated mainly by AMPK activation. Finally, we were able to demonstrate that preconditioning with dexmedetomidine, a centrally acting α_2–AR agonist, attenuated I/R injury-induced renal dysfunction, acute tubular necrosis and inflammatory response partially through the p38-CD44 pathway, whereas postconditioning with dexmedetomidine provided no tissue protection. We also found that neither pre- nor postconditioning with a peripheral α_2–AR agonist fadolmidine ameliorated kidney I/R injury, suggesting the importance of central α_2–ARs. Taken together, the data from our experimental studies conducted in an established murine model of kidney I/R injury support the notion that compounds mimicking the cellular effects of CR, activators of kidney AMPK, as well as centrally acting α_2–AR agonists may represent a novel therapeutic approach in patients susceptible to AKI and perhaps to those undergoing kidney transplantation.

1 Introduction

Acute kidney injury (AKI) is the generic term that refers to a sudden decrease in renal function, resulting in retention of waste products (Lameire *et al.*, 2005). The incidence of AKI in hospitalized patients is 2--7% and in intensive care units (ICUs) even greater than 10%. The mortality and morbidity rates are high; in ICUs over half of the patients undergoing renal replacement therapy die and patients that survive have a high risk of developing chronic kidney disease (CKD)(Waikar *et al.*, 2008). The best way to prevent AKI is to maintain appropriate levels of relative blood volume and cardiac output (CO) and avoid nephrotoxic agents. No specific medication for AKI is in clinical use (Lameire *et al.*, 2005). One of the most common causes of AKI is ischaemia-reperfusion (I/R) injury. I/R injury results from impairment of oxygen (O_2) and nutrient supply to the kidneys, as well as from accumulation of metabolic waste products. This, in turn, leads to acute tubular and endothelial cell death by apoptosis and necrosis. The initial injury is further worsened through the subsequent reperfusion period, inflammation, damage by oxidative stress, endothelial cell injury and vascular dysfunction (Bonventre and Yang, 2011). Mitochondria seem to be the most vulnerable organs damaged by I/R injury (Hasegawa *et al.*, 2010). The repair process may also be maladaptive and lead to incomplete tubular repair, chronic inflammation and increased fibrosis in the kidneys, leading to CKD (Bonventre and Yang, 2011).

Caloric restriction (CR), without malnutrition, extends the lifespan in various species, including rodents and primates. It also delays the onset of age-associated phenotypes, including cardiovascular diseases (CVDs), cancer and diabetes (Fontana *et al.*, 2010). Some studies indicate that CR may also provide protection against ischaemic injuries (Morris *et al.*, 2011). Sirtuins (silent information regulator 2 [Sir2] proteins) (SIRT1--7) are evolutionarily conserved nicotine adenine dinucleotide+ (NAD+)-dependent deacetylases and are known to mediate the health-promoting effects of CR (Hao and Haase, 2010; Morris *et al.*, 2011). SIRT1 (Haigis and Sinclair, 2010; Hao and Haase, 2010) and induction of endothelial nitric oxide synthase (eNOS) (Nisoli *et al.*, 2005) play central roles in mediating the beneficial effects of CR and these two molecules also regulate each other, forming an interesting positive feedback loop (Nisoli *et al.*, 2005, Canto and Auwerx, 2012). SIRT1 activation also seems to be beneficial in the context of AKI. Pretreatment with the SIRT1 activator resveratrol (*trans*-3,5,4'-trihydroxystilbene) (RSV) reduces acute I/R injury in rats (Bertelli *et al.*, 2002; Chander and Chopra, 2006) and activation of SIRT1 protects the mouse renal medulla from oxidative stress (He *et al.*, 2010). Nevertheless, it is still not clear how CR or SIRTs mediate their possible renoprotective effects.

Autophagy reprocesses the cell´s substances for biosynthetic or metabolic needs and is a general cellular response to stress (Mizushima *et al.*, 2008). CR is known to stimulate autophagy (Groenendyk *et al.*, 2010, Madeo *et al.*, 2010), which increases SIRT1 expression and attenuates hypoxia-induced mitochondrial and renal damage (Kume *et al.*, 2010). There is growing evidence that induction of autophagy protects cells by eliminating damaged and potentially dangerous organelles, such as leaky mitochondria (Cybulsky, 2010).

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a metabolic master switch that is also activated when cellular energy levels are low. AMPK and SIRT1 regulate each other in many ways. AMPK plays an important role in the regulation of ion transport, podocyte functioning and diabetic renal hypertrophy in the kidneys (Hallows et al., 2010). AMPK is also a key regulator of lipid and glucose metabolism. Previous studies have provided evidence that AMPK is rapidly activated by acute renal ischaemia. However, the functional significance

of this AMPK activation remains undefined. Mount *et al.* (2005) showed that although renal ischaemia activates AMPK within 1 min, the downstream target for AMPK, namely eNOS, was not phosphorylated. It is also unclear whether long-term pharmacological activation of AMPK provides protection against renal I/R injury.

AICAR (5-amino-4-imidazolecarboxamide riboside-1-β-D-ribofuranoside) is an adenosine analogue that directly binds to AMPK, leading to allosteric modification and activation of AMPK. AICAR is taken up by adenosine transporters and subsequently phosphorylated to ZMP (5-aminoimidazole-4-carboxamide-1-β-D-furanosyl 5'-monophosphate) in the cell (Wong *et al.*, 2009). ZMP in turn mimics AMP in AMPK signalling (Merrill *et al.*, 1997). Previous studies have provided evidence that AICAR protects against I/R injury in several tissues, such as heart and liver (Bullough *et al.*, 1994, Alkhulaifi and Pugsley, 1995, Galinanes *et al.*, 1995, Mathew *et al.*, 1995, Peralta *et al.*, 2001). Lin *et al.* (2004) were the first to demonstrate that combination therapy with AICAR and the antioxidant acetyl cysteine attenuates I/R-induced AKI. However, the effects of AICAR treatment alone on I/R-induced AKI remain unclear.

The alpha2 adrenoceptor (α_2-AR) agonist dexmedetomidine (Dex) is in clinical use as a sedative for intensive care unit (ICU) patients who require only mild sedation and it also seems to enhance renal function in certain clinical cases (Kulka *et al.*, 1996; Frumento *et al.*, 2006). Animal studies have indicated that Dex may provide protection against ischaemic injuries by affecting various intracellular kinases, oxidant status and adhesion molecules (Maier *et al.*, 1993; Kuhmonen *et al.*, 1997; Jolkkonen *et al.*, 1999; Gu *et al.*, 2011b; Ibacache *et al.*, 2012; Kilic *et al.*, 2012; Tufek *et al.*, 2013)

The aim of this thesis project was to increase the knowledge of the pathophysiological mechanisms of I/R- induced AKI and identify new targets for drug development. We postulated that we could attenuate I/R-induced AKI by activating AMPK and/or other metabolic sensors, as well as autophagy, either through specific activators or through CR. We also wanted to test the hypothesis that α_2-AR agonists may provide renoprotection through metabolic reprogramming.

2 Review of the literature

2.1 Kidney anatomy and physiology

Mammalian kidneys develop from the intermediate mesoderm. Humans normally have two kidneys located retroperitoneally between the transverse processes of the thoracic 12--lumbar 3 (T12--L3) vertebrae. The kidneys receive blood from the paired renal arteries and drain into the paired renal veins, excreting urine into a ureter that empties into the urinary bladder (Diagram 1). The functions of the kidneys include filtration and excretion of metabolic waste products and regulation of fluid, electrolyte and acid-base balances. The kidneys also reabsorb glucose and amino acids. Interstitial fibroblasts in the kidney secrete a cytokine called erythropoietin (EPO), which stimulates red-blood cell production and also has other biological functions. The juxtaglomerular cells in the kidneys also regulate blood pressure (BP) by secreting renin, an important factor of the renin-angiotensin-aldosterone system (RAS) and the proximal tubule cells of the kidneys produce calcitriol, which is a hormonally active form of vitamin D. The cells of the renal cortex take part in gluconeogenesis. Various cells show differing metabolic profiles in the kidney. In the inner medulla, the cells have fewer mitochondria and are dependent predominantly on glycolytic metabolism. The tubular cells in the cortex have abundant mitochondria, suggesting that they have more oxidative metabolism and use fatty acids (an important energy source for kidneys), ketone bodies and lactate as metabolic substrates (Berndt, 1976; Hallows *et al.*, 2010; Bovee, 1986).

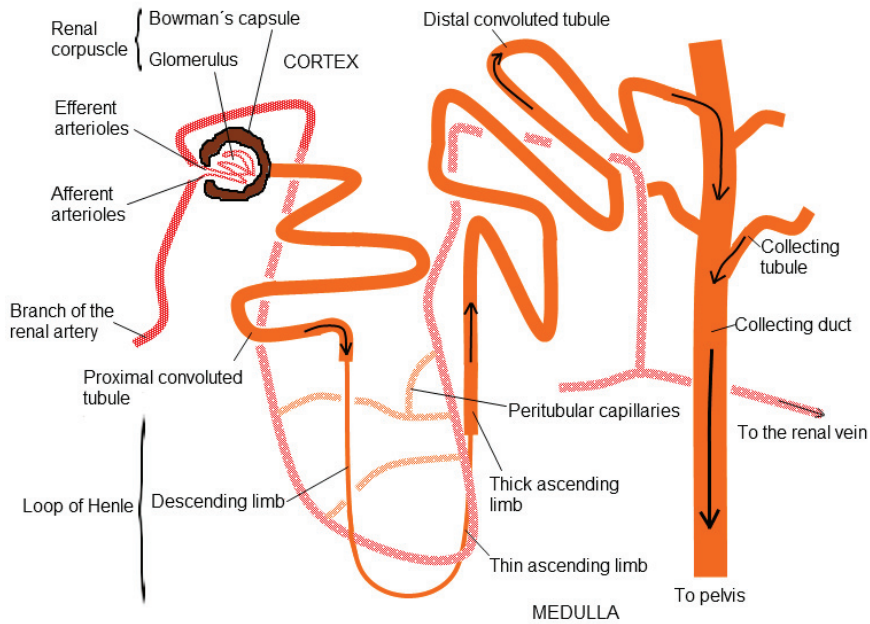

Diagram 1. Structure of a nephron. The urine-producing functional structures of the kidney are called nephrons and each nephron is composed of an initial filtering component, the renal corpuscle, and a tubule specialized for reabsorption and secretion, the renal tubule. Several different cell types are found in the kidneys, including glomerular parietal cells and glomerular podocytes, proximal tubular brush-border cells, Loop of Henle thin segment cells, thick ascending limb cells, distal tubular cells, collecting duct cells, interstitial cells, and endothelial and smooth-muscle cells in the renal arteries and their branches.

2.2 Acute kidney injury

AKI is the generic term for a sudden decrease in renal function, resulting in retention of nitrogenous (urea and creatinine) and non-nitrogenous waste products. Frequently, this situation is also accompanied by metabolic disturbances, such as metabolic acidosis and hyperkalaemia, changes in body fluid balance and effects on many other organ systems. AKI can be classified from severe (i.e. requiring dialysis) to slight increases in serum creatinine (S-creatinine) concentration (e.g. 44μmol/l x 2). The widely used Risk, Injury, Failure, Loss and End-stage renal failure (RIFLE) criteria use S-creatinine and urine output to classify AKI into three severity categories (risk, injury and failure) and two clinical outcome categories (loss and end-stage renal disease) (Lameire *et al.*, 2005, Devarajan, 2006, Choudhury, 2010, Lameire, 2013).

There is no specific medication for AKI (Lameire *et al.*, 2005). AKI occurs in 2--7% of all patients admitted to hospital and is associated with a 2- to 15-fold increase in mortality (Waikar *et al.*, 2008). Although the pathophysiology of AKI is complex and the aetiologies diverse, there is now accumulating evidence to indicate that hypotension, hypoperfusion, hypoxia, oxidative stress and renal vasoconstriction contribute to the pathogenesis (Kunzendorf *et al.*, 2010). The causes that present as AKI are numerous and can be divided into prerenal, intrinsic and postrenal (Lameire *et al.*, 2005). Intrinsic causes damage the kidneys directly (e.g. acute glomerulonephritis) and postrenal acute renal failure is caused by an obstruction in the urinary tract below the kidneys. Prerenal causes lead to true or relative hypovolaemia, decrease blood flow to the kidney and lead to ischaemia. The reperfusion period worsens injury through inflammation and induction of oxidative stress. For example, haemorrhagic shock, renal transplantation or cardiovascular surgery are potential prerenal causes that may lead to true hypovolaemia. Reduction in the effective circulating volume may be due to low CO, systemic vasodilatation or intrarenal vasoconstriction (Lameire *et al.*, 2005). Hypovolaemia activates the neurohumoral vasoconstrictive systems that attempt to maintain the BP and CO (Badr and Ichikawa, 1988). The kidney has its own autoregulation system that keeps renal blood flow and glomerular filtration rate (GFR) at adequate levels. This system functions until the mean systemic arterial blood pressure (MAP) drops below 75-80 mmHg (Sharfuddin and Molitoris, 2011). If the BP falls, the preglomerular arterioles dilate. This is mediated by the generation of nitric oxide (NO) (De Nicola *et al.*, 1992) and prostaglandins (Dzau *et al.*, 1984). Vasoconstriction of the postglomerular arterioles by angiotensin II (Ang II) also aids in preserving the glomerular capillary hydrostatic pressure (Lameire *et al.*, 2005).

The tubuloglomerular feedback mechanism, mediated by communication between the macula densa and the glomerular microvasculature, preserves the GFR and fluid delivery to the distal nephrons (Lameire *et al.*, 2005). Drugs that disturb kidney autoregulation (e.g. nonsteroidal anti-inflammatory drugs (NSAIDs)) can also lead to AKI (Gambaro and Perazella, 2003).

2.2.1 Pathophysiology of ischaemia-reperfusion-induced AKI

I/R injury is a common cause of AKI (Liano and Pascual, 1996, Mehta *et al.*, 2004) and there are several causes that may lead to ischaemia in the kidneys. For example anaesthesia, cardiac surgery, haemorrhage and gastrointestinal fluid losses may all lead to I/R-induced AKI (Thadhani *et al.*, 1996). In the beginning, all these causes lead to inadequate amounts of effective intravascular blood volume and impairment of kidney blood flow. This leads to impairment of O_2 and nutrient supply to the kidneys, as well as to accumulation of metabolic waste products,

which cause cell damage. If the reperfusion period follows, it is accompanied by inflammation and oxidative stress, as well as endothelial and vascular dysfunction (Bonventre and Yang, 2011). (Diagram 2)

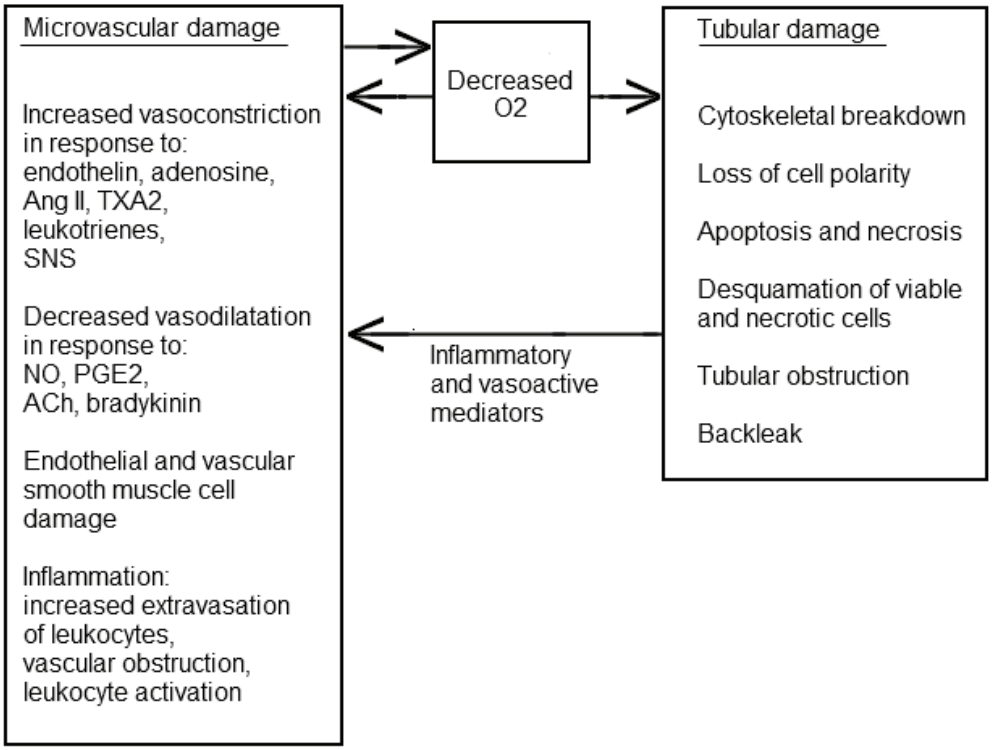

Diagram 2. Schematic picture of the pathophysiology of I/R-induced AKI (modified from picture; AKI. *Norbert Lameire, Wim Van Biesen, Raymond Vanholder.* Lancet 2005; 365: 417–30). ACh denotes acetylcholine, Ang II angiotensin II, NO nitric oxide, O_2 oxygen, SNS sympathetic nervous system, TXA2 thromboxane A2.

2.2.1.1 Vascular and endothelial components of injury

During I/R-induced AKI, the total blood flow to the kidneys is impaired, but there are also regional alterations in renal blood flow. In animal models, the blood flow to different parts of the kidneys is reduced disproportionately to the reduction in total kidney perfusion (Le Dorze *et al.*, 2009). It is known that I/R injury leads to enhanced vasoconstriction, endothelial-leukocyte interactions and activation of the coagulation system, which in turn lead to impairment of the microcirculation and regional ischaemia, especially in the outer medulla. Local oedema of the outer medulla, as a result of ischaemic injury, further decreases the blood flow. The vascular endothelial and smooth-muscle cells play crucial roles in this vascular dysfunction (Bonventre and Yang, 2011). The endothelial cells contribute to vascular tone, leukocyte functioning and smooth-muscle responsiveness (Sprague and Khalil, 2009). In I/R injury, the endothelium is injured and small arterioles vasoconstrict more than do vessels from the normal kidney in response to increased levels of circulating vasoactive substances (e.g. Ang II), as well as in

response to sympathetic nerve stimulation (Bonventre and Yang, 2011). Damaged endothelial cells also produce less NO and other vasodilatory substances and vasodilatation in response to these vasodilatory substances is decreased (Conger, 1983). Leukocyte-endothelial adhesion and leukocyte activation lead to increased amounts of vasoactive cytokines (e.g. tumour necrosis factor alpha [TNF-α], interleukins IL-1β, IL-6, IL-12, IL-15, IL-18, IL-32, and endothelin), which impair the blood flow even more (Bonventre and Yang, 2011). Tubuloglomerular feedback also likely contributes to preglomerular arteriolar vasoconstriction, which reduces the glomerular forces available for filtration (Blantz *et al.*, 2007).

In I/R-induced AKI, the expression of intercellular adhesion molecule 1 (ICAM-1) and other cell adhesion molecules (e.g. cluster of differentiation 44 [CD44]) on endothelial cells is increased, which increases the number of endothelium-leukocyte interactions, as well as the expression of counterreceptors on leukocytes (Kelly *et al.*, 1996). This leads to activation of the leukocytes, obstruction of capillaries and postcapillary venules, further activation and transmigration of leukocytes, production of cytokines and further promotes the inflammatory response (Bonventre and Yang, 2011). Endothelial damage leads to loss of the glycocalyx, disruption of the actin cytoskeleton, alteration of endothelial cell-cell contacts and breakdown of the perivascular matrix, which increase vascular permeability and loss of fluid into the interstitium (Basile, 2007, Rabelink *et al.*, 2010). Swelling of the endothelial cells may further impair the blood flow (Bonventre and Yang, 2011).

I/R injury also reduces the number of microvessels in the outer medulla. This may be due to the downregulation of angiogenic factors (e.g. vascular endothelial growth factor [VEGF]) and increased amount of angiogenesis inhibitors (Basile *et al.*, 2008). The reduced number of vessels leads to chronic hypoxia (Basile, 2007), which likely further increases the tubular injury and development of fibrosis. This, in turn, may further decrease the availability of O_2 and nutrients to the tubules, enhance tubular stress and epithelial cell injury, interfere with regenerative processes and lead to further fibrosis (Bonventre and Yang, 2011).

2.2.1.2 Inflammation

Inflammation plays an important role in I/R-induced AKI and both innate and adaptive immune responses contribute to the pathophysiology. The (nonantigen-specific) innate component is responsible for the early response to injury. Neutrophils, monocytes/macrophages, dendritic cells (DCs), natural killer (NK) cells and natural killer T (NKT) cells all take part in this response. The adaptive component, activated by specific antigens, is initiated within hours and lasts several days after injury. The initial ischaemic injury is followed by induction of inflammation through increase in several cytokines (*e.g.*, TNF-α, IL-6, IL-1β, transforming growth factor β [TGF-β]) and chemotactic cytokines (chemokines) (*e.g.*, monocyte chemotactic protein 1 [MCP-1], IL-8 and Regulated on Activation, chemokine (C-C motif) ligand 5 [CCL5]/ Normal T cell Expressed and Secreted [RANTES]) produced by tubular and endothelial cells (Bonventre and Yang, 2011). Tubular cells also express Toll-like receptors (TLRs), complement and complement receptors, and costimulatory molecules, which regulate T lymphocyte activity (Jang *et al.*, 2009).

Chemokines recruit leukocytes at the site of injury and are also important in angiogenesis and fibrosis. On the other hand, they can also have anti-inflammatory functions. Chemokines are induced by cytokines (TNF-α and IL-1β), complement activation, reactive oxygen species (ROS), the nuclear factor kappa B (NF-κB) system and TLR-related pathways. For example, chemokine (C-X_3-C motif) [CX_3C], chemokine (C-X-C motif) ligand 1 [CXCL1] (or IL-8), chemokine

(C-C motif) ligand 3 [CCL3] (MIP-1α) and CCL5 have been associated with pathogenesis of I/R-induced AKI (Akcay *et al.*, 2009, Bonventre and Yang, 2011). Studies in humans have also demonstrated that the levels of the proinflammatory cytokines IL-6 and IL-8 in the plasma predict mortality in patients with AKI (Simmons *et al.*, 2004). Increased amounts of chemokines, in turn, lead to infiltration of leukocytes into the injured kidneys (Akcay *et al.*, 2009, Bonventre and Yang, 2011).

The complement system plays an important role in the inflammatory process in I/R-induced AKI. In kidneys, activation of the complement system is mediated by the alternative pathway (Bonventre and Yang, 2011).

There are also several anti-inflammatory factors that inhibit the inflammatory response during I/R-induced AKI (Bonventre and Yang, 2011). Ischaemia increases epithelial cell haem oxygenase 1, which downregulates the inflammatory response and protects against I/R injury (Nath, 2006). Tamm-Horsfall protein (THP) also has protective effects; it seems to undergo its activity by downregulating the expression of TLR4 in proximal tubular cells (El-Achkar *et al.*, 2008).

AKI is often accompanied by hepatic dysfunction and systemic inflammation. Studies in mice have shown that IL-17A is released from the small intestine after AKI, which leads to increased inflammation and subsequent small intestine and liver injuries (Park *et al.*, 2011). Development of liver injury frequently leads to other extrarenal complications, such as respiratory failure, intestinal barrier destruction and systemic inflammatory response syndrome (SIRS). These secondary complications are also important causes of mortality (Elapavaluru and Kellum, 2007). The inflammatory response associated with I/R injury also induces production of ROS, which in turn act as secondary messengers and harm cell organelles directly (Bonventre and Yang, 2011).

2.2.1.3 Reactive oxygen species

Cellular respiration and metabolism continuously generate low levels of ROS, including superoxide (O_2^-). O_2^- in turn acts as a precursor for potentially more dangerous ROS, such as hydrogen peroxide (H_2O_2) and hydroxyl OH^- radicals. ROS monitor O_2 tension and mediate signal transduction from membrane receptors in many physiological processes. Low concentrations of ROS also control vascular tone through vascular smooth-muscle cell relaxation. Normally, the three isoforms of vascular superoxide dismutase (SOD) eliminate small concentrations of O_2^- (Lakshmi *et al.*, 2009).

In oxidative stress, excess ROS overwhelm the endogenous antioxidant system. The acute response initiated by I/R injury in the kidneys is characterized by increased production of ROS and induction of proinflammatory cytokines, leading to inflammatory response and acute tubular necrosis (ATN) (Bonventre and Yang, 2011). ROS are known to cause renal tubule cell injury by oxidation of proteins, peroxidation of lipids, DNA damage and induction of apoptosis. Oxidative stress is also increased in humans with AKI. This was observed in the depletion of plasma protein thiols and increased carbonyl formation (Devarajan P., 2006, Sabbahy and Vaidya, 2010).

Mitochondria seem to be among the first organs damaged in I/R-induced AKI (Hasegawa *et al.*, 2010) and are also one of the main sources of ROS (Gutterman, 2005). Both mitochondrial oxidative damage and mitochondrial dysfunction play crucial roles in the pathogenesis of kidney I/R injury (Bonventre and Yang, 2011). (Diagram 3)

Increased O_2^- can also be derived from nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine reductase, uncoupled eNOS, the mitochondrial electron- transport chain (also under physiological conditions), lipoxygenases, cytochrome P450 monooxygenases and cyclooxygenases. Under prolonged periods of oxidative stress, the antioxidative system may collapse and become downregulated. This leads to further impaired removal of ROS (Taniyama and Griendling, 2003, Heistad, 2006). It is believed that the progression from AKI to CKD is largely driven by ROS. In line with this, pretreatment with an SOD-mimicking molecule decreased kidney fibrosis after I/R injury (Sabbahy and Vaidya, 2010).

ROS increase degradation of NO, and increased interaction of NO with O_2^- leads to formation of peroxynitrite ($ONOO^-$). O_2^- and $ONOO^-$ both contribute to the development of endothelial dysfunction (Munzel *et al.*, 2005). ROS also inhibit other endothelium-dependent vasodilators, which further increases vascular tone. This is likely to be especially harmful in I/R-induced AKI, since blood flow to the kidney is already decreased.

The increased amounts of ROS also activate redox-sensitive transcription factors (Rojas *et al.*, 2006; Sabbahy and Vaidya, 2010) and boost the expression of proinflammatory genes (Irani, 2000). This leads to altered gene expression and increases the expression of proinflammatory proteins, thus also affecting mitochondrial biogenesis and functioning, cell proliferation, apoptosis and the cell cycle. ROS also induce premature senescence by activating cell-cycle inhibitors (Costopoulos *et al.*, 2008, Andrades *et al.*, 2009).

