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- Till min familj -

ABSTRACT

The acute surgical stress response induces a short-term inflammatory and insulin resistant condition, commonly producing hyperglycemia, which is strongly associated to morbidity and mortality in postsurgical patients. The chronic state of renal insufficiency, on the other hand, represents a long-term stress associated with alarmingly high mortality rates, primarily due to cardiovascular complications, and is similarly related to chronic inflammation, oxidative stress, and insulin resistance. The underlying mechanisms are however incompletely understood. A growing wealth of data emphasizes how mutual relationships between immunity and metabolism may contribute to disease, as well as the intrinsic properties of metabolically active organs, such as adipose tissue and skeletal muscle. Hence, in this translational study, we aimed to identify inflammatory and metabolic mediators being dysregulated and potentially contributing to homeostatic disturbances after surgery and in chronic kidney disease (CKD) patients. In **paper I**, we evaluated the expression changes of 45 inflammatory and insulin signaling genes in skeletal muscle during major abdominal surgery of eight non-diabetic patients. In **paper II,** mRNA measurements of 21 genes were performed in 12 patients undergoing surgery on two separate adipose tissue depots: abdominal subcutaneous and omental adipose tissue. Overall, although some tissue specific alterations were observed, striking similarities were noted between the tissues. For example, we observed significantly increased mRNA levels of inflammatory signaling genes (e.g. interleukin 6 (*IL6*), suppressor of cytokine signaling 3 (*SOCS3*) and nicotinamide phosphoribosyltransferase (*NAMPT*)) in all tissues examined, but tumor necrosis factor (*TNF*) was only up-regulated in skeletal muscle. Insulin signaling pathway genes (e.g. insulin receptor substrate 1 (*IRS1*) and glucose transporter 4 (*SLC2A4*)), were only significantly affected in the adipose tissue depots. **Paper III and IV** focused on abdominal subcutaneous adipose tissue. Gene expression levels were compared between CKD stage 5 patients and non-uremic controls to reveal uremic specific alterations. In accordance with surgical patients, adipose tissue from CKD patients showed a significant up-regulation of inflammatory pathway genes such as *IL6* and *SOCS3*. Additionally, leptin and the oxidative stress-related genes uncoupling protein-2 (*UCP2*) and cytochrome b-245, alpha polypeptide (*CYBA*) were found to be down-regulated in relation to controls. Interestingly, we found reduced mRNA levels of the bone-associated factor osteoprotegerin (OPG), with reported implications for inflammation, vascular diseases and mortality, in uremic adipose tissue as compared to control tissue, despite higher OPG serum protein concentrations in patients and no apparent immunohistochemical differences. These observations suggest that aberrant gene expression of inflammatory and oxidative stress genes, as well as genes implicated in the control of vascular calcification, may be important features of the uremic adipose tissue, which may have significant effects on the uremic phenotype. In summary, these studies contribute to our understanding of human gene expression alterations in association to clinical conditions producing acute and chronic stress, respectively, which may have implications for inflammatory and metabolic complications observed after surgery and in CKD. These results may also be relevant for other disorders in which pronounced inflammation and metabolic disturbances exacerbate the disease state, such as obesity and diabetes.

LIST OF PUBLICATIONS

I. **Witasp A,** Nordfors L, Schalling M, Nygren J, Ljungqvist O, Thorell A. Increased expression of inflammatory pathway genes in skeletal muscle during surgery. Clinical Nutrition 2009; 28(3): 291-298.

II. **Witasp A,** Nordfors L, Schalling M, Nygren J, Ljungqvist O, Thorell A. Expression of inflammatory and insulin signaling genes in adipose tissue in response to elective surgery. Journal of Clinical Endocrinology and Metabolism 2010; doi: 10.1210/jc.2009-2588.

III. **Witasp A,** Carrero JJ, Heimbürger O, Lindholm B, Hammarqvist F, Stenvinkel P, Nordfors L. Increased expression of pro-inflammatory genes in abdominal subcutaneous fat in chronic kidney disease stage 5 patients. Submitted for publication.

IV. **Witasp A*,** Carrero JJ*, Hammarqvist F, Qureshi AR, Heimbürger O, Schalling M, Lindholm B, Nordfors L, Stenvinkel P. Expression of osteoprotegerin in human fat tissue; implications for chronic kidney disease. Submitted for publication.

* Both authors contributed equally to this work.

ADDITIONAL PUBLICATIONS

I. Axelsson J, Møller HJ, **Witasp A,** Qureshi AR, Carrero JJ, Heimbürger O, Bárány P, Alvestrand A, Lindholm B, Moestrup SK, Stenvinkel P. Changes in fat mass correlate with changes in soluble sCD163, a marker of mature macrophages, in patients with CKD. American Journal of Kidney Diseases 2006; 48(6): 916-25.

II. Axelsson J*, **Witasp A*,** Carrero JJ, Qureshi AR, Suliman ME, Heimbürger O, Bárány P, Lindholm B, Alvestrand A, Schalling M, Nordfors L, Stenvinkel P. Circulating levels of visfatin/pre-B-cell colony-enhancing factor 1 in relation to genotype, GFR, body composition, and survival in patients with CKD. American Journal of Kidney Diseases 2007; 49(2): 237-44.

* Both authors contributed equally to this work.

III. **Witasp A**, Nordfors L, Lindholm B, Stenvinkel P. Use of single-nucleotide polymorphisms in the search for genetic modifiers of the uremic phenotype. J Ren Nutr. 2007;17(1):17-22. Review

IV. Carrero JJ, **Witasp A**, Stenvinkel P, Qureshi AR, Heimbürger O, Bárány P, Suliman ME, Anderstam B, Lindholm B, Nordfors L, Schalling M, Axelsson J. Visfatin is increased in chronic kidney disease patients with poor appetite and correlates negatively with fasting serum amino acids and triglyceride levels. Nephrology Dialysis Transplantation 2010; 25(3): 901-6.

CONTENTS

LIST OF ABBREVIATIONS

ADIPOQ	Adiponectin
ADIPOQR	Adiponectin receptor
AGT	Angiotensinogen
AHSG	Alpha-2-HS-glycoprotein
BMI	Body mass index
CKD	Chronic kidney disease
CRP	C-reactive protein
C_t	Threshold cycle
CVD	Cardiovascular disease
CYBA	Cytochrome b-245, alpha polypeptide
DM	Diabetes mellitus
EMR1	Egf-like module containing mucin-like, hormone receptor-like 1
ESRD	End-stage renal disease
FFA	Free fatty acids
GFR	Glomerular filtration rate
GLUT4	Glucose transporter 4
HD	Hemodialysis
hsCRP	High sensitivity CRP
IGF1R	Insulin-like growth factor 1 receptor
IKBKB	Inhibitor of kappa light polypeptide gene enhancer in B cells, kinase beta
IL	Interleukin
ILR	Interleukin receptor
IRS1	Insulin receptor substrate 1
LEP	Leptin
LEPR	Leptin receptor
MSR1	Macrophage scavange receptor 1
NAMPT	Nicotinamide phosphoribosyltransferase
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells
OPG	Osteoprotegerin
PBEF1	Pre-B-cell colony enhancing factor 1
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PD	Peritoneal dialysis
PIK3R1	Phosphoinositide-3-kinase, regulatory subunit 1 p85 alpha
PPARD	Peroxisome proliferator-activated receptor delta
ROS	Reactive oxygen species
RRT	Renal replacement therapy
SAT	Subcutaneous adipose tissue
SERPINE1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
SGA	Subjective global assessment
SLC2A	Solute carrier family 2, facilitated glucose transporter

SOCS3	Suppressor of cytokine signaling 3
STAT3	Signal transducer and activator of transcription 3
TG	Triglycerides
TNF	Tumor necrosis factor
UCP2	Uncoupling protein 2
VAT	Visceral adipose tissue
VCAM-1	Vascular cell adhesion molecule-1

1 INTRODUCTION

The focus of this thesis is stress exposures, here represented by the acute challenge of a surgical trauma or the chronic state of renal impairment, and their role on metabolic dysfunctions. For this purpose, the current thesis involves analyses of patients undergoing major gastrointestinal surgery as well as patients with chronic renal failure in an attempt to identify common molecular stress mechanisms that distort vital physiological systems in these patients. The rationale behind this study outline of including two seemingly different patient populations, exposed to two distinct categories of stress conditions, is that both groups experience pronounced inflammatory activities and similar metabolic disturbances.

It is a well known fact that the acute surgical stress response induces inflammation and a transient state of insulin resistance. Similarly the chronic state of renal insufficiency, representing long-term stress is related to chronic inflammation, oxidative stress, and insulin resistance. In these scenarios it is likely that various inflammatory and metabolic mediators are dysregulated, contributing to both systemic and local disturbances. Hence, a selection of such putative mediators is being studied in this thesis in the context of two important metabolically active tissues; adipose tissue and skeletal muscle. In particular, adipose tissue with its intrinsic properties constitutes an excellent example of how immunological and metabolic pathways are highly integrated and how deviations from their delicate balance may result in pathophysiological conditions.

Efforts to minimize stress-related complications are associated with beneficial effects on recovery and outcome of the patient, and it is therefore crucial to gain increased knowledge of the underlying molecular mechanisms. The thesis strategy of conducting parallel gene expression studies on different patient populations subjected to acute or long-term stress, as well as on different tissues, provides new possibilities for translational comparisons and the identification of a common mechanistic platform. Our ultimate goal is that the data generated in this thesis could contribute to a deepened understanding of the molecular events preceding metabolic aberrations that influence patient outcome in various types of physical stress.

1.1 STRESS RESPONSE AFTER ELECTIVE SURGERY AND IN CKD PATIENTS

1.1.1 Acute stress through surgical injury

It is estimated that 5% of the Swedish population undergo elective surgery each year, making it one of the most commonly performed medical treatments and the most common physical trauma (Thorell et al., 1999b). Metabolic alterations in response to elective surgical procedures, such as major abdominal surgery and hip replacement, have been studied to some extent over the last decades (Ljungqvist et al., 2005). Similar to other types of trauma, such as accidental trauma, burn injury and sepsis, elective surgery evokes a switch from a normal state to "post-injury metabolism". Post-injury metabolism is characterized by a state of catabolism and suppression of anabolic reactions with the overall purpose to secure supply of glucose to vital energy demanding organs such as the brain (Cuthbertson, 1942). This catabolic state promotes hepatic glycogen breakdown with release of glucose to the bloodstream, protein breakdown and reduced protein synthesis in skeletal muscle, as well as adipose tissue lipolysis. The subsequent release of free amino acids and fatty acids from muscle and adipose tissue, respectively, will serve as important substrates for glucose production (gluconeogenesis), further contributing to the glucose supply to vital glucose-dependent organs.

During the catabolic phase the metabolic effects of insulin are attenuated and glucose tolerance is impaired (Brandi et al., 1990). In all, this generates a hyperglycemic condition and it has been shown that there is a positive correlation between the degree of hyperglycemia and the magnitude of the trauma exposed to the patient (Stoner et al., 1979). This is important since hyperglycemia increases the risk for complications such as postoperative wound infections (Fietsam et al., 1991) and impairs recovery (Van den Berghe et al., 2003). Moreover, a pronounced or prolonged stress-induced catabolism, increasing the risk for complications, may, in turn, increase the inflammatory and endocrine stress responses, which may further delay recovery. Due to similarities between stress-induced hyperglycemia and diabetes, post-injury metabolism has been referred to as "diabetes of injury" (Khaodhiar et al., 1999). Since outcome after surgery depends on how well the patients can cope with metabolic disturbances (Hill et al., 1993; van den Berghe et al., 2001) more knowledge about the molecular events provoked by the surgical injury, contributing to the metabolic aberrations, is needed. Considering that plasma concentrations of classical stress response hormones, such as glucagon, adrenaline, noradrenaline, cortisol and growth hormone, are only modestly increased immediately after surgery (Thorell et al., 1993; Thorell et al., 1996) the pursuit for additional mediators has been an ongoing mission during the last decade.

1.1.2 Chronic stress via uremic imbalance

One of the primary functions of the kidneys is to remove metabolic waste products and noxious substances from the plasma via urine excretion, meaning that when renal function is impaired these substances accumulate in the circulation. Various pathological conditions cause renal insufficiency, which may either occur temporarily or turn into an irreversible, chronic condition. The Third National Health and Nutrition

Examination Survey (NHANES III) has estimated that up to 11% of the general US adult population suffer from chronic kidney disease (CKD) in different stages (Coresh et al., 2003). Such comprehensive epidemiological data is not available for the Swedish population, but as of December 2008 it was estimated that 8029 patients were on renal replacement therapy (RRT) such as dialysis or transplantation (Svenskt Njurregister). However, it should be emphasized that this applies only to patients with terminal CKD, termed CKD stage 5 or end-stage renal disease (ESRD), when the glomerular filtration rate (GFR) of the kidneys has reached below 15 ml/min/1.73 m^2. The main bulk of CKD patients are in fact in CKD stage 1 to 4, defined by a GFR > 90 ml/min/1.73 m^2; 60-89 ml/min/1.73 m^2; 30-59 ml/min/1.73 m^2; or 15–29 ml/min/1.73 m^2, respectively, and by definition not yet eligible of RRT. Only 0.2% of the NHANES III study population were in CKD stage 5, as compared to 3.3% in CKD stage 1, 3.0% in CKD stage 2, 4.3% in CKD stage 3 and 0.2% in CKD stage 4 (Coresh et al., 2003). In addition, RRT is usually not initiated in patients with CKD 5 before GFR has fallen below 10 ml/min/1.73 m^2. Therefore, the number of patients on RRT markedly underestimates the number of patients with CKD-5.

