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Being a thesis for the degree of doctor of medicine (philosophiae doctor)

FAT TISSUE, ADIPOKINES AND CLINICAL COMPLICATIONS OF CHRONIC KIDNEY DISEASE

Jonas Axelsson
I swear by Apollo Physician, by Asclepius, by Health, by Panacea, and by all the Gods and Goddesses, making them my witnesses, that I will carry out, according to my ability and judgement, this oath and this indenture...I will use treatment to help the sick according to my ability and judgement, but never with a view to injury and wrongdoing... In whatsoever house I enter, I will enter to help the sick, and I will abstain from all intentional wrongdoing and harm, especially from abusing the bodies of man and woman, bond or free. And whatsoever I shall see or hear in the course of my profession in my intercourse with men, if it be what should not be published abroad, I will never divulge, holding such things to be holy secrets. Now if I carry out this oath, and break it not, may I gain forever reputation among all men for my life and for my art.

The Physician’s Oath, Hippocrates (460-377 B.C.)
Love - is anterior to Life -
Posterior – to Death –
Initial of Creation, and
The Exponent of Earth.
Emily Dickinson (1830 – 1886)

For my parents, Inger and Bo Axelsson,
and for my grandparents;
Ella and Gustav Petterson & Ingrid and Anders Axelsson.
Patients with chronic kidney disease (CKD), irrespective of initial etiology, have a risk of cardiovascular morbidity (CVD) and mortality that is many-fold higher than that of a similar person without CKD. While traditional risk factors, such as hyperlipidemia, certainly contribute, these factors cannot by themselves explain the burden of CVD. Recent data suggest that endocrine signaling of fat mass may lead to the complex of CVD risk factors known as the metabolic syndrome. In the following work, we test the hypothesis that an uremic metabolic syndrome caused by aberrant adipose tissue signaling may contribute to inflammation and metabolic disturbances in CKD.

**Study I.** In a cross-sectional study of 187 incident patients starting dialysis therapy, we measured several inflammatory cytokines and adipokines and related these to total and truncal fat mass as estimated by dual-energy x-ray absorptiometry (DEXA). We found that truncal, but not non-truncal, fat mass was associated with circulating levels of both interleukin(IL)-6 and C-reactive protein (CRP).

**Study II.** In a study of 197 incident CKD patients evaluated shortly before the start of dialysis, we showed that patients with elevated fat mass, although having elevated inflammatory markers, are less likely to need recombinant erythropoietin (epoetin), and that epoetin dose is associated with circulating levels of leptin.

**Study III.** This is a post hoc, cross-sectional study comparing 239 CKD patients with varying degrees of renal function impairment with an age- and gender-matched randomly selected control group of 24 individuals. We explored the role of decreased renal function on the adipokine resistin, apparently able to inhibit hepatic insulin action in mice. We also investigate possible links with inflammation (CRP, IL-6) and the insulin resistance (QUICKI, HbA1c%) present in patients with CKD. While resistin is strongly related to renal function and inflammation, we could find no links to surrogate markers of insulin resistance.

**Study IV.** In a longitudinal study of 158 CKD patients with varying degrees of renal failure, we explored the relationship between changes in body fat (DEXA) and tissue resident macrophages (assessed by measuring sCD163), as well as links to systemic inflammation (IL-6, CRP). We concluded that an increase in fat mass is associated with increased sCD163, a surrogate marker of macrophages, and more inflammation in CKD patients.

**Study V.** Again using a cross-sectional design of 189 prevalent CKD patients, we investigated putative links between the adipokine visfatin and insulin resistance in CKD. We conclude that while a reduced renal function and inflammation both independently predict visfatin levels, visfatin is not associated with QUICKI or HbA1c%. However, increased circulating visfatin was associated with endothelial adhesion molecules (sVCAM-1) and a higher mortality rate.

**Study VI.** Using a Mendelian randomization approach in a cohort of 198 incident CKD patients starting dialysis, we show significant differences in serum lipids between functional genotypes of α2-Heremans-Schmid glycoprotein (AHSG) as well as a significant association between AHSG and fat mass (DEXA).

**Conclusions.** In patients with chronic renal disease, total and, especially, truncal fat mass is associated with increased circulating levels of several cytokines and adipokines that have may influence cardiovascular health and outcome. Thus, further studies are needed to evaluate the impact of fat mass and adipokines on outcome in this patient group.

**Keywords.** Chronic kidney disease, end-stage renal disease, insulin resistance, inflammation, cardiovascular disease, adipokines, leptin, epoetin, resistin, visfatin, AHSG, CD163, outcome.
I. Truncal fat mass as a contributor to inflammation in end-stage renal disease.

II. Body fat mass and serum leptin levels influence epoetin sensitivity in patients with end-stage renal disease.
Axelsson J, Qureshi AR, Heimbürg O, Lindholm B, Stenvinkel P, Barany P.

III. Serum resistin levels are associated with residual renal function and inflammation, but not with insulin resistance, in patients with chronic kidney disease.
*Kidney Int.* 2006 Feb;69(3):596-604

IV. Changes in fat mass are associated with changes in CD163, a marker of activated macrophages, in patients with chronic kidney disease.
*In Manuscript.*

V. Circulating levels of visfatin/pre-B cell colony-enhancing factor are influenced by genotype and are associated with markers of endothelial dysfunction and survival in patients with chronic kidney disease.
*In Manuscript.*

*In Manuscript.*
Thus there are two books from whence I collect my Divinity; besides that written by one of God, another of his servant Nature, that universal and public Manuscript, that lies expanded unto the eyes of all; those that never saw him in the one have discovered him in the other.

William Shakespeare (1564 – 1616)
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INTRODUCTION

The recently discovered role of adipose tissue as not only an inert storage depot but also a source of adipokines has opened up a brand new area for research. These adipokines may act as autogenic regulators of body fat depots, modulating gastrointestinal activities, metabolic changes and central nervous mechanisms which have been speculated to play a central role in the development of the complications often observed in patients with metabolic complications such as insulin resistance, cardiovascular disease (CVD) and sarcopenia (loss of muscle mass) [1]. Furthermore, there are intimate links between adipokines and pro-inflammatory cytokines, such as IL-6, as well as between fat and muscle tissue [1]. Considering the dramatic effect the loss of renal function has on the clearance of these substances [2], the systemic effects of adipokines in CKD patients may be greater than in the general population.

Is it good to be fat if you have CKD?

In contrast to findings in the general population, a number of studies have documented that a high body mass index (BMI) is associated with a better outcome in the chronic kidney disease (CKD) patient population [3, 4]. However, as discussed in this study, BMI is not a very precise parameter of nutritional status and does not accurately describe body composition (eg. differentiate between muscle mass and fat mass). Indeed, in a study of 70 028 patients that initiated dialysis in the United States from 1995-1999, it was demonstrated that a protective effect from a high BMI is only present in patients with a normal or high muscle mass [4]. However, some of the data presented in the present study would indeed appear to support a positive effect of adipose tissue, at least in the setting of end-stage renal disease (ESRD).
However, as we have only performed cross-sectional studies in two cohorts, further mechanistic studies are clearly needed to ascertain if CKD patients, are, on balance, harmed by the adverse metabolic consequences of an increased fat mass, or if the pathophysiology of CKD makes an increased adipokine level on balance beneficial despite the presumed detrimental effect of an increase in systemic inflammation associated with increased fat mass.

Our results indicate that an increased fat mass in CKD, like in other patients groups, may indeed have adverse metabolic consequences, including an increase in systemic inflammation, but that the effects of a decreased muscle mass are more important in determining short-term outcome. We suggest the possibility that a deeper and more mechanistic understanding of adipocytes signaling could – at long last - suggest new therapeutic targets for this very exposed patient group.
We must trust to nothing but facts: These are presented to us by Nature, and cannot deceive. We ought, in every instance, to submit our reasoning to the test of experiment, and never to search for truth but by the natural road of experiment and observations.

Antoine Lavoisier (1743 – 1794)
Renal disease epidemiology

The global population of patients with chronic kidney diseases (CKD) that have entered end-stage renal disease (ESRD), i.e. require treatment with renal replacement therapy (RRT), was estimated to have reached almost 1.7 million at the end of 2003, and continues to grow at a significantly higher rate than the world population. Of the 1.7 million ESRD patients, 1.3 million were undergoing dialysis treatment, and over 300,000 people were living with kidney transplants [5]. The limited amount of available data, mainly derived from registries in the United States, suggests that CKD is also a significant epidemiologic problem in the stages before RRT. The Third National Health and Nutrition Examination Survey (NHANES III), collecting data among a large representative sample of the United States population (18,723 participants of different age, sex, and ethnic groups examined between 1988 and 1994), still represents the most comprehensive source of epidemiologic data regarding CKD in the conservative phase [6]. This survey estimated that up to 11% (19 million) of the general US adult population could have some degree of CKD, including more than 8 million individuals with glomerular filtration rates (GFR) of less than 60 mL/min. The percentage of the overall US population with serum creatinine (SCr) values >1.5 mg/dL (>132 μmol/L) was 9.7% in men and 1.8% in women; it was also found that older age, together with male sex, was associated with higher SCr levels. More than 30% of men and nearly 10% of women aged >70 years were estimated to have SCr levels >1.5 mg/dL in this survey [6]. This analysis also estimated that 5.9 million people could have stage 1 CKD with normal renal function.
In the developed countries, renal injury is today caused mainly by the vasculopathy associated with diabetes mellitus, autoimmune inflammation damaging the kidney (such as chronic glomerulonephritis) and hereditary kidney diseases (such as polycystic kidney disease). Toxic damage (e.g. from heavy metals) and hypertension used to be common causes of renal failure, but are today seldom seen by the nephrologists due to better preventative medicine and stricter environmental laws. Detection of patients at risk is important to implement measures to slow down progression of CKD and avoid secondary complications. As it is clear that most of these CKD patients die before they reach ESRD [5], it might be that by taking the warranted preventive measures, the number of ESRD patients might still further increase exponentially.

