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RENAI FUNCTION AND MARKERS OF CARDIOVASCULAR RISK

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Abstract

Patients with chronic renal insufficiency (CRI), also at its early stages, have an elevated risk of cardiovascular disease (CVD). Many well-established risk factors of CVD co-occur in CRI, e.g. dyslipidemia and hypertension. The present studies investigated the association between renal function and selected CVD risk factors.

The fractional catabolic rate of low-density lipoprotein apolipoprotein B (LDL FCR) has previously been found to be reduced in patients with severe CRI or on dialysis. This study investigated the LDL FCR in 57 patients with moderate to severe CRI and not on dialysis. Although the mean LDL FCR was comparable between the CRI patients and healthy controls, among the renal patients the LDL FCR was correlated with renal function, whereas it was significantly reduced only in patients with advanced CRI (estimated glomerular filtration rate < 15 mL/min/1.73 m²).

Leptin is a protein regulating food intake and energy expenditure and it is also involved in lipid metabolism. Hyperleptinaemia is a known feature of CRI patients; they are thought to be leptin resistant. The association between leptin and the lipoprotein profile was studied in 73 CRI patients with moderate to severe CRI and not on dialysis. Leptin was associated with lipid and lipoprotein concentrations in the renal patients, as in the control subjects, pointing towards a poorer lipoprotein profile with higher leptin levels.

Hypertensive subjects in whom nocturnal blood pressure (BP) declines by less than 10% (non-dippers) show more organ damage than those in whom it falls by more than 10% (dippers). Here, non-dipping was found in 19% of middle-aged subjects (226 males, 234 females) evaluated with ambulatory BP monitoring. The non-dippers had significantly lower renal function as compared with the dippers, and dipping status was a significant predictor of the variation in eGFR. Furthermore, an increased risk of non-dipping was observed among subjects with only minor decreases in renal function.

Carotid intima-media thickness (cIMT) can be used as a surrogate marker of early atherosclerosis. The present study investigated the association between renal function and cIMT in middle-aged subjects (247 males, 258 females). Renal function was independently associated with cIMT among males and also among postmenopausal women. The increased cIMT was seen in conjunction with mild renal impairment.

In conclusion, the catabolism of LDL correlated with the renal function among CRI patients, but it was significantly reduced only in patients with advanced CRI. Leptin concentrations correlated with the lipoprotein profile in CRI patients. Among general middle-aged subjects, even a mild decrease in renal function was associated with derangements in BP regulation and with increased carotid atherosclerosis.

Keywords: atherosclerosis, blood pressure, leptin, lipids, renal insufficiency
To my family
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Kuopio, May 2010

Helena Kastarinen
Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABPM</td>
<td>Ambulatory blood pressure monitoring</td>
</tr>
<tr>
<td>Apo</td>
<td>Apolipoprotein</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>Chol/HDL</td>
<td>Ratio of total cholesterol to high-density lipoprotein cholesterol</td>
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<tr>
<td>cIMT</td>
<td>Carotid intima-media thickness</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>CRI</td>
<td>Chronic renal insufficiency</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
</tr>
<tr>
<td>ESRD</td>
<td>End-stage renal disease</td>
</tr>
<tr>
<td>FCR</td>
<td>Fractional catabolic rate</td>
</tr>
<tr>
<td>HD</td>
<td>Hemodialysis</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>IDL</td>
<td>Intermediate-density lipoprotein</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>Leptin/BMI</td>
<td>Ratio of leptin concentration to body mass index</td>
</tr>
<tr>
<td>MDRD</td>
<td>Modification of Diet in Renal Disease</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PD</td>
<td>Peritoneal dialysis</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TG</td>
<td>Triglyceride(s)</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very-low-density lipoprotein</td>
</tr>
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</table>
List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals I-IV:


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1 Introduction

Patients with chronic renal insufficiency (CRI) are at a substantially elevated risk of developing atherosclerosis (Lindner et al. 1974, Weiner et al. 2004). Approximately 40% of these patients have cardiovascular disease (CVD) even before they reach end-stage renal failure that requires dialysis therapy (Foley et al. 1995). Cardiac mortality is elevated by more than 100-fold among dialysis patients aged 45 years or younger compared with that in the general population (Foley et al. 1998). In the general population, mild renal impairment has also been associated with an increased risk for death, cardiovascular events, hospitalization and stroke (Go et al. 2004, Henry et al. 2002, Nakayama et al. 2007, Wannamethee et al. 1997). This indicates that CVD begins to develop early in the course of CRI. In a recent meta-analysis, it was found that the absolute risk for death increased exponentially with decreasing renal function, and this increase in risk was greater in younger age cohorts (Tonelli et al. 2006).

Despite the improvements achieved in the management of renal insufficiency, the survival of renal patients remains reduced as compared with control subjects. CVD is the most important cause of morbidity and mortality among patients with CRI (Foley et al. 1998). Many – if not most – known or potential risk factors of CVD are present in patients with CRI: hypertension, dyslipidemia, derangements in glucose metabolism, increased oxidative stress, thrombosis and inflammation, evidence of endothelial damage, alterations in phosphate and calcium homeostasis (Baigent et al. 2000, Foley et al. 2005, Schiffrin et al. 2007). Many of these risk factors and CVD itself may also predispose to renal disease and once established to a more rapid loss of renal function, thus forming a circulus vitiosus (Attman et al. 1999, Chade et al. 2005, Elsayed et al. 2007, Freedman et al. 1995, Shlipak et al. 2009, Vaziri 2006).

Recently, CRI has been recognized and appreciated as a public health problem (Schieppati & Remuzzi 2005). According to data from the United States, the prevalence of CRI is 13% in the adult population (Coresh et al. 2007). Diabetes and hypertension constitute the most common causes of end-stage renal disease (ESRD), but atherosclerotic renovascular disease is becoming more prevalent, especially among older patients (Foley & Collins 2007). Therefore, measures aimed to reduce the ever-expanding prevalence of diabetes, hypertension and atherosclerosis are of utmost importance also for the prevention of renal insufficiency. It is also noteworthy that most patients with early stage
CRI die from CVD even before they require renal replacement therapy (DuBose 2007).