The etiology of kidney failure is largely heterogeneous, the most common causes in Sweden being glomerulonephritis and diabetic nephropathy (Svenskt Njurregister). However, irrespective of primary disease this population is greatly burdened by high rates of morbidities and increased mortality risk. It is noticeable that the survival of a patient with CKD-5 is comparable to that of a patient with malignant disease and, in fact, a large number of patients die, primarily due to cardiovascular diseases (CVD), even before they reach advanced CKD stages (Foley et al., 2005; National Kidney Foundation, 2002). Although there are still many unknowns, it has been suggested that age, smoking, hypertension, left ventricular hypertrophy, dyslipidemia and diabetes mellitus (Muntner et al., 2005), as well as the prevailing state of oxidative stress and low-grade chronic inflammation, probably triggered by the renal insufficiency, contribute to the elevated CVD risk in CKD patients (Himmelfarb et al., 2002; Shlipak et al., 2005). It is also of interest to note that patients reaching advanced chronic kidney disease display a spectrum of pathological alterations characteristic for the metabolic syndrome, including chronic low-grade inflammation (Stenvinkel et al., 2005), and accelerated atherosclerosis (Wang et al., 2005) as well as metabolic aberrations such as dyslipidemia (Beddhu et al., 2005) and insulin resistance (DeFronzo et al., 1981).

1.2 INFLAMMATION DUE TO SURGICAL INJURY OR UREMIC IMBALANCE

1.2.1 Inflammation and inflammatory mediators

Inflammation is traditionally described as the acute response to infection and tissue injury, manifested as swelling (tumor), redness (rubor), pain (dolor) and heat (calor) (Larsen and Henson, 1983). This chain of reactions to withstand noxious stimuli, such as pathogens and injury, is highly essential for the individual's survival. The associated immunological response is complex, involving a vast network of cells at the site of injury, systemic immune cells and inflammatory mediators produced by these cells.

Cytokines constitute an important group of secreted polypeptides or glycoproteins, acting primarily by paracrine and/or autocrine mechanisms. Cytokines such as tumor necrosis factor (TNF), interleukin (IL) 1 and 6 bind to specific cell surface receptors which, via signal transduction pathways exert their diverse roles in the immune response, including activation of the endothelium, leukocytes and the acute-phase response (Spink and Cohen, 1997). Moreover, cytokines influence differentiation, proliferation and survival of immune cells and the production of other cytokines, thus contributing to the maintenance of crucial homeostatic mechanisms. Under some circumstances, however, these immunological mechanisms may be detrimental, which is seen in the exaggerated response leading to sepsis, or when a sustained response develops into a chronic inflammatory state such as rheumatoid arthritis, with consequences such as tissue destruction, organ failure or metabolic aberrations (Medzhitov, 2008; Sattar et al., 2003). The relation between metabolic diseases, such as obesity and diabetes, and inflammation has recently attracted much interest. In this context, the link between inflammation and insulin resistance is particularly intriguing (Wellen and Hotamisligil, 2005).

1.2.2 Surgical injury imposes an acute inflammatory reaction

Surgery involves by definition a tissue injury and the associated inflammatory response is required in order to enable proper wound healing and restoration of homeostasis. During major surgery, the induction of a series of inflammatory responses mediated by cytokines such as IL1B, IL6, IL8 and TNF has been registered. The cytokine response occurs rapidly after injury, reflecting active gene transcription and translation by the injured or stimulated cells (Lin et al., 2000). Two of the earliest and most potent mediators of the host response to trauma are TNF and IL6 (Lin et al., 2000). TNF mediates its biological effects mainly through activation of the tumor necrosis factor receptor 1 (TNFRSF1A) (Lin et al., 2000) and crucial TNF-mediated events include the activation of the proinflammatory transcription factor NFKB and the subsequent stimulation of other cytokine cascades, such as the IL6 pathway. IL6 is consistently increased in the circulation after injury (Van Snick, 1990), being detectable already within 30 minutes after abdominal surgery (Baigrie et al., 1992; Ohzato et al., 1992; Shenkin et al., 1989). The increments in IL6 levels are related to the degree of the surgical trauma. Thus, the duration (Cruickshank et al., 1990; Shenkin et al., 1989) as well as the magnitude of the surgical procedure (Baigrie et al., 1992; Cruickshank et al., 1990) seem to determine circulating IL6 levels. The effects of IL6 are mediated by the activation of the IL6 receptor complex and subsequent signal transducing molecules, JAK/STAT, regulating gene transcription and thus essential cellular activities (Spink and Cohen, 1997).

A connection between several cytokines, elevated after trauma or surgery, and stress metabolism have been reported (Abraham and Regan, 1985; Baigrie et al., 1992; Cruickshank et al., 1990; Okusawa et al., 1988; Shenkin et al., 1989; Spinas et al., 1986). Cytokines may also contribute to oxidative stress in the trauma situation by up-regulating non-insulin dependent glucose transporters which induce glucose overload in cells in the presence of hyperglycemia (Shikhman et al., 2001). Glucose overload, resulting in glucose toxicity and increased formation of reactive oxygen species (ROS), mediates deleterious effects such as altered gene expression and release of additional

pro-inflammatory mediators in non-insulin dependent cells (Brownlee, 2003). Thus, cytokines as well as mediators of oxidative stress and oxidative stress responsive transcription factors are likely candidate genes in pathways resulting in metabolic derangements associated with surgical stress.

1.2.3 CKD imposes a chronic low-grade inflammation

CKD is associated with a state of low-grade chronic inflammation characterized by systemically elevated proinflammatory cytokines, such as IL6 and TNF, or altered levels of acute-phase proteins, such as C-reactive protein (CRP) (Stenvinkel et al., 2002; Stenvinkel et al., 1999), which increase along with declining renal function (Eustace et al., 2004). This suggests either a decreased clearance or increased production of proinflammatory mediators, potentially triggered by the renal impairment *per se* (Eustace et al., 2004). Additionally, infections, comorbidities, genetic and dietary factors may contribute to the inflammatory state (Carrero et al., 2008). Regardless of the underlying causes however, inflammation is an independent predictor of all-cause and cardiovascular mortality in CKD, and thus likely a main contributor to the number of premature deaths in CKD (Pecoits-Filho et al., 2004; Stenvinkel et al., 2008).

CRP is one of the most commonly measured inflammatory mediators and elevated CRP levels have been observed in pre-dialysis (Stenvinkel et al., 1999) as well as in dialysis patients (Ducloux et al., 2002). Although traditionally considered as merely a marker of inflammation it is now clear that CRP is an independent risk factor for both all-cause and cardiovascular mortality in the CKD population (Ducloux et al., 2002; Iseki et al., 1999; Menon et al., 2005; Yeun et al., 2000; Zimmermann et al., 1999). Circulating IL6 levels are also independently associated with mortality in CKD patients, including both hemodialysis and pre-dialysis patients (Barreto et al., 2010), and have therefore been suggested to be an equally important, or even stronger, predictor of mortality than CRP in CKD patients (Honda et al., 2006; Panichi et al., 2004). Importantly, it has been proposed that inflammation enhances the severity of CKD-related complications such as protein-energy wasting, vascular calcification and insulin resistance, which in turn are well-established risk factors for cardiovascular complications (Carrero and Stenvinkel, 2009). In all, it is noticeable that a persistent inflammation is closely linked to the pathophysiological alterations observed in patients with CKD, ultimately worsening the disease progress and outcome.

1.3 METABOLIC DYSFUNCTIONS AFTER ELECTIVE SURGERY AND IN CKD PATIENTS

1.3.1 Insulin action and impaired insulin sensitivity

The insulin pathway is one of the most important anabolic pathways, fundamental for the organism's capacity to prepare for starvation and, hence, for long-term survival (Frayn, 2003). Overall, insulin supports storage of carbohydrates, proteins and fat and at the same time counteracts breakdown of these substrates (**Figure 1**). The three major insulin-responding organs are the liver, skeletal muscle and adipose tissue. In response to elevated concentrations of blood glucose, increased concentrations of insulin induce

translocation of glucose-transporting proteins (GLUT4) from an intracellular microsomal pool to the cell surface, which facilitates glucose transport across the cell membrane. In the liver, insulin stimulates synthesis of glycogen and suppresses glycogen breakdown and hepatic gluconeogenesis. Glucose transport across the cell membrane via GLUT4 is a rate-limiting step for insulin-mediated glucose uptake (Katz et al., 1988; Yki-Jarvinen et al., 1990) and animal models of insulin resistance have shown that the reduction in glucose uptake is associated with a deteriorated glucose transport (Strommer et al., 1998) and impaired insulin-stimulated GLUT4 translocation (King et al., 1992).

During normal conditions, the fasting blood glucose concentrations are typically maintained between 4.5-5.0 mmol/l. However, in situations of insulin resistance blood glucose concentrations may rise to hyperglycemic levels due to impairment of these energy-storing pathways. For example, patients diagnosed with diabetes are characterized by fasting plasma glucose concentrations above 7.0 mmol/l. If untreated, sustained hyperglycemia produces overflow of substrates in the mitochondrial respiratory chain complexes and, consequently, overproduction of ROS. As a result, key metabolic pathways are inhibited, gene expression is altered and pro-inflammatory mediators are released, in all causing deleterious effects on the organism which ultimately may develop into microvascular changes in the retina, renal glomerulus or peripheral nerves (Brownlee, 2003).

It should be emphasized that insulin resistance is not only present in the diabetic condition but also in various pathological states, such as obesity, rheumatoid arthritis (Sattar et al., 2003), hepatitis C (Knobler et al., 2003) and in critically ill patients (van den Berghe et al., 2001) as well as in, as discussed in the present thesis, elective surgery (Thorell et al., 1994) and CKD patients (DeFronzo et al., 1981) (**Table 1**). Regardless

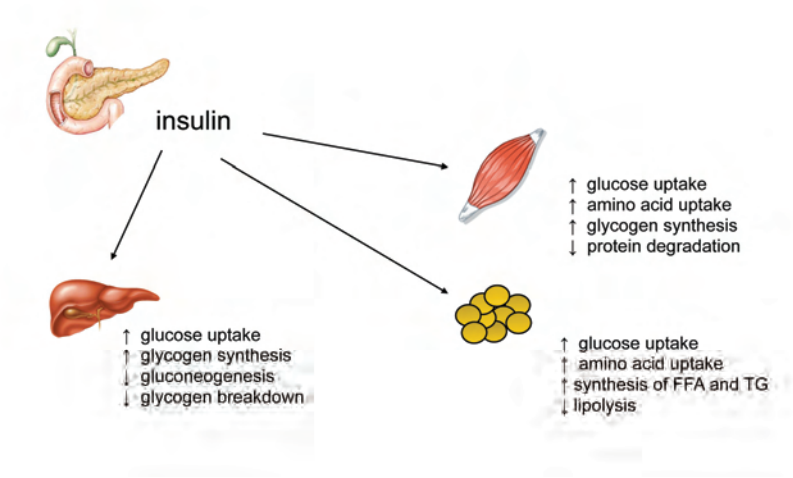

Figure 1. Metabolic effects of insulin. FFA, free fatty acids; TG, triglycerides.

of previous history of diabetes, patients undergoing surgical treatment or suffering from chronic renal failure develop impaired insulin sensitivity, which is associated to impaired recovery (Thorell et al., 1999b) and increased risk for cardiovascular complications (Shinohara et al., 2002), respectively. A series of studies to improve the understanding of postoperative insulin resistance have been conducted on elective surgery patients during the last decades (Thorell et al., 1993, 1994; Thorell et al., 1999a; Thorell et al., 1999b), and efforts have been made to characterize the CKD-associated insulin resistance (DeFronzo et al., 1981; Friedman et al., 1991). Hyperinsulinaemic clamp methods in combination with tracers and biochemical techniques have been exploited to study this in more detail. The underlying molecular mechanisms are, however, not fully understood.

1.3.2 Postoperative insulin resistance in elective surgery patients

1.3.2.1 Resistance to the effects of insulin after surgery

Postoperative insulin resistance is a transient state, lasting for two to three weeks after surgery. Although preoperative insulin sensitivity is highly variable between patients, the relative change is surprisingly uniform with approximately 50% reduction in whole body insulin sensitivity after a given surgical procedure, such as open cholecystectomy (Thorell et al., 1994). Although a marked reduction in insulin-stimulated glucose uptake occurs as early as two hours after completion of surgery (Thorell et al., 1999a) the relative reduction in insulin sensitivity appears to be most pronounced on the first postoperative day (Thorell et al., 1994). Postoperative resistance to the effects of insulin on glucose metabolism is manifested by reduced peripheral tissue glucose uptake and at the same time a hampered glucose transport in skeletal muscle and impaired glycogen synthesis (Thorell et al., 1999a). A decline in insulin-induced glucose transport in adipose tissue and an increase in lipolysis and fat oxidation contribute further to the impaired glucose utilization after surgery (Nordenstrom et al., 1989). In addition, hepatic glucose production is increased after surgery (Nygren et al., 1997) and lipid mobilization and utilization are enhanced (Nygren et al., 1998).

Table 1. Deviations from normal insulin action in T2DM, post-surgery and in chronic kidney disease.

	Type 2 diabetes	Postoperative insulin resistance	Chronic kidney disease
Whole body insulin sensitivity	↓	↓	↓
Insulin secretion	↔/↓	↔	↔/↓
Non-oxidative glucose disposal	↓	↓	↓
Liver glucose production	↑	↑	↔
Skeletal muscle glucose utilization	↓	↓	↓
Adipose tissue glucose utilization	↓	↓	↓

1.3.2.2 Consequences

As a result, these deteriorations in glucose homeostasis may result in elevated blood glucose concentrations, causing hyperglycemia, which is a major culprit in the development of complications and delayed recovery after surgical trauma (Ljungqvist et al., 2005; Van den Berghe et al., 2003) In elective surgery patients, postoperative insulin resistance is an independent predictor of length of hospital stay and the degree of the induced insulin resistance is proportional to the magnitude of the surgical trauma (Thorell et al., 1993). Both blood loss and the type of surgery predict the relative changes in insulin sensitivity. On the other hand, the relative reduction in insulin sensitivity has been shown to be independent of variables such as age, BMI, duration of surgery and preoperative insulin sensitivity (Thorell et al., 1993, 1994; Thorell et al., 1999a; Thorell et al., 1999b). Ever since it was shown that critically ill postoperative patients, undergoing intensive insulin therapy to achieve normoglycaemia, experienced a dramatically improved outcome compared to patients receiving conventional treatment (van den Berghe et al., 2001; Van den Berghe et al., 2003), it has been clear that the development of therapeutic treatments to minimize the effects of insulin resistance and hyperglycemia is imperative in the operative care.