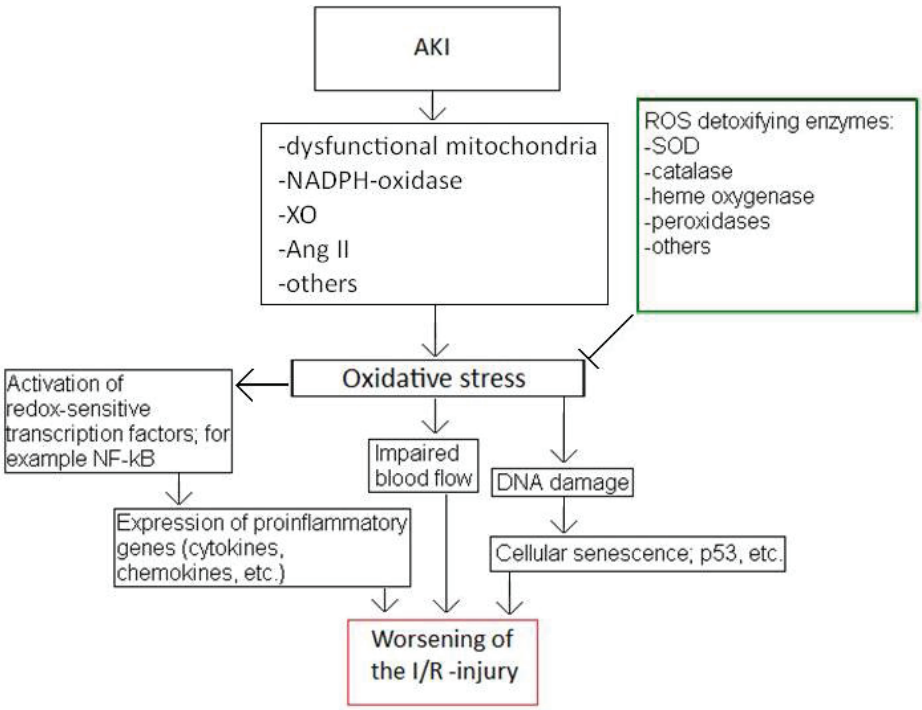

Diagram 3. Effects of oxidative stress after AKI.
AKI = acute kidney injury; Ang II = angiotensin II; I/R = ischaemia-reperfusion; ROS = reactive oxygen species; SOD = superoxide dismutase; XO = xanthine oxidase

2.2.1.4 Tubular injury

Proximal tubule. In most animal models of I/R-induced AKI, the epithelial cell damage associated with I/R injury is most apparent in the proximal tubule (Bonventre and Yang, 2011). Recent studies using biomarkers of proximal tubule injury, such as kidney injury molecule 1 (KIM-1) (measured in urine) have also revealed significant proximal tubule injury in humans (Vaidya *et al.*, 2008). Many patients also have clear signs of tubule epithelial injury on biopsy (Bonventre and Yang, 2011). Another biomarker, currently in clinical use, neutrophil gelatinase-associated lipocalin (NGAL), is produced mainly in the distal tubule (and also in many other organs), but it is also filtered and reabsorbed by the normal proximal tubule (Vaidya *et al.*, 2008). The initial ischaemia destroys cytoskeletal integrity and cell polarity. These cytoskeletal alterations occur rapidly and are dependent on the severity and duration of ischaemic injury (Sutton and Molitoris, 1998). The proximal tubule brush border is damaged and polarity of the cells is lost, with mislocalization of adhesion molecules and other membrane proteins (e.g. Na^+K^+-adenosine triphosphatase [ATPase] and β-integrins). Cytokines induce disruption of cell-matrix adhesion, and cell-cell interactions at adherent and tight junctions are also damaged (Bonventre and Yang, 2011).

Epithelial cells communicate via tight junctions and adhesion junctions. These junctions are regulated by the cytoskeleton, which is regulated by the Rho family of guanosine triphosphatases (GTPases), which in turn are activated in response to ischaemia. Inhibition of some downstream effectors of Rho GTPases attenuates I/R-induced AKI (Prakash *et al.*, 2008, Bonventre and Yang, 2011). Heat-shock proteins (Wang *et al.*, 2011) and autophagy (Kimura *et al.*, 2011) seemingly also play crucial roles in proximal tubule cell survival after I/R injury in rodents.

In severe injury, only the basement membrane remains as a barrier between the filtrate and the peritubular interstitium. The increase in permeability leads to backleakage of glomerular filtrate from the tubular lumen to the intersitium (Bonventre and Yang, 2011). Damaged cells and their debris form proinflammatory casts together with proteins present in the tubular lumen. These casts can obstruct the tubule, increase intratubular pressure (Zuk *et al.*, 2001) and can also be detected in the urine as a hallmark of AKI in humans (Zuk *et al.*, 2001; Bonventre and Yang, 2011)

Distal tubule. Distal tubular cells are more resistant to hypoxia, ischaemia, and oxidative injury and remain intact during I/R-induced AKI (Bonventre and Yang, 2011). The medullary thick ascending limb has a greater capacity to convert from oxidative to glycolytic metabolism (Bagnasco *et al.*, 1985). Mitogen-activated protein kinase (MAPK) pathway activation, antiapoptotic proteins and various growth factors may also contribute to the relative resistance of distal tubular cells to ischaemic injury (Bonventre and Yang, 2011).

2.2.1.5 Repair

Kidneys are capable of recovering from I/R injury that increases cell death. However, I/R-induced AKI can also lead to acceleration of CKD especially in humans with underlying CKD (Ishani *et al.*, 2009). The turnover rate of human proximal tubule cells (Nadasdy *et al.*, 1994) increases after I/R-induced AKI when cell death is increased by necrosis and apoptosis. However, this repair process may also be maladaptive and I/R-induced AKI can lead to incomplete tubular repair, chronic tubulointerstitial inflammation, proliferation of fibroblasts and increased formation of extracellular matrix (ECM) (Bonventre and Yang, 2011). For example, chronic hypoxia, resulting

from loss of peritubular microvessels (Basile, 2007), and chronic activation of macrophages (Duffield, 2010), may also contribute to the development of fibrosis after I/R injury.

2.2.2 Current treatments

Currently, there is no specific medication for AKI in clinical use. The best way to prevent AKI is to take care of the relative blood volume and CO and avoid nephrotoxins. With elderly patients, heart-failure patients, patients with liver disease, previous renal insufficiency, renal-artery stenosis or diabetes, renal blood flow and/or autoregulation may already be decreased, which makes them even more vulnerable to relative hypovolaemia and nephrotoxins (Lameire *et al.*, 2005). This suggests that all drugs that disrupt kidney autoregulation (e.g. NSAIDs, angiotensin-converting enzyme [ACE] inhibitors and Ang II receptor blockers) should be avoided and plasma concentrations of nephrotoxic drugs (e.g aminoglycosides and cyclosporine) should be measured. Allopurinol may be useful for patients with leukaemia and lymphoma, because it decreases the synthesis of uric acid (Ribeiro and Pui, 2003). Forced alkaline diuresis protects the renal tubules in rhabdomyolysis and prevents blockage of renal tubules by uric acid or methotrexate (Lameire *et al.*, 2005).

2.2.2.1 Volume expansion

In clinical studies, the overall management of patients includes the use of fluids, which makes it difficult to assess the therapeutic value of volume expansion alone. It is also not clear whether crystalloids or colloids should be used in critically ill patients (with or without AKI) (Lameire *et al.*, 2005). For example, in the Saline versus Albumin Fluid Evaluation (SAFE) trial, there were no differences between saline and albumin (Finfer *et al.*, 2004). Hydration prevents ATN after surgery and ATN induced by contrast media, platinum or amphotericin B (Lameire *et al.*, 2005). Nevertheless, if administrated too much, volume expansion may cause pulmonary oedema, especially when kidney function is impaired (Lameire *et al.*, 2005; Prowle *et al.*, 2010)

2.2.2.2 Drugs

Prophylaxis with N-acetylcysteine may lower the risk of contrast nephropathy (Duong *et al.*, 2005). However, acetylcysteine interferes with the tubular handling of creatinine directly, which means that in this case the decrease in S-creatinine level does not prove that acetylcysteine protects kidneys (Hoffmann *et al.*, 2004).

Loop diuretics (e.g. furosemide) have no renoprotective effects in patients with AKI (Schetz, 2004; Sampath *et al.*, 2007). However, mannitol may be useful in rhabdomyolysis and kidney-transplant surgery (Schetz, 2004). These observations spark interest, because loop diuretics can be used to increase diuresis in oliguric AKI. Still, diuretics do not improve prognosis (Karajala *et al.*, 2009). Dopamine is a renal vasodilator and increases urine output. It is widely used, especially in ICU patients. However, dopamine, or its analogues, do not protect the kidneys (Choudhury, 2010, Zacharias *et al.*, 2013). Norepinephrine has also been widely studied and may have minor beneficial effects (Choudhury, 2010). Calcium channel blockers and ACE inhibitors seem to offer no protection against AKI and treatment with EPO has also failed to show any advantage (Zacharias *et al.*, 2013). Other treatments for AKI that are currently being investigated include bioartificial kidneys, plasma therapies and stem-cell therapies (Lameire *et al.*, 2005, Choudhury, 2010).

2.2.2.3 Supportive treatment

The current preventive treatment of I/R-induced AKI is summarized in Panel 1. In patients with AKI, it is important to avoid potassium, which used to cause mortality in AKI patients before invention of dialysis and rapid laboratory tests (Lameire *et al.*, 2005).

Panel 1. Management priorities in patients with AKI (modified from Lameire *et a*l., 2005)

• Correct prerenal and postrenal factors
• Review medications and stop nephrotoxins
• Optimize CO and renal blood flow
• Restore and/or increase urine flow
• Monitor fluid intake and output; measure bodyweight daily
• Treat acute complications (hyperkalaemia, hyponatraemia, acidosis, hyperphosphataemia, pulmonary oedema)
• Nutritional support
• Treat infections
• Expert nursing care (management of catheter care and skin in general; psychological support)
• Initiate dialysis before uraemic complications emerge
• Give drugs in doses appropriate for GFR

The daily fluid intake of an oliguric patient should be limited and dietary sodium should be restricted to 2 g/day. The caloric requirement in AKI is high. Carbohydrate intake should be sufficient (more than 100 g/day) to avoid breakdown of endogenous protein for glucose. The protein requirements are dependent on the clinical status; 40 g/day high-quality protein can be given and the protein content increased if necessary (Lameire *et al.*, 2005).

Dialysis may be necessary, because it allows larger quantities of protein to be given. Berbel *et al.* (2011) concluded that AKI patients undergoing renal replacement therapy should receive 25-30 kcal/kg and a minimum intake of 1.5 g/kg of proteins daily.

Enteral or parenteral alimentation may be necessary in postoperative patients or in those with anorexia or vomiting. The use of essential amino acids or their keto analogues in postoperative or trauma patients has been suggested, but may not improve the outcome of AKI (Lameire *et al.*, 2005). Hyperglycaemia is known to have many adverse effects; elevated blood glucose levels are also associated with increased occurrence of AKI after cardiac surgery (Song *et al.*, 2013).

2.2.2.4 Renal replacement therapy

Dialysis should be started before complications occur. The criteria for dialysis are given in Panel 2.

Panel 2. Proposed criteria for dialysis with AKI (modified from Lameire *et a*l., 2005)

• Oliguria: urine output < 200 ml in 12 h
• Anuria: urine output < 50 ml in 12 h
• Hyperkalaemia: potassium concentration > 6.5 mmol/l
• Acidaemia: pH < 7.0
• Azotaemia: urea concentration > 30 mmol/l
• Uraemic encephalopathy
• Uraemic neuropathy/myopathy
• Uraemic pericarditis
• Plasma sodium abnormalities: concentration >155 mmol/l or <120 mmol/l
• Hyperthermia
• Drug overdose with dialysable toxin

2.2.3 Prognosis

The prognosis varies, depending on the stage of AKI. The in-hospital mortality rate with AKI patients has varied between 20% and 25% (Waikar *et al.*, 2008). However, if renal replacement therapy is needed, the mortality rate can be as high as 66% (Waikar *et al.*, 2008). AKI is also often accompanied by multiorgan failure (MOF), which further increases mortality (Lameire *et al.*, 2005). A slight decline in the mortality rates of AKI has been observed in recent decades (Waikar *et al.*, 2008).

AKI is irreversible in 5% of all and in 16% of elderly patients (Lameire *et al.*, 2005). Many of these patients will eventually develop CKD (Coca *et al.*, 2009; Hsu *et al.*, 2009). In children with AKI, kidney dysfunction develops in early adulthood (Lameire *et al.*, 2005). The risk of CKD is higher with older patients (Ishani *et al.*, 2009).

2.3 Caloric restriction and underlying pathways

2.3.1 Health-promoting effects of caloric restriction and prolonged lifespan

It was observed almost 80 years ago that CR, without malnutrition, prolongs the lifespan in several species, including yeast, flies, worms, fish, rodents and rhesus monkeys (*Macaca mulatta* [Zimmermann]) (Fontana *et al.*, 2010). CR has positive effects in chronic conditions, e.g. cancer, neurodegenerative diseases, age-associated renal injury, obesity, diabetes, as well as atherosclerosis. The health-promoting effects of CR have been demonstrated in many organisms, including primates (Colman *et al.*, 2009; Fontana *et al.*, 2010). CR lasting between 3 months and 1 year also seems to attenuate ischaemic injuries in rodent models of cardiac and cerebral ischaemia (Yu and Mattson, 1999; Chandrasekar *et al.*, 2001; Ahmet *et al.*, 2005). On the cellular

level, it is known that CR reduces oxidative stress and enhances autophagy (Speakman and Mitchell, 2011).

There are at least four pathways that have been implicated in mediating the effects of CR; 1.insulin like growth factor 1 (IGF-1)/insulin signalling pathway, 2. SIRT pathway, 3. AMPK pathway and 4. target of rapamycin (TOR) pathway (Diagram 4) (Speakman and Mitchell, 2011).

Budding yeast gets older' mainly because of the accumulation of extrachromosomal ribosomal DNA (rDNA) circles (ERCs). It was discovered that Sir2, the first SIRT family member, is a suppressor of ERC formation and regulates the lifespan. This was the first time SIRTs were linked with the positive effects of CR. It was observed that increased copy numbers of the *SIR2* gene not only increase the lifespan in yeast but also delay aging in *Caenorhabditis elegans* (Maupas) and *Drosophila melanogaster (Meigen)*. It was concluded that Sir2 and its orthologues are crucial modulators in the regulation of longevity in both lower and higher organisms. Sir2 and its homologues also mediate CR-induced extension of the yeast replicative lifespan, which may be different under nondividing conditions (Smith *et al.*, 2007).

The normal process of aging in kidneys includes structural and functional changes, e.g. glomerulosclerosis and tubulointerstitial fibrosis, a decrease in GFR and renal blood flow, and progressive loss of multiple tubular transport functions. CR slows down this aging process. SIRT1 is also expressed in the kidney and seems to be cytoprotective (Hao and Haase, 2010). However, its role in CR-mediated renoprotection is still unclear.

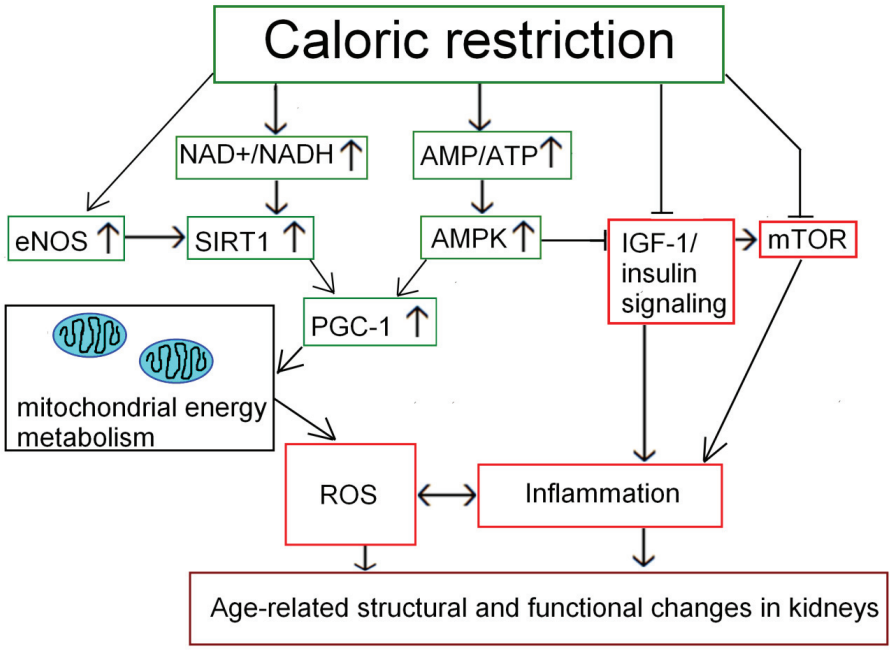

Diagram 4. Potential molecular mechanisms of the anti-aging actions of caloric restriction in kidneys. AMP=adenosine monophosphate, ATP= adenosine triphosphate, AMPK= adenosine monophosphate kinase, eNOS= endothelial nitric oxide synthase, IGF-1=insulin-like growth factor 1, mTOR=mammalian target of rapamycin, NAD+= nicotinamide adenine dinucleotide, NADH= nicotinamide adenine dinucleotide hydrogen, PGC-1= peroxisome proliferator-activated receptor-γ coactivator 1, ROS=reactive oxygen species, SIRT1= silent information regulator 1.

2.3.2 Sirtuins

SIRTs are evolutionarily conserved NAD^+-dependent class III histone deacetylases. They also have monoadenosine diphosphate (ADP) ribosyltransferase activity, especially two mammalian SIRTs, SIRT4 and SIRT6 (Diagram 5). SIRTs regulate gene transcription, DNA repair and recombination, chromosomal stability and mediate the health-promoting effects of CR. Seven Sir2 homologues, (SIRT1--7), have been identified in mammals. SIRT1, SIRT6 and SIRT7 are primarily located in the nucleus, whereas SIRT2 is found in the cytoplasm and SIRT3, SIRT4 and SIRT5 reside in the mitochondria (Haigis and Sinclair, 2010).

The activity of SIRTs is regulated differently in various tissues by CR. For example, Chen and coworkers (2008) showed recently that CR reduces SIRT1 activity in the liver, whereas SIRT1 expression in skeletal muscle and white adipose tissue is markedly induced by CR.

SIRT1 couples protein deacetylation with NAD^+ hydrolysis, generating nicotinamide (NAM) and 2'-*O*-acetyl-ADP-ribose. The catalytic activity of SIRTs is dependent on NAD^+. The intracellular NAD^+/nicotinamide adenine dinucleotide hydrogen (NADH) ratio, in turn, is dependent on cellular energy and the redox state. SIRTS are typically activated during different stressful states, such as CR. In most cases, this tissue- and context-specific activation of SIRTs increases cellular resistance to different metabolic, oxidative and hypoxic disorders. Activation of SIRT1 is associated with longevity and the attenuation of metabolic disorders. In the kidney, SIRT1 protects cells and participates in the regulation of BP and sodium balance (Hao and Haase, 2010).

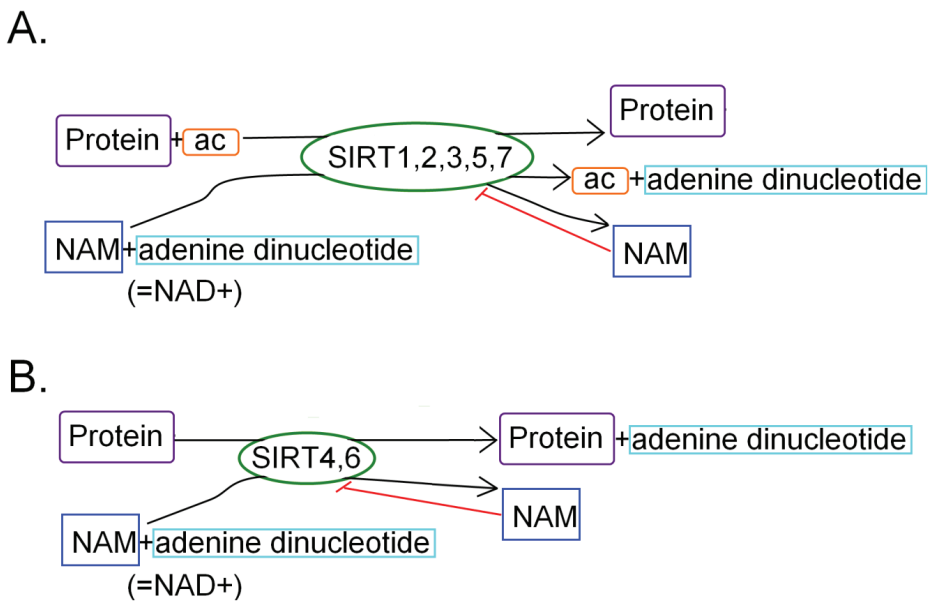

Diagram 5. Actions of sirtuins. Schematic picture how sirtuins deacetylate (e.g.) proteins (Panel A) and have also ADP-ribosyl transferase activity (Panel B). ac=acetyl group, NAD+= nicotinamide adenine dinucleotide, NAM=nicotinamide, SIRT=silent information regulator.

2.3.2.1 SIRT1

SIRT1 mediates and is also needed for mediating the beneficial effects of CR (Chen *et al.*, 2005, Boily *et al.*, 2008). It mainly functions by deacetylating several targets, which include histones, transcription factors and enzymes (Hao and Haase, 2010).

Regulation of SIRT1 is complex. CR and conditions that cause oxidative stress and DNA damage increase the expression of SIRT1 (Cohen *et al.*, 2004, Rodgers *et al.*, 2005). High-fat diets, insulin resistance, high glucose and senescence, in turn, decrease the expression of SIRT1. The increase in SIRT1 expression in response to acute stress is controlled and counterbalanced in many ways and involves regulation at the promoter, messenger RNA (mRNA) and protein levels (Hao and Haase, 2010).

SIRT1 enzymatic activity is associated with the consumption of NAD^+ and the production of NAM, which in turn, inhibits its catalytic activity. Nicotinamide phosphoribosyltransferase NAMPT lowers the concentration of inhibitory NAM and increases the levels of NAD^+. NAD+ also increases SIRT1 expression (Morris *et al.*, 2011).

Further evidence for the health benefits of SIRT1 activation comes from resveratrol (RSV) and its analogue studies (Chen *et al.*, 2009; Della-Morte *et al.*, 2009). Small amounts of RSV also occur naturally, e.g. in red grapes. However, there is no evidence that it would be beneficial to humans (Morris *et al.*, 2011).

During CR, SIRT1 helps to maintain glucose levels through stimulation of gluconeogenesis and depression of glycolysis in the liver. These effects are mediated through deacetylation and activation of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) and forkhead box protein O1 (FOXO1) (Rodgers *et al.*, 2005). SIRT1 also represses peroxisome proliferator-activated receptor-γ (PPAR-γ) in white adipose tissue and thus mobilizes fatty acids during CR (Picard *et al.*, 2004). There are ongoing clinical trials with SIRT activators. It is hoped that they will aid in maintaining glucose levels of diabetic patients within a reasonable range, which would be beneficial for the kidneys as well (Hao and Haase, 2010).

SIRT1 plays a crucial role in the regulation of vascular tone. It decreases the amount of Ang II type 1 (AT_{1A}) receptors (Miyazaki *et al.*, 2008) and increases vasodilation by deacetylating eNOS (Mattagajasingh *et al.*, 2007). The latter increases endothelial NO levels. In line with this, inhibition of endothelial SIRT1 leads to decreased levels of NO and impaired vasodilation (Mattagajasingh *et al.*, 2007).

2.3.2.2 SIRT1 and the kidney

In rodents, CR increases the SIRT1 levels in multiple tissues, including the kidney (Cohen *et al.*, 2004, Rodgers *et al.*, 2005). CR is also associated with less injury in age-related and diabetic nephropathy models (Nangaku *et al*, 2005). This is probably due to a combination of systemic and local effects that includes improved glucose and lipid metabolism, decreased amounts of advanced glycosylation end products (AGEs), ROS reduction, increase in NO bioavailability and attenuation of Ang II (Hao and Haase, 2010). SIRT1 is likely to be one of the key signal molecules at the cellular level. In line with this, mice with complete *SIRT1* deficiency fail to adapt to CR (Chen *et al.*, 2005). Interestingly, Kume *et al.* (2010) also recently showed that CR protected against hypoxia in aged kidneys through SIRT1-dependent mitochondrial autophagy.

He *et al.* (2010) also recently showed the importance of SIRT1 in the kidney during oxidative stress in which activation of SIRT1 protected the mouse renal medulla from oxidative

injury. In the heart, SIRT1 attenuates I/R injury through activation of antioxidant mechanisms and downregulation of proapoptotic molecules (Hsu *et al.*, 2010; Nadtochiy *et al.*, 2011a, 2011b).

SIRT1 activation enhances resistance to apoptosis in human embryonic kidney cells (Cohen *et al.*, 2004). Activation of SIRT1 also protects cardiac cells, neurons and cells in the pancreatic islets after ischaemic injuries, oxidative stress or cytokine-mediated injury (Hao and Haase, 2010). In the rat kidney, RSV treatment also ameliorates I/R-induced AKI (Bertelli *et al.*, 2002; Chander and Chopra, 2006). SIRT1 also protects mesangial cells from apoptosis (Kume *et al.*, 2007).

SIRT1 is abundantly expressed in renal medullary interstitial cells and seems to protect them (Hao and Haase, 2010; He *et al.*, 2010). These cells are constantly under oxidative stress, have relatively little blood flow and little O_2 (Pallone, 2006). The results of Hao and Haase (2010) show that SIRT1 is also expressed in podocytes, but only low levels are found in the renal cortex. Furthermore, Hao and Haase showed that SIRT1 protects primary renal medullary interstitial cells from oxidative stress and that if a single allele of SIRT1 is deleted, it worsens the injury in the renal medulla after unilateral ureteral obstruction. Activation of SIRT1 also protects the kidney in this case (Hao and Haase, 2010).

SIRT1 also regulates sodium balance by decreasing the transcription of the epithelial sodium channel in the inner medullary collecting duct. Aldosterone, in turn, decreases the levels of mRNA encoding *SIRT1* (Zhang *et al.*, 2009).

2.3.2.3 SIRT3

SIRT3 has been implicated in the extension of the lifespan in humans. Its activity and expression are also regulated through the intracellular NAD/NADH ratio. SIRT3 levels are reduced in a sedentary lifestyle and elevated after endurance exercise, which also delayed signs of senescence in patients over 65 years of age (Pillai *et al.*, 2010). SIRT3 is enriched in the kidneys, but its role in (patho)physiological processes in the kidneys is unclear (Palacios *et al.*, 2009).

In the murine liver and kidney, SIRT3 is expressed in two different isoforms: the long form (44 kDa) and the short form (28 kDa). The long form is mainly located in the mitochondria and is the primary mitochondrial deacetylase. Numerous mitochondrial proteins have already been recognized as substrates of SIRT3 (Bao *et al.*, 2010).

Ang II regulates inflammation, cell growth and proliferation. Disruption of the AT_{1A} receptor increases the lifespan in mice. The positive effects of knocking out Ang II signalling in kidneys seem to be mediated through induction of NAMPT and SIRT3, and subsequent boosting of mitochondria (Benigni *et al.*, 2009). In line with this, the absence of SIRT3 leads to marked reduction in adenosine triphosphate (ATP) (Ahn *et al.*, 2008).

In the heart, SIRT3 protects cardiomyocytes from oxidative stress by activating transcription factors and increasing the expression of antioxidants (Chen *et al.*, 2013). Exogenous NAD, at least in neurons and cardiomyocytes, could probably boost the activity of SIRTs and thus protect cells (Pillai *et al.*, 2010).

2.3.2.4 NAMPT

NAMPT is the rate-limiting enzyme in the NAD^+ salvage pathway, which means that it plays a critical role in the regulation of SIRT1 activity. It lowers the concentration of inhibitory NAM and increases the levels of NAD^+, which in turn increases the activity of SIRT1. NAD^+ is also consumed by other reactions that do not involve SIRTs (Hao and Haase, 2010). CR increases

both NAMPT and SIRT1 activities. NAMPT exists in intracellular and extracellular forms (iNAMPT and eNAMPT). eNAMPT is found in plasma and also regulates glucose homeostasis (Fukuhara *et al.*, 2005). Patients with CKD have increased serum levels of NAMPT, which may also contribute to fibrogenesis (Yilmaz *et al.*, 2008).

2.3.3 AMPK

AMPK is a ubiquitously expressed heterotrimeric kinase that acts as a highly conserved ultrasensitive energy sensor and participates in the regulation of energy-generating and energy-consuming pathways. AMPK regulates cellular energy metabolism, cellular nutrient (glucose and fatty-acid) uptake, protein synthesis, gene transcription, inflammation, ion transport, autophagy, cellular polarity and NO synthesis (Hallows *et al.*, 2010).

AMPK consists of a catalytic α subunit and regulatory β and γ subunits. Each of these subunits exists as multiple isoforms (α_1, α_2, β_1, β_2, γ_1, γ_2, γ_3)(Oakhill *et al.*, 2009). This gives rise to 12 different heterotrimer combinations, and splice variants further increase the diversity. AMPK is also found in the rat kidney, where the α_1 and β_2 subunits are predominant and the γ_1 and γ_2 subunits are also expressed (Cammisotto *et al.*, 2008). Mouse kidneys show the same type of distribution, except that β_1 is predominant (Hallows *et al.*, 2010).