The present studies investigated the association between renal function and the two principal risk factors for CVD: namely lipid and blood pressure (BP) derangements. The metabolism of low-density lipoprotein (LDL), the major lipid risk factor for CVD, and the effect of leptin on the lipid profile were studied in patients with CRI. The association between mildly reduced renal function and BP was studied in healthy subjects, as was the relationship between renal function and atherosclerosis in the carotid arteries.
2 Review of the literature

2.1 Characterization and classification of chronic renal insufficiency (CRI) and chronic kidney disease (CKD)

CRI is characterized by multiple abnormalities in body homeostasis. Common findings are anaemia due to lack of erythropoietin, deficiency of active vitamin D leading to hypocalcaemia and increased production of parathyroid hormone (PTH), as well as increased concentrations of creatinine, urea, phosphorus and potassium due to decreased excretion. Metabolic acidosis is a common finding in advanced renal insufficiency. Disturbances can also be seen among different hormone levels as well as in glucose and lipid metabolism. The ability of the kidneys to regulate the amount of water in the body is also disturbed. Increased BP is almost inevitably seen in advanced renal insufficiency, and there are many mechanisms behind this elevation in pressure. Increased excretion of protein into the urine may or may not be seen in conjunction with renal insufficiency, depending on the type of renal disease. There are several diseases that influence the kidneys, for example both types of diabetes, hypertension, atherosclerosis, glomerulonephritis and polycystic kidney disease.

CRI is treated by diet and drugs in the mild to severe stages, whereas in ESRD, renal replacement therapies (hemodialysis (HD), peritoneal dialysis (PD) and renal transplantation) are needed in order to prolong life.

Patients with chronic kidney disease (CKD) can be stratified according to the type and severity of renal dysfunction (Table 1). Patients with CKD exhibit markers of renal damage (e.g. proteinuria or hematuria) or reduced renal function, whereas patients with CRI have established renal insufficiency. The degree of renal function can be estimated using various laboratory methods. The golden standard is to use an isotope (inulin, chromium or technetium), but the most commonly used estimates are serum or plasma creatinine or urea, which however require careful interpretation. A more valid estimate can be obtained using various formulas to calculate glomerular filtration rate (GFR) from serum/plasma/urine creatinine. Such formulas are the Cockcroft-Gault equation and the MDRD-equation (Levey et al. 2003). Recently a new formula (CKD-EPI) was introduced in order to further improve the ability to estimate the GFR in subjects with near-normal kidney function (Levey et al. 2009). Serum levels of cystatin C can also be used to evaluate renal function both as such and as a supplemental factor in
equations (Séronie-Vivien et al. 2008, Tidman et al. 2008). In the medical literature, CKD as a concept is commonly used as an equivalent of estimated (eGFR) or measured GFR less than 60 ml/min/1.73 m² (CKD stages 3–5), even though this can cause confusion. In the present thesis, the patients with CKD stage 3–5 will be referred to as patients with CRI.

### Table 1. The commonly used classification of CKD.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR, mL/min per 1.73 m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or increased GFR</td>
<td>≥ 90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mildly decreased GFR</td>
<td>60–89</td>
</tr>
<tr>
<td>3</td>
<td>Moderately decreased GFR</td>
<td>30–59</td>
</tr>
<tr>
<td>4</td>
<td>Severely decreased GFR</td>
<td>15–29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure</td>
<td>&lt; 15 (or dialysis)</td>
</tr>
</tbody>
</table>

Adapted from Levey et al. 2003.

### 2.2 Cardiovascular disease (CVD) in patients with CKD

#### 2.2.1 The prevalence of CVD in patients with CKD

CRI is associated with premature atherosclerosis and increased cardiovascular morbidity and mortality in patients on HD, PD and also in patients who have undergone renal transplantation or who are on conservative treatment (Foley et al. 1998, Goodman et al. 2000, Lindner et al. 1974). In addition to severe CRI, also mild or moderate renal impairment (CKD stage 2–3) has been found to increase the risk of death, various cardiovascular events and hospitalization (Astor et al. 2008, Culleton et al. 1999, Deo et al. 2008, Desbien et al. 2008, Fried et al. 2003, Go et al. 2004, Henry et al. 2002, Irie et al. 2006, Ishizaka et al. 2008, Kawamoto et al. 2008a, Koren-Morag et al. 2006, Manjunath et al. 2003a, Manjunath et al. 2003b, Mann et al. 2001, McCullough et al. 2007, Muntner et al. 2002, Reis et al. 2002, Wannamethee et al. 1997, Zhang et al. 2007). Peripheral arterial disease is known to be common in patients with CRI (Leskinen et al. 2002). CVD accounts for 40 to 55% of deaths of dialysis patients (Finnish Registry for Kidney Diseases 2000, Foley et al. 1995). Clinical and echocardiographic CVD has been found to be present in a considerable proportion of patients that start ESRD therapy with 14% having coronary artery disease, 19% angina pectoris, 31% cardiac failure, 8% peripheral vascular disease, 15% systolic dysfunction and 74% left ventricular hypertrophy (LVH) (Foley et al. 1995). In a study in predialysis patients (CKD
stage 4–5), the prevalence of congestive heart failure was 15%, angina 25%, peripheral vascular disease 14%, and furthermore, 10% and 11% of the patients had myocardial infarction and stroke, respectively, in their medical history (Holland & Lam 2000). In another study including conservatively treated patients with CKD stage 2–5, 34% had a history of vascular disease, and 21% had LVH on electrocardiogram (Wheeler et al. 2003). In a recent study with incident HD patients, it was shown that prior coronary heart disease (CHD) increased the risk of non-fatal myocardial infarction by 57% and the risk of cardiac death by 16% as compared with subjects without prior CHD (Trivedi et al. 2009).

Arterial damage can be classified according to the site of the calcifications. Arterial intima calcification is associated with the development of plaques and occlusive lesions. Arterial media calcification, also called Mönckeberg’s arteriosclerosis, is commonly associated with aging, the presence of diabetes or ESRD, and it is often found in the muscle-type conduit arteries, such as the femoral and the tibial arteries. In its typical form, arterial media calcification does not obstruct the arterial lumen, but it has been found to be a strong marker of CVD risk in patients with non-insulin-dependent diabetes mellitus (Lehto et al. 1996) and in HD patients (London et al. 2003). Arterial media calcification in patients with CKD has recently been reviewed by McCullough et al. (2008).

Mild renal impairment (CKD stage 2) has been found to increase CVD risk in patients with already prevalent CVD, hypertension and diabetes (Mann et al. 2003). In primary hypertensive patients, mild or moderate renal dysfunction (CKD stage 2–3) was associated with preclinical end-organ damage (Leoncini G et al. 2004), and renal function was associated with cardiovascular risk after acute myocardial infarction (Anavekar et al. 2008).