1.3.2.3 Insulin resistance and inflammation

Because plasma levels of insulin remain relatively unaltered following surgery, the metabolic aberrations observed are most likely not due to a hampered insulin secretion but to disturbances in the insulin signal transduction pathways (Thorell et al., 1996). Presently it is also believed that stress hormones such as catecholamines, cortisol and growth hormone, released in response to surgical injury, are not the sole mediators of postsurgical insulin resistance in modern surgical practice. Although infusion of these hormones has been shown to induce a metabolic stress response, including insulin resistance (Deibert and DeFronzo, 1980; Kollind et al., 1987; Pacy et al., 1990; Rizza et al., 1982), previous studies of our group have shown that plasma concentrations of these hormones were only modestly elevated or unaffected after surgery (Thorell et al 1993, Thorell et al 1994). Instead, the importance of proinflammatory mediators has been highlighted as significantly elevated concentrations of proinflammatory cytokines are found in the circulation after surgery (Gletsu et al., 2006; Kremen et al., 2006; Thorell et al., 1996), which correlate to the postoperative reduction in insulin sensitivity (Thorell et al., 1996). Recent evidence for a complex interaction between the immunological response and metabolic control has emerged and it is now clear that proinflammatory cytokines play a pivotal role in metabolic aberrations such as insulin resistance (Hirosumi et al., 2002; Shoelson et al., 2003; Uysal et al., 1997; Wellen and Hotamisligil, 2005). The role for inflammatory mediators in the postsurgical-stress phase is however still unclear and, in addition, the sites of action are not comprehensively understood.

1.3.3 Metabolic complications in patients with CKD

1.3.3.1 Insulin resistance

Diabetic nephropathy is a leading cause of renal failure in Sweden (Schon et al., 2004) as well as in other western populations (Chen et al., 2003) and the number of diabetic CKD patients are expected to increase as the prevalence of diabetes is steadily increasing world wide (National Kidney Foundation, 2002). However, irrespective of the proportion of patients diagnosed with clinical diabetes, CKD patients may display impaired insulin secretion, metabolism and sensitivity (DeFronzo et al., 1983). Insulin resistance may develop at early stages of renal dysfunction (Eidemak et al., 1995; Fliser et al., 1998; Sechi et al., 2002; Sit et al., 2006) and correlates negatively with the decline in renal function (Kobayashi et al., 2005). The causes of insulin resistance are largely unknown but factors such as uremic toxicity, hormonal imbalances (particularly hyperparathyroidism), vitamin D status, metabolic acidosis, anemia and physical inactivity are suggested to contribute (Akmal et al., 1985; Bergstrom et al., 1998; DeFronzo and Beckles, 1979; Mak, 1996; Sit et al., 2005; Tonshoff et al., 2005). Some factors have been recognized as contributors to the insulin resistance in uremia, although much remains uncertain regarding the underlying cellular mechanisms of uremia-induced deterioration of insulin action. Uremia-associated insulin resistance has been described as an impaired insulin action in peripheral tissues but with preserved hepatic insulin sensitivity (DeFronzo et al., 1981). For example, the ability of insulin to stimulate peripheral glucose disposal by muscle is markedly impaired. It was previously shown that insulin-mediated stimulation of glucose metabolism was reduced by 50% in skeletal muscle of uremic patients, compared to control subjects, despite normal insulin binding, insulin receptor autophosphorylation and tyrosine kinase activity, as well as an unaltered abundance of GLUT4 proteins (Friedman et al., 1991). The ability of insulin to bind to its receptor was also reported to be unaffected in skeletal muscle (Cecchin et al., 1988; Contreras et al., 1992), liver (Kauffman and Caro, 1983) and adipocytes (Truglia et al., 1988) of uremic rats. On the other hand, the nonoxidative glucose disposal appears to be deteriorated (Castellino et al., 1992; Foss et al., 1996).

1.3.3.2 Oxidative stress and vascular calcification

An elevated state of oxidative stress is linked to uremia-associated inflammation and insulin resistance (Locatelli et al., 2003; Stenvinkel et al., 1999). Recently it was experimentally shown that ROS generation, triggered by oxidative stress, may diminish insulin sensitivity in adipocytes by reducing the insulin-stimulated glucose transport (D'Apolito et al.). Insulin resistance (Shinohara et al., 2002) as well as oxidative stress (Himmelfarb et al., 2002) are identified as significant risk factors for vascular disease (Halliwell, 1993) and predicts cardiovascular morbidity and mortality in CKD patients. Vascular complications are highly prevalent and represent a leading cause of premature deaths in the CKD population (Foley et al., 1998). The network of mediators and inhibitors of vascular and soft tissue calcification are not fully elucidated but recent studies have suggested that, among other factors, inflammatory mediators may hamper the action of calcification inhibitors, including matrix Gla protein, alpha-2-HS-glycoprotein (AHSG) and osteoprotegerin (OPG) (Stenvinkel et al., 2005). In CKD

patients, low AHSG serum levels associate with vascular calcification and all-cause and cardiovascular mortality (Ketteler et al., 2003) whereas circulating OPG levels are increased (Kazama et al., 2002) and associate with both aortic calcification (Barreto et al., 2005) and mortality (Morena et al., 2006).

1.3.3.3 Metabolic syndrome in CKD

Due to the rapidly growing number of obese individuals, the number of diabetic patients is increasing, causing rising numbers of patients with kidney disease and increased prevalence of metabolic syndrome in patients with CKD (Chen et al., 2003; Chen et al., 2004; Palaniappan et al., 2003). Considering that the metabolic syndrome is an adverse condition that normally takes years to develop in a non-uremic individual (Despres and Lemieux, 2006) it is feasible to assume that the physiological imbalance in patients with CKD could accelerate the pathogenesis progression of the metabolic syndrome and contribute to the elevated risk for cardiovascular death. In CKD patients, abdominal obesity seems to be causally associated to hyperadipokinemia, inflammation and insulin resistance as well as to other metabolic syndrome components, suggesting a role for uremic adipose tissue in these complications (Carrero et al., 2009). Moreover, since previous studies have demonstrated altered gene expression of adipose tissue specific genes in CKD patients compared to controls (Marchlewska et al., 2004; Nordfors et al., 1998) it may be that uremic adipose tissue possesses different properties compared to non-uremic adipose tissue and therefore it is of current interest to further study uremic adipose tissue in view of metabolic alterations in CKD.

1.4 SKELETAL MUSCLE AND ADIPOSE TISSUE IN METABOLISM AND INFLAMMATION

1.4.1 Skeletal muscle

Skeletal muscle is considered as the quantitatively most important organ for insulin-stimulated glucose uptake (Zurlo et al., 1990). In skeletal muscle, glucose may either enter the glycolysis pathway to generate energy, or undergo conversion to glycogen to be stored as an energy resource. Insulin stimulates glycogen synthase and inhibits the enzyme glycogen phosphorylase, hence promoting storage of glucose as glycogen and inhibiting glucose production via breakdown of glycogen. Free fatty acids (FFAs) may also be transported into muscle to be used as fuel or stored as triacylglycerol (Frayn, 2003). Skeletal muscle may also act as a source of inflammatory mediators as it has been shown to produce cytokines during exercise (Pedersen and Hoffman-Goetz, 2000) as well as in inflammatory muscle disorders such as dermatomyositis and polymyositis (Figarella-Branger et al., 2003). Moreover, TNF is overexpressed in muscle tissues in obese, insulin resistant and diabetic patients (Saghizadeh et al., 1996).

1.4.2 Adipose tissue

Although adipose tissue provides mechanical cushioning and thermal insulation of other organs, its most important function is to control the storage and release of energy. Adipose tissue harbors the body’s main depository of chemical energy, stored in the adipocytes as triacylglycerol. Insulin is a major signal for lipid storage under conditions

of excess energy, such as after a meal. Conversely, at times of energy deficit or high energy demands, stimulation of lipolysis in the adipocyte produces FFAs, which are released to the bloodstream. A deterioration in this regulation may have detrimental effects since a sustained exposure of excessive fatty acids in the bloodstream induces tissue insulin resistance and hampers insulin secretion from the pancreatic beta-cells (Frayn, 2003). Furthermore, next to skeletal muscle, adipose tissue is the second most important peripheral organ for insulin-mediated glucose uptake (via GLUT4) and adipose tissue specific ablation of GLUT4 in mice was recently shown to cause insulin resistance, highlighting its crucial role in whole-body insulin sensitivity (Zisman et al., 2000).

It is now clear that adipose tissue is a far more complex organ than merely being a chemical energy reservoir. A variety of hormones and cytokines are produced and secreted from the adipose tissue and influence a wide aspect of bodily functions such as energy expenditure, food intake, insulin sensitivity, and inflammation (Frayn et al., 2003). These secretory factors have been referred to as adipokines, some of which have the capacity to act peripherally whereas some act on the local level as autocrine or paracrine mediators. One of the best characterized adipokines is leptin, a hormone that is produced in proportion to fat mass and is implicated in the regulation of appetite, energy homeostasis (Friedman and Halaas, 1998) and inflammation (Ahima and Flier, 2000). Another well-known adipokine is adiponectin, which is only secreted from adipocytes and exerts remote-acting effects in the body. It is reported to have anti-inflammatory, antiatherogenic (Goldstein and Scalia, 2004) and insulin sensitizing effects, as well as to regulate lipid and energy metabolism (Berg et al., 2002). Serum levels of adiponectin are also found to be reduced in obese and individuals with type 2 diabetes mellitus (Arita et al., 1999; Hotta et al., 2000). In addition to leptin and adiponectin, several other adipose tissue-derived mediators, such as resistin and visfatin (also termed pre-B-cell colony enhancing factor 1, PBEF1, or nicotinamide phosphoribosyltransferase, NAMPT), have been categorized as adipokines with roles intersecting immunity and metabolism.

In addition to the fat-storing adipocytes, adipose tissue harbors other cell types such as pre-adipocytes, endothelial cells, and macrophages. Such cells contribute primarily to the secretory functions and to a less extent to the energy regulating activities of adipose tissue (Frayn, 2003). Macrophages with their immunomodulatory functions are of particular interest as they significantly contribute to the adipose tissue cytokine release (Weisberg et al., 2003). Both TNF and IL6 are expressed by adipose tissue and have been in focus for intense research during the last years due to their discovered roles in altered adipose-tissue function and adipogenesis, as well as the state of low-grade inflammation in obesity, insulin resistance and metabolic syndrome (Bastard et al., 2002; Gustafson et al., 2007; Hotamisligil et al., 1995; Hotamisligil et al., 1993; Vozarova et al., 2001). Having too much of adipose tissue, as in obesity, or too little, as after sustained starvation, is associated with severe metabolic and immunological abnormalities and constitute striking example on how these pathways seem to be pathophysiologically interrelated and linked to the to the effect of altered adipose tissue distribution and/or activity (Wellen and Hotamisligil, 2005).

1.4.2.1 Subcutaneous vs. visceral adipose tissue

The two main fat depots in the body are located intra-abdominally, visceral (e.g. omental) adipose tissue (VAT), and peripherally, subcutaneous adipose tissue (SAT). These two compartments have been suggested to be biologically distinct (Atzmon et al., 2002; Vohl et al., 2004). VAT is located in close proximity to the portal vein to which it releases metabolic fuels and hormones that are transported to the liver (Gustafson et al., 2007), and VAT has therefore been considered to have a larger impact on metabolic processes. It has also been proposed that VAT accumulation may exert more harmful effects than that of SAT since increased VAT is an independent, overall mortality risk factor (Pischon et al., 2008). Nonetheless, since the body's total amount of SAT may be three to four times the volume of the VAT, the secretory role of SAT is possibly equally important to VAT (Gustafson et al., 2007).

1.4.2.2 Cross-talk between adipose tissue and bone

The existence of a bone-fat cross-talk has been proposed (Lee et al., 2007) based on a growing number of studies pointing towards interactive roles of adipose tissue and bone. *In vitro* studies suggest that osteoblasts and adipocytes share a common progenitor cell (Zuk et al., 2002) and adipokines, such as adiponectin (Shinoda et al., 2006) and leptin (Ducy et al., 2000; Gordeladze et al., 2002) are reported to influence bone remodeling, whereas bone-derived proteins, e.g. osteocalcin, may play a role in adipose tissue biology, metabolic control and body weight regulation (Ferron et al., 2008; Gomez-Ambrosi et al., 2008; Lee et al., 2007). Moreover, it has been shown that the bone remodeling factor osteopontin is expressed in omental fat tissue in a direct proportion to the degree of obesity (Gomez-Ambrosi et al., 2008). These observations appear to relate adipose tissue biology to bone remodeling and metabolism, although a detailed understanding is lacking.

In context of uremic adipose tissue and vascular calcification/ossification, this bone-fat crosstalk may be particularly interesting. OPG and AHSG are, as was previously mentioned, involved in the regulation of vascular system and bone (Schafer et al., 2003; Schinke et al., 1996; Szweras et al., 2002; Yasuda et al., 1998) and associated to vascular disease in patients with CKD (Barreto et al., 2005; Ketteler et al., 2003; Morena et al., 2006). OPG expression was first demonstrated in bone, vasculature, the left main bronchus and midgut in mice (Simonet et al., 1997) but more recent studies have found gene expression of *OPG* also in the 3T3L1 adipocyte cell line and adipose tissue depots of Sprague-Dawley rats (An et al., 2007) as well as in omental adipose tissue in obese individuals (Fain et al., 2008). Further, elevated OPG protein concentrations were detected in human obese subcutaneous adipose tissue (Skopkova et al., 2007) and higher circulating OPG levels have been linked to abdominal obesity (Oh et al., 2005), whereas decreased OPG concentrations associate with weight-reduction (Holecki et al., 2007). However, the role of AHSG in fat is largely unknown and, to the best of our knowledge, *AHSG* and *OPG* expression in human uremic SAT has not yet been investigated.

2 AIMS

The general aim of this thesis is to improve the knowledge regarding disease-related stress-alterations in fundamental inflammatory and metabolic signaling pathways.