Exercise, CR and ischaemia decrease the level of ATP and increase the cellular concentrations of AMP. AMP binds to the γ subunit of AMPK and activates it (Scott *et al.*, 2004). However, AMP also activates AMPK by other means (Kahn *et al.*, 2005). Binding of AMP allows AMPK to be further phosphorylated, which increases its activity by approximately 100-fold (Hawley *et al.*, 1995). AMP binding also inhibits dephosphorylation of AMPK by protein phosphatases, which may be the main mechanism by which AMP binding causes increased phosphorylation of AMPK (Sanders *et al.*, 2007). High amounts of ATP inhibit all the above-mentioned mechanisms (Hardie and Hawley, 2001). Activated AMPK increases energy production and decreases energy consumption by phosphorylating its downstream targets (Hallows *et al.*, 2010).

In the kidneys, AMPK participates in the regulation of energy metabolism, ion transport, podocyte functioning, renal hypertrophy, ischaemia, inflammation and diabetes (Hallows *et al.*, 2010). AMPK regulates the functioning of many ion transporter proteins, which consumes large amounts of energy in the kidney (Hallows, 2005). In the inner medulla, cells have fewer mitochondria and their metabolism is mainly glycolytic (Meury *et al.*, 1994). In contrast, the tubular cells in the cortex have abundant mitochondria, more oxidative metabolism and use fatty acids, ketone bodies and lactate as metabolic substrates (Balaban and Mandel, 1988).

In endothelial cells, AMPK regulates fatty-acid oxidation, NO production, inflammation and angiogenesis. It has been proposed that this may contribute to decreased atherogenesis and improved endothelial functioning (Fisslthaler and Fleming, 2009). For example, shear stress (Chen *et al.*, 2009), ATP levels (Mount *et al.*, 2005), various hormones (Chen *et al.*, 2003, 2009), vasoactive substances (Mount *et al.*, 2008) and ROS (Zou *et al.*, 2003) regulate AMPK activity in endothelial cells.

2.3.3.1 LKB1 and other AMPK regulators

Liver kinase B1 (LKB1) is one of at least three potential upstream AMPK kinases (Woods *et al.*, 2003) and is also expressed in the kidney (Denison *et al.*, 2009). It phosphorylates a threonine residue (Thr^{172}) on the catalytic α subunit of AMPK (Sanders *et al.*, 2007). LKB1 has a long (LKB1L) and a short (LKB1S) splice variant; LKB1L is the variant found in the kidneys (Denison

et al., 2009). AMP does not activate the LKB1 complex itself, but makes AMPK a better substrate for LKB1 (Sanders *et al.*, 2007). It also activates 13 other downstream kinases that are collectively called AMPK-related kinases (ARKs). Their functions are incompletely understood (Alessi *et al.*, 2006). It has been suggested that LKB1 may be constitutively active and that increase in the AMP/ATP ratio activates AMPK by making the already phosphorylated AMPK resistant to the activity of protein phosphatases (Sanders *et al.*, 2007).

The calcium/calmodulin-dependent kinase kinases (CaMKKs) are another class of AMPK activators, they also phosphorylate Thr^{172} on the α subunit of AMPK. Regulation of AMPK by CaMKKβ is regulated in response to changes in calcium concentration. The AMP/ATP ratio does not affect this regulation (Hurley *et al.*, 2005). In some cells, TGF-β-activated kinase1 (TAK-1) may be an upstream kinase of AMPK (Xie *et al.*, 2006).

In the kidneys, ischaemia increases the AMP/ATP ratio and activates AMPK within 1 min. After 5 min, AMPK is activated even more. After the acute phase of I/R injury, AMPK is mainly activated in the cortical tubules (Mount *et al.*, 2005). The physiological relevance of this activation is not clear.

The downstream targets of AMPK are still not clear, at least not in the ischaemic kidney, since it does not appear to phosphorylate eNOS (Mount *et al.*, 2005). The upstream AMPK kinase(s) in the ischaemic kidney are still not known. The role of AMPK activation may also differ between the ischaemia and reperfusion periods. In the heart, AMPK is activated during ischaemia and this activation seems to be beneficial in most situations (Russell *et al.*, 2004; Arad *et al.*, 2007). However, in acute stroke activation of AMPK seems to be harmful (Li *et al.*, 2007) . The net effect of AMPK activation is strongly dependent on tissue and timing.

Dietary salt, diabetes, adiponectin and ischaemia also regulate AMPK in the kidney (Hallows *et al.*, 2010). Adiponectin activates AMPK in the podocytes and thus reduces albuminuria (Cammisotto and Bendayan, 2008). In the tubular cells, it also activates AMPK and reduces glycogen accumulation (Cammisotto *et al.*, 2008). Reduced AMPK activity also contributes to the pathogenesis of diabetic renal hypertrophy (Lee *et al.*, 2007).

Diabetes and high glucose levels decrease AMPK activity in the kidneys (Cammisotto *et al.*, 2008). This may also be related to decreased adiponectin levels (Lee *et al.*, 2007) and/or activation of the mammalian target of rapamycin (mTOR) pathway (Guo and Zhao, 2007). Hyperglycaemia also deactivates LKB1 (Lieberthal and Levine, 2009). On the other hand, it is not known how the AMPK activators affect renal gluconeogenesis; e.g. in the liver, metformin (which is known to activate AMPK without an increase in AMP/ATP –ratio) reduces it (Lee *et al.*, 2010). Metformin seems also provide some renoprotection against I/R kidney injury in mice (Seo-Mayer *et al.* 2011).

AMPK activity in the kidneys changes with age (Zhou *et al.*, 2009). Jin *et al.* (2004) showed that older rats seem to have more phosphorylated AMPK (AMPK-P), but the total amount of AMPK is lower. In young rats, induction of oxidative stress caused a two-fold increase in the expression of active AMPK. In aged rats, there was no change in AMPK phosphorylation (Jin *et al.*, 2004, Percy *et al.*, 2009).

2.3.3.2 AICAR

AICAR is an adenosine analogue and has a short half-life. It binds to AMPK, which causes allosteric modification and activates AMPK. In cells, AICAR is phosporylated to ZMP which in turn acts similarly as AMP and activates AMPK by binding to its γ subunit (Merrill *et al.*,

1997). This allosteric activation also increases AMPK affinity for upstream AMPK kinases, which phosporylate AMPK at αThr^{172}. This phosporylation by upstream kinases is responsible for most AMPK activity (Gaskin *et al.*, 2009). In a canine model of autologous renal transplantation, combination therapy with the AMPK activator AICAR and the antioxidant N-acetyl cysteine attenuates I/R injury (Lin *et al.*, 2004). The relative contributions of these two drugs to renoprotection are unclear. In the 1980s and 1990s, AICAR was studied as a possible candidate for diminishing cardiac injury during coronary-artery bypass grafting (Holdright *et al.*, 1994), but this development project was stopped due to adverse effects (increased serum uric acid levels and crystalluria) and development of other treatments.

AICAR seems to suppress the production of ROS (Kim *et al.*, 2008), inhibits proinflammatory NF-κB signalling (Katerelos *et al.*, 2010) and inhibits inflammation in mouse mesangial cells (Peairs *et al.*, 2009) through AMPK activation. However, it also employs several other mechanisms of activity that may be independent of the activation of AMPK.

AICAR inhibits cytokines in adipose tissue (Lihn *et al.*, 2004) and also prevents postischaemic leukocyte rolling and adhesive interactions with endothelial cells, thus attenuating reperfusion injury (Gaskin *et al.*, 2007, 2009). AICAR is also known to increase production of NO in human aortic endothelial cells (Morrow *et al.*, 2003).

In the renal proximal tubules, I/R injury is known to damage predominantly mitochondria (Hasegawa *et al.*, 2010). Long-term treatment with AICAR increases muscle mitochondrial content. The probable route of this activity is activation of AMPK and subsequent activation of PGC-1α (Winder *et al.*, 2000), which is an important regulator of mitochondrial biogenesis and functioning (Gerhart-Hines *et al.*, 2007, Spiegelman, 2007).

AICAR increases glucose uptake into the muscles and decreases the blood glucose levels (Holmes *et al.*, 1999). Strict glycaemic control is beneficial for critically ill patients, but its effects on the kidneys after I/R injury are not as well documented.

AICAR is also known to increase the expression of human microsomal fatty-acid omega-hydroxylase CYP4F2 (cytochrome P450, family 4, subfamily F, polypeptide 2). These fatty- acid hydroxylases remove excess fatty acids that can disrupt mitochondrial functioning (Hsu *et al.*, 2010). AICAR also increases adiponectin gene expression (Lihn *et al.*, 2004) and there is evidence that adiponectin also activates AMPK (Yamauchi *et al.*, 2002; Wu *et al.* 2003).

AICAR inhibits autophagy by a mechanism independent of AMPK activity and inhibits the proteosomal degradation of proteins by an AMPK-dependent mechanism (Viana *et al.*, 2008). AICAR pretreatment also protects cardiomyocytes by inhibiting endoplasmic reticulum (ER) stress (Terai *et al.*, 2005).

2.3.4 AMPK and SIRT1 regulate each other

AMPK and SIRT1 regulate each other and share many common target molecules (Ruderman *et al.*, 2010). SIRT1 seems to increase the activity of LKB1 in human embryonic kidney 293T cells (and presumably also in others), which in turn activates AMPK (Lan *et al.*, 2008). On the other hand, activation of AMPK apparently increases transcription of NAMPT which in turn increases the NAD^+/NADH ratio and decreases the concentration of NAM, both of which lead to activation of SIRT1 (Fulco *et al.*, 2008). It has also been suggested that AMPK may be able to alter the NAD^+/NADH ratio and activate SIRT1 independently of NAMPT (Canto *et al.*, 2009). One of the common targets for AMPK and SIRT1 is PGC-1α, of which phosphorylation by AMPK makes it more prone to deacetylation by SIRT1. This enhances its ability to activate its own promoter (Terai *et al.*, 2005).

2.3.5 mTOR

mTOR regulates protein translation and is an important integrator and transducer of intracellular signalling pathways. Some studies indicate that the life-extension benefits of CR may also be regulated through the mTOR pathway (Bishop and Guarente, 2007, Kennedy *et al.*, 2007)). In kidneys, increased expression of mTOR prevents I/R injury (Ma *et al.*, 2009). I/R-induced AKI also alters the transcription of several mTOR pathway-associated genes (Grigoryev *et al.*, 2006).

Activation of the mTOR pathway is also associated with renal hypertrophy and is apparently activated under conditions in which AMPK activity is decreased, such as diabetes and high glucose levels (Hallows *et al.*, 2010).

2.3.6 eNOS and nitric oxide

Endothelum-derived NO maintains vascular tone, functions as a neurotransmitter, mediates cellular defence and regulates mitochondrial functioning and amount of ROS. NO is also an important antiatherosclerotic molecule, due to its anti-inflammatory, antiapoptotic, antiplatelet and antiproliferative activities. NO has been implicated in many CVDs and almost all known CVD risk factors appear to be associated with decreased endothelial generation of NO (Moncada and Higgs, 2006; Pechanova and Simko, 2007).

NO is generated by the enzyme nitric oxide synthase (NOS). Three isoforms of NOS have been identified: neuronal (nNOS) or NOS1, inducible (iNOS) or NOS2 and endothelial (eNOS) or NOS3. nNOS is expressed in neurons and eNOS in endothelial cells, cardiac myocytes and blood platelets. Cytokines induce iNOS, which is not expressed constitutively. eNOS generates NO and L-citrulline from L-arginine, O_2 and NADPH (Govers and Rabelink, 2001).

NO is a free radical. It has one unpaired electron in the highest orbital and thus reacts rapidly with other molecules containing unpaired electrons. The haem group of haemoglobin acts as an intravascular scavenger and reacts normally with endogenous NO. The half-life of NO *in vivo* is less than 1 s, because normally NO diffuses rapidly into red-blood cells, where it is converted into nitrate. However, if O_2^- and NO are formed within a few cell diameters of each other, they will form the free radical, $ONOO^-$ (Munzel *et al.*, 2005).

CR-induced increase in eNOS activity promotes mitochondrial biogenesis (Nisoli *et al.*, 2005), and Canto and Auwerx (2012) recently showed that eNOS is also induced by SIRT1 deacetylation (Canto and Auwerx, 2012). On the other hand, eNOS also induces SIRT1 gene expression during CR, forming a positive regulatory loop (Nisoli *et al.*, 2005; Canto and Auwerx, 2012). eNOS-Ser1177 is also a target of AMPK. Nevertheless, eNOS is not phosphorylated in the kidneys during acute ischaemia (Hallows *et al.*, 2010, Mount *et al.*, 2005).

2.3.7 Angiogenesis

Kidney I/R injury reduces the number of microvessels in the outer medulla possibly due to the downregulation of angiogenic factors (e.g. VEGF) and increased amount of angiogenesis inhibitors. The reduced number of vessels leads to chronic hypoxia, which likely further increases the tubular injury and development of fibrosis (Bonventre and Yang, 2011).

In damaged tissues, the subsequent process of neovascularization (angiogenesis) is regulated by various signals that are mediated through transmembrane receptor tyrosine kinases and nonreceptor tyrosine kinases (Maulik, 2004). On the other hand, CR also increases angiogenesis through circulating factors (Csiszar *et al.*, 2013), and SIRT1 apparently plays a crucial role in

this process (Potente and Dimmeler, 2008). In line with this, the SIRT1 activator RSV seems to have both pro- and antiangiogenic effects, depending on the situation (Chen and Tseng, 2007). Chemokines (Frangogiannis, 2007) and thrombospondins (TSPs) (Hugo and Daniel, 2009) are important regulators of angiogenesis and it is known that I/R-induced AKI increases the amount of TSPs in the kidneys (Hugo and Daniel, 2009).

2.4 Autophagy

Autophagy reprocesses cellular substances for biosynthetic or metabolic needs and is a general cellular response to stress (Mizushima *et al.*, 2008). It enables recycling of macromolecules to provide nutrients and energy. In this process, portions of the cytoplasm are sequestered within vesicles called autophagosomes, which are then delivered to lysosomes for degradation (Rubinsztein *et al.*, 2011). Formation and elongation of autophagosomes is a complex process. However, the elongation phase involves the protein microtubule-associated protein 1 light chain 3/autophagy-related protein 8 (MAP1-LC3/Atg8), which is further processed to LC-3B (LC3-II) and remains in completed autophagosomes until fusion with the lysosomes. The specific association of LC3-II with autophagosomes makes it a very good marker for studying autophagy (Ravikumar *et al.*, 2010).

CR stimulates autophagy (Madeo *et al.*, 2010; Groenendyk *et al.*, 2010) and SIRT1 seems to mediate this effect (Rubinsztein *et al.*, 2011). Although the exact mechanism is still unclear, SIRT1 is known to deacetylate several autophagy genes (*atg5, atg7* and *atg8*) (Lee *et al.*, 2008). Kume *et al.* (2010) recently proposed that SIRT1 promotes autophagy through FOXO3, which is an important transcription factor (Kume *et al.*, 2010). *Atg* genes (e.g. *Beclin* and *LC-3B*) and proteins are also crucial factors in the molecular machinery of autophagy in kidneys (Periyasamy-Thandavan *et al.*, 2009).

Other factors linked with the regulation of autophagy include the α_2 agonist clonidine (Williams *et al.*, 2009) and p38 MAPK (Tang *et al.*, 2008).

Mitochondrial oxidative damage and mitochondrial dysfunction play crucial roles in the pathogenesis of kidney I/R injury (Bonventre and Yang, 2011). There is growing evidence that induction of autophagy protects cells by eliminating damaged and potentially dangerous organelles, such as leaky mitochondria (Cybulsky, 2010; Kume *et al.*, 2010) and could thus also provide protection against I/R-induced AKI. In line with this, Finckenberg *et al.* (2011) recently showed that also CR protects against Ang II-induced mitochondrial remodelling and cardiac hypertrophy through induction of autophagy. However, these results should still be interpreted with caution, because induction of autophagy protected cells in some cases, at least in cardiac I/R injury, but too much induction may even be harmfull (Gustafsson and Gottlieb, 2009). Depending on the level of cellular stress, autophagy can either directly induce cell death or act as a protective mechanism of cell survival (Periyasamy-Thandavan *et al.*, 2009).

2.5 Alpha2 adrenoceptors and their agonists

All catecholamine receptors belong to the superfamily of G protein-coupled receptors, which means that they transduce signals from extracellular ligands to cellular effector molecules via guanine nucleotide-binding proteins (G proteins). The activity of adrenaline and noradrenaline (NA) are mediated via adrenergic receptors, which are divided into α- and β-adrenoceptors (α-

and β-ARs) according to their activity in smooth-muscle relaxation. α-ARs are further divided into α_1- and α_2-ARs. It was first suggested that α_2-ARs would be presynaptic (inhibiting NA release) and α_1-ARs postsynaptic (stimulatory end-organ responses). However, it is now known that there are also postsynaptic α_2-ARs in tissues. After identification of selective drugs for α_1- and α_2-ARs, the division of adrenoceptors into subtypes was based on pharmacological criteria. The α_1-ARs were defined as those activated by phenylephrine and prazosine and α_2-ARs as those activated by clonidine and medetomidine and antagonized by yohimbine and idaxozan. The subclassification of ARs is based on a molecular biological classification. The α_1-, α_2- and β-ARs are each divided into three subtypes. It is now known that three human genes encode unique human α_2-AR subtypes (α_2A, α_2B, α_2C) and that α_2D-ARs (found in bovines, guinea pigs, rats and mice) are species variants or homologues of human α_2A-AR (Haapalinna, 2006).

α_2-ARs are found ubiquitously in the central, peripheral and autonomic nervous systems, as well as in vital organs and blood vessels, and mediate many of the biological effects of endogenous adrenaline and NA (Kaur and Singh, 2011). Receptors situated in the given neuron, responding to transmitters released by the same neuron, are called autoreceptors. The best known role for α_2-ARs is the (auto)regulation of NA release, which occurs in many neurons. However, there are also α_2-ARs on various neurons other than noradrenergic neurons (e.g. dopaminergic, serotonergic, histaminergic and cholinergic) and activation of these 'heteroreceptors' decreases the firing rate and neurotransmitter release from these neurons (Haapalinna, 2006).

Human and rat kidneys contain all three types of α_2-AR (Cussac *et al.*, 2002), and rats also have α_2D-ARs, which is a rodent species variant of human α_2A-AR (Lehtimaki *et al.*, 2008). Studies with isolated perfused kidneys show that rat renal plasma membranes contain both α_1- and α_2-ARs in a ratio of approximately 1:3 (Schmitz *et al.*, 1981). Meister *et al.* (1994) showed that α_2A/D and α_2C receptor mRNAs are found in the inner medulla associated with collecting tubules and α_2B receptor mRNA in the proximal tubules in the outer medulla radiating into the cortex (Meister *et al.*, 1994). Huang *et al.* (1996) also detected α_2B-ARs in the basolateral membrane of the proximal convoluted and straight tubules (Huang *et al.*, 1996). In rat proximal tubular cells, the α_2B-AR is coupled with G protein, and its stimulation by Dex inhibits the accumulation of cyclic AMP (cAMP) (Cussac *et al.*, 2002). In other cases, α_2-AR-coupled G proteins cause membrane hyperpolarization via opening of K^+ channels and inhibiting Ca^{2+} influx. Consequently, α_2-ARs have several important physiological functions (Lehtimaki *et al.*, 2008).

The systemic effects of α_2-ARs include hypotension, bradycardia, sedation, analgesia and decrease in intraocular pressure (Haapalinna, 2006). Dex is a highly selective α_2-AR agonist (Hamasaki *et al.*, 2002) and has eight times higher affinity for α_2-AR than does clonidine (Kaur and Singh, 2011). Neither clonidine nor Dex is totally selective for any one of the α_2-AR subtypes, but Dex seems to have higher α_2A-AR and α_2C-AR affinity than clonidine. The sedative activity of Dex comes mainly through the α_2A-ARs of the locus coeruleus of the brain stem and analgesic activity through the α_2A-ARs of the spinal cord. In the heart, α_2-AR agonists decrease tachycardia (through blocking of the cardioaccelerator nerve) and induce bradycardia via α_2A-AR (through vagomimetic activity). In peripheral blood vessels, α_2-AR agonists cause sympatholysis-mediated vasodilatation and smooth-muscle cell receptor-mediated vasoconstriction (Kaur and Singh, 2011). Kawano *et al.* (2012) have recently shown that Dex directly inhibits vascular K_{ATP} channels, which seems to be an underlying mechanism of Dex-induced peripheral vasoconstriction.

2.5.1 Dexmedetomidine

Dex has sedative, analgesic and sympatolytic properties (Mantz *et al.*, 2011). There are also some notions that Dex protects tissues, including kidneys, against ischaemic injuries in animal models (Maier *et al.*, 1993; Kuhmonen *et al.*, 1997; Jolkkonen *et al.*, 1999; Gu *et al.*, 2011b; Mantz *et al.*, 2011; Kilic *et al.*, 2012; Ibacache *et al.*, 2012; Tufek *et al.*, 2013). It is already in clinical use as a sedative for ICU patients who require only mild sedation and also seems to enhance renal function in some clinical cases (Kulka *et al.*, 1996; Frumento *et al.*, 2006). Its sedative effect is based on the reduction of the activity of the NAergic neurons in the locus coeruleus. However, it also has other systemic and haemodynamic effects, including decreased heart rate (HR) and lowered BP. Its sympatolytic activity is mediated via central and peripheral mechanisms and it also binds to α_2 receptors in the spinal cord, which enhances its analgesic effect. A great advance in its clinical use (as a sedative) is that Dex maintains the patient´s respiratory drive quite well (Coursin *et al.*, 2001).

The distribution phase of Dex is fast (distribution half-life 5–10 min). The terminal half-life is approximately 2–3 h. Dex is highly protein-bound (94%), and the steady-state volume of distribution is 1.33 l/kg. Dex is almost completely biotransformed in the liver through cytochrome P450-mediated metabolism and glucuronidation. A small amount of unchanged form is excreted in the urine or faeces (Bhana *et al.*, 2000; Lee *et al.*, 2012).

2.5.1.1 Dexmedetomidine and the kidneys

There are already data showing that Dex may protect the kidneys in clinical use. At least after thoracic surgery, 24-h Dex infusion (0.4 μg kg^{-1} h^{-1})-induced diuresis and calculated creatinine clearance values were significantly better in the Dex group than in the control group. The Dex group also received fewer diuretic agents than did the control group (Frumento *et al.*, 2006). In dogs, Dex 10 μg/kg intravenous (i.v.) decreased CO by 50%. However, blood flow through the arteriovenous anastomoses and skin decreased by 70--90%, but renal blood flow only by 30%. The cerebral and left ventricular blood flows were maintained considerably better, indicating that Dex causes redistribution of CO, predominantly reducing blood flow to the less vital organs and shunt flow (Lawrence *et al.*, 1996). In a rat model of intra-abdominal sepsis, Dex 50 μg/kg intraperitoneal (i.p.) attenuated sepsis-induced kidney injury and apoptosis (Koca *et al.*, 2013).

2.5.1.2 Dexmedetomidine and ischaemia-reperfusion injury

Dex attenuated myocardial infarcts (Ibacache *et al.*, 2012) and protected against ischaemic brain (Kuhmonen *et al.*, 1997; Jolkkonen *et al.*, 1999; Maier *et al.*, 1993), spinal cord (Bell *et al.*, 2013), intestine (Kilic *et al.*, 2012), liver (Tufek *et al.*, 2013) and kidney (Gu *et al.*, 2011b) injuries, as well as I/R-induced AKI-related lung injuries (Gu *et al.*, 2011a) in animal models. It was suggested that Dex may establish its protection against ischaemia through its anti-inflammatory (Gu *et al.*, 2011b) and/or antioxidative properties (Tufek *et al.*, 2013), by the activation of cellular prosurvival kinases (Ibacache *et al.*, 2012; Bell *et al.*, 2013) and/or by decreasing the release of NA (Maier *et al.*, 1993; Jolkkonen *et al.*, 1999). A recent study of Tufek *et al.* 2013 showed that Dex 100 μg/kg i.p. reduces oxidative stress also in kidneys after hepatic I/R injury in rats.

In the study of Gu *et al.* (2011b), pre- and posttreatment with 25 ug/kg i.p. Dex attenuated I/R-induced AKI in mice. Pretreatment also increased the survival rates after nephrectomy. It was suggested that Dex protects kidneys through α_2-AR-dependent decrease in the activity of the

inflammatory high-mobility group protein B1-Toll-like receptor 4 (HMGB1-TLR4) circuit (Gu *et al.*, 2011b). Previous clinical studies showed that α_2-AR agonists can attenuate I/R-induced AKI (Kulka *et al.*, 1996; Frumento *et al.*, 2006).

Taoda *et al.* (2001) showed that Dex inhibits the release of NA in rat kidneys via activation of α_2C-ARs. Activation of other α_2-AR subtypes (2A and 2B) may also lead to reduction of NA release, at least in mouse kidneys (Taoda *et al.*, 2001). During I/R injury, this may lead to decreased sympathetic activity and thus also enhance blood flow and protect the kidneys.

Renal sympathetic efferent neurons are highly activated in human hypertension. Denervating the human kidney is an old therapeutic concept, but only new, minimally invasive, device-based approaches have enabled renal denervations with relatively small risks. This procedure reduces NA spillover from the kidneys to the circulation by 47%, decreases renin secretion and increases renal blood flow (Schlaich *et al.*, 2012).

2.5.1.3 Haemodynamic effects of dexmedetomidine

The overall cardiovascular effects of Dex are known and can be derived from the pharmacological effects of α_2-ARs (Lehtimaki *et al.*, 2008). In clinical studies, a brief biphasic, dose-dependent, cardiovascular response was reported after the administration of Dex (Leino *et al.* 2009). A Dex bolus increases BP, which causes a reflex drop in HR. It has been suggested that the stimulation of α_2B receptors in vascular smooth muscle causes the increase in BP (Lehtimäki *et al.*, 2008). This initial response lasts for 5--10 min, after which BP decreases, due to the inhibition of central sympathetic outflow. NA release is also decreased through presynaptic α_2 receptors, which also decreases BP and HR (Afonso and Reis, 2012).

2.5.2 Fadolmidine

Fadolmidine (MPV-2426 hydrochloride, 3-(1H-imidazol-4ylmethyl)-indan-5ol hydrochloride) (Fado) is another α_2-AR agonist. Pharmacokinetics and pharamacodynamics of fadolmidine have already been widely studied in previous preclinical studies. Fado has high affinity and full agonist efficacy for all three subtypes of human α_2-AR (α_2A, α_2B and α_2C). It also activates rodent α_2D-ARs, as well as human α_1A- and α_1B-ARs. Weak α_1-AR agonism has also been observed in rat vas deferens (Lehtimaki *et al.*, 2008). Fado is a less lipophilic and more polar molecule than Dex, which makes it act more peripherally (Lehtimäki *et al.*, 2008, Leino *et al.*, 2009). This is also likely to explain why Dex might have some central effects (e.g. sedation) that fado does not have. Fado is known to have antinociceptive effects when administered intrathecally and it also lowers intraocular pressure. Its analgesic potency (effective dose 50 [ED_{50}] = 0.73 μg) is greater than that of clonidine (ED_{50} = 6.4 μg) or Dex (ED_{50} = 2.2 μg) (Lehtimäki *et al.*, 2008). The antinociceptive effects of Fado can be reversed by a selective α_2-AR antagonist, atipamezole (Pertovaara and Wei, 2000).

