

From the Divisions of Baxter Novum and Renal Medicine,  
Department of Clinical Science, Intervention and Technology,  
Karolinska Institutet, Stockholm, Sweden

# DETERMINANTS AND CLINICAL IMPLICATIONS OF CIRCULATING FATTY ACIDS IN INDIVIDUALS WITH CHRONIC KIDNEY DISEASE

Xiaoyan Huang 黄晓彦



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## ABSTRACT

Patients with chronic kidney disease (CKD) have a high risk of cardiovascular morbidity and mortality. Adding to traditional risk factors, *e.g.*, Framingham risk factors, novel risk factors including inflammation, insulin resistance (IR) and metabolic syndrome (MetS) are being detected in patients with advanced CKD. Previous research demonstrates a promising possibility of improving patient outcomes by dietary manipulation, which could be an essential part of multi-faceted interventions. This thesis tries to increase our understanding of circulating fatty acids as a reflection of dietary intake in patients with CKD, with special emphasis on their clinical determinants and outcome implications.

*Study 1* identifies fatty acids in serum cholesterol esters and adipose tissue that are adequate biomarkers of habitual intake in CKD. We found that linoleic acid (LA), eicosapentaenoic acid, docosahexaenoic acid, and palmitic acid in serum cholesterol esters and adipose tissue are good indicators of the habitual dietary fat intake in elderly men with CKD. Dietary fish intake reflects well the intake of *n*-3 polyunsaturated fatty acids (PUFA) of marine origin.

*Study 2* investigates the implications of circulating essential PUFA, as a reflection of long-term dietary intake, on the inflammatory risk profile and clinical outcome of dialysis patients. LA in plasma phospholipids is inversely associated with interleukin-6 and all-cause mortality in dialysis patients. Associations between *n*-3 PUFA, inflammation and mortality were not observed.

*Study 3* investigates clinical determinants and outcome implications of estimated stearoyl-CoA desaturase-1 (SCD-1) activities of the liver and adipose tissue, as indicators of saturated fat intake, in dialysis patients. We found that both hepatic and adipose tissue SCD-1 activity indices independently relate with interleukin-6 and predict mortality in dialysis patients.

*Study 4* assesses cross-sectional relationships between serum fatty acid patterns, MetS, IR and inflammation in CKD. A serum fatty acid pattern reflecting low LA and high saturated fatty acids strongly associates with MetS, IR and C-reactive protein, while another pattern reflecting high *n*-3 PUFA is not linked with these risk factors, in two independent cohorts of elderly individuals with CKD.

**Keywords:** chronic kidney disease; competing risk; dialysis; factor analysis; inflammation; linoleic acid; mortality; *n*-3 polyunsaturated fatty acids; saturated fatty acids; stearoyl-CoA desaturase-1.

## LIST OF PUBLICATIONS

- I. **Huang X**, Sjögren P, Cederholm T, Ärnlöv J, Lindholm B, Risérus U, and Carrero JJ. *Serum and adipose tissue fatty acid composition as biomarkers of habitual dietary fat intake in elderly men with chronic kidney disease*. Nephrology Dialysis Transplantation. 2013, in press.
- II. **Huang X**, Stenvinkel P, Qureshi AR, Risérus U, Cederholm T, B á ány P, Heimbürger O, Lindholm B, and Carrero JJ. *Essential polyunsaturated fatty acids, inflammation and mortality in dialysis patients*. Nephrology Dialysis Transplantation. 2012 Sep;27(9):3615-20.
- III. **Huang X**, Stenvinkel P, Qureshi AR, Cederholm T, B á ány P, Heimbürger O, Lindholm B, Risérus U\*, and Carrero JJ\*. *Clinical determinants and mortality predictability of stearyl-CoA desaturase-1 activity indices in dialysis patients*. Journal of Internal Medicine. 2013 Mar;273(3):263-72.  
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## LIST OF ABBREVIATIONS

AHA	American Heart Association
ALA	Alpha-linolenic acid
CKD	Chronic kidney disease
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DM	Diabetes mellitus
DNL	<i>de novo</i> lipogenesis
EPA	Eicosapentaenoic acid
ESRD	End-stage renal disease
FFA	Free fatty acids
GFR	Glomerular filtration rate
GLC	Gas-liquid chromatography
HD	Hemodialysis
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model of assessment - insulin resistance
ICD	International Classification of Diseases
IL-6	Interleukin-6
IR	Insulin resistance
LA	Linoleic acid
LDL	Low-density lipoprotein
MetS	Metabolic syndrome
MIA-1	Malnutrition, Inflammation, and Atherosclerosis 1 year
MUFA	Monounsaturated fatty acids
NCEP: ATP III	National Cholesterol Education Program Adult Treatment Panel III
PD	Peritoneal dialysis
PEW	Protein-energy wasting
PIVUS	Prospective Investigation of the Vasculature in Uppsala Seniors
PUFA	Polyunsaturated fatty acids
RCT	Randomized controlled trials
SCD-1	Stearoyl-CoA desaturase-1
SFA	Saturated fatty acids
SGA	Subjective global assessment
UAER	Urinary albumin excretion rate
ULSAM	Uppsala Longitudinal Study of Adult Men



# 1 INTRODUCTION

## 1.1 CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD), usually defined as albuminuria and/or decreased glomerular filtration rate (GFR),<sup>1,2</sup> is highly prevalent worldwide and is recognized as a threat to public health.<sup>3,4</sup> Epidemiological studies demonstrate that the prevalence of CKD has reached epidemic proportions affecting 10–13% of the populations in many countries.<sup>5-12</sup> As shown in a systematic review, CKD is age related: While 7.2% of subjects older than 30 years have CKD, the prevalence ranges from 23.4% to 35.8% in those older than 64 years.<sup>13</sup>

One potential outcome of CKD is end-stage renal disease (ESRD),<sup>14</sup> which requires renal replacement therapy in the form of hemodialysis (HD), peritoneal dialysis (PD), or kidney transplantation. However, even before developing ESRD, CKD patients are at a strikingly increased risk for hospitalization and premature death,<sup>15</sup> in particular from cardiovascular causes,<sup>16-18</sup> thus strongly linking CKD to cardiovascular disease (CVD).<sup>19</sup> Moreover, CKD is accompanied by extremely high morbidity, low quality of life, decreased productivity, family pressure, mental disorders and high costs.<sup>4</sup>

Motivated by the dismal outcomes associated with CKD, a number of randomized controlled trials (RCT) have been conducted in this vulnerable population. Evidence has shown the efficacy of a few management options for CKD. For instance, control of hypertension and proteinuria with angiotensin-converting-enzyme inhibitors reduces risk of progression to ESRD;<sup>20,21</sup> reduction of low-density lipoprotein (LDL) lowers cardiovascular risk;<sup>22,23</sup> antioxidants prevents cardiovascular events in HD patients;<sup>24,25</sup> and frequent HD treatment results in a better survival rate.<sup>26</sup> Nevertheless, the past two decades have unfortunately also witnessed many more RCT failing to show a survival benefit of new treatment strategies in CKD patients, such as planned early initiation of dialysis,<sup>27</sup> increased dialysis dose,<sup>28,29</sup> online hemodiafiltration,<sup>30</sup> intensified nutrition,<sup>31</sup> homocysteine lowering therapy,<sup>32,33</sup> normalization of hemoglobin with erythropoietin,<sup>34-36</sup> lipid lowering with statins,<sup>37,38</sup> treatment with angiotensin-converting-enzyme inhibitors or calcium channel blocker,<sup>39,40</sup> and correction of secondary hyperparathyroidism.<sup>41,42</sup>

The reasons for such frustrating results have not been fully elucidated, and it is possible

that the risk profile is different in CKD compared with the general population. Adding to traditional risk factors, *e.g.*, Framingham risk factors (age, male sex, obesity, smoking, hypertension, dyslipidemia, and diabetes), novel risk factors are being detected in patients with advanced CKD and may contribute to our efforts in identifying the real cardiovascular culprits.<sup>43</sup> These factors include persistent inflammation,<sup>44</sup> protein-energy wasting (PEW),<sup>45</sup> metabolic syndrome (MetS),<sup>46</sup> insulin resistance (IR),<sup>47</sup> endothelial dysfunction,<sup>48</sup> oxidative stress,<sup>49</sup> and vascular calcification.<sup>50</sup> Each of these is not only highly prevalent in CKD but also more strongly linked to CVD than in the general population.<sup>43</sup> Causal relationships between these new markers and CVD in CKD patients remain to be established.

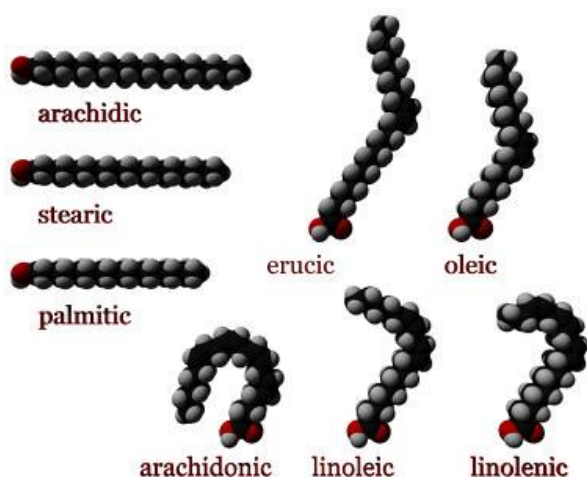
## **1.2 DIETARY FATS IN CHRONIC KIDNEY DISEASE**

Food is ingested and assimilated by humans in an effort to produce energy, maintain life, or stimulate growth. Hippocrates once said “*Let thy food be thy medicine & thy medicine be thy food*”. Researchers recognize unique health benefits of individual nutrients/dietary patterns as a whole. Observational and interventional evidence in CKD has shown favorable implications of a healthy diet on GFR,<sup>51,52</sup> blood pressure,<sup>53</sup> metabolic acidosis,<sup>52,54-56</sup> nutritional status,<sup>52</sup> and inflammation.<sup>57</sup> Although confirmation in large RCT is needed, these preliminary findings suggest a promising possibility of improving patient outcomes by dietary manipulation, which can at least be an essential part of multi-faceted interventions.<sup>58</sup>

Whereas limited evidence supports that reduction in total dietary fat intake *per se* can decrease CVD, replacement of dietary saturated fat and trans-fat with unsaturated fat has been recommended for prevention of CVD in the general population.<sup>59</sup> Earlier studies indicate that the dietary content of polyunsaturated fatty acids (PUFA) is often decreased in HD patients.<sup>60,61</sup> Also, HD patients consume fish, a major source of eicosapentaenoic acid (EPA; 20:5 *n*-3) and docosahexaenoic acid (DHA; 22:6 *n*-3), in quantities far below American Heart Association (AHA) recommendations,<sup>62</sup> and the levels of *n*-3 PUFA in HD patients are lower than in non-CKD controls.<sup>63</sup> On the other hand, the majority of HD patients consume too much fat - particularly saturated fatty acids (SFA) - in their diets.<sup>64-66</sup> As apparent from these studies, suboptimal dietary fat quality seems common in CKD patients and this may contribute to the CVD risk profile.

### 1.3 FATTY ACIDS: GENERAL CONSIDERATIONS

A fatty acid is a carboxylic acid with a long aliphatic chain. There are three main types of fatty acids in humans: SFA, monounsaturated fatty acids (MUFA), and PUFA. The latter two are further classified into *n*-3, *n*-6, and *n*-9 (or omega-3, -6, and -9) subfamilies, depending on the location of first double bond counting from the terminal methyl carbon toward the carbonyl carbon.<sup>67</sup> The structure of several common fatty acids is shown in **Figure 1**.