The specific aims are:

- To evaluate alterations in the transcription profile of genes in inflammatory, metabolic and insulin signaling pathways in skeletal muscle after elective surgery (Paper I).
- To evaluate alterations in the transcription profile of genes in inflammatory and insulin as well as in adipokine signaling pathways in subcutaneous and omental adipose tissue following surgical injury (Paper II).
- To investigate potential aberrations in selected inflammatory, insulin signaling and oxidative stress related pathways associated to CKD by gene expression analysis in uremic adipose tissue (Paper III).
- To investigate the presence of the bone-regulating factors AHSG and OPG in human uremic adipose tissue (Paper IV).

3 SUBJECTS AND METHODS

The following sections provide an overview of materials and methods used. Detailed descriptions can be found in the corresponding publications and manuscripts included in this thesis.

3.1 STUDY PARTICIPANTS

This thesis is part of a translational project involving the analyses of two distinct patient materials: elective surgery patients (Paper I and II) and CKD-5 patients (Paper III and IV). In addition, non-uremic subjects were included as control groups in Paper III and IV. Basic characteristics of the patients and controls are summarized in **Table 2**. All studies were approved by the Ethics Committee of the Karolinska Institutet, Sweden, and all patients and controls gave their consent to participate after being informed about the purpose and nature of the study.

3.1.1 Elective surgery patients (Paper I and II)

Patients undergoing major gastrointestinal surgery at the Centre for Gastrointestinal disease, Ersta Hospital, Stockholm, participated in Paper I and II. Eight patients were included in Paper I and four additional patients were included in Paper II. The clinical causes for surgery were malignant disease or inflammatory bowel disease (Paper I: malignant disease n=7 and inflammatory bowel disease n=1, Paper II: malignant disease n=9 and inflammatory bowel disease n=3). Detailed diagnoses of each patient are given in Paper I and II. Exclusion criteria for the two studies were known metabolic or disseminated malignant disease, usage of medication known to affect glucose metabolism or age above 70 years.

3.1.2 CKD patients (Paper III and IV)

Paper III and IV included incident dialysis patients with CKD-5 (GFR < 15 ml/min) that were recruited at the Karolinska University Hospital, Huddinge, shortly before initiating dialysis. Patients contributing with biopsies were recruited prior to peritoneal dialysis (PD) catheter insertion. All patients were included in a prospective study of CKD-5 patients in Stockholm, ongoing since 1994, called the Malnutrition, Inflammation and Atherosclerosis (MIA) study (Stenvinkel et al., 1999). The main causes of CKD in this cohort are chronic glomerulonephritis (approximately 25%), diabetic nephropathy (approximately 20%) and polycystic kidney disease (approximately 15%). The study exclusion criteria were age below 18 years or above 70 years, clinical signs of acute infection, active vasculitis or liver disease at the time of inclusion, unavailability of data and samples, or unwillingness to participate in the study.

3.1.3 Non-renal controls (Paper III and IV)

Patients undergoing elective hernia repair or laparoscopic cholecystectomy without known renal, cardiovascular or diabetic disease were included as non-renal controls in Paper III and IV. Nine control subjects were initially recruited for Paper III, and additional eleven control subjects were recruited for Paper IV, all from Karolinska

University Hospital, Huddinge. Patients presenting signs of systemic inflammation prior to surgery were not included in the studies.

Table 2. Brief description of included study participants

	Paper I.	**Paper II.**	**Paper III.**		**Paper IV.**	
	Surgery patients	Surgery patients	CKD-5 patients	Controls	CKD-5 patients	Controls
N	8	12	37	9	38	20
Age	63 (46-69)	65 (46-72)	58 (22-73)	62 (29-73)	58 (22-73)	56 (40-77)
Females/males	4/4	7/5	15/22	4/5	16/22	11/9
Body mass index (kg/m^2)	25.5 (16.5-29.8)	24.8 (16.5-29.8)	23.7 (19.1-30.1)	28.1 (23.9-32.8)	23.5 (13.3-29.0)	26.4 (21.6-34.9)
Baseline C-reactive protein (mg/L)	10 (10-15)	10 (10-46)	4.2 (0.2-55)	3.5 (2.0-13.9)	3.9 (0.2-55)	3.4 (0.4-42)

Data presented as mean (range).

3.2 TISSUE SAMPLING

3.2.1 Sampling in elective surgery patients (Paper I and II)

Blood samples were drawn preoperatively and at day 1 and 2 after surgery to obtain serum and plasma. At the day of surgery, sampling of approximately 1 g each of rectus abdominis muscle (skeletal muscle), and adipose tissue from abdominal wall (subcutaneous fat) and greater omentum (omental fat) were performed immediately at initiation of surgery and shortly before closing of the wound. All specimens were immediately stored at -70°C pending further analyses.

3.2.2 Sampling in CKD patients and non-renal controls (Paper III and IV)

At the day of surgery or PD catheter insertion, fasting blood samples were drawn from patients and control subjects for generation of plasma and serum. At initiation of appropriate surgical procedure and following incision in the abdominal wall, approximately 1 g of subcutaneous adipose tissue was obtained. Serum/plasma and tissue samples were kept and stored at -70°C if not analyzed immediately.

3.3 CLINICAL MEASUREMENTS

3.3.1 Biochemical assessments (Paper I-IV)

In Paper I and II, serum CRP concentrations of elective surgery patients were measured at Karolinska Institutet using routine methods. In Paper III and IV, biochemical assessments of blood parameters such as creatinine, serum albumin, triglycerides and high sensitivity C-reactive protein (hsCRP) were measured in CKD-5 patients and non-uremic controls using standardized methods at the Department of Clinical Chemistry,

Karolinska University Hospital, Huddinge. Measurements of specific biochemical markers were performed with commercially available assays or standard enzymatic procedures.

3.3.2 Clinical characteristics (Paper I-IV)

BMI was calculated as the weight in kilograms divided by the square of the height in meters. In Paper III and IV, glomerular filtration rates (GFR) were estimated by taking the mean of urea and creatinine clearance (CKD-5 patients) or from the plasma level of cystatin C (non-renal controls). Further, clinical history of CVD (presence of ischemic cardiac disease, peripheral vascular disease and/or cerebrovascular disease) or diabetes was obtained from medical records.

3.4 GENE EXPRESSION ANALYSES

3.4.1 RNA extraction and cDNA synthesis (Paper I-IV)

Total RNA was extracted with Aurum^{TM} Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad Laboratories, Hercules, CA, USA) (Paper I and II) or RNeasy Lipid Tissue Mini Kit (QIAGEN Sciences, Maryland, USA) (Paper III and IV). Quantity and integrity of the isolated RNA were determined with NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA). For synthesis of cDNA, SuperScriptTM III First-Strand Synthesis System for RT-PCR, with random hexamers (Invitrogen, Carlsbad, CA, USA) was used.

3.4.2 Quantitative real-time polymerase chain reaction (Paper I-IV)

For Paper I, II and III ABI TaqMan® Low Density Custom Arrays (Applied Biosystems, Foster City, CA, USA) were designed to contain assays for the measurement of multiple target and endogenous control genes. Single TaqMan® Gene Expression Assays with primers and probes (Applied Biosystems, Foster City, CA, USA) were used in Paper IV. Real-time quantitative polymerase chain reaction (PCR) of target and endogenous control genes were performed in reactions containing cDNA, appropriate gene expression assay and ABI TaqMan® Universal Master Mix No AmpErase® UNG on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). All gene amplifications were performed in triplicates or duplicates. The real-time data were collected as threshold cycle (Ct) values from which the relative gene expression quantities (arbitrary units) were calculated according to a modified delta-Ct method (Vandesompele et al., 2002). This method allows correction for sample to sample variation in RNA quality and PCR efficiency by normalizing to multiple endogenous control genes.

3.5 IMMUNOHISTOCHEMISTRY (PAPER IV)

Sections (20 µm) from fresh frozen adipose tissue were cut in a cryostat and mounted on positively charged slides (Superfrost Plus, Thermo Scientific, Braunschweig, Germany). All slides were postfixed for ten minutes in 4% formaldehyde solution followed by an overnight incubation at 4°C with the primary antibody (mouse anti-human OPG antibody, no IMG-103A, Imgenex, San Diego, CA, 1:200 dilution). Slides

serving as negative controls were incubated in the absence of the primary antibody. Following incubation with a biotinylated secondary antibody (horse anti-mouse IgG) and the ABC staining system (Vectastain ABC kit, Vector Laboratories Inc., Burlingame, CA) 3, 3'-Diaminobenzidine (DAB; Sigma-Aldrich, St Louis, MO) were applied to the slides to allow detection of primary antibody binding. Slides were counterstained with hematoxylin (Vector Laboratories Inc., Burlingame, CA) prior to examination.

3.6 STATISTICAL ANALYSES (PAPER I-IV)

As most variables were not normally distributed all statistical analyses were performed with non-parametric methods and descriptive data is presented either as median (range) or median (25^{th}-75^{th} percentile), with statistical significance set at the level of $p<0.05$. Thus, Wilcoxon signed-rank test for matched pairs and Wilcoxon two sample rank sum test/Kruskal-Wallis were used for comparisons of continuous variables between sampling points (Paper I and II) and groups (Paper III and IV), respectively. Univariate correlations were determined with the Spearman's rank correlation in Paper I-IV and in addition, multivariate regression analysis as well as Kaplan Meier analysis for survival were performed in Paper IV. Statistical platform JMP IN Version 5.1 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

4 RESULTS AND DISCUSSION

4.1 PAPER I: INCREASED EXPRESSION OF INFLAMMATORY PATHWAY GENES IN SKELETAL MUSCLE DURING SURGERY

In this study, we aimed to evaluate if the expression of 45 genes, representing a carefully selected variety of inflammatory and metabolic mediators, is regulated in skeletal muscle during elective surgery. Previous studies of our group have shown that insulin-mediated skeletal muscle glucose utilization is markedly impaired after surgery (Nygren et al., 1998; Thorell et al., 1999a) and it is of great interest to find out whether the pathological events taking place could be preceded by transcriptional changes.

4.1.1 Inflammatory pathway genes

Of the inflammatory candidate genes studied we observed an up-regulation of the IL6 and TNF pathways, as illustrated by significantly elevated mRNA levels of *IL6*, the IL6 receptor (*IL6R*), and suppressor of cytokine signaling 3 (*SOCS3*) as well as enhanced expression of tumor necrosis factor (*TNF*), TNF receptor 1A (*TNFRSF1A*) and nuclear factor of kappa light polypeptide gene enhancer in B-cells (*NFKB1*). In addition, mRNA levels of IL1 receptor 1 (*IL1R1*), nicotinamide phosphoribosyltransferase (*NAMPT*), and angiotensinogen (*AGT*), were elevated during surgery. Fold-changes in gene expression between baseline and surgery are presented in **Table 3**. The systemic inflammatory status after surgery, as assessed by plasma CRP concentrations at the day of surgery and postoperative day 1 and 2, were not significantly correlated to the changes in mRNA levels of genes involved in inflammatory signaling.

4.1.2 Insulin signaling, glucose homeostasis and metabolic pathway genes

Further, to elucidate whether surgical injury also influences the rate of transcription of genes encoding molecules involved in glucose transport as well as early, mRNA levels of intermediate and final components of the insulin signal transduction pathway, insulin receptor substrate 1 (*IRS1*), phosphoinositide-3-kinase (*PIK3*) and GLUT4, encoded by the solute carrier family 2, facilitated glucose transporter member 4 (*SLC2A4*) gene, were measured. In this particular setting, however, no significant alterations in these genes were observed. The expression of other potentially interesting genes was found to be altered following surgery. The mRNA levels of serpin peptidase inhibitor member 1 (*SERPINE1*, or plasminogen inhibitor-1) (6.9-fold, p=0.008), recently shown to interfere with insulin signaling *in vitro*, (Lopez-Alemany et al., 2003) were increased, as were peroxisome proliferator-activated receptor delta (*PPARD)* (2.0-fold, p=0.016), which is implicated in basic lipid metabolism (Basu-Modak et al., 1999).

Hence, a total of 11 genes displayed changes in expression after surgery as compared to baseline. In addition, there were no significant differences in mRNA changes between males and females.

Table 3. Relative mRNA quantities (arbitrary units) at Baseline and Surgery of genes that were significantly altered after surgery.

Pathway	Gene Symbol	Baseline (n = 8)	Surgery (n = 8)	Fold Change	p-value
Modulators of inflammation	*AGT*	0.974 (0.143-1.70)	4.34 (0.855-9.23)	4.46	0.023
	IL1R1	1.57 (1.00-3.05)	8.37 (1.50-19.4)	5.31	0.039
	IL6	1.20 (0.364-3.83)	119 (6.47-487)	99.5	0.008
	IL6R	1.15 (0.831-2.29)	2.88 (1.16-9.39)	2.52	0.039
	NFKB1	1.41 (0.807-1.69)	2.31 (1.31-9.68)	1.64	0.023
	NAMPT	0.916 (0.647-1.28)	6.17 (1.47-15.1)	6.74	0.008
	SOCS3	0.689 (0.332-2.40)	80.2 (7.35-372)	116	0.008
	TNF	1.19 (0.506-3.06)	18.5 (1.39-47.5)	15.5	0.008
	TNFRSF1A	1.33 (0.0127-3.03)	1.82 (0.0218-8.41)	1.37	0.008
Metabolism	*PPARD*	1.08 (0.812-1.67)	2.17 (0.922-7.28)	2.00	0.016
	SERPINE1	0.829 (0.152-2.72)	5.75 (0.832-88.1)	6.94	0.008

Relative mRNA quantification was measured in triplicates and presented as median (range). Wilcoxon signed-rank test for matched pairs was performed, and genes with p-value <0.05 are reported.

4.1.3 Conclusion

In conclusion, our data demonstrate that prominent alterations in gene expression of central pro-inflammatory cytokines, IL6 and TNF, occur in skeletal muscle during elective surgery. This highlights the possibility of an augmented activity of local inflammatory mediators in surgical stress, which previously have been causally linked to insulin resistance (Hotamisligil et al., 1993; Klover et al., 2003; Rotter et al., 2003).

4.2 PAPER II: EXPRESSION OF INFLAMMATORY AND INSULIN SIGNALING GENES IN ADIPOSE TISSUE IN RESPONSE TO ELECTIVE SURGERY

These studies were aimed at evaluating adipose tissue gene expression profiles, potentially important for surgery-induced metabolic aberrations, such as adipokine and insulin signaling pathway genes. Therefore, mRNA quantification analyses of 21 genes were performed in adipose tissue sampled at initiation and end of surgery. Both subcutaneous and omental adipose tissue were exploited since different adipose tissue depots have been shown to display diverse gene expression profiles (Atzmon et al., 2002; Vohl et al., 2004).