In the study of Leino *et al.* (2009), Fado decreased MAP at low doses (0.3 μg and 1 μg), whereas at higher doses (3 μg, 10 μg and 30 μg) there was an intitial peak in BP. In both cases, HR decreased simultaneously. At the 1-μg dose, which is already analgesic, Fado showed only minor haemodynamic effects (Leino *et al.*, 2009). It is likely that this initial transient peak in BP was the result of peripheral vasoconstriction caused by Fado. However, it remains partly open whether this response was mediated only by α_2-ARs or whether the α_1-ARs also showed some effect (Lehtimaki *et al.*, 2008).

3 Aims of the study

I/R (ischaemia-reperfusion) injury is a common cause of AKI (acute kidney injury). AKI often occurs in the context of MOF (multi organ failure) and sepsis and is associated with high mortality rates. Currently, there are no specific curative treatments available for I/R injury-induced AKI. The aim of the present study was to increase our understanding of the pathophysiological processes underlying acute ischaemic kidney injury and repair and to identify tentative drug targets for AKI. In particular, we investigated here whether three different treatment strategies with tissue-protective and anti-inflammatory properties, namely CR (caloric restriction), AMPK (adenosine monophosphate kinase) activation by AICAR (5-amino-4-imidazolecarboxamide riboside-1-β-D-ribofuranoside) and α_2-AR (α_2-adrenoceptor) activation by α_2-AR agonists could ameliorate kidney I/R injury. The specific objectives of this thesis were the following:

1. To investigate whether preconditioning of the kidneys by CR could ameliorate kidney I/R injury and how the renoprotective effects of CR are mediated at the cellular level.

2. To investigate whether metabolic remodelling of the kidney with the AMPK activator AICAR ameliorates kidney I/R injury and whether the salutary effects of AICAR are mediated through antioxidative and anti-inflammatory mechanisms.

3. To explore the mechanisms and time course behind the renoprotective effects of the centrally acting α_2-AR agonist Dex and whether metabolic reprogramming is involved.

4 Materials and methods

4.1 Experimental animals and ethical issues (I-III)

The investigation conforms to the Guide For the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The protocols were approved by the Provincial State Office of Southern Finland/ National Animal Experiment Board (approval number STH059A). The rats were kept under a 12-h light/12-h dark cycle. In Study I, the rats in the CR groups had free access to water, but were given access to only 70% of the food their littermates consumed daily. In all other studies, all rats had free access to food and water. The rats were purchased from Charles River Laboratories (Research Models and Services, Sulzfeld, Germany). Wistar (WT) rats were used in the first study and Sprague Dawley (SD) rats in the second and third studies.

4.2 Study design (I-III)

4.2.1 Murine model of ischaemia-reperfusion injury (I-III)

An established model of renal I/R injury was used in all three studies (Kennedy and Erlich, 2008). The rats were anaesthetized with isoflurane and a single dose of buprenorphine (0.1 mg/kg intramuscular [i.m.], given 5 min before surgery) was used as an analgesic. Bilateral renal ischaemia was induced by clamping the renal pedicles for 40 min with microvascular clamps. Control animals were subjected to sham operations without renal pedicle clamping. The rats were hydrated with warm saline during the operation and the body temperature was maintained constantly at 37 °C. The wounds were sutured after removing the clips. The rats were anaesthetized with isoflurane 24 h after the operation, and blood samples were collected from the inferior vena cava for biochemical measurements. The kidneys were excised, washed with ice-cold saline, blotted dry and weighed. The left kidney was used for Western blotting, polymerase chain reaction (PCR) and histological examinations. Tissue samples for histology were fixed in 10% formaline and processed to paraffin with routine methodology. Samples for immunohistochemistry were snap-frozen in -38 °C isopentane. For protein and gene expression studies, renal samples were snap-frozen in liquid nitrogen. The samples were stored at -80 °C. For more details see the original publications.

4.2.2 Design of Study I

Twentyeight 6--7-week-old male WT rats were divided into three groups: 1) Sham-operated controls (n = 9), 2) I/R group (n = 10) and 3) I/R group + CR initiated 2 weeks before the I/R operation (n = 9). CR was calculated from the food intake of pair-fed WT controls fed *ad libitum*. The CR rats were only given access to 70% of the food their littermates consumed daily, whereas the control rats had free access to chow (Harlan Laboratories, Boxmeer, The Netherlands) and drinking water. To further investigate the molecular mechanisms of CR, we conducted an additional study with the following four groups (n = 6 in each group): 1) I/R group, 2) I/R group + CR, 3) I/R group + CR treated with the autophagy inhibitor 3-methyladenine (3-MA) (30 mg/kg i.v. given 1 h before the operation) and 4) I/R group + CR treated with the SIRT1 inhibitor sirtinol (1 mg/kg i.v. given 1 h before the operation). The drug dosages of 3-MA and sirtinol were selected from previous *in vivo* studies performed in rats (Kennedy and Erlich, 2008, Jiang *et al.*, 2010).

4.2.3 Design of Study II

6--7-week-old SD rats were divided into five groups: 1) Sham-operated controls treated with sodium chloride (NaCl) 0.9% 5 ml/kg i.v (n = 17), 2) I/R group treated with NaCl 0.9% 5 ml/kg i.v (n = 19), 3) I/R group treated with AICAR , Bepharm Ltd, Shanghai, China) 50 mg/kg i.v (n = 8), 4) I/R group treated with AICAR 160 mg/kg (n = 9) and 5) I/R group treated with AICAR 500 mg/kg i.v (n = 8). An additional study with two groups (n = 6), the sham group and sham treated with AICAR 500 mg/kg i.v., was performed to further evaluate the effects of AICAR on the serum biochemical markers. The AICAR was suspended in NaCl 0.9% and injected i.v. 60 min before the operation.

4.2.4 Design of Study III

In Study III, four different protocols were performed.

In the first protocol, the effects of dexmedetomidine preconditioning on kidney I/R injury were evaluated, using 6--7-week-old SD rats divided into four groups (n = 8--11 per group): 1. Sham-operated group, 2. I/R group (40 min bilateral ischaemia followed by 24 h of reperfusion, 3. I/R group + Dex (1 μg/kg i.v. given 60 min before the surgery) and 4. I/R group + Dex (10 μg/kg i.v.). The Dex was given as an i.v. bolus 60 min before the surgery. In additional experiments, the effects of Dex infusions (0.1 μg kg^{-1} min^{-1} or 0.3 μg kg^{-1} min^{-1} started 60 min before surgery and continued until the 30-min reperfusion period) (n = 10 in both groups) on kidney I/R injury were also examined. Blood samples were taken during the follow-up period for pharmacokinetic (PK) analyses.

To explore whether Dex establishes its renoprotective effects through inhibition of necroptosis (a form of programmed necrosis), we also conducted an additional study with the following four groups (n =10 in each group): 1) I/R group, 2) I/R group + Dex (10 μg/kg i.v 60 min before ischaemia), 3) I/R group + the necroptosis inhibitor necrostatin-1 (Nec-1) (1.65 mg/kg i.p. given 15 min before ischaemia) and 4) I/R group + Dex + Nec-1.

In the second protocol, the effects of Dex postconditioning on kidney I/R injury were evaluated. The rats were again divided into four groups (this time n = 5--10 per group): 1. Sham-operated group, 2. I/R group (40-min bilateral ischaemia followed by 24 h of reperfusion, 3. I/R group + Dex (1 μg/kg i.v. given immediately after the 40-min ischaemic period) and 4. I/R group + Dex (10 μg/kg i.v.).

In the third protocol, the effects of pre- and postconditioning with the peripheral α_2-AR agonist fadolmidine on kidney I/R injury were examined, as outlined in protocols 1 and 2. Fado preconditioning was given as an i.v. bolus 60 min before the surgery at a dosage of 1 μg/kg or 10 ug/kg (n = 7--18 per group). Fado postconditioning (n = 5--10 per group) was given as an i.v. bolus (1 and 10 μg/kg) given immediately after the 40-min ischaemic period.

In the fourth protocol, the haemodynamic effects of Dex and Fado, 1 μg/kg and 10 μg/kg preconditioning bolus groups and 0.1 μg kg^{-1} min^{-1} and 0.3 μg kg^{-1} min^{-1} infusion groups (n = 5 or 6 per group) on kidney I/R injury were evaluated.

4.3 Blood pressure and heart rate measurement (III)

The haemodynamic effects (i.e. HR and BP) of Dex and Fado preconditioning on kidney I/R injury were continuously recorded, using an intra-arterial catheter inserted into the carotid artery and a Powerlab system (ADInstruments GmbH, Spechbach, Germany).

4.4 Biochemical determinations (I-III)

Creatinine, urea and electrolytes from serum samples as well as serum lipids and liver enzymes were measured by routine laboratory techniques (ADVIA 1650 Chemistry System, Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA and Konelab 30i, ThermoFisher Scientific, Vantaa, Finland).

For the analysis of protein carbonyls (II), renal samples were analysed with an OxiSelect(TM) Protein Carbonyl enzyme-linked immunosorbent assay (ELISA) Kit (Cell Biolabs, Inc., San Diego, CA, USA), according to the manufacturer`s instructions.

Dex plasma concentrations (III) were determined in a separate pharmacokinetic (PK) study, in which blood samples were taken at 0.25, 0.5, 2 and 3 h from the tail vein of conscious rats. The Dex concentrations were determined by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The estimate for plasma concentration at 1 h was performed, using nonlinear fit in GraphPad Prism software (v6.02).

Drug concentrations after the various Dex infusion rates were measured from plasma samples taken just before the reperfusion phase (100 min after beginning of infusion) (n = 7 in each infusion group and n = 2 or 3 for each time point after the i.v. bolus). For the bolus and infusion PK studies, the lower limits of quantification were 0.0200 and 0.100 ng/ml for Dex.

4.5 Kidney histology (I-III)

For histological examination, 4-µm-thick paraffin sections were cut and stained with haematoxylin-eosin (n = 8--19 per group). The renal samples were visually examined by a pathologist with a Leica DMR microscope (Leica Microsystems AG, Heerbrugg, Switzerland) and morphological changes from the entire cross-sectional area of the cortex and medulla were assessed according to the ATN--scoring system adopted from Dragun *et al.* (2001) (magnification x 200, ≥ 20 fields per kidney section quantified using the ATN-scoring system). Evaluation of histopathological changes included loss of the tubular brush border, tubular dilatation, cast formation and cell lysis. Tissue damage was quantified in a blinded manner and scored according to the percentage of damaged tubules in the sample: 0, no damage; 1, less than 25% damage; 2, 25--50% damage; 3, 50--75% damage; and 4, more than 75% damage.

4.6 Immunohistochemistry (I-III)

For immunohistochemistry, frozen kidneys were processed, processed with primary and secondary antibodies and semiquantitative scoring of inflammatory cells and kidney nitrotyrosine expression was performed (n = 8--19), as described in detail elsewhere (Helkamaa *et al.*, 2003). The relative amount of antibody-positive signalling in the cortical and medullary areas per sample was determined with computerized densitometry (Leica IM500 and Leica QWIN software, Leica Microsystems). See the original publications for details.

4.7 Western blotting (I-III)

Proteins from kidney samples were isolated with lysis buffer and complete protease inhibitors (Roche Diagnostics, Neuilly-Sur-Seine, France). Tissue samples were homogenized, using a Bertin Precellys 24 homogenizer (Bertin Technologies, Aix en Provence, France). Nuclear proteins for assessment of SIRT1 were extracted with Microcon´s YM-10 filters and Centrifugal Filter Units (Millipore, Bedford, MA, USA), according to the instructions of the manufacturer.

The protocol was first described by Schreiber *et al*., (1989). The samples were electrophoretically separated and proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Immobilon-P®, Millipore). The membranes were probed with the primary and secondary antibodies. See the original publications for the details of the protocol. The relative protein expressions in separate samples from the membranes were quantified with a fluorescent image analyser (FUJIFILM Corp, Tokyo, Japan).

For the detection of AMPK, AMPK-P, eNOS, eNOS-P and PGC-1α, the PVDF membrane was blocked in Odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE, USA) and IRDye 680LT goat antirabbit (1/7500, LI-COR Biosciences) and IRDye 800CW goat antimouse (1/10 000, LI-COR Biosciences) secondary antibodies were used. The protein expressions were detected by direct infrared fluorescence on the Odyssey CLx infrared imaging system (LI-COR Ltd, Cambridge, UK), according to the instructions of the manufacturer.

4.8 Quantitative RT-PCR (I-II)

Quantitative (real-time) reverse-transcription polymerase chain reaction (qRT-PCR) was performed, using the LightCycler instrument (Roche Diagnostics) for detection of monocyte chemotactic protein-1 (MCP-1), NADPH oxidase 1 (Nox1), mitochondrial superoxide dismutase (MnSOD), catalase and ribosomal 18S mRNA, as described elsewhere (Louhelainen *et al*., 2007). The following primers were used: MCP-1 sequence (5´-3´) forward GCAGGTCTCTGTCAGGCTTCT, reverse GGCTGAGACAGCACGTGGAT; Nox1-2 forward GGAGTTGCAGGAGTCCTCATTTT, reverse TTCTGCCGGGAGCGATAA; MnSOD forward TTAACGCGCAGATCATGCA, reverse CCTCGGTGACGTTCAGATTGT; catalase forward TCAGCGACCGAGGGAT, reverse GGGCTGGGCTCAATGC; and 18s forward ACATCCAAGGAAGGCAGCAG, reverse TTTTCGTCACTACCTCCCCG. See the original publications for the details of the protocol.

4.9 Microarray (III)

Kidney samples from the I/R injury group with and without Dex preconditioning (n = 3 in both groups) were preserved in liquid nitrogen. Total RNA was isolated with TRIzol Reagent (Gibco, Invitrogen) and purified with the RNeasy mini kit (Qiagen, Valencia, CA, USA), according to the manufacturers' instructions. The concentration and integrity of total RNA were analysed with a spectrophotometer and 2100 Bionanalyser (Agilent Technologies, Santa Clara, CA, USA). Further sample processing and hybridization to Sureprint G3 Rat GE 8x60K microrrays (Agilent Technologies), representing over 30 000 rat transcripts, was performed by the Biomedicum Functional Genomics Unit (www.helsinki.fi/fugu/). The data were normalized to the median with the Robust Multi-Array Analysis (RMA) reprocessing algorithm and analysed with Genespring 11 software (Agilent Technologies). Differentially expressed probe sets were selected, based on filtering by parametric statistical analysis, not assuming equal variances (Welch-type t test) with $P < 0.05$ as a threshold for significance, followed by filtering for fold change (±1.2-fold) between the groups compared. The functional annotation describing the categorical data for gene ontology and pathway information for the lists of genes were performed with DAVID (Database for Annotation, Visualization and Integrated Discovery, 2008) (Dennis *et al.*, 2003). The data were also analysed through the use of Ingenuity Pathway Analysis (IPA) (Ingenuity® Systems, www.ingenuity.com).

4.10 Statistical analysis (I-III)

The data are presented as the mean ± standard error of the mean (SEM). Statistically significant differences in mean values were tested by analysis of variance (ANOVA) and the Bonferroni's post-hoc test, or by the Student's T-test, when appropriate. The differences were considered significant at $P < 0.05$.

5 Results

5.1 Renal function and morphology (I-III)

5.1.1 Kidney function

Acute kidney I/R injury was associated with a 7.1-8.5-fold increase in S-creatinine concentration and a 7.0-8.5-fold increase in S-urea concentration as compared to sham-operated controls.

A 2-week CR period (70% *ad libitum*) before the I/R injury prevented AKI-induced kidney dysfunction with the maximal 17% decrease in S-creatinine concentration (Figure 1A). CR did not affect the S-urea concentration (Figure 1B).

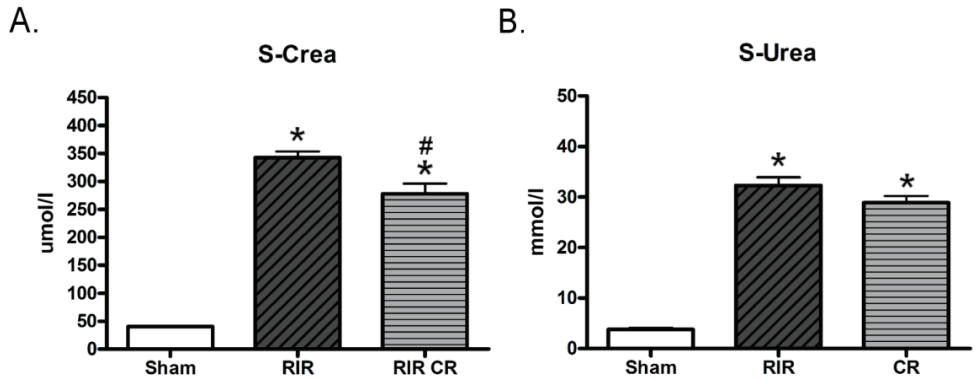

Figure 1. Effects of CR on serum creatinine (Panel A) and serum urea concentrations (Panel B) in rats with renal ischaemia-reperfusion (RIR) injury. Sham denotes sham-operated rats and RIR, rats with renal I/R injury. RIR CR denotes rats with I/R injury treated with CR initiated 2 weeks before the I/R operation. Means ± SEM are given, n = 9 or 10 in each group. *P < 0.05 vs. Sham; #P < 0.05 vs RIR.

AMPK activator AICAR 500 mg/kg i.v. decreased the S-creatinine and S-urea concentrations by 35% and 25%, respectively, whereas the lower AICAR doses did not significantly influence the S-creatinine and S-urea levels (Figure 2).

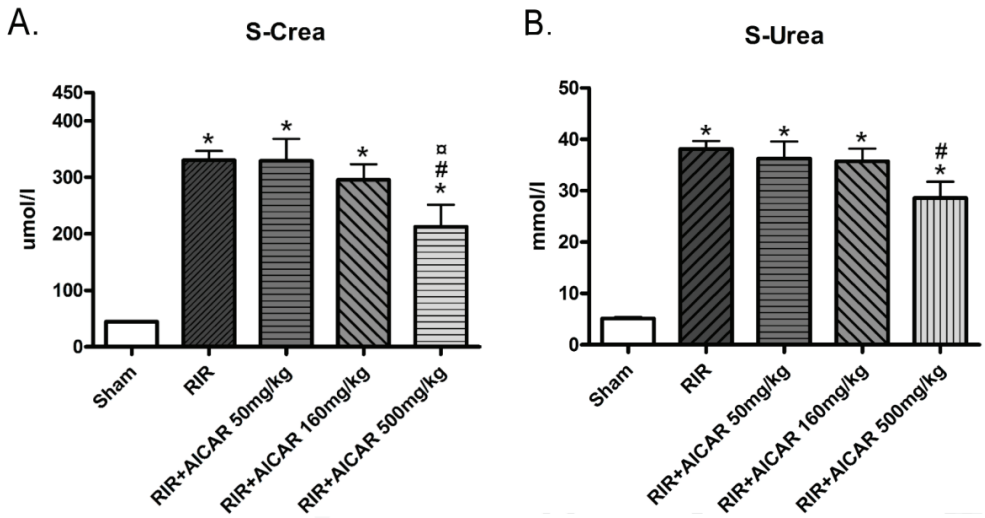

Figure 2. Effects of AICAR on serum creatinine (Panel A) and serum urea concentrations (Panel B) in rats with renal ischaemia-reperfusion injury. RIR+AICAR50 denotes rats with I/R injury treated with AICAR at a dose of 50 mg/kg i.v.; RIR+AICAR160, rats with I/R injury treated with AICAR at 160 mg/kg i.v.; RIR+AICAR500, rats with I/R injury treated with AICAR at 500 mg/kg i.v. AICAR denotes 5-amino-4-imidazolecarboxamide riboside-1-β-D-ribofuranoside. For other abbreviations see Figure legend 1. Means ± SEM are given, n = 8--19 in each group. *P < 0.05 vs. Sham; #P < 0.05 vs RIR; ¤ P < 0.05 vs RIR+AICAR50.

Preconditioning with an α_2-adrenoceptor agonist Dex-10 μg/kg bolus decreased the S-creatinine concentration by 30% and S-urea concentration by 23%, while the smaller dose (1 μg/kg) did not influence kidney function (Figure 3). Continuous Dex infusions (0.1 μg kg^{-1} min^{-1} or 0.3 μg kg^{-1} min^{-1}) did not affect the S-creatinine or S-urea levels.

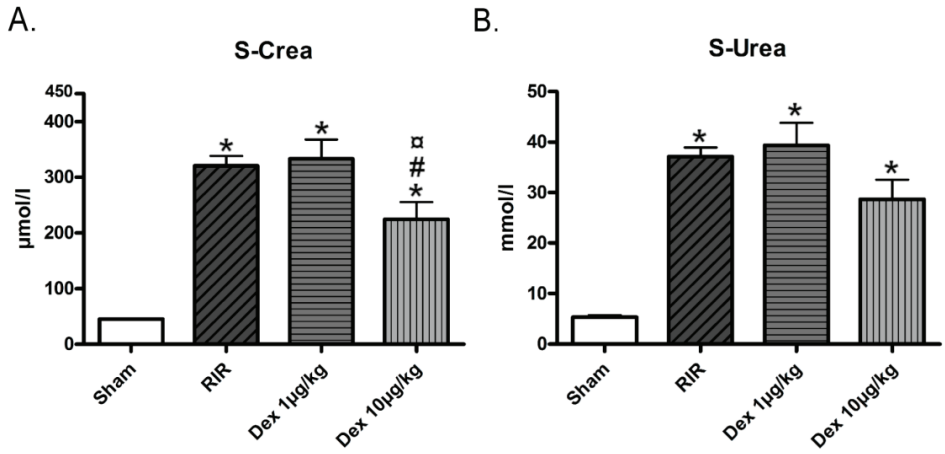

Figure 3. Effects of Dex preconditioning on serum creatinine (Panel A) and serum urea concentrations (Panel B) in rats with renal ischaemia-reperfusion injury. Dex denotes rats with I/R injury treated with dexmedetomidine. For other abbreviations see Figure legend 1. Means ± SEM are given, n = 7--11 in each group. *P < 0.05 vs. Sham; #P < 0.05 vs RIR. ¤P < 0.05 vs Dex 1-μg/kg bolus.

A Dex 1-μg/kg or 10-μg/kg i.v. bolus administrated 40 min after the ischaemic period (postconditioning) did not affect the S-creatinine or S-urea levels.

Fado preconditioning with a 10-μg/kg bolus decreased S-creatinine 19% and S-urea by 17%, compared with the I/R injury group; however, the differences were not statistically significant. A smaller (1-μg/kg) Fado bolus did not affect the S-creatinine or S-urea. A Fado 1-μg/kg or 10-μg/kg bolus administered 40 min after the ischaemic period (postconditioning) did not affect the S-creatinine or S-urea levels.

5.1.2 Kidney morphology

Histopathological analysis of the kidneys harvested 24 h after I/R showed marked injury of the renal parenchyma comprising vast necrosis of the tubuloepithelial cells, tubular dilatation and cast formation. A 2-week CR period, AICAR 500 mg/kg i.v. and Dex preconditioning all ameliorated I/R injury-induced ATN. Dex postconditioning or Fado (preconditioning or postconditioning) did not affect I/R injury-induced ATN. See the original publications.

5.2 Oxidative/nitrosative stress (I-II)

Acute kidney I/R injury was associated with a 2.2-12.5-fold increase in renal nitrotyrosine expression as compared to sham-operated controls. A 2-week CR preconditioning period normalized the amount of nitrotyrosine and also AICAR 160 mg/kg significantly ameliorated renal nitrosative stress (Figure 4). I/R AKI or AICAR treatment did not affect the expression of Nox1 or protein carbonyl levels in the kidneys. The expression of catalase was lower in I/R injury groups, as compared with the sham-operated controls. AICAR did not affect this decrease. See the original publications for details.

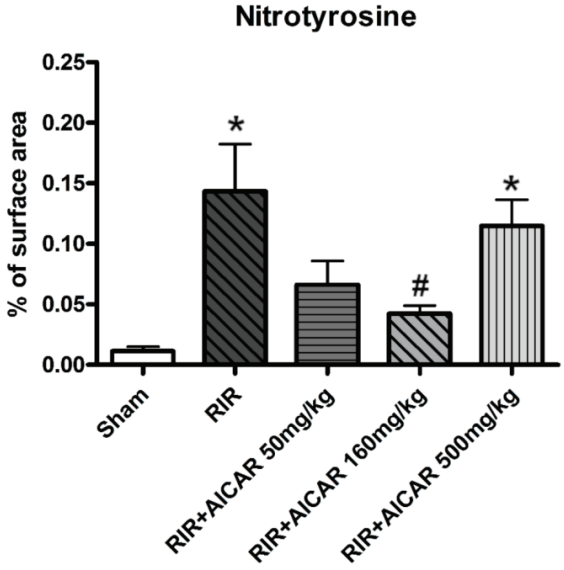

Figure 4. Effects of AICAR on nitrosative stress measured as kidney nitrotyrosine expression in rats with renal ischaemia-reperfusion injury. Means ± SEM are given, n = 8--19 in each group. *P < 0.05 vs. Sham; #P < 0.05 vs RIR. For abbreviations see Figure legend 1.

5.3 Inflammation (I-III)

5.3.1 Inflammatory mediators

I/R AKI was associated with a 4.0-23-fold increase in the number of ED1-positive inflammatory cells in the kidney. Pre-conditioning with Dex 10 μg/kg ameliorated the I/R injury-induced monocyte/macrophage infiltration (Figure 5). A 2-week CR period did not affect the ED1 cell count. In Study II the number of renal ED1-positive cells in the sham-operated group and in the groups with the two highest AICAR doses was statistically at the same level. See the original publications.

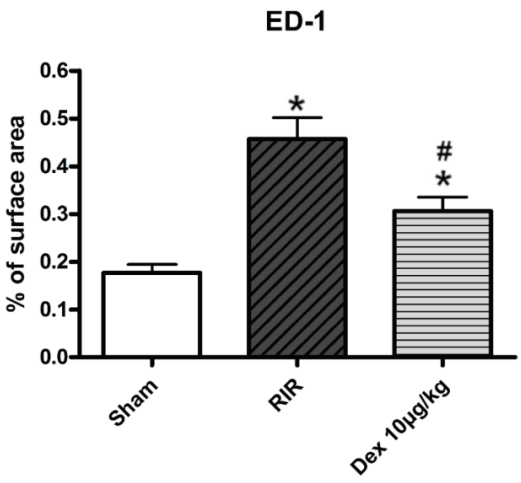

Figure 5. Effects of Dex 10 µg/kg preconditioning on monocyte/macrophage infiltration in rats with renal ischaemia-reperfusion injury. Means ± SEM are given, n = 10 or 11 in each group. *P < 0.05 vs. Sham; #P < 0.05 vs RIR. For abbreviations see Figure legend 1.

5.3.2 Inflammatory pathways and chemokines

I/R AKI was associated with a 1.1-1.6-fold increase in expression of TLR-4 in rat kidneys. CR and Dex showed no notable effects on TLR-4 expression.

In Study I, AKI was associated with a 59-fold increase in MCP-1 [also known as Chemokine (C-C motif) ligand 2, (CCL2)] mRNA levels ($p < 0.05$). CR partially counteracted this increase, so that in the CR group kidney MCP-1 mRNA expression showed only a 9.3-fold increase, compared with the sham group (Figure 6A). Pharmacological inhibition of autophagy by 3-MA showed a statistically insignificant increase in MCP-1 levels in the RIR CR group, whereas the SIRT1 inhibitor sirtinol showed no effect (Figure 6B).