### Renal pathology

Most chronic nephropathies lack a specific treatment and progress relentlessly to ESRD. However, research has indicated possible

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**Table 1. Staging of CKD according to the National Kidney Foundation. Reprinted with permission.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (mL/min/1.73 m²)</th>
<th>Action*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or ↑ GFR</td>
<td>≥90</td>
<td>Screening, CKD risk reduction</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mild ↓ GFR</td>
<td>60–89</td>
<td>Estimating progression</td>
</tr>
<tr>
<td>3</td>
<td>Moderate ↓ GFR</td>
<td>30–59</td>
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</tr>
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</table>

Shaded area identifies patients who have chronic kidney disease; unshaded area designates individuals who are at increased risk for developing chronic kidney disease. Chronic kidney disease is defined as either kidney damage or GFR <60 mL/min/1.73 m² for ≥3 months. Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies.

* Includes actions from preceding stages.

Abbreviations: GFR, glomerular filtration rate; CKD, chronic kidney disease; CVD, cardiovascular disease
preventive methods such as pharmacologic control of blood pressure, reduction of proteinuria, smoking cessation, tight glucose control for diabetes, and many other measures that may postpone dialysis for many patients with CKD. Remission of disease and regression of structural damage to the kidney are the goals to achieve in the future, and is still a hot topic of research, even after more than 50 years. Students of renal pathophysiology have known for decades that regardless of where injury begins, if it persists, the kidney will gradually self-destruct through a final common pathway involving interstitial nephritis and fibrosis [7].

Essentially, glomerular hypertension leads to proteinuria and downstream cytokine effects. Nephritogenic T lymphocytes are engaged, adding more cytokine activity through interstitial nephritis. Tubular epithelial units thus decondensate and their basement membranes disrupt to permit epithelial-mesenchymal transition, forming fibroblasts. These fibroblasts migrate, proliferate, and lay down unwanted collagens, leading to fibrosis of interstitial tissues and ultimately ESRD.

**Polypeptide clearance in renal disease**

While the kidney is normally an important place of polypeptide metabolism and excretion as well as a source of important peptide hormones such as erythropoietin, current technology for dialysis treatment does not remove polypeptides larger than 3-5 kDa [8]. Also, even this removal is dependent on the degree of ultrafiltration.
and is totally unselective as to the type of polypeptides removed, unlike normal physiological kidneys. This appears to lead to the accumulation of either active or metabolized polypeptide fragments, as evident from the fact that almost any cytokine measured is elevated in CKD and correlates with GFR.
Sometimes give your services for nothing, calling to mind a previous benefactor or present satisfaction. And if there be an opportunity of serving one who is a stranger in financial straights, give full assistance to all such. For where there is love of man, there is also love of the art. For some patients, though conscious that their condition is perilous, recover their health simply through their contentment with the goodness of the physician. And it is well to superintend the sick to make them well, to care for the healthy to keep them well, also to care for one’s own self, so as to observe what is seemly.

Hippocrates (460-377 B.C.)
GREATLY INCREASED MORTALITY IN PATIENTS WITH CKD

High mortality in chronic kidney disease

Despite the remarkable advances in dialysis therapies, CKD patients are at marked increased risk for CVD [9], suggesting that they are subject to a process of accelerated atherogenesis due to uremia-associated factors. In fact, even subtle kidney dysfunction should be considered a medical condition predisposing to increased cardiovascular risk [10]. Despite recent improvements in dialysis technology the majority of maintenance dialysis patients die within a 5-year period - a survival worse than that of the majority of patients with cancer disease.

Traditional risk factors proven to contribute to atherosclerosis in the general population, such as dyslipidemia, diabetes mellitus, and hypertension, are also highly prevalent in CKD patients [11]. However, it is evident that the high CVD-risk in CKD is incompletely accounted for by traditional risk factors. The search for non-traditional uremia-related risk factors that may be involved in the pathogenesis of CVD in the CKD patient has been an area of intense study. It is becoming increasingly appreciated that CKD is characterized by a state of low-grade chronic inflammation that seems to be linked to wasting, vascular calcification, oxidative stress, and endothelial dysfunction [9, 11]. Indeed, a wide array of inflammatory biomarkers, such as C-reactive protein (CRP), interleukin (IL)-6 and white blood cell count have been shown to be robust predictors of outcome in this patient group [9].

Individual differences in complications of CKD

Whereas a majority of CKD patients in various European and North American studies have serologic evidence of an activated acute-phase
response, CKD patients of East-Asian origin appear to have a lower prevalence of elevated CRP and CVD [12]. Moreover, about 20% of all CKD patients have levels of CRP and IL-6 that could be regarded as normal, or even subnormal. The reason(s) why some CKD patients have normal IL-6 and CRP levels are unknown but it can be speculated that some, if not many, inter-individual differences can be explained by genetic susceptibility patterns [13].

**CKD as a state of chronic systemic inflammation**

Evidence suggest that persistent inflammation (and oxidative stress) appears early on in the process of a deteriorating kidney function [14]. Primed peripheral polymorphonuclear leucocytes seem to be a key mediator of low-grade inflammation and oxidative stress in mild CKD [15]. In ESRD patients elevated median CRP levels have been documented both close to the start of dialysis [16] and in patients receiving both peritoneal and hemodialysis [17]. Furthermore, while large inter-individual differences exist, chronic inflammation is such a common phenomenon in European [12] and North-American [18] CKD populations that its role as an atherosclerotic mediator and prognostic indicator is of vital interest to the field of nephrology.

*Figure 2. Prevalence of elevated serum CRP in dialysis patients*

![Figure 2. Prevalence of elevated serum CRP in dialysis patients](image-url)
Numerous studies have shown that elevated CRP predicts both all-cause and cardiovascular mortality in both CKD [19] as well as in ESRD patients treated by hemodialysis (HD) [20-22] or peritoneal dialysis (PD) [17]. In PD patients an elevated CRP was shown to be an independent predictor of non-fatal myocardial infarction [23] and increased incidence of CVD [17]. Other inflammatory markers, including IL-6 [24-26] and fibrinogen [27], also have been shown to predict mortality in this population. Indeed, Panichi et al [26] have demonstrated that interleukin(IL)-6 is a stronger predictor than CRP of survival in HD-patients. Their data are corroborated by own recent findings showing that out of four putative biochemical risk markers (CRP, IL-6, S-albumin and AHSG) IL-6 may be the most reliable predictor of CVD and mortality in ESRD [28].

**Strong associations between wasting, inflammation and atherosclerosis**

Although hypoalbuminemia and malnutrition are associated to both inflammation and oxidative stress and are important predictors of mortality in CKD patients [29], malnutrition as such does not appear to be the direct cause of mortality in CKD patients. One possible explanation for this may be interactions between atherosclerotic CVD, inflammation and malnutrition in CKD patients [16]. Our previous studies suggest that there is a vicious circle of malnutrition (mainly protein-energy malnutrition), inflammation and atherosclerosis (MIA) in CKD patients in which elevated levels of pro-inflammatory cytokines may play a central role. Human adipose tissue has recently been shown to be a hormonally active system that secretes various adipocytokines such as leptin, IL-6, resistin, visfatin and adiponectin. Additionally, the hormonal activity of adipose tissue is thought to differ according to body location with visceral fat being the most active, playing an
important role in the development of insulin resistance and premature atherosclerosis; two common features of CKD [30].

**Insulin resistance in renal disease**

In patients with CKD, a syndrome of insulin resistance is present even in the earliest stage of renal dysfunction, and several components of this syndrome are associated with cardiovascular events [31]. Furthermore, insulin resistance correlates linearly with the decline in renal function and with increasing apolipoprotein (Apo) levels [32]. The exact cause(s) for this uremic metabolic syndrome remain unknown, but may include adipokine signaling [33, 34], retention of uremic toxins, hyper-parathyroidism, systemic inflammation [35] and hyperlipidemia [35].

Uraemic insulin resistance was initially considered to be peripheral, as a result of the low muscle uptake of glucose [36]. Normal insulin binding, insulin receptor autophosphorylation and tyrosine kinase activity, and a normal expression of glucose transporter (GLUT)-4 in skeletal muscle from uraemic patients [37] pointed to an alteration in the insulin-dependent activation of the glucose transport process. Castellino et al. [38] measured substrate oxidation during clamps in uremic subjects, and concluded that the reduction in glucose disposal involved the non-oxidative glucose disposal rate, pointing to a defect in glycogen synthase activity analogous to that seen in the other main types of insulin resistance. Such a defect has been reported in the muscles of uraemic rats [39], but has not been detected in the muscles of uraemic humans [39].

In more recent studies, results of doubly labeled oral glucose tolerance tests performed in non-diabetic uraemic patients and healthy controls matched for age and body mass index have been performed to evaluate
the respective importance of the splanchnic and peripheral defects of glucose metabolism on uraemic glucose tolerance [40]. Under these more physiological conditions, the main contributor to the uraemic glucose intolerance was a high rate of glucose appearance, mediated by an increased endogenous glucose production and a reduced splanchnic uptake of glucose. A reduction in glucose disposal did not contribute to the higher plasma glucose levels [40]. Glucose non-oxidative disposal tended to be higher, probably as a result of the effect of hyperglycaemia.

Additionally, defective insulin secretion in some uraemic patients is another contributor to impaired glucose tolerance in the presence of insulin resistance. Alvestrand et al. [41] showed that a reduced glucose-induced insulin secretion differentiates glucose-intolerant from similarly insulin-resistant non-glucose-intolerant uraemic patients. Secondary hyperparathyroidism leading to high calcium entry into the β cell may reduce insulin secretion as well as contribute to the abnormal pulsatility of insulin secretion in some uraemic patients [42].