4.2.1 Inflammatory pathway genes

Data on all relative mRNA quantities measured at baseline and surgery, as well as fold changes between baseline and surgery, are presented in **Table 4**. In line with our studies in skeletal muscle (Witasp et al., 2009) we found that patients undergoing major

abdominal surgery displayed striking increments in mRNA levels of IL6 pathway genes *IL6*, signal transducer and activator of transcription 3 (*STAT3*) and *SOCS3*, as well as of *NAMPT* in subcutaneous adipose tissue. In addition, *IKBKB*, adiponectin and leptin receptor were found to be down-regulated. Similar changes were also found in omental adipose tissue: mRNA levels of *IL6*, *SOCS3*, *NAMPT* and *NFKB1* were up-regulated, whereas adiponectin and leptin receptor mRNA levels were decreased after surgery. Relating this to the patient's systemic inflammatory status, changes in mRNA levels of *NFKB1* were negatively (rho=-0.68, p=0.015) correlated to preoperative concentrations of CRP in omental adipose tissue but not in subcutaneous adipose tissue. However, no correlations between mRNA changes and CRP concentrations at postoperative day 1 and 2 were observed.

In parallel with the inflammatory mediators, it is of interest to quantify mRNA levels of macrophage markers in adipose tissue after surgery. Thus, mRNA expression of macrophage marker genes *CD68*, macrophage scavenger receptor 1 (*MSR*1) and Egf-like module containing, mucin-like, hormone receptor-like 1 (*EMR1*) were analyzed in both subcutaneous and omental adipose tissues, of which *CD68* and *MSR1* mRNA levels did not significantly change after surgery whereas EMR1 mRNA levels were undetectable in a majority of the samples and were therefore omitted from the statistical analyses.

4.2.2 Insulin signaling and glucose homeostasis pathway genes

Interestingly, and in contrast to skeletal muscle, surgery seemed to down-regulate insulin-signaling pathway genes in adipose tissue. *IRS1* mRNA levels were decreased 1.6-fold (p=0.043) and 2.1-fold (p=0.012) in subcutaneous and omental adipose tissue, respectively, whereas *PIK3R1* (2.0-fold, p=0.007) and *SLC2A4* (1.8-fold, p=0.012) were only affected in omental adipose tissue. The decrease in mRNA levels of these genes was however not, after correction for multiple testing, correlated to the increments in mRNA quantities of inflammatory pathway genes.

4.2.3 Association between baseline characteristics and gene expression

Baseline characteristics such as age, gender and BMI did not seem to have a large impact on the transcriptional alterations during surgery. Age was not found to be correlated to mRNA changes of any of the studied genes following surgery and only *SOCS3* displayed a more pronounced increase in mRNA concentrations in omental adipose tissue in males compared to females (156 [52.6-213] fold vs. 57.8 [-23.8-87.7] fold, p=0.028) after surgery. BMI was correlated to changes in omental but not in subcutaneous adipose tissue: *IKBKB* was negatively correlated to BMI (rho= -0.59, p= 0.042), whereas *IL6* and *NAMPT* mRNA changes were positively correlated to BMI (rho=0.66, p=0.019 and rho=0.85, p=0.001). When evaluating parameters of the operational procedure in relation to gene expression, no correlations were found between the alterations in mRNA levels and preoperative treatment with carbohydrates, nor did it correlate with the amount of insulin infused at the day of surgery. Only in subcutaneous adipose tissue *SOCS3* mRNA changes (rho=-0.63, p=0.027) were found to be negatively correlated with the duration of surgery.

4.2.4 Conclusion

Taken together, the data from the current study demonstrate dynamic changes occurring in adipose tissue after surgery, characterized by increased output from inflammatory pathway genes, which we previously found to be regulated in skeletal muscle (Witasp et al., 2009), but decreased output from insulin signaling pathway genes. No marked differences in the expression of inflammatory genes were observed between subcutaneous and omental adipose tissue depots, whereas more insulin signaling pathway genes were affected in omental adipose tissue. This suggests that both adipose tissue depots may be implicated in the integration of inflammatory and metabolic pathways in metabolic derangements associated with surgery. These data are novel within this research field and as such encourage further investigations of molecular processes induced by surgical stress.

Table 4. Fold changes in relative expression (arbitrary units) of adipokine and insulin/glucose uptake signaling genes in subcutaneous and omental adipose tissue during surgery.

Pathway	Gene	Subcutaneous adipose tissue		Omental adipose tissue	
		Fold Change[a]	P-Value[b]	Fold Change[a]	P-Value[b]
Endocrine	*ADIPOQ*	-1.1	0.034	-2.0	0.007
signaling	*ADIPOR1*	-1.4	N.S.	-1.6	N.S.
	ADIPOR2	1.1	N.S.	-1.5	N.S.
	LEP	-1.3	N.S.	-1.3	N.S.
	LEPR	-1.4	0.001	-1.5	0.009
Inflammatory	*TNF*	1.7	N.S.	1.1	N.S.
signaling	*TNFRSF1A*	1.3	N.S.	-1.2	N.S.
	IKBKB	-1.4	0.005	-1.7	N.S.
	NFKB1	1.1	N.S.	1.5	0.027
	IL6	23	0.001	113	0.016
	IL6R	-1.1	N.S.	1.2	N.S.
	STAT3	1.4	0.009	1.8	N.S.
	SOCS3	70	0.001	82	0.021
	NAMPT	4.4	0.001	9.2	0.009
	CD68	-1.2	N.S.	-1.5	N.S.
	MSR1	1.5	N.S.	-1.6	N.S.
Insulin	*IRS1*	-1.6	0.043	-2.1	0.012
signaling/	*PIK3R1*	-1.4	N.S.	-2.0	0.007
glucose uptake	*SLC2A4*	-1.7	N.S.	-1.8	0.012
	SLC2A1	1.3	N.S.	1.7	N.S.

N=12, triplicate relative mRNA quantity measurements.

[a] Changes in relative mRNA quantification expressed as surgery measurements over baseline measurements.

[b] Differences between relative mRNA quantification at baseline and surgery, analysed by Wilcoxon signed-rank test for matched pairs.

N.S., not significant

4.3 PAPER III: INCREASED EXPRESSION OF PRO-INFLAMMATORY GENES IN ABDOMINAL SUBCUTANEOUS FAT IN CHRONIC KIDNEY DISEASE STAGE 5 PATIENTS.

In the third paper, the study objective was to identify gene expression differences between CKD-5 patients and non-uremic patients, our hypothesis being that the uremic milieu in CKD patients provokes an unfavorable shift in the transcriptional output in important inflammatory signaling organs, such as adipose tissue. Therefore, 21 candidate genes, primarily adipokines and genes with a relevant role in glucose homeostasis, insulin signaling/sensitivity, oxidative stress and inflammation, were quantified at the mRNA level in abdominal subcutaneous adipose tissue (SAT) of CKD-5 patients and controls.

4.3.1 Inflammatory pathway genes and relation to inflammatory markers

Relative mRNA quantities of *IL6* measured in SAT were markedly higher (p=0.025) in patients compared to controls, as were *SOCS3* (p=0.039) (**Table 5)**, and these two were positively correlated (rho=0.54, p=0.02). On the other hand, *IL6R* mRNA concentrations were slightly reduced (p=0.049) and did not correlate to mRNA concentrations of *IL6*. Neither *IL6* nor *IL6R* mRNA levels were correlated to circulating plasma concentrations of IL6 or hsCRP. There were no expression differences in the remaining inflammatory pathway genes studied between CKD patients and controls.

4.3.2 Oxidative stress and adipokine genes

mRNA quantities and fold-differences are presented in **Table 5**. There was a two-fold decrease in cytochrome b-245, alpha polypeptide (*CYBA*) mRNA levels (p=0.0014) and significantly lower levels of mRNA encoding the uncoupling protein 2 (*UCP2*) (-1.5-fold) in patients compared to controls. Moreover, reduced levels of leptin (*LEP*) (-2.3-fold) and leptin receptor (*LEPR*) (-1.3-fold) mRNAs were found in patients compared to controls and there was a positive correlation between *LEP* and *LEPR* mRNA quantities (rho=0.49, p=0.0027), of which only *LEP* mRNA correlated with circulating concentrations of leptin (rho= 0.74, p<0.001). mRNA quantities of the remaining adipokine genes studied were not different between patients and controls.

4.3.3 Insulin signaling and glucose homeostasis pathway genes

None of the studied insulin signaling and glucose homeostasis pathway genes were differentially expressed between CKD patients and controls.

4.3.4 Association between baseline characteristics and gene expression

A brief description of clinical characteristics of the studied CKD patients and control subjects are presented in **Table 2**, a more detailed version may be found in Table 1 in the corresponding manuscript included in this thesis. Age and gender distributions were

similar in CKD patients and controls but patients were more inflamed by means of higher levels of inflammatory surrogate markers VCAM-1 (1050 [877-1230] vs. 593 [526-640] ng/ml, p<0.0001) and IL-6 (6.9 [4.3-10.7] vs. 2.7 [2.3-4.3] ng/ml, p=0.0009) and the median BMI of CKD patients were significantly lower than that of controls (23.7 [21.8-25.9] vs. 28.1 [26.9-29.4] kg/m^2, p=0.0004). A normalization of the gene expression for BMI was therefore performed and it was found that the mRNA concentrations of *IL6*, *SOCS3*, *CYBA*, *LEP* and *UCP2* remained significantly different between patients and controls (**Figure 2**). In the CKD-5 patient group, BMI was positively correlated to *CYBA* (rho=0.33, p=0.047) and *LEP* (rho=0.47, p=0.004) mRNA levels. Moreover, age was negatively correlated to *NAMPT* (rho=-0.40, p=0.01) and *SLC2A4* (rho=-0.37, p=0.02) but positively correlated to *CD68* (rho=0.50, p=0.002) and *CYBA* (rho=0.38, p=0.02) mRNA quantities. *ADIPOQ* (1.36 vs. 1.93, p=0.0094), *ADIPOR2* (1.09 vs. 1.58, p=0.0033), *IKBKB* (1.57 vs. 1.84, p=0.0045), *SLC2A1* (0.87 vs. 1.44, p=0.0424), *SLC2A4* (0.89 vs. 2.07, p=0.0030) and *PIK3R1* (1.32 vs. 1.59, p=0.0257) mRNA levels were all elevated in females compared to males, despite the fact that there were no gender differences in baseline clinical characteristics. Patients with clinical signs of CVD had lower *ADIPOR2* mRNA levels than patients without CVD (1.09 vs. 1.51, p=0.0329).

Table 5. Gene expression of selected adipokines and other candidates implicated in energy homeostasis, inflammation, insulin/glucose signaling and oxidative metabolism in subcutaneous adipose tissue in non-uremic controls and CKD-5 patients.

Gene	Controls (N=9)	CKD-5 patients (N=37)	Fold difference	P-value
ADIPOQ	1.44 (1.08-2.38)	1.55 (1.23-2.14)	1.07	N.S.
ADIPOR1	1.53 (1.25-2.15)	1.50 (1.22-2.32)	-1.02	N.S.
ADIPOR2	1.30 (0.92-1.42)	1.36 (1.03-1.71)	1.05	N.S.
LEP	1.10 (0.78-1.42)	0.48 (0.29-0.92)	-2.29	0.01
LEPR	1.02 (0.95-1.42)	0.81 (0.64-1.09)	-1.26	0.009
UCP2	0.87 (0.73-1.00)	0.57 (0.45-0.67)	-1.53	0.0002
CD68	0.75 (0.46-1.16)	0.52 (0.33-0.99)	-1.41	N.S.
TNF	0.61 (0.33-1.01)	0.80 (0.46-1.59)	1.31	N.S.
TNFRSF1A	0.48 (0.21-0.78)	0.35 (0.01-0.51)	-1.37	N.S.
IKBKB	1.68 (1.43-2.18)	1.65 (1.22-1.93)	-1.02	N.S.
NFKB1	1.32 (1.10-1.85)	1.01 (0.89-1.41)	-1.32	N.S.
IL6	1.23 (0.70-1.62)	4.00 (1.82-6.73)	3.25	0.02
IL6R	1.25 (1.04-1.84)	1.01 (0.65-1.31)	-1.24	0.04
SOCS3	1.17 (0.68-1.12)	2.20 (1.27-3.36)	1.88	0.03
STAT3	1.05 (0.93-1.24)	0.95 (0.79-1.08)	-1.10	N.S.
NAMPT	2.19 (1.22-4.07)	2.47 (1.72-3.29)	1.13	N.S.
IRS1	1.66 (1.03-2.05)	1.58 (0.97-2.70)	-1.05	N.S.
PIK3R1	2.20 (1.35-4.03)	1.51 (1.23-2.12)	-1.47	N.S.
SLC2A4	0.83 (0.59-1.44)	1.40 (0.79-2.02)	1.69	N.S.
SLC2A1	1.04 (0.74-1.49)	1.06 (0.75-1.79)	1.01	N.S.
CYBA	1.27 (0.97-1.63)	0.65 (0.45-1.03)	-1.95	0.001

N.S., not significant

4.3.5 Conclusion

In summary, seven of the investigated genes displayed a significantly altered expression profile in uremic tissues in relation to non-uremic tissues. Thus, the presence of renal failure appears to confer altered transcriptional outputs of specific proinflammatory and oxidative stress pathway genes in adipose tissue, of which the major findings were the significantly elevated mRNA concentrations of the *IL6*, *IL6R* and *SOCS3* in CKD-5 patients. Considering the strong associations between adipose tissue characteristics, inflammation and metabolic complications (Wellen and Hotamisligil, 2005) as well as the impact of inflammation and oxidative stress on outcome and CKD complications (Stenvinkel et al., 2008) (**Figure 3**) the current findings may be of importance for a better understanding of the uremic phenotype.