**Figure 1.** Three dimensional representations of several fatty acids.

Fatty acids have multiple biological functions. During fasting, free fatty acids (FFA; the non-esterified form) are released from triacylglycerols to provide an efficient source of energy, yielding large quantities of ATP.<sup>68,69</sup> FFA can act as second messengers required for the translation of external cellular signals. Within cells, fatty acids can act to amplify or modify signals that control enzyme activities. FFAs are also involved in regulating gene expression.<sup>70</sup> Such effects can be highly specific to particular fatty acids. On the other hand, esterified fatty acids are the basic building blocks of lipids, *e.g.*, phospholipids, and confer distinctive and crucial physical and metabolic properties to the latter. In particular, the presence of SFA and unsaturated fatty acids ensures that there is a proper balance between rigidity and flexibility of the cell membranes.<sup>71</sup> In addition, there are more dynamic functions of fatty acids, *e.g.*, anti-inflammatory effects.<sup>72,73</sup>

Major dietary sources of fatty acids are summarized in **Table 1**. Linoleic acid (LA; 18:2 *n*-6) and alpha-linolenic acid (ALA; 18:3 *n*-3) are essential fatty acids that cannot be synthesized endogenously by mammals and therefore must be obtained from the diet.

**Table 1.** Major dietary sources of fatty acids.

Common name	Chemical structure*	Dietary sources
<i>Saturated fatty acids</i>		
Palmitic acid	16:0	Meats, cheeses, butter, palm oil
Stearic acid	18:0	Animal fat, cocoa butter and shea butter
<i>Monounsaturated fatty acids</i>		
Palmitoleic acid	16:1 <i>n</i> -7	Macadamia oil, sea buckthorn oil
Oleic acid	18:1 <i>n</i> -9	Sunflower oil, safflower oil
<i>Polyunsaturated fatty acids</i>		
<i>n</i> -6 subfamily		
Linoleic acid	18:2 <i>n</i> -6	Sunflower seed, corn, soya, sesame, canola, safflower and their oils
Gamma-linolenic acid	18:3 <i>n</i> -6	Evening primrose oil
Dihomo-gamma-linolenic acid	20:3 <i>n</i> -6	Meats, chicken
Arachidonic acid	20:4 <i>n</i> -6	Meat, eggs
<i>n</i> -3 subfamily		
Alpha-linolenic acid	18:3 <i>n</i> -3	Rapeseed, soybeans, walnuts, flaxseed, perilla, chia, hemp and their oils
Eicosapentaenoic acid	20:5 <i>n</i> -3	Oily fish, seafood, seaweed, krill oil, seal oil
Docosapentaenoic acid	22:5 <i>n</i> -3	Seal meat and oils
Docosahexaenoic acid	22:6 <i>n</i> -3	Oily fish, seafood, seaweed, krill oil, seal oil

*Note:* \*, presented as C:D *n*-x. C is the number of carbon atoms and D is the number of double bonds in the fatty acid. A double bond is located on the  $x^{th}$  carbon-carbon bond, counting from the terminal methyl carbon toward the carbonyl carbon.<sup>67</sup>

Both EPA and DHA are long-chain *n*-3 PUFA of marine origin. Although they can be synthesized from dietary ALA via elongation and desaturation endogenously, the efficiency of the conversion from ALA to EPA/DHA is rather poor.<sup>74,75</sup> SFA and MUFA are considered non-essential fatty acids, because apart from dietary input, they can be synthesized by *de novo* lipogenesis, elongation and desaturation.<sup>76</sup>

#### 1.4 METHODOLOGY TO EVALUATE FATTY ACID INTAKE

There are various methods used to evaluate dietary intake of fatty acids in nutritional epidemiology. Dietary assessment methods have several limitations that may weaken both the accuracy and precision of the measurement, such as under-reporting of respondents,<sup>77</sup> interviewer bias, and lack of well-matched food composition databases.<sup>78</sup> Alternatively, fatty acid biomarkers in blood or tissues could be accurate and convenient for estimating long-term dietary fatty acid intake.<sup>78</sup> Previous studies in

many populations have suggested that fatty acid proportions in serum cholesterol esters, phospholipids, as well as adipose tissue are good indicators of the corresponding habitual intake of fatty acids of exogenous origin, including EPA and DHA.<sup>79-81</sup> However, results regarding the effect of chronic disease status (CVD, hypertension, diabetes) on diet-biomarker correlations are mixed.<sup>79,81</sup>

Because both dietary intake and biomarkers of fatty acid intake are associated with GFR in community studies,<sup>82,83</sup> it is conceivable that renal diseases may modify these associations. Although some studies in CKD patients have used serum fatty acids as biomarkers of dietary intake,<sup>62,84</sup> it is presently unknown if these biomarkers validly do so in the context of CKD. This issue is further developed in *Study 1*.

## 1.5 FATTY ACIDS AND INFLAMMATION

The inflammatory process is modulated by various mediators, including compounds generated from fatty acid precursors. *n*-3 PUFA have been investigated *in vitro*, *in vivo*, and in clinical studies and considered to exert pleiotropic anti-inflammatory properties in several diseases.<sup>85</sup> Eicosanoids are pro-inflammatory signaling molecules derived from either *n*-6 or *n*-3 PUFA. The eicosanoids derived from *n*-3 PUFA are less pro-inflammatory than those derived from the *n*-6 family.<sup>86,87</sup> Additional anti-inflammatory effects of *n*-3 PUFA include attenuation of endothelial adhesiveness, activation of leukocytes and resident macrophages, leukocyte-endothelial interaction, leukocyte transmigration, and the release of substances that lead to tissue injury.<sup>85</sup> Conversely, SFA can directly cause inflammation; they increase the expression and secretion of inflammatory cytokines<sup>88-90</sup> and induce nuclear factor-kappa B activation.<sup>91</sup>

Given the evidence relating CKD to persistent low-grade inflammation,<sup>92,93</sup> some observational studies have investigated the link between PUFA and inflammation in CKD patients. Whereas observational evidence does not show an association between *n*-3 PUFA and inflammation in dialysis subjects,<sup>94</sup> several RCT, as summarized in **Table 2**, have shown that supplementation with *n*-3 PUFA nevertheless has the potential to reduce inflammatory markers in CKD patients.<sup>61,95-103</sup> Even though the latent anti-inflammatory property of *n*-6 PUFA, specifically LA, has been suggested in the general population,<sup>104-107</sup> data in CKD are so far rare.<sup>96</sup> The association between these essential fatty acids and inflammation in dialysis patients and patients with moderate CKD is further explored in *Study 2* and *Study 4*, respectively.

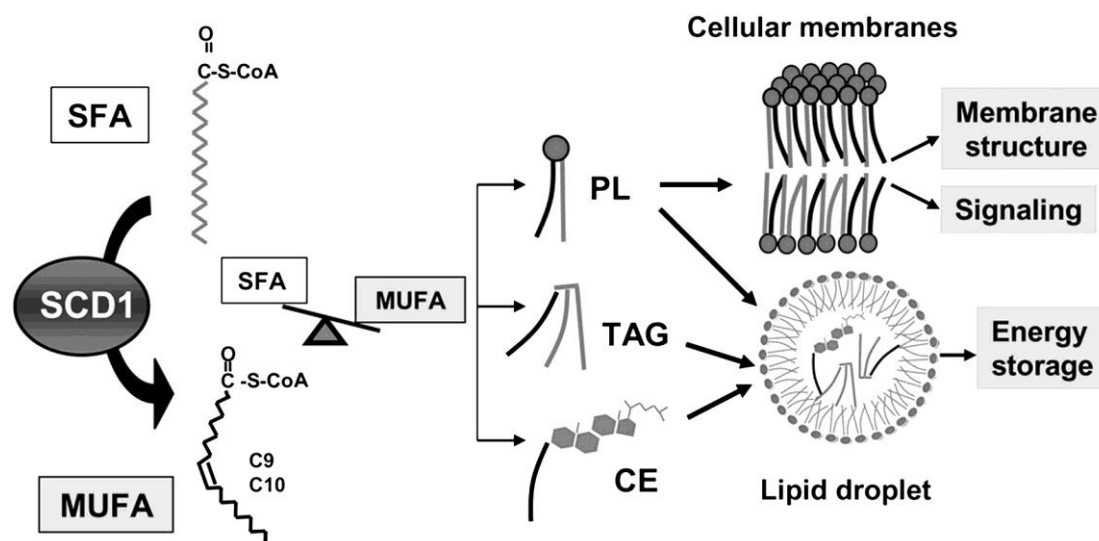
**Table 2.** Randomized controlled trials of *n*-3 polyunsaturated fatty acid supplementation on inflammation in chronic kidney disease patients sorted by chronological order.

Study	Patients*	Duration	Intervention	Outcome
Peck <i>et al.</i> <sup>61</sup>	n=8, HD	8 weeks	Capsules, 6 g/d fish oil	↑ PGE <sub>2</sub> (0.10 > P > 0.05)
Lossl <i>et al.</i> <sup>95</sup>	n=8, HD	12 weeks	Capsules, 5.2 g/d <i>n</i> -3 PUFA	↓ LTB <sub>4</sub> ↑ LTB <sub>5</sub>
Begum <i>et al.</i> <sup>96</sup>	n=12, HD	16 weeks	Capsules, 4.4 g/d <i>n</i> -3 PUFA	↓ LTB <sub>4</sub>
Saifullah <i>et al.</i> <sup>97</sup>	n=15, HD	3 months	Capsules, 1.3 g/d <i>n</i> -3 PUFA	↓ CRP
Madsen <i>et al.</i> <sup>98</sup>	n=22, CKD stages 3-4	8 weeks	Capsules, 2.4 g/d <i>n</i> -3 PUFA	↓ CRP (P=0.06)
Moreira <i>et al.</i> <sup>99</sup>	n=31, HD with CRP < 50 mg/L	8 weeks	A canned sardine sandwich/HD session (3 times per week)	↓ CRP only in sensitivity analyses
Himmelfarb <i>et al.</i> <sup>100</sup>	n=31, HD	8 weeks	Capsules, 0.8 g/d DHA	↓ IL-6, WBC, neutrophil fraction of WBC
Ewers <i>et al.</i> <sup>101</sup>	n=40, HD	2x6 weeks (crossover trial)	Capsules, 3 g/d <i>n</i> -3 PUFA	↓ CRP
Bowden <i>et al.</i> <sup>102</sup>	n=18, HD	6 months	Soft-gel pills, 0.96 g/d EPA and 0.6 g/d DHA	↓ CRP
Kooshki <i>et al.</i> <sup>108</sup>	n=17, HD	10 weeks	Capsules, 1.24 g/d EPA and 0.84 g/d DHA	↓ sICAM-1
Daud <i>et al.</i> <sup>103</sup>	n=32, HD with serum albumin < 39 g/L	6 months	Capsules, 1.8 g EPA and 0.6 g DHA/HD session (3 times per week)	No effect on CRP

Note: \*, in the interventional group. Abbreviations: CKD, chronic kidney disease; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HD, hemodialysis; IL, interleukin; LTB, leukotriene B; PGE, prostaglandin E; PUFA, polyunsaturated fatty acids; sICAM-1, soluble intercellular adhesion molecule type 1; WBC, white blood cells.

Non-essential fatty acids are subject to endogenous conversion, the process of which is catalyzed by elongases and desaturases.<sup>109</sup> Among them, stearoyl-CoA desaturase (SCD)-1, expressed both in the liver and adipose tissue, converts dietary or *de novo* SFA into MUFA and is regulated by feedback inhibition (**Figure 2**).<sup>110-112</sup> Thus, elevated SCD-1 activity signifies excess SFA intake. It has been reported that SCD-1 activity decreases after feeding with *n*-6 PUFA.<sup>113,114</sup> Indeed, increased SCD-1 activity has been implicated in various risk factors including inflammation.<sup>104,105</sup> However, the

role of SCD-1 in uremic inflammation has not yet been studied. This issue is further developed in *Study 3*.