2.2.2 Overview of the risk factors for CVD in the general population and in patients with CKD

In the general population, the traditional risk factors for atherosclerosis include lipoprotein abnormalities such as elevated total and LDL cholesterol and triglycerides (TG), low high-density lipoprotein (HDL) cholesterol, increased ratio of total cholesterol to HDL cholesterol (Chol/HDL), and increased lipoprotein (a) (Lp(a)). Other risk factors include hypertension, smoking, chronic inflammation, lipid oxidation, increased levels of homocysteine, and disturbances in glucose metabolism, deficient endothelial function and thrombosis as well as obesity, and lack of exercise. Age, sex and genetic factors also have an impact as
regulators of many of the above-mentioned risk factors. (Ariyo et al. 2003, Berliner et al. 1995, Danesh et al. 2000, Endemann & Schiffrin 2004, Hopkins et al. 2005, Kendrick & Chonchol 2008, Luc et al. 2002, Lusis 2000). During recent years, many new risk factors or biomarkers for CVD have emerged. The traditional risk factors are predictive also in renal patients, and they have been shown to be more strongly related with CVD in elderly persons with CKD stage 2–4 (Muntner et al. 2005, Shlipak et al. 2005, Stenvinkel et al. 2008).

The risk factor burden for the development of CVD in CKD is significant, and the number of cardiovascular risk factors tends to increase with the CKD stage (Foley et al. 2005). In a study that assessed cardiovascular risk factors in CKD patients not on dialysis, hypertension was prevalent in 76%, and diabetes in 15%. Furthermore, the patients had lower LDL cholesterol and lower HDL cholesterol as well as higher TG concentrations compared with healthy control subjects (Landray et al. 2001, Wheeler et al. 2003).

2.2.3 Lipoprotein abnormalities as risk factors for CVD in CKD

In CRI patients, total and LDL cholesterol concentrations frequently remain comparable with that of the general population, whereas increases in the TG concentrations and decreases in the HDL cholesterol are often seen. In a recent study on Framingham offspring, participants with CRI had higher prevalences of elevated TG concentration (40% versus 30% in subjects without CRI) and low HDL cholesterol concentration (43% versus 29% in subjects without CRI). Furthermore, participants with CRI had higher prevalences of elevated LDL cholesterol concentrations and the use of lipid-lowering therapy compared with participants without CRI, although the differences were statistically insignificant (Parikh et al. 2006). In HD patients, TG and intermediate-density lipoprotein (IDL) cholesterol concentrations were independent risk factors for aortic atherosclerosis (Shoji et al. 1998), and high TG and low HDL concentrations were associated with a rapid progression of coronary artery calcification (Tamashiro et al. 2001). However, in a study of patients on PD, lower total cholesterol was associated with increased risk of all-cause mortality, and higher TG concentrations were found to be harmful (Habib et al. 2006). In new HD patients, increased cholesterol concentrations in the absence of inflammation/malnutrition were associated with increased CVD mortality, whereas in the presence of inflammation/malnutrition this trend was reversed (Liu et al. 2004). In CRI patients not yet on dialysis, LDL cholesterol concentrations
did not exhibit any significant relationship with CVD mortality after adjustments for malnutrition, inflammation and cachexia. The authors concluded that lower lipid concentrations were associated with higher mortality in these patients, but that this inverse association was explained in part by the presence of the malnutrition-inflammation-cachexia syndrome (Kovesdy et al. 2007). Nevertheless, this reciprocal epidemiology theory by which lipid lowering may not be of benefit among renal patients has raised interesting speculations.

Lp(a) concentrations have been found to predict CVD in the general population (Ariyo et al. 2003, Danesh et al. 2000, Emerging risk Factors Collaboration 2009). The concentrations of Lp(a) have been found to associate with increased CVD also in patients on HD (Docci et al. 1994, Kronenberg et al. 1994), whereas in patients with CKD stages 3–4, Lp(a) levels were not predictive of CHD or CVD mortality risk (Muntner et al. 2005, Shlipak et al. 2005).

2.2.4 Hypertension as a risk factor for CVD in CKD

Hypertension is frequently secondary to renal parenchymal disease and it occurs already in patients with even mild reductions in GFR (Phillips 2005). Furthermore, in a study in glomerulonephritic patients with normal GFR, ambulatory BP was already elevated and associated with greater left ventricular wall thickness (Stefanski et al. 1996). Renal impairment was also shown to reduce the variability in the 24-hour BP (Manios et al. 2009). In patients on renal replacement therapy (including transplanted patients), 87% of patients were taking antihypertensive medication (Finnish Registry for Kidney Diseases 2007). In a study assessing cardiovascular risk factors in CKD patients not on dialysis, hypertension was present in 76% of the subjects (Landray et al. 2001, Wheeler et al. 2003). Other studies have shown a prevalence of 35–71% of hypertension in subjects with CRI not on dialysis (Muntner et al. 2005, Parikh et al. 2006). Hypertension has been shown to be associated with cardiovascular mortality in dialysis patients as well. In a survival-analysis of 1435 dialysis patients, high systolic or diastolic BP was a risk factor for overall and cardiovascular mortality (Degoulet et al. 1980). Furthermore, in incident HD patients, uncontrolled hypertension was a main risk factor for cardiovascular mortality (Lucas et al. 2003). In patients with CRI not on dialysis, hypertension doubled the risk of CHD (Muntner et al. 2005).
2.2.5 Other risk factors for CVD in CKD

Smoking has been found to be less common among subjects with CRI than in subjects without renal impairment (prevalence of 7% versus 14%, respectively) in a community-based study (Parikh et al. 2006). The studies examining smoking as a cardiovascular risk factor in renal patients have however produced contrasting results. In HD patients, smoking was not a significant risk factor for CVD (Rostand et al. 1982), although, in subjects with CRI but not yet on renal replacement therapy, smoking did increase the risk for CVD by 1.6 to 1.8-fold (Muntner et al. 2005, Shlipak et al. 2005).

