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Assessment and Monitoring of Nutritional Status in Chronic Kidney Disease Patients

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ABSTRACT

Patients with chronic kidney disease (CKD) often suffer from malnutrition and protein-energy wasting (PEW) resulting in poor nutritional status which is a powerful predictor of mortality. As it is not clear how well nutritional markers used in the clinical care of these patients accurately reflect nutritional status we evaluated in post-hoc, cross-sectional observational studies several common markers of malnutrition and PEW, and assessed their correlations to each other and to survival in patients with CKD stage 5 (CKD 5).

In **Study I** self-rated appetite along with anthropometrics and biochemical markers of nutritional status were measured and related to all-cause mortality in 523 CKD 5 patients. This study shows that self-rated appetite is not an independent predictor of survival in most patients with CKD 5.

In **Study II** serum albumin, and other biochemical markers of nutritional status, clinical anthropometrics, and dual-energy x-ray absorptiometry, were assessed in CKD 5 patients. Analyzing data from 458 incident and 383 prevalent dialysis patients, we found that serum albumin correlates poorly with other markers of nutritional status. Thus, its value as a reliable marker of nutritional status appears limited.

In **Study III** serum insulin-like growth factor (IGF)-1 and biochemical, clinical, and densitometric markers of nutritional status and mineral and bone metabolism were evaluated in 365 incident dialysis patients. This study shows that low serum IGF-1 associates with a sarcopenic body composition and with markers of disturbed bone metabolism, while also predicting an increased risk of mortality.

In **Study IV** we assessed temporal changes in the appetite regulating peptide hormones pancreatic polypeptide (PP), glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide 1 (GLP-1) following a fat- and carbohydrate-rich meal in 6 hemodialysis patients and 9 healthy controls. This study shows that fasting levels of both PP and GIP levels and the postprandial PP response are elevated in HD patients as compared to controls. We speculate that this may be one mechanism whereby CKD engenders poor appetite.

In **Study V** several common markers of nutritional status were analyzed in 399 incident dialysis patients and 289 prevalent dialysis patients and, using multivariate regression models, related to results of subjective global assessment (SGA). This study shows that serum levels of albumin, creatinine, and cholesterol as well as handgrip strength are in general only weakly or not at all associated with PEW as assessed by SGA following correction for cofounders.

LIST OF PUBLICATIONS

I. **Gama-Axelsson T**, Lindholm B, Bárány P, Heimbürger O, Stenvinkel P, Qureshi AR. *Self-rated appetite as a predictor of mortality in patients with stage 5 chronic kidney disease*. J Ren Nutr 2013; 23:106-113.

II. **Gama-Axelsson T,** Heimbürger O, Stenvinkel P, Bárány P, Lindholm B, Qureshi AR. *Serum albumin as predictor of nutritional status in patients with ESRD.* Clin J Am Soc Nephrol 2012; 7:1446-1453.

III. Jia T, **Gama-Axelsson T,** Heimbürger O, Bárány P, Lindholm B, Stenvinkel P, Qureshi AR. *IGF-1 and Survival in ESRD*. Clin J Am Soc Nephrol. 2014 Jan;9(1):120-7.

IV. **Gama-Axelsson T***, Quiroga B*, Axelsson J, Anderstam B, Lindholm B, Qureshi AR and Carrero JJ*. Postprandial responses of circulating pancreatic polypeptide (PP) and incretin levels in hemodialysis patients following a standardized meal. In submission.*
* Contributed equally to the study.

V. **Gama-Axelsson T**, Jia T, Lindholm B, Heimbürger O, Carrero JJ, Barany P, Stenvinkel P and Qureshi AR. *A comparative analysis of readily available markers of nutritional status in incident and prevalent dialysis patients. In manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ALAT	Alanine-aminotransferase
APD	Automated peritoneal dialysis
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CAPD	Continuous ambulatory peritoneal dialysis
CCK-8	Cholecystokinin-8
CI	Confidence intervals
CKD	Chronic kidney disease
CKD5-D	Chronic kidney disease stage 5 on dialysis
CKD5-ND	Chronic kidney disease stage 5 not on dialysis
CNS	Central nervous system
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
DEXA	Dual-energy X-ray absorptiometry
DW	Dry weight
ELISA	Enzyme-linked immunosorbent assay
ESRD	End-stage renal disease
FBM	Fat body mass
FGF-23	Fibroblast growth factor-23
GFR	Glomerular filtration rate
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide 1
HD	Hemodialysis
HGS	Hand grip strength
HR	Hazard ratio
IDPN	Intradialytic parenteral nutrition
IGF-1	Insulin-like growth factor-1
IL-6	Interleukin 6
IQR	Interquartile range
LBM	Lean body mass
PD	Peritoneal dialysis
PP	Pancreatic polypeptide
PTH	Parathyroid hormone
MIA	Malnutrition, inflammation, and atherosclerosis
MIMICK-1	Mapping of inflammation markers in chronic kidney disease-1 (study in prevalent hemodialysis patients)
MIMICK-2	Mapping of inflammation markers in chronic kidney disease-2 (study in prevalent peritoneal dialysis patients)
PEW	Protein-energy wasting
ROC	Receiver operator characteristics
RRT	Renal replacement therapy
SD	Standard deviation
SGA	Subjective global nutritional assessment

1 THESIS SUMMARY

Both malnutrition (defined as a state of nutrient deficiency secondary to diet patterns) and protein-energy wasting (defined as a state of body protein catabolism and insufficient energy intake secondary to chronic renal disease), usually occurring together in chronic kidney disease and here used as interchangeable entities, are common in chronic kidney disease patients, where they also are strongly associated with low-grade inflammation and an adverse outcome (1-7). The main goal of this thesis was to investigate the relative usefulness of clinically available tools to accurately identify and monitor protein-energy wasting and malnutrition in chronic kidney disease as identifying patients at risk is a goal in itself and also a prerequisite for interventional therapies and prioritization of resources. By studying a broad spectrum of renal diseases of patients with reduced glomerular filtration rates but not yet on dialysis, as well as patients on both hemodialysis and peritoneal dialysis, we increase the practical applicability of our results in the clinical setting. Unfortunately, our data do not support the use of any one single marker for the unequivocal identification of malnourished chronic kidney disease patients, and even less makes it likely that commonly used methods can be clinically useful for risk stratification. Instead the results of our studies suggest that a panel combining several markers and including ideally also subjective global assessment should be used if possible. Thus, further studies are urgently needed to identify and validate better and more reliable tools to assess and monitor nutritional status in various chronic kidney disease populations.

2 INTRODUCTION

2.1 CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD), often leading to a gradual and irreparable loss of renal function, is common enough to be considered a worldwide public health threat (8). In the United States alone, 14.5% of adults are likely to have some degree of CKD (9). A similar prevalence has been reported in Europe, Australia and Asia (9-11). Having multiple etiologies, CKD is defined as a state of kidney damage and/or decreased glomerular filtration that lasts for at least 3 months (8). Long relatively unnoticed, the worldwide trend of increasing body weight, hypertension and insulin resistance in the population has been followed by a similar but delayed increase in CKD prevalence (12). This increase has also led to an increased awareness, and a common nomenclature developed to facilitate clinical and scientific evaluation. According to this, CKD can be divided into 5 stages (**Table 1**) depending on the presence of kidney damage, i.e., albuminuria, and loss of kidney function as assessed by glomerular filtration rate (GFR). CKD is usually characterized by a progressive course of worsening renal function eventually leading to stage 5 CKD, also called end-stage renal disease (ESRD), which requires renal replacement therapy (RRT). Currently, two major kinds of RRT are clinically available, dialysis and kidney transplantation. Dialysis is furthermore available as hemodialysis (HD, entailing extracorporeal filtration of blood) or peritoneal dialysis (PD, which utilises the peritoneal membrane as a filter).

Table 1. Classification of CKD according to KDIGO guidelines (8).

Prevalence of CKD*	Stage	Description	GFR (mL/min/1.73m^2)
3.1%	CKD 1	Kidney damage with normal GFR	≥90
4.1%	CKD 2	Kidney damage with mildly decreased GFR	60-89
7.6%	CKD 3	Moderate decrease in GFR	30-59
0.25%	CKD 4	Severe decrease in GFR	15-29
0.25%	CKD 5	Kidney failure	<15 or dialysis

*Prevalence of CKD in the American population (13)

2.2 Renal replacement therapy

While a kidney transplant is the preferred option of RRT, the limited availability of organs and the risks of the operative procedure mean that most patients that reach CKD 5 are started on dialysis. While renal transplantation is by far the best method of RRT, fully replacing kidneys in case of a well-functioning graft, dialysis has its drawbacks and limitations. Both the dialyzer membrane used for HD and the peritoneal membrane used for PD are less selective than the kidney's glomerular filtration barrier. Therefore, many essential nutrients are lost into the dialysate and the dialysis procedure may cause an inflammatory response stimulating protein catabolism. Nevertheless, dialysis has the capability of improving several indices of nutritional status.

2.3 How CKD alters nutritional status

Patients with CKD present a variety of metabolic and nutritional abnormalities (14-16). An early diagnosis and proper treatment of these conditions, including dietary interventions, can slow down the progression of disease symptoms (17) and may also ameliorate disease complications such as hyperkalemia, acidosis and sarcopenia (18). Metabolic and nutritional abnormalities arise in CKD from both pathophysiological (e.g. uremic toxicity, altered metabolism) and iatrogenic (e.g. polypharmacy and the prescription of a low protein diets to slow disease progression) causes. Patients with renal disease are also more vulnerable to malnutrition due to their intake of numerous medications, a more restrictive diet, disabilities and disease-related social issues. With the start of dialysis, some of these abnormalities are improved, but others remain or worsen, while new factors also likely contribute to increase the prevalence of malnutrition and protein-energy wasting (PEW) in this population (19).

2.4 Malnutrition *versus* PEW

Over the years, many different nomenclatures have developed to describe the various nutritional disturbances seen in patients with CKD. This can lead to confusion and misunderstandings (20). In the following, I will use the terms malnutrition and PEW as outlined below and in **Table 2**.

Malnutrition in the context of CKD occurs when an inappropriate dietary intake of one or more macro- or micro-nutrients results due to an inadequate diet. Meanwhile, wasting is the result of metabolic abnormalities that cannot be corrected solely by an improved diet (21). Thus, malnutrition may indicate both a state of under-nutrition and one of energy excess (22, 23). For instance, malnutrition may be diagnosed both in a sarcopenic dialysis patient with a too low calorie intake, as well as in an obese insulin-resistant patient with CKD stage 3 and a very high energy intake.

PEW is meanwhile defined as a state of gradual and non-functional loss of muscle and fat tissue, eventually resulting in cachexia (19, 20). In addition, decreased functional capacity due to metabolic stresses is often present. Also, the described state is not merely caused by an inadequate dietary intake, but rather the result of disease processes such as acidosis (24, 25), inflammation-driven catabolism (26), nutrient losses in the dialysate (27, 28), along with endocrine disturbances, such as hyperparathyroidism (29), hyper-glucagonemia (30) and peripheral insulin resistance (31). PEW is currently the recommended term to describe the commonly observed alterations of nutritional status seen with CKD (20) although the term malnutrition is still widely used. PEW may be seen as a broader concept that includes also malnutrition; in reality, both PEW and "pure" malnutrition are usually present in CKD patients with poor nutritional status.

Further differentiating the two syndromes, PEW is but mostly caused by alterations in physiological processes such as energy expenditure, inflammation, central nervous system (CNS) signaling and endocrine disorders, resulting in inappropriate catabolism of muscle more than of fat (19, 20, 32). Further complicating the picture, a low protein diet is advocated for many CKD stage 4 and 5 subjects, as it has been demonstrated to reduce uremic symptoms and to slow the rate of renal function decline (20). However, a low protein diet may also result in a negative protein balance, especially in subjects not taking amino acid and/or keto acid supplementation.

Table 2. Differences between malnutrition and PEW.