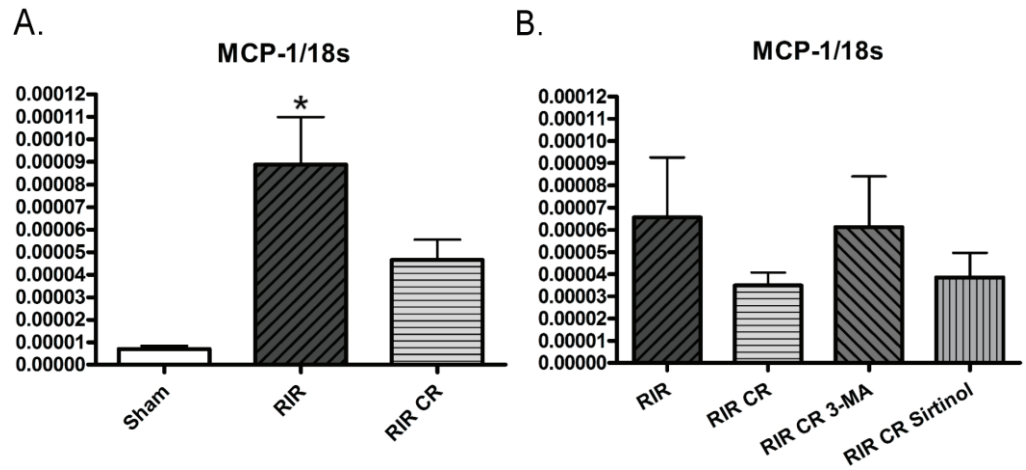

Figure 6. Effects of CR on kidney MCP-1 mRNA expression in rats with renal ischaemia- reperfusion injury. The mRNA levels measured by qRT-PCR are given in Panels A and B. 3-MA denotes 3-methyladenine. For other abbreviations see Figure legend 1. Means ± SEM are given, n = 6--10 in each group. *P < 0.05 vs. Sham.

5.4 Pathways of metabolism and survival (I-III)

5.4.1 SIRT1

I/R AKI was associated with a 2.6-26 -fold increase in kidney SIRT1 expression, compared with the sham-operated controls. CR furthermore increased the I/R-induced SIRT1 expression by

4.6-fold, compared with the sham group ($p < 0.001$). The I/R injury-induced increase in kidney SIRT1 expression was prevented with AICAR treatment in a dose-dependent manner. Dex did not significantly affect kidney SIRT1 expression. See the original publications.

5.4.2 AMPK

Kidneys harvested 24 hours after I/R injury showed a 40-50% decrease in the ratio of phosphorylated/total AMPK expression as compared to sham-operated rats. The total expression of AMPK in the kidney remained unaltered in Studies I and II and was increased increased 150% in Study III. CR did not noticeably affect the AMPK expression or AMPK-P/AMPK ratio after I/R, whereas AMPK activator AICAR 500 mg/kg significantly increased the phosphorylation level of AMPK in the kidneys. Dex did not significantly affect the AMPK. See the original publications.

5.4.3 eNOS

In Study I acute kidney I/R injury was associated with a 77% decrease, and in Study II a 19% decrease, in kidney eNOS expression as compared to sham-operated controls. CR partially, statistically non-significantly, increased kidney eNOS expression (1.9-fold increase in eNOS expression compared to I/R injury group), whereas kidney eNOS expression in CR group did not differ from that of sham-operated controls ($p=0.61$). AICAR 160mg/kg further decreased expression of eNOS as compared to I//R injury group ($p<0.05$). In Study III acute kidney I/R injury was associated with a 1.9-fold increase in total expression of eNOS in rats kidneys. I/R injury did not have any effect on eNOS-P expression level (study III). Dex treatment was associated to a 1.8-fold increase in the expression of eNOS-P as compared to sham group ($p<0.05$), but didn´t change amount of total eNOS. See the original publications.

5.4.4 PGC-1α

In Study I, I/R induced AKI decreased expression of PGC-1α was by 44%, compared the sham-operated control group. CR counteracted this decrease by increasing PGC-1α expression by 45%, compared with the I/R AKI group. Expression of kidney PGC-1α ($p = 0.68$) in the CR group did not differ from that in the sham group. See the original publications.

5.4.5 p38 MAPK

In study III, kidney I/R injury increased the p38 MAPK expression by 1.6-fold and the phosphorylated form of p38 MAPK by 1.4-fold, compared with the sham-operated animals. Dex further increased the expression of both forms of p38 MAPK. See the original publications.

5.4.6 Autophagy

I/R AKI decreased the autophagy marker light chain 3B (LC-3B) 14 kDa/16 kDa ratio by only 0.4-fold in the kidneys, compared with the sham group. CR prevented this decrease in the ratio, indicating that it boosted autophagy (Figure 7A). The same effect was also seen with the active 14-kDa form alone. AKI decreased the expression level of the LC-3B 14-kDa form by only 0.3-fold, compared with the sham-operated animals and CR normalized it (Figure 7B).

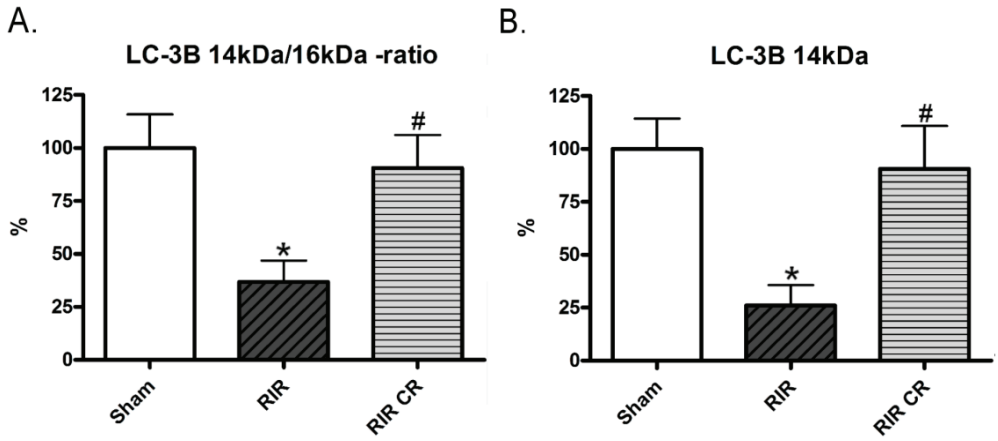

Figure 7. Effects of CR on kidney LC-3B 14 kDa/16 kDa ratio (Panel A) and LC-3B 14 kDa expression (Panel B) in rats with renal ischaemia-reperfusion injury. The LC-3B 14 kDa/16 kDa ratio and LC-3B 14-kDa expression were measured by Western blotting (20 µg total protein of the whole-cell lysate per lane). Means ± SEM are given, n = 6 or 7 in each group. *P < 0.05 vs. Sham; #P < 0.05 vs RIR. For abbreviations see Figure legend 1.

Also Dex 10µg/kg i.v. preconditioning bolus attenuated the kidney I/R injury-induced decrease in the LC-3B 14 kDa/16 kDa ratio, as well as in the LC-3B 14-kDa expression level. See the original publications for more details.

5.4.7 I/R and dexmedetomidine –induced changes in gene expression

Dex treatment altered the expression of 306 genes, compared with the RIR group; 245 of these genes were downregulated with Dex treatment and 61 genes were upregulated. There were 17 enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways among all the regulated genes, of which ECM receptor interaction, hypertrophic cardiomyopathy (HCM), TGF-β signalling, dilated cardiomyopathy, focal adhesion, regulation of the actin cytoskeleton, chemokine signalling and pathways in cancer development were most significantly enriched (Table 1).

Ingenuity analysis revealed inhibition of RAC and nuclear factor erythroid 2-related factor (NRF2) pathways, whereas the 'aryl hydrocarbon receptor' (AHR) pathway was activated. Dex decreased the expression of Rab27b, Rasa2 and cell division control protein 42 homologue (CDC42)-binding protein kinase alpha isoform B, which are all associated with RAC signalling. Expression of two RAB GTPase (also members of the RAS superfamily)-associated genes, RAB GTPase-activating protein 1 (Rabgap1) and RAB GTPase-binding effector protein 1 (Rabep1), also decreased with Dex treatment. Moreover, Ingenuity analysis also revealed that Dex treatment decreased the expression of inflammatory marker CD44 mRNA levels.

Table 1. The enriched KEGG pathways among all regulated genes.

KEGG pathway	Number of genes	P-Value	
			down-regulated genes up-regulated genes
Hypertrophic cardiomyopathy (HCM)	5	9.0E-3	Igf1 Golga4 Itgb1 Itga2 Itgb6
ECM-receptor interaction	5	8.0E-3	Cd44 Golga4 Itgb1 Itga2 Itgb6
TGF-beta signaling pathway	5	9.8E-3	Bmpr1b Bmpr2 Dcn Cul1 Inhbb
Dilated cardiomyopathy	5	1.1E-2	Igf1 Golga4 Itgb1 Itga2 Itgb6
Focal adhesion	7	1.2E-2	Igf1 Golga4 Itgb1 Itga2 Itgb6 Pgf Pik3ca
Regulation of actin cytoskeleton	7	1.6E-2	Wasf1 Golga4 Itgam Itgb1 Itga2 Itgb6 Pik3ca
Chemokine signaling pathway	6	2.6E-2	Fgr Ccl12 Ccl2 Ccl3 Ccl7 Pik3ca
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	4	3.6E-2	Golga4 Itgb1 Itga2 Itgb6
Aminoacyl-tRNA biosynthesis	3	5.8E-2	Nars Rars Eprs
Arginine and proline metabolism	3	9.5E-2	Aldh1a7 Glul Gamt
mTOR signaling pathway	3	9.5E-2	Igf1 Pgf Pik3ca
Leukocyte transendothelial migration	4	1.0E-1	Txk Itgam Itgb1 Pik3ca
Spliceosome	4	1.2E-1	Ddx46 Thoc1 Plrg1 Snrpf
NOD-like receptor signaling pathway	3	1.2E-1	Ccl12 Ccl2 Ccl7
Hematopoietic cell lineage	3	1.8E-1	Cd44 Itgam Itga2
Small cell lung cancer	3	2.0E-1	Itgb1 Itga2 Pik3ca
Pathways in cancer	6	2.0E-1	Igf1 Itgb1 Itga2 Msh2 Pgf Pik3ca

5.5 Haemodynamic and pharmacokinetic measurements (III)

5.5.1 Haemodynamic effects

The mean arterial pressure (MAP) baseline before administration of dexmedetomidine or fadolmidine averaged 107.1 ± 11.3 mmHg and heart rates 405 ± 35 beats per minute (BPM). There were no differences between the groups (ANOVA $P = 0.09$ and $P = 0.32$, respectively).

Dex decreased MAP more than did Fado (Figure 8). The percentage change in MAP was dose-dependent with both drugs in the bolus groups (Figure 8A). Dex infusions (0.1 μg kg^{-1} min^{-1} and 0.3 μg kg^{-1} min^{-1}) decreased MAP to a similar extent, compared with the higher Dex bolus, whereas neither of the Fado infusions influenced MAP (Figure 8B). The percentage change in MAP 0--50 min after administration of the drugs with the Dex 10-μg/kg bolus differed from that in all other bolus groups (Figure 8A).

The higher bolus dose of Fado also had a slight hypotensive effect (Figure 8A). The largest percentage change in MAP with the bolus groups was caused by the Dex 10-μg/kg bolus 8 min after administration of the drugs and with the infusions in the Dex 0.3- μg kg^{-1} min^{-1} group after 18 min.

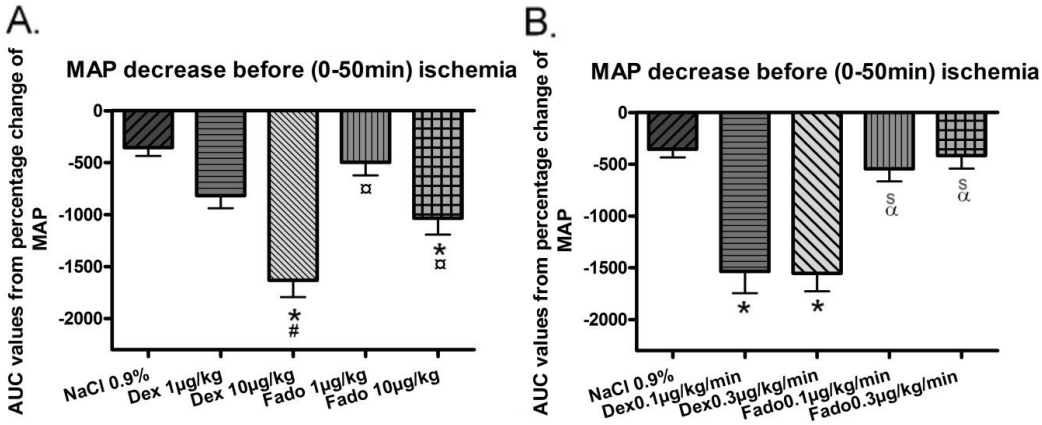

Figure 8. Effects of Dex and Fado on MAP before the ischaemic period. Percentage changes of MAP from the baseline, represented by the AUC values, during 0--50-min time period after administration of the drugs for the bolus groups, are given in Panel A and for the infusion groups in Panel B. n = 5 or 6 in each group. *P < 0.05 vs. NaCl 0.9%; #P < 0.05 vs Dex 1-μg/kg bolus; ¤P < 0.05 vs Dex 10-μg/kg bolus; SP < 0.05 vs Dex 0.1- μg kg^{-1} min^{-1} infusion; α P < 0.05 vs Dex 0.3- μg kg^{-1} min^{-1} infusion. AUC = area under the curve, Dex = dexmedetomidine, MAP = mean arterial blood pressure, min = minutes, NaCl 0.9% = sodium chloride 0.9%. For other abbreviations see Figure legend 1.

5.5.2 Dexmedetomidine plasma concentrations

The plasma Dex concentrations 15 min, 30 min, 120 min and 180 min after the 10-μg/kg bolus administration were 2.8 ng/ml, 0.80 ng/ml, 0.28 ng/ml and 0.16 ng/ml, respectively. Calculated from the measured time points, the plasma Dex concentration at time point 60 min (corresponding to the time point when the ischaemic period was initiated) and time point 40 min (corresponding to the time point when reperfusion was initiated) were 0.38 ng/ml and 0.33 ng/ml, respectively. The plasma Dex concentrations at time point 90 min after continuous

infusion with infusion rates of 0.1 μg kg^{-1} min^{-1} and 0.3 μg kg^{-1} min^{-1} were 0.58 ng/ml and 1.3 ng/ml, respectively.

5.6 Serum biochemistry (I-III)

Acute kidney I/R injury was associated with 55-98%, 26%, and 340-530% increases in the serum concentrations of alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase, respectively, as compared to sham-operated controls, indicating AKI-induced hepatic injury (Tables 2-4). Acute kidney injury also markedly increased the serum levels of SGGT (serum gamma-glutamyl transferase, Studies I-III) and SGLDH (serum glutamate dehydrogenase, Study II). Two weeks pretreatment with CR before the I/R injury decreased serum alanine aminotransferase, aspartate aminotransferase and SGTT levels (Table 2). The highest dose of AICAR decreased serum aspartate aminotransferase level but did not restore other markers of I/R-injury-induced hepatic damage/dysfunction (Table 3). Also pretreatment with dex before the I/R injury decreased serum alanine aminotransferase, aspartate aminotransferase and SGTT levels (Table 4).

AICAR treatment alone without I/R injury increased the serum concentrations of ALAT by1.3-fold and ASAT by 1.4-fold, compared with the sham-operated controls. Other biochemical markers studied were not markedly influenced by AICAR treatment alone (See study II Supporting Information Table S1).

Statistically significant results are shown in tables 2-4. See the original publications for other biochemical markers measured.

Table 2. Effects of two weeks CR on serum biochemistry in rats with kidney I/R injury. Sham denotes sham-operated rats; RIR, rats with renal I/R injury; CR, rats with I/R injury treated prior with two weeks caloric restriction (70% ad libitum). Means ± SEM are given, n= 9-10 in each group. **P*<0.05 vs Sham; #*P*<0.05 vs RIR.

Variable	Sham n=10	RIR n=9	RIR+CR n=9	ANOVA p-value
s-ALAT (U/l)	45,8±3,3	71,0±5,9*	63,3±13,5#	0,0004
s-ASAT (U/l)	106±6,1	667±41,6*	612±195,8*#	<0.0001
s-GGT (U/l)	0,0±0,0	27,0±3,9*	16,93±5,2*#	<0.0001
s-K (mmol/l)	4,3±0,1	6,4±0,4*	6,9±0,5*	<0.0001
s-Pi (mmol/l)	3,1±0,1	4,3±0,2*	3,4±0,2#	<0.0001
s-Trigly (mmol/l)	0,8±0,1	1,3±0,1*	0,83±0,1#	0,0007
s-Gluc (mmol/l)	7,1±0,3	6,1±0,2	7,6±0,5#	0,0029

Table 3. Effects of AICAR treatment on serum biochemistry in rats with kidney I/R injury. Sham denotes sham-operated rats; RIR, rats with renal I/R injury; RIR+AICAR50, rats with I/R injury treated with AICAR at the dose 50 mg/kg i.v.; RIR+AICAR160, rats with I/R injury treated with AICAR at the dose 160 mg/kg i.v.; RIR+AICAR500, rats with I/R injury treated with AICAR at the dose 500 mg/kg i.v. Means ± SEM are given, n= 8-19 in each group. **P*<0.05 vs Sham; #*P*<0.05 vs RIR.

Variable	Sham n=17	RIR n=19	RIR+ AICAR50 n=8	RIR+ AICAR160 n=9	RIR+ AICAR500 n=8	ANOVA p-value
s-ALAT (U/l)	39.3±2.8	77.7±4.2*	67±7.1*	59±4.0*	62±6.2*	0.2413
s-ASAT (U/l)	120.4±5.9	612.5±42.1*	648±96.3*	505±52.1*	374±49.2*#	<0.0001
s-GGT (U/l)	0.0±0	27.3±2.6*	26.1±6.0*	19.2±2.8*	13.2±2.5*#	<0.0001
s-GLDH (U/l)	10.0±0.8	198.8±19.8*	195±28.0*	201±24.4*	129±31.7*	<0.0001
s-K (mmol/l)	4.7±0.1	6.9±0.3*	6.4±0.3*	6.1±0.2*	5.6±0.3#	<0.0001
s-Pi (mmol/l)	2.9±0.1	4.7±0.3*	4.3±0.3*	4.2±0.3*	3.7±0.3	<0.0001

Table 4. Effects of dexmedetomidine treatment on serum biochemistry in rats with kidney I/R injury. Sham denotes sham-operated rats; RIR, rats with renal I/R injury; dex, rats with I/R injury treated with dexmedetomidine at dose 10ug/kg i.v.. Means ± SEM are given, n= 6-11 in each group. **P*<0.05 vs Sham; #*P*<0.05 vs RIR.

Variable	Sham n=7-10	RIR n=8-11	RIR+dex n=6-10	ANOVA p-value
s-ALAT (U/l)	46,2±3,0	85,2±5,0*	56,6±3,2#	<0.0001
s-ASAT (U/l)	129±7,2	571±44,4*	316,1±39,5*#	<0.0001
s-GGT (U/l)	<1,0	29,3±3,6*	16,1±3,6*#	<0.0001
s-K (mmol/l)	4,6±0,1	6,6±0,3*	5,0±0,1#	<0.0001
s-Cl (mmol/l)	97,3±0,9	89,0±1,8*	98,2±1,6#	0,0005
s-Pi (mmol/l)	2,9±0,1	4,9±0,3*	3,6±0,3#	<0.0001

5.7 Summary of the main findings (I-III)

A 2-week CR, AICAR and pretreatment with Dex all ameliorated kidney function and attenuated acute tubular necrosis after I/R induced AKI. Inflammation and oxidative stress in kidneys were also diminished. Treatment with AICAR increased amount of phosphorylated AMPK in kidneys. CR increased kidney SIRT1 expression and maintained also kidney autophagy levels. Also Dex preconditioning maintaned levels of autophagy and increased expression of p38 MAPK in kidneys. CR, AICAR and Dex all attenuated kidney I/R injury- associated hepatic injury. See Table 5 for more details.

Table 5. Effects of CR, AICAR and Dex treatments on kidneys in rats with kidney I/R injury. CR denotes rats with I/R injury treated before injury with 2-week caloric restriction; AICAR, rats treated with AICAR; Dex, rats treated with dexmedetomidine; ATN, acute tubular necrosis; NA (not available), parameter was not examined in the particular study; ↑ = increased; ↓ = decreased; ↑/↓ = no change, compared with the I/R injury group. The degree of change is based on the author´s interpretation.

Variable	CR	AICAR	Dex
Kidney function	↑	↑	↑
ATN	↓	↓	↓
Inflammation	↓	↓↓	↓↓
Oxidative stress	↓	↓	NA
Phosphorylation ratio of AMPK	↑/↓	↑	↑/↓
SIRT1	↑	↓	↑/↓
eNOS	↑	↓	↑/↓
eNOS-P	NA	NA	↑
PGC-1a	↑	NA	NA
Autophagy	↑	NA	↑
p38-P	NA	NA	↑
Kidney I/R injury- associated hepatic injury	↓↓	↓	↓↓

6 Discussion

I/R-induced AKI is a common problem among hospitalized patients, mortality rates are high and currently there is no specific medication in use (Waikar et al., 2008). The pathophysiology of I/R-induced AKI is complex and still incompletely understood (Bonventre and Yang, 2011). This thesis project was undertaken to find new ways to alleviate AKI and shed light on its pathophysiology.

6.1 Methodological aspects

6.1.1 Experimental animal model

An established murine (rat) model, which was developed to study I/R-induced AKI and has many similarities to human I/R-induced AKI, was used in all experiments (Kennedy and Erlich, 2008). In this animal model, the surgical operations can be performed relatively quickly and the duration of ischaemia and collection of samples can also be performed at exact time points. The model is widely used and there are already abundant background data available (e.g. changes seen in clinical chemistry and kidney histology) from it. In contrast to mice, rats are larger and thus the surgical procedure is easier. With rats, there are also many well-established strains available.

There are some structural, haemodynamic and molecular differences among rodent, larger animal and human kidneys (Kennedy and Erlich, 2008). In our RIR model, bilateral renal ischaemia was induced by clamping the renal arteries for 40 min with microvascular clamps. Pig kidneys may be more like human kidneys than those of mice, rats and dogs. However, practical reasons such as availability, ethical issues and the fact that pigs are not as amenable to genetic modification as rodents limit the use of this model.

It should also be noted that humans rarely experience the isolated ischaemic events studied here, and very often they also have other clinical conditions, such as hypertension, diabetes, congestive heart failure or drug abuse, which also damage the kidneys. This also warrants further studies with 'unhealthy' animals, e.g. diabetic Goto-Kakizaki (GK) and hypertensive double-transgenic (dTGR) rats.

The prognosis after 40 min of total ischaemia and a slight increase in the S-creatinine value (seen with many hospitalized patients) varies greatly. In ICUs, over 50% of patients undergoing renal replacement therapy die (Waikar *et al.*, 2008). In mice after unilateral nephrectomy and unilateral 37-min ischaemia, the mortality rates exceeded 90% in 4 days (Mitchell *et al.* 2010). Milder damages are more challenging to study and knowledge of the long-term outcome in animal models is scarce. However, it is also known that slight damages, especially if repeated, may eventually lead to CKD in both rodents (Kennedy and Erlich, 2008) and humans (Waikar *et al.*, 2008).

6.1.2 Kidney function and histology

S-creatinine and S-urea levels are the most widely used clinical markers of kidney function. Although insulin clearance and some other methods may be more accurate in estimating the GFR, they are to date not as easily available (Frank *et al.*, 2012); thus, S-creatinine and S-urea levels were also used as primary markers of kidney function in our studies.

Kidney histology was examined with a microscope and morphological changes were assessed with a widely used ATN--scoring system adopted from Dragun *et al.* (2001). However,

it should be noted, that tubular necrosis in murine models is often more marked than is usually seen in human biopsy specimens (Kennedy and Erlich, 2008).

6.2 Renoprotective effects of caloric restriction

CR is known to prolong the lifespan (Fontana *et al.*, 2010) and has health-promoting effects in many chronic diseases (Colman *et al.*, 2009; Fontana *et al.*, 2010). It may also protect against more acute ischaemic injuries (Yu and Mattson 1999; Chandrasekar *et al.*, 2001; Ahmet *et al.*, 2005). Study I with CR was conducted to determine whether it protects against I/R-induced AKI and how it establishes its possible renoprotective effects at the cellular level. New potential targets for drug development were also of interest.

We found out that in an animal model, CR ameliorated I/R-induced AKI-related kidney dysfunction, ATN and nitrosative stress. It also induced SIRT1 expression and enhanced autophagy, as well as counteracting I/R-induced decreases in eNOS and PGC-1α expression levels in kidneys. We showed that I/R-induced AKI is associated with decreased expression of LC-3B, an important marker of autophagy, and CR counteracted this decrease. On the other hand, the autophagy inhibitor 3-MA counteracted the renoprotective effects of CR, which further suggests a crucial role for autophagy induction as a mechanism explaining the renoprotective effects of CR at the cellular level. It is known that induction of autophagy eliminates damaged organelles, e.g. dysfunctional mitochondria, which may otherwise increase the amount of hazardous waste products, such as ROS in the cell (Mizushima *et al.*, 2008). The CR-induced attenuation of nitrosative stress seen in our study could thus be explained through enhanced autophagy and decreased amounts of dysfunctional mitochondria in the kidneys. Jiang *et al.* (2012) recently showed that autophagy protects tubular cells in AKI.

It is worthwhile noting that in Study I, the SIRT1 inhibitor sirtinol did not influence renal function in CR rats with I/R injury. However, sirtinol could have failed to inhibit SIRT1 properly, because SIRT1 is located mainly in the nuclear compartment. A recent study by Fan *et al.* (2013) strongly supports a protective role for SIRT1 in I/R-induced AKI, and previous studies with CR have shown that in many tissues SIRT1 is activated by CR and that this activation may also be beneficial in the context of kidney diseases (Hallows *et al.*, 2010). Furthermore, the SIRT1 activator RSV attenuates I/R-induced AKI in rats (Bertelli *et al.*, 2002; Chander and Chopra, 2006), and activation of SIRT1 protects the mouse renal medulla from oxidative stress (He *et al.*, 2010). Taken together, these notions support the idea that increased expression of SIRT1 has protective effects in the kidneys.

SIRT1 is located mainly in the nuclear compartment, but it also activates cytoplasmic enzymes, such as eNOS (Canto and Auwerx, 2012), which was upregulated by CR in our study, compared with the I/R group. CR is also known to activate eNOS (Nisoli *et al.*, 2005). On the other hand, during CR eNOS induces SIRT1 gene expression, which forms an interesting positive regulatory loop (Nisoli *et al.*, 2005; Canto and Auwerx, 2012). In our study, the further increase in SIRT1 expression by CR after kidney I/R injury could also have been mediated by NO produced by eNOS. Increased levels of NO have protective effects against I/R-induced AKI (Liu *et al.*, 2007; Chen *et al.*, 2008; Milsom *et al.*, 2010), and also ischaemic preconditioning (IPC) protects kidneys against ischaemia by increasing NO through eNOS (Tawa *et al.*, 2010; Tsutsui *et al.*2013).

Tsutsui *et al.* (2013) suggested that increased NO suppresses sympathetic nerve activity and reduces NA concentrations in the kidneys during the I/R period. NO also acts as a nitrite reductase under ischaemic conditions (Milsom *et al.*, 2010), and other studies also indicate that

activating eNOS and increasing NO during I/R is beneficial (Liu *et al.*, 2007; Chen *et al.*, 2008). It is thus possible that in our study, CR induced eNOS and increased the amount of NO, which protected the kidneys either through suppressing renal sympathetic nerve activity or reduction of nitrite. NO may also have acted as a vasodilator and thus preserved the blood flow to the postischaemic kidneys.

PGC-1α regulates mitochondrial biogenesis (Shoag and Arany, 2010), and induction of PGC-1α can decrease formation of ROS in the mitochondria (Patten and Arany, 2012). CR-induced eNOS also increases mitochondrial biogenesis (Nisoli *et al.*, 2005). Induction of the PGC-1α and eNOS levels by CR may thus also have decreased the amount of dysfunctional mitochondria and ROS in the kidneys in our study, which may also explain the attenuation of nitrosative stress.