**Figure 2.** Regulation of monounsaturated fatty acid/saturated fatty acid balance in mammalian cell lipids by stearoyl-CoA desaturase-1. Abbreviations: CE, cholesterol esters; MUFA, monounsaturated fatty acids; PL, phospholipids; SCD1, stearoyl-CoA desaturase-1; SFA, saturated fatty acids; TAG, triacylglycerols. Reprinted with permission.<sup>115</sup>

## 1.6 FATTY ACIDS, INSULIN RESISTANCE AND THE METABOLIC SYNDROME

MetS currently affects approximately 25% of the adult population.<sup>116</sup> It increases the risk of CKD<sup>46,117-120</sup> as well as mortality risk in CKD patients.<sup>121</sup> IR is a key feature of MetS, in combination with other metabolic disorders.<sup>116</sup> IR develops with the decline in GFR<sup>47,122</sup> and, in turn, may predict a rapid progression of CKD.<sup>120</sup> In addition, chronic low-grade inflammation is also closely connected with both MetS and IR, and have been suggested as an important causal factor for these glucometabolic derangements.<sup>123</sup>

Studies in non-CKD populations suggest that energy-dense, high-fat diets promote IR and MetS.<sup>124,125</sup> Dietary fat quality, rather than quantity, may be more important in increasing these risks: whereas SFA intake seems to aggravate MetS and IR<sup>126</sup>, dietary *n*-6 PUFA from vegetable sources have been linked to improved insulin sensitivity and reduced risk of developing MetS and.<sup>127,128</sup> Marine *n*-3 PUFA have also been associated with favorable effects on MetS, such as lowering of triglycerides,<sup>129</sup> but evidence for improving insulin-glucose metabolism is weak.<sup>130</sup> In the context of CKD,

whether fatty acids associate with IR and MetS is presently unknown. This issue is further developed in *Study 4*.

### **1.7 FATTY ACIDS, CARDIOVASCULAR EVENTS AND SURVIVAL**

Results from observational studies addressing the association between fatty acids and outcomes in CKD patients are elusive.<sup>131-133</sup> In one prospective cohort study, the consumption of fish in HD patients was associated with an approximately 50% lower rate of mortality over 3 years.<sup>134</sup> Also, HD patients within the highest tertile of erythrocyte DHA content had a reduced mortality risk.<sup>135</sup> In a recent nested case-control study, a strong and independent association between higher *n*-3 PUFA levels and a lower risk of sudden cardiac death throughout the first year of dialysis in incident HD patients was observed.<sup>136</sup> However, another study in prevalent HD patients did not find a significant association between erythrocyte *n*-3 PUFA proportions and mortality.<sup>137</sup> This negative result is in line with results from a study using dietary *n*-3 PUFA estimations in HD patients<sup>94</sup> Dietary modifications towards high *n*-3 PUFA intake have the potential to reduce mortality in populations at high CVD risk. Two large RCT, the GISSI-Prevenzione<sup>138</sup> and JELIS trials,<sup>139</sup> showed that *n*-3 PUFA supplementation was associated with a significant reduction in deaths from cardiac causes in non-CKD populations. Yet, in renal patients, only two trials investigated the potential of *n*-3 PUFA supplementation to reduce hard endpoints. The OPACH study showed that *n*-3 PUFA supplementation significantly reduces the number of myocardial infarctions as a secondary outcome in HD patients.<sup>140</sup> Similarly, the FISH study observed that fish oil supplementation improves cardiovascular event-free survival and thrombotic events as secondary outcomes in patients with new synthetic arteriovenous HD grafts.<sup>141</sup>

There has been emerging evidence on the association between *n*-6 PUFA (specifically LA) and mortality in the general population.<sup>142-147</sup> Nonetheless, no studies to date investigated this relationship in CKD patients. The association between these essential fatty acids (LA, EPA and DHA) and mortality in dialysis patients is further developed in *Study 2*.

Considering its risk implications on excess body and liver fat deposition,<sup>114,148</sup> hypertriglyceridemia,<sup>149</sup> IR,<sup>150</sup> diabetes mellitus (DM),<sup>151</sup> inflammation,<sup>104,105</sup> and endothelial dysfunction,<sup>105</sup> it is plausible to hypothesized that increased SCD-1 activity



may increase risk of mortality. Data from a community-based cohort indeed suggests so.<sup>145</sup> However, the mortality predictability of SCD-1 has not been explored in CKD. This association is further developed in *Study 3*.

## 2 AIMS

The overall aim of this thesis was to increase our understanding of circulating fatty acids as a reflection of dietary intake in patients with CKD, with special emphasis on their clinical determinants and outcome implications.

The specific aims were:

- To identify fatty acids in serum cholesterol esters and adipose tissue that are adequate biomarkers of habitual intake in individuals with CKD (*Study 1*).
- To investigate the implications of circulating essential PUFA, as a reflection of long-term dietary intake, on the inflammatory risk profile and clinical outcome of dialysis patients (*Study 2*).
- To investigate clinical determinants and outcome implications of estimated hepatic and adipose tissue SCD-1 activities in dialysis patients (*Study 3*).
- To assess cross-sectional relationships between serum fatty acid patterns, the metabolic syndrome, insulin sensitivity and inflammation in individuals with moderate CKD (*Study 4*).

## **3 MATERIALS AND METHODS**

### **3.1 PARTICIPANTS**

This thesis was developed with data obtained from three observational cohorts: the Malnutrition, Inflammation, and Atherosclerosis 1 year (MIA-1), the Uppsala Longitudinal Study of Adult Men (ULSAM), and the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohorts. MIA-1 had been coordinated by the Division of Renal Medicine, Department of Clinical Sciences, Intervention and Technology, Karolinska Institutet. Patient phenotype was analyzed *post hoc* using collected data and, when necessary, by making new analyses from frozen samples. ULSAM and PIVUS studies were performed in collaboration with Uppsala University, which is responsible for cohort collection and management.

#### **3.1.1 MIA-1**

The MIA cohort is an ongoing patient cohort described in detail elsewhere.<sup>44</sup> Briefly, 434 ESRD patients with  $\text{GFR} < 15 \text{ mL}/\text{min}/1.73\text{m}^2$ , enrolled at Karolinska University Hospital at Huddinge from 1994 to 2009, were evaluated close to the start of dialysis and were followed prospectively. Exclusion criteria included age  $< 18$  or  $> 70$  or 75 years, signs of overt infection, and unwillingness to participate. These patients were then invited to perform a second clinical assessment after approximately one year of dialysis therapy. Patient recruitment occurred between April 1996 and October 2010. From 434 patients included, 255 attended the second visit, comprising the MIA-1 cohort. Reasons for not attending the second assessment included death ( $n=45$ ), kidney transplantation ( $n=58$ ) and unwillingness or inability to participate ( $n=76$ ).

#### **3.1.2 ULSAM**

ULSAM is a community-based cohort initiated in 1970; all 50-year-old men born between 1920 and 1924 and living in Uppsala, Sweden, were invited to a health survey at the Department of Public Health and Caring Sciences/Geriatrics, Uppsala University (described in detail at <http://www.pubcare.uu.se/ULSAM/>). Participants returned for subsequent examinations at age 60, 70, 77, and 82 years. This thesis was based on the third examination cycle of the ULSAM cohort, when participants were approximately 70 years of age (visits performed during 1991 to 1995;  $n=1221$ ).

### 3.1.3 PIVUS

PIVUS is a community-based cohort initiated in 2001 at the Department of Medicine, Uppsala University (described in detail at <http://www.medsci.uu.se/pivus/>). All 70-year-old individuals living in Uppsala, Sweden, between 2001 and 2004 were eligible for the PIVUS study. A random sample of 1016 subjects was included with the primary aim to investigate the predictive power of different measurements of endothelial function and arterial compliance.

## 3.2 STUDY PROTOCOLS

Because of specific exclusion criteria in each study and some missing values of the main outcomes assessed (due to impossibility to make an assessment or lack of plasma available), the number of individuals and main parameters considered in each of the studies vary as summarized in **Table 3**.

**Table 3.** Basic description of the individual studies.

Study	Cohort	Subjects	Exposure	Outcome
1	ULSAM	506	Dietary fatty acid intake	Fatty acid compositions of serum CE and AT
2	MIA-1	222	PUFA in plasma PL	IL-6, all-cause mortality
3	MIA-1	222	Estimated hepatic and AT SCD-1 activities	IL-6, all-cause mortality
4	ULSAM PIVUS	274 187	Serum fatty acid patterns	Metabolic syndrome, glucose disposal, HOMA-IR, CRP

*Note:* Abbreviations: AT, adipose tissue; CE, cholesterol esters; CRP, C-reactive protein; HOMA-IR, homeostasis model of assessment - insulin resistance; IL-6, interleukin-6; MIA-1, Malnutrition, Inflammation, and Atherosclerosis 1 year; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; PL, phospholipids; PUFA, polyunsaturated fatty acids; SCD-1, stearoyl-CoA desaturase-1; ULSAM, Uppsala Longitudinal Study of Adult Men.

### 3.2.1 Study 1

This is a cross-sectional analysis including individuals with a serum cystatin C-estimated GFR  $<60$  mL/min/1.73m<sup>2</sup> ( $n=543$ ) from the ULSAM cohort. Exclusion criteria were incomplete data on 7-day dietary records ( $n=36$ ) and abnormal values of reported energy intake ( $<3200$  or  $>18,000$  kJ/d;  $n=1$ ). **Study 1** therefore comprises 506 participants with CKD according to the current Kidney Disease Outcomes Quality Initiative definition.<sup>1</sup> Fatty acid compositions of serum cholesterol esters and adipose

tissue were analyzed in two random sub-samples of 248 and 318 CKD men, respectively. The Ethics Committee of Uppsala University, Sweden, approved the study (Dnr 251/90).

### **3.2.2 Study 2**

This is a prospective observational study using data from the MIA-1 cohort. From the 255 MIA-1 dialysis subjects, we excluded 6 patients with “dialysis vintage” (preceding time on dialysis) <4 or >18 months and 26 additional patients without sufficient plasma for fatty acid analysis. No differences were observed in general and demographic characteristics between the included 223 patients and non-included patients. After fatty acid analysis was performed, one patient was excluded due to inconsistent chromatographic results *a priori*. **Study 2** therefore comprises 222 dialysis patients. The Ethics Committee of Karolinska Institutet, Sweden, approved the study (Dnr 008/98, 415/03, 2010/1112).

### **3.2.3 Study 3**

This is a prospective observational study using data from the MIA-1 cohort. Similar to **Study 2**, 222 dialysis patients were included in this analysis. However, in **Study 3**, additionally two patients were excluded due to inconsistent chromatographic results of free fatty acid composition. Thus, the analysis related with FFA included 220 dialysis patients *a priori*. The Ethics Committee of Karolinska Institutet, Sweden, approved the study (Dnr 008/98, 415/03, 2010/1112).

### **3.2.4 Study 4**

This is a cross-sectional analysis including individuals with CKD from two independent community-based cohorts: the ULSAM and PIVUS studies. In ULSAM, a total of 543 individuals were identified as having CKD on the basis of a cystatin C-estimated GFR <60 mL/min/1.73m<sup>2</sup> in accordance with the current Kidney Disease Outcomes Quality Initiative.<sup>1</sup> Fatty acid composition of serum cholesterol esters was available in 274 individuals who were included in the present analysis. In PIVUS, a total of 187 PIVUS individuals with a cystatin C based GFR <60 mL/min/1.73m<sup>2</sup> were included in the present analysis, and data on fatty acid composition of serum cholesterol esters was available in all of them. The Ethics Committee of Uppsala University, Sweden, approved the study (Dnr 251/90, 00-419, 2011/045, 2011/045/1).