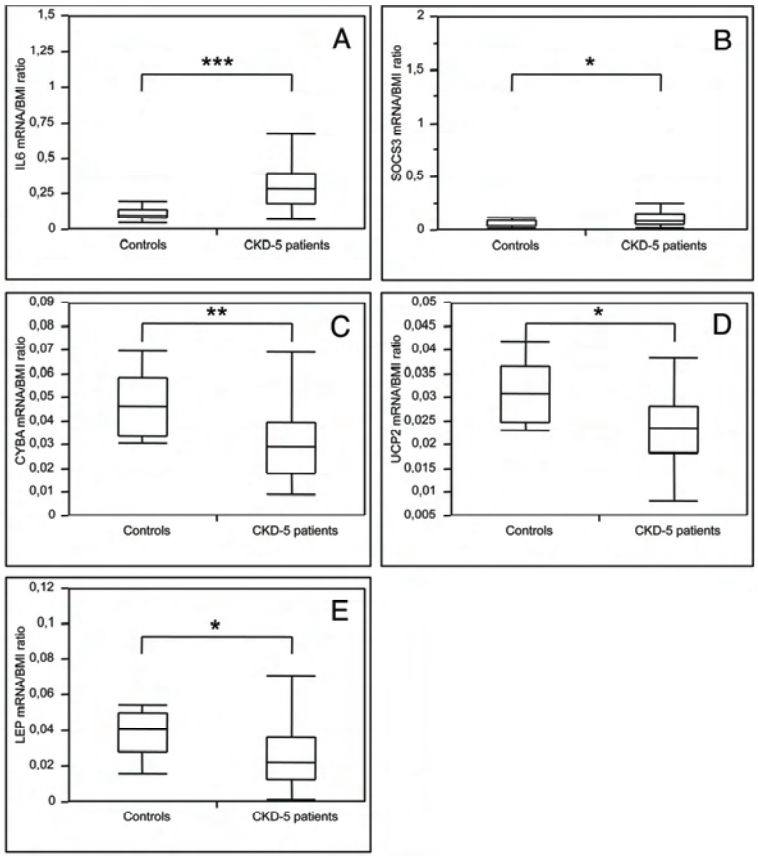

Figure 2. mRNA levels (relative expression, arbitrary units) of five genes A. *IL6*, B. *SOCS3, C. CYBA,* D. *UCP2* and E. *LEP* remained significantly different between CKD-5 patients (N=37) and non-uremic controls (N=9) after normalization to BMI. mRNA/BMI ratio shown as the median and quartile. *, **, *** = P-values <0.05, <0.01, <0.001 by Wilcoxon rank-sum test. BMI, body mass index; IL6, interleukin 6; SOCS3, suppressor of cytokine signaling 3; CYBA, cytochrome b-245, alpha polypeptide; UCP2, uncoupling protein 2; LEP, leptin.

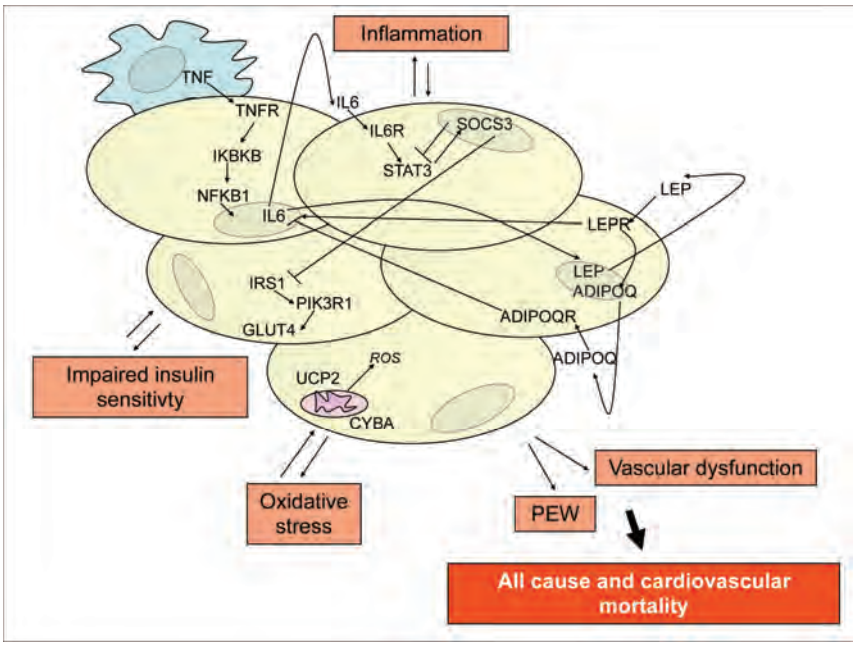

Figure 3. Simplified overview of the investigated genes, primarily adipokines and genes with relevance for glucose homeostasis, insulin signaling/sensitivity, oxidative stress and inflammation, in relation to adipose tissue signaling and CKD complications.
PEW, protein energy wasting

4.4 PAPER IV: EXPRESSION OF OSTEOPROTEGERIN IN HUMAN FAT TISSUE; IMPLICATIONS FOR CHRONIC KIDNEY DISEASE

The fourth and final study included in this thesis was conducted with the focus on the recently proposed cross-talk between adipose tissue biology, bone remodelling and metabolism (Lee et al., 2007) and we therefore aimed to investigate whether bone-regulating proteins osteoprotegerin (OPG) and alpha-2-HS-glycoprotein (AHSG) are expressed in uremic SAT. Analyses were performed on CKD-5 patients and non-uremic controls with clinical and biochemical characteristics as presented in **Table 6**. Age and gender were similar between the groups, whereas patients displayed lower BMI and S-albumin levels but higher concentrations of surrogate markers of inflammation, such as VCAM-1 and IL-6.

4.4.1 Detection of OPG but not AHSG mRNAs in SAT

Relative mRNA quantities of *AHSG* and *OPG* were measured and presence of *OPG* but not of *AHSG* mRNA could be detected in adipose tissue from both patients and controls. *OPG* mRNA quantities were significantly lower in patients compared to controls (0.5 [0.4-0.9] vs. 1.1 [0.9-1.8] P<0.0001) (**Figure 4**). When comparing the ratio of *OPG* mRNA to BMI between the groups it was found that, regardless of the BMI normalization, CKD patients display lower *OPG* mRNA levels (0.02 [0.02-0.04]

vs. 0.04 [0.03-0.07], P<0.0002). As *AHSG* mRNA was not detected in SAT we did not perform any further analyses of AHSG.

4.4.2 OPG in relation to clinical measurements

Circulating OPG concentrations were, in contrast to mRNA levels, significantly higher in patients as compared to controls (9.3 [7.1-11] vs. 4.2 [3.5-5.0] pmol/l, p<0.0001) (**Table 6, Figure 4**) and correlated with age (rho=0.60, p<0.0001) but did not differ with regard to sex, CVD or diabetes mellitus (data not shown) in patients. Moreover, serum OPG levels were correlated with BMI (rho= 0.45; P=0.007) and were increased in overweight (BMI>25 kg/m^2) as compared with lean CKD-5 patients (10.8 vs. 9.1 pmol/l, p=0.04). In a multivariate model, the association between circulating OPG and BMI was independent of age, gender, inflammation (assayed as CRP levels) and diabetes (**Table 7**). Still, no correlations between concentrations of adipose tissue *OPG* mRNA and serum OPG were found in any of the two study groups.

4.4.3 Immunohistochemical staining of OPG in adipose tissue

Immunohistochemical staining of SAT from both CKD-5 patients and non-uremic controls showed positive immunolabeling of OPG (**Figure 5**). There was no apparent difference in immunolabeling intensity between patient and control tissue.

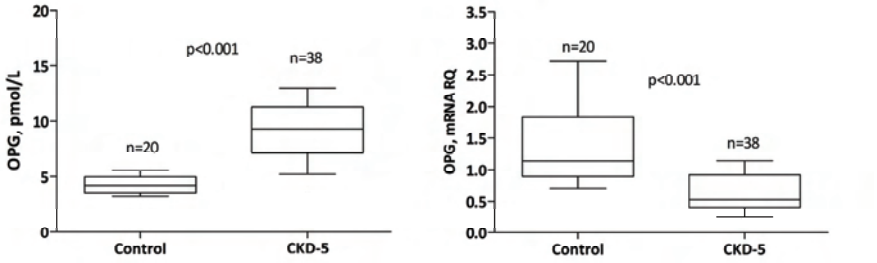

Figure 4. Serum OPG levels (A) and expression levels (relative quantity [RQ]) of OPG mRNA in subcutaneous adipose tissue (B) from healthy controls and CKD-5 patients.

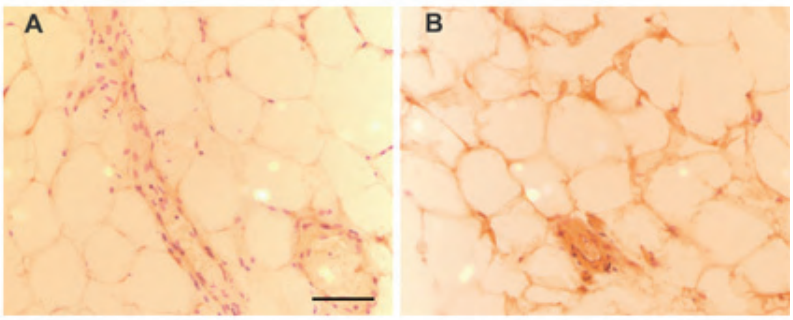

Figure 5. Immunostaining of OPG in subcutaneous fat of CKD-5 patients and controls. Negative controls, not treated with primary antibody, showed no immunoreactivity (A). Immunoreactivity for OPG was mainly localized to mature adipocytes (B). Scale bar: 20µm (applies to both panels).

4.4.4 Multivariate analysis

A larger cohort consisting of 97 CKD-5 patients were used for further statistical analyses in order to confirm the independent association of BMI with serum OPG levels found in the 38 patients. A multiple regression analyses showed that age and inflammation were independent predictors of serum OPG concentrations, whereas BMI was not (**Table 8**). Still, also in this group there were significant differences in median serum OPG levels between patients grouped according to BMI (18-24.9 kg/m^2 8.9 pmol/l, >25 kg/m^2 10.7 pmol/l and >30 kg/m^2 9.5 pmol/l, p=0.02, **Figure 6**). In addition, high OPG levels were, as shown in previous studies, associated with poorer survival during the follow-up period. During this period, 34 (35%) patients died, of which 8 (24%) died due to cardiovascular complications. A Kaplan Meier survival analysis showed that patients with OPG levels within the upper distribution tertile had, as expected, a poorer survival (log rank test 4.1, p=0.04, **Figure 7**).

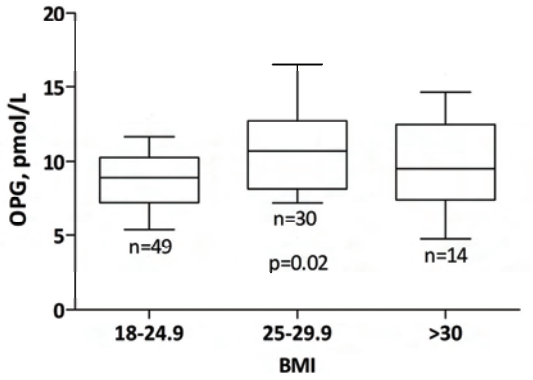

Figure 6. Comparison of serum OPG concentrations between normal (BMI 18-24.9), overweight (BMI 25-29.9) and obese (BMI >30) CKD-5 patients.

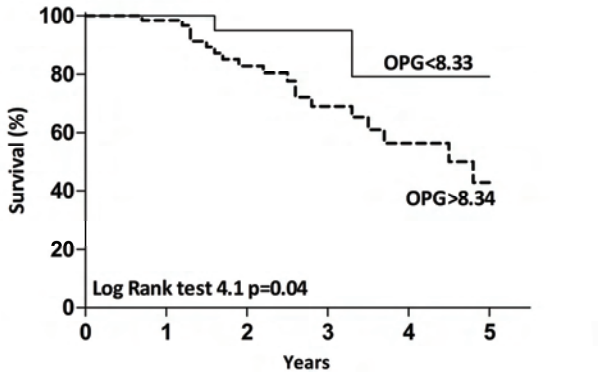

Figure 7. Kaplan Meier survival analysis showing that CKD-5 patients (n=97) with OPG levels within in the highest tertile of distribution have a worse all-cause 5-year survival.

4.4.5 Conclusion

In conclusion, data from the present study showed that subcutaneous adipose tissue in uremic patients expresses *OPG* mRNA, but not *AHSG*, and is immunopositive for OPG. Adipose tissue *OPG* mRNA levels were lower in CKD-5 patients than in controls and, conversely, serum OPG concentrations were higher in patients. Further, serum OPG concentrations were increased in overweight compared to lean CKD patients, although we failed to confirm that BMI was an independent predictor of OPG levels. Nevertheless, the data on OPG in uremic adipose tissue are novel in view of adipose tissue biology in CKD. The relation between OPG and calcification/ossification processes in uremia is of interest to study further in more detail in the future. It remains to be seen whether OPG contributes to the recently proposed cross-talk between fat and bone-associated factors.

Table 6. Clinical and biochemical characteristics of CKD-5 patients and controls.

	Controls	**CKD-5 biopsied**	**CKD-5 total**
N	20	38	97
Age (years)	56 (48-66)	58 (50-65)	58 (51-68)
Gender (% males)	45	57	64
Glomerular filtration rate (ml/min)	94 (86-115)	7 (6-9)***	7 (6-9)***
Diabetes (%)	0	46	40
Cardiovascular Disease (%)	0	44	43
Body mass index (kg/m^2)	26.4 (24.4-29.0)	23.5(21.2-25.5)***	24.1(22.1-27.6)***
S-albumin (g/L)	37(36-40)	35 (33-37)**	34 (30-36)**
C-reactive protein (mg/L)	3.4 (1.2-8.0)	3.9 (1.2-9.9)	4.6 (1.6-14.1)
Interleukin-6 (pg/mL)	2.7 (2.1-4.4)	6.8 (3.9-9.9)***	6.8 (3.7-11.4)***
VCAM-1 (ng/ml)	614 (542-647)	1050 (871-1230)***	1349(1043-1499) [1]***
Osteoprotegerin (pmol/L)	4.2 (3.5-5.0)	9.3 (7.1-11)***	9.5 (7.6-12)***

Data presented as median (25-75 quartile) or percentage.