6.3 Renoprotective effects of AICAR

In Study I we showed that I/R injury decreased the AMPK-P/AMPK ratio in the kidneys, compared with the sham-operated animals. CR protected the kidneys, but it did not manifest its effects through AMPK phosphorylation. However, other studies have indicated that activation of AMPK may be beneficial during ischaemia (Bullough *et al.*, 1994; Alkhulaifi and Pugsley, 1995; Galinanes *et al.*, 1995; Mathew *et al.*, 1995; Peralta *et al.*, 2001), which led us to test whether or not we could ameliorate kidney I/R injury through the AMPK activator AICAR.

In Study II preconditioning with AICAR indeed dose-dependently increased the amount of AMPK-P, improved kidney function, ameliorated ATN, nitrosative stress and infiltration of inflammatory cells into the kidneys after I/R-induced AKI. In I/R-induced AKI, the AMP/ATP ratio in the kidneys increases and AMPK is activated within 1 min. The significance of this activation has been unclear (Hallows *et al.*, 2010). We showed that 24 h after I/R injury, AMPK phosphorylation is decreased. We also showed that phosphorylation of AMPK with AICAR attenuates I/R-induced AKI. AICAR was given i.v. 1 h before I/R injury, so it was likely that AMPK was already phosphorylated during the ischaemia, which suggests that activation of AMPK protects kidneys against I/R-induced AKI.

AICAR treatment also prevented the I/R-induced increase in renal SIRT1 expression. Increased SIRT1 expression may be a protective mechanism in I/R-induced AKI, but it is no longer necessary when AMPK is pharmacologically activated and nitrosative stress and accumulation of inflammatory cells in the kidney are diminished.

AICAR also has other mechanisms of activity in addition to activation of AMPK. However, previous studies have shown that AICAR inhibits inflammation (Peairs *et al.*, 2009; Katerelos *et al.*, 2010) and decreases the amount of ROS (Kim *et al.*, 2008) through AMPK activation, which makes it likely that AMPK activation was also the main mechanism of activity in our study.

Lee *et al.* (2009) recently showed that preconditioning with a single dose of AICAR 100 mg/kg i.p. decreases S-creatinine and S-urea levels, as well as I/R-induced ATN, and protects kidneys against subsequent reperfusion injury in SD rats. In their study, Lee *et al.* showed that AICAR dose-dependently phosphorylated AMPK in the kidneys and the renoprotective effects diminished when AMPK was inhibited. These results suggest that AICAR was also the main protective agent in the study by Lin *et al.* (2004), in which combination therapy with AICAR and N-acetyl cysteine attenuated kidney I/R injury and improved kidney transplant function after cold preservation. Together, these studies suggest that AMPK activators are a potential new way to improve kidney function and attenuate ATN associated with I/R-induced AKI.

6.4 Renoprotective effects of α_2-adrenoceptor agonists

Studies I and II showed that activation of the metabolic sensors AMPK and SIRT1, as well as induction of autophagy, seems to be beneficial in the context of I/R-induced AKI. There is also some evidence from clinical studies that α_2-AR agonists may protect kidneys (Kulka *et al.*, 1996; Frumento *et al.*, 2006). However, the mechanism of activity remains unclear, which led us to examine whether we could ameliorate kidney I/R injury by the α_2-AR agonists Dex and/or Fado and whether they establish their possible renoprotective activities through AMPK and/or SIRT1 and/or induction of autophagy.

In Stydy III, Dex (10 μg/kg i.v. 1 h before ischaemia) preconditioning (but not postconditioning) indeed ameliorated kidney I/R injury and inflammatory response. Dex 1 μg/kg also attenuated ATN, indicating that it also provided some renoprotection against I/R injury, although this was not enough to decrease the S-creatinine or S-urea levels. Dex did not establish its effects through AMPK or SIRT1, but it prevented I/R-induced decrease in LC-3B level, indicating that it boosted autophagy. This likely led to increased recycling of dysfunctional cellular organelles. Further studies are warranted to reveal the exact steps of the autophagic response that Dex affects. This would also shed more light on the overall pathogenesis of I/R-induced AKI.

We also determined that the peripheral α_2-AR agonist Fado treatment did not protect the kidneys, which indicates that the renoprotective effects of Dex were mediated (also) through central mechanisms. Nociceptive signalling activates the sympathetic nervous system and it is known that the renal sympathetic nervous system plays a crucial role in the development of I/R-induced AKI (Fujii *et al.*, 2003). Dex inhibits the release of NA in rat kidneys via activation of α_2-ARs and reduces renal sympathetic nerve activity and plasma catecholamines by a central sympathoinhibitory effect (Xu *et al.*, 1998; Taoda *et al.*, 2001). Dex can therefore decrease the release of lactate from the tissues and facilitate the recovery from ischaemic events through these central sympatholytic properties, which may explain why Dex did and Fado did not protect the kidneys in our study (Willigers *et al.* 2003). However, local effects, such as stimulation of kidney α_2B-ARs (Cussac *et al.*, 2002) and α_2-AR-mediated increase of NO in the glomerulus and proximal tubule (Thomson and Vallon, 1995; Nakayama et al., 2006), may contribute to the renoprotective effects of Dex. Although Dex may also have other effects, previous studies in which Dex protected tissues against ischaemia support the idea that it establishes its effects mainly through (central or peripheral) α_2-AR stimulation (Gu *et al.*, 2011b; Ibacache *et al.*, 2012).

In Study III, Dex increased the active form of renal p38 MAPK. It is known that MAPKs can be activated through α_2-ARs and that p38 MAPK protects the rat heart against I/R injury and promotes autophagy (Mocanu *et al.*, 2000; Nakano *et al.*, 2000; Bonventre, 2002; Cussac *et al.*, 2002; Tang *et al.*, 2008). Dex may have thus protected the kidneys through induction of these prosurvival kinase pathways. eNOS is a downstream mediator of p38 MAPK, and expression of eNOS was also induced by Dex. As the study of Thomson and Vallon (1995) also indicates, α_2-AR stimulation increases NO in the kidneys, which could explain why preconditioning with Dex protected the kidneys in our study.

Inhibition of necroptosis (a form of programmed necrosis) seems to be beneficial in the context of RIR injury (Linkermann *et al.*, 2012). We wanted to know whether Dex could also establish its activities through inhibition of necroptosis, so we performed an additional study with the death receptor-interacting protein kinase 1 (RIPK1) inhibitor, Nec-1. Nec-1 improved kidney function slightly, but not significantly, and the renoprotective effects of Dex clearly

exceeded those of Nec-1. Cotreatment with Dex and Nec-1 also protected the kidneys, and Nec-1 slightly (but not significantly) increased the renoprotective effect of Dex. Together, these results clearly show that Dex establishes its activities by means other than inhibition of renal necroptosis.

To further explore how Dex establishes its renoprotective effects, we performed a gene analysis of over 30 000 genes, which revealed that Dex also suppressed several signalling cascades promoting hypertrophic ('hypertrophic cardiomyopathy' pathway) and inflammatory (TGFβ pathway) signalling in the kidneys (Spurgeon *et al*., 2005). Among the most interesting findings was that Dex decreased CD44 mRNA. Cell adhesion and leukocyte infiltration to injured tissue is mediated through the CD44 receptor (Chase *et al*., 2012). Dex decreased CD44 expression, which likely explains the reduction in ED-1-positive cells in the kidneys after I/R injury in our study. This is in line with the previous study by Gu *et al*. (2011b), which also indicates that Dex protects kidneys through suppression of the inflammatory HMBG1-TLR4 pathway. In our study, Dex slightly decreased kidney TLR-4 expression after I/R injury, but the difference was not statistically significant. However, it should be noted that the experimental settings were different in these two studies. We also noted that Dex activated the organoprotective AHR pathway, which consists of a family of transcriptional regulators. These regulators are activated in response to hypoxia and inhibit NF-κB, which in turn also decreases the inflammatory response.

RAC signalling regulates production of ROS and over-expression of RAC worsens I/R injury in heart (Talukder *et al.*, 2013). Interestingly, Shan *et al*. (2010) showed that disruption of RAC signalling protects against I/R injury in the heart. In our study, Dex decreased expression of several genes (*Rab27b, Rasa2* and *CDC42-binding protein kinase alpha isoform B*) associated with RAC signalling, which may have also led to attenuation of kidney injury.

Moreover, many kinase signalling pathways, including p38 MAPK, seem to regulate Nrf2 activation and facilitate its accumulation in the nucleus (Park *et al.*, 2013). Nrf2, in turn, regulates the expression of several antioxidant and cytoprotective genes, and its activation is known to be protective during ischaemic AKI in mice (Liu *et al.*, 2009). Thus, Dex may also establish some of its (antioxidative and anti-inflammatory) protective effects through the α_2B-adrenergic receptors-p38 MAPK-Nrf2 pathway. However, in this study Dex also attenuated I/R injury and decreased the amount of ED-1-positive cells. This is also likely to lead to reduced amounts of oxidative stress/ROS. N-acetylcystein reduces the amount of ROS, which further inhibits Nrf2 activation (Leonard *et al.*, 2006). Thus, the lower amount of ROS likely explains why the Nrf2-signalling route was not activated in Study III.

Dex and Fado both lowered BP slightly when the preconditioning bolus was given, and continuous Dex infusions also caused a significant drop. Fado and Dex have high affinity and full agonist efficacy for all three α_2-AR subtypes and Fado is also a full agonist for α_1-AR, which explains the observed transient rise in BP following the bolus (Lehtimäki *et al*., 2008). However, BP-dependent effects apparently played a minor role in Study III, because Dex infusion caused the highest hypotensive effect, but did not affect the S-creatinine levels. A haemodynamically induced IPC effect also seems unlikely, because the Dex 0.1 μg kg^{-1} min^{-1} infusion caused the largest hypotensive effect, but provided no renoprotection. However, autoregulation of blood flow in the kidneys functions until the MAP level drops below 75–80 mmHg (Sharfuddin and Molitoris, 2011), which suggests that an IPC effect was also possible in our study.

These results suggest that central (and possibly also peripheral) α_2-AR stimulation before, and during, the ischaemic period improves kidney function and attenuates I/R-induced development of ATN. This activity is mediated through preservation of normal levels of

autophagy, activation of the p38 MAPK-CD44 pathway and suppression of the proinflammatory signalling pathways in the kidneys.

6.5 Mechanisms of activity

The results of this thesis project show that I/R-induced AKI can be attenuated through CR, the AMPK activator AICAR and the α_2-AR agonist Dex. The mechanisms of activity seem to differ somewhat, but induction of autophagy, activation of AMPK, induction of SIRT1, PGC-1α and eNOS, as well as diminished inflammatory signalling and reduced nitrosative/oxidative stress apparently play key roles (Diagram 6).

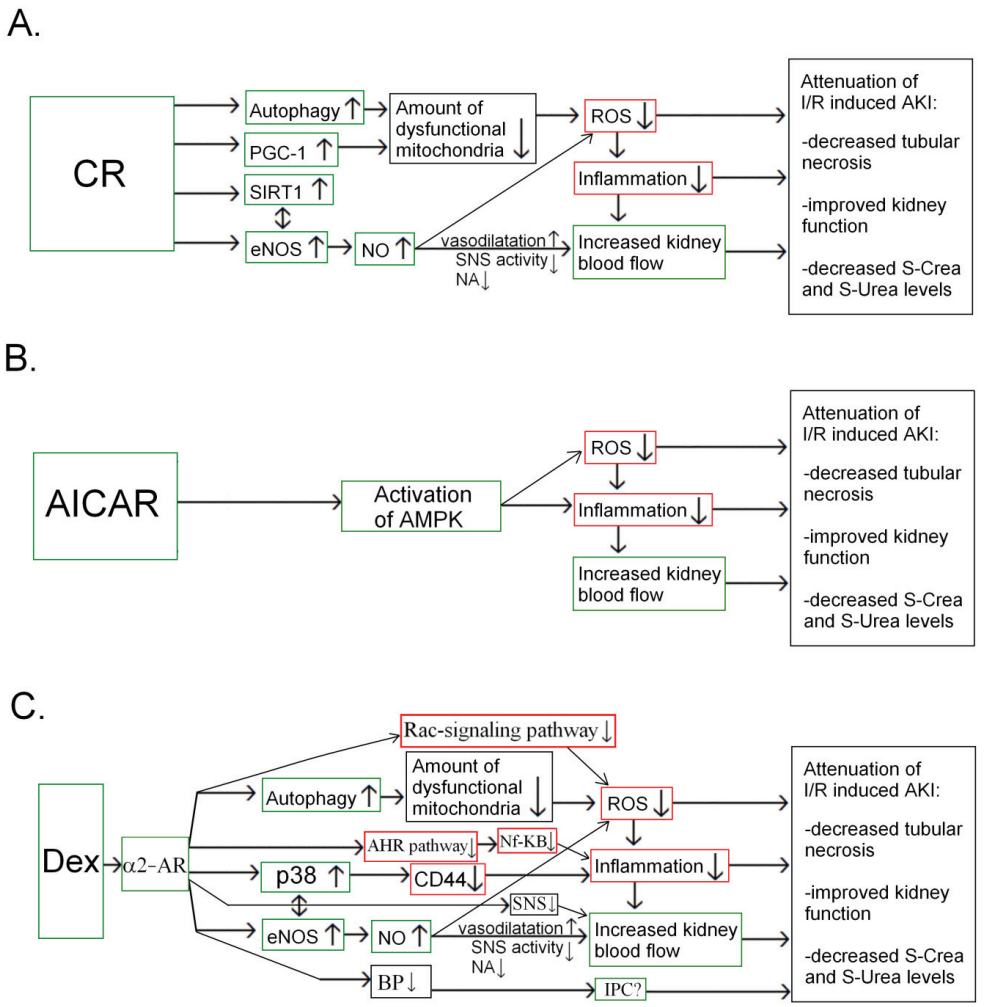

Diagram 6. Mechanisms of activity. The proposed mechanisms of how CR (Panel A), AICAR (Panel B) and Dex (Panel C) attenuate I/R-induced AKI. AHR = aryl hydrocarbon receptor, AICAR = 5-amino-4-imidazolecarboxamide riboside-1-β-D-ribofuranoside, AKI = acute kidney injury, AMPK = adenosine monophosphate kinase, BP = blood pressure, CR = caloric restriction, Dex = dexmedetomidine, IPC = ischaemic preconditioning, I/R = ischaemia-reperfusion, NA = noradrenaline, NF-κB = nuclear factor kappa B, NO = nitric oxide, PGC-1α = peroxisome proliferator-activated receptor gamma coactivator 1α, ROS = reactive oxygen species, SIRT1 = silent information regulator 1, SNS = sympathetic nervous system, α_2-AR = α_2-adrenoceptor.

6.6 Clinical relevance

These studies were undertaken to investigate the molecular and pathophysiological mechanisms of I/R-induced AKI. Since there is currently no specific treatment for I/R-induced AKI, the pathways involved may represent important targets for new drug development finding new ways to alleviate this injury. These studies revealed that drugs mimicking the effects of CR, activating AMPK or acting through central α2-ARs are new potential ways to alleviate I/R-induced AKI and should be investigated more thoroughly.

CR provides protection against acute tissue injury in the kidneys (Mitchell *et al.,* 2010), heart (Chandrasekar *et al*., 2001; Ahmet *et al*., 2005) and brain (Yu and Mattson, 1999) in various animal models. We showed that CR preconditioning ameliorates I/R AKI through enhanced autophagy and counteraction of I/R-induced decreases in the renal expression of eNOS and PGC-1α. The study by van Ginhoven *et al.* (2010) showed that CR is feasible with human transplant donors (van Ginhoven *et al.*, 2010). However, in most clinical cases, CR and/or fasting before ischaemic injury could not be performed. In Finland, kidney transplants come mainly from brain-dead donors (CR preconditioning is no longer possible), while with living patients increased catabolism (related to CR) may lead to degradation of muscle proteins and impair the physical condition of (especially elderly) patients. It should be also noted that in kidney transplantation surgery kidney ischemia model is "cold ischemia", whereas in our I/R model it was warm bilateral ischemia. Taken together, it is unlikely that these results have any direct clinical relevance in transplantation surgery, although further studies are warranted.

However, Study I provides new targets for drug treatments in I/R-induced AKI. Preservation of PGC-1α and eNOS levels with CR suggests that PGC-1α and eNOS activators may also be beneficial in the treatment of I/R-induced AKI. Drugs that directly increase mitochondrial biogenesis, enhance their functioning or capture mitochondrial ROS should also be studied further in the context of AKI. For example, the antioxidant α-lipoic acid seems a very promising agent (Wang *et al*., 2013). Induction of SIRT1, in turn, suggests that sirtuin activators may also be beneficial in the context of AKI. This new class of drugs is currently being studied as a new potential way to treat type 2 diabetes (Pulla *et al*., 2012).

CR, as well as preconditioning with Dex 10 μg/kg i.v., also counteracted I/R-induced decrease in autophagy. This preservation of normal autophagy levels likely helps to clear off hazardous waste products and facilitates the kidneys' ability to recover after I/R-induced AKI. This suggests that inductors of autophagy may also be potential new drugs for the treatment of ischaemic injuries.

Study II also provides a new (in the context of renal ischaemia) potential target for drug development. AICAR phosphorylated AMPK dose-dependently and attenuated I/R-induced AKI. This suggests that activation of AMPK during ischaemia in the kidneys is a protective mechanism, and activators of kidney AMP kinase may thus represent a novel therapeutic approach to the treatment of AKI. Further studies with AMPK activators are warranted.

Dex is already in clinical use as a sedative for ICU patients who require only mild sedation, and some previous clinical studies indicate that it may also enhance renal function (Kulka *et al.*, 1996; Frumento *et al.*, 2006). Our findings that Dex downregulates the inflammatory response and alleviates I/R-induced AKI support the use of Dex as a sedative, especially if the patient is susceptible to AKI. A very recent study by Ji *et al.* (2013), in which the use of Dex was associated with a significant reduction in the incidence of AKI in patients undergoing cardiac surgery, supports this notion. Further clinical studies with α2-AR agonists are needed.

The effect of these treatments on the long-term outcome of AKI should also be studied. It would be beneficial to determine whether Dex treatment of ICU patients reduces the incidence and/or degree of subsequent CKD. The possible adverse effects appearing over a longer time period, as well as with repetitious treatments (e.g. with Dex), should be evaluated.

These studies thus show that pharmacological agents mimicking the effects of CR, AMPK activators and α2-AR agonists may provide new promising ways to alleviate I/R-induced AKI.

7 Conclusions

The aim of the present study was to increase our knowledge of the pathophysiological mechanisms underlying ischaemic AKI and to identify tentative drug targets for AKI. Using a well-established murine model of kidney I/R injury combined with biochemical, histological and immunohistochemical assessments of kidney damage, we evaluated the potential of three different treatment strategies, namely preconditioning with CR, AMPK activation by AICAR, and α_2-AR activation by the α_2-AR agonist Dex, and examined their mechanisms of activity at the cellular level. The main conclusions of this series of experimental studies are as follows:

I Preconditioning of the kidneys by CR for 2 weeks ameliorates I/R injury-induced inflammatory response, oxidative stress and ATN. The renoprotective effects of CR are mediated, at least in part, via induction of SIRT1 expression in the kidney, promotion of autophagy and counteraction of I/R-induced decreases in the expression of eNOS and PGC-1α.

II The AMPK activator AICAR ameliorates I/R injury-induced AKI in a dose-dependent manner when given systemically.

III Preconditioning, but not postconditioning, with the centrally acting α_2-AR agonist Dex ameliorates I/R injury-induced renal dysfunction, inflammatory response and ATN. In contrast, neither pre- nor postconditioning with the peripheral α_2-AR agonist Fado ameliorates kidney I/R injury, suggesting the importance of central α_2-ARs.

These findings suggest that compounds mimicking the cellular effects of CR, activators of AMPK and centrally acting α_2-AR agonists may represent a novel therapeutic approach to treat ischaemic AKI.

8 Acknowledgements

This study was carried during the 2007-2014 period in the Institute of Biomedicine, Pharmacology, University of Helsinki. The thesis was funded by grants from Helsinki University Science Foundation and Emil Aaltonen´s and Ida Montin´s –foundations.

I would like to express my gratitude to my supervisors Professor Eero Mervaala, MD, PhD and Docent Piet Finckenberg. The thesis wouldn´t have been possible without them. Their advices and support were beyond a price. Eero gave also a great example how to finish important projects properly and get valuable things done.

I would also like to express my gratitude to Professor Esa Korpi, the previous Head of the Institute of Biomedicine for the opportunity to work in the Institute of Biomedicine, Pharmacology. I would also like to thank Docent Risto Lapatto MD, PhD for his great advices and supervising in the beginning of this project.

I especially want to thank the official reviewers of this thesis, Docent Petteri Piepponen and Docent Sanna Lehtonen, as well as my opponent Professor Markku Koulu.

My co-authors Jouko Levijoki, Elina Mervaala, Markus Storvik, Satu Sankari, Juha Kaivola and Ken Lindstedt. Our great technicians Anneli von Behr and Nada Bechara-Hirvonen, as well as Päivi Leinikka who performed all the surgical procedures with animals (no one could have done it better). I would also like to thank other members of our cardiovascular group, Eveliina Tauriainen, Lucy Shi, Saara Merasto, Essi Martonen and Erik Vahtola. Thank you all for your co-operation and help with this project and thank you that I have had this chance to know you.

9 REFERENCES

Afonso, J, Reis, F (2012). Dexmedetomidine: current role in anesthesia and intensive care. *Rev Bras Anestesiol* 62: 118-33.

Ahmet, I, Wan, R, Mattson, MP, Lakatta, EG & Talan, M (2005). Cardioprotection by intermittent fasting in rats. *Circulation* 112: 3115-21.

Ahn, BH, Kim, HS, Song, S, Lee, IH, Liu, J, Vassilopoulos, A, Deng, CX, Finkel, T (2008). A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. *Proc Natl Acad Sci U S A* 105: 14447-52.

Akcay, A, Nguyen, Q & Edelstein, CL (2009). Mediators of inflammation in acute kidney injury. *Mediators Inflamm*: 137072.

Alessi, DR, Sakamoto, K & Bayascas, JR (2006). LKB1-dependent signaling pathways. *Annu Rev Biochem* 75: 137-63.

Alkhulaifi, AM, Pugsley, WB (1995). Role of acadesine in clinical myocardial protection. *Br Heart J* 73: 304-5.

Andrades, M, Ritter, C, de Oliveira, MR, Streck, EL, Fonseca Moreira, JC & Dal-Pizzol, F (2009). Antioxidant Treatment Reverses Organ Failure in Rat Model of Sepsis: Role of Antioxidant Enzymes Imbalance, Neutrophil Infiltration, and Oxidative Stress. *J Surg Res*, 10.

Arad, M, Seidman, CE & Seidman, JG (2007). AMP-activated protein kinase in the heart: role during health and disease. *Circ Res* 100: 474-88.

Araujo, M, Welch, WJ (2006). Oxidative stress and nitric oxide in kidney function. *Curr Opin Nephrol Hypertens* 15: 72-7.

Badr, KF, Ichikawa, I (1988). Prerenal failure: a deleterious shift from renal compensation to decompensation. *N Engl J Med* 319: 623-9.

Bagnasco, S, Good, D, Balaban, R & Burg, M (1985). Lactate production in isolated segments of the rat nephron. *Am J Physiol* 248: F522-6.

Balaban, RS, Mandel, LJ (1988). Metabolic substrate utilization by rabbit proximal tubule. An NADH fluorescence study. *Am J Physiol* 254: F407-16.

Bao, J, Lu, Z, Joseph, JJ, Carabenciov, D, Dimond, CC, Pang, L, Samsel, L, McCoy, JP Jr, Leclerc, J, Nquyen, P, Gius, D, Sack, MN (2010). Characterization of the murine SIRT3 mitochondrial localization sequence and comparison of mitochondrial enrichment and deacetylase activity of long and short SIRT3 isoforms. *J Cell Biochem* 110: 238-47.

Basile, DP (2007). The endothelial cell in ischemic acute kidney injury: implications for acute and chronic function. *Kidney Int* 72: 151-6.

Basile, DP, Fredrich, K, Chelladurai, B, Leonard, EC & Parrish, AR (2008). Renal ischemia reperfusion inhibits VEGF expression and induces ADAMTS-1, a novel VEGF inhibitor. *Am J Physiol Renal Physiol* 294: F928-36.

Bell, MT, Puskas, F, Bennett, DT, Herson, PS, Quillinan, N, Fullerton, DA, Reece, TB (2013). Dexmedetomidine, an alpha-2a adrenergic agonist, promotes ischemic tolerance in a murine model of spinal cord ischemia-reperfusion. *J Thorac Cardiovasc Surg*, 10.

Benigni, A, Corna, D, Zoja, C, Sonzogni, A, Latini, R, Salio, M, Conti, S, Rottoli, D, Longaretti, L, Cassis, P, Moriqi, M, Coffman, TM, Remuzzi, G (2009). Disruption of the Ang II type 1 receptor promotes longevity in mice. *J Clin Invest* 119: 524-30.

Berbel, MN, Pinto, MP, Ponce, D & Balbi, AL (2011). Nutritional aspects in acute kidney injury. *Rev Assoc Med Bras* 57: 600-6.

Berndt, WO (1976). Renal function tests: what do they mean? A review of renal anatomy, biochemistry, and physiology. *Environ Health Perspect* 15: 55-71.

Bertelli, AA, Migliori, M, Panichi, V, Origlia, N, Filippi, C, Das, DK, Giovannini, L (2002). Resveratrol, a component of wine and grapes, in the prevention of kidney disease. *Ann N Y Acad Sci* 957: 230-8.

Bhana, N, Goa, KL & McClellan, KJ (2000). Dexmedetomidine. *Drugs* 59: 263,8; discussion 269-70.

Bishop, NA, Guarente, L (2007). Genetic links between diet and lifespan: shared mechanisms from yeast to humans. *Nat Rev Genet* 8: 835-44.

Blantz, RC, Deng, A, Miracle, CM & Thomson, SC (2007). Regulation of kidney function and metabolism: a question of supply and demand. *Trans Am Clin Climatol Assoc* 118: 23-43.

Boily, G, Seifert, EL, Bevilacqua, L, He, XH, Sabourin, G, Estey, C, Moffat, C, Crawford, S, Saliba, S, Jardine, K, Xuan, J, Evans, M, Harper, ME, McBurney, MW (2008). SirT1 regulates energy metabolism and response to caloric restriction in mice. *PLoS One* 3: e1759.

Bonventre, JV (2002). Kidney ischemic preconditioning. *Curr Opin Nephrol Hypertens* 11: 43-8.

Bonventre, JV, Yang, L (2011). Cellular pathophysiology of ischemic acute kidney injury. *J Clin Invest* 121: 4210-21.

Bovee, KC (1986). Renal function and laboratory evaluation. *Toxicol Pathol* 14: 26-36.

Bullough, DA, Zhang, C, Montag, A, Mullane, KM & Young, MA (1994). Adenosine-mediated inhibition of platelet aggregation by acadesine. A novel antithrombotic mechanism in vitro and in vivo. *J Clin Invest* 94: 1524-32.

Cammisotto, PG, Bendayan, M (2008). Adiponectin stimulates phosphorylation of AMP-activated protein kinase alpha in renal glomeruli. *J Mol Histol* 39: 579-84.

Cammisotto, PG, Londono, I, Gingras, D & Bendayan, M (2008). Control of glycogen synthase through ADIPOR1-AMPK pathway in renal distal tubules of normal and diabetic rats. *Am J Physiol Renal Physiol* 294: F881-9.

Canto, C, Auwerx, J (2012). Targeting sirtuin 1 to improve metabolism: all you need is NAD(+)? *Pharmacol Rev* 64: 166-87.

Canto, C, Gerhart-Hines, Z, Feige, JN, Lagouge, M, Noriega, L, Milne, JC, Elliot, PJ, Puigserver, P and Auwerx, J (2009). AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature* 458:1056–1060.

Chander, V, Chopra, K (2006). Protective effect of nitric oxide pathway in resveratrol renal ischemia-reperfusion injury in rats. *Arch Med Res* 37: 19-26.

Chandrasekar, B, Nelson, JF, Colston, JT & Freeman, GL (2001). Calorie restriction attenuates inflammatory responses to myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 280: H2094-102.