### 3.3 METHODS

#### 3.3.1 Clinical examination

All investigations were performed under standardized conditions as described elsewhere.<sup>44,152,153</sup> Body mass index was calculated as the ratio of the body weight (in kg) to the height (in m<sup>2</sup>). Waist circumference was measured midway between the lowest rib and the iliac crest. Smoking status was defined as smoking versus nonsmoking. Regular physical activity was defined as the reporting of regular or athletic leisure-time exercise habits according to four physical activity categories (sedentary, moderate, regular, and athletic).<sup>154</sup> Supine blood pressure was measured twice in the right arm after 10 minutes' rest, and means were calculated. Subjective global assessment (SGA) was used to evaluate the overall nutritional status. SGA relies on clinical judgment accrued from grading scales calculated from a brief history and physical examination.<sup>155</sup> The history examination focuses on gastrointestinal symptoms (anorexia, nausea, vomiting and diarrhea) and weight loss in the preceding 6 months. The physical examination includes loss of subcutaneous fat over the triceps and mid-axillary line of lateral chest wall, muscle wasting in the deltoids and quadriceps, and the presence of ankle edema. These features are classified as 0 = normal, 1 = mild, 2 = moderate, 3 = severe. On the basis of a weighing of these data, patients are classified into two groups: normal nutritional status, with a SGA score = 1; and PEW, with a SGA score >1.<sup>156</sup>

Previous CVD was defined as history of any CVD as recorded in the Swedish Hospital Discharge Registry [International Classification of Diseases (ICD-8) codes 390 to 458 or ICD-9 codes 390 to 459]. DM was defined as fasting plasma glucose  $\geq 7.0$  mmol/L, 2-hour postload glucose levels  $\geq 11.1$  mmol/L, or the use of oral hypoglycemic agents or insulin.<sup>157</sup> Hypertension was defined as systolic blood pressure  $\geq 140$  mmHg, diastolic blood pressure  $\geq 90$  mmHg, or use of antihypertensive medications. Hyperlipidemia was defined as serum cholesterol  $> 6.5$  mmol/L and/or serum triglycerides  $> 2.3$  mmol/L and/or treatment with lipid-lowering medications. We adopted the modified National Cholesterol Education Program Adult Treatment Panel III (NCEP: ATP III) criteria, with three or more of the following criteria being defined as MetS:<sup>116</sup> (1) abdominal obesity: waist circumference  $> 102$  cm for men,  $> 88$  cm for women; (2) hypertriglyceridemia: serum triglycerides  $\geq 1.7$  mmol/L or on lipid lowering drug treatment; (3) decreased high-density lipoprotein (HDL): serum HDL concentrations  $< 1.04$  mmol/L for men,  $< 1.3$  mmol/L for women, or on lipid lowering

medication; (4) hypertension: systolic blood pressure  $\geq 130$  mmHg or diastolic blood pressure  $\geq 85$  mmHg or under anti-hypertensive drug treatment, and; (5) hyperglycemia: fasting plasma glucose concentrations  $\geq 5.6$  mmol/L or on anti-glycemic medication or previously diagnosed type 2 DM.

### **3.3.2 Dietary assessment**

In ULSAM, dietary habits were evaluated with an optically readable form of a 7-day dietary record based on a validated pre-coded menu book,<sup>158</sup> which was prepared and previously used by the Swedish National Food Administration.<sup>159</sup> The participants were given oral instructions by a dietitian on how to perform the dietary registration, and the amounts consumed were reported in household measurements or specified as portion sizes. The daily intake of energy, various fatty acids, fish, and alcohol was calculated by using a database from the Swedish National Food Administration. This method permitted estimation of the intake of major specific fatty acids, *e.g.*, palmitic and stearic acids in the SFA class. Fatty acid intake was expressed in two different ways: as absolute intake (g/d), and as a percentage of total fat intake by weight [(g/g total fat) \* 100], with the latter being comparable with biomarker measurements.

Stringent criteria to identify adequate reporters of energy intake were applied according to the Goldberg cut-off.<sup>160</sup> In this procedure, an acceptable range of energy intake is determined for each subject in relation to estimated energy expenditure taking the level of physical activity and calculated basal metabolic rate into consideration, *i.e.*, producing a 95% confidence interval for energy intake required for weight maintenance. Subjects with reported energy intake within the 95% confidence interval were regarded as adequate reporters, rendering a subpopulation of 250 individuals for verification of the associations reported in the whole material ( $n=506$ ).

### **3.3.3 Laboratory analyses**

After an overnight fast, blood samples were obtained. Plasma and serum were separated and kept frozen at  $-70$  °C, if not analyzed immediately. In the MIA-1 study, triglyceride, total cholesterol, HDL, high-sensitive C-reactive protein (CRP), and albumin concentrations were analyzed using certified methods in the Department of Laboratory Medicine at Karolinska University Hospital. The Friedewald equation<sup>161</sup> was used to calculate LDL from total cholesterol, HDL, and triglyceride. Serum

concentrations of interleukin (IL)-6 were quantified by immunometric assays on an Immulite Analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA).

In ULSAM and PIVUS, the assays were performed at the Department of Clinical Chemistry, University Hospital, Uppsala, which is accredited according to the Swedish Board for Accreditation and Conformity Assessment (Swedac) standard ISO/IEC 17025. Serum triglyceride and HDL concentrations were assayed by enzymatic techniques. Fasting blood glucose concentration was determined by an oxidase method and insulin by radioimmunoassay. CRP measurements were performed by latex enhanced reagent (Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec analyzer (Dade Behring). Serum cystatin C (ULSAM: N Latex Cystatin C, Dade Behring, Deerfield, IL, USA; PIVUS: Gentian, Moss, Norway) was used to estimate GFR.<sup>162,163</sup> Individuals with CKD were further divided into stage 3A and more advanced stage of CKD on the basis of a GFR cut-off value of 45 mL/min/1.73m<sup>2</sup>. Urinary albumin excretion rate (UAER) was calculated on the amount of albumin in the urine collected during the night. The assay employed a commercially available radioimmunoassay kit (Albumin RIA 100, Pharmacia, Uppsala, Sweden). Microalbuminuria was defined as UAER  $\geq$ 30 mg/24h.

### **3.3.4 Fatty acid compositions and desaturase activities**

Fatty acid compositions of plasma phospholipids (MIA-1), FFA (MIA-1), serum cholesterol esters (ULSAM and PIVUS), and adipose tissue (PIVUS) were analyzed by gas-liquid chromatography (GLC) at the Unit for Clinical Nutrition Research, Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden. Subcutaneous AT was collected with biopsy from the upper, outer quadrant of the buttocks.<sup>164</sup> The samples were stored at -70 °C for some weeks until analyses.

As previously described,<sup>165</sup> an extraction with chloroform was conducted. The dry extracts were dissolved in a few drops of chloroform and applicated on thin liquid chromatography plates for separation of the lipids. The lipid esters were trans-methylated and the methyl esters were extracted. The fatty acid methyl esters were dissolved in hexane and separated by GLC. The Hewlett Packard GLC system used for the analyses consisted a GC 5890, automatic sampler 7671A, integrator 3392A, and 25 m Quadrex Fused Silica capillary column OV-351. The fatty acids were identified by comparison of the retention times of separation was controlled by Nu



Check Prep GLC reference standard GLC-68A. The coefficients of variation (CV) for all fatty acids were 1–5.5%, except for stearic acid, with a CV of 9.9%.<sup>166</sup> Fatty acids are given as the relative percentage of the sum of the fatty acids analyzed.

Direct measurement of SCD-1 activities in humans is complicated and not feasible in large cohort studies.<sup>110,167</sup> We therefore estimated hepatic and adipose tissue SCD-1 activities by using product-to-precursor fatty acid ratios (palmitoleic acid/palmitic acid). Preceding studies show a high degree of correlation between serum fatty acid biomarker-derived indices and tissue-derived indices, both liver and adipose tissue, with correlation coefficients of 0.86<sup>168</sup> and 0.63,<sup>169</sup> respectively. The palmitoleic acid/palmitic acid ratio (16:1 *n*-7/16:0) is preferred over the ratio oleic acid/stearic acid (18:1 *n*-9/18:0), since the latter is biased by high dietary intake of oleic acid.<sup>170</sup> Dietary intake of palmitoleic acid, on the other hand, is very low in a Western-type Swedish diet and mostly represents a small amount of dietary fats in a typical Swedish diet.<sup>171</sup> Thus, plasma palmitoleic acid is almost exclusively derived from endogenous conversion from palmitic acid by SCD-1 and, in the present study, palmitoleic acid/palmitic acid was determined in plasma phospholipids and FFA to reflect SCD-1 activities in the liver and in adipose tissue, respectively.<sup>113,169</sup>

### 3.3.5 Insulin resistance

We used both the euglycemic hyperinsulinemic clamp technique and homeostasis model of assessment - insulin resistance (HOMA-IR) to evaluate IR in the ULSAM cohort, while IR in the PIVUS cohort was solely assessed by the latter. Insulin sensitivity, *i.e.*, assessed as the insulin-mediated glucose disposal (M) was estimated by euglycemic clamp as described by DeFronzo *et al.*,<sup>172</sup> slightly modified with insulin (Actrapid Human, Novo, Copenhagen, Denmark) being infused at a constant rate of 56 mU/body surface area (m<sup>2</sup>)/min during 120 minutes. This rate was estimated to suppress hepatic glucose output almost completely also in participants with type 2 DM. The target plasma glucose concentration was 5.1 mmol/L. M was calculated as the amount of glucose per kg of body weight (bw) taken up during the last 60 minutes of the study and expressed as mg/kg bw/min. HOMA-IR was computed with the formula: fasting plasma glucose (mmol/L)\*fasting serum insulin (mU/L)/22.5.<sup>173</sup>

### 3.3.6 Follow-up

All patients in the MIA-1 cohort were prospectively followed-up for up to 5 years, or until April 30<sup>th</sup>, 2011, death or kidney transplantation, whichever event occurred first. Causes of death were extracted from medical records by a physician blind to the study results. Death due to CVD included: fatal myocardial ischemia or infarction, cardiac arrest or unknown sudden death, acute as well as chronic heart failure, cerebrovascular accidents, cerebral hemorrhage, and ruptured aortic aneurysm.

### 3.3.7 Statistical analysis

Values were expressed as mean  $\pm$  standard deviation, median (interquartile range; IQR) or percentage of total, as appropriate. Logarithmic transformation was applied for non-normally distributed continuous variables. All tests were two-tailed and  $P < 0.05$  was considered significant. Because  $P$  values were not adjusted for multiple testing, they have to be considered as descriptive. All statistical analyses were performed using statistical software STATA version 12 (Stata Corporation, College Station, TX, USA).

Comparisons between the two groups were evaluated by the Student's unpaired  $t$  tests for normally distributed continuous variables, the nonparametric Mann–Whitney tests for non-normally distributed continuous variables, and  $\chi^2$  tests for nominal variables.

As many values were not normally distributed, Spearman's rank correlation was used to determine univariate correlations. Multivariable linear or logistic regression analyses were performed to assess independent associations, after the adjustment of potential confounders. Data are presented as standard coefficients (std. beta) or odds ratios, as well as 95% confidence intervals.

Because kidney transplantation and death before transplantation are mutually exclusive events, *i.e.*, the occurrence of either one prevents the occurrence of the other, traditional Cox regressions may be biased; we therefore calculated the cumulative incidence of death before kidney transplant using the competing risk approach.<sup>174</sup> Data are presented as hazard ratios and 95% confidence intervals.

Other specific statistical analyses are discussed in each of the studies presented in this thesis.