In a study that assessed cardiovascular risk factors in CRI patients not on dialysis, diabetes was present in 15% of the subjects (Landray et al. 2001, Wheeler et al. 2003), and in an elderly general population, subjects with CRI had a 17% prevalence of diabetes (Shlipak et al. 2005). In another study in the general population, those with CRI had more often diabetes than those who had better renal function (24% versus 12%, respectively) (Parikh et al. 2006). Insulin resistance has been found to develop early in renal disease (CKD stage 1–2) (Dzúrik et al. 1995, Fliser et al. 1998, Onat et al. 2007). Reduced renal function has been associated with the presence of the metabolic syndrome in cross-sectional studies (Kawamoto et al. 2008b, Peralta et al. 2006,). In subjects with CRI, diabetes tripled the risk of CVD (Muntner et al. 2005). In population based studies, obesity has been found to be more prevalent than or as common in subjects with CRI as in subjects without CRI (Muntner et al. 2005, Parikh et al. 2006, Shlipak et al. 2005). Obesity evoked a 1.5-fold increase in CVD risk after adjustments for age, race and gender, but with further adjustments with smoking habits, diabetes, hypertension and cholesterol concentrations, the risk increase proved insignificant (Muntner et al. 2005), as was confirmed in another study (Shlipak et al. 2005).

Use of alcohol has been found less common in subjects with CRI compared with subjects without CRI, whereas the use of more than two alcoholic drinks per week was found to reduce CVD mortality and abstention was found to increase CVD mortality (Shlipak et al. 2005). Physical activity was less prevalent in subjects with CRI, and low physical activity was associated with elevated risk of CVD mortality even after adjustment for all other traditional risk factors also in these elderly subjects (Shlipak et al. 2005), whereas in another study on middle-aged subjects with CRI, low physical activity did not turn out to be a significant risk factor for major CHD events (Muntner et al. 2005).
There are also other CKD related risk factors for CVD mortality, such as vitamin D deficiency, disturbed PTH homeostasis, anaemia and malnutrition, increased oxidative stress, hyperhomocystinaemia as well as the underlying renal disease and factors related to the treatment of CKD (Hörl 2002, Kendrick & Chonchol 2008, Shoji & Nishizawa 2005, Stenvinkel et al. 2008, van der Zee et al. 2009). Alterations in the mineral metabolism and the treatment of these changes also contribute to the development of vessel wall disturbances in patients with CKD (Goodman et al. 2000). Vitamin D has multiple metabolic effects in addition to its central role in calcium-phosphorus and PTH homeostasis, and deficiency of vitamin D has been found to associate with incident CVD in the general population (Melamed et al. 2008, Wang et al. 2008). Also in patients with CKD stage 2–5, low levels of vitamin D were associated with elevated mortality (Barreto et al. 2009, Inaguma et al. 2008). High serum phosphorus concentrations as well as high values of the calcium-phosphorus ion product were independent risk factors for death in patients with ESRD (Block et al. 1998, Block et al. 2004). One possible mechanism behind this observation could be a disturbance in the FGF23-Klotho axis promoted by hyperphosphataemia (Kuro-o 2010). Increased FGF-23 levels have been found to be independently associated with mortality among patients starting dialysis treatment (Gutiérrez et al. 2008).

Subjects with severe CRI are more likely to have anaemia due to the lack of erythropoietin. Reduced haemoglobin levels are also more prevalent in patients with moderate CRI compared with subjects without CRI (Muntner et al. 2005, Shlipak et al. 2005). In elderly patients with CRI, anaemia was not a significant risk factor for CVD mortality (Shlipak et al. 2005), but in a middle aged CRI population, anaemia increased the risk of a major CVD event by 2-fold (Muntner et al. 2005). Furthermore, anaemia is a well known risk factor for the development of LVH, also in early CRI (CKD stage 2) (Levin et al. 1999).

Oxidative stress is elevated in CRI. There are thought to be several mechanisms contributing to this finding, e.g. decreased antioxidant levels and increased free radical production (Hasselwander & Young 1998, Stenvinkel et al. 2008, Tetta et al. 1999). Oxidized LDL plays a major role in the development of atherosclerosis (Steinberg 2009). However, studies assessing the oxidation of LDL in patients with CRI have yielded contrasting results (Annuk et al. 2001, Bergesio et al. 2001, Hasselwander & Young 1998, Loughrey et al. 1994, Maggi et al. 1994a, Maggi et al. 1994b, O’Byrne et al. 2001, Roob et al. 2001, Westhuyzen et al. 1997). Oxidized LDL has been associated with the magnitude of atherosclerosis and cardiovascular mortality in patients with advanced CRI.
Malnutrition has been suggested to be an important factor to explain the high CVD mortality among dialysis patients (Degoulet et al. 1982). Albuminuria has also been shown to increase CVD risk in the general population (Arnlöv et al. 2005), and in subjects with CKD stage 1–3 (Brantsma et al. 2008).


2.3  Endogenous lipid metabolism

2.3.1  Lipids and lipoproteins

In the human body, lipids are needed as a source of energy, as components of cell membranes, and as precursors for steroid hormones and bile acids. The major lipids found in human plasma are the TG, phospholipids and cholesterol esters. The plasma lipoproteins are water-soluble macromolecules, which consist of a hydrophobic core of cholesterol esters and TG and an outer coating of free cholesterol, phospholipids and apolipoproteins (apo) (Ginsberg 1998, Mahley et al. 1984). Lipoproteins are classified by their densities after ultracentrifugation, and these can be further subdivided into subfractions (Table 2).
Table 2. The main plasma lipoprotein classes and their properties.

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Density (g/mL)</th>
<th>Main apolipoproteins</th>
<th>Main lipid being carried</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicron</td>
<td>&lt; 0.95</td>
<td>Apo-AI, Apo-AIV, ApoB-48, ApoC-II, ApoC-III, ApoE</td>
<td>TG</td>
<td>absorbed dietary</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TG and cholesterol</td>
</tr>
<tr>
<td>VLDL</td>
<td>&lt; 1.006</td>
<td>ApoB-100, ApoC, ApoE</td>
<td>TG</td>
<td>hepatic synthesis</td>
</tr>
<tr>
<td>IDL</td>
<td>1.006–1.019</td>
<td>ApoB-100, ApoC, ApoE</td>
<td>cholesterol</td>
<td>VLDL degradation</td>
</tr>
<tr>
<td>LDL</td>
<td>1.019–1.063</td>
<td>ApoB-100</td>
<td>cholesterol</td>
<td>IDL degradation</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>1.051–1.082</td>
<td>ApoB-100, apo(a)</td>
<td>cholesterol</td>
<td>hepatic synthesis</td>
</tr>
</tbody>
</table>

Adapted from Gotto et al. 1986 and Mahley et al. 1984.