	Context	Clinical signs	Biochemical signs	Treatment response
Malnutrition	Occurs when insufficient dietary intake results from an inadequate diet, leading to a scarcity of one or more nutrients.	Associated with the degree of uremic symptoms. Food intake low**.** Energy expenditure low-normal. Preferential loss of fat over lean body mass.	Normal or low s-albumin	Reversed by adequate dialysis and nutritional support
PEW	A state of loss of muscle and fat tissues together with metabolic changes not amenable to dietary intervention in the context of CKD.	Associated with degree of co-morbidities and inflammation. Increased energy expenditure. Fat is underutilized - muscle is wasted	Markedly low s-albumin, pre-albumin, s-creatinine High CRP	Correction of acidosis, inflammation, nutritional interventions, adequate, dialysis doses

2.5 Prevalence of malnutrition/PEW in different CKD populations

In scientific studies, the prevalence of malnutrition, defined differently between studies, in early to moderate CKD is usually lower, while as CKD advances it increases dramatically in prevalent dialysis patients (14-16, 20). Furthermore, transplanted patients (T) often improve their nutritional status and ameliorate PEW symptoms.

Table 3. Shows a number of studies describing the prevalence of PEW in several patients groups from several countries.

Table 3. Shows a number of studies describing the prevalence of PEW in several patients groups from several countries. BMI, body mass index; LBM, lean body mass; MIS, malnutrition inflammation score; nPNA, normalized protein nitrogen appearance and SGA, subjective global assessment; T, transplanted patients.

Study	Year	Country	Sample size	Age	Method	Prevalence
Chruściel et al. (33)	2001	Poland	109 T	40±11	SGA	21%
					BMI< 21	23.3%
Djukanović et al. (34)	2003	Yugoslavia	452 T	13-54 y	BMI <21	15%
Vasselai et al. (35)	2008	Brazil	45 PD	53±15	SGA	35.6%
Sanches et al. (36)	2008	Brazil	122 CKD 3-4	55 ± 11	SGA	18%
Campbell et al. (37)	2008	Australia	56 CKD 3-4	70±14	SGA	12%
de Mutsert et al. (38)	2009	Netherlands	1601 dialysis	59±15	SGA	28%
Cordeiro et al. (39)	2009	Sweden	173 HD	65(51-74)	SGA	43%
Rambod et al. (40)	2009	USA	809 HD	53±15	MIS>5	46.8
Szeto et al.(41)	2010	China	314 PD	60±12	MIS>6	60.2%
					SGA	28.7%
Miyamoto et al. (42)	2011	Sweden	280 dialysis	56(35-68)	SGA	30.3%
Leinig et al. (43)	2011	Brazil	199 PD	57±13	SGA	64.7%

2.6 Linking PEW and malnutrition to outcomes

Decreased indices of nutritional status have been associated with increased risk of death (44). Indeed, decreased muscle mass and low protein and energy intakes are linked to worse outcome (44). The importance of PEW as a determinant of poor outcome in CKD population is reflected by the 'reverse epidemiology phenomenon'. For example, in patients with late stage CKD, low (rather than high) cholesterol and low (rather than high) BMI, predict poor outcome (45). By contrast, in the general population those with hypercholesterolemia and obesity have an increased risk of co-morbidities and mortality.

As obesity is rapidly growing in the general population (and is one risk factor for kidney disease), it is not surprising that many CKD patients are overweight or obese. Indeed, obesity is a risk factor for CKD progression (46).

Despite weight status, PEW is equally harmful in obese as it is in lean CKD patients (47). Although many possible explanations have been proposed for these contradictory associations, the most convincing one is that PEW, a risk factor for increased mortality, associates with a decrease in cholesterol and BMI in these patients. Obviously, to prevent, diagnose – as soon as it develops -and treat PEW effectively in CKD subjects must be a priority.

2.7 Causes of malnutrition and PEW in CKD

2.7.1 Renal causes of malnutrition and PEW

The kidneys are central to various metabolic processes, including the recycling of amino-acids from small circulating peptides, the re-uptake of glucose and the metabolism of waste products from the urea cycle. As renal function decreases, the accumulation of waste compounds occurs, resulting in a toxic concentration of potentially harmful substances. This state of uremic intoxication, also known as uremia, is both detrimental to systemic homeostasis and has detrimental consequences for more complex metabolic processes such as appetite regulation and food intake (48, 49) (**Figure 1**).

2.7.2 Chronic low-grade inflammation

While malnutrition is doubtlessly an important cause of PEW in CKD, much research demonstrate chronic low-grade inflammation as a causal factor of equal importance (32). It has been reported that inflammation is present in 30 to 50% of ESRD patients (50); furthermore, inflammation is an important cause of hypoalbuminaemia (51), which is a risk factor for mortality in these patients (52-55). Inflammation has been shown to decrease protein production and to increase protein breakdown, resulting in a highly negative protein balance (56). Indeed, Stenvinkel et al. found markedly higher prevalence of inflammation in patients with cardiovascular disease (CVD) and malnutrition terming this commonly observed triad of concurrent complications as malnutrition, inflammation and arteriosclerosis (MIA) syndrome (1, 50), and others have made similar observations (3).

2.7.3 Metabolic acidosis

Metabolic acidosis is a common metabolic disturbance in CKD patients that predispose to bone disease, altered protein metabolism, skeletal muscle wasting, and progressive glomerular filtration rate loss (57). In addition, acidosis induces increased protein catabolism with degradation of the essential, branched-chain amino acids and muscle protein (58). Moreover, metabolic acidosis is also known to suppress synthesis of proteins such as albumin (58), and, as noted above low levels of albumin as well as catabolism are strong predictors of mortality. Indeed, metabolic acidosis, generally manifested by reduced serum bicarbonate levels, is also correlated with increased mortality (59, 60). Interestingly, Kovesdy et al. found that not only low levels of bicarbonate (<22 mmol/L) are associated with mortality, but also high levels (>29 mmol/L) (61).

2.7.4 Co-morbidities

Like many other diseases associated with aging and unhealthy living, CKD rarely occurs in isolation but rather is often accompanied by various co-morbid conditions. Indeed, as many as 86% of patients with CKD may have at least one co-morbidity (62). CKD is often the end-result of diet-related diseases such as diabetes, hypertension, and atherosclerosis. In fact, diabetes which is now the leading cause of CKD and ESRD in many countries including the US (63) may also increase the risk of PEW. Indeed, Pupim et al. reported that diabetic ESRD patients are more likely to develop PEW than non-diabetic patients, as the diabetic patients had a greater skeletal muscle protein loss (64). In addition, hypertension is the second main cause of development of CKD (65) and the majority of patients with stage 4–5 CKD have high blood pressure. Moreover, cardiovascular disease (CVD) is present in a large proportion of CKD patients (65). In these patients, both diabetes and hypertension are associated with CKD progression and cardiovascular death (66). These and several other disturbances predispose CKD patients to deteriorations of nutritional status and development of PEW.

2.7.5 Medications

Often CKD patients have multiple medication requirements and need to comply with intense dialysis sessions and often strict dietary recommendations. In addition, these

patients commonly need to take numerous medications to treat other existing co-morbidities (67). The interactions of these drugs can alter taste or may interact with nutrient metabolism, which increases the risk of malnutrition.

2.7.6 Dialysis treatment

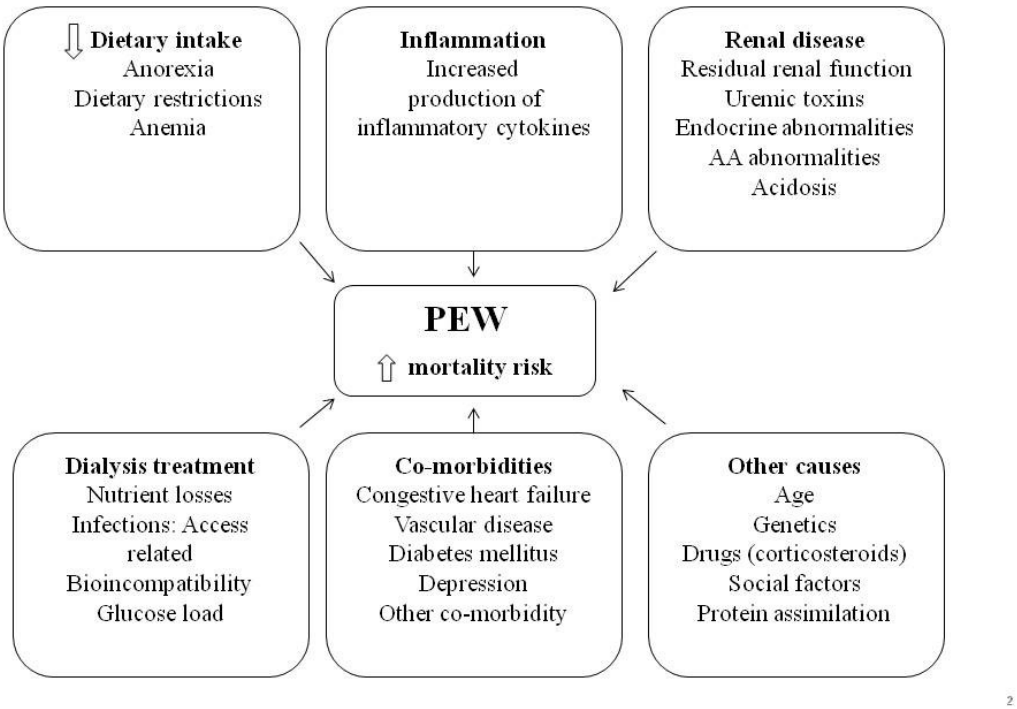
Dialysis treatment has undoubtedly prolonged CKD patients´ lifespan and improved uremic intoxication commonly found in pre-dialysis patients. However, these benefits come with potential side-effects.

A detailed review of the literature (68) have shown that PD therapy induces a decreased turnover rates and a reduced efficiency of protein turnover. This condition is especially harmful under stress as nutrient intake may be insufficient especially during superimposed catabolic illnesses (68). In HD patients, many factors that influence protein metabolism predispose these patients to increased catabolism and loss of lean body mass. It has been shown that the HD procedure in itself can induce protein catabolism – these adverse effects are likely the result of decreased protein production and increased protein breakdown (69, 70). Indeed, low plasma amino acid levels during HD sessions are thought to be one of the possible explanations for increased catabolism (70).

2.7.7 Loss of appetite

Loss of appetite, or anorexia, often results in an insufficient intake of nutrients, and is common in CKD where it significantly contributes to malnutrition (72). Anorexia commonly occurs when the GFR is less than 10%-15% of normal (20), with a reported prevalence of 35-50% in diverse populations of CKD patients (73-75). In addition, older kidney patients may face an extra obstacle to a healthy and well-balanced diet, if they suffer from odontological problems. It is not uncommon for these patients to suffer from dental problems such as higher number of decayed, missing or filled teeth (76) and an increased prevalence of periodontitis (77). These issues can evolve into chewing and/or biting problems, making patients less likely to consume high-fiber foods such as bread, fruits or vegetables and therefore risking their essential nutrient intake.

Figure 1. Multifactorial causes of PEW.



As CKD progresses, anorexia develops gradually (78). The exact mechanisms remain unclear, but are thought to involve uremic intoxication, inflammation and hormonal derangements. Among the hormonal changes described are both increases in circulating levels of anorexigens such as leptin, as well as decreases in orexigens such as ghrelin (79). Finally, as patients progress to ESRD and dialysis is introduced, an improvement in appetite is often seen (49).

2.7.8 Social aspects

Social aspects related to CKD also contribute to a worse nutritional status. The burden of living with kidney disease is aggravated when emotional problems such as depression, cognitive impairments and social isolation occur. It has been shown that depressive behavior and depression in CKD patients is closely linked to the presence of PEW (80). In fact, dialysis patients with poor appetite have been reported to be more often depressed (73). Furthermore, social factors associated with aging and chronic disease, such as loneliness and poverty, are all highly prevalent in CKD patients (20) and likely contribute to malnutrition and PEW.