**Oxidative stress and endothelial dysfunction**

Oxidative stress (or oxidant-derived tissue injury) takes place when the production of oxidants exceeds the local antioxidant capacity [43]. As CKD is a state with strikingly increased oxidation and impaired antioxidation systems [43], it may be assumed that the effects of the oxidative stress on the acceleration of atherosclerosis are exaggerated. Myeloperoxidase (MPO), an important enzyme found in neutrophils that is involved in the reactive oxygen species (ROS)-production may modulate vascular signaling during inflammation [43] and may be a key link between inflammation, oxidative stress and endothelial dysfunction in CKD [9]. Beside nutritional modulation and anti-
oxidative treatment strategies genetic variations in the MPO gene may also contribute to CVD via enhanced MPO activity [44]. Endothelial dysfunction, which has been identified as a key mechanism by which inflammation and oxidative stress, mediate their effect on the vasculature, is well documented in CKD patients [45]. Although the mechanisms underlying the impaired function of the endothelium in CKD are not well understood, inflammation, and oxidative stress have all been suggested as causative, partly interacting factors [9].

**Vascular calcification**

Vascular calcification is another common feature of CKD that is related to both CVD and outcome [46]. Although the underlying mechanisms are complicated and include several factors, such as Ca x PO$_4$ balance, diabetes and low bone turn-over, evidence suggest that inflammation, and oxidative stress also contributes to the vascular calcification process. Indeed, inflammation seems to have important inhibitory effects on circulating calcification inhibitors, such as AHSG, matrix Gla protein and osteoprotegerin [47]. Of these calcification inhibitors, α2-Heremans-Schmid glycoprotein (AHSG; a negative acute phase reactant synthesized in the liver) may be the most interesting [47]. Indeed, mice lacking the AHSG gene develop early ectopic tissue calcification, while AHSG has been shown to correlate with CVD in both the general population [48] and patients with CKD, where we also found a correlation with survival [49, 50]. Of interest, a recent study also showed that the metabolic syndrome is more common in non-renal patients with elevated AHSG [51], and the AHSG gene has also been associated with differences in body composition amongst healthy individuals [52].
Renal anemia and erythropoiesis

Recombinant human erythropoetin (epoetin) is used to treat anemia in patients with CKD when the endogenous production in the kidney fails. However, dose requirements vary considerably between patients, even accounting for physician preferences [53]. Factors that influence the degree of anemia include erythropoietin deficiency, inflammation, iron deficiency, body mass index and secondary hyperparathyroidism [53]. Interestingly, fat mass is the main source for leptin, while the leptin receptor is expressed not only in the central nervous system, but also in peripheral tissues, including in haematopoietic and immune cells [54]. Therefore, the physiological role of leptin may not be limited to the regulation of food intake and energy expenditure. Indeed, the leptin receptor bears structural homology to members of the class I cytokine family (including interleukin [IL]-6 and granulocyte colony-stimulating factor [GCSF]) and recent data have demonstrated that leptin is able to modulate the immune response [55]. Furthermore, leptin stimulates human erythroid development in vitro [56, 57] and in patients with anorexia nervosa, weight gain is accompanied by rising leptin levels and stimulation of hematopoiesis [58]. During epoetin treatment decreasing serum leptin levels have been observed [59, 60], suggesting a possible mechanism for the observed increase in appetite in these patients.
Excessively corpulent and excessively lean persons are alike condemnable. A body which is neither too stout or too lean, but strikes the mean as regards plumpness, is the best. However, a lean frame should have the preference to a stout one.

Sushruta (ca. 500 B.C.)

The body is most fully developed from thirty to thirty-five years of age, the mind at about forty-nine.

Aristoteles (384 – 322 B.C.)
THE ADIPOSE TISSUE AS AN ENDOCRINE ORGAN

The epidemic of obesity
During the past two decades, the prevalence of obesity has risen dramatically and the world-wide epidemic of obesity has recently received much attention. Obesity is now acknowledged by the World Health Organization as one of the top 10 global health problems [61], and it has been reported that more than 50% of the US adult population are overweight (body mass index, BMI >25 kg/m$^2$) and the prevalence of obesity (BMI $\geq$30 kg/m$^2$) has increased markedly in the US (from 12% in 1991 to 18% in 1998 [62]). Obesity is considered as a major threat to health due to its association with several complications including type-2 diabetes mellitus, hypertension, hyperlipidemia, coronary artery disease, sleep apnea, gallbladder disease and premature death [63].

Although the increased prevalence of obesity is considered to be the result of both an increased caloric intake and a more sedentary life style with reduced physical activity, there is also a strong genetic influence on body weight, and heritability studies have suggested that 70% of the variability in body weight in our energy-rich society may be due to genetic factors [64].

Adipose tissue biology
The adipocytes are a remarkable cell type in several respects. It stores excess energy in the form of lipids and is thus able to dramatically change its size in accordance with changing metabolic needs. This ability gives adipose tissue an almost unlimited capacity for growth, making it perhaps the only tissue in the body with the ability to so drastically increase its size without an underlying transformed cellular
phenotype. Adipose tissue is responsive to both central and peripheral metabolic signals and is itself capable of secreting a number of proteins – termed adipokines. These adipocyte-specific or enriched proteins have been shown to produce a variety of local, peripheral, and central effects that will be discussed below. Adipose tissue is thus able to integrate signals from other organs with energy stores and respond by regulating the secretion of multiple proteins. As an active participant in whole body energy homeostasis, adipose tissue controls several other systems associated with energy expenditure and feeding behavior [65]. However, while adipocytes are capable of an almost unlimited increase in size, the secretory profile of larger adipocytes becomes altered and increasingly dysregulated compared to adipocytes of smaller size [66]. Although the total number of adipocytes is also increased with increasing fat mass, the mayor source of increased mass is the development of very large adipocytes - which may partially account for the inability of adipose tissue to function properly in and contribute to some of the problems associated with obesity.

The molecular mechanisms underlying many of these associations are not yet completely characterized. Paradoxically, similar disorders are also observed in lipodystrophic patients and genetic mouse models that completely lack adipose tissue [67]. These studies have demonstrated that adipose tissue is an active endocrine organ that secretes numerous proteins necessary for normal physiologic homeostasis [68]. The metabolic abnormalities observed with lipodystrophy may be due to the lack of adipokines and/or to the increase in fatty acids in cells other than adipocytes. The latter is generally referred to as "lipotoxicity" as normal cellular function and insulin signaling can be impaired in nonadipose cells with increased intracellular accumulation of fatty acids [35]. The systemic effects of decreased insulin sensitivity associated with obesity may be a reflection of the lipotoxic effects of
fatty acids as well as an imbalance of adipokines. Indeed, providing lipodystrophic mice with adipose tissue implants, thus supplying a sink for fatty acids as well as a supply of adipokines, reversed many of the metabolic abnormalities seen in these mice [68]. Additionally, the exogenous treatment of these lipodystrophic mice with select adipokines alone had similar effects [69]. This may be due in part to the ability of adipokines to reduce fatty acids in nonadipose tissue, although adipokines have other functions on insulin sensitivity independent of this action [69]. Importantly, lipotoxicity and the dysregulation of adipokines should not be viewed as independent, but rather intertwined processes that are each capable of mutually influencing or even causing the other.

Figure 3. Showing the similar developmental origin of adipocytes and macrophages – as well as their proposed interplay \textit{in vivo}. 

\textbf{Cytokines such as:}
- IL-6
- TNF-\(\alpha\)
- Resistin

\textbf{External stimuli including:}
- Fatty acids
- Glucose
- Oxidative stress

\textbf{Stores lipids and regulates metabolic homeostasis.}

\textbf{Nuclear receptors (LXR, PPAR\(\gamma\))}

\textbf{TNF-\(\alpha\), IL-6}

\textbf{Adipokines such as:}
- Leptin
- Adiponectin
- Omentin

\textbf{Adipocyte}

\textbf{Macrophage}

\textbf{Acts in host defense and scavenges debris.}
Adipose tissue signaling and dialysis

As discussed above, adipose tissue is a complex organ with functions far beyond the mere storage of energy. All of these adipokines are likely to have important physiological functions in CKD but may also be important contributors to systemic inflammation and metabolic disturbances in this patient group [65, 70]. In PD patients this is of special significance, as the initiation of therapy is often associated with an increase in fat mass, likely, at least partly, related to glucose absorption from the dialysis fluid [71].

Figure 4. Illustration of the proposed hypothesis. Adipose tissue is an endocrine organ with pleiotropic signaling.
In the present work, our aim was to demonstrate plausible links between adipose tissue signaling and clinical complications of CKD.

More specifically, we aimed to:

A. Quantify the impact of body composition on circulating levels of pro-inflammatory cytokines and to investigate the possible causes for any observed correlations using CD163, a surrogate marker of macrophage numbers.

B. Study the possible effect of the adipokine leptin on renal anemia and epoetin sensitivity.

C. Investigate links between the cytokine resistin and inflammation, renal function and insulin resistance in CKD.

D. Characterize the biological significance of the cytokine visfatin in CKD, specifically as regards impact on glucose metabolism.

E. Assess the possible links between AHSG, known to inhibit vascular calcification, and the metabolic syndrome in CKD.
Subjects

All patients were recruited from the outpatient clinic and renal wards of the Karolinska University Hospital at Huddinge by one of the study physicians. The patients were asked to be part of one of two ongoing prospective studies of renal patients; the MIA study of incident CKD 5 patients (n=320 as of May 2006) started in 1994 and still ongoing with a follow-up time of up to 11 years (mean 47±1 months, range 1-113 months), and the PRIMA study investigating prevalent CKD 3-4 patients (n=55) and started in 2001 (mean follow-up time of 40±2 months, range 2-49 months). Thus, patients were analyzed post-hoc using collected data and de novo analysis of frozen samples. The studies exclusion criteria were age below 18 years, or above 70 years for MIA and 80 years for PRIMA, clinical signs of acute infection, active vasculitis or liver disease at the time of evaluation, or unwillingness to participate in the study. The patients where divided into groups according to the degree of renal failure using the staging recommended by the K/DOQI Guidelines [72].