Lp(a) is an LDL-like particle consisting of an additional apolipoprotein, apolipoprotein(a) (Berg 1963, Scanu 1990). Chylomicrons and very low-density lipoprotein (VLDL) particles transport TGs, whereas LDL and HDL are carriers of cholesterol. The apolipoprotein constituents vary between the lipoprotein classes and determine the specific roles of these lipoproteins in the lipid metabolism, for example in the transport and redistribution of lipids in different tissues. This review will concentrate specifically on LDL.

2.3.2 Metabolism of apo B-containing lipoproteins

The diet and the bile are the major sources of intestinal lipids, which are packaged into chylomicrons within the enterocytes and transported via the lymph to the circulation. In the plasma, the TG in chylomicrons is hydrolyzed by endothelial lipoprotein lipase (LPL), which is activated by apoC-II. This then results in the formation of cholesterol-rich remnant particles, which are removed from the circulation to the liver by remnant or LDL receptors that recognize apoE. The cholesterol in the liver is derived either from the lipoproteins (chylomicron remnants, VLDL remnants, LDL and HDL) or from hepatic cholesterol synthesis. β-Hydroxy-β-methylglutaryl-coenzymeA reductase (HMG CoA reductase) is a glycoprotein found in the endoplasmic reticulum of all cells, especially in the liver, small intestine, adrenals and gonads. This enzyme possesses the ability to
synthesize cholesterol via the conversion of acetate to mevalonic acid, and its activity is down-regulated by its end-product, cholesterol. The hepatocytes synthesize VLDL and these lipoproteins are secreted into the plasma. LPL hydrolyzes the TGs in VLDL to free fatty acids and glycerols and thus renders them smaller in size (VLDL remnants) and it gives rise to IDL particles. Part of the circulating VLDL remnants or IDL is recognized by the LDL receptor (B/E receptor) in liver. Most of the IDL is catabolized into LDL by hepatic lipase (HL). LDL is the major cholesterol-carrying particle in plasma, and it is transported from the circulation into the liver and peripheral tissues by LDL receptors. LDL receptor-related protein is a member of the LDL receptor gene family, and it recognizes many ligands, including chylomicron remnants (Herz & Strickland 2001; for a more detailed introduction to general lipoprotein metabolism see Ginsberg 1998, Goldstein & Brown 2009, Vaziri 2006).

HDL is synthesized in both the liver and the small intestine and it appears as bilayered discs. Lecithin: cholesterol acyltransferase (LCAT) esterifies the cholesterol in the HDL, and with further apolipoprotein changes, the HDL particle becomes spherical. HDL plays a major role in the mobilization of cholesterol from the tissues (reverse cholesterol transport), as cholesterol ester transfer protein (CETP) transfers cholesterol esters to other lipoproteins and new free cholesterol is attached to HDL (Ginsberg 1998, Vaziri 2006).

**Determinants of the LDL cholesterol concentration**

The major determinants of the plasma concentrations of LDL cholesterol are the production of apoB-100, the conversion of VLDL to LDL, and LDL receptor mediated clearance. Additional factors include features of cholesterol absorption, and of enterohepatic cholesterol metabolism and genes affecting the proteins involved in the LDL metabolism.

**Production of apoB-100 and LDL cholesterol**

ApoB-100 is produced by the liver and secreted in VLDL. The concentrations of apoB-100 and LDL cholesterol have been found to be regulated mainly by the apoB-100 synthetic rate (Kesäniemi & Grundy 1982). The LDL synthesis is dependent on the rate of the VLDL synthesis and the proportion of VLDL that is converted into LDL (Kesäniemi et al. 1985, Reardon et al. 1978). In familial hypercholesterolemia (FH), direct secretion of LDL was demonstrated many
years ago (Soutar et al. 1977). The absolute amount of apo B per LDL is constant and similar to that in VLDL, suggesting that each LDL particle is derived from the catabolism of a single VLDL particle (Sigurdsson et al. 1975). The primary function of LDL is to transport cholesterol to the peripheral target cells (Ginsberg 1998).

**Metabolism and clearance of apoB-100 and LDL cholesterol**

The major determinant of the LDL cholesterol concentration in plasma is the rate of its clearance in addition to the rate of LDL synthesis. Normally, the fractional catabolic rate of LDL apo B (LDL FCR) is 0.3–0.4 (30–40% of the intravascular pool is catabolized per day) with around half passing through the receptor-mediated pathway (Dietschy et al. 1993, Kesäniemi & Grundy 1982, Kesäniemi et al. 1985, Langer et al. 1972, Teng et al. 1986). In addition, some LDL may be removed by scavenger receptors, which are responsible for the clearance of modified LDL, or by non-receptor-mediated pathways (Dietschy et al. 1993, Greaves & Gordon 2009). It was previously shown that patients with FH exhibit low LDL FCRs (0.19 pools/day), whereas the mean LDL FCR was 0.34 pools/day in obese subjects and 0.41 pools/day in patients with CHD (Kesäniemi et al. 1985, Teng et al. 1986). There is a report that in patients with non-insulin-dependent diabetes mellitus, LDL FCR is decreased by 28% (Duvillard et al. 2000).

**LDL receptor**

The LDL receptor is a single-chain transmembrane protein with five distinct domains which recognize both apoB-100 and apoE (B/E receptors) and thereby bind LDL by means of a high-affinity, saturable mechanism. The bound LDL is incorporated into the cell, and LDL becomes dissociated from its receptor, which then recycles to the cell surface, whereas the LDL undergoes lysosomal digestion. ApoB is degraded and cholesterol esters are hydrolyzed. The free cholesterol then controls the rate of cholesterol synthesis within the cell by down-regulating the enzyme HMG CoA reductase. Excess free cholesterol is re-esterified within the cell by acyl: cholesterol acyltransferase (ACAT). The rate of synthesis of LDL receptors is regulated by a feedback mechanism linked to the cholesterol content of the cell. One major role of the LDL receptor is to provide a constantly available source of cholesterol for cell membrane synthesis throughout the body.
Furthermore, cholesterol is supplied to various organs which require the sterol as a substrate for their metabolic products, e.g. bile acids, sex hormones and corticosteroids. LDL receptors are mainly expressed in the liver because of its size, but they can also be found on the surface of most other cells, especially in the gonads and the adrenals. Mutations of the gene encoding the LDL receptor result in impaired degradation of LDL and eventually FH. In addition to the genetic factors, other modifications of LDL or the LDL receptor as well as features of dietary fat can influence LDL clearance (Goldstein & Brown 2009, Hörkkö et al. 1992, Hörkkö et al. 1994a, Kesäniemi et al. 1987, Stone et al. 1994).