2.8 Assessment of PEW in CKD patients

2.8.1 Historical background

While consensus exists as to the importance of identifying and treating malnourished CKD patients (81, 82), the necessary tools for such intervention are currently lacking. Over the years, many methods have been proposed to identify malnourished and wasted CKD patients, as well as to grade the severity of their state (15, 83-92). Unfortunately, none has proven both easy to use in the clinical setting, easy to interpret and accurately linked to patient outcomes and composite nutritional status. Thus, there is currently no single tool that can be used to identify high-risk individuals; with so many concurrent metabolic and nutritional abnormalities, such a tool has remained elusive. The National Kidney Foundation Clinical Practice Guidelines for Nutrition in Chronic Renal failure (93) thus, recommends that nutritional status should not be evaluated with only a single measure alone, but instead using a combination of valid, complementary measures. Indeed, according to an expert panel directed by the International Society of Renal Nutrition and Metabolism (20) the current recommended procedure to diagnose PEW requires the analysis of serum chemistry (s-albumin, s-prealbumin and s-cholesterol), body mass (assessed by BMI, weight variation and percent of body fat), signs of muscle wasting overtime (reduced mid-arm muscle circumference and creatinine appearance) as well as unintentional low dietary intake (for at least 2 months).

These measures may include biological assessment of protein-energy status, subjective global assessment, dietary intake assessment and anthropometry measurements. However, the ideal way to measure PEW not always corresponds to the hectic reality in daily clinical practice. Therefore, SGA has been used as a surrogate of PEW in many studies (38, 94, 95)

2.8.2 Biomarkers of nutritional status

Biochemical parameters are normally used to assess and monitor nutritional status in CKD patients. However, none of the currently favored biomarkers have been demonstrated to accurately reflect nutritional status in CKD (1, 53, 96). Instead, strong associations between several biomarkers and mortality seem to derive, at least in

part, from their close association with inflammation (2), and in part from the important role of the kidney in metabolizing circulating peptides (97).

2.8.2.1 Serum albumin

The amount of serum albumin is determined by its synthesis, breakdown and volume of distribution (98). In CKD patients, factors such as overhydration, proteinuria and losses into the dialysate and urine may cause a decrease in plasma protein concentration. Counter-regulatory mechanisms may also influence the serum albumin concentration. Whereas in the short term, protein deficiency decreases the rate of albumin synthesis (99), compensation in the long term may occur through a decrease in albumin breakdown and a shift of albumin from the extravascular to the intravascular space. Additionally, albumin has a relatively long half-life and is present in large quantities (100), limiting the impact of a decreased protein intake on concentrations of albumin. Even in some extreme cases of malnutrition, such as marasmus, serum albumin levels in these patients remain normal (101). In kwashiorkor, on the contrary, serum albumin levels are usually low (102, 103). However, it is important to keep in mind that kwashiorkor is typically accompanied by infections and severe protein intake deficiency (86, 104). In a study by Kaysen *et al.* (105) low serum albumin levels in dialysis patients were mainly associated with inflammation. Thus, it seems that hypoalbuminemia in CKD patients is rather associated with a chronic inflammatory state than insufficient food intake (106). Despite the prevailing acceptance of albumin as a nutritional status biomarker in CKD, circulating levels of albumin are in fact - as will be shown in Study II in this thesis - *not* an appropriate accurate indicator of nutritional status (107).

2.8.2.2 Serum creatinine

Creatinine is the breakdown product of creatine phosphate in muscle. Under stable kidney function, creatinine is typically produced at a relatively regular rate by the body depending on the total amount of muscle mass (108). Low lean muscle mass has been associated with increased risk of mortality in CKD and dialysis patients (71, 109). Lean muscle mass in these patients is commonly assessed by the serum creatinine level due to its easy availability, cost-effectiveness and reliability nature (110).

2.8.2.3 Insulin-like growth factor-1 (IGF-1) and binding proteins

IGF-I is a peptide hormone, produced predominantly by the liver in response to pituitary GH (growth hormone), which is involved in a number of physiological processes. The production of IGF-I signals the availability of nutrients needed for its anabolic actions. IGF-I has a considerably short long half-life of only 2 to 6 hours and is regulated by nutrition and dysregulated in states of under- and over-nutrition, its serum concentrations falling in malnutrition and responding promptly to re-feeding (111). Therefore, its usefulness as nutritional biomarker has been proposed (112). Compared to other commonly used markers of nutritional status, such as serum albumin and serum transferrin, IGF-1 was found to correlate better with anthropometric measurements markers of nutritional status in HD patients (113). However, due to its high cost, the use of IGF-1 is limited in the research setting while its high costs definitely is a barrier for its use in clinical practice (114). In Study III we found that IGF-1 levels correlated with several markers of body composition and nutritional status, and with mortality.

2.8.2.4 C-reactive protein (CRP)

CRP is a marker of inflammation - not a direct nutritional marker (114). However, it has an important function in the overall assessment of nutritional status in CKD patients as it is an acute phase reactant that inversely correlates with the concentrations of visceral proteins (115). In practice, one should remember that very low levels of visceral proteins, for instance serum albumin, are often due to presence of inflammation rather than low protein intake or protein depletion (114). Thus, both in the clinical and research setting it is recommended to check CRP levels in conjunction with other nutritional markers and make a decision for possible nutritional intervention, as well as intervention against factors causing CRP to rise.

2.8.2.5 Cholesterol

Cholesterol is a lipoprotein that functions as a precursor for the synthesis of steroid hormones, bile acids, and vitamin D. Serum cholesterol (and several other blood lipids and lipoproteins; such as, total cholesterol, LDL cholesterol, HDL cholesterol and

triglycerides) are indicators of patients´ nutritional status. Indeed, s-cholesterol has been proposed as assessment criterion for malnutrition and PEW (20).

Contrary to the general population (116), a high s-cholesterol level in the CKD and dialysis population is associated with improved survival (3, 117). However, this association seems to only be true in patients who are inflamed and/or malnourished (118, 119), which suggest that low levels of s-cholesterol may be a surrogate marker of inflammation and/or malnutrition.

2.8.3 Clinical and anthropometric markers of nutritional status

2.8.3.1 Subjective global assessment (SGA)

With limited value of serum albumin and other circulating surrogate biomarkers of nutritional status, subjective global assessment (SGA) (120) has been proposed as an easy, inexpensive, and handy subjective method of assessing nutritional status in CKD patients. SGA is currently considered to be a reliable marker of malnutrition and PEW in uremic patients (93, 121). Additionally, SGA uses scoring of a patient´s medical history and physical status as revealed by physical examination to distinguish well-nourished, mildly malnourished and severely malnourished patients (or using finer grading). The attributes scored in the SGA assessment include the following: gastrointestinal symptoms, dietary intake, body weight, disease state and functional capacity. Due to its strong correlations with PEW (122) and outcomes (123), SGA is recommended as a component of longitudinal monitoring of chronic dialysis patients by, amongst others, the National Kidney Foundation Kidney Disease/Dialysis Outcomes and Quality Initiative (K/DOQI) (93).

Although several studies show that SGA is a useful tool in CKD (83) (84), it should be kept in mind that SGA is still a subjective score that may be biased by inter- and intra-personal differences. As such, it is perhaps best employed in a longitudinal manner by the same trained investigator to follow patients over time.

2.8.3.2 Malnutrition inflammation score (MIS)

As inflammation is closely related with nutritional status and PEW in CKD patients, the MIS (124) scoring system was developed in order to better assess this complex syndrome. Therefore, by using components from the SGA questionnaire (weight

change, dietary intake, GI symptoms, functional capacity, co-morbidity, subcutaneous fat, and signs of muscle wasting), as well as adding biochemical parameters that are influenced by inflammation status (s-albumin and total iron-binding capacity, TIBC) and body mass index (BMI). In total, there are 10 parameters in the MIS, each including four degrees of severity (where 0 = normal and 3= severely abnormal) and adding each one of the MIS components to have the final score (0= normal nutritional status and 30 = severely malnourished) (124).

2.8.3.3 Other nutritional scoring systems

In addition to SGA and MIS, alternative scoring systems to assess nutritional status have been developed. Among those, Mini Nutritional Assessment (MNA) (125) and Mini-Nutritional Assessment–Short Form (MNA-SF) (126) have been developed as nutritional assessment tools for the geriatric population. In addition, these tools have also been used in CKD and dialysis patients (125, 127); however, the usefulness of the MNA-SF is still questionable (128)

2.8.3.4 Dietary records

As previously discussed, insufficient food intake associates with malnutrition and the development of PEW (19). Therefore, it is of paramount importance to assess dietary intake in CKD patients. Many methods have been used throughout the years in an attempt to estimate dietary intake; the methods described below are the most commonly used in clinical and research practice.

Food diary consists of carefully written records of food and beverage intake as well as the amount consumed during each meal and snacks (129). It is recommended that the recording is done after each eating occasion in order to avoid memory bias. In addition, food diaries are usually kept for seven consecutive days or three non-consecutive days (for example, two week days and one day during weekend), as to have a more representative sample of patient´s diet.

24-Hour dietary recall usually consists of an interview where the patient is asked to recall the previous 24-hours food and beverage intakes (including the amount and time of consumption) (129)**.** In addition, the success of these interviews relies to a large extent on the experience of the interviewer who ideally should be a dietitian or a health care worker trained to perform such tasks**.**

Food frequency questionnaire (FFQ) consists of a list of foods (depending on the nutrients or foods of interest) with selection of options relating to the frequency of consumption of each of the foods listed (for example, times per day, daily, weekly, or monthly) (130). Furthermore, FFQs inquire about dietary intake given within a specified time frame (such as, the past month, 6 months or 1 year). This questionnaire was elaborated to collect dietary information from large sample of individuals. It is usually self-administered, but sometimes an interviewer can administer in person or through telephone interview (131).

The dietary records described above are usually used as a proxy for dietary intake; all of the currently used dietary records suffer from a number of limitations. First, these methods are by nature subjective and very much dependent on patient´s memory and willingness to cooperate. Second, patients are very likely to underreport foods that are considered unhealthy and overreport those that are recommended (132). Third, in general these methods contain a great amount of measurement errors which can make the data collected unreliable (133, 134). Therefore, one should be aware that assessing one´s diet is a difficult task as objective tools to collect information about food and beverage intake remains to be created.

Protein intake can be estimated also by more objective means using equations that relate total nitrogen losses to dietary protein intake assuming steady state. In non-dialyzed CKD patients and PD patients, collection of 24-h urine (and in PD patients also dialysate) and measurement of urea in removed fluids and in plasma allow calculation of the protein equivalent of nitrogen appearance (PNA) providing an estimate of dietary protein intake. In HD patients, assessment of PNA using urea kinetic modeling can be performed without actually measuring urea removal.

2.8.3.5 Body composition

Theoretically, body composition and the contents of fat, protein and water should reflect long-term dietary intake and PEW. Over time, and also depending on the severity of the disease, changes in body composition are inevitable especially in CKD patients in whom body composition can change more rapidly than in healthy older adults. CKD affects how the body metabolizes fat, keeps water balance, and is able to maintain muscle, bone and other components of lean body mass. While several methods have been employed in assessing body composition in CKD patients, these

evaluations need to standardized and validated in these patients – as they can suffer from the large variation of body composition overtime.

2.8.3.6 Anthropometrics

Anthropometric measurements such as body weight and height (and BMI, see below) should as a rule always be included when assessing nutritional status. Skin fold thickness is a more specific anthropometric tool widely used in the clinical practice due to its low cost, simplicity and non-invasive nature. It is used to estimate both fat mass and fat-free mass (135). In combination with measurement of mid-arm circumference one can also calculate mid-arm muscle circumference which is a surrogate marker of lean body mass. However, skin fold thickness measurements suffer from a large intra- and inter-observer variability, and are only modestly reliable (136).

Another marker used to determine body fat mass is waist circumference. This is again a simple and easy method, which has been suggested to predict survival in CKD patients (137). However, as fat mass does not necessarily reflect PEW in CKD, waist circumference is likely not a good marker of the nutritional deficiencies associated with PEW.

2.8.3.7 Body mass index (BMI)

BMI is calculated as body weight in kilograms divided by the square of height in meters. According to the World Health Organization (WHO) guidelines, obesity is defined as a BMI ≥30.0 kg/m^2, and overweight as a BMI of ≥25.0 kg/m^2. Normal weight is defined as a BMI of 18.5–24.9 kg/m^2, which is within the normal range according to the WHO, and underweight as a BMI <18.5 kg/m^2. In CKD patients, BMI may not reflect real nutritional status, as gross imbalance in fluid status in these patients may cloud the results. Also, loss of muscle mass is characteristic in PEW; however, a relatively well-preserved fat mass still usually remain, resulting in small changes in BMI that can be disguised by imbalances in fluid homeostasis (47). Finally, overweight ESRD patients may also suffer from PEW (47). For these reasons, BMI alone does not accurately reflect nutritional status or PEW in CKD patients.