## 4 MAIN RESULTS AND DISCUSSION

### 4.1 STRENGTHS AND LIMITATIONS

#### 4.1.1 Strengths

This thesis has a number of strengths, starting with the detailed phenotype of our patient materials. The use of fatty acid compositions is an asset, as it avoids the problems of under-/over-reporting of dietary recalls. Factor analysis further captures inner relationships between the spectrum of fatty acids, grasping the concept of dietary fat quality and facilitating the interpretation of findings. Gold standard methods, *i.e.*, fatty acid composition of adipose tissue<sup>175</sup> and the euglycemic clamp technique,<sup>172</sup> improve the validity of the data. The 7-day dietary record is the most preferred dietary assessment method, and the use of Goldberg cut-offs to control for reporting bias represents a further strength.<sup>77</sup> Another advantage is a long follow-up time without any patient being lost to follow-up. Also, we corrected in survival analyses for the competing risk of transplantation; restoration of renal function cancels the prospective risk of dying. Lastly, we in the present thesis focus on either essential fatty acid biomarkers (representing their dietary intake) or non-essential fatty acids (representing endogenous metabolism) in specific research questions *a priori*. This approach is biologically reasonable, since circulating fatty acids, even within a same biochemical family (SFA, MUFA, *n*-3 and *n*-6 PUFA), can be derived from distinct sources and may not be metabolically equivalent.<sup>78,176</sup> Consistent with this concept, fatty acids expressed as these groups or ratios (PUFA/SFA and *n*-6/*n*-3 PUFA) used in some previous studies may be neither useful nor relevant in humans,<sup>78,177</sup> and is not supported by RCT.<sup>178,179</sup>

#### 4.1.2 Limitations

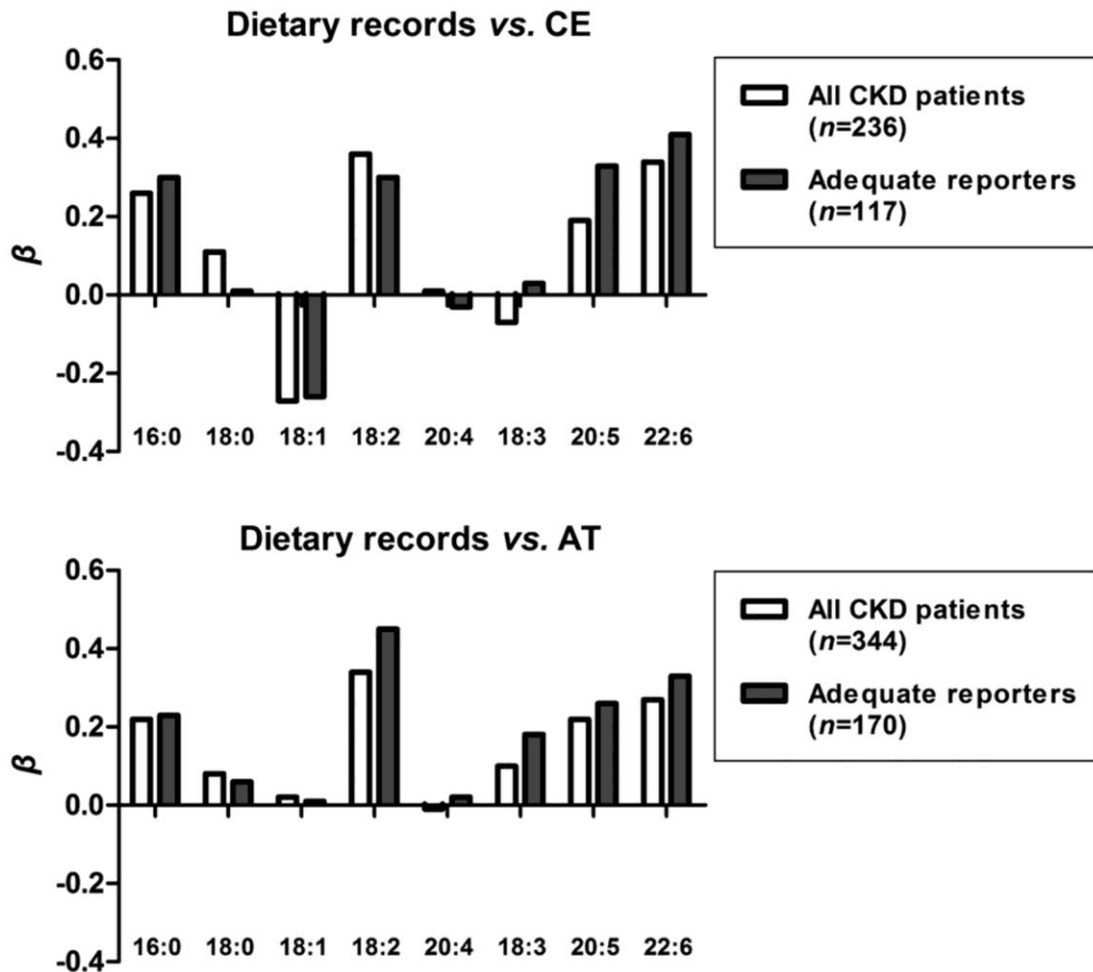
Our results should be interpreted considering the studies' limitations. First of all, the cross-sectional nature of analyses does not allow inferring causality from the results. However, in studies on etiology, diagnosis, prognosis, or adverse effects, observational studies are more valid than RCT.<sup>180</sup> Second, although the inclusion of individuals with similar both age/dialysis duration and geographical distribution reduces important confounding, our results may not necessarily be extrapolated to the general CKD/dialysis population. Third, there may be unmeasured or unknown confounders we cannot take into account, *i.e.*, residual confounding. In this regard, we did not have information regarding the possible intake of fish oil supplements. Fourth, although

serum fatty acids can be used as indicators of dietary intake, some fatty acids are subjected to endogenous conversion.<sup>113</sup> Circulating fatty acids may also represent the intake of certain foods that can contain other beneficial nutrients, *e.g.*, fiber, which may contribute to the observed effects.<sup>57</sup> Thus, the lack of dietary intake data to corroborate the biomarkers (MIA-1) is acknowledged as a limitation. Finally, in **Study 3**, we rely on estimations of SCD-1 indices, though they are considered to reflect hepatic and adipose tissue SCD-1 activities accurately<sup>168,169</sup> and have been widely adopted.<sup>169,181-183</sup> Nonetheless, direct measurement of SCD-1 activities in humans is complicated and not feasible in large cohort studies.<sup>167</sup>

There are further limitations from a statistical point of view. Our sample sizes are relatively small and the number of events in survival analysis is limited, potentially introducing type II (false negative) errors in decisions for which our patient materials were not adequately powered. We should also acknowledge the possibility of type I (false positive) errors in the case of random findings due to multiple testing. Because *P* values were not adjusted accordingly, they have to be considered as descriptive. Nonetheless, the fact that we mostly performed hypothesis-driven tests could somewhat reduce this possibility as an explanation to our findings and, importantly, the replication of our findings in an independent cohort (**Study 4**) would argue against type 1 error. In some cases, we might have introduced risk of over-adjustment.<sup>184</sup> We have applied a shrinkage factor with Firth correction<sup>185,186</sup> in **Study 1** and tried, to our best, to avoid the impact of collinearity by adjusting for factors pathophysiologically unrelated.<sup>187</sup>

## **4.2 FATTY ACID COMPOSITIONS AS BIOMARKERS OF HABITUAL INTAKE**

In **Study 1**, we showed that LA and DHA in serum cholesterol esters were strongly correlated with their corresponding intake in individuals with CKD stage 3-4, as presented in **Figure 3**. Palmitic acid and EPA presented moderate  $\beta$  values. On the other hand, stearic acid, ALA and arachidonic acid (20:4 *n*-6) were not associated with the dietary intake whilst oleic acid was negatively correlated with its proportion in the diet. In adipose tissue, the correlations with dietary fatty acids were similar, except that ALA was moderately associated, and oleic acid was not significantly associated, with their counterparts in dietary records. The strength of the associations between dietary fatty acids and their corresponding cholesterol ester and adipose tissue biomarkers were maintained or even improved in the subpopulation of adequate reporters.

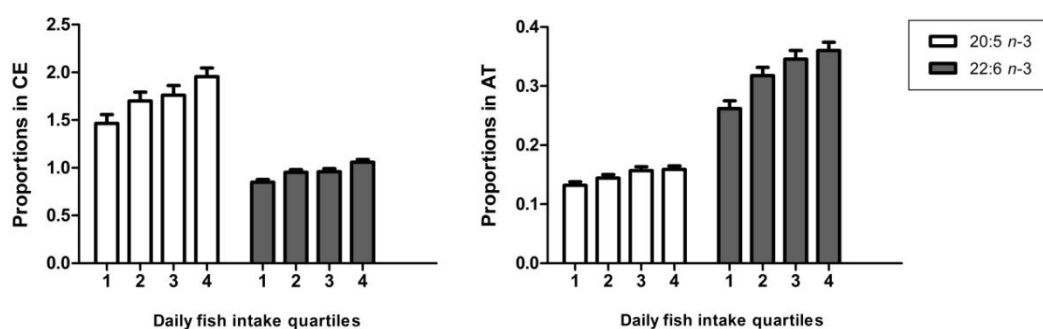


**Figure 3.** Relations between individual fatty acid proportions in dietary records versus serum cholesterol esters (CE) and adipose tissue (AT) respectively, expressed as standard coefficients ( $\beta$ ) in multivariable regression models, both in all individuals with chronic kidney disease as well as in adequate reporters only. Models were adjusted for body mass index, smoking, alcohol intake, physical activity, cardiovascular disease, diabetes, hypertension, hyperlipidemia, estimated glomerular filtration rate, and urinary albumin excretion rate. Reprinted with permission.<sup>188</sup>

For non-essential fatty acids, the relationships between individual fatty acid proportions in dietary records and both serum cholesterol ester and adipose tissue compositions were weaker or absent (**Figure 3**), accordant with those in populations without CKD.<sup>79-81,189</sup> However, palmitic acids were fairly good markers of dietary intake in the current population, although less strongly correlated than observed for LA and DHA. The correlations of SFA are weakened partly due to the fact that endogenous metabolism, including *de novo* lipogenesis (DNL), elongation and desaturation, affects the levels of these fatty acids.<sup>76</sup> Apart from diet, SFA generated from carbohydrates through the process of DNL is another source of palmitic and stearic acids in the blood and tissues. In Western populations with relatively high fat intake, however, that DNL dilutes SFA

pools has been considered to be of minor importance.<sup>76</sup> Furthermore, SCD-1 both in the liver and adipose tissue converts palmitic and stearic acids to synthesize palmitoleic and oleic acids, with oleic acid being the preferred substrate.<sup>167</sup> It is therefore not surprising that there was a lack of direct association with the major MUFA oleic acid. The significantly negative association of oleic acid was however unexpected and difficult to explain. One might speculate that hepatic SCD-1 activity is suppressed in response to high intake of PUFA,<sup>114</sup> food sources of which also contain substantial amounts of oleic acid.<sup>76</sup> It is thus possible that high intake of vegetable oils (partly represented as high dietary oleic acid content) may in turn inhibit endogenous synthesis of oleic acid, thereby decreasing its levels in the body, and *vice versa*.

As expected from its biology, the relationships between dietary intake and biomarkers for most essential PUFA were indeed the strongest in our study. This agrees with similar reports in non-CKD individuals,<sup>79,80</sup> and these biomarkers can be used as indicators of compliance in supplementation studies.<sup>114,190-192</sup> However, for ALA, we did not observe strong associations between dietary fatty acid intake and the biomarker, not even when considering adequate reporters. These results were unexpected and the reason is unclear, but in similar studies the agreement of ALA seems also poorer than for the other essential PUFA.<sup>79,81</sup> The smaller proportion of ALA and the relatively higher within-person variability in its measurement may have contributed to these results.