The acetyl LDL or scavenger receptor is found in macrophages and hepatic endothelial cells and it binds and degrades chemically modified LDL including acetylated and oxidized LDL (Greaves & Gordon 2009). The scavenger receptor has been proposed to play a key role in atherogenesis. Modifications of LDL have in turn been suggested to influence the scavenger receptor mediated LDL clearance. In an in vitro study, a minor modification of LDL resulted in reduced recognition whereas a more extensive modification caused increased recognition by human macrophages (Gonen et al. 1983).

The coordinated action of HMG CoA reductase with the LDL receptor expression ensures an adequate supply of cholesterol to cells. Pharmacological agents that inhibit HMG CoA reductase reduce endogenous cholesterol synthesis and thereby stimulate LDL receptor activity, resulting in increased LDL uptake and a decline in plasma LDL cholesterol concentrations.

2.3.3 Lipoprotein metabolism in CKD with special emphasis on LDL apo B metabolism

The review will focus on renal patients on conservative or dialysis treatment, and therefore the nephrotic syndrome, proteinuria or renal transplantation will not be discussed.

Abnormalities in plasma lipids and lipoproteins in CRI

CRI is characterized by multiple abnormalities both in the plasma lipoproteins and the apolipoproteins. The common features of dyslipoproteinemia in CRI are presented in Table 3. In general, patients on dialysis treatment present with lower concentrations of HDL and higher concentrations of total TGs, VLDL or IDL.
cholesterol as compared to healthy controls. These changes are already present in mild renal insufficiency, but there is a tendency towards increased severity with further impairment of the renal function.

Table 3. The common features of dyslipoproteinemia in CRI.

<table>
<thead>
<tr>
<th>Lipid or lipoprotein</th>
<th>Change</th>
<th>Apolipoprotein</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>↑</td>
<td>ApoA-I</td>
<td>↓</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>↑→↓↔ 1</td>
<td>ApoA-II</td>
<td>↓</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>↑</td>
<td>ApoB</td>
<td>↑→↓↔ 2,3,5</td>
</tr>
<tr>
<td>IDL cholesterol</td>
<td>↑</td>
<td>ApoC-II</td>
<td>↓</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>↑→↓↔ 1,2,5</td>
<td>ApoC-III</td>
<td>↑</td>
</tr>
<tr>
<td>small dense</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chylomicrons</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


The plasma concentrations of apolipoproteins are also altered in renal patients as presented in Table 3. As the plasma lipid levels are frequently normal in CRI patients, the significance of apolipoprotein derangements as markers of dyslipoproteinemia has been emphasized. In general, it has been proposed that a decrease in the apoA-I to apoC-III ratio should be considered as a marker of abnormal lipoprotein metabolism in CRI (Attman & Alaupovic 1991, Samuelsson et al. 1994).

Pathophysiology of renal dyslipoproteinemia

The ultimate pathophysiology of the lipid abnormalities in CRI (renal dyslipoproteinemia) remains poorly understood. The effects of comorbidities and their treatment (diabetes mellitus, proteinuria, infections, malignancies, liver diseases, obesity, treatment with steroids, lipid lowering drugs, treatment for uraemia) are also difficult to distinguish from the effect of reduced renal function. The decreased levels of apoA-I were found to be a result of increased rate of catabolism in HD patients, whereas the reduced apoA-II levels were the result of a decreased rate of production (Okubo et al. 2004). Lp(a) metabolism is partly regulated by renal uptake (Kronenberg et al. 1997), which can account for some of the increased Lp(a) concentrations observed in patients with CRI (Oida et al.)
Many derangements in the enzymes of the lipid metabolism have been found in patients with CRI, and the major alterations are presented in Table 4.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Alteration</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCAT</td>
<td>↓</td>
<td>decrease in HDL cholesterol</td>
</tr>
<tr>
<td>CETP</td>
<td>↑↔</td>
<td>decrease in HDL cholesterol</td>
</tr>
<tr>
<td>LPL</td>
<td>↓</td>
<td>decreased catabolism of TG-rich lipoproteins</td>
</tr>
<tr>
<td>HL</td>
<td>↓</td>
<td>increase in IDL, increase in HDL and chylomicron remnant TG</td>
</tr>
<tr>
<td>ACAT</td>
<td>↑</td>
<td>decrease in HDL cholesterol</td>
</tr>
<tr>
<td>HTGL</td>
<td>↓¹</td>
<td>accumulation of TG-rich LDL</td>
</tr>
<tr>
<td>DGAT</td>
<td>↓</td>
<td>decreased TG content in VLDL</td>
</tr>
</tbody>
</table>


In a study conducted in rats, hepatic LDL receptor related protein was found to be down-regulated in CRI, which can partly account for the elevation of plasma concentrations of chylomicron remnants and also other lipoproteins in CRI (Kim & Vaziri 2005). PTH affects lipid metabolism, particularly the lipolytic activity, and secondary hyperparathyroidism has been pointed out as one mechanism behind uremic dyslipidemia (Drüeke & Lacour 1985, Lin et al. 1994). The metabolism of postprandial lipoprotein has been shown to be abnormal in dialysis patients resulting in increased levels of chylomicron remnants for a prolonged period of time and accumulation of chylomicrons in the circulation (Weintraub et al. 1992). The exact mechanism of the defective chylomicron remnant uptake by the liver remains unclear.

**LDL apo B metabolism in CRI**

HMG CoA reductase is the rate-limiting enzyme for cholesterol biosynthesis. In a study conducted in rats, the expression or the activity of the HMG-CoA reductase gene did not differ between the CRI and sham-operated control animals, even though the former were hypercholesterolemic (Liang & Vaziri 1997). In patients with CRI, LDL apo B production rates were equal to those of the control subjects.
(Hörkkö et al. 1994b), whereas in HD or PD patients, significantly lower LDL apo B production rates were observed (Hörkkö et al. 1995).

In kinetic studies in patients with severe CRI not yet on dialysis, the clearance of LDL was significantly decreased as compared with control subjects. Moreover, this phenomenon was seen both in autologous-LDL and control-LDL (LDL from control subjects studied in the CRI patients) suggesting that the defect is present in both the LDL particle and the LDL receptor (Hörkkö et al. 1994b). Furthermore, LDL isolated from dialysis patients or one and two-thirds nephrectomized animals displayed reduced clearance in vivo from animal plasma (Hörkkö et al. 1994a, Shapiro 1993). In HD patients, the clearance of LDL was found to be similar or reduced as compared with control subjects, whereas the LDL FCR was markedly decreased in PD patients (Hörkkö et al. 1995, Ikewaki et al. 2005). The above-mentioned kinetic studies were performed in patients with severe CRI (CKD stage 4–5), while data is lacking on patients with less advanced renal disease.