[1] n = 59

*, **, *** = P-values <0.05, <0.01, <0.001 by Wilcoxon test denoting differences vs control group.

No differences were found between the biopsied and the full CKD group

Abbreviations: VCAM1, vascular cell adhesion molecule 1; ns, not significant.

Table 7. Multiple regression analysis estimating factors associated to serum OPG levels in 38 CKD-5 patients.

	Estimate	SE	T- Value	Adjusted R-Square	P value
Intercept	1.76230	0.60675	2.90		0.0074
Age	1.51070	0.37773	4.00	0.28	0.0005
Male gender	1.23091	0.36519	3.37	0.06	0.0024
Inflammation	1.43508	0.52378	2.74	0.03	0.0110
Diabetes Mellitus	0.67309	0.37370	1.80	0.06	0.0833
Body mass index	0.83471	0.35061	2.38	0.10	0.0249

The adjusted R^2 was 0.53. Age was grouped according to the median (58 years), whereas inflammation (CRP>10 mg/mL) and body mass index (<18 and >24.9 kg/m^2) were grouped by established cut-offs.

Table 8. Multiple regression analysis estimating factors associated to serum OPG levels in 97 CKD-5 patients.

	Estimate	SE	T- Value	Adjusted R-Square	P value
Intercept	7.36611	0.62178	11.85		<0.0001
Age	1.93515	0.60194	3.21	0.1272	0.0018
Male gender	0.58080	0.62750	0.93	-0.0102	0.3572
Inflammation	1.42821	0.64565	2.21	0.0469	0.0296
Diabetes Mellitus	1.00176	0.60199	1.66	0.0170	0.0997
Body mass index	1.04623	0.59281	1.76	0.0302	0.0811

The adjusted R^2 was 0.18. Age was grouped according to the median (58 years), whereas inflammation (CRP>10 mg/mL) and body mass index (<24.9 and >25 kg/m^2) were grouped by established cut-offs.

5 GENERAL DISCUSSION

5.1.1 Overall aim

The human body is equipped with a plethora of tightly regulated control mechanisms to prevent inappropriate responses and maintain physical homeostasis. Nevertheless, under various stress situations this balance may become distorted, causing harm to the individual. The studies included in this thesis concern responses raised against inflicting stress stimuli of distinct characters, specifically surgical injury and chronic renal failure. Although clearly different in their origin, both the acute stress induced via the surgical procedure and the long-term stress developed during the course of chronic renal failure result in increments in systemic inflammatory markers and metabolic changes, which may be detrimental for the patient's recovery or disease progression. The current thesis is therefore aimed at illuminating molecular patterns, which could distinguish a common core of mechanisms important for the two investigated stress models. To limit our investigations we selected two metabolically important organs, skeletal muscle and adipose tissue, which have been shown to also possess inflammatory properties, as seen in other pathophysiological conditions, e.g. inflammatory myopathies (Figarella-Branger et al., 2003) and obesity (Wellen and Hotamisligil, 2005). Further, encouraged by the fact that other studies successfully have exploited gene expression approaches to reveal strong correlations between adipose tissue expression of inflammatory/immune-response genes and metabolic aberrations associated to obesity (Emilsson et al., 2008; You et al., 2005), we performed targeted gene expression analyses on both tissues to dissect central stress-activated/interacting pathways.

5.1.2 Summary of results

Table 9 summarizes the observed gene expression patterns of key inflammatory mediators and insulin/glucose pathway genes in the different tissues and study groups of Paper I, II and III. Notably, surgical stress (Paper I and II) seems to influence a similar cluster of inflammatory candidate genes in both skeletal muscle and adipose tissue. However, and interestingly, except for a decrease in *IRS1* mRNA expression in subcutaneous adipose tissue, alterations in the insulin signaling pathway were only seen in omental fat. Although adipose tissue has lately been reported to serve as a source of proinflammatory factors in obesity, atherosclerosis, type 2 diabetes mellitus and critical illness (Kremen et al., 2006; Mazurek et al., 2003), consequently attracting much attention the inflammatory characteristics of adipose tissue, skeletal muscle has been reported to play an inflammatory role in disease as well (Figarella-Branger et al., 2003). Indeed, pronounced inflammatory activities were found to take place in skeletal muscle after surgery in Paper I. Unfortunately, corresponding gene expression analyses have not yet been performed in skeletal muscle in CKD patients, precluding comparative evaluations between the stress models. In future studies, however, it will be of great interest to investigate if the common inflammatory pattern illustrated in **Table 9** is also found in uremic skeletal muscle. Abdominal subcutaneous adipose tissue was further studied in Paper III and IV, comparing gene expression between CKD-5 patients and non-uremic controls to indentify uremic specific alterations. Surprisingly few

differences were noted between uremic and non-uremic subcutaneous adipose tissue but, as was seen after surgery, adipose tissue from CKD patients showed a significant up-regulation of inflammatory pathway genes such as *IL6* and *SOCS3*. Additionally, leptin and the oxidative stress-related genes *UCP2* and *CYBA* were found to be down-regulated in relation to controls (not shown in Table 9). Finally, not summarized in table 6 are the findings of Paper IV in which we demonstrated that mRNA levels of the bone-associated factor *OPG* are reduced in adipose tissue in uremic patients as compared to controls, despite higher OPG serum protein concentrations in patients and no apparent differences between groups when analyzed immunohistochemically. OPG has implications for inflammation, vascular diseases and mortality (An et al., 2007; Barreto et al., 2005; Yasuda et al., 1998) and the fact that it is expressed in adipose tissue opens up for new areas of research.

Table 9. Comparisons between Paper I, II and III

		Surgical patients			**CKD-5 patients**
		Paper I.	**Paper II.**		**Paper III.**
		Skeletal muscle	**Subcutaneous adipose tissue**	**Omental adipose tissue**	**Subcutaneous adipose tissue**
Pathway	Gene	Change[1]	Change[1]	Change[1]	Change[2]
IL6	IL6	↑	↑	↑	↑
	IL6R	↑	N.S.	N.S.	↓
	STAT3	N.S.	↑	N.S.	N.S.
	SOCS3	↑	↑	↑	↑
TNF	TNF	↑	N.S.	N.S.	N.S.
	TNFRSF1A	↑	N.S.	N.S.	N.S.
	IKBKB	N.S.	↓	↓	N.S.
	NFKB1	↑	N.S.	↑	N.S.
Insulin/glucose	IRS1	N.S.	↓	↓	N.S.
	PIK3R1	N.S.	N.S.	↓	N.S.
	SLC2A4	N.S.	N.S.	↓	N.S.
	SLC2A1	N.S.	N.S.	N.S.	N.S.

[1] mRNA levels after surgery vs. baseline, significance assessed with Wilcoxon signed-rank test for matched pairs

[2] mRNA levels in CKD-5 patients vs. non-uremic controls, significance assessed with Wilcoxon two sample rank sum test

N.S., not significant

5.1.3 Inflammatory and oxidative stress mediators

An interesting observation is the marked increase in *IL6* mRNA in all of the investigated tissues in Paper I, II and III. Because TNF has long been considered as a link between inflammation and insulin resistance both in adipose tissue (Hotamisligil et al., 1995; Hotamisligil et al., 1993) and skeletal muscle (Saghizadeh et al., 1996) we initially believed that TNF could be a potential "key" mediator in stress-induced metabolic aberrations. However, our findings prompted us to reevaluate this hypothesis as mRNA levels of *IL6*, as opposed to *TNF*, were dramatically up-regulated in both stress-models and in all investigated tissues. This is perhaps not surprising as it has been found that circulating IL6 concentrations can reach 10- to 1000-fold the normal level in sepsis and after major surgery (Martin et al., 1997) and that most CKD patients have elevated systemic IL6 levels (Stenvinkel et al., 2002). Importantly, IL6 differs from other cytokines as regards to sites of action since it is acting not only in an autocrine or paracrine but also endocrine fashion, making it a particularly potent proinflammatory mediator (Yudkin et al., 2000). Indeed, IL6 is associated to detrimental complications such as hypertension, adiposity, insulin resistance and increased risk of cardiovascular disease in CKD patients (Stenvinkel et al., 2002). Moreover, we (Thorell et al., 1996) and others (Gletsu et al., 2006) have shown that concentrations of circulating IL6 are positively correlated to the reduction in insulin sensitivity after surgery, and that worsening of insulin resistance is associated with increasing adipose tissue content of IL6 (Gletsu et al., 2006).

Inflammation is also related to oxidative stress in a wide range of biological processes, e.g diabetes, triggering tissue-damage and other potentially pathogenic mechanisms (Brownlee, 2005). In CKD patients oxidative stress constitutes an important link between uremia, inflammation and elevated mortality risk and provides a better prognostic value as opposed to traditional cardiovascular risk factors such as hypertension and hypercholesterolemia (Halliwell, 1993). It is therefore worth noting that our finding of reduced mRNA levels of *UCP2* and *CYBA* in adipose tissue of CKD-5 patients could suggest an unfavorable shift in the transcriptional output of oxidative stress genes in uremic fat. Oxidative stress in adipose tissue may be of particular importance since it has been shown to reduce insulin sensitivity by hampering insulin-stimulated glucose transport in 3T3-L1 adipocytes (D'Apolito et al., 2010). Insulin resistance, in turn, further contributes to the elevated risk for cardiovascular complications and mortality in CKD patients (Shinohara et al., 2002).

5.1.3.1 Adipose tissue and skeletal muscle in inflammation

Previous studies have shown that *IL6* and *IL8* mRNAs were barely detectable in peripheral blood leukocytes after surgery, despite elevated concentrations in blood, thus indicating that cells in the surgical wound produce and secrete IL6 and IL8 (Sakamoto et al., 1994) as well as, as suggested by these studies, by adipose tissue and skeletal muscle. Consequently, it could be expected that local inflammatory activities contribute more to systemic IL6 and therefore are more important for the insulin desensitizing processes in peripheral tissues. IL6 production is suggested to be stimulated by cellular, metabolic or inflammatory stress (Sabio et al., 2008) and the current studies illustrate that the stress imposed by the uremic imbalance or the surgical injury indeed stimulates a locally increased proinflammatory transcription, further contributing to the

inflammatory burden and stress reactions. The primary sources of cytokines are not completely determined but it has been estimated that adipose tissue contributes with as much as 30% of the total circulating concentrations of IL6, and studies in healthy individuals have shown that the production of IL6, as well as systemic concentrations, correlated positively with percent body fat (Mohamed-Ali et al., 1997). There are also reasons to believe that adipose tissue is a source of inflammatory mediators in the uremic state, since our group has previously reported relations between increased fat mass and serum IL6 concentrations (Axelsson et al., 2004), corroborating other studies reporting an association between inflammation biomarkers and fat mass in CKD (de Araujo Antunes et al., 2009; Roubicek et al., 2009). Additionally, our findings of increased *IL6* expression in skeletal muscle are corroborated by studies reporting skeletal muscle cytokine production both in healthy subjects (Pedersen and Hoffman-Goetz, 2000) and chronically ill individuals (Figarella-Branger et al., 2003). Furthermore, TNF overexpression has been reported in skeletal muscle in obese, insulin resistant and diabetic humans (Saghizadeh et al., 1996) further supporting that this tissue has important inflammatory properties in various states of disease.

5.1.3.2 Macrophage markers in adipose tissue

The issue of macrophages residing in adipose tissue is highly relevant in this discussion and has recently gained a lot of attention. Previous studies have shown an enhanced transcription of inflammatory genes in nonfat cells (Fain et al., 2008) of which macrophages appear to be responsible for almost all adipose tissue TNF expression and significant amounts of IL6 expression (Fain et al., 2004; Weisberg et al., 2003). When evaluating the expression of macrophage specific genes (*CD68* and *MSR1*) in the current studies we did indeed detect mRNA levels of these markers in both subcutaneous and omental fat but they were not significantly increased after surgery, nor did we observe any differences in the expression between CKD patients and controls. These findings corroborate a previous study showing presence, but no increase, of CD68-positive cells in adipose tissue in cardiac surgery patients (Kremen et al., 2006). On the contrary, another study on CKD patients demonstrated an increased subcutaneous adipose tissue mRNA expression of *CD68* as well as infiltration of CD68 immunopositive cells, compared to non-uremic patients (Roubicek et al., 2009). Although, in our limited investigations, we were unable to find any indications of an increased infiltration of macrophages, this issue should not be neglected, but rather meriting further studies especially regarding the macrophage specific contribution of inflammatory or oxidative stress-related molecules.

5.1.4 Insulin signaling intermediates

The down-stream insulin signaling pathway genes selected for our studies (Paper I, II and III) did not show altered mRNA levels in skeletal muscle (surgery patients), *IRS1* mRNA concentrations were affected in subcutaneous fat after surgery, but not in CKD-5 patients, whereas *IRS1*, *PIK3* and GLUT4 (gene: *SLC2A4*) mRNA concentrations were all altered in omental adipose tissue following surgery. Nevertheless, it is clear from recent studies that glucose utilization is impaired in peripheral tissues after surgery (Thorell et al., 1999a) as well as in patients with CKD (DeFronzo et al., 1981). Among the identified aberrations associated with peripheral insulin resistance after surgery are defects in insulin-stimulated GLUT4 translocation in skeletal muscle

(Thorell et al., 1999a), but without impaired signaling from the insulin receptor to PIK3 (Strommer et al., 1998). In uremia, glucose uptake is impaired without perturbations in the insulin binding and activation or depletion of the GLUT4 protein (Friedman et al., 1991). Our results might imply that (1) the surgical and uremic stress affect the expression of other insulin signaling pathway genes, (2) the transcription of the investigated genes is not regulated in skeletal muscle or subcutaneous fat, under these circumstances, or, (3) that impaired activity of these insulin signaling pathway mediators does not contribute to the progression/aggravation of the pathophysiological conditions in these patients. Further studies are needed to gain better knowledge of the cellular mechanisms contributing to insulin resistance in postsurgical as well as CKD patients.