In the MIA study, the patients had a mean age of 54±1 at inclusion (range 19-70 years). The causes of CKD were chronic glomerulonephritis in about 25% of patients, diabetic nephropathy in about 20% patients, polycystic kidney disease in about 15% patients and other, or unknown, etiologies in 40% of the patients. Most patients started dialysis therapy (either HD or PD) shortly after enrolment in the study. Patients on HD mostly received 3-4 hours of therapy three times per week using a high-flux synthetic membrane, while PD patients had glucose and/or polyglucose-based solutions with three or four daily exchanges. Eighteen percent of MIA patients were diagnosed as type-1 diabetics, defined as insulin-dependent from the outset, while 15%
were diagnosed as type-2 diabetics, defined as initially non-insulin dependent diabetes mellitus. In accordance with current therapy recommendations, most of the patients initially taking oral antiglycemic agents or on restricted diets had been switched to insulin therapy at the time of inclusion in the study. The majority of patients were on antihypertensive medications (angiotensin-converting enzyme inhibitors and/or angiotensin II receptor antagonists; 52%, betablockers; 59%, calcium-channel blockers; 42%) and other commonly used drugs in CKD, such as phosphate and potassium binders, diuretics, erythropoiesis-stimulating agents, iron substitution and vitamin B, C, and D supplementation. Only 19% of the patients were on lipid-lowering medication (HMG-CoA-reductase inhibitors).

In the PRIMA study, the mean age at inclusion was 59±2 years (range 27–80 years), while the causes of CKD were chronic glomerulonephritis in about 25% of patients, diabetic nephropathy in about 25% patients, polycystic kidney disease in about 15% patients and other, or unknown, etiologies in 35% of the patients. Ten percent of PRIMA patients were diagnosed as type-1 diabetics, defined as insulin-dependent from the outset, while 19% were diagnosed as type-2 diabetics, defined as initially non-insulin dependent diabetes mellitus. In accordance with current therapy recommendations, most of the patients initially taking oral antiglycemic agents or on restricted diets had been switched to insulin therapy at the time of inclusion in the study. The majority of patients were on antihypertensive medications and other commonly used drugs in CKD.

Additionally, a population-based randomly selected group of 30 control subjects (67% males) with a mean age of 62±2 years (range 37-79 years) were used in some studies for comparative analyses of
biochemical and metabolic parameters. The control subjects were investigated according to a similar protocol as the patient group. The random selection of subjects in the Stockholm region was performed by Statistics Sweden (SCB). No other exclusion criteria than unwillingness to participate in the study was applied in the selection of the control group. Thus, 14 of the controls were smokers, 5 had a medical history of CVD, 7 were on medications for hypertension, one had leukaemia and one control subject was considered malnourished by subjective global assessment (SGA).

Table 2, below summarizes the characteristics of patients and controls used in each study. As different secondary exclusion criteria related to the availability of data and samples were used in each study, the numbers vary between studies.
Table 2: Baseline characteristics of patients and population-based, matched controls of the studies.

<table>
<thead>
<tr>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
<th>Study 4</th>
<th>Study 5</th>
<th>Study 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD</td>
<td>CKD</td>
<td>CKD</td>
<td>Controls</td>
<td>CKD</td>
<td>Controls</td>
</tr>
<tr>
<td>n</td>
<td>197</td>
<td>166</td>
<td>239</td>
<td>24</td>
<td>158</td>
</tr>
<tr>
<td>% of CKD 5 with DNA</td>
<td>N/A</td>
<td>N/A</td>
<td>82%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52±1</td>
<td>57±1</td>
<td>54±1</td>
<td>59±2</td>
<td>64±2</td>
</tr>
<tr>
<td>% males</td>
<td>62%</td>
<td>64%</td>
<td>67%</td>
<td>64%</td>
<td>68%</td>
</tr>
<tr>
<td>CKD 3-4 / CKD 5 (%)</td>
<td>0% / 100%</td>
<td>0% / 100%</td>
<td>15% / 85%</td>
<td>N/A</td>
<td>24% / 76%</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>7±1</td>
<td>7±1</td>
<td>13±4</td>
<td>90±3</td>
<td>19±3</td>
</tr>
<tr>
<td>Diabetes mellitus (type-1/-2)</td>
<td>12% / 16%</td>
<td>10% / 18%</td>
<td>16% / 18%</td>
<td>0% / 0%</td>
<td>11% / 17%</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5±0.4</td>
<td>23.8±0.5</td>
<td>24.7±0.5</td>
<td>24.8±0.6</td>
<td>24.9±0.4</td>
</tr>
</tbody>
</table>

Data are mean±SEM. CKD = chronic kidney disease, GFR = glomerular filtration rate, BMI = body mass index, N/A = not applicable.
Biochemical measurement methods

After an overnight fast, venous blood samples were drawn, kept on ice, and stored immediately at -70°C for biochemical analyses. Plasma samples were centrifuged within 30 minutes of sampling. GFR was either estimated by the mean of creatinine and urea clearance, calculated from 24-h urinary samples collected from the CKD 5 patients, or determined by iohexol clearance in the CKD 3-4 patients as well as in the controls.

Routine hsCRP measurements were done using nephelometry at the Department of Clinical Chemistry. Plasma analysis for some cytokines were also performed on an IMMULITE® system (DPC Corp, Los Angeles, CA, USA) using commercially available assays and according to the manufacturer instructions. This included hs-CRP (for complementary work, analytical sensitivity was 1 pg/mL) and TNF-α (analytical sensitivity 1 pg/mL). The levels of serum cholesterol and triacylglycerols were analyzed by standard enzymatic procedures (Roche Diagnostics GmbH). High-density (HDL) cholesterol was determined after precipitation of apolipoprotein (Apo) B-containing lipoproteins by phosphotungstic acid.

Plasma resistin levels were measured by a commercially available high sensitivity photometric enzyme-linked immunosorbent assay (ELISA) (LINCO Research, St Charles, MS, USA), as was s-ICAM1 and s-sVCAM1 (R&D Systems Europe Ltd, Abingdon, UK; sensitivity 0.35 ng/mL, intra-assay CV 4%, inter assay CV 7%), serum visfatin (Phoenix Pharmaceuticals Inc., Bellmont, CA, USA; sensitivity 0.11 ng/mL; intra-assay CV 3.9%, inter assay CV 5.6%), IL-6 (sensitivity 0.2 pg/mL; intra-assay CV 3.5%, inter assay CV 5.1%) and TNF-α (sensitivity 0.039 pg/mL; intra-assay CV 3.6%, inter assay CV 5.4%) (both from R&D Systems Ltd, Abbington, U.K), as well as human
AHSG (Epitope Diagnostics Inc., San Diego, CA, USA; detection limit 0.025 g/L; intra-assay CV 5.5%, interassay CV 6.8%). The plates were read using ELISA VERSAmax reader™ (Molecular Devices Corporation, Sunnyvale, CA, USA). Leptin (sensitivity 0.5 ng/mL), and adiponectin (sensitivity is 1 ng/mL, specificity against human C1q <0.01 %, within-day and between-day precision are 2-4 % and 9 %, respectively) were analyzed with radioimmune assays (RIA) using commercial kits and according to manufacturer instructions (LINCO Research, St Charles, MS, USA). Where applicable, data were analyzed with the SoftmaxPRO® software (Molecular Devices Corporation, Sunnyvale, CA, USA).

Albumin was routinely determined at the Department of Clinical Chemistry, Karolinska University Hospital. Levels of Apo A1 (Apo A) and Apo B were determined using an immunonephelometric procedure (Behring AG, Marburg, Germany), whereas the remaining biochemical analyses were also done using routine methods at the Department of Clinical Chemistry at Karolinska University Hospital at Huddinge.

**Determination of the concentration of soluble CD163**

Plasma soluble CD163 was measured with ELISA as previously described [73]. Briefly, rabbit anti-sCD163, 4 mg/L was coated onto micro-titer wells. After washing, 100 µL of sample (diluted 1:50 in PBS with albumin, pH 7.2) was added and incubated for 1 h. The wells were then washed and 100 µL of monoclonal anti-sCD163 (GHI/61, diluted 1:500) was added and incubated for 1 h. After a second wash, 100 µL of peroxidase-labeled antibody (goat anti-mouse immunoglobulins, DAKO P447, diluted 1:4000) was added and incubated for 1 h. The wells were washed, and 100 µL of a H2O2/1,2-phenylenediamine
dihydrochloride substrate solution was added. After 15 min 50 µL of 1 mol/L H₂SO₄ was added and the plates read at 492/620 nm. Control-samples and standards of purified sCD163 were co-analysed in each run.

**Metabolism and nutrition**

Nutritional status was recorded at the time of inclusion, concurrent with the drawing of blood samples, as assessed by SGA [74]. Body mass index (BMI) was calculated as weight (in kg) / (height [in m])². In non-diabetic patients, insulin resistance was calculated by quantitative insulin-sensitivity check index (QUICKI: 1 / [log (fasting plasma insulin (µU/mL)) + log(fasting plasma glucose (mmol/L))]) [75] as well as by the homeostasis model assessment for insulin resistance (HOMA-IR: fasting serum insulin [µU/ml] * fasting plasma glucose [mmol/l] / 22.5) [76], both of which have recently been validated in renal patients [77]. Insulin resistance was not assessed in diabetic subjects.