Chemical modifications of the lipoproteins may influence their metabolism, and one possible mechanism behind this is the carbamylation of LDL, which results from constant exposure and reaction with urea-derived cyanate. The concentration of carbamylated LDL was shown to be significantly increased in HD patients as compared with control subjects (Apostolov et al. 2005), and carbamylated LDL prevented the binding of LDL to the cell surface receptors in human fibroblasts (Weisgraber et al. 1978). Moreover, extensive carbamylation of LDL was shown to accelerate the LDL clearance, while a minor alteration decreased the LDL removal (Hörkkö et al. 1992). One could speculate that a minor modification does not cause LDL to be recognized by the scavenger receptor, but reduces its interaction with the B/E receptor finally resulting in an increase in the residence time in the subendothelial tissue, which may increase the atherogenicity of the LDL particle (Gonen et al. 1985). Like other serum proteins, lipoproteins are non-enzymatically glycated in the presence of glucose to form advanced glycosylation end products (AGEs). A marked increase in the level of AGEs has been detected in renal patients (Meerwaldt et al. 2009), and it has been shown that circulating AGEs react with plasma lipoproteins to prevent their recognition by tissue LDL receptors (Bucala et al. 1994). Although oxidative stress is increased in renal impairment, studies assessing the oxidation of LDL in patients with CRI have yielded contrasting results (Annuk et al. 2001, Bergesio et al. 2001, Hasselwander & Young 1998, Loughrey et al. 1994, Maggi et al. 1994a,
2.4 Leptin

2.4.1 Overview of the biology of leptin

Leptin is a 16 kDa (167 amino acid) protein secreted primarily by the white adipose tissue (Greek leptos – meaning thin). Leptin regulates food intake and energy expenditure and its levels are strongly directly correlated with the body mass index (BMI), percent body fat and total fat mass. It was identified in 1994 in ob/ob mice, which are morbidly obese due to inherited lack of leptin (Zhang et al. 1994). In these animals, supplementation of leptin resulted in a reduction of body weight and subsequent correction of their metabolic abnormalities. Leptin is comprised of four α-helices and two short β-strands, and it has an intra-chain disulfide bond. The size of the body fat stores and the adipocyte size regulate the leptin expression transcriptionally. Leptin expression and serum levels increase after food intake, whereas leptin expression is suppressed during food restriction. Leptin gene expression is regulated in a reciprocal fashion by PPARγ (peroxisome proliferator-activated receptor) (decreased) and C/EPBα (CAAT/enhancer-binding protein) (induced), two transcription factors which control adipocyte differentiation. Leptin is also regulated by genetic factors, and numerous hormonal and environmental factors. There are at least six isoforms of the leptin receptor, and they are found in different tissues throughout the body and though different levels of expression. Leptin circulates in two forms, i.e. free and bound leptin (Sinha et al. 1996). The main binding protein is identical to the extracellular domain of the leptin membrane receptor (Tartaglia 1997). Leptin is involved in carbohydrate and lipid metabolism, the reproductive system, the inflammatory and immune reactions, the sympathetic nerve activity, the haematopoiesis and the angiogenesis. The biology of leptin has been reviewed by Auwerx & Staels 1998, Beltowski 2006a, Peelman et al. 2004, and Reidy & Weber 2000.

Humans with a complete deficiency of leptin due to mutations in the leptin gene are morbidly obese from infancy and display multiple hormonal abnormalities. In these children, treatment with recombinant human leptin has beneficial effects on the appetite, fat mass, hyperinsulinemia, and hyperlipidemia.
In patients with severe lipodystrophy and low leptin levels, leptin treatment improved glycemic control and lipid concentrations (Oral et al. 2002).

### 2.4.2 Leptin and plasma lipids and lipoproteins

In subjects with genetically low leptin levels, treatment with leptin resulted in a marked fall in the TG concentrations (Oral et al. 2002). In a study in the general population, leptin levels did not correlate with total, LDL or HDL cholesterol or TGs (Ostlund et al. 1996), whereas in some other studies, positive correlations were found between leptin and total cholesterol or TGs, and a negative correlation between leptin and HDL (Chan et al. 2005, Couillard et al. 1998, Iribarren et al. 2007, Lawlor et al. 2007, Sattar et al. 2009, Wallace et al. 2001, Wannamethee et al. 2007).

There may be several mechanisms by which leptin can influence lipid metabolism both directly or indirectly through central or peripheral actions. In mice with diet-induced lipodystrophy and decreased leptin levels, leptin infusion resulted in suppression of the hepatic TG synthesis (Nagao et al. 2008). In fat Zucker rats with increased leptin levels, the basal expression of hepatic LDL receptors was lower than in lean rats, but accumulation of LDL apo B100 was not increased. The increased concentration of plasma TG in obese rats was due to plasma VLDL accumulation (Liao et al. 1997). Chronic leptin administration or transgenic overexpression of leptin decreased HDL and TGs in mice models (Matsuoka et al. 2001, Silver et al. 1999). In leptin deficient mice, leptin was found to promote hepatic HDL clearance by up-regulating the scavenger receptor type B1 which in turn led to decreases in the plasma HDL concentrations (Lundåsen et al. 2003). Decreased HDL levels may result in impaired cholesterol removal from the peripheral tissues. In human monocytes, leptin was found to be able to increase the endogenous cholesterol synthesis in vitro, this being more pronounced in hypercholesterolemic cells. Furthermore, this effect was involved in the enhancement of HMG CoA reductase activation (Kosztáczky et al. 2007). In humans with the metabolic syndrome, leptin was positively associated with desmosterol, a surrogate marker of cholesterol synthesis (Hallikainen et al. 2007). Leptin levels have been found to correlate with the levels of oxidized LDL in postmenopausal women (Porreca et al. 2004), pointing to a link between leptin and atherosclerosis. An association analogous to this was found between leptin levels and urinary isoprostane levels as a marker of oxidative stress, although in
that study the association turned insignificant after adjustment for BMI (Nakanishi et al. 2005).