2.8.3.8 Hand grip strength (HGS)

Muscle strength in CKD patients is often evaluated by muscle dynamometry such as hand grip strength (HGS) which associates with lean body mass as assessed by anthropometry, dual-energy X-ray absorptiometry (DEXA, see below) and creatinine kinetics and with nutritional status as assessed by SGA score. In addition, the combined assessment of body composition (lean body mass) and muscle function has begun to become more prevalent as composite marker of nutritional status (138). Indeed, a dynamometric HGS measurement standardized to age and gender has emerged as an easily performed bedside test (139) that is considered to be a reliable and useful marker of both nutritional status and future mortality risk (1, 92).

2.8.4 Other methods to assess nutritional status

2.8.4.1 Bioelectrical impedance and conductance

Bioelectrical impedance analysis (BIA) has been proposed as a noninvasive and simple technique to measure body hydration status of patients, especially as regards the determination of "dry" body weight in HD patients (140). Also, BIA has also been suggested as a valuable tool in subjects undergoing PD (141). BIA is based on the assumption that human body may be viewed as a number of parallel connected resistors. By connecting electrodes to various body parts (typically arms and legs) with a conductive gel, electrical current passes through the body at various frequencies and then the conductance is measured. Current standard BIA models used in CKD view the body as 5 interconnecting cylinders, 2 for the arms, 2 for the legs, and 1 for the trunk, instead of the more common assumption previously that the whole body can be seen as just one cylinder. BIA models are prone to errors of simplification which depend on body size, shape and regional fluid accumulation.

2.8.4.2 Dual-energy x-ray absorptiometry

A more reliable tool to estimate body composition is dual energy X-ray absorptiometry (DEXA). DEXA passes low-energy x-rays through the body to measure fat mass, fat-free mass and bone mineral density (142). While DEXA is relatively good at measuring fat mass (136), it is not used routinely in the clinical setting due to its high cost and its inaccuracy in severely overhydrated patients (143). Furthermore, the

amount of radiation, although small, may be a concern in patients exposed to DEXA repeatedly over many years.

2.8.4.3 Other methods

There are also several specialized methods that can be used to assess nutritional status in clinical research such as ultrasonography, magnetic resonance imaging, computed tomography and total body potassium and total body nitrogen but these methods are used less often and usually serve as tools for validation of more common markers.

2.9 Studies on prevention and treatment PEW

Throughout this thesis, we discussed the fatal consequences of PEW and poor nutritional status; thus, the importance of assessing and monitoring it. In this session, we will discuss the current available therapies used to prevent and treat PEW.

2.9.1 Treatment of co-morbidities

Interventions that target common co-morbidities such as CVD, diabetes and infections are of utmost importance in the prevention and treatment of PEW in CKD patients. In addition, underlying disturbances linked to uremia such as metabolic acidosis, fluid retention and inflammation to name a few are helpful in reducing catabolism and thereby resolve to some extension PEW and malnutrition. Indeed, when reduced serum bicarbonate is corrected, it has been shown to improve nutrition status and slow progression toward ESRD (18). Among measures to alleviate inflammation, nutritional supplementation with ω-3 fatty acid has shown the potential to improve systemic inflammation and has been tested in several intervention studies involving HD patients, resulting in improved outcomes (144, 145)

In addition, treatment of co-morbidities, in CKD patients, helps prevent progression of CKD to dialysis or renal transplantation, cardiovascular events and premature death. Since cardiovascular death is overrepresented in this population (146), treatment of cardiovascular risk factors must be a priority. For example, appropriate management of diabetes and control of blood pressure are highly advisable (147), as well as treatment of hyperlipidemia and reduction of proteinuria (147)

2.9.2 Early nutritional intervention

Initiatives to improve food intake in CKD patients are of paramount importance. In addition, in order to compensate for nutrient losses during dialysis and to further improve nutritional status of dialysis patients, intradialytic nutritional support has been developed. Apart from the nutritional support, these interventions take the form of intravenous (in HD) and intra-peritoneal (in PD) infusions. Intra-dialytic parenteral nutrition (IDPN) is an acknowledged method of protein and energy supplementation in HD patients. Typically composed of a mixture of amino acids, dextrose and lipids, IDPN has been shown to increase protein synthesis, decrease protein degradation, resulting in a highly positive protein balance (148). However, recent studies have demonstrated no additional benefit of IDPN over oral nutritional support (149). Therefore, current guidelines limit the use of IDPN to wasted subjects in whom oral nutritional support turned out to be ineffective in improving nutritional status (150).
Nutritional counseling is a mainstay of therapy, with the initial goal of protein restriction in pre-dialysis CKD patients while because of the catabolic influence of the dialysis procedure switching to increasing protein and calorie intake as dialysis is started (150). In the research setting, appetite stimulation (151) has proven to be effective for short periods, but these methods are seldom tried in the clinic. For example, subcutaneous ghrelin and the administration of melanocortin-receptor antagonists have both been reported to reduce uremic anorexia and lead to increased food intake (152, 153) . On the other hand, administration of nutritional supplements (154) as well as daily dialysis sessions (155) are increasingly used in the clinic. In **Table 4**, we discuss some nutritional intervention studies aiming to improve nutritional parameters in randomized trials in patients HD and PD patients.

Table 4. Randomized nutritional interventions to improve nutritional status in HD and PD patients*. Adapted from (81).

Study	Intervention	Patients (n)	Results
Eustace et al. (2000)(156)	EAA (3.6 g with meals three-times daily) vs placebo for 3 months	HD/PD (47) Albumin concentration ≤38 g/l	S-albumin levels in HD patients (EAA vs placebo) ↑ by 2.2 g/l Improvement in HGS and SF-12® Correlation between baseline CRP levels and improvement in albumin levels.

Gonzalez-Espinoza et al (2005)(157)	Egg albumin-based ONS; open-label controlled trial with 6-month follow-up	PD (28) Case (13) Control (15)	S-albumin levels increased from 26.4 g/l to 30.5 g/l in the study group vs 26.6 to 28.0 g/l in the controls DPI, DEI and nPNA ⬆ more in the study group than in the controls Malnutrition ⬇ 6% in the controls vs 28% in the study group Main predictor of s- albumin levels: egg albumin-based ONS and DPI.
Leon et al. (2006)(158)	Targeting several nutritional barriers (ONS was a small component of intervention) for 12 months	HD (180) Albumin concentration <37 g/l	Albumin levels: +2.1 g/l in intervention group vs +0.6 g/l in controls DEI: +4.1 kcal/kg per day in intervention group vs –0.6 kcal/kg per day in control DPI: +0.13 g/kg per day in intervention group vs –0.06 g/kg per day in controls.
Cano et al. (2007)(149)	ONS vs ONS + IDPN for 1 year ONS: 5.9 kcal and 0.39 g protein per day IDPN: 6.6 kcal and 0.26 g protein per day	HD (186) ONS (93) ONS + IDPN (93)	After 3 months: ⬆ BMI, s- albumin levels, and prealbumin levels in both groups ⬆ s-prealbumin levels of >30 mg/l within 3 months predicted improved 2-year survival in all patients.
Fouque et al. (2008)(159)	CKD-specific ONS (Renilon®, Nutricia, Schiphol, The Netherlands) vs standard care for 3 months	HD (86) Case (46) Control (40) Baseline albumin concentration 35.2 g/l in both groups	⬆ DPI ($P < 0.01$) and DEI, and improved subjective global assessment and quality of life in the group receiving ONS No difference in albumin or prealbumin levels between groups, but change in albumin and prealbumin levels correlated with protein intake Phosphatemia was unaffected and use of phosphate binders remained stable or ⬇

*Table adapted from Kalantar-Zadeh et al. Nat rev nephrol. 2011 May 31;7(7):369-84. Abbreviations: c-reactive protein; DEI, dietary energy intake; DPI, dietary protein intake; EAA, essential amino acid; IPDN, intradialytic parenteral nutrition; nPCR, normalized protein catabolic rate; nPNA, non-protein nitrogen appearance; ONS, oral nutritional supplement; R, coefficient of correlation.

3 RESEARCH AIMS

The overall aim of the investigations summarized in this thesis is to analyze which tools are more efficient in assessing nutritional status in patients with CKD and to evaluate the association of identified nutritional risk markers with the high mortality rates seen in patients with CKD.

The specific aims of the studies were:

- To investigate the impact of dialysis on appetite and the correlation between appetite loss and mortality in CKD5 patients starting on dialysis as well as in prevalent dialyzed CKD5 patients (Study I);
- To study serum albumin and its correlation with markers of nutritional status in dialysis patients (Study II);
- To study the predictive role of IGF-1 in non-dialyzed CKD stage 5 patients and the mortality risk associated with IGF-1 change after initiation of dialysis treatment in these patients (Study III)
- To analyze the impact of a standardized test meal on pancreatic polypeptide (PP) and duodenal incretin responses in HD patients (Study IV).
- To compare readily available markers of PEW to SGA and assess their validity in incident and prevalent dialysis patients (Study V).

4 PATIENTS AND METHODS

4.1 PARTICIPANTS AND CLINICAL COHORTS

4.1.1 Malnutrition, inflammation, and atherosclerosis (MIA) cohort

The MIA cohort is a cohort consisting of incident patients with CKD stage 5 (GFR<15 mL/min) sampled close to the start of renal replacement therapy (either HD or peritoneal dialysis) from the renal program of the Karolinska University Hospital at Huddinge, Sweden. Patients are further followed up till death or transplantation. Also, patients are invited to attend additional visits approximately after 1 year and 2 years of dialysis. This ongoing prospective cohort study started in 1994, and a descriptive protocol has been described in more detail (160). The study exclusion criteria were age below 18 years or above 70 years, clinical signs of acute infection, active vasculitis or liver disease at the time of evaluation, or unwillingness to participate. This cohort constitutes the patient material included in *Studies I, II, III and V* (**Table 5**). The Ethics Committee of the Karolinska Institute, Sweden, approved the study (Dnr. 273/94).

4.1.2 Mapping of inflammatory markers in chronic kidney disease (MIMICK-1) cohort

The MIMICK-1 cohort is a patient population material consisting of prevalent patients undergoing HD at the Karolinska University Hospital at Huddinge, Stockholm (including as well its satellite dialysis units at Kungsholmen Södersjukhuset), Sophiahemmet, Danderyds Hospital and Uppsala Academic Hospital. This study originally aimed at investigating the variability of inflammatory parameters in prevalent HD patients over time. Recruitment of the patients occurred from October 2003 through March 2004. All patients who were receiving regular HD therapy at any of the units were invited to participate (n=254); 6 patients declined, and one patient with HIV infection was excluded. The 247 eligible patients were then followed up for 12 weeks. Eleven patients were excluded because of insufficient baseline clinical information; seven were excluded because of insufficient hs-CRP measurements; and one patient died. The remaining 228 patients were further followed up for assessment of overall and cardiovascular mortality in relation to biochemical markers, and constitutes the basis

of *Studies I, II and V*, which only contain baseline measurements. The Ethics Committee of the Karolinska Institutet, Sweden, approved the study (Dnr. 03/415).

4.1.3 Mapping of inflammatory markers in chronic kidney disease (MIMICK-2) cohort

The MIMICK-2 cohort comprises 84 patients from a cross-sectional study with follow-up that originally aimed at monitoring inflammatory markers in all prevalent PD patients who were being controlled at the Karolinska University Hospital and Danderyds Hospital in Stockholm. All participants were prevalent PD patients who had been on continuous ambulatory peritoneal dialysis (CAPD) or automated peritoneal dialysis (APD) for at least 3 months. Patients were recruited from March 2008 to April 2011. This cohort constitutes the basis of study V. The Ethics Committee of the Karolinska Institutet, Sweden, approved the study (Dnr. 03/415).