**Dual-energy x-ray absorptiometry**

Lean body mass (LBM) and truncal fat mass (FM) were estimated by dual energy x-ray absorptiometry (DEXA) using the DPX-L device (Lunar Corp., Madison, WI, USA). With this technique, fat and LBM distribution are directly estimated without making assumptions about the two-compartment model. DEXA has proved superior to other simple non-invasive methods for determining body composition in renal failure, especially if repeated measurements are made [78]. However, it must be kept in mind that, although the state of hydration does not affect the estimate of fat mass with DEXA, it does affect that of LBM.
Genotyping and the concept of Mendelian randomization

All genetic analysis were performed using Pyrosequencing® (Biotage AB, Uppsala, Sweden), a well established and highly accurate method for SNP genotyping, which has been validated against the gold standard 5’nuclease (TaqMan®) assay [79]. DNA was extracted using QIAamp® DNA kit. Samples were stored at -20°C. Sequence amplification was performed by the polymerase chain reaction (PCR) on a PTC-225 Thermocycler (MJ Research Inc., Cambridge, MA, USA). PCR primers for each sequence were designed using the software Primer Designer 4 for Windows, version 4.1©, and one primer in each primer pair was biotinylated. All oligonucleotides were synthesized by Thermo Electron Corp.® (Waltham, MA, USA). The PCR reaction volume was 50 µl, containing 20-50 ng of DNA, 10 pmoles of each forward and reverse primer, 0.2 mM of each dNTP, 0.3 U of DyNAzyme™ II (DNA Polymerase, Finnzymes, CA, USA), 10mM of Tris-HCl, 1.5 mM of MgCl₂, 50 mM of KCl and 0.1 % Triton X-100. Sequence primers were placed adjacent to the SNP and the Pyrosequencing® reaction was performed according to manufacturer instructions.

As these were mainly clinical studies, genotype data was used in a Mendelian randomization approach to attempt to separate causality and association for the studied protein products. This concept, based upon the random assortment of genes from parents to offspring at gamete formation, is emerging as a useful method for studying the nature (causal or not) of observed associations. Similarly to randomized trials, association studies between gene polymorphisms with a well-established function may be useful for excluding confounding as an explanation for a given epidemiological relationship.
The rationale behind this concept is that transmission of genes occurs in a random way; therefore, offspring have an equal chance of inheriting either of the 2 alleles that their parents have at any particular locus, a phenomenon independent from environmental factors. This is particularly useful in cross-sectional studies, where it may help in interpreting associations that could be produced as both the effect of a gene or result of an environmental exposure.

**Figure 5:** The concept of Mendelian randomization. By using the Mendelian randomization approach the confounding effects of environmental exposures might be overcome since the association between a disease and a specific gene polymorphism is not generally susceptible to reverse causation or confounding. Conceptually, the Mendelian randomization approach is similar to the technique used in randomized trial association.

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**Statistical analysis**

Results are expressed as mean and standard error of mean (normally distributed variables) or median and range (non-normal distribution) unless otherwise indicated, with \( p<0.05 \) indicating significance. Normality of distribution for each variable was assessed using the Shapiro-Wilk test [80]. Comparisons between groups of normally
distributes variables was done using Student’s t-test or ANOVA, while comparisons between groups of non-normally distributed variables were made using Wilcoxon (2 groups) or Kruskal-Wallis (more than 2 groups) tests. Comparisons between groups for nominal variables were made using the Chi-square test. As many values were not normally distributed, correlations between variables were calculated according to Spearman Rank. Partial nonparametric correlations using Spearman’s Rank were used to adjust for sCD163. Patient survival was calculated using non-adjusted and adjusted Kaplan-Meier survival curves sometimes using division according to the cut-off point determined using receiver operating characteristics (ROC) curve function. Due to the high mortality rate, analysis was limited to 60 months, at which time more than 50% of the patients had died. All analyses were performed using statistical software SAS version 8.2 (SAS Campus Drive, Cary, NC, USA).

**Ethical considerations**

All human studies were conducted according to the Helsinki declaration and approved by the Ethics Committee of the Karolinska Institutet. Handling of data was done in according with Swedish law, as was the utilization of biobanks. All required permits were obtained prior to initiation of the studies.
METHODOLOGICAL CONSIDERATIONS

Selection bias
It should be kept in mind that the incident and prevalent patients used in the present study are not representative of the general CKD population in developed countries. First, by applying the selection criteria of age we excluded many older patients from participation. Indeed, the mean age at inclusion of the MIA cohort is 52±2 years – lower than the average age of incident patients starting RRT in Sweden, which is 56±1 years [81]. Furthermore, due to local therapy preferences, the MIA cohort has a intention-to-treat PD incidence of about 50%, much more than the 24% prevalence reported for the whole country [81].

Secondly, it is well known that certain patient groups, including those with another native language than the physician in charge of

Figure 6: The clinical dilemma: Doing clinical research.
Adapted from Ann Intern Med. 1997 Mar 1;126(5):389-91.
recruitment, tend to decline participation in clinical studies [82]. This leads to an unquantified selection bias that may limit the validity of results.

**Clinical characterization**

The collection of clinical data is prone to at least two sources of error – patient error and measurement error. Measurement errors occur with every method, and are usually systematic, e.g. the slight deviation of a scale’s measured weight from true body weight. Thus, this type of error can generally be controlled through careful calibration and validation.

More difficult to detect is patient error. In all of the papers comprising the present study, we relied to some degree on patient reporting, including interviews for smoking habits and SGA. While validation studies indicate that SGA is accurate in characterizing patient’s nutritional status [74], the inter-interviewer agreement is only around 70% - mainly due to differences in the patient’s response to the questions posed [83]. While we relied on the same trained interviewer to conduct all of the SGA assessments, we can thus not exclude that certain data are skewed due to patient error. Also, by relying on diagnostic information from the charts, it is likely that we underestimated the prevalence of atherosclerotic disease and diabetes type-2. Indeed, in papers IV-VI we found a significant number of patients that were diagnosed with type-2 diabetes de novo after application of WHO diagnostic criteria.

**Subjective and objective end-points**

The use of “cardiovascular mortality” instead of all-cause mortality as an endpoint in clinical investigations is hazardous. Firstly, data obtained from death certificates or medical records are haphazard,
biased and inaccurate [84, 85]. Assessment of the actual cause of death is inherently problematic and often reliant on an autopsy, which is now infrequently performed. Indeed, in a recent report from the Framingham Heart study [86], only 67% of 942 patients listed as having died of cardiovascular causes were deemed by an independent physician panel to actually have done so.

Thus, in the present study, we have relied on the more robust all-cause mortality – a convenient measurement in CKD where several studies show that more than 70% of patients die from cardiovascular or inflammatory causes [10].

**Anthropometric measurements**

Several methods have been used to monitor lean body mass in CKD, e.g., anthropometrics, creatinine kinetics, multifrequency bioimpedance (BIA) and dual-energy x-ray absorptiometry (DEXA). Of these, DEXA seems to be the most reliable, especially if serial measurements are made [78]. By using DEXA, a reliable estimation of the amount of body fat mass can also be done.

Body mass index (BMI) is another anthropometric measure that has been extensively used in the general population. While height remains fairly constant throughout adult life, weight will vary partly due to gain or loss of fat mass, but also with hydration status, muscle growth and sarcopenia. That this is especially true in renal patients becomes clear if a second dimension, that of total body water content (TBW), is considered. For any given BMI we thus observe a large variation of TBW and thus implicitly of fat mass. Indeed, several well-done studies using either bioelectrical impedance analysis (BIA) or dual-energy x-ray absorptiometry have shown large variations in total body water [87, 88] or fat mass [87] in renal patients with the same BMI.
Subjective global assessment (SGA) is widely available and seems to be a reliable predictor of poor outcome in both sexes [89]. It is a combined subjective and objective test of the patient’s medical history and physical examination, including recent weight loss, dietary intake, gastrointestinal symptoms and visual assessment of subcutaneous fat [74, 90, 91]. In addition, several large prospective studies have demonstrated that SGA is a reliable predictor of poor outcome in dialysis patients suggesting that it provides a meaningful assessment of nutritional status [92-94]. Although SGA has several advantages, such as its low cost, rapid performance and strong predictive value for mortality it should be appreciated that visceral proteins are not assessed and that its sensitivity, precision and reproducibility over time have not been well studied. As discussed above, there is a significant degree of inter-interviewer variability in scoring. Thus, while the studies presented here used a variety of nutritional estimates in an attempt to show a composite picture of patient nutritional status, we can not exclude the chance that more exact measurements of body composition (eg. magnetic resonance imaging) had yielded other results.

**Diagnostic grouping**

When categorizing patients according to the presence or absence of a certain diagnosis, we relied on the information provided by the patient’s medical chart. Thus, patients with one or more incidences of angina pectoris, myocardial infarction, cerebrovascular lesion, peripheral arterial insufficiency or aortic aneurysm were all grouped as CVD. Obviously, such a grouping is very crude, as the grouped diseases have different and in many cases not yet completely elucidated etiologies. Furthermore, as we relied on patient charts, we can not exclude that we have missed some events that took place at
other hospitals or that were misdiagnosed when they presented. Indeed, in Paper V and VI, we have used the WHO criteria [95] in addition to chart information of diabetes diagnosis – yielding a further 20 type-II diabetics whose fasting blood glucose at inclusion qualified them for this diagnosis but who had never been diagnosed before.

**Measurement of GFR**

In the present study, GFR was estimated using the mean of urea and creatinine clearances as assessed by 24-hour urine collection in the MIA patients, while PRIMA patients and controls were evaluated by iohexol clearance. The greatest disadvantage of clearance measurements relying on urine collection is the difficulty in controlling patient compliance and the large daily variations in source pool, generation rate, extrarenal and tubular excretion, and the various methodological concerns when measuring creatinine. A less common problem is neurogenic bladder dysfunction (not uncommon in diabetics) resulting in inaccurate GFR estimations in a small number of patients.