Chase, SD, Magnani, JL & Simon, SI (2012). E-selectin ligands as mechanosensitive receptors on neutrophils in health and disease. *Ann Biomed Eng* 40: 849-59.

Chen, CJ, Fu, YC, Yu, W & Wang, W (2013). SIRT3 protects cardiomyocytes from oxidative stress-mediated cell death by activating NF-kappaB. *Biochem Biophys Res Commun* 430: 798-803.

Chen, CJ, Yu, W, Fu, YC, Wang, X, Li, JL & Wang, W (2009). Resveratrol protects cardiomyocytes from hypoxia-induced apoptosis through the SIRT1-FoxO1 pathway. *Biochem Biophys Res Commun* 378: 389-93.

Chen, D, Bruno, J, Easlon, E, Lin, SJ, Cheng, HL, Alt, FW, Guarante, L (2008). Tissue-specific regulation of SIRT1 by calorie restriction. *Genes Dev* 22: 1753-7.

Chen, D, Steele, AD, Lindquist, S & Guarente, L (2005). Increase in activity during calorie restriction requires Sirt1. *Science* 310: 1641.

Chen, H, Montagnani, M, Funahashi, T, Shimomura, I & Quon, MJ (2003). Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem* 278: 45021-6.

Chen, H, Xing, B, Liu, X, Zhan, B, Zhou, J, Zhu, H, Chen, Z (2008). Ozone oxidative preconditioning protects the rat kidney from reperfusion injury: the role of nitric oxide. *J Surg Res* 149: 287-95.

Chen, Y, Tseng, SH (2007). Review. Pro- and anti-angiogenesis effects of resveratrol. *In Vivo* 21: 365-70.

Chen, Z, Peng, IC, Sun, W, Su, MI, Hsu, PH, Fu, Y, Zhu, Y, DeFea, K, Pan, S, Tsai, MD, Shyy, JY (2009). AMP-activated protein kinase functionally phosphorylates endothelial nitric oxide synthase Ser633. *Circ Res* 104: 496-505.

Choudhury, D (2010). Acute kidney injury: current perspectives. *Postgrad Med* 122: 29-40.

Coca, SG, Yusuf, B, Shlipak, MG, Garg, AX & Parikh, CR (2009). Long-term risk of mortality and other adverse outcomes after acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 53: 961-73.

Cohen, HY, Miller, C, Bitterman, KJ, Wall, NR, Hekking, B, Kessler, B, Howitz, KT, Gorospe, M, de Cabo, R, Sinclair, DA (2004). Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 305: 390-2.

Colman, RJ, Anderson, RM, Johnson, SC, Kastman, EK, Kosmatka, KJ, Beasley, TM, Allison, DB, Cruzen, C, Simmons, HA, Kemnitz, JW, Weindruch, R (2009). Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 325: 201-4.

Conger, JD (1983). Vascular abnormalities in the maintenance of acute renal failure. *Circ Shock* 11: 235-44.

Costopoulos, C, Liew, TV & Bennett, M (2008). Ageing and atherosclerosis: Mechanisms and therapeutic options. *Biochem Pharmacol* 75: 1251-61.

Coursin, DB, Coursin, DB & Maccioli, GA (2001). Dexmedetomidine. *Curr Opin Crit Care* 7: 221-6.

Csiszar, A, Sosnowska, D, Tucsek, Z, Gautam, T, Toth, P, Losonczy, G, Colman, RJ, Weindruch, R, Anderson, RM, Sonntaq, WE, Unqvari, Z (2013). Circulating factors induced by caloric restriction in the nonhuman primate Macaca mulatta activate angiogenic processes in endothelial cells. *J Gerontol A Biol Sci Med Sci* 68: 235-49.

Cussac, D, Schaak, S, Gales, C, Flordellis, C, Denis, C & Paris, H (2002). alpha(2B)-Adrenergic receptors activate MAPK and modulate proliferation of primary cultured proximal tubule cells. *Am J Physiol Renal Physiol* 282: F943-52.

Cybulsky, AV (2010). Endoplasmic reticulum stress in proteinuric kidney disease. *Kidney Int* 77: 187-93.

De Nicola, L, Blantz, RC & Gabbai, FB (1992). Nitric oxide and angiotensin II. Glomerular and tubular interaction in the rat. *J Clin Invest* 89: 1248-56.

Della-Morte, D, Dave, KR, DeFazio, RA, Bao, YC, Raval, AP & Perez-Pinzon, MA (2009). Resveratrol pretreatment protects rat brain from cerebral ischemic damage via a sirtuin 1-uncoupling protein 2 pathway. *Neuroscience* 159: 993-1002.

Denison, FC, Hiscock, NJ, Carling, D & Woods, A (2009). Characterization of an alternative splice variant of LKB1. *J Biol Chem* 284: 67-76.

Dennis, G, Jr, Sherman, BT, Hosack, DA, Yang, J, Gao, W, Lane, HC, *et al.* (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 4: 3.

Devarajan P. (2006). Update on Mechanisms of Ischemic Acute Kidney Injury. . *J Am Soc Nephrol*: 1503-20.

Dragun, D, Hoff, U, Park, J, Qun, Y, Schneider, W, Luft, F, Haller, H (2001). Prolonged cold preservation augments vascular injury independent of renal transplant immunogenicity and function. *Kidney Int* 60: 1173.

Duffield, JS (2010). Macrophages and immunologic inflammation of the kidney. *Semin Nephrol* 30: 234-54.

Duong, MH, MacKenzie, TA & Malenka, DJ (2005). N-acetylcysteine prophylaxis significantly reduces the risk of radiocontrast-induced nephropathy: comprehensive meta-analysis. *Catheter Cardiovasc Interv* 64: 471-9.

Dzau, VJ, Packer, M, Lilly, LS, Swartz, SL, Hollenberg, NK & Williams, GH (1984). Prostaglandins in severe congestive heart failure. Relation to activation of the renin--angiotensin system and hyponatremia. *N Engl J Med* 310: 347-52.

El-Achkar, TM, Wu, XR, Rauchman, M, McCracken, R, Kiefer, S & Dagher, PC (2008). Tamm-Horsfall protein protects the kidney from ischemic injury by decreasing inflammation and altering TLR4 expression. *Am J Physiol Renal Physiol* 295: F534-44.

Elapavaluru, S, Kellum, JA (2007). Why do patients die of acute kidney injury? *Acta Clin Belg Suppl* (2): 326-31.

Fan, H, Yang, HC, You, L, Wang, YY, He, WJ & Hao, CM (2013). The histone deacetylase, SIRT1, contributes to the resistance of young mice to ischemia/reperfusion-induced acute kidney injury. *Kidney Int* 83: 404-13.

Finckenberg, P, Eriksson, O, Baumann, M, Merasto, S, Lalowski, MM, Levijoki, J, Haasio, K, Kytö, V, Muller, DN, Luft, FC, Oresic, M, Mervaala, E (2011). Caloric Restriction Ameliorates Angiotensin II-Induced Mitochondrial Remodeling and Cardiac Hypertrophy. *Hypertension*, 10.

Finfer, S, Bellomo, R, Boyce, N, French, J, Myburgh, J, Norton, R, SAFE Study Investigators (2004). A comparison of albumin and saline for fluid resuscitation in the intensive care unit. *N Engl J Med* 350: 2247-56.

Fisslthaler, B, Fleming, I (2009). Activation and signaling by the AMP-activated protein kinase in endothelial cells. *Circ Res* 105: 114-27.

Fontana, L, Partridge, L & Longo, VD (2010). Extending healthy life span--from yeast to humans. *Science* 328: 321-6.

Forbes, JM, Hewitson, TD, Becker, GJ & Jones, CL (2000). Ischemic acute renal failure: long-term histology of cell and matrix changes in the rat. *Kidney Int* 57: 2375-85.

Frangogiannis, NG (2007). Chemokines in ischemia and reperfusion. *Thromb Haemost* 97: 738-47.

Frumento, RJ, Logginidou, HG, Wahlander, S, Wagener, G, Playford, HR & Sladen, RN (2006). Dexmedetomidine infusion is associated with enhanced renal function after thoracic surgery. *J Clin Anesth* 18: 422-6.

Fujii, T, Kurata, H, Takaoka, M, Muraoka, T, Fujisawa, Y, Shokoji, T, Nishiyama, A, Abe, Y, Matsumura, Y (2003). The role of renal sympathetic nervous system in the pathogenesis of ischemic acute renal failure. *Eur J Pharmacol* 481: 241-8.

Fukuhara, A, Matsuda, M, Nishizawa, M, Segawa, K, Tanaka, M, Kishimoto, K, Matsuki, Y, Murakami, M, Ichisaka, T, Murakami, H, Watanabe, E, Takagi, T, Akiyoshi, M,Ohtsubo, T, Kihara, S, Yamashita, S, Makishima, M, Funahashi, T, Yamanaka, S, Hiramatsu, R, Matsuzawa, Y, Shimomura, I (2005). Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 307: 426-30.

Fulco, M, Cen, Y, Zhao, P, Hoffman, EP, McBurney, MW, Sauve, AA and Sartorelli V (2008). Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. *Dev Cell* 14: 661–673.

Galinanes, M, Zhai, X, Bullough, D, Mullane, KM & Hearse, DJ (1995). Protection against injury during ischemia and reperfusion by acadesine derivatives GP-1-468 and GP-1-668. Studies in the transplanted rat heart. *J Thorac Cardiovasc Surg* 110: 752-61.

Gambaro, G, Perazella, MA (2003). Adverse renal effects of anti-inflammatory agents: evaluation of selective and nonselective cyclooxygenase inhibitors. *J Intern Med* 253: 643-52.

Gaskin, FS, Kamada, K, Yusof, M, Durante, W, Gross, G & Korthuis, RJ (2009). AICAR preconditioning prevents postischemic leukocyte rolling and adhesion: role of K(ATP) channels and heme oxygenase. *Microcirculation* 16: 167-76.

Gaskin, FS, Kamada, K, Yusof, M & Korthuis, RJ (2007). 5'-AMP-activated protein kinase activation prevents postischemic leukocyte-endothelial cell adhesive interactions. *Am J Physiol Heart Circ Physiol* 292: H326-32.

Gerhart-Hines, Z, Rodgers, JT, Bare, O, Lerin, C, Kim, SH, Mostoslavsky, R, Alt, FW, Wu, Z, Puiqserver, P (2007). Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. *EMBO J* 26: 1913-23.

Govers, R, Rabelink, TJ (2001). Cellular regulation of endothelial nitric oxide synthase. *Am J Physiol Renal Physiol* 280: F193-206.

Grigoryev, DN, Liu, M, Cheadle, C, Barnes, KC & Rabb, H (2006). Genomic profiling of kidney ischemia-reperfusion reveals expression of specific alloimmunity-associated genes: Linking "immune" and "nonimmune" injury events. *Transplant Proc* 38: 3333-6.

Groenendyk, J, Sreenivasaiah, PK, Kim do, H, Agellon, LB & Michalak, M (2010). Biology of endoplasmic reticulum stress in the heart. *Circ Res* 107: 1185-97.

Gu, J, Chen, J, Xia, P, Tao, G, Zhao, H & Ma, D (2011a). Dexmedetomidine attenuates remote lung injury induced by renal ischemia-reperfusion in mice. *Acta Anaesthesiol Scand* 55: 1272-8.

Gu, J, Sun, P, Zhao, H, Watts, HR, Sanders, RD, Terrando, N, Xia, P, Maze, M, Ma, D (2011b). Dexmedetomidine provides renoprotection against ischemia-reperfusion injury in mice. *Crit Care* 15: R153.

Guo, Z, Zhao, Z (2007). Effect of N-acetylcysteine on plasma adiponectin and renal adiponectin receptors in streptozotocin-induced diabetic rats. *Eur J Pharmacol* 558: 208-13.

Gustafsson, AB, Gottlieb, RA (2009). Autophagy in ischemic heart disease. *Circ Res* 104: 150-8.

Gutterman, DD (2005). Mitochondria and reactive oxygen species: an evolution in function. *Circ Res* 97: 302-4.

Haapalinna, A. (2006). The effects of atipamezole on brain neurochemistry and behaviour on laboratory rodents - possible implications for the treatment of neurodegenerative diseases with an alpha2-adrenoceptor antagonist.

Haigis, MC, Sinclair, DA (2010). Mammalian sirtuins: biological insights and disease relevance. *Annu Rev Pathol* 5: 253-95.

Hallows, KR (2005). Emerging role of AMP-activated protein kinase in coupling membrane transport to cellular metabolism. *Curr Opin Nephrol Hypertens* 14: 464-71.

Hallows, KR, Mount, PF, Pastor-Soler, NM & Power, DA (2010). Role of the energy sensor AMP-activated protein kinase in renal physiology and disease. *Am J Physiol Renal Physiol*, 10.

Hamasaki, J, Tsuneyoshi, I, Katai, R, Hidaka, T, Boyle, WA & Kanmura, Y (2002). Dual alpha(2)-adrenergic agonist and alpha(1)-adrenergic antagonist actions of dexmedetomidine on human isolated endothelium-denuded gastroepiploic arteries. *Anesth Analg* 94: 1434-40.

Hao, CM, Haase, VH (2010). Sirtuins and their relevance to the kidney. *J Am Soc Nephrol* 21: 1620-7.

Hardie, DG, Hawley, SA (2001). AMP-activated protein kinase: the energy charge hypothesis revisited. *Bioessays* 23: 1112-9.

Hasegawa, K, Wakino, S, Yoshioka, K, Tatematsu, S, Hara, Y, Minakuchi, H, Sueyasu, K, Washida, N, Tokuyama, H, Tzukerman, M, Skorecki, K, Hayashi, K, Itoh, H (2010). Kidney-specific overexpression of Sirt1 protects against acute kidney injury by retaining peroxisome function. *J Biol Chem* 285: 13045-56.

Hawley, SA, Selbert, MA, Goldstein, EG, Edelman, AM, Carling, D & Hardie, DG (1995). 5'-AMP activates the AMP-activated protein kinase cascade, and Ca2+/calmodulin activates the calmodulin-dependent protein kinase I cascade, via three independent mechanisms. *J Biol Chem* 270: 27186-91.

He, W, Wang, Y, Zhang, MZ, You, L, Davis, LS, Fan, H, Yang, HC, Fogo, AB, Zent, R, Harris, RC, Breyer, MD, Hao, CM (2010). Sirt1 activation protects the mouse renal medulla from oxidative injury. *J Clin Invest* 120: 1056-68.

Heistad, DD (2006). Oxidative stress and vascular disease: 2005 Duff lecture. *Arterioscler Thromb Vasc Biol* 26: 689-95.

Helkamaa, T, Finckenberg, P, Louhelainen, M, Merasto, S, Rauhala, P, Lapatto, R, Cheng, Z, Reenilä, I, Männistö, P, Müller, D, Luft, F, Mervaala, E (2003). Entacapone protects from angiotensin II-induced inflammation and renal injury. *J Hypertens*. 21:2353-63.

Hoffmann, U, Fischereder, M, Kruger, B, Drobnik, W & Kramer, BK (2004). The value of N-acetylcysteine in the prevention of radiocontrast agent-induced nephropathy seems questionable. *J Am Soc Nephrol* 15: 407-10.

Holdright, D, Sparrow, J, Wright, C, Steiner, J, Fox, K (1994). Effect of acadesine, a new metabolic agent, on exercise-induced myocardial ischemia in chronic stable angina. *Cardiovasc Drugs Ther* 8: 193-7.

Holmes, BF, Kurth-Kraczek, EJ & Winder, WW (1999). Chronic activation of 5'-AMP-activated protein kinase increases GLUT-4, hexokinase, and glycogen in muscle. *J Appl Physiol* 87: 1990-5.

Hsu, CP, Zhai, P, Yamamoto, T, Maejima, Y, Matsushima, S, Hariharan, N, Shao, D, Takagi, H, Oka, S, Sadoshima, J (2010). Silent information regulator 1 protects the heart from ischemia/reperfusion. *Circulation* 122: 2170-82.

Hsu, CY, Chertow, GM, McCulloch, CE, Fan, D, Ordonez, JD & Go, AS (2009). Nonrecovery of kidney function and death after acute on chronic renal failure. *Clin J Am Soc Nephrol* 4: 891-8.

Huang, L, Wei, YY, Momose-Hotokezaka, A, Dickey, J & Okusa, MD (1996). Alpha 2B-adrenergic receptors: immunolocalization and regulation by potassium depletion in rat kidney. *Am J Physiol* 270: F1015-26.

Hugo, C, Daniel, C (2009). Thrombospondin in renal disease. *Nephron Exp Nephrol* 111: e61-6.

Hurley, RL, Anderson, KA, Franzone, JM, Kemp, BE, Means, AR & Witters, LA (2005). The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. *J Biol Chem* 280: 29060-6.

Ibacache, M, Sanchez, G, Pedrozo, Z, Galvez, F, Humeres, C, Echevarria, G, Duaso, J, Hassi, M, Garcia, L, Díaz-Araya, G, Lavandero, S (2012). Dexmedetomidine preconditioning activates pro-survival kinases and attenuates regional ischemia/reperfusion injury in rat heart. *Biochim Biophys Acta* 1822: 537-45.

Irani, K (2000). Oxidant signaling in vascular cell growth, death, and survival : a review of the roles of reactive oxygen species in smooth muscle and endothelial cell mitogenic and apoptotic signaling. *Circ Res* 87: 179-83.

Ishani, A, Xue, JL, Himmelfarb, J, Eggers, PW, Kimmel, PL, Molitoris, BA, Collins, AJ (2009). Acute kidney injury increases risk of ESRD among elderly. *J Am Soc Nephrol* 20: 223-8.

Jang, HR, Ko, GJ, Wasowska, BA & Rabb, H (2009). The interaction between ischemia-reperfusion and immune responses in the kidney. *J Mol Med (Berl)* 87: 859-64.

Ji, F, Li, Z, Young, JN, Yeranossian, A & Liu, H (2013). Post-bypass dexmedetomidine use and postoperative acute kidney injury in patients undergoing cardiac surgery with cardiopulmonary bypass. *PLoS One* 8: e77446.

Jiang, M, Liu, K, Luo, J & Dong, Z (2010). Autophagy is a renoprotective mechanism during in vitro hypoxia and in vivo ischemia-reperfusion injury. *Am J Pathol* 176: 1181-92.

Jiang, M, Wei, Q, Dong, G, Komatsu, M, Su, Y & Dong, Z (2012). Autophagy in proximal tubules protects against acute kidney injury. *Kidney Int* 82: 1271-83.

Jin, Q, Jhun, BS, Lee, SH, Lee, J, Pi, Y, Cho, YH, Baik, HH, Kanq, I (2004). Differential regulation of phosphatidylinositol 3-kinase/Akt, mitogen-activated protein kinase, and AMP-activated protein kinase pathways during menadione-induced oxidative stress in the kidney of young and old rats. *Biochem Biophys Res Commun* 315: 555-61.

Jolkkonen, J, Puurunen, K, Koistinaho, J, Kauppinen, R, Haapalinna, A, Nieminen, L, Sivenius, J (1999). Neuroprotection by the alpha2-adrenoceptor agonist, dexmedetomidine, in rat focal cerebral ischemia. *Eur J Pharmacol* 372: 31-6.

Kahn, BB, Alquier, T, Carling, D & Hardie, DG (2005). AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab* 1: 15-25.

Karajala, V, Mansour, W & Kellum, JA (2009). Diuretics in acute kidney injury. *Minerva Anestesiol* 75: 251-7.

Katerelos, M, Mudge, SJ, Stapleton, D, Auwardt, RB, Fraser, SA, Chen, CG, Kemp, BE, Power, DA (2010). 5-aminoimidazole-4-carboxamide ribonucleoside and AMP-activated protein kinase inhibit signalling through NF-kappaB. *Immunol Cell Biol* 88: 754-60.

Kaur, M, Singh, PM (2011). Current role of dexmedetomidine in clinical anesthesia and intensive care. *Anesthesia: Essays and Researches*.

Kawano, T, Yamazaki, F, Chi, H, Kawahito, S, Equchi, S (2012). Dexmedetomidine directly inhibits vascular ATP-sensitive potassium channels. *Life Sci* 90:272-7.

Kelly, KJ, Williams, WW,Jr, Colvin, RB, Meehan, SM, Springer, TA, Gutierrez-Ramos, JC, Bonventre, JV (1996). Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. *J Clin Invest* 97: 1056-63.

Kennedy, BK, Steffen, KK & Kaeberlein, M (2007). Ruminations on dietary restriction and aging. *Cell Mol Life Sci* 64: 1323-8.

Kennedy, SE, Erlich, JH (2008). Murine renal ischaemia-reperfusion injury. *Nephrology (Carlton)* 13: 390-6.

Kilic, K, Hanci, V, Selek, S, Sozmen, M, Kilic, N, Citil, M, Yurtlu, DA, Yurtlu, BS (2012). The effects of dexmedetomidine on mesenteric arterial occlusion-associated gut ischemia and reperfusion-induced gut and kidney injury in rabbits. *J Surg Res*, 10.

Kim, JE, Kim, YW, Lee, IK, Kim, JY, Kang, YJ & Park, SY (2008). AMP-activated protein kinase activation by 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) inhibits palmitate-induced endothelial cell apoptosis through reactive oxygen species suppression. *J Pharmacol Sci* 106: 394-403.

Kimura, T, Takabatake, Y, Takahashi, A, Kaimori, JY, Matsui, I, Namba, T, Kitamura, H, Niimura, F, Matsusaka, T, Soga, T, Rakugi, H, Isaka, Y (2011). Autophagy protects the proximal tubule from degeneration and acute ischemic injury. *J Am Soc Nephrol* 22: 902-13.

Koca, U, Olguner, CG, Ergur, BU, Altekin, E, Tasdogen, A, Duru, S, Girgin, P, Gündüz, K, Cilaker Mıcılı, S, Güzeldağ, S, Akkuş, M (2013). The effects of dexmedetomidine on secondary acute lung and kidney injuries in the rat model of intra-abdominal sepsis. *ScientificWorldJournal* 2013: 292687.

Kuhmonen, J, Pokorny, J, Miettinen, R, Haapalinna, A, Jolkkonen, J, Riekkinen, PS, Sivnius, J (1997). Neuroprotective effects of dexmedetomidine in the gerbil hippocampus after transient global ischemia. *Anesthesiology* 87: 371-7.

Kulka, PJ, Tryba, M & Zenz, M (1996). Preoperative alpha2-adrenergic receptor agonists prevent the deterioration of renal function after cardiac surgery: results of a randomized, controlled trial. *Crit Care Med* 24: 947-52.

Kume, S, Haneda, M, Kanasaki, K, Sugimoto, T, Araki, S, Isshiki, K, Isono, M, Uzu, T, Guarente, L, Kashiwagi, A, Koya, D (2007). SIRT1 inhibits transforming growth factor beta-induced apoptosis in glomerular mesangial cells via Smad7 deacetylation. *J Biol Chem* 282: 151-8.

Kume, S, Uzu, T, Horiike, K, Chin-Kanasaki, M, Isshiki, K, Araki, S, Sugimoto, T, Haneda, M, Kashiwagi, A, Koya, D (2010). Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. *J Clin Invest* 120: 1043-55.

Kunzendorf, U, Haase, M, Rolver, L & Haase-Fielitz, A (2010). Novel aspects of pharmacological therapies for acute renal failure. *Drugs* 70: 1099-114.

Lakshmi, SV, Padmaja, G, Kuppusamy, P & Kutala, VK (2009). Oxidative stress in cardiovascular disease. *Indian J Biochem Biophys* 46: 421-40.

Lameire, N (2013). The definitions and staging systems of acute kidney injury and their limitations in practice. *Arab J Nephrol Transplant* 6: 145-52.

Lameire, N, Van Biesen, W & Vanholder, R (2005). Acute renal failure. *Lancet* 365: 417-30.

Lan, F, Cacicedo, JM, Ruderman, N and Ido, Y (2008). SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation. *J Biol Chem* 283: 27628–27635.

Lawrence, CJ, Prinzen, FW & de Lange, S (1996). The effect of dexmedetomidine on nutrient organ blood flow. *Anesth Analg* 83: 1160-5.

Le Dorze, M, Legrand, M, Payen, D & Ince, C (2009). The role of the microcirculation in acute kidney injury. *Curr Opin Crit Care* 15: 503-8.

Lee, S, Chang, Y, Na, K, Lee, K, Suh, K, Kim, S, Chang, Y, Shin, Y, Bang, B (2009). The Preconditioning with AICAR Protects Against Subsequent Renal Ischemia Reperfusion Injury. *Korean J Nephrol* 28: 96-102.

Lee, IH, Cao, L, Mostoslavsky, R, Lombard, DB, Liu, J, Bruns, NE,Tsokos, M, Alt, F, Finkel, T (2008). A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci U S A* 105: 3374-9.

Lee, MJ, Feliers, D, Mariappan, MM, Sataranatarajan, K, Mahimainathan, L, Musi, N, Foretz, M, Viollet, B, Weinberg, JM, Choudhury, GG, Kasinath, BS (2007). A role for AMP-activated protein kinase in diabetes-induced renal hypertrophy. *Am J Physiol Renal Physiol* 292: F617-27.

Lee, MJ, Feliers, D, Sataranatarajan, K, Mariappan, MM, Li, M, Barnes, JL, Choudhury, GG, Kasinath, BS (2010). Resveratrol ameliorates high glucose-induced protein synthesis in glomerular epithelial cells. *Cell Signal* 22: 65-70.

Lee, S, Kim, BH, Lim, K, Stalker, D, Wisemandle, W, Shin, SG, Janq, IJ, Yu, KS (2012). Pharmacokinetics and pharmacodynamics of intravenous dexmedetomidine in healthy Korean subjects. *J Clin Pharm Ther* 37: 698-703.

Lehtimaki, J, Leino, T, Koivisto, A, Viitamaa, T, Lehtimaki, T, Haapalinna, A, Kuokkanen, K, Virtanen, R (2008). In vitro and in vivo profiling of fadolmidine, a novel potent alpha(2)-adrenoceptor agonist with local mode of action. *Eur J Pharmacol* 599: 65-71.

Leino, T, Viitamaa, T, Haapalinna, A, Lehtimaki, J & Virtanen, R (2009). Pharmacological profile of intrathecal fadolmidine, a alpha2-adrenoceptor agonist, in rodent models. *Naunyn Schmiedebergs Arch Pharmacol* 380: 539-50.

Leonard, MO, Kieran, NE, Howell, K, Burne, MJ, Varadarajan, R, Dhakshinamoorthy, S, Porter, AG, O'Farrelly, C, Rabb, H, Taylor, CT (2006). Reoxygenation-specific activation of the antioxidant transcription factor Nrf2 mediates cytoprotective gene expression in ischemia-reperfusion injury. *FASEB J* 20: 2624-6.

Li, J, Zeng, Z, Viollet, B, Ronnett, GV & McCullough, LD (2007). Neuroprotective effects of adenosine monophosphate-activated protein kinase inhibition and gene deletion in stroke. *Stroke* 38: 2992-9.

Liano, F, Pascual, J (1996). Epidemiology of acute renal failure: a prospective, multicenter, community-based study. Madrid Acute Renal Failure Study Group. *Kidney Int* 50: 811-8.

Lieberthal, W, Levine, JS (2009). The role of the mammalian target of rapamycin (mTOR) in renal disease. *J Am Soc Nephrol* 20: 2493-502.

Lihn, AS, Jessen, N, Pedersen, SB, Lund, S & Richelsen, B (2004). AICAR stimulates adiponectin and inhibits cytokines in adipose tissue. *Biochem Biophys Res Commun* 316: 853-8.

Lin, A, Sekhon, C, Sekhon, B, Smith, A, Chavin, K, Orak, J, Sinqh, I, Sinqh, A (2004). Attenuation of ischemia-reperfusion injury in a canine model of autologous renal transplantation. *Transplantation* 78: 654-9.