4.1.4 Post study

The Post intervention study (161, 162) was conducted between November 2005 and February 2008. In the study presented in this thesis, 6 clinically stable non-diabetic patients who had been on HD for at least 6 months and treated for at least 4 h three times per week using biocompatible membranes, as well as 9 HS with no known chronic disease or ongoing inflammation were included. Exclusion criteria were age under 20 or over 70 years at recruitment, pregnancy or a plan to become pregnant within the next 6 months, signs of infection and/or antibiotics prescribed during the last 4 weeks, clinically manifesting organ failure other than ESRD at the time of enrolment, alanine-aminotransferase (ALAT) > 2 times the normal range and/or neuropsychiatric illness that could affect informed consent or compliance with the protocol. Of the 10 patients, eight had at least one kidney biopsy before reaching ESRD, with four diagnosed as having hypertensive kidney disease (tubulointerstitial changes), three having glomerular scarring consistent with previous glomerulonephritis and one presenting with obstructive nephropathy after multiple kidney stones. Of the patients, three were taking β-blockers, four were taking calcium-channel blockers and six were on angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers, while none of the patients were prescribed statins. None of the patients were on anti-inflammatory drugs or anti-diabetics. This

material provided the basis for Study IV. The Ethics Committee of the Karolinska Institutet, Sweden, approved the study (Dnr. 2005/311-31/1; Eudra Clinical Trial Number 2005-004403-12).

4.1.5 Prevalent HD patients

Of a total of 164 prevalent patients, 128 (76 males and 52 females, median age 65 years range 26-84 years) agreed to participate in a previous study on nutritional status in HD patients (8). The causes of renal failure in the 128 patients were diabetic nephropathy (n=23), chronic glomerulonephritis (n=38), polycystic kidney disease (n=13), pyelonephritis and interstitial nephritis (n=14) and other diseases or unknown etiology (n=40). Thirteen of the diabetic patients were insulin-dependent. Seventy-seven patients had signs of cardiovascular and/or peripheral vascular disease (grouped as CVD). Of these, 20 had suffered one or more myocardial infarctions, 15 had ischemic heart disease but no prior myocardial infarction, one had an aortic aneurysm and 11 had peripheral ischemic atherosclerotic vascular disease. Nineteen patients suffered from chronic heart failure. Six patients had cerebrovascular disease with neurological symptoms, following one or more attacks of stroke; all these patients also had signs of CVD. Of the 23 diabetic patients, 17 had CVD. Ninety-six patients had no residual renal function, defined as renal urea clearance <0.5 ml/min. This material was included in Study II. The Ethics Committee of the Karolinska Institutet, Sweden, approved the study (Dnr. 126/87).

4.1.6 Prevalent CAPD patients

All CAPD patients (n=36) at Huddinge University Hospital were recruited to participate in a previous study on nutritional status in PD (9). The patients were divided into two groups, based on the SGA Group I (n=11) had a normal nutritional status, group II (n=25) had either mild to moderate (n=16) or severe (n=9) malnutrition. All patients were investigated in the morning, before the first dialysate exchange after an overnight oral fast. Twenty-one patients (group I n=6 and group II n=15) were treated with a single anti-hypertensive drug or various combinations of the following anti-hypertensive agents: angiotensin- converting enzyme inhibitors, beta-blockers and calcium-blockers. Six patients were treated with diuretics. There were nine diabetics and all were insulin dependent. Most of the patients were

supplemented with sodium bicarbonate and calcium carbonate to prevent acidosis and hyperphosphatemia respectively. Twenty-one patients had signs of cardiovascular and/or peripheral vascular disease (grouped as CVD). Of these, eight had suffered one or more myocardial infarctions, four had ischemic heart disease but no prior myocardial infarction and five had peripheral ischemic atherosclerotic vascular disease. Two patients suffered from chronic heart failure. Two patients had cerebrovascular disease with neurological symptoms, following one or more attacks of stroke; these patients also had signs of CVD. Of the nine diabetic patients, seven had CVD. Thirty patients had repeated episodes of peritonitis. Of these, twelve suffered four or more episodes of peritonitis, eight patients in group II and four patients in group I. This material was included in Study II. The Ethics Committee of the Karolinska Institutet, Sweden, approved the study (Dnr. 199/86).

4.1.7 Healthy controls

A population-based group of subjects (n= 79, 72% males, 53 (47-76) years) serving as controls for an ongoing study on CKD stage 3-4 patients was included for comparative reasons in the current thesis. This control group consisted of individuals from the Stockholm region who were randomly selected by Statistics Sweden (a government agency) and who accepted to participate, as volunteers, in the group. This material was included in Study V. The Ethics Committee of the Karolinska Institutet, Sweden, approved the study (Dnr. 40/02).

4.2 CLINICAL AND PHYSICAL EXAMINATION

Each patient's medical chart was reviewed, extracting data pertaining to underlying kidney disease, history of CVD, diabetes, other co-morbid conditions, common medication and survival. CVD was defined by clinical history or signs of ischemic cardiac disease, and/or presence of peripheral vascular disease and/or cerebrovascular disease. Smoking habits were recorded as current smokers, former smokers and non-smokers. GFR in the MIA cohort was estimated as the mean of urea and creatinine clearances. In the MIMICK cohort, total time on HD was annotated as vintage time.

Survival was determined from the day of examination and sample collection, with no loss of follow-up of any patient. Cardiovascular mortality was defined as death as a

result of coronary heart disease, sudden death, stroke, or complicated peripheral vascular disease. Causes of death were registered by a nephrologist unaware of other clinical or biochemical data of the patients and to the objectives of the studies.

4.2.1 Nutritional status and PEW

The presence of PEW and malnutrition was evaluated by SGA questionnaire (120). This assessment was completed either at the time of or within 1 week of blood sample collection and after the HD session in the MIMICK cohort. SGA includes six different components: three subjective assessments that are answered by the patients and concern the patient's history of weight loss, incidence of anorexia, and incidence of vomiting, and three assessments that are performed by the evaluators and are based on the subjective grading of muscle wasting, the presence of edema, and the loss of subcutaneous fat. On the basis of these assessments, each patient received a nutritional status score: 1 = normal nutritional status, 2 = mild malnutrition, 3 = moderate malnutrition and 4 = severe malnutrition. For the purpose of the studies, PEW and/or malnutrition was defined as SGA>1.

The evaluation of self-rated appetite (Study I), was assessed from the SGA questionnaire. Regarding the self-rated appetite assessment, all patients were asked to grade their appetite themselves according to the following scale: 1=good, 2=sometimes bad, 3=often bad, and 4=always bad.

4.2.2 Intervention standardized test meal

In the interventional study (Study IV), patients and healthy controls, following an overnight fast of at least eight hours, were sampled (on the day after a dialysis session in the case of patients). A dietician-designed meal consisting of 75 g of milk fat, 5 g of carbohydrates and 6 g of proteins was administered during ten minutes as a milkshake, immediately followed by 75 g of glucose mixed with 500 mL of purified water during five minutes. The meal was designed to mimic a heavy dinner in calories and nutrient composition.

4.2.3 Anthropometric evaluation

Body composition was assessed in the cohorts and in the healthy controls by using skinfold thicknesses of biceps, triceps, subscapular, and supra-iliac with a conventional skinfold caliper (Cambridge Scientific Instruments, Cambridge, MD). Lean body mass (LBM) and fat body mass (FBM) were estimated by means of dual-energy x-ray absorptiometry (in the MIA cohort) using the DPX-L device (Lunar Corp, Madison, WI) or according to a theoretical formula (in the MIMICK cohort) based on skinfold thicknesses and body density (135). Handgrip strength was measured using a Harpenden Handgrip Dynamometer (Yamar, Jackson, MI, USA) in the dominant hand (in the incident dialysis patients) or in the hand without fistula (in the prevalent HD group) and normalized with measurements from healthy subjects (7). These assessments were completed either at the time of or within 1 week of blood sample collection and after the HD session in the MIMICK cohort.

The body mass index (BMI) was calculated as weight (kg)/(height [m])^2 and classified according to the WHO specifications.

4.2.4 Blood samples

In Study I, II, III and V, blood samples were collected after an overnight fast (in the MIA and MIMICK2 cohorts) or before the dialysis session (in the MIMICK1 cohort) after the longest interdialytic period. Plasma samples were kept frozen at –70 °C if not analyzed immediately. In these cohorts, determinations of serum creatinine, serum albumin (bromcresol purple), haemoglobin, hypochromic red blood cells, total, LDL- and HDL-cholesterol, triglycerides, white blood cells count, high sensitivity C-reactive protein (hs-CRP) (nephelometry) and parathyroid hormone (PTH), phosphate, and calcium were performed by routine procedures at the Department of Clinical Chemistry, Karolinska University Hospital Huddinge. Other biochemical markers were analysed at the Research Laboratory of the Department of Renal Medicine, Huddinge. Serum IGF-1, IGF-1 binding protein-3 (IGFBP-3), and plasma Interleukin 6 **(**IL-6) were measured on an Immulite Automatic Analyzer (DPC Corp., Los Angeles, CA). The intra-assay coefficient of variation for IGF-1 was 4.3%, and the interassay coefficient of variation was 6.9%. IGFBP-1 was analyzed by ELISA (IEMA Test; Medix Biochemica, Kauniainen, Finland). Intact fibroblast growth factor-23 (FGF-23) was measured with ELISA (Kainos

Laboratories International, Tokyo, Japan). Serum osteoprotegerin (OPG) was analyzed by ELISA (R&D Systems Inc., Minneapolis, MN).

In Study IV, blood samples were collected at 0 min, 30 min, 60 min, 120 min and 240 minutes after meal intake in both HD patients and HS. Plasma and serum were extracted immediately and subsequently frozen at -80°C until analysis. The plasma levels of pancreatic polypeptide (PP) and cholecystokinin-8 (CCK-8) were measured by enzyme-linked immunosorbent assay (ELISA) with kits from Millipore Corporation (St. Charles, MO, USA) and Wuhan EIAab Science Co., Ltd (Wuhan, China), respectively. Serum total glucose-dependent insulinotropic polypeptide, GIP (1–42 amide and 3–42 amide) and plasma active glucagon-like peptide 1, GLP-1(7–36 amide and 7–37 amide) levels were measured by high sensitivity photometric ELISA (Millipore, St Charles, MO, USA). Each measure was repeated two times for each peptide and only accepted coefficient of variation (CV) lower that 10%. PP, GIP and GLP-1 had CV lower than 10%, but not CCK8 so it was excluded from the study. Serum concentrations of insulin were determined using an Immulite Automatic Analyzer (Siemens Medical Solutions Diagnostic Products, Los Angeles, CA, USA). Serum glucose and triglycerides were measured using routine methods at the Department of Laboratory Medicine, Karolinska University Hospital.

4.2.5 Statistical analyses

All statistical analyses were performed using statistical software SAS version 9.4 (SAS Campus Drive, Cary, NC, USA 27513). Normally distributed variables were expressed as mean ± SD and non-normally distributed variables were expressed as median and range (minimum and maximum) or interquartile range (10^{th}-90^{th} or 25^{th}-75^{th} percentile, IQR). Also, categorical values were expressed as number and percentage, unless otherwise indicated. Statistical significance was set at the level of $p< 0.05$.

Comparisons between two groups were assessed with the Student's unpaired t-test and Mann-Whitney test or χ^2 test, as appropriate. Differences among more than two groups were analyzed by analysis of variance (ANOVA) using one-way ANOVA or Kruskal-Wallis test, as appropriate. As many values were not normally distributed, Spearman's rank correlation (ρ) was used to determine univariate correlations.

Multivariate associations were performed by multiple regressions, stepwise multiple regressions and multinomial logistic regression analyses.

To evaluate the sensitivity and specificity of selected parameters as predictors of mortality, Receiving Operator Characteristics (ROC) curve analyses were performed (163). The optimum cut-off value, with the combination of the highest sensitivity and specificity, was calculated.