2.4.3 Leptin and atherosclerosis

In vivo studies in mice have demonstrated that leptin is involved in atherogenesis (Bodary et al. 2005), and in human studies, leptin levels were positively associated with the risk of CHD or coronary artery calcification, although contrasting results also exist (Brennan et al. 2007, Couillard et al. 1998, Iribarren et al. 2007, Söderberg et al. 1999, Wallace et al. 2001). As reviewed earlier (Beltowski 2006a, Fantuzzi & Mazzone 2007, Peelman et al. 2004), leptin may be linked with atherogenesis by several direct or indirect actions, for instance by effects on the endothelium, on haemostatic factors or on traditional risk factors of atherosclerosis such as hypertension or lipid metabolism. For example, a significant association has been found between leptin levels and BP independent of body mass (Barba et al. 2003, Beltowski 2006b).

2.4.4 Leptin and kidneys

Hyperleptinaemia is a known feature of CRI, and is observed in patients with different stages of CKD on conservative treatment, HD or PD (Dagogo-Jack et al. 1998, Heimbürger et al. 1997, Merabet et al. 1997, Nordfors et al. 1998, Sharma et al. 1997). In adults with normal renal function, the leptin concentration decreased by 12% when it passed through the kidney circulation, whereas in patients with mild or moderate renal insufficiency there was no clearance of leptin in the kidneys (Sharma et al. 1997). In rat models, the kidneys appear to account for most of the whole body clearance of leptin (Cumin et al. 1997, Zeng et al. 1997). After a successful kidney transplantation, a decline occurred in the leptin concentrations, further emphasizing the major role of the kidneys in the leptin metabolism (Kokot et al. 1998). Serum free leptin concentrations were elevated in HD patients, whereas serum bound leptin concentrations were normal as compared with healthy controls (Widjaja et al. 2000). It has also been shown that elevated leptin concentrations down-regulate the expression of the ob gene, but that this feedback inhibition can be overcome by the inflammatory cytokines which are present in CKD (Nordfors et al. 1998). CRI has been proposed to be a state of leptin resistance, as most patients do not have apparent undernourishment (Rodríguez-Carmona et al. 2000). In patients on dialysis, particularly those on
PD, however, leptin concentrations displayed a negative correlation with the nutritional status pointing to a possible role of leptin in the poor nutrition associated with uraemia (Aguilera et al. 2002, Johansen et al. 1998). Although elevated, leptin concentrations remain correlated with BMI or percent body fat in renal patients as they are in the general population (Coen et al. 2003, Dagogo-Jack et al. 1997, Heimbürger et al. 1997, Menon et al. 2004, Nordfors et al. 1998, Pérez Fontán et al. 1999, Rodríguez-Carmona et al. 2000, Stenvinkel et al. 1997). The gender difference, i.e. higher leptin levels in females, has also been found to remain intact among renal patients (Coen et al. 2003, Menon et al. 2004, Nakazono et al. 1998, Ostlund et al. 1996, Pérez Fontan et al. 1999). HD with polysulfone membrane-dialyzers in high-flux dialysis decreased serum leptin levels as compared with cellulose membranes (Nakazono et al. 1998). Patients on PD have even more elevated leptin concentrations than patients on HD or on conservative treatment (Díez et al. 2005, Johansen et al. 1998, Pérez Fontán et al. 1999, Rodriguez-Carmona et al. 2000, Taskapan et al. 2007). In PD, the body is confronted with an almost continuous load of glucose, resulting in chronic hyperinsulinaemia. It is noteworthy that insulin has been shown to regulate plasma leptin concentrations (Utriainen et al. 1996), and in PD patients, intraperitoneally administered insulin therapy resulted in a reduction in plasma leptin concentrations as compared with subcutaneous insulin (Nevalainen et al. 2000).

Given the role that the kidneys play in the catabolism of leptin, it would be anticipated that the leptin concentrations would correlate with renal function. In some earlier studies, a weak yet significant negative correlation was found, in some studies even after partial adjustment for BMI, percent body fat or gender (Menon et al. 2004, Nordfors et al. 1998, Rodríguez-Carmona et al. 2000, Sarnak et al. 2002, Shoji et al. 1997). In most reports on CRI patients not yet on dialysis, renal function has not correlated with the leptin levels or the correlation turned insignificant when adjusted for BMI (Chan et al. 2004, Clausen et al. 1999, Heimburger et al. 1997, Iida et al. 1996, Pérez Fontán et al. 1999, Young et al. 1997).

### 2.4.5 Leptin and lipids in CKD

Associations between leptin and lipoprotein or lipid concentrations have been studied in renal patients. In CRI patients not yet on dialysis, the concentrations of leptin were found to positively correlate with cholesterol and TGs (Rodriguez-
Carmona et al. 2000), although other studies including similar patients failed to
detect a significant correlation (Diez et al. 2005, Heimbürger et al. 1997). In HD
or PD patients, leptin showed a positive correlation with total and LDL
cholesterol as well as TGs (Aguilera et al. 2002, Nakazono et al. 1998, Taskapan
et al. 2007). There are also reports in which leptin did not correlate with the lipid
or the lipoprotein concentrations in dialysis patients (Diez et al. 2005, Rodríguez-
Carmona et al. 2000). In most of the afore-mentioned studies, the conclusions
were drawn on the basis of univariate analyses. It is notable that the association
between leptin and the lipoprotein profile has not been studied in CKD patients
with less severe renal impairment.

2.4.6 Leptin and cardiovascular risk in CKD

There are limited data on the associations between leptin concentrations,
lipoprotein metabolism and cardiovascular risk in renal patients. In one study
examining dialysis patients, increased leptin concentrations were associated with
increased risk for cardiovascular events but only in patients with a BMI higher
than 25 kg/m² (Mallamaci et al. 2005); yet in patients with severe CRI (most
treated with dialysis) leptin levels were not correlated with the prevalence of
vascular disease (Aguilera et al. 2002, Diez et al. 2005). As discussed earlier,
leptin has several effects on the human body. In HD patients, an inverse
relationship between serum PTH and the leptin concentration has been reported
(Zoccali et al. 2004), whereas in another study on HD patients, the serum leptin
concentration was inversely related to bone resorption and bone formation (Coen
et al. 2003). Adynamic bone disease with its typical features such as low serum
PTH concentrations and decreased bone metabolism has recently been suggested
as a potential risk factor for CVD (Andress 2008).

2.5 Diurnal blood pressure variation

2.5.1 Measurement of blood pressure

BP refers to the force that blood exerts against the vessel wall. Systolic BP is