**Figure 4.** Mean eicosapentaenoic acid (20:5 *n*-3) and docosahexaenoic acid (22:6 *n*-3) proportions in serum cholesterol esters (CE) and adipose tissue (AT) according to daily fish intake (energy adjusted) quartiles. Bars represent standard errors. *P* for trend <0.01 for all. Modified from.<sup>188</sup>

As shown in **Figure 4**, total energy intake adjusted daily fish intake was positively associated with the proportions of EPA and DHA in cholesterol esters ( $\beta = 0.21$  and

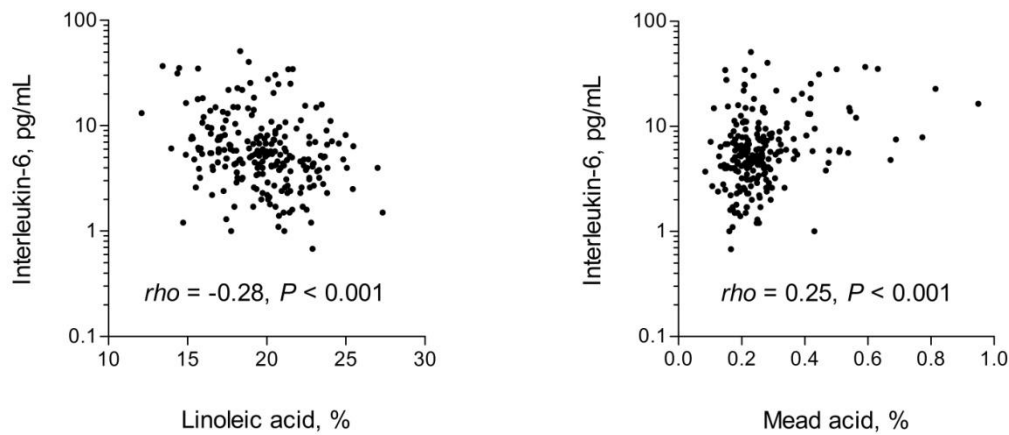
0.26) and adipose tissue ( $\beta = 0.18$  and  $0.18$ ). Such findings are consistent with a previous report showing a positive association between the frequency of fish servings and *n*-3 PUFA index (the sum of erythrocyte EPA and DHA contents) in 75 HD patients.<sup>62</sup> This suggests that dietary fish intake is a proxy of EPA and DHA intake in this population.

We also found that the associations between dietary and biomarker fatty acids held constant across eGFR (above and below 45 mL/min/1.73m<sup>2</sup>) or UAER (above and below 30 mg/24h) groups, suggesting that moderate renal failure does not modify the associations. Likewise, one previous investigation indicates that the status of other chronic diseases, *e.g.*, CVD, hypertension and DM, does not modify these relationships either.<sup>79</sup> Nevertheless, we must take into consideration that the included patients were mostly within CKD stage 3, and further studies may be necessary including patients with a broader GFR distribution.

In summary, our results suggest that LA, EPA, DHA, and palmitic acid in serum cholesterol esters and adipose tissue are good indicators of the habitual dietary intake of fatty acids in elderly men with CKD. Dietary fish intake well reflect intake of *n*-3 PUFA of marine origin in this population. The weak or lack of association with other fatty acids limits their use as biomarkers and thus fatty acid composition does not capture the intake of all fatty acids. Taken together, specific fatty acid biomarkers could be a valid and objective tool to use in epidemiological studies which aim at linking dietary fat quality and diet-related conditions in CKD. At the same time, they can be considered to measure compliance in dietary intervention studies.

### 4.3 FATTY ACIDS AND INFLAMMATION

In *Study 2*, we observed a negative relationship between plasma LA and IL-6 in dialysis patients (**Figure 5**). An opposite association was found for Mead acid, whose elevation in the blood is regarded as an indication of LA deficiency.<sup>193</sup> Results in *Study 4* also confirm this concept: in two independent cohorts of elderly individuals with moderate CKD, a serum fatty acid pattern (generated by factor analysis with a varimax rotation) representing low LA/high SFA was strongly and independently associated with CRP.

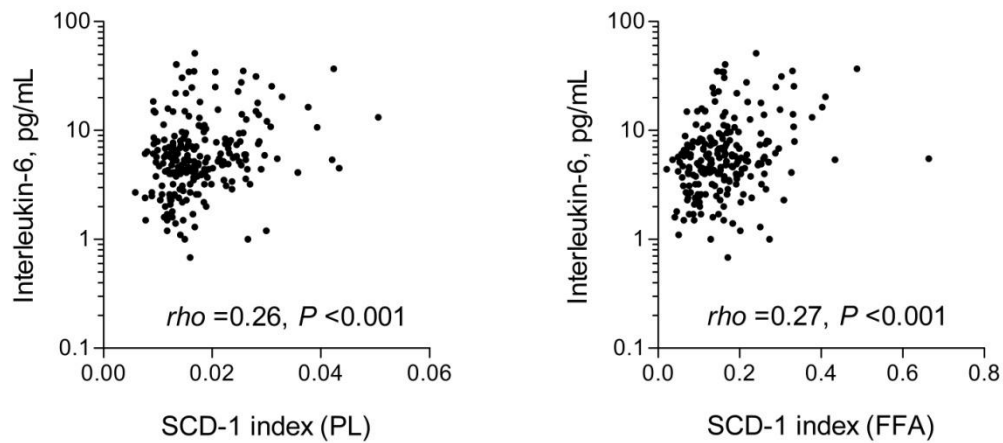


**Figure 5.** Correlations between linoleic acid, Mead acid and serum interleukin-6 concentrations in 222 dialysis patients. Reprinted with permission.<sup>194</sup>

These findings are in agreement with previous community reports showing that the plasma level of LA inversely correlated with pro-inflammatory biomarkers.<sup>104-107</sup> Notably, a recent RCT in abdominally obese individuals showed that dietary substitution of butter (SFA-rich) by sunflower oil (LA-rich) improves inflammatory status in compliant individuals.<sup>114</sup> Data on effects of *n*-6 PUFA supplementation in CKD patients are almost nonexistent, with only Begum *et al.*<sup>96</sup> demonstrating a trend toward a decrease in leukotriene B<sub>4</sub> (a pro-inflammatory eicosanoid) production. LA suppresses the production of adhesion molecules, chemokines, and interleukins *in vitro*.<sup>195</sup> Arachidonic acid, one of the LA metabolites, is also favorably linked with circulating pro-inflammatory and anti-inflammatory markers in humans.<sup>107</sup> SFA can directly cause inflammation; they increase the expression and secretion of inflammatory cytokines<sup>88-90,196</sup> and induce nuclear factor-kappa B activation.<sup>91</sup>

In **Study 3**, SCD-1 indices in both plasma phospholipids and FFA, reflecting the enzyme activities in the liver and adipose tissue, were directly correlated with IL-6 in dialysis patients, as shown in **Figure 6**. Such a link is supported by findings in animals, cell studies<sup>197,198</sup> and community-based cohorts.<sup>104,105</sup> Since SCD-1 increases in response to dietary SFA intake,<sup>113</sup> these observations also support the notion that SFA have pro-inflammatory functions as aforementioned. However, SCD-1 *per se* may also cause inflammation in liver and adipose tissue,<sup>197</sup> a finding supported by observations from SCD-1 knockout mice which are protected from inflammation in macrophages, endothelial cells, and adipose tissue.<sup>198</sup>





**Figure 6.** Correlations of stearoyl-CoA desaturase-1 (SCD-1) activity indices in plasma phospholipids (PL) and free fatty acids (FFA) with serum interleukin-6 concentrations in dialysis patients. Modified from.<sup>199</sup>

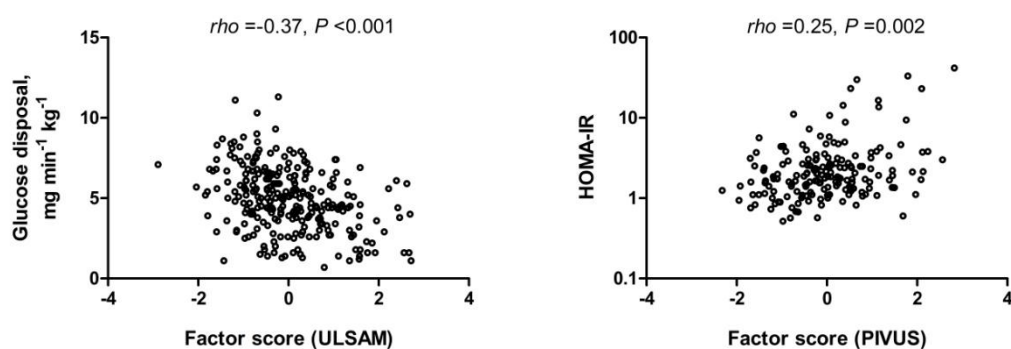
We did not observe an association between circulating *n*-3 PUFA and inflammatory markers in CKD (*Study 4*) and dialysis patients (*Study 2*). However, this is in agreement with findings in Swedish community studies<sup>104,105</sup> and with a previous American report in dialysis patients using *n*-3 PUFA intake estimated from dietary records.<sup>94</sup> The absent relationship between *n*-3 PUFA and uremic inflammation may be explained by the stronger links that *n*-3 PUFA shared with PEW. Nevertheless, our results do not contradict the notion that supplementation with *n*-3 PUFA has the potential to reduce systemic inflammation in CKD patients.<sup>133,200</sup> It has been reported that dialysis patients have reduced plasma *n*-3 PUFA levels<sup>62,201</sup> and thereby, circulating levels may not suffice to exert their anti-inflammatory effects. In fact, clinical trials generally show that supplementation with *n*-3 PUFA has the potential to reduce inflammation (as summarized in **Table 2**), indirectly supporting the speculation from observational research that reduced circulating *n*-3 PUFA in dialysis patients exert few anti-inflammatory properties. On the other hand, it is also possible that, in the context of a Swedish diet where fish intake is relatively adequate compared with for instance that in an American diet,<sup>63,171</sup> these links are thus not fully evident. Further studies in CKD populations should confirm these relationships.

Taken together, these findings support the concept that LA may suppress while SFA may induce the CKD-related inflammatory status.

#### 4.4 FATTY ACIDS AND INSULIN RESISTANCE

In *Study 4*, the fatty acid pattern representing low LA/high SFA strongly correlates with IR, depicted by low glucose disposal or high HOMA-IR values, in both ULSAM and PIVUS cohorts of CKD subjects (**Figure 7**). Consistent with our finding, earlier reports in non-CKD populations indeed showed that individuals with a low proportion of serum LA have impaired fasting glycemia<sup>202</sup> and increased risk of developing DM.<sup>151</sup> Also, interventional studies have shown that whereas a diet enriched in LA improves insulin sensitivity, a diet high in SFA is likely to result in IR.<sup>126,170</sup> A recent study demonstrated that a diet rich in PUFA in insulin resistant men acutely reduces triacylglycerol-derived skeletal muscle fatty acid uptake, accompanied by improved postprandial insulin sensitivity.<sup>203</sup>