Survival analyses were made with the Kaplan-Meier survival curve or the Cox proportional hazard model. The relative risks for mortality were determined by multivariate Cox regression analysis and presented as hazard ratio (HR; 95% confidence intervals (CI))

4.3 STUDY PROTOCOLS

Table 5. Description of the studies included in the thesis

Study	Cohort	Subjects	Outcome
I	MIA MIA (1 year follow-up)	280 243	Changes in self-rated appetite status; all-cause mortality
II	MIA MIMICK-1 HD CAPD	458 222 125 36	Predictive value of s-albumin as a marker of nutritional status
III	MIA MIA (1 year follow-up)	365 207	Changes in IGF-1 levels after initiation of dialysis; its correlation with bone metabolism, nutritional status and mortality.
IV	POST STUDY CONTROL	6 9	Effect of test meal in appetite regulating hormones (PP, GIP and GLP-1)
V	MIA MIMICK-1 MIMICK-2 CONTROL	399 211 78 79	Predictive value of commonly used markers of PEW (HGS, s-creatinine, s-albumin, cholesterol)

Study I is a post-hoc, cross-sectional observational study of prevalent patients with CKD stage 5, including both those on maintenance dialysis (CKD5-D) and those not yet on dialysis (CKD5-ND). Self-rated appetite, along with anthropometrics and biochemical markers of nutritional status, were measured and related to all-cause 4-year mortality as assessed by Kaplan–Meier survival curves and the Cox proportional

hazards model. Study I shows that self-rated appetite is not an independent predictor of 48-months survival in most patients with CKD 5-D.

Study II evaluates, again in a post-hoc, cross-sectional observational study, the use of serum albumin as a risk marker for malnutrition in two relatively large cohorts of incident and prevalent CKD stage 5 patients. Serum albumin measured using a common methodology (bromocresol purple) was related to other markers of nutritional status, including other biochemical markers, clinical anthropometrics, and densitometry (dual-energy x-ray absorptiometry). Analyzing data from 458 incident and 383 prevalent dialysis patients, we found that serum albumin correlates poorly with other markers of nutritional status. Thus, its value as a reliable marker of nutritional status appears limited.

Study III evaluates, in a post-hoc, cross-sectional observational study, the use of IGF-1 as a risk marker for malnutrition and PEW measured using SGA in a large cohort of prevalent dialysis patients. Serum IGF-1 was related to other biochemical, clinical, and densitometric markers of nutritional status and mineral and bone metabolism in 365 clinically stable patients and its' predictive value for 5-year mortality risk was tested using log-rank test. The study shows that low serum IGF-1 associates with a sarcopenic body composition and with markers of disturbed bone metabolism, while also predicting an increased risk of mortality.

Study IV is a post-hoc analysis of data from a randomized, controlled intervention testing the impact of a fat- and carbohydrate-rich meal on biochemical markers of oxidative stress and inflammation. Using stored samples, we assessed changes in the appetite regulating peptide hormones pancreatic polypeptide (PP), glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide 1 (GLP-1) in HD patients and healthy controls during the 4 first 4 hours after consuming a meal. Six HD patients and 9 healthy subjects (HS) received a standardized test meal. Fasting and postprandial circulating levels of peptide hormones were measured using commercial enzyme-linked immunoassays (ELISAs), while glucose and triglycerides were assessed using conventional clinical chemistry methods. The study shows that fasting levels of both PP and GIP levels are both elevated in HD patients as compared to controls, as is the postprandial PP response. We speculate that this may be one mechanism whereby CKD engenders poor appetite.

Study V uses three cohorts (399 incident CKD5 patients starting on dialysis, 211 prevalent HD and 78 prevalent PD patients) to compare, in a post-hoc, cross-sectional observational study, common markers of nutritional status for assessing PEW as defined by SGA score. Using three separate multivariate regression models, we tested the relative predictive values for changes in SGA to predict changes in common clinical and biochemical markers of PEW. We report that serum levels of albumin, creatinine, and cholesterol as well as handgrip strength are in general only weakly or not at all associated with PEW as assessed by SGA following correction for cofounders.

5 RESULTS AND DISCUSSIONS

5.1 OVERVIEW

Both malnutrition (defined as a state of nutrient deficiency secondary to diet patterns) and protein-energy wasting (PEW, defined as a state of body protein catabolism and insufficient energy intake secondary to chronic renal disease), usually occurring together in CKD and here used as interchangeable entities, are common in CKD patients, where they also are strongly associated with low-grade inflammation and an adverse outcome (1-7). The main goal of this thesis was to investigate the relative usefulness of clinically available tools to accurately identify and monitor PEW and malnutrition in CKD as identifying patients at risk is a goal in itself and also a prerequisite for interventional therapies and prioritization of resources. By studying a broad spectrum of renal diseases of patients with reduced glomerular filtration rates but not yet on dialysis, as well as patients on both HD and PD, we increase the practical applicability of our results in the clinical setting. Unfortunately, our data do not support the use of any one single marker for the unequivocal identification of malnourished CKD patients, and even less makes it likely that commonly used methods can be clinically useful for risk stratification. Instead the results of our studies suggest that a panel combining several markers and including ideally also SGA should be used if possible. Thus, further studies are urgently needed to identify and validate better and more reliable tools to assess and monitor nutritional status in various CKD populations.

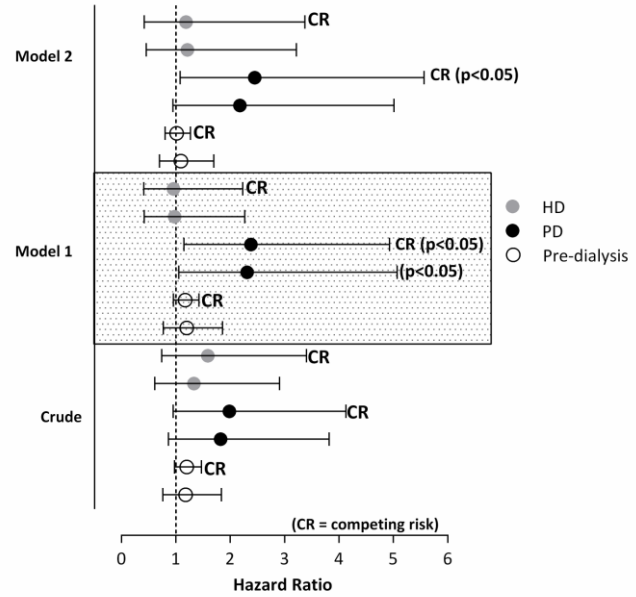
5.2 MEASURING SELF-RATED APPETITE TO ASSESS NUTRITIONAL STATUS

Anorexia is thought to be an important driver of malnutrition and PEW, with studies suggesting a prevalence of subjective anorexia of between 35% and 50% in different CKD populations (73-75). Anorexia appears to be more common in CKD patients with a low GFR . Both Kalantar-Zadeh et al. (164) and Carrero et al. (165) investigated maintenance HD patients and found that simply reporting a moderately or severely decreased appetite as part of SGA questioning was associated with a greater risk of mortality over 15 and 19 months respectively of follow-up, even after adjustment for age, sex, inflammation, dialysis vintage, and co-morbidity (164, 165). Interestingly,

both studies (164, 165) and clinical experience suggests that most CKD patients improve their appetite following the start of dialysis (49), suggesting that the already mentioned studies select patients whose anorexia is in fact therapy resistant or who may be under-treated. To our knowledge, no systematic studies of the relation between self-rated appetite and mortality have been reported in CKD stage 5 patients not yet on dialysis (CKD5-ND).

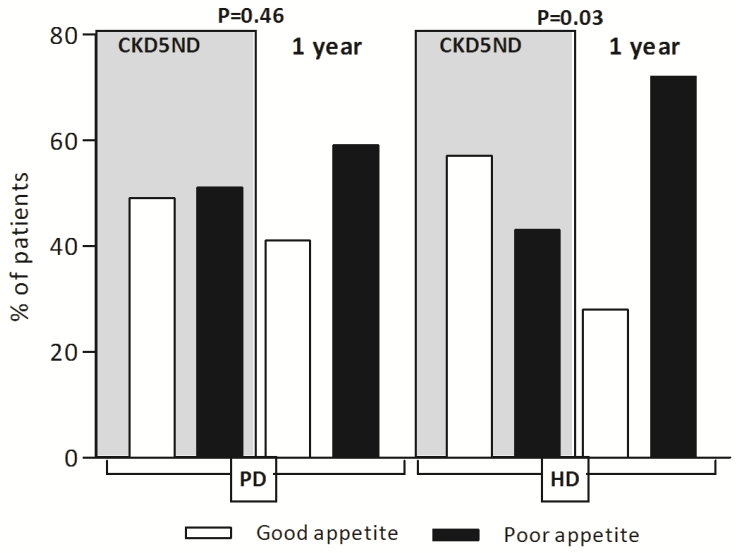
In *Study I*, we investigated the impact of dialysis on appetite, predictors of appetite loss, and the correlation between appetite loss and mortality in a cohort of CKD5-ND patients starting on dialysis, as well as in prevalent dialyzed CKD stage 5 patients (CKD5-D) undergoing maintenance PD or HD . Our main finding in *Study I* was that among CKD5-ND patients, mortality during the four-year follow-up period did not differ between those with a self-rated poor appetite and those with a good appetite at baseline. When analyzing patients on dialysis therapy, a poor appetite predicted mortality risk in PD patients, but not in HD patients (**Figure 2**).

Figure 2. Cox proportional hazards from three models (non-adjusted crude model, Model 1 with adjustment for age and gender; and Model 2 with adjustment also for co-morbidities (DM and CVD) and dialysis vintage or GFR) of mortality risk during 48 months of follow-up in CKD5-ND (n=280), PD (n=127) and HD (n=116) patients. CR, competing risk analysis.



Of interest, when analyzing changes in self-rated appetite in those patients who were investigated longitudinally (both before start and on maintenance dialysis), the proportion of patients who reported a poor appetite after the start of dialysis increased significantly amongst HD patients, whereas the changes in appetite in PD patients were not significant (**Figure 3**). This discrepancy is surprising for two reasons. First, it opposes the prevailing view that initiation of HD is associated with an improvement in appetite along with an increased food intake due to the relaxations of dietary restrictions. Second, patients on PD have been reported to be at greater risk of anorexia due to feelings of fullness due to the presence of dialysate in the abdomen, as well as a central glucose load that may reduce the drive to eat (166). Indeed, PD patients have been reported to have a lower food intake than both HD patients and healthy controls (167). A poorer appetite in PD patients may be a surrogate marker of more intensive therapy and/or a high glucose load, possibly indicating that hypertonic solutions were needed to increase a poor peritoneal ultrafiltration, factors all linked to a poor survival in PD patients (168) (169, 170). It is also possible that the finding that self-rated appetite did not decrease in patients on PD reflects a selection bias, as patients starting PD had less co-morbidity than those starting HD.

Figure 3. Appetite status in 151 CKD5-ND patients who had been investigated both before start of dialysis and after about one year of dialysis treatment with PD (n=91) or HD (n=60).



5.3 MEASURING BIOCHEMICAL MEDIATORS OF APPETITE TO ASSESS NUTRITIONAL STATUS

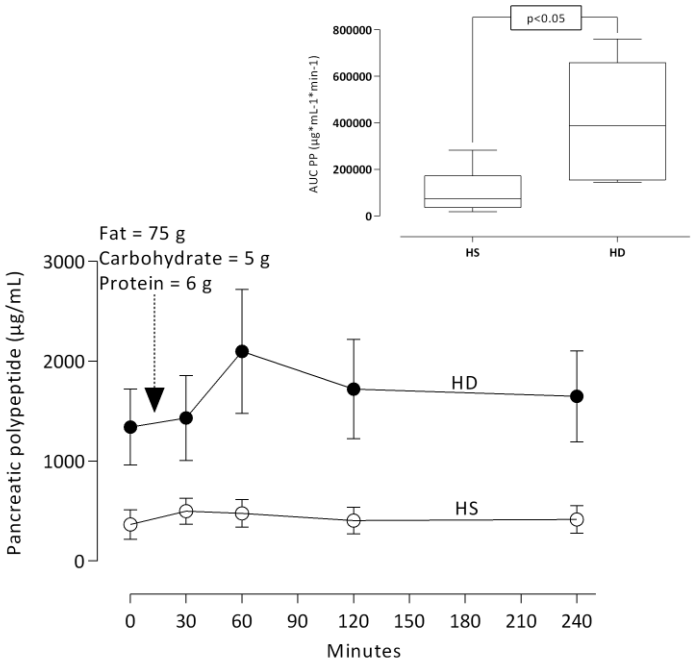
Previous studies have demonstrated that CKD is often associated with abnormal circulating levels of bio-molecules that have been implicated in the regulation of energy expenditure, appetite regulation and body composition. These include leptin, ghrelin (171-173) and adiponectin (171, 173, 174), leading us (79, 175) to hypothesize that poor appetite in uremic patients may be partly due to an accumulation of anorexigenic and catabolic substances, some of which may be at least partly removed through dialysis.