Linkermann, A, Bräsen, JH, Himmerkus, N, Liu, S, Huber, TB, Kunzendorf, U, Krautwald, S (2012). Rip1 (receptor-interacting protein kinase 1) mediates necroptosis and contributes to renal ischemia/reperfusion injury. *Kidney Int.* 81:751-61.

Liu, M, Grigoryev, DN, Crow, MT, Haas, M, Yamamoto, M, Reddy, SP, Rabb, H (2009). Transcription factor Nrf2 is protective during ischemic and nephrotoxic acute kidney injury in mice. *Kidney Int* 76: 277-85.

Liu, X, Chen, H, Zhan, B, Xing, B, Zhou, J, Zhu, H, Chen, Z (2007). Attenuation of reperfusion injury by renal ischemic postconditioning: the role of NO. *Biochem Biophys Res Commun* 359: 628-34.

Louhelainen, M, Vahtola, E, Kaheinen, P, Leskinen, H, Merasto, S, Kytö, V, Finckenberg, P, Colucci, W, Levijoki, J, Pollesello, P, Haikala, H, Mervaala, E (2007). Effects of levosimendan on cardiac remodeling and cardiomyocyte apoptosis in hypertensive Dahl/Rapp rats. *Br J Pharmacol* 150:851–861.

Ma, D, Lim, T, Xu, J, Tang, H, Wan, Y, Zhao, H, Hossain, M, Maxwell, PH, Maze, M (2009). Xenon preconditioning protects against renal ischemic-reperfusion injury via HIF-1alpha activation. *J Am Soc Nephrol* 20: 713-20.

Madeo, F, Tavernarakis, N & Kroemer, G (2010). Can autophagy promote longevity? *Nat Cell Biol* 12: 842-6.

Maier, C, Steinberg, GK, Sun, GH, Zhi, GT & Maze, M (1993). Neuroprotection by the alpha 2-adrenoreceptor agonist dexmedetomidine in a focal model of cerebral ischemia. *Anesthesiology* 79: 306-12.

Mantz, J, Josserand, J & Hamada, S (2011). Dexmedetomidine: new insights. *Eur J Anaesthesiol* 28: 3-6.

Mathew, JP, Rinder, CS, Tracey, JB, Auszura, LA, O'Connor, T, Davis, E, Smith, BR (1995). Acadesine inhibits neutrophil CD11b up-regulation in vitro and during in vivo cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 109: 448-56.

Mattagajasingh, I, Kim, CS, Naqvi, A, Yamamori, T, Hoffman, TA, Jung, SB, DeRicco, J, Kasuno, K, Irani, K (2007). SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 104: 14855-60.

Maulik, N (2004). Angiogenic signal during cardiac repair. *Mol Cell Biochem* 264: 13-23.

Mehta, RL, Pascual, MT, Soroko, S, Savage, BR, Himmelfarb, J, Ikizler, TA, Paganini, EP, Chertow, GM; Program to Improve Care in Acute Renal Disease (2004). Spectrum of acute renal failure in the intensive care unit: the PICARD experience. *Kidney Int* 66: 1613-21.

Meister, B, Dagerlind, A, Nicholas, AP & Hokfelt, T (1994). Patterns of messenger RNA expression for adrenergic receptor subtypes in the rat kidney. *J Pharmacol Exp Ther* 268: 1605-11.

Merrill, GF, Kurth, EJ, Hardie, DG & Winder, WW (1997). AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *Am J Physiol* 273: E1107-12.

Meury, L, Noel, J, Tejedor, A, Senecal, J, Gougoux, A & Vinay, P (1994). Glucose metabolism in dog inner medullary collecting ducts. *Ren Physiol Biochem* 17: 246-66.

Milsom, AB, Patel, NS, Mazzon, E, Tripatara, P, Storey, A, Mota-Filipe, H, Sepodes, B, Webb, AJ, Cuzzocrea, S, Hobbs, AJ, Thiemermann, C, Ahluwalia, A (2010). Role for endothelial nitric oxide synthase in nitrite-induced protection against renal ischemia-reperfusion injury in mice. *Nitric Oxide* 22: 141-8.

Mitchell, JR, Verweij, M, Brand, K, van de Ven, M, Goemaere, N, van den Engel, S, Chu, T, Forrer, F, Müller, C, de Jong, M, van IJcken, W, IJzermans, JN, Hoeijmakers, JH, de Bruin, RW (2010). Short-term dietary restriction and fasting precondition against ischemia reperfusion injury in mice. *Aging Cell* 9: 40-53.

Miyazaki, R, Ichiki, T, Hashimoto, T, Inanaga, K, Imayama, I, Sadoshima, J, Sunagawa, K (2008). SIRT1, a longevity gene, downregulates angiotensin II type 1 receptor expression in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 28: 1263-9.

Mizushima, N, Levine, B, Cuervo, AM & Klionsky, DJ (2008). Autophagy fights disease through cellular self-digestion. *Nature* 451: 1069-75.

Mocanu, MM, Baxter, GF, Yue, Y, Critz, SD & Yellon, DM (2000). The p38 MAPK inhibitor, SB203580, abrogates ischaemic preconditioning in rat heart but timing of administration is critical. *Basic Res Cardiol* 95: 472-8.

Moncada, S, Higgs, EA (2006). Nitric oxide and the vascular endothelium. *Handb Exp Pharmacol* (176 Pt 1): 213-54.

Morris, KC, Lin, HW, Thompson, JW & Perez-Pinzon, MA (2011). Pathways for ischemic cytoprotection: Role of sirtuins in caloric restriction, resveratrol, and ischemic preconditioning. *J Cereb Blood Flow Metab*, 10.

Morrow, VA, Foufelle, F, Connell, JM, Petrie, JR, Gould, GW & Salt, IP (2003). Direct activation of AMP-activated protein kinase stimulates nitric-oxide synthesis in human aortic endothelial cells. *J Biol Chem* 278: 31629-39.

Mount, PF, Hill, RE, Fraser, SA, Levidiotis, V, Katsis, F, Kemp, BE, Power, DA (2005). Acute renal ischemia rapidly activates the energy sensor AMPK but does not increase phosphorylation of eNOS-Ser1177. *Am J Physiol Renal Physiol* 289: F1103-15.

Mount, PF, Lane, N, Venkatesan, S, Steinberg, GR, Fraser, SA, Kemp, BE, Power, DA (2008). Bradykinin stimulates endothelial cell fatty acid oxidation by CaMKK-dependent activation of AMPK. *Atherosclerosis* 200: 28-36.

Moura, E, Afonso, J, Hein, L & Vieira-Coelho, MA (2006). Alpha2-adrenoceptor subtypes involved in the regulation of catecholamine release from the adrenal medulla of mice. *Br J Pharmacol* 149: 1049-58.

Munzel, T, Daiber, A & Mulsch, A (2005). Explaining the phenomenon of nitrate tolerance. *Circ Res* 97: 618-28.

Nadasdy, T, Laszik, Z, Blick, KE, Johnson, LD & Silva, FG (1994). Proliferative activity of intrinsic cell populations in the normal human kidney. *J Am Soc Nephrol* 4: 2032-9.

Nadtochiy, SM, Redman, E, Rahman, I & Brookes, PS (2011a). Lysine deacetylation in ischaemic preconditioning: the role of SIRT1. *Cardiovasc Res* 89: 643-9.

Nadtochiy, SM, Yao, H, McBurney, MW, Gu, W, Guarente, L, Rahman, I, Brookes, PS (2011b). SIRT1-mediated acute cardioprotection. *Am J Physiol Heart Circ Physiol* 301: H1506-12.

Nakano, A, Cohen, MV, Critz, S & Downey, JM (2000). SB 203580, an inhibitor of p38 MAPK, abolishes infarct-limiting effect of ischemic preconditioning in isolated rabbit hearts. *Basic Res Cardiol* 95: 466-71.

Nangaku, M, Izuhara, Y, Usuda, N, Inagi, R, Shibata, T, Sugiyama, S, Kurokawa, K, van Ypersele de Strihou, C, Miyata, T (2005). In a type 2 diabetic nephropathy rat model, the improvement of obesity by a low calorie diet reduces oxidative/carbonyl stress and prevents diabetic nephropathy. *Nephrol Dial Transplant* 20: 2661–2669.

Nath, KA (2006). Heme oxygenase-1: a provenance for cytoprotective pathways in the kidney and other tissues. *Kidney Int* 70: 432-43.

Nisoli, E, Tonello, C, Cardile, A, Cozzi, V, Bracale, R, Tedesco, L, Falcone, S, Valerio, A, Cantoni, O, Clementi, E, Moncada, S, Carruba, MO (2005). Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 310: 314-7.

Oakhill, JS, Scott, JW & Kemp, BE (2009). Structure and function of AMP-activated protein kinase. *Acta Physiol (Oxf)* 196: 3-14.

Palacios, OM, Carmona, JJ, Michan, S, Chen, KY, Manabe, Y, Ward, JL,3rd, Goodyear, LJ, Tonq, Q (2009). Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. *Aging (Albany NY)* 1: 771-83.

Pallone, TL (2006). Is oxidative stress differentially regulated in the renal cortex and medulla? *Nat Clin Pract Nephrol* 2: 118-9.

Park, EJ, Kim, YM, Park, SW, Kim, HJ, Lee, JH, Lee, DU, Canq, KC (2013). Induction of HO-1 through p38 MAPK/Nrf2 signaling pathway by ethanol extract of Inula helenium L. reduces inflammation in LPS-activated RAW 264.7 cells and CLP-induced septic mice. *Food Chem Toxicol* 55: 386-95.

Park, SW, Chen, SW, Kim, M, Brown, KM, Kolls, JK, D'Agati, VD, Lee, HT (2011). Cytokines induce small intestine and liver injury after renal ischemia or nephrectomy. *Lab Invest* 91: 63-84.

Patten, IS, Arany, Z (2012). PGC-1 coactivators in the cardiovascular system. *Trends Endocrinol Metab* 23: 90-7.

Peairs, A, Radjavi, A, Davis, S, Li, L, Ahmed, A, Giri, S, Reilly, CM (2009). Activation of AMPK inhibits inflammation in MRL/lpr mouse mesangial cells. *Clin Exp Immunol* 156: 542-51.

Pechanova, O, Simko, F (2007). The role of nitric oxide in the maintenance of vasoactive balance. *Physiol Res* 56 Suppl 2: S7-S16.

Peralta, C, Bartrons, R, Serafin, A, Blazquez, C, Guzman, M, Prats, N, Xaus, C, Cutillas, B, Gelpí, E, Roselló-Catafau, J (2001). Adenosine monophosphate-activated protein kinase mediates the protective effects of ischemic preconditioning on hepatic ischemia-reperfusion injury in the rat. *Hepatology* 34: 1164-73.

Percy, CJ, Brown, L, Power, DA, Johnson, DW & Gobe, GC (2009). Obesity and hypertension have differing oxidant handling molecular pathways in age-related chronic kidney disease. *Mech Ageing Dev* 130: 129-38.

Periyasamy-Thandavan, S, Jiang, M, Schoenlein, P & Dong, Z (2009). Autophagy: molecular machinery, regulation, and implications for renal pathophysiology. *Am J Physiol Renal Physiol* 297: F244-56.

Pertovaara, A, Wei, H (2000). Attenuation of ascending nociceptive signals to the rostroventromedial medulla induced by a novel alpha2-adrenoceptor agonist, MPV-2426, following intrathecal application in neuropathic rats. *Anesthesiology* 92: 1082-92.

Picard, F, Kurtev, M, Chung, N, Topark-Ngarm, A, Senawong, T, Machado De Oliveira, R, Leid, M, McBurney, MW, Guarente, L (2004). Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature* 429: 771-6.

Pillai, VB, Sundaresan, NR, Kim, G, Gupta, M, Rajamohan, SB, Pillai, JB, Samant, S, Ravindra, PV, Isbatan, A, Gupta, MP (2010). Exogenous NAD blocks cardiac hypertrophic response via activation of the SIRT3-LKB1-AMP-activated kinase pathway. *J Biol Chem* 285: 3133-44.

Potente, M, Dimmeler, S (2008). Emerging roles of SIRT1 in vascular endothelial homeostasis. *Cell Cycle* 7: 2117-22.

Prakash, J, de Borst, MH, Lacombe, M, Opdam, F, Klok, PA, van Goor, H, Meijer, DK, Moolenaar, F, Poelstra, K, Kok, RJ (2008). Inhibition of renal rho kinase attenuates ischemia/reperfusion-induced injury. *J Am Soc Nephrol* 19: 2086-97.

Prowle, JR, Echeverri, JE, Ligabo, EV, Ronco, C & Bellomo, R (2010). Fluid balance and acute kidney injury. *Nat Rev Nephrol* 6: 107-15.

Pulla VK, Battu MB, Alvala M, Sriram D, Yogeeswari P (2012). Can targeting SIRT-1 to treat type 2 diabetes be a good strategy? A review. *Expert Opin Ther Targets.* Aug;16(8):819-32.

Rabelink, TJ, de Boer, HC & van Zonneveld, AJ (2010). Endothelial activation and circulating markers of endothelial activation in kidney disease. *Nat Rev Nephrol* 6: 404-14.

Ravikumar, B, Sarkar, S, Davies, JE, Futter, M, Garcia-Arencibia, M, Green-Thompson, ZW, Jimenez-Sanchez, M, Korolchuk, VI, Lichtenberg, M, Luo, S, Massey, DC,Menzies, FM, Moreau, K, Narayanan, U, Renna, M, Siddiqi, FH, Underwood, BR, Winslow, AR, Rubinsztein, DC (2010). Regulation of mammalian autophagy in physiology and pathophysiology. *Physiol Rev* 90: 1383-435.

Ribeiro, RC, Pui, CH (2003). Recombinant urate oxidase for prevention of hyperuricemia and tumor lysis syndrome in lymphoid malignancies. *Clin Lymphoma* 3: 225-32.

Rodgers, JT, Lerin, C, Haas, W, Gygi, SP, Spiegelman, BM & Puigserver, P (2005). Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* 434: 113-8.

Rojas, A, Figueroa, H, Re, L & Morales, MA (2006). Oxidative stress at the vascular wall. Mechanistic and pharmacological aspects. *Arch Med Res* 37: 436-48.

Rubinsztein, DC, Marino, G & Kroemer, G (2011). Autophagy and aging. *Cell* 146: 682-95.

Ruderman, NB, Xu, XJ, Nelson, L, Cacicedo, JM, Saha, AK, Lan, F, Ido, Y (2010). AMPK and SIRT1: a long-standing partnership? *Am J Physiol Endocrinol Metab* 298: E751-60.

Russell, RR,3rd, Li, J, Coven, DL, Pypaert, M, Zechner, C, Palmeri, M, Giordano, FJ, Mu, J, Birnbaum, MJ, Young, LH (2004). AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury. *J Clin Invest* 114: 495-503.

Sabbahy, ME, Vaidya, VS (2010). Ischemic kidney injury and mechanisms of tissue repair. *Wiley Interdiscip Rev Syst Biol Med*, 10.

Sampath, S, Moran, JL, Graham, PL, Rockliff, S, Bersten, AD & Abrams, KR (2007). The efficacy of loop diuretics in acute renal failure: assessment using Bayesian evidence synthesis techniques. *Crit Care Med* 35: 2516-24.

Sanders, MJ, Grondin, PO, Hegarty, BD, Snowden, MA & Carling, D (2007). Investigating the mechanism for AMP activation of the AMP-activated protein kinase cascade. *Biochem J* 403: 139-48.

Schetz, M (2004). Should we use diuretics in acute renal failure? *Best Pract Res Clin Anaesthesiol* 18: 75-89.

Schlaich, MP, Hering, D, Sobotka, PA, Krum, H & Esler, MD (2012). Renal Denervation in Human Hypertension: Mechanisms, Current Findings, and Future Prospects. *Curr Hypertens Rep*, 10.

Schmitz, JM, Graham, RM, Sagalowsky, A & Pettinger, WA (1981). Renal alpha-1 and alpha-2 adrenergic receptors: biochemical and pharmacological correlations. *J Pharmacol Exp Ther* 219: 400-6.

Schreiber, E, Matthias, P, Muller, M & Schaffner, M (1989). Rapid detection of octamer binding proteins with 'mini-extracts' prepared from a small number of cells. *Nucleic. Acids Res.* 17:6419.

Scott, JW, Hawley, SA, Green, KA, Anis, M, Stewart, G, Scullion, GA, Norman, DG, Hardie, DG (2004). CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. *J Clin Invest* 113: 274-84.

Seo-Mayer, PW, Thulin, G, Zhang, L, Alves, DS, Ardito, T, Kashgarian, M, Caplan, MJ (2011). Preactivation of AMPK by metformin may ameliorate the epithelial cell damage caused by renal ischemia. *Am J Physiol Renal Physiol* 12;301(6).

Shan, L, Li, J, Wei, M, Ma, J, Wan, L, Zhu, W, Li, Y, Zhu, H, Arnold, JM, Peng, T (2010). Disruption of Rac1 signaling reduces ischemia-reperfusion injury in the diabetic heart by inhibiting calpain. *Free Radic Biol Med* 49: 1804-14.

Sharfuddin, AA, Molitoris, BA (2011). Pathophysiology of ischemic acute kidney injury. *Nat Rev Nephrol* 7: 189-200.

Shoag, J, Arany, Z (2010). Regulation of hypoxia-inducible genes by PGC-1 alpha. *Arterioscler Thromb Vasc Biol* 30: 662-6.

Simmons, EM, Himmelfarb, J, Sezer, MT, Chertow, GM, Mehta, RL, Paganini, EP, Soroko, S, Freedman, S, Becker, K, Spratt, D, Shyr, Y, Ikizler, TA; PICARD Study Group (2004). Plasma cytokine levels predict mortality in patients with acute renal failure. *Kidney Int* 65: 1357-65.

Smith, DL,Jr, McClure, JM, Matecic, M & Smith, JS (2007). Calorie restriction extends the chronological lifespan of Saccharomyces cerevisiae independently of the Sirtuins. *Aging Cell* 6: 649-62.

Song, JW, Shim, JK, Yoo, KJ, Oh, SY & Kwak, YL (2013). Impact of intraoperative hyperglycaemia on renal dysfunction after off-pump coronary artery bypass. *Interact Cardiovasc Thorac Surg* 17: 473-8.

Speakman, JR, Mitchell, SE (2011). Caloric restriction. *Mol Aspects Med* 32: 159-221.

Spiegelman, BM (2007). Transcriptional control of mitochondrial energy metabolism through the PGC1 coactivators. *Novartis Found Symp* 287: 60,3; discussion 63-9.

Sprague, AH, Khalil, RA (2009). Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochem Pharmacol* 78: 539-52.

Spurgeon, KR, Donohoe, DL & Basile, DP (2005). Transforming growth factor-beta in acute renal failure: receptor expression, effects on proliferation, cellularity, and vascularization after recovery from injury. *Am J Physiol Renal Physiol* 288: 568-77.

Sutton, TA, Molitoris, BA (1998). Mechanisms of cellular injury in ischemic acute renal failure. *Semin Nephrol* 18: 490-7.

Talukder, MA, Elnakish, MT, Yang, F, Nishijima, Y, Alhaj, MA, Velayutham, M, Hassanain, HH, Zweier, JL (2013). Cardiomyocyte-specific overexpression of an active form of Rac predisposes the

heart to increased myocardial stunning and ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 304: H294-302.

Tang, G, Yue, Z, Talloczy, Z, Hagemann, T, Cho, W, Messing, A, Sulzer, DL, Goldman, JE (2008). Autophagy induced by Alexander disease-mutant GFAP accumulation is regulated by p38/MAPK and mTOR signaling pathways. *Hum Mol Genet* 17: 1540-55.

Taniyama, Y, Griendling, KK (2003). Reactive oxygen species in the vasculature: molecular and cellular mechanisms. *Hypertension* 42: 1075-81.

Taoda, M, Adachi, YU, Uchihashi, Y, Watanabe, K, Satoh, T & Vizi, ES (2001). Effect of dexmedetomidine on the release of [3H]-noradrenaline from rat kidney cortex slices: characterization of alpha2-adrenoceptor. *Neurochem Int* 38: 317-22.

Tawa, M, Fukumoto, T, Yamashita, N, Ohkita, M, Ayajiki, K, Okamura, T, Matsumura, Y (2010). Postconditioning improves postischemic cardiac dysfunction independently of norepinephrine overflow after reperfusion in rat hearts: comparison with preconditioning. *J Cardiovasc Pharmacol* 55: 6-13.

Terai, K, Hiramoto, Y, Masaki, M, Sugiyama, S, Kuroda, T, Hori, M, Kawase, I, Hirota, H (2005). AMP-activated protein kinase protects cardiomyocytes against hypoxic injury through attenuation of endoplasmic reticulum stress. *Mol Cell Biol* 25: 9554-75, 10.1128/MCB.25.21.9554-9575.2005.

Thadhani, R, Pascual, M & Bonventre, JV (1996). Acute renal failure. *N Engl J Med* 334: 1448-60.

Thomson, SC, Vallon, V (1995). Alpha 2-adrenoceptors determine the response to nitric oxide inhibition in the rat glomerulus and proximal tubule. *J Am Soc Nephrol* 6: 1482-90.

Tsutsui, H, Tanaka, R, Yamagata, M, Yukimura, T, Ohkita, M & Matsumura, Y (2013). Protective effect of ischemic preconditioning on ischemia/reperfusion-induced acute kidney injury through sympathetic nervous system in rats. *Eur J Pharmacol*, 10.

Tufek, A, Tokgoz, O, Aliosmanoglu, I, Alabalik, U, Evliyaoglu, O, Ciftci, T, Güzel, A, Yıldırım, ZB (2013). The protective effects of dexmedetomidine on the liver and remote organs against hepatic ischemia reperfusion injury in rats. *Int J Surg* 11: 96-100.

Vaidya, VS, Waikar, SS, Ferguson, MA, Collings, FB, Sunderland, K, Gioules, C, Bradwin, G, Matsouaka, R, Betensky, RA, Curhan, GC, Bonventre, JV (2008). Urinary biomarkers for sensitive and specific detection of acute kidney injury in humans. *Clin Transl Sci* 1: 200-8.

van Ginhoven, TM, de Bruin, RW, Timmermans, M, Mitchell, JR, Hoeijmakers, JH & Ijzermans, JN (2010). Pre-operative dietary restriction is feasible in live-kidney donors. *Clin Transplant*, 10.

Viana, R, Aguado, C, Esteban, I, Moreno, D, Viollet, B, Knecht, E, Sanz, P (2008). Role of AMP-activated protein kinase in autophagy and proteasome function. *Biochem Biophys Res Commun* 369: 964-8.

Waikar, SS, Liu, KD & Chertow, GM (2008). Diagnosis, epidemiology and outcomes of acute kidney injury. *Clin J Am Soc Nephrol* 3: 844-61.

Wang, Z, Gall, JM, Bonegio, RG, Havasi, A, Hunt, CR, Sherman, MY, Schwartz, JH, Borkan, SC (2011). Induction of heat shock protein 70 inhibits ischemic renal injury. *Kidney Int* 79: 861-70.

Wang L, Wu CG, Fang CQ, Gao J, Liu YZ, Chen Y, Chen YN, Xu ZG (2013). The protective effect of α-Lipoic acid on mitochondria in the kidney of diabetic rats.*Int J Clin Exp Med*.;6(2):90-7.

Williams, A, Sarkar, S, Cuddon, P, Ttofi, EK, Saiki, S, Siddiqi, FH, Jahreiss, L, Fleming, A, Pask, D, Goldsmith, P, O'Kane, CJ, Floto, RA, Rubinsztein, DC (2008). Novel targets for Huntington's disease in an mTOR-independent autophagy pathway. *Nat Chem Biol* 4: 295-305.

Willigers, HM, Prinzen, FW, Roekaerts, PM, de Lange, S & Durieux, ME (2003). Dexmedetomidine decreases perioperative myocardial lactate release in dogs. *Anesth Analg* 96: 657-64.

Winder, WW, Holmes, BF, Rubink, DS, Jensen, EB, Chen, M & Holloszy, JO (2000). Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol* 88: 2219-26.

Wong, A, Howie, J, Petrie, J, Lang, C (2009).AMP-activated protein kinase pathway: a potential therapeutic target in cardiometabolic disease. *Clin Sci (Lond)*:116:607-20.

Woods, A, Johnstone, SR, Dickerson, K, Leiper, FC, Fryer, LG, Neumann, D, Schlattner, U, Wallimann, T, Carlson, M, Carling, D (2003). LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol* 13: 2004-8.

Wu, X, Motoshima, H, Mahadev, K, Stalker, TJ, Scalia, R & Goldstein, BJ (2003). Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. *Diabetes* 52: 1355-63.

Xie, M, Zhang, D, Dyck, JR, Li, Y, Zhang, H, Morishima, M, Mann, DL, Taffet, GE, Baldini, A, Khoury, DS, Schneider, MD (2006). A pivotal role for endogenous TGF-beta-activated kinase-1 in the LKB1/AMP-activated protein kinase energy-sensor pathway. *Proc Natl Acad Sci U S A* 103: 17378-83.

Xu, H, Aibiki, M, Seki, K, Ogura, S & Ogli, K (1998). Effects of dexmedetomidine, an alpha2-adrenoceptor agonist, on renal sympathetic nerve activity, blood pressure, heart rate and central venous pressure in urethane-anesthetized rabbits. *J Auton Nerv Syst* 71: 48-54.

Yamauchi, T, Kamon, J, Ito, Y, Tsuchida, A, Yokomizo, T, Kita, S, Sugiyama, T, Miyagishi, M, Hara, K, Tsunoda, M, Murakami, K, Ohteki, T, Uchida, S, Takekawa, S, Waki, H, Tsuno, NH, Shibata, Y, Terauchi, Y, Froguel, P, Tobe, K, Koyasu, S, Taira, K, Kitamura, T, Shimizu, T, Nagai, R, Kadowaki, T (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423: 762-9, 10.

Yamauchi, T, Kamon, J, Minokoshi, Y, Ito, Y, Waki, H, Uchida, S, Yamashita, S, Noda, M, Kita, S, Ueki, K, Eto, K, Akanuma, Y, Froguel, P, Foufelle, F, Ferre, P, Carling, D,Kimura, S, Nagai, R, Kahn, BB, Kadowaki, T (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 8: 1288-95.

Yilmaz, MI, Saglam, M, Carrero, JJ, Qureshi, AR, Caglar, K, Eyileten, T, Sonmez, A, Cakir, E, Yenicesu, M, Lindholm, B, Stenvinkel, P, Axelsson, J (2008). Serum visfatin concentration and endothelial dysfunction in chronic kidney disease. *Nephrol Dial Transplant* 23: 959-65.

Yu, ZF, Mattson, MP (1999). Dietary restriction and 2-deoxyglucose administration reduce focal ischemic brain damage and improve behavioral outcome: evidence for a preconditioning mechanism. *J Neurosci Res* 57: 830-9.

Zacharias, M, Mugawar, M, Herbison, GP, Walker, RJ, Hovhannisyan, K, Sivalingam, P, Conlon, NP (2013). Interventions for protecting renal function in the perioperative period. *Cochrane Database Syst Rev* 9.

Zhang, D, Li, S, Cruz, P & Kone, BC (2009). Sirtuin 1 functionally and physically interacts with disruptor of telomeric silencing-1 to regulate alpha-ENaC transcription in collecting duct. *J Biol Chem* 284: 20917-26.

Zhou, G, Sebhat, IK & Zhang, BB (2009). AMPK activators--potential therapeutics for metabolic and other diseases. *Acta Physiol (Oxf)* 196: 175-90.

Zou, MH, Hou, XY, Shi, CM, Kirkpatick, S, Liu, F, Goldman, MH, Cohen, RA (2003). Activation of 5'-AMP-activated kinase is mediated through c-Src and phosphoinositide 3-kinase activity during hypoxia-reoxygenation of bovine aortic endothelial cells. Role of peroxynitrite. *J Biol Chem* 278: 34003-10.

Zuk, A, Bonventre, JV & Matlin, KS (2001). Expression of fibronectin splice variants in the postischemic rat kidney. *Am J Physiol Renal Physiol* 280: F1037-53.