**Biochemical analysis**

Most biochemical measurements were performed using standard techniques such as ELISA. However, while these techniques have proven robust in the general population, they are not validated in the specific uremic setting. Thus we cannot exclude that our measurements are affected by protein metabolites that accumulate in CKD due to decreased renal clearance, as was the case previously with PTH, were accumulation of inactive C-terminal metabolites led to great confusion among scientists [96]. In principle, any antibody-based method could be affected by accumulated metabolites, but no studies have investigated if such an interaction exists or to what extent.
In papers III-VI, we analyzed novel peptides in frozen serum and plasma. While every precaution has been taken to ensure good storage conditions at -70°C, the limited number of aliquots (3-5 per patient) means that some of the older samples have been repeatedly thawed and refrozen. In an attempt to detect possible degradation of the contents, we correlated the measured levels to the number of months since sampling. Using this simple test, we found a correlation between time in the freezer and visfatin concentrations ($r^2=0.12$, $p<0.05$). Following exclusions of patients that had been recruited before 2000 as well as patients in whom we had no aliquots that had never been thawed, there was no correlation between serum visfatin concentration and time in the freezer.

It should also be noted that patient characterization is based on a single measurement of CRP or other cytokines, all of which are known to be highly variable over time. Despite this, there are to date dozens of studies showing that even a single cytokine measurement is a robust predictor of outcome – indicating the chronic nature of inflammation in the CKD patient group [97].

**Time-dependency**

The first patient included in the present work was recruited for the MIA study in 1994. As therapy recommendations have changed with time, it is not implausible that the year of inclusion may constitute a confounder in some aspects of our analysis. While every attempt has failed to turn up such a bias, we believe that the use of statins is higher in patients recruited during the last 5 years when compared to those recruited earlier. Moreover, the use of epoetin in CKD patients is also more common today than it was 10 years ago. Thus, the change of European Best Practice Guidelines for epoetin was revised in 2004,
which has resulted in a significantly higher percentage of patients reaching the target.

**Limitations of genetic studies and the concept of Mendelian randomization**

Genetic epidemiology should not be considered a panacea for epidemiological studies of patients with CKD. The concept of Mendelian randomization rests on the premise that linkage disequilibrium does not affect the phenotype (the outcome) we are interested in, i.e. that alleles located near those we are testing do not contribute to determine the phenotype in question. However, we should be aware that it is well established in human studies that variation in linkage disequilibrium at both short and long distances in the human genome may be considerable.

It was emphasized that another problem may be that patterns of linkage disequilibrium may vary between populations, a phenomenon often called into question to justify different results in studies of gene-

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**Table 3: Mendelian randomization studies**

**Benefits**
- Suggests causality.
- Is independent of many confounders.
- Safe and easy.
- Easy to duplicate in other populations.

**Limitations**
- Failure to establish reliable genotype—intermediate phenotype or genotype—disease associations.
- Confounding of genotype—intermediate phenotype—disease associations.
- Pleiotropy and the multi-function of genes.
- Lack of suitable polymorphisms for studying pathway of interest.
disease associations [98]. Furthermore, application of the concept of equivalence between the effect of genes and exposures that influence a common pathogenetic pathway demands precise knowledge of gene function. Particularly in multiethnic studies, it should be considered that some genes have a much different prevalence in disparate populations (eg. Asians vs. Caucasians or African Americans).

Indeed, due to the current lack of knowledge about linkage disequilibrium patterns, care is needed when making inferences in studies of genetic association, and particular care should be given at stratification when dealing with study populations of different ethnicities. Finally, nonreplication of association studies is a notorious problem. This generally depends on insufficient statistical power coupled with publication bias. Thus, association studies should be not only properly designed, but also adequately powered to test the associations between genes and clinical outcomes. Notwithstanding these limitations and method caveats, Mendelian randomization opens an entirely new perspective for the interpretation of observational studies. Gene-disease associations may offer important clues to the study of intriguing questions raised by the many apparently paradoxical associations described in patients with CKD.

**Statistical considerations**

*Errors of probability testing*. In statistics, a false negative, also called a type II error, exists when a test incorrectly reports that a result was not detected, when it was really present. Alternatively, a type II error can be thought of as acceptance of the null hypothesis even though it is actually false. The probability that an observed negative result is a false negative versus a true negative may be calculated using Bayes'
The key concept of Bayes' theorem is that the true rates of false positives and false negatives are not a function of the accuracy of the test alone, but also the actual rate within the population. In the present study, type II errors can for example occur due to underpowered studies of certain subgroups (e.g. females in Paper I). Where applicable, we have attempted to minimize type II error by using multiple methods with different specificity and sensitivity to study a putative relationship, as well as by a well-defined hypothesis and a robust outcome measurement such as all-cause mortality.

A false positive, also called a type I error, exists when a test incorrectly reports that it has found a positive result where none really exists. Alternatively, a type I error can be thought of as an incorrect rejection of the null hypothesis - accepting the alternative hypothesis even though the null hypothesis was true. While this type of error is more seldom seen in medical research, the large number of analysis that can be run does create the risk of chance findings. Indeed, given a significance level of $p<0.05$, one in every twenty analysis should yield a false positive result. Also, if there is a diagnostic value demarcating the choice of two means, moving it to decrease type I error will increase type II error (and vice-versa).

*Statistical measures of absolute reliability.* The use of the SEM statistic in the present study is associated with several assumptions. First, it is assumed that there is a “population” of measurements for each individual, and that this population is normally distributed and that there are no carry-over effects between repeated tests. The use of the SEM also denotes that heteroscedasticity (i.e. different levels of variance in the population – such as would be the case if the quantification method has different levels of accuracy in the measured interval) is not present in the data. Therefore, if for example an SEM of 3.5 mL/min is
calculated, it is assumed that this amount of absolute error is the same for individuals recording high values (such as healthy controls) in the sample as those scoring low values (ie. CKD 5 patients).

In addition, many findings are based upon the use of dichotomized and continuous variables together, resulting in a loss of predictive power of the dichotomized variables by more than 50% [99]. Another issue is multicollinearity. When different domains of the same phenomenon, such as inflammation, are studied in the same model, the variables tend to correlate with each other, thus violating the assumptions of a multivariate regression model [100].

**Bonferroni correction.** Some people argue that a more stringent criteria for significant than p<0.05 should be used when more than one statistical test is performed in any analysis of data. However, the Bonferroni method is concerned with the general null hypothesis (that all null hypotheses are true simultaneously), which is rarely the case in hypothesis driven research. The main weakness of Bonferroni correction is that the interpretation of a finding depends on the number of other tests performed. Furthermore, the likelihood of type II errors is increased, so that truly important differences are deemed non-significant. Indeed, the majority of epidemiologists reject the Bonferroni adjustment, and recommend simply describing what tests of significance have been performed, and why [101].

**Post-hoc analysis.** Finally, it should be noted that we have mainly performed cross-sectional, post hoc, studies – preventing us from drawing conclusions about cause and effect.
Positive negative findings
Several methodologists have pointed out that the high rate of nonreplication of research discoveries is a consequence of the convenient, yet ill-founded strategy of claiming conclusive research findings solely on the basis of a single study assessed by formal statistical significance, typically for a $p$-value less than 0.05 [102]. Research is clearly not most appropriately represented and summarized by $p$-values, but, unfortunately, there is a widespread notion that medical research articles should be interpreted based only on $p$-values. “Negative” research is also very useful. “Negative” is actually a misnomer, and the misinterpretation is widespread.

<table>
<thead>
<tr>
<th>Table 4. Summary of empirical evidence of the prevalence of methodological concerns in published reports of randomized trials. (Adapted from JAMA. 1995 Feb 1;273(5):408-12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failing to specify eligibility criteria                                             25% of 364 reports.</td>
</tr>
<tr>
<td>Not reporting adequate method of randomization.                                                                68% of 206 reports (52% of 80 reports in internal medicine)</td>
</tr>
<tr>
<td>Not reporting the mechanism of assigning interventions.                                                        89% of 196 reports (44% of 80 reports in internal medicine).</td>
</tr>
<tr>
<td>Not stating if the study was blind or not                                                                   51% of 506 reports.</td>
</tr>
<tr>
<td>Incorrect analysis of multiple observations.                                                                  63% of 192 reports.</td>
</tr>
<tr>
<td>Inadequate information on adverse reactions.                                                                  61% of 192 reports.</td>
</tr>
<tr>
<td>Inadequate methods of comparison of subgroups.                                                                58% of 50 reports.</td>
</tr>
</tbody>
</table>

Finally, power is related to the effect size. Thus research findings are more likely true in scientific fields with large effects, such as the impact of smoking on cancer or cardiovascular disease (relative risks 3–20), than in scientific fields where postulated effects are small, such as genetic risk factors for multigenetic diseases (relative risks 1.1–1.5) [103], where the effect size may be in the tenth or even hundredth decimal.
Philosophy is written in this grand book, the universe, which stands continually open to our gaze. But the book cannot be understood unless one first learns to comprehend the language and read the letters in which it is composed. It is written in the language of mathematics, and its characters are triangles, circles, and other geometric figures without which it is humanly impossible to understand a single word of it; without these, one wanders about a dark labyrinth.

Galileo Galilei (1564 – 1642)
**Paper I and IV: Body fat mass influences systemic inflammation**

These two studies demonstrate relations between regional fat mass and inflammatory biomarkers in CKD patients. Although markedly elevated plasma levels of pro-inflammatory cytokines, such as IL-6, have been documented in the majority of ESRD patients [104] we do not fully understand the exact cause(s) of the hypercytokinemia in the uremic patient group. In obese individuals, recent evidence suggest that other tissues, such as adipose tissue, may contribute to a chronic inflammatory state [105, 106]. In Paper I, increased body fat mass (and in particular increased truncal fat mass) was associated with increased levels of IL-6 suggesting that fat mass is a significant source of IL-6 production also in CKD patients.