5.1.5 Comparisons between subcutaneous and omental fat

The design of Paper II makes it possible to compare the mRNA expression between subcutaneous and omental (visceral) adipose tissue and, at least in our hands, the most prominent difference appears to be the reduced expression of the insulin signaling genes in omental adipose tissue, since no marked differences were observed as regards to expression of inflammatory mediators between subcutaneous and omental adipose tissue. It is plausible that omental adipose tissue plays a more pronounced role in postsurgical stress, but the literature does not provide a comprehensive answer to this. Some studies suggest that visceral adipose tissue has a larger impact than subcutaneous adipose tissue on proinflammatory signaling and metabolic aberrations (Arner, 1999; Wajchenberg et al., 2002). However, abdominal subcutaneous adipose tissue expression of inflammatory cytokines has been linked to metabolic comorbidities associated with obesity (You et al., 2005) and, moreover, decreased insulin-induced glucose-transport has been reported in isolated subcutaneous fat cells after surgery (Nordenstrom et al., 1989). Thus, although the knowledge regarding adipose tissue and its role in human physiology is expanding, the specific roles of different adipose tissue depots is not fully understood. Though it may very well be that visceral/omental adipose tissue is characterized by a more active production of inflammatory and metabolic factors, it is still important to keep in mind that it probably has an overall smaller contributory effect compared to subcutaneous adipose tissue since the body's total amount of subcutaneous adipose tissue may exceed the amount of visceral adipose tissue by three to four times (Gustafson et al., 2007).

5.1.6 Possible interactive mediators in other organ systems

The discussion above has so far illustrated how inflammatory and metabolic aberrations may contribute to disease, as well as the intrinsic properties of metabolically active organs, such as adipose tissue and skeletal muscle, that enable integration of divergent pathways (Nielsen and Pedersen, 2008; Wellen and Hotamisligil, 2005). Importantly, since the body's different physiological systems and tissues are interconnected and largely dependent on one another, disturbances may be propagated between different tissues as well as between local and systemic actors. This view encouraged us to further explore potential cross-talks between distinct tissues and therefore we expanded our analyses on adipose tissue in CKD patients in Paper IV. Particularly interesting in view of the vascular complications in CKD patients are the emerging data suggesting an intriguing bone-fat cross-talk with potential implications for vascular biology (Ducy et

al., 2000; Gordeladze et al., 2002; Lee et al., 2007). The current study investigated the bone-proteins AHSG and OPG and we detected *OPG*, but not *AHSG*, expression in uremic adipose tissue. The mRNA levels were lower in CKD patients as compared to controls, despite higher OPG serum protein concentrations in patients and no apparent differences between groups when analyzed immunohistochemically. It is clear that CKD patients suffer from a cluster of complications such as malnutrition, oxidative stress, endothelial dysfunction and insulin resistance. These are linked to chronic inflammation and contribute to atherosclerosis, vascular ossification and the alarmingly high CVD-related mortality in this patient group (Stenvinkel et al., 2008; Stenvinkel et al., 2005). Therefore it is an urgent issue to search for additional mediators, and potential treatable targets, contributing to tissue aberrations and chronic inflammation. With a role in bone-turnover (Yasuda et al., 1998), inflammation (An et al., 2007) and vascular calcification (Barreto et al., 2005) OPG is highly interesting in this aspect. These preliminary results emphasize the need for further research regarding interactions between adipose tissue and bone in uremia. Such interactions might have implications for pathophysiological mechanisms linking adipose tissue with bone disorders and vascular calcification.

5.1.7 Comparison between changes in acute and chronic stress

The main strength of the current thesis is the translational design, which provides unique opportunities to address the question of common pathogenic mechanisms between two clinically relevant stress situations. Insulin resistance appears as one central and common denominator in acute and chronic stress. Present in a wide spectrum of diseases, ranging from hepatitis C (Knobler et al., 2003) to rheumatoid arthritis (Sattar et al., 2003) it may be viewed as an universal metabolic change during disease and there are good reasons to propose, based on a large body of data, that it is a crucial parameter for the outcome of acutely as well as chronically ill patients (Shinohara et al., 2002; van den Berghe et al., 2001). An important remark is that the complications, i.e. cardiovascular and infectious complications, muscle weakness, renal insufficiency and polyneuropathy, that occur within hours/days in acutely ill patients and which are being prevented by ameliorating the insulin resistant condition (Van den Berghe et al., 2006; Van den Berghe et al., 2003) are similar to the ones that develop in chronic insulin resistance in patients with type 2 diabetes after years/decades (Brownlee, 2001), as well as in uremic patients (Shinohara et al., 2002; Siew et al., 2007). This further puts emphasis on the overlapping pathophysiologial processes of acute and chronic stress, and clearly demonstrates a potential benefit of exploring common and essential response mechanisms via comparative and translational studies.

The time aspect of the different stressors, i.e. the fact that surgical patients are only acutely exposed to the surgical trauma whereas CKD patients suffer from an irreversible and sustained pathophysiological condition, should inevitably be considered in this discussion. The short-term compensatory response to a tissue injury caused by surgery likely causes more dynamic and pronounced alterations, producing acute inflammation and a transient decrease in insulin sensitivity to allow the redistribution of glucose from tissues with insulin-dependent glucose uptake, such as skeletal muscle, to vital organs such as the brain and heart, and to immune cells that increase their energy demand during tissue repair (Medzhitov, 2008). On the other

hand, patients reaching CKD suffer a life-long condition and the body needs to mobilize strategies to cope with, and adapt to, the chronic uremic imbalance and increased amounts of noxious substances in the circulation. In this situation it may be speculated that tissues being exposed to this uremic milieu undergo stress-adaptations, and that these adaptations may involve production of mediators that could either exacerbate or ameliorate the uremic condition. Moreover, CKD is associated to low-grade, chronic, inflammation, similar to what is found in obesity and diabetes, and it has been suggested that this persistent inflammation is partly a separate condition, referred to as para-inflammation (Hotamisligil, 2006; Medzhitov, 2008). This para-inflammation is considered to share similarities with the classical inflammatory response but is suggestively triggered by nutrients or intrinsic cues (Hotamisligil, 2006; Medzhitov, 2008) and it is not unlikely that such an intrinsic cue could be the toxic uremic milieu. While it is clear that the uremic milieu induces a fast progression of vascular calcification (Suliman et al., 2008), it is also reasonable to assume that it could accelerate the development of disorders such as the metabolic syndrome, which normally takes years to develop in a non-uremic individual (Despres and Lemieux, 2006).

One universal goal in research and clinical work regarding these pathophysiological states should be to better understand the intimate relation between stress, immunity and metabolism. Unequivocal experimental evidence shows that inflammation is a trigger of insulin resistance. Studies in rodent models have shown that the presence or absence of various inflammatory mediators modulates the insulin responsiveness (Hirosumi et al., 2002; Shoelson et al., 2003; Uysal et al., 1997), pointing towards inflammation as a primary cause of insulin resistance. In addition, metabolic dysfunction triggers inflammation, worsening the metabolic function even further. Moreover, because insulin also possesses anti-inflammatory properties, a state resistant to insulin aggravates the inflammatory response, in all creating a vicious circle (Dandona et al., 2004). Future studies in which these issues are addressed should be instrumental for delineating the order of events. Nevertheless, it should be underscored that despite the fact that the surgical tissue injury and the uremic tissue stress have different biological implications they share common characteristics, such as increments of crucial inflammatory mediators and reduction in insulin sensitivity, which have deteriorating effects on the individual.

5.1.8 Study considerations

5.1.8.1 Gene expression analyses

Gene expression is a fundamental regulatory mechanism, controlling important biological functions and was therefore chosen as a key variable in the present thesis. Still, one might question the rationale of studying cellular alterations at the transcription level. For example, do the transcriptional outputs change fast enough in order to enable detection of alterations during surgery? As the ability of supplying fuel to all organs of the body is crucial for survival, there is a need for sensitive control mechanisms and high flexibility in the regulation of the metabolic systems. Metabolic challenges require immediate activation of adequate signal transduction pathways and enzymes. In addition to these immediate responses there is also a more prolonged adaptation taking place, which may develop within the course of a couple of hours, or sometimes within a

few days, to produce larger quantities of the required mediators. Gene expression changes is a way to enhance and prolong the response (Frayn, 2003). Thus, in the case of surgery, the immediate and transient effect of stress hormones may be extended by increased transcription and, consequently, enhanced effects of cytokines. For example, since IL6 is released into plasma already within 30 min after abdominal surgery (Baigrie et al., 1992; Ohzato et al., 1992; Shenkin et al., 1989), a potential up-regulation of *IL6* should be detectable immediately following surgery, as in our present studies. Nonetheless, findings in gene expression studies may be inconclusive since mRNA levels do not always correctly reflect the actual protein levels. Also, gene expression analyses can only give information about biological changes that are reflected in the transcriptional network and do not take into account post-transcriptional alterations that may induce changes in cellular activities. However, such changes could potentially affect the expression by influencing processes such as transcript stability, rates of transcription, transport of RNA from the nucleus and alternative splicing.

5.1.8.2 Insulin resistance measurements

Although one important aim in Paper I and II was to explore possible underlying mechanisms of postoperative insulin resistance, no actual measurements of insulin sensitivity were performed. However, the surgical model, i.e. major elective surgery, exploited in the present study has repeatedly and convincingly proven to be insulin resistance-inducing in a series of studies from our group (Thorell et al., 1993, 1994; Thorell et al., 1999a; Thorell et al., 1999b). In fact, by measuring insulin sensitivity in more than 100 patients undergoing major surgery, we have not been able to identify a single subject who displays increased insulin sensitivity postoperatively. We were therefore confident that the patients undergoing major abdominal surgery included in the current studies would also become insulin resistant, and we therefore did not find it necessary to confirm this by subjecting the patients to further blood sampling and repeated clamp studies. However, this precludes the possibility to put changes in gene expression in relation to insulin sensitivity, which could be considered as a limitation of the study.

5.1.8.3 Statistical methods

All studies are limited in size and thus statistical correlations should be interpreted with caution regarding causality. The small number of observations also increase the risk of Type I error. We are also well aware of the risk of conducting Type II errors due to multiple testing in the case of our multiplex gene expression studies.

5.1.8.4 The study design

Because the studies included in the current thesis are of an observational character, the possibility of making causative associations are precluded and no definitive answers regarding the mechanisms involved in the different situations of stress are given. Nevertheless, our intention was to present the data in a straightforward way that could provide novel information to the current literature in this research area and possibly provide routes for further investigation. Above all, the translational approach of the current thesis provides an amazing opportunity to explore common and essential response mechanisms in conjunction with acute and chronic stress on a unique patient

material that allow analyses concerning the intimate relation between stress, immunity and metabolism.

6 CONCLUSIONS

In summary, the main results obtained from this thesis suggest the following:

- The effects of surgical stress on gene expression differ between different tissue depots (Paper I and II).

- The acute stress inflicted by three hours of elective surgery induces alterations in the transcription profile of abdominal skeletal muscle. These are mainly characterized by increments in mRNA concentrations of inflammatory pathway genes (Paper I).

- During a surgical stress situation, similar alterations in mRNA levels of inflammatory pathway genes occur in abdominal subcutaneous adipose tissue and in omental adipose tissue (Paper II).

- Inflammatory activities in peripheral insulin responding tissues may play a role in the progression of postsurgical stress aberrations (Paper I and II).

- mRNA levels of central insulin signaling genes are decreased in omental adipose tissue after surgery. This is particularly interesting in view of metabolic dysregulation associated to postsurgical complications, such as insulin resistance and hyperglycemia (Paper II).

- Under the sustained stress condition imposed by chronic renal failure inflammatory genes are up-regulated in the uremic as compared to the non-uremic abdominal subcutaneous adipose tissue (Paper III). These inflammatory genes are the same as are being up-regulated in acute surgical stress (Paper I, II and III).

- Uremic adipose tissue demonstrates a distinct expression pattern of oxidative stress related pathway genes (Paper III).

- Both uremic and non-uremic abdominal subcutaneous adipose tissue produce *OPG* mRNA and is positive for OPG immunohistochemical staining. The presence of bone-regulating factors such as OPG in uremic adipose tissue may have implications for bone-fat cross-talks and, further, for vascular calcification (Paper IV).

- The studies on CKD-5 patients may illustrate some potential aberrations important for the intrinsic properties of uremic fat and phenotype (Paper III and IV).

7 FUTURE PERSPECTIVES

The ultimate question is if these findings matter. The current thesis illuminates a core of similarly regulated mediators, which may participate in the progression of inflammatory and metabolic complications observed after surgery and in CKD. We believe that these observations contribute to the understanding of alterations occurring in skeletal muscle and adipose tissue in association to acute and chronic stress. Whereas no final evidence is provided, the present data emphasize that the impact of the mutual interplay between immunity and metabolism and its implications for acute and chronic stress should not be neglected. Moreover, the data suggest that skeletal muscle and adipose tissue are potential sites for integration of stress regulating pathways. Learning more about the body's different physiological systems and tissues, and how they are interconnected and largely dependent on one another may also be relevant for other common disorders, eg. diabetes, in which the normal function of adipose tissue and skeletal muscle is impaired and pronounced inflammation and metabolic disturbances exacerbate the disease state. In the future, further studies to gain experimental, epidemiological and clinical evidence for the data presented in the current thesis are warranted to expand the knowledge in this field of translational research.

Moreover, studies on epigenetic changes, such as DNA methylation, could add information regarding the stress response "memory". For example, epigenetic changes may be induced by the surgical stress, producing prolonged gene expression changes, or, vice versa, the surgery induced gene expression changes could trigger epigenetic alterations, further augmenting the stress reaction. Delineating the chain of events by gaining information on the time course of the introduced changes is required to acquire a complete picture of the molecular mechanisms causing postsurgical complications. Similarly, it is of interest to investigate if the uremia in the CKD patients induces epigenetic changes that alter gene expression, which potentially influence the disease progression. Combining such genetic information with clinical data not only provide a more profound understanding of the underlying mechanisms of the metabolic alterations but may also be used for clinical applications, such as early risk factor stratification, permitting selection of individualized treatment regimes. Preventive actions aimed at regulating the inflammatory and metabolic changes may have beneficial effects in the clinical setting, promoting postoperative recovery and ameliorating complications in CKD patients, as well as improving the overall metabolic control.

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