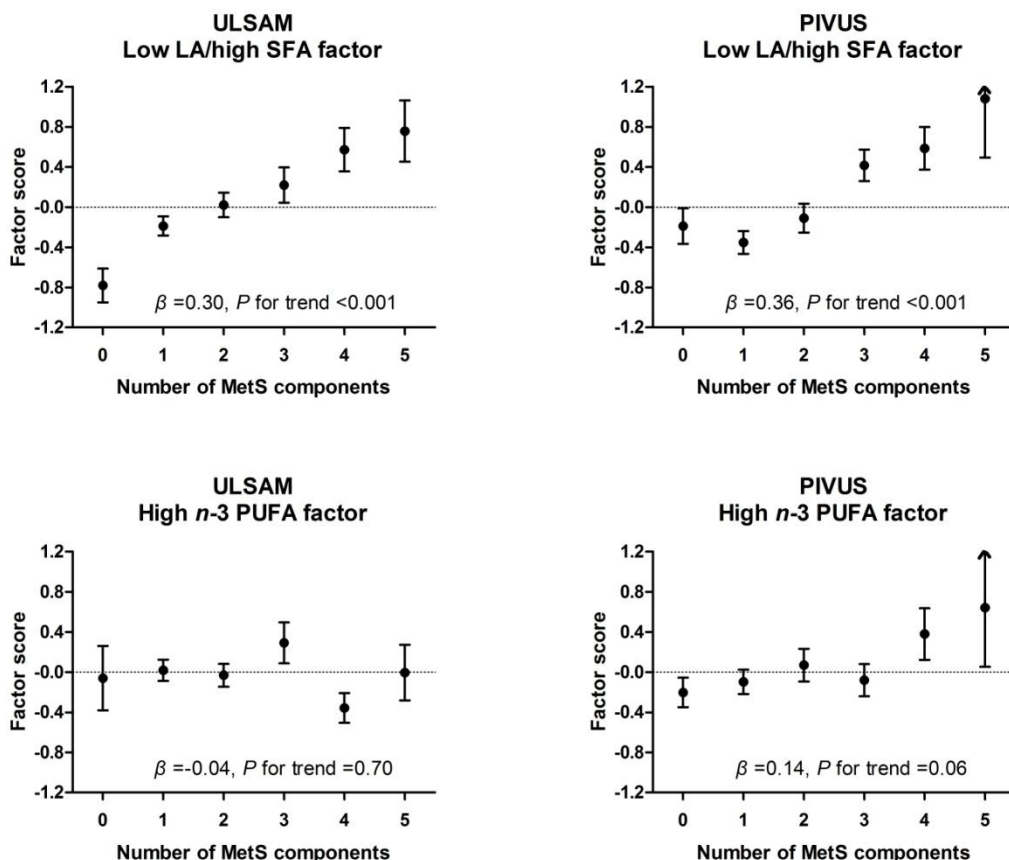
In the PIVUS study, a factor representing high *n*-3 PUFA was positively linked with HOMA-IR. However, a similar result was not confirmed in ULSAM, by using either glucose disposal or HOMA-IR. The association of the high *n*-3 PUFA factor reported in PIVUS may be attributed to the fact that a moderate positive loading from arachidonic acid, which usually comes from dietary animal sources,<sup>204</sup> was also present. Indeed, we did not observe such associations when EPA or DHA were studied individually.



**Figure 7.** Correlations of low linoleic acid/high saturated fatty acid factors with glucose disposal in the Uppsala Longitudinal Study of Adult Men (ULSAM) cohort or with homeostasis model of assessment - insulin resistance (HOMA-IR) in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort. Participants (*n*=12 in ULSAM and *n*=16 in PIVUS) on medication for diabetes (orally or via injections) were excluded in the analyses of glucose disposal and HOMA-IR.

## 4.5 FATTY ACIDS AND METABOLIC SYNDROME

In *Study 4*, 68 (25%) and 61 (33%) CKD individuals in the ULSAM and PIVUS studies, respectively, met at least three NCEP: ATP III criteria and were considered to have MetS. Factor scores of the two derived factors (low LA/high SFA and high *n*-3 PUFA factors) were plotted by the number of MetS components, as presented in **Figure 8**. Increasing scores of the low LA/high SFA factors in both cohorts were strongly and positively associated with the number of MetS components. A borderline increasing trend was revealed for the *n*-3 PUFA factor in PIVUS, but could not be confirmed in ULSAM. In multivariable logistic regression models (**Table 4**), every standard deviation decrease in the low LA/high SFA factors (thereby depicting an increase in LA intake and a reduction in SFA intake) reduced the odds to have MetS in the two studies. Likewise, across decreasing low LA/high SFA factor tertiles, the odds to have MetS were incrementally smaller. No significant relationship with MetS was



**Figure 8.** Factor scores across increasing number of metabolic syndrome components in the Uppsala Longitudinal Study of Adult Men (ULSAM) and the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohorts. Data are presented as mean  $\pm$  standard errors. Standardized coefficients ( $\beta$ ) and *P* values for trend were derived from linear regression analyses. Abbreviations: LA, linoleic acid; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

observed for scores of the *n*-3 PUFA factor in both cohorts. To test the robustness of these results and the representativeness of the generated factors, analyses were repeated using single fatty acid proportions (proportions of serum cholesterol ester LA, EPA, and DHA). LA as a single fatty acid was negatively associated, while EPA and DHA were not associated, with the presence of MetS in the two cohorts.

The MetS strongly predicts total and cardiovascular mortality in the community<sup>205</sup> as well as in individuals with CKD.<sup>121</sup> MetS may also represent a risk factor for CKD.<sup>46,119,121</sup> Our current results are of clinical interest since we identified links between modifiable dietary fat patterns and the presence of MetS in high-risk individuals with impaired renal function. Strengthening this observation, we also found strong associations between this dietary pattern, IR and inflammation, both key pathogenic links underlying the clustering of abnormalities in MetS.<sup>116</sup> This finding is consistent with previous observations in the community and in individuals with DM, CVD or MetS.<sup>127,128</sup>

**Table 4.** Multivariable logistic regression models predicting for the presence of the metabolic syndrome in individuals with chronic kidney disease according to the generated serum fatty acid patterns.

	OR (95% CI)	
	ULSAM	PIVUS
<b>Factor 1 (Low LA/high SFA)</b>		
Continuous (per SD decrement)	0.60 (0.44, 0.81)	0.45 (0.30, 0.67)
Grouped as		
Low LA (high scores)	Ref.	Ref.
Medium LA (medium scores)	0.52 (0.25, 1.06)	0.52 (0.24, 1.13)
High LA (low scores)	0.22 (0.09, 0.51)	0.16 (0.06, 0.43)
<i>P</i> for trend	<b>0.002</b>	<b>0.001</b>
<b>Factor 2 (High <i>n</i>-3 PUFA)</b>		
Continuous (per SD increment)	0.84 (0.61, 1.15)	1.27 (0.91, 1.76)
Grouped as		
Low <i>n</i> -3 PUFA (low scores)	Ref.	Ref.
Medium <i>n</i> -3 PUFA (medium scores)	1.48 (0.72, 3.05)	0.65 (0.29, 1.47)
High <i>n</i> -3 PUFA (high scores)	0.65 (0.29, 1.44)	1.06 (0.49, 2.30)
<i>P</i> for trend	0.1	0.5

*Note:* All models were adjusted for smoking status, physical activity, and estimated glomerular filtration rate and, in the PIVUS cohort, also sex. Abbreviations: CI, confidence interval; LA, linoleic acid; OR, odd ratio; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; PUFA, polyunsaturated fatty acids; SD, standard deviation; SFA, saturated fatty acids; ULSAM, Uppsala Longitudinal Study of Adult Men.

## 4.6 FATTY ACIDS AND MORTALITY

Sixty-one (27%) dialysis patients in the MIA-1 cohort died during a median follow-up period of 18.4 (IQR: 5.5 - 37) months. The main causes of death were CVD-related ( $n = 37$ , 61% of total deaths). One hundred and sixteen (52%) individuals underwent kidney transplantation. Due to the different biological interpretations of plasma fatty acids, we focused on essential fatty acids (as indicators of exogenous input) in *Study 2* and on non-essential fatty acids (as estimates of endogenous metabolism) in *Study 3*, respectively.

In *Study 2*, each percentage of increase in the proportion of LA significantly reduced the mortality risk before kidney transplantation. Adjusting within the causal pathway (Model 2, plus IL-6 concentrations) did not modify the results (**Table 5**). The inverse association between circulating LA levels and the risk of all-cause mortality we observed adds to the growing evidence in the general population that an increase in circulating LA associates with reduced cardiovascular risk and improved outcomes.<sup>142-145</sup> Mead acid, whose elevation in the blood is regarded as an indication of LA deficiency,<sup>193</sup> was directly associated with mortality, indirectly reinforcing the consequences of LA deficiency. Apart from the possible causal pathways we have discussed (inflammation, IR, and MetS), mechanisms by which LA may link to

**Table 5.** All-cause mortality risk before kidney transplantation (competing risk models) associated to proportions of plasma phospholipid polyunsaturated fatty acids (per 1% of increase) in 222 dialysis patients.

	HR (95% CI)		
	Crude	Model 1	Model 2
Linoleic acid	0.89 (0.81, 0.98)	0.88 (0.79, 0.98)	0.89 (0.79, 0.99)
Mead acid (*10)	1.31 (1.08, 1.59)	1.35 (1.19, 1.52)	1.33 (1.17, 1.52)
$\alpha$ -linolenic acid (*10)	0.93 (0.71, 1.23)	0.86 (0.63, 1.17)	0.89 (0.65, 1.23)
LC $n$ -3	0.89 (0.71, 1.11)	0.91 (0.72, 1.15)	0.91 (0.72, 1.16)

*Note:* Because of small proportions, the levels of Mead acid and  $\alpha$ -linolenic acid were multiplied by 10 to show meaningful risks estimates, thus depicting the risk associated to 0.1% increase; Model 1 is adjusted for sex, age, comorbidities (composite score of diabetes mellitus and cardiovascular disease), dialysis modality, and protein-energy wasting (by subjective global assessment); Model 2 is adjusted for factors detailed in Model 1 plus interleukin-6. No interactions (the polyunsaturated fatty acids \* sex, the polyunsaturated fatty acids \* dialysis modality, and the polyunsaturated fatty acids \* protein-energy wasting) were observed. Abbreviations: CI, confidence interval; HR, hazard ratio; LC  $n$ -3, long-chain  $n$ -3 polyunsaturated fatty acids (the sum of eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids). Reprinted with permission.<sup>194</sup>

reduced mortality could include retarding the reduction of GFR,<sup>82</sup> reducing liver fat,<sup>114</sup> as well as lowering of cholesterol<sup>206,207</sup> and blood pressure.<sup>208</sup>

As shown in **Table 5**, *n*-3 PUFA did not associate with mortality, a result which however agrees with findings from the general Swedish population where *n*-3 PUFA intake is relatively high<sup>145</sup> but also with previous evidence in dialysis patients using either erythrocyte *n*-3 PUFA content<sup>137</sup> or dietary-estimated *n*-3 PUFA.<sup>94</sup> Kutner *et al.*<sup>134</sup> reported that higher fish intake associated with decreased mortality risk in incident dialysis subjects. Divergences in results may be attributed to methodological issues (food frequency questionnaires *vs.* plasma PUFA content) as well as study design. Also, Hamazaki *et al.*<sup>135</sup> showed that Japanese dialysis patients within the highest tertile of erythrocyte DHA content had a reduced mortality risk. Again, variances in the source of *n*-3 PUFA content between that and our study (erythrocyte *vs.* plasma phospholipids), the use of categories in studies with reduced sample size and especially the likely differences in fish intake between Swedish and Japanese populations, limit the relevance of comparisons. While further research is needed to confirm our results, regional, cultural and individual dietary differences may preclude a general conclusion regarding the association between PUFA and risk profile. Thus, our findings should be contemplated within the context of Western-type and Swedish diet. Despite the present study being the largest of its kind in dialysis patients, it should be noted that the magnitude of the reduced risk estimates was in fact similar between *n*-6 and *n*-3 PUFA.

In **Study 3**, due to the lack of clinically defined cut-off points for SCD-1 activity indices, we estimated them on the basis of receiver operator characteristic curve analyses for prediction of all-cause mortality. The clinical cut-off values of maximum sensitivity and highest specificity were used in further analyses to define high and low SCD-1 activities in this dialysis population.

In the competing-risk Cox models presented in **Table 6**, patients with high phospholipid and FFA SCD-1 activity indices presented significantly higher mortality risk before kidney transplantation, as compared with those with low SCD-1 indices. Similar direct associations were observed when SCD-1 indices were tested as continuous variables.

**Table 6.** All-cause mortality risk before kidney transplantation (competing risk models) associated to stearoyl-CoA desaturase-1 activity indices in plasma phospholipids and free fatty acids in the dialysis patients.