IL-6 production by human subcutaneous adipose tissue has previously been demonstrated *in vivo* [105] and it has been estimated that about 20% of the total circulating concentration of IL-6 originates from fat tissue [105]. Moreover, recent studies demonstrate that adipose tissue in obesity is characterized by macrophage infiltration [107, 108] and that weight loss is associated with a reduction in circulating levels of inflammatory biomarkers, such as IL-6 [106, 109].
Figure 7: Data from Paper I. Significant associations between interleukin-6 and (A) truncal fat mass (DEXA) and (B) Apolipoprotein A-I in serum of 187 CKD 5 patients.
Figure 8: Data from Paper IV. Significant associations between fat mass (DEXA) and CD163, a surrogate marker of macrophage numbers in 158 patients with CKD.
This is supported by Paper IV, where we report correlations between body fat, circulating levels of a number of adipokines and cytokines and sCD163, a specific marker of monocytes/macrophages [110] both cross-sectionally and longitudinally. It may be that adipocytes can directly secrete pro-inflammatory cytokines along with the diverse family of adipokines [25]. However, current evidence favors an alternative hypothesis [7, 26] suggesting that activated macrophages resident in fat tissue under certain conditions release pro-inflammatory cytokines such as IL-6 and TNF-α that in turn activate a systemic inflammatory response [27]. Study IV supports the latter theory by showing that circulating levels of sCD163 correlate with both fat mass, circulating levels of pro-inflammatory cytokines and increased circulating levels of endothelial adhesion molecules. Furthermore, following adjustment for sCD163 the previously significant relationship between fat mass and inflammatory biomarkers (such as IL-6 or CRP) disappears, while the relationships between fat and leptin, as well as between fat and insulin resistance, remains significant.

It is notable that the relationship between fat mass and inflammatory biomarkers seemed to differ between truncal and non-truncal fat mass. Truncal fat mass is closely related to the visceral fat mass, which is the fat-tissue depot considered to be the most metabolically active and that has been identified as a key factor in the development of insulin resistance, type II diabetes and premature atherosclerosis [30, 111]. Importantly, visceral and subcutaneous adipose tissue depots are biologically distinct [112] and omental adipose tissue releases 2-3 times more IL-6 than subcutaneous fat tissue [113]. Clearly, in future studies evaluating BMI and obesity as risk factors for outcome, inflammation and cardiovascular events in CKD patients a distinction between fat mass of visceral and subcutaneous origin should be made.
It may seem paradoxical that we found associations between plasma IL-6 levels and both increased fat mass (indicating adequate energy stores) and surrogate markers of sarcopenia (indicating protein malnutrition). However, the processes of protein and energy malnutrition may not always evolve simultaneously. Thus, the presence of obesity and increased truncal fat mass may not necessarily imply adequate protein intake and nutritional status. Indeed, we (Honda et al. In manuscript. 2006) have found markedly elevated plasma IL-6 levels in ESRD patients considered to be malnourished by SGA in patient groups with both high (>25 kg/m\(^2\)) and low (<20 kg/m\(^2\)) BMI. Indeed, a “J-shaped” risk curve is seen CKD patients when analyzing the impact of obesity on survival, and it may be that while the enhanced generation of pro-inflammatory cytokines from the fat tissue contributes to the sarcopenia, other factors, including energy stores, sex hormones and adipokines, also associated with fat mass actually have a beneficial effect on outcome in CKD. This is supported by the recent studies of “reverse epidemiology” by Kalantar-Zadeh et al. [3] showing a survival benefit of a high BMI in large epidemiological studies.

However, in cross-sectional studies the true associations between IL-6 and body composition may not be revealed as ESRD patients with a wide range of co-morbid conditions, duration of disease and age are studied. Clearly, long-term prospective studies are needed to evaluate the evolution of inflammatory biomarkers and their relationship to changes in regional body composition as renal function declines.
**Paper VI: Links between AHSG and blood lipids**

In Paper VI, we report associations between both circulating AHSG and $AHSG$ genotypes linked to high levels of AHSG and a pro-atherogenic lipid profile with high triglyceride levels and low HDL-cholesterol. Circulating AHSG was also significantly associated with an increased truncal fat mass.

![Figure 9](image)

*Figure 9. Data from Paper VI. The significant correlation between total fat mass (DEXA) and serum AHSG in 198 patients with CKD.*

Adipokines may act as autogenic regulators of body fat depots, modulating gastrointestinal activities, metabolic changes and central nervous mechanisms which have been speculated to play a central role in the loss of muscle mass (sarcopenia) often observed in ESRD patients [104].

However, it may be that adipose tissue regulates more than just appetite. A possible role for AHSG in influencing susceptibility to the metabolic syndrome was first suggested by *in vitro* studies
demonstrating that AHSG inhibits, in a dose-dependent manner, the insulin-stimulated tyrosine kinase activity of the insulin receptor, insulin receptor autophosphorylation, and insulin substrate 1 phosphorylation [114]. These effects were corroborated *in vivo* in rat liver and skeletal muscle following acute injection of human recombinant AHSG [115] and in AHSG-null mice, which exhibit significantly enhanced insulin sensitivity and are resistant to weight gain on a high-fat diet [116].

![Graph](image)

**Figure 10.** Data from Paper VI showing the significant difference in serum AHSG between patients with and without diabetes mellitus in 198 patients starting RRT.

In humans, serum AHSG levels have been reported to be associated with insulin resistance and fat accumulation in the liver [51], as well as with resistance to weight-gain through increased adipocyte β2-adrenoceptor function [52, 117]. Also, it was recently reported that in the 711 nondiabetic outpatients with coronary artery disease in the Heart and Soul Study, a high circulating AHSG concentration was association with the metabolic syndrome even after adjustment for potential confounding variables [48]. In the same study, higher fetuin-A
quartiles were also strongly and independently associated with higher LDL and triglyceride concentrations, and lower HDL concentrations [48].

Renal failure *per se* leads to changes in blood lipid levels, including decreased plasma levels of Apo A due to specific changes in the metabolism of Apo A-containing lipoproteins and decreased HDL levels [118]. Whereas hypercholesterolemia is a well-established risk factor for cardiovascular mortality and morbidity in the general population, hypocholesterolemia is associated with a significantly higher risk of death from CVD in ESRD patients than hypercholesterolemia [119]. Also, a previous study has demonstrated an important direct role for inflammatory cytokines
in cholesterol-mediated LDL receptor regulation in mesangial cells, enabling unregulated intracellular accumulation of unmodified LDL [120]. Indeed, we found (Figure 7) a significant correlation between serum IL-6 and both HDL-cholesterol (negative association) and LDL-cholesterol (positive association). Thus, also in this area further studies are needed to elucidate the complex interplay between inflammation and blood lipids, and the potential role of targeted treatment strategies that elevate circulating HDL in inhibiting deleterious cytokine-mediated effects on LDL receptor regulation and vascular function in ESRD patients.

While these data reflect that AHSG is an important candidate among the factors that influence metabolic dysregulation, perhaps the most intriguing implication of the present study is the antithetical role of AHSG in influencing outcomes both under normal conditions, where elevated circulating AHSG seems to engender metabolic derangements, and under extreme circumstances, such as CKD, where low circulating AHSG is an important predictor of elevated mortality, hypothetically through an increased risk of ectopic tissue ossification [49]. Thus, we hypothesized that AHSG may be one link between the conflicting traditional and non-traditional risk factors linked to CVD in CKD, offering novel insights into the complex pathophysiology and hope of new tools for risk assessment and treatment. Indeed, while an elevated fat mass is an important predictor of death in the general population, some studies in CKD patients have showed a beneficial effect of even morbid obesity in this population [3]. Paper VI suggests that while exposed to the nefarious consequences of an increased fat mass, the selected group of obese CKD patients that reach ESRD [121] would in balance benefit from the associated elevated levels of AHSG acting to reduce vascular calcification – likely a more important cause of
mortality in CKD than the metabolic derangements of morbid obesity and an elevated circulating AHSG.

**Paper II: Pleiotropic signaling, leptin and erythropoiesis**

In Paper II, we show that serum leptin levels may also be an independent predictor of epoetin requirements in uremia (even after the adjustment for inflammation) and point out that there is some evidence to suggest that leptin may have hematopoietic properties [122]. So far, relatively few studies have investigated the plausible pleiotropic effects of adipokines [70]. Leptin, the first adipokine described (1994), was shown to modulate feeding behavior in rats [123]. Leptin signaling in the CNS has recently also been shown to be an important cause of anorexia in uremic rats [124]. In PD patients, we have shown [125] that serum leptin levels increase with initiation of PD, are inversely related to inflammation and predict longitudinal changes in lean body mass. However, leptin may also be able to modulate bone modeling through central mechanisms [126], as well as the apparent ability of leptin to

Table 6. Data from Study II showing the independent relationship between body fat mass (A, C) and serum leptin (B, D) with epoetin sensitivity expressed as epoetin/Hb/kg body weight in 166 CKD patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Patients (N = 166)</th>
<th>Patients Prescribed Epoetin (n = 115)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model A (r² = 0.14)</td>
<td>Model B (r² = 0.17)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>F Ratio</td>
<td>P</td>
</tr>
<tr>
<td>Age (y)</td>
<td>1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Smoker (yes/no)</td>
<td>0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Polycystic kidney disease (yes/no)</td>
<td>5.5</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Log serum ferritin (ng/mL)</td>
<td>4.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Log serum parathyroid hormone (pg/mL)</td>
<td>4.0</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Log serum IL-6 (ng/mL)</td>
<td>0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Body fat mass (kg)</td>
<td>5.8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Log serum leptin (ng/mL)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

NOTE. The initial models included all factors significantly associated with the dependent variable in univariate analysis, as well as age and sex. Epoetin sensitivity was calculated as log (epoetin [IU/wk] + 1)/blood Hb level [g/dL]body weight [kg]. Abbreviation: NS, not significant.
promote collagen formation in tissues [127], both of which may have as yet unexplored links to vascular calcification and bone disease in uremia. Indeed, a recent study by Parhami et al [128] showed that leptin is able to enhance vascular calcification in vitro and in mice, suggesting a much more direct connection between this hormone and cardiovascular disease in uremia.