Physiologically, the short-term regulation of appetite and satiety happens at a gastrointestinal level through the central and local effects of peptide hormones secreted by intestinal cells, as well as through the action of the nervous system (176). Peptide hormones released by the gut to induce satiety and thus limit food intake include PP, GIP, GLP-1, and CCK (177). These and other peptides secreted by gastrointestinal cells are also important regulators of gastric motility, and have previously been reported to be elevated in dialysis patients (178). Thus, this mode of appetite regulation is of particular interest in the CKD population, whose meal size is reduced and who eat more slowly, while experiencing an increased feeling of satiety already before meals (179). Also, some of the anorexigenic peptides have recently been reported to be elevated in the blood of CKD patients receiving dialysis (180, 181). Although impaired tubular metabolism of these molecules likely plays a role in their increased concentrations, the consequences of this increase as well as the putative role of other anorexigens acting through the gut remain unknown (78, 182-184).

Therefore, we investigated in a post-hoc analysis of samples from a previous interventional trial the response of PP and other incretins (GIP and GLP-1) to a fat and carbohydrate – rich meal in HD patients (Study IV) (161, 162). In the 6 HD patients investigated, the basal concentrations of blood PP were much higher in fasting HD patients than in fasting 9 healthy subjects (**Figure 4**). Furthermore, following a meal, blood concentrations of PP increased significantly more in the HD patients than did blood concentrations in the healthy subjects (in whom changes in

PP were negligible). Suggesting a physiological significance rather than a reflection of reduced renal clearance, the other gastrointestinal peptides studied, GIP and GLP-1, did not exhibit a similar postprandial increase in either group.

Figure 4. Area under the curve (AUC) of PP concentrations in healthy subjects (HS) and H



While dynamic changes of these molecules in response to feeding have not to our knowledge been studied previously in HD patients, it has been speculated that an observed elevation of PP may be one of multiple etiologies contributing to the high prevalence of anorexia in HD patients (48, 78, 165, 170, 185). Similar to our findings, Henriksen *et al.* (186) found that PP levels were significantly higher in 10 patients with moderately decreased renal function as compared to 10 healthy controls. Physiologically, PP is secreted by endocrine cells at the periphery of pancreatic islets (187). The presence of protein and fat derived from dietary intake is the major stimulus for the release of PP. Administering synthetic PP to human test subjects resulted in a decreased appetite and food intake, whereas little effect was found on gastric emptying (188). Taken together with this finding, our results suggest that analysis of PP could potentially be of value when investigating anorexia as a cause for PEW in CKD patients. Unfortunately, we were not able to measure PP levels in

the cohort studies (*Study I-III, and Study IV*) while in *Study IV* the original protocol did not assess self-rated appetite. Future studies are warranted to ascertain if patients with elevated circulating levels of PP also report a poor appetite, and what happens to PP levels if appetite changes.

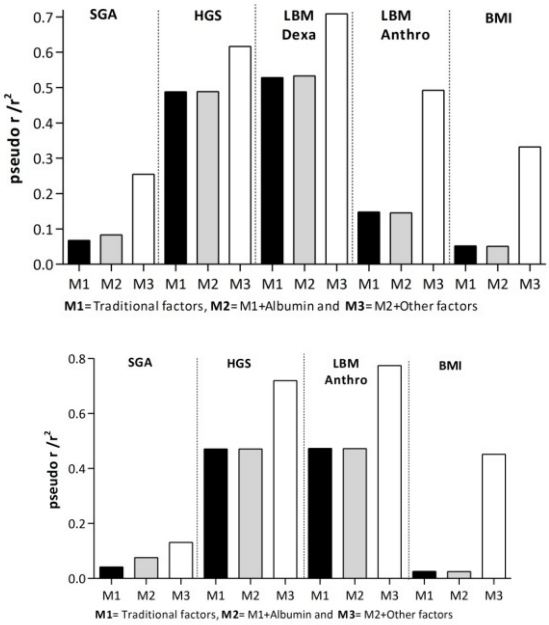
5.4 MEASURING S-ALBUMIN TO ASSESS NUTRITIONAL STATUS IN CKD

Despite the large body of evidence demonstrating the uselessness of serum albumin levels as an accurate marker of nutritional status in CKD (14) (189), it is still the most widely used method to assess nutritional status in both the clinical and research settings (86). It was traditionally assumed that s-albumin concentrations in the blood primarily reflected hepatic amino acid availability, and that it thus would function as an indicator of nutritional status (86). However, while blood albumin levels are predominantly low in CKD patients, this is not a reflection of malnutrition for several reasons (107). First, albumin is an acute phase reactant; thus, s-albumin levels in dialysis patients are strongly associated with inflammation (105). Second, blood albumin concentration varies with the amount of intravascular fluid; therefore overhydration, which is common in these patients, can disguise the real concentration of s-albumin. Third, the impact of hepatic protein restriction on circulating albumin is limited by albumin´s considerable half-life (up to 20 days) and abundance (100). Thus, even in extreme cases of malnutrition - such as marasmus and anorexia nervosa - s-albumin levels tend to remain normal (101). Indeed, in the Minnesota study (190) an induced, prolonged starvation elicited multiple compensatory changes in the healthy participants, but s-albumin levels declined only marginally. Thus, given the above findings and data demonstrating a similar plasma albumin degradation rate in CKD patients as in healthy individuals (191), it would be surprising if malnutrition was the main factor influencing serum albumin in CKD. Instead, as summarized above, circulating albumin levels in CKD patients are likely most strongly related to common co-morbidities, fluid overload and, especially, low grade inflammation (192).

Another important determinant of blood albumin particular to CKD is illustrated in *Study II*, where we report that urinary albumin excretion is the strongest predictor of s-albumin levels in incident dialysis patients with residual renal function. The same study did not show any strong associations between nutritional status and s-albumin in either incident or prevalent dialysis patients. Whereas s-albumin was weakly

associated with SGA score, there were negligible associations of s-albumin also with other markers of nutritional status, such as HGS and LBM (**Figure 5**).

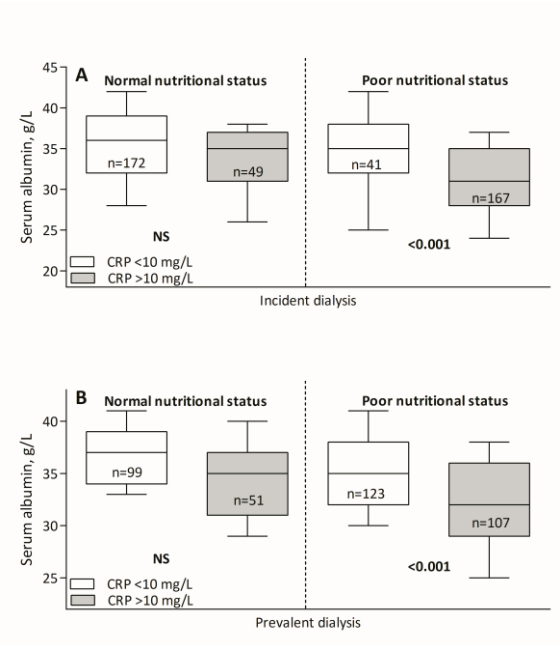
Figure 5. The predictive strength (expressed as pseudo r for SGA and r^2 for HGS, LBM_{DEXA}, $LBM_{Anthropometrics}$ and BMI) of available information of traditional clinical factors (Model 1), Model 1 + serum albumin (Model 2) and when using all available nutritional information (Model 3), for the prediction of the variability of different nutritional markers in incident and prevalent dialysis patients.



Traditional factor = age, gender, diabetes and CVD. Other factor= all additional available information about nutritional status: SGA, hand grip strength, lean body mass (estimated by two methods), BMI, hsCRP and smoking.

Instead, *Study II* suggests that urinary albumin losses, and inflammation (as assessed by hsCRP) are much more important determinants of s-albumin levels in the dialysis setting (**Figure 6**). Indeed, while inflammation, usually assessed as hsCRP, is well described to associate with malnutrition and atherosclerosis in the MIA syndrome (32, 160), there are direct causal pathways linking a low albumin to inflammation (165). Furthermore, many studies have shown that inflammation is consistently associated with low levels of s-albumin in uremia, regardless of nutritional state (53, 54).

Figure 6. Box plot of serum albumin by nutritional status in inflamed and non-inflamed, incident (Panel A; n=429) and prevalent dialysis patients (Panel B; n=380).



5.5 MEASURING SERUM CREATININE TO PREDICT NUTRITIONAL STATUS IN CKD

Clinically, besides serum albumin, the biomarker most commonly used for assessing nutritional status in CKD patients is serum creatinine (193). As a proxy of lean body mass, s-creatinine is believed to reflect patient´s nutritional status. While this may seem unintuitive given the role of s-creatinine as a key marker of glomerular filtration, it has been shown that it indeed correlates with markers of nutrition (194). In *Study V*, we were able to compare these two common biomarkers, and report that s-creatinine predicted SGA score to the same (albeit low) degree as did serum albumin, but that the former was less affected by inflammation (assessed as hsCRP) than was the latter. By now there is enough evidence in literature (110, 194, 195) reporting the successful use of s-creatinine as a cost-effective, reliable and commonly available biomarker of muscle mass in stable dialysis patients. For example, a recent review by Patel et al. (110) reported that s-creatinine correlates well with several other markers of muscle in stable dialysis patients. However, few of these studies have investigated s-creatinine as a predictor of nutritional status rather than of muscle mass (194), while changes in s-creatinine during and between dialysis sessions makes easy interpretation and use of s-creatinine as a routine marker of nutrition difficult. In *Study V*, we found based on bivariate correlations in incident dialysis patients and

prevalent HD patients, that s-creatinine was weakly associated with signs of PEW (SGA>1) in male and female patients.

It is possible that measuring creatinine appearance in urine would represent a further improvement; however, this was not explored in the current study.

5.6 ANTHROPOMETRIC MEASUREMENTS TO ASSESS NUTRITIONAL STATUS

Anthropometrics has been used to assess nutritional status clinically in several populations (193, 196, 197) . Over time, a multitude of different measurement methods have been developed and proposed – including BMI, HGS, skinfold thickness, arm circumference, waist circumference and others. Unfortunately, most anthropometric measurements have to be used with caution when used to assess nutritional status or PEW in CKD patients as markers of body composition, such as lean muscle and bone mass, fat tissue are often altered in these patients due to numerous factors other than nutritional and metabolic alterations commonly observed in patients with CKD such as fluid retention.

When such a correlation was evaluated in *Study V*, we were only partly able to replicate the previously reported association between a low HGS and SGA>1 in CKD (14). In this study, we were able to show a robust association only in incident dialysis patients, with a weak association in prevalent PD patients and no association at all in prevalent HD patients. This discrepancy may be partially explained by the age difference amongst cohorts: incident dialysis 55(35-68) years; prevalent HD 66(42-80) years; prevalent PD 64(43-80) years and controls 53(47-76) years, as well as the association between HGS and s-creatinine in two of the cohorts, potentially leading to over adjustment in our multivariate model.

Finally, SGA remains one of the most well established and commonly used methods for identifying patients with PEW in clinical research settings (85). However, in daily clinical practice its use is limited by its lack of specificity (e.g. self-reported loss of appetite and visual signs of sarcopenia), subjective nature, the time it takes to perform and its' dependence on trained dietitians or nurses, as well as the confounding introduced by reported inter-individual (and also intra-individual) variation amongst assessors. The problem is well illustrated by Ek et al., who in 90 patients aged 70 years of age or older employed two independent observers to administer SGA. When the data was analyzed, the main determinant of SGA was

found to be the experience of the assessor (198). For these and other reasons, it is important to continue the search for reproducible and easily available techniques to assess malnutrition and PEW separately and specifically.

5.7 CAN IGF-1 BE A BETTER BIOMARKER OF NUTRITIONAL STATUS IN CKD?

As discussed, we lack a reliable tool or biomarker to assess and monitor PEW in CKD. Serum IGF-1 has long been used as a marker of hypothalamic anabolic signaling in the setting of pediatric CKD, especially during treatment with exogeneous anabolic drugs (199). IGF-1 levels in the blood have also been proposed as a marker of nutritional status and food intake in patients with ESRD (15, 113). Indeed, IGF-I has been reported to be a useful marker of under-nutrition in prevalent HD patients (113). However, to our knowledge no large-scale studies have evaluated IGF-1 as a marker of PEW in CKD patients.

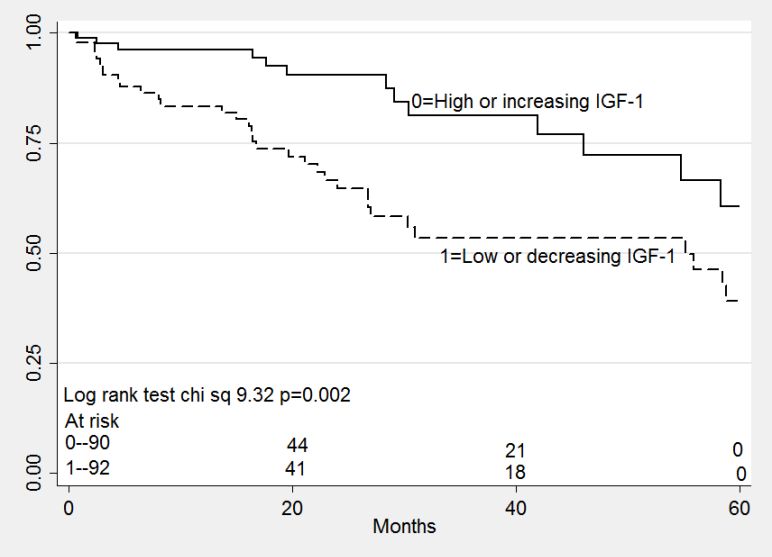
Physiologically, IGF-1 functions as the downstream effector molecule mediating growth hormone (GH) action in body tissues. In the short-term, IGF-1 signaling regulates protein and carbohydrate metabolism whereas its long-term actions concern cell proliferation, differentiation and anti-apoptotic mechanisms (200). Serum levels of IGF-1 are directly determined by GH release, which in turn is a factor of age (201), genetic traits (202), nutritional and health status (203, 204). In a setting of insufficient food intake, IGF-1 levels are low (205) but rise rapidly upon re-feeding (206, 207). Also, in populations such as postmenopausal women, serum IGF-1 concentrations correlate with lean body mass (208). Furthermore, in the HERITAGE Family Study, an IGF-1 gene polymorphism was associated with both body fat mass and fat free mass (209). Indeed, a previous study from our group (210) found that IGF-1 appear to correlate well with markers of PEW.

Given the encouraging data and the clear rationale, we investigated the association between circulating IGF-1 and PEW of CKD patients in *Study III*. We report an inverse association between IGF-1 and PEW as assessed by SGA. In addition, we found that body fat mass associated with IGF-1 variation during the first year on dialysis, but could not find an association of IGF-1 with lean body mass. However, like s-albumin, IGF-1 levels were also influenced by inflammation. Indeed, in Study III, we found that IGF-1 levels were negatively related to inflammation (IL-6, ß=-0.13, $p<0.05$). Taken

together, these findings suggest that the value of s-IGF-1 as a marker of nutritional status in CKD patients may be limited.

Also of interest in *Study III*, there was a significant increase in IGF-1 during the first year on dialysis therapy, and those patients who exhibited a persistently high or increasing level of IGF-1 had a lower risk of all-cause mortality than did those with a low or decreasing IGF-1 (**Figure 7**). These effects were independent of pre-existing risk factors including age, CVD, diabetes, inflammation and PEW. Thus, it would appear that the predictive value of IGF-1 on mortality in dialysis patients is not necessarily related to PEW. To the best of our knowledge, just a few previous studies have evaluated the predictive value of IGF-1 on mortality in renal disease patients. These have reported that serum IGF-1 predicts short-term mortality in intensive care unit patients with acute kidney injury (211). Also, consistent with *Study III*, a cross-sectional study including 127 prevalent HD patients with up to 36 months of follow-up reported an inverse association between IGF-1 levels and all-cause mortality (7), while a low IGF-1 has been associated with mortality in 64 HD patients followed for 24 months (212).

Figure 7.The Kaplan-Meier curves of five year survival of 207 patients who had persistently high or increased IGF-1 levels or persistently low or decreased IGF-1 levels during their first year on dialysis.



Clearly, the mechanism(s) whereby a high IGF-1 may reflect a positive prognosis in CKD patients remains to be elucidated. One may assume that the role of IGF-1 as a key

mediator of anabolism is important. However, it is of interest to note that one study reported that short-time administration of recombinant human IGF-1 (rhIGF-1) could improve glomerular filtration rate and promote anabolism in PD patients with a poor nutritional status (213). A similar effect has been reported in a rat model of acute ischemic injury, where rhIGF-1 was apparently able to accelerate the recovery of renal function and reduce the rate of tissue catabolism (214). However, the efficacy and long-term clinical usefulness of GH or rhIGF-1 therapy in CKD patients is still very much unclear.

6 WHERE DO WE GO FROM HERE?

The present thesis attempts to find out which tools current in use to assess and monitor nutritional status and PEW reflect "true" nutritional status in the clinical and research setting. Due to budget restraints and the limited time that can be devoted to nutritional assessments, an ideal marker of nutritional status should not only be accurate, but also inexpensive, easy to use, and widely available (**Table 6**). As the cross-sectional nature of our studies precludes conclusions regarding causality, ideally the next steps ought to be to initiate longitudinal, interventional and mechanistic research attempting to identify marker(s) that appropriately reflect changes in nutritional status.

Malnutrition and PEW have been shown to lead to poor outcome – not only in patients with kidney disease, but also in other diseased population as well as in the general population. Due to its multifactorial etiology, it is not always simple to detect and thus treat such maladies. Therefore, it is recommended that more clear and feasible guidelines are developed that are easy to follow and compatible with the reality of most clinical practices. The latest guidelines to diagnose and monitor PEW have been developed by a committee of renal nutrition experts (20). Despite detailed description on the used criteria to assess PEW, adherence to this guideline seems to be supremely difficult. Indeed, most studies use only one suggested criteria (usually, SGA, MIS or s-albumin) to measure PEW (35, 39, 42, 44); even though, it has been agreed that PEW is best detected by use of multiple tools including but not limited to circulating biomarkers, markers of body composition such as BMI, percentage of body fat, mid-arm circumference, creatinine appearance, or description of current dietary intake. Therefore, there is a need for a longitudinal study with frequent follow-up (such as every third month), assessed by experienced renal dieticians; such studies are lacking at present.

Despite that several intervention studies have shown that nutritional supplementation can ameliorate and even revert PEW (81), none have lasted more than 12 months. In addition, while one year may be enough to see some changes in body composition

and other parameters of nutritional status, it is still unknown what would be the long-term effects of these interventions and if continuous use should be advocated. In addition, muscle wasting, a common consequence of PEW, can be mitigated by increased frequency of physical activity especially anaerobic exercises. It has been reported that a clinical intervention combining administration of an anabolic steroid (nandrolone decanoate) (215) and resistance training during dialysis sessions three times a week for 12 weeks produced anabolic effects and improved physical functioning in HD patients (216). Moreover, there is enough evidence in the literature to show that exercise training for patients with CKD has multiple benefits beyond the improvement of physical function (217). However, these intervention studies also only lasted up to 18 months. Therefore, the need of longitudinal randomized interventions including optimum nutritional intake and adequate physical activities levels must be a priority in order to discover which therapy combination will ensure the best results in improving nutritional status and PEW as well as increase chances of survival.

Table 6. Characteristics of good marker of nutritional status in CKD patients

Characteristics of good marker of nutritional status
Simple to measure, inexpensive and available locally
Correlates with SGA and other markers of nutritional status
Marker unaffected by disease (except malnutrition)
Strong prognostic value
Uninfluenced by immediate food intake

7 CONCLUSION

One main conclusion of this thesis is the relatively poor correlation of existing biomarkers of nutritional state to the current standard assessment method – SGA. This suggests an urgent need to find an objective method consistent both with clinical risk and epidemiological outcomes to assess nutritional status in patients with CKD. Furthermore, our studies suggest that while SGA certainly captures aspects of both malnutrition and PEW in CKD, the exact aspects as well as their potential overlap remain to be clarified. However, until a better tool is validated, the careful evaluation of well-trained renal dietitians employing approaches such as SGA remains the best available method to detect and follow nutritional abnormalities in the CKD population.

- **Study 1:** The subjective variable self-reported appetite may be of limited value in the clinical setting as an independent predictor of mortality risk in CKD5-ND patients, and in dialysis patients it may be a weaker predictor than previously thought.

- **Study 2:** As expected, s-albumin correlates poorly with other validated and more reliable methods of assessment in nutritional status. Indeed, as previously published by us and others, s-albumin is more a marker of inflammation and disease. In this publication, we aimed to demystify the fame of s-albumin as a strong and reliable marker of malnutrition. It is not, and should not be used as such!

- **Study 3:** In incident dialysis patients, low serum IGF-1 associates with body composition and markers of mineral and bone metabolism, and it predicts increased mortality risk.

- **Study 4** The fasting PP and GIP levels, and the postprandial PP response, were markedly higher in HD patients compared to HS whereas postprandial responses of glucose, triglycerides, GIP and GLP-1 were similar in patients

and HS. We speculate that an elevated PP response may be involved in the commonly observed prolonged feeling of fullness and poor appetite of this patient population.

- **Study 5:** Using three separate multivariate regression models to investigate the relative predictive value for SGA of changes in common clinical and biochemical parameters, we confirm in this study that readily available markers of PEW, s-albumin, s-creatinine, cholesterol and HGS, are in general only weakly or not at all independently associated with PEW as assessed by SGA.

8 STRENGTHS AND LIMITATIONS

8.1 STRENGTHS

Our analyses have a number of strengths, mainly the detailed phenotype of our patient materials and a long follow-up time. Another advantage, not always considered in epidemiological studies, is that we censored for transplantation in our survival analysis; restoration of renal function cancels the prospective risk of dying. The cross-sectional nature of the studies in the present thesis does not allow us to infer causality from the results. However, in studies on etiology, diagnosis, or prognosis and adverse effects, observational studies are much more valid than randomized controlled trials (218).

8.2 LIMITATIONS

There are several limitations of these studies. First of all, it should be noted that there is no easily available gold standard method that unequivocally could define nutritional status – and such method may not even exist. Thus we and others have to rely on imperfect methods which are influenced by factors other than nutrition. It is important to acknowledge that body weight, skinfold thicknesses and DEXA may all be affected by hydration status in the studied patients (219). In fact, BMI may not reflect real nutritional status, as gross imbalance in fluid status in CKD patients may cloud the results. Furthermore, SGA is widely used in clinical and research practice and has been shown to predict outcome in dialysis patients (220); however, as a subjective tool, SGA cannot verify questions that may have been under- or over-reported. In addition, despite relying on well-trained nurses to perform the SGA assessments, one should keep in mind that the existence of intra- and inter-individual divergences may exist. Indeed, the inter-interviewer agreement of SGA is about 70% (122). Despite being validated against the gold standard method for nutritional assessment of body protein stores, total body nitrogen level, SGA could only differentiate severely malnourished patients from patients with normal nutrition whereas the exact degree of malnutrition could not be ascertained (122). In the studies contained in this thesis, we have not used the malnutrition gradation. Instead we dichotomized this variable into presence (SGA>1) of malnutrition or PEW signs, or not.

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