	Adjusted models	
	HR (95% CI)	P value
<b>Phospholipids (n=222)</b>		
High SCD-1 index [reference: low]	2.29 (1.28, 4.11)	<b>0.006</b>
SCD-1 index (per SD increase)	1.28 (1.00, 1.64)	<b>0.05</b>
<b>Free fatty acids (n=220)</b>		
High SCD-1 index [reference: low]	2.36 (1.38, 4.03)	<b>0.002</b>
SCD-1 index (per SD increase)	1.42 (1.19, 1.70)	<b>&lt;0.001</b>

*Note:* In each lipid fraction, hazard ratios are presented either as categories (dichotomized into high and low SCD-1 according to ROC cut-off values -0.020 in phospholipids and 0.164 in free fatty acids-) or presented as a continuous variable depicting risk associated to each standard deviation increase. All models are adjusted for age, sex, comorbidities (composite score of diabetes mellitus and cardiovascular disease), dialysis modality, protein-energy wasting and interleukin-6. Abbreviations: CI, confidence intervals; HR, hazard ratios; SCD-1, stearoyl-CoA desaturase-1; SD, standard deviation. Modified from.<sup>199</sup>

The strong and direct implication of both SCD-1 indices on mortality is in line with a previous Swedish community study, where hepatic SCD-1 index measured in serum cholesterol esters also predicted mortality.<sup>145</sup> Reasons behind this association may involve deleterious effects associated with high SCD-1 activity, such as promotion of hepatic lipogenesis and steatosis,<sup>209</sup> IR,<sup>150,210</sup> endothelial dysfunction,<sup>105</sup> and atherosclerosis.<sup>211</sup> Because SCD-1 activity increases in humans in response to high SFA intake and low unsaturated fat intake,<sup>113,114</sup> replacing palmitic acid or refined carbohydrates by MUFA or PUFA in the diet might be a useful dietary strategy to reduce SCD-1 activities. It is however unclear whether such an intervention could be of clinical importance, and it also remains to be shown in humans whether SCD-1 *per se* has adverse health effects. On the other hand, there is convincing evidence that replacing dietary palmitic acid by *n*-6 PUFA reduces cardiovascular events in humans.<sup>212</sup> In this context it has been speculated that increased SCD-1 is an adaptive response to excess intake of SFA and/or sugars, and that such response will prevent toxic effects of high cellular levels of palmitic acid by converting it to MUFA.<sup>167</sup>

To conclude, we find that both circulating LA level (inversely) and estimated SCD-1 activities (directly) predict all-cause mortality in dialysis patients. Marine *n*-3 PUFA was not found associated with mortality risk in this population.

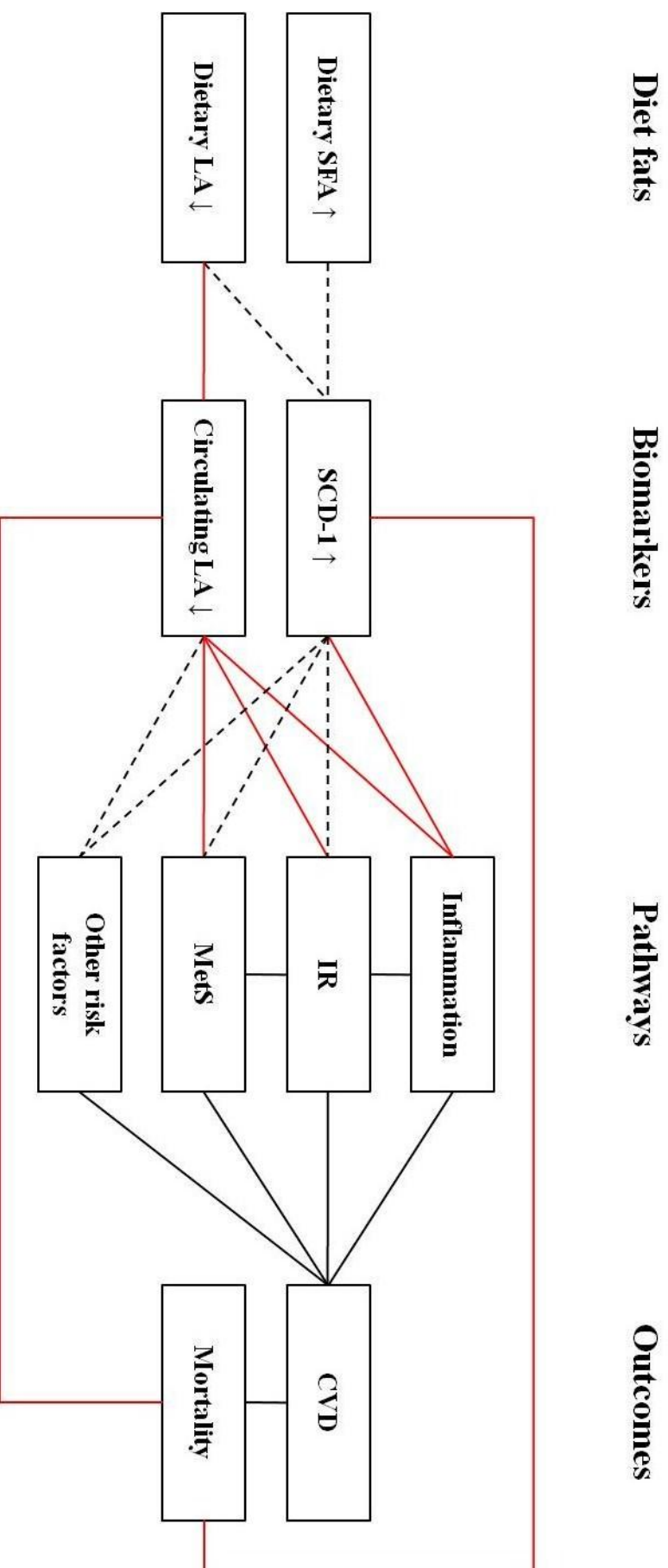
## 5 CONCLUSIONS

This thesis reports associations of circulating fatty acids and related biomarkers with the risk profile and outcomes in patients with CKD. The main conclusions are:

1. LA, EPA, DHA, and palmitic acid in serum cholesterol esters and adipose tissue are good indicators of the habitual dietary fat intake in elderly men with CKD. Dietary fish intake reflects well the intake of *n*-3 PUFA of marine origin in this population.
2. A serum fatty acid pattern reflecting low LA and high SFA is associated with inflammatory status, IR, and presence of MetS in CKD patients.
3. Increased indices of hepatic and adipose tissue SCD-1 activities strongly correlate with inflammation in dialysis patients.
4. Low circulating LA level predicts all-cause mortality in dialysis patients.
5. Hepatic and adipose tissue SCD-1 activity indices are directly associated with mortality risk in dialysis patients.
6. *n*-3 PUFA of marine origin (EPA and DHA) are not associated with inflammation, IR, the presence of MetS, or mortality in Swedish cohorts of individuals with CKD.

The main findings of the thesis are schematically summarized in **Figure 9**.





**Figure 9.** Dietary determinants, potential risk and outcome implications of circulating linoleic acid and stearoyl-CoA desaturase-1 in chronic kidney disease. Red lines indicate the associations proposed in this thesis; dashed lines indicate the associations established in the non-CKD population. Abbreviations: CVD, cardiovascular disease; IR, insulin resistance; LA, linoleic acid; MetS, metabolic syndrome; SCD-1, stearoyl-CoA desaturase-1; SFA, saturated fatty acids.

## 6 DIRECTIONS OF FUTURE RESEARCH

The present thesis attempts to shed light on a variety of possible connections and intriguing hypotheses that would, ultimately, lead to new therapeutic strategies in CKD. As the cross-sectional design of our studies precludes conclusions regarding causality, the next obvious steps would be to initiate longitudinal, interventional and mechanistic research attempts.

Interestingly, results of *n*-3 PUFA in our Swedish cohorts presented in this thesis are neutral. *n*-3 PUFA levels differ substantially across regional and cultural areas<sup>63</sup> and, in the context of CKD, other comorbidities such as PEW (a condition implicated in so called reverse epidemiology) may also complicate their risk implications. In future studies, all these differences ought to be taken into account to achieve a general conclusion regarding the association between *n*-3 PUFA and the CKD risk profile.

Even though many interventional studies have tested the effects of *n*-3 PUFA/fish oil supplementation, particularly on proteinuria, blood lipoproteins, and inflammatory markers, a large number of them may not be sufficiently powered, not only due to the small sample size but also the relatively short duration. The turnover of fatty acids within each lipid pool and the incorporation of *n*-3 PUFA into the lipid pools occur at different rates.<sup>78</sup> In future supplemental studies, one should thus prolong the duration as appropriate to capture changes of fatty acid composition, *e.g.*, 2 months in erythrocyte membrane, and even longer for higher doses, and choose appropriate biomarkers to reflect varying periods of dietary intake of *n*-3 PUFA efficiently.<sup>213,214</sup> Also, gender differences,<sup>215</sup> differential dose effect,<sup>216</sup> and the interaction with *n*-6 PUFA<sup>217,218</sup> warrants attention. In CKD, specifically, the coexistence of high phosphate content in fish cannot be ignored.

Our results raise the hypothesis that dialysis patients could benefit from increased intake of vegetable oils, the primary source of LA in the Western-type diet. Such strategies are being tested in non-CKD individuals and previous controlled trials suggest cardiovascular benefits when substituting SFA in the diet specifically for PUFA,<sup>219</sup> effects mostly mediated by anti-hyperlipidemic mechanisms,<sup>206</sup> but perhaps also by a reduction in liver fat and inflammation.<sup>114</sup> For this reason, a science advisory from the AHA<sup>146</sup> supports an *n*-6 PUFA intake of at least 5% to 10% of

energy in the context of other AHA lifestyle and dietary recommendations.<sup>220</sup> Whereas most RCT to date have been designed to investigate the effects of *n*-3 PUFA,<sup>133,200</sup> our data suggest that more research focus should be given to *n*-6 PUFA from vegetable oils in the context of CKD. In addition, other potential effects of LA are inconsistent or largely unknown.<sup>82,83,221</sup> It would be attractive to investigate, for instance, whether it exerts reno-protective effects.

We observed relationships of SCD-1 indices with inflammation and mortality risk, but it remains undetermined whether SCD-1 *per se* has adverse health effects in humans.<sup>222</sup> SCD-1 may cause inflammation in liver and adipose tissue,<sup>197</sup> a finding supported by observations from the SCD-1 knockout mouse.<sup>198,210</sup> Nevertheless, increased SCD-1 activity may be an adaptive response to excess intake of SFA and/or sugars, and such response may prevent toxic effects of high cellular levels of palmitic acid by converting it to MUFA.<sup>167</sup> In fact, the SCD-1 knockout mouse also develops severe vascular inflammation and atherosclerosis when exposed to a typical Western diet.<sup>210</sup> Pharmacological inhibitors of the SCD-1, if available in the future, should be tested in a clinical setting, but with caution. An RCT replacing SFA with PUFA, while maintaining adequate protein and energy intake in CKD patients, is a less aggressive, but more feasible means.

Previous research on dietary practice in the CKD population mainly focuses on single nutrients. Likewise, our studies in this thesis explore the role of fatty acids exclusively. Due to the complexity and intercorrelation of food components (we eat meals rather than isolated nutrients), however, dietary patterns may better capture inner relationships among nutrients and facilitate the interpretation of risk implications.<sup>223</sup> The Mediterranean dietary pattern<sup>224</sup> is increasingly gaining attention in the general population.<sup>225</sup> We have found that adherence to this dietary pattern is associated with lower risk of CKD in a community-based cohort of elderly men and a greater adherence to this diet independently predicted better survival in those with manifest CKD (Huang *et al.* In submission). Future studies of this type, *i.e.*, investigating the overall dietary quality, may be of greater practical relevance than studies focusing on single nutrients and should be encouraged.

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