**Paper III and V: Resistin and visfatin are not associated with insulin resistance in CKD**

In Paper III and V we investigated the putative correlation between increased circulating levels of resistin and visfatin and surrogate markers of insulin resistance in CKD, but find no such link [129, 136]. Indeed, while resistin levels are elevated and strongly associated with both GFR and inflammatory biomarkers in CKD, the significant relationship between plasma resistin levels and insulin resistance was lost following the correction for GFR, suggesting that resistin is not a likely mediator of insulin resistance in patients with CKD [129]. Despite this, glucose intolerance was found to common in the study population, as it is in other studies showing strong links between CKD and the development of insulin resistance [36, 39]. This intolerance has been attributed to peripheral insulin resistance rather than to reduced insulin production or liver dysmetabolism [36]. Furthermore, insulin resistance is intimately linked to several of the most common complications of CKD, including inflammation [35, 43], endothelial dysfunction [132], oxidative stress [35, 36, 133], dyslipidemia [43, 120], muscle wasting [134] and increased risk of infections [135]. However, while insulin therapy is common in CKD patients, little progress has been made in elucidating the possible causes of the altered glucose metabolism in uremia.
In Paper V we thus evaluated visfatin/PBEF-1, which was recently described to be able to induce insulin receptor signaling in mice, and to be expressed in mouse adipose tissue. Similarly to the case for FIZZ-1, we found that serum levels of visfatin were elevated with a decreasing GFR and positively correlated with systemic inflammation – but we could again find no links with surrogate markers of insulin resistance [136], leading us to yet again conclude that there are telling differences between mice and men also in the area of adipose tissue biology.

However, much research remains to be done in this area, including mechanistic and prospective studies. Already, we believe that it is clear that renal function is an important factor to take into account in clinical studies relating to insulin sensitivity also in individuals without CKD as well as in patients with diabetes mellitus, who often have an impaired renal function.

**Paper V: Visfatin, or pre-B Cell Colony Enhancing Factor (PBEF)-1, as a cytokine**

In Paper V, we also analyzed the association between visfatin and inflammation. Corresponding to findings from analysis of other adipokines and cytokines in CKD, including adiponectin [137], IL-6 [138], and resistin [129], we found that serum visfatin was inversely correlated with GFR. This suggests that reduced clearance by the kidneys is one contributing factor to the hypercytokinemia associated with renal disease. Indeed, physiological clearance of cytokines through renal catabolism contributes an important fraction of the total metabolic clearance of polypeptide hormones [139], and Nakamura et al [140] have shown that reduced renal function is associated with progressively altered clearance of IL-6 *in vivo*. Thus, it is not surprising that we also saw correlations between visfatin and most of the other cytokines, a correlation that disappeared following correction for GFR.
[136]. However, other factors may also be involved in mediating the elevated circulating levels of visfatin in CKD. In vitro trials have suggested that IL-6 is a negative regulator of visfatin gene expression in 3T3-L1 [141] adipocytes [142], while studies in humans have shown that visfatin is mainly released by macrophages resident in the adipose tissue, rather than by the adipocytes themselves, suggesting an association with other adipocytokines such as migration inhibitory factor (MIF)-1. As many renal diseases are organ manifestations of a systemic inflammatory disease, involving immuno-competent cells, it is interesting to speculate that the regulatory visfatin may also play a more central role in some instances of CKD. However, we present no data supporting such a role, and are aware of no published studies that have investigated this hypothesis.
FINAL DISCUSSION AND FUTURE DIRECTIONS

In the present work, we perform some of the first association studies looking at the clinical impact of adipose tissue signaling in CKD. While we report several intriguing relationships, the next step is obviously to conduct interventional and mechanistic studies. Thus, it is so far unknown what the effects of changes in fat mass are on adipokines and outcome in CKD. Furthermore, it is not known if the elevated levels of adipokines seen in most studies comprise inactive metabolites or active proteins – or if this increase is a physiological response to uremia or a pathophysiological result of decreased renal clearance. Further studies are also needed to elucidate the role of novel adipokines such as omentin, obestatin and retinol binding protein (RBP)-4 in the setting of CKD.

**Detrimental effects of fat**
- Increased systemic inflammation
- Dyslipidemia
- Insulin resistance
- Obstructive sleep apnoea
- Psychosocial effects
- Altered eating pattern
- Aberant immune response?

**Beneficial effects of fat**
- Well-preserved energy stores
- Various adipokines with local and systemic ameliorating effects.
- Association with hight AHSG

**Modulators of fat mass signaling**
- Distribution of fat (central vs. subcutaneous)
- Selection pressure resulting in selected survivor group
  - Genetic confounders
  - Comorbidities
  - Life-style factors (eg. diet, exercise)
Also, although there is little doubt that obesity in the general population constitutes one of the most important risk factors for the metabolic syndrome and CVD, the impact of obesity on CKD and its’ complications are not yet clear. While there are some studies showing the expected association with increased morbidity and mortality [143], most epidemiological studies investigating the survival effect of an increased BMI or other surrogate markers of fat mass show a survival advantage even for morbidly obese patients with CKD [3]. Thus, it may be speculated that the “uremic-metabolic syndrome” described in CKD patients is actually alleviated rather than compounded by adipokine signaling – physiological or patophysiological. Regardless of the results of future scientific studies into the role of adipocytes, the intimate links between adipose tissue, inflammation and immunity should be explored in an effort to bring about novel therapies for this afflicted patient group.
SUMMARY OF CONCLUSIONS

The present study comprises six original papers. We conclude that:

✦ An increased fat mass is associated with increased levels of IL-6, CRP and the macrophage marker sCD163 in patients with advanced CKD.

✦ In longitudinal analysis, patients that gain fat during the first year of dialysis also significantly increase their circulating sCD163.

✦ An increased fat mass is also associated with elevated levels of the vascular calcification inhibitor AHSG in patients with CKD 5. Meanwhile, genetic polymorphisms associated with higher levels of transcription of AHSG in other studies correlate with high serum triglycerides and low serum HDL-cholesterol in the same population.

✦ Both an increased fat mass and the associated increased serum leptin levels correlate with a decreased need for epoetin therapy in CKD 5.

✦ The adipokines/cytokines visfatin and resistin are both elevated with decreasing GFR and associated to inflammatory parameters such as IL-6 and CRP. However, while resistin was not associated with insulin resistance in CKD, we found significantly elevated visfatin in patients with diabetes mellitus. Despite this, visfatin did not correlate with surrogate markers of insulin resistance in non-diabetics.

✦ Both increased serum sCD163 and visfatin were associated with a worse survival in CKD, even after adjustment for age and sex.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AHSG</td>
<td>α2-Heremans-Schmid glycoprotein</td>
</tr>
<tr>
<td>Apo</td>
<td>Apolipoprotein</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>ESRD</td>
<td>End-stage renal disease</td>
</tr>
<tr>
<td>FIZZ-1</td>
<td>Found in inflammatory zone-1 / resistin</td>
</tr>
<tr>
<td>FM</td>
<td>Fat mass</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>p</td>
<td>Probability value of chance</td>
</tr>
<tr>
<td>PBEF-1</td>
<td>Pre-B-cell colony enhancing factor 1 / visfatin</td>
</tr>
<tr>
<td>Rho</td>
<td>Spearman Rank correlation coefficient</td>
</tr>
<tr>
<td>RRT</td>
<td>Renal replacement therapy</td>
</tr>
<tr>
<td>SGA</td>
<td>Subjective global assessment of nutrition</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor α</td>
</tr>
</tbody>
</table>
When the storms rage around us, and the state is threatened by shipwreck, we can do nothing more noble than to lower the anchor of our peaceful studies into the ground of eternity.

Johannes Kepler (1575 – 1630)

If there is any doubt as to whether a person is dead or alive, apply lightly roasted onion to his nostrils, and if he is alive, he will immediately scratch his nose.

John of Mirfield (1362 – 1407)
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And finally, my heartfelt gratitude to all the people within the Karolinska Institutet who helped me to steer when sailing close to the wind of bureaucracy on the last leg of the race!

If I have seen further, it is by standing on the shoulders of Giants.

Sir Isaac Newton (1642 – 1727)
Prayer indeed is good, but while calling on the gods a man should himself lend a hand.

Hippocrates (460–377 B.C.)


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A drug is a substance that, when injected into a rat, produces a scientific paper.

Anonymous
The problem of economic loss due to sickness is a very serious matter for many families with and without incomes, and therefore, an unfair burden upon the medical profession.

F.D. Roosevelt (1882 – 1945)
Whoever is to acquire a competent knowledge of medicine, ought to be possessed of the following advantages: a natural disposition; instruction; a favorable position of study; early tuition; love of labour; leisure. First of all, a natural talent is required; for, when Nature opposes, everything else is in vain.

Hippocrates (460 – 377 B.C.)
We are tending to become a standardized country, and it is perhaps on standardization that industrial progress is founded. But standardization of our education system is apt to stamp out individualism and defeat the very ends of education by leveling the product down rather than up. The qualities that really count in this world are quite beyond pigeonholing, quite beyond measurement by scales, tape, or mental tests, quite beyond rating by any known system of examination, all of which fail in giving us an estimate of that most precious of all qualities, personality. The capacity of a man himself is only revealed when, under stress and responsibility, he breaks through his educational shell, and he may then be a splendid surprise to himself no less than to his teachers.

Harvey Cushing (1869 – 1939)
While I thought that I was learning how to live, I have been learning how to die.

Leonardo da Vinci (1452 – 1519)
If we had nothing but pecuniary rewards and worldly honors to look to, our profession would not be one to be desired. But in its practice you will find it to be attended with peculiar privileges; second to none in intense interest and pure pleasures. It is our proud office to tend the fleshy tabernacle of the immortal spirit, and our path, if rightly followed, will be guided by unfettered truth and love unfeigned.

Joseph, Lord Lister (1827 – 1912)

The people in this world put on a tremendous show, and doctors have the front row seats.

Carl Augustus Hamann (1868 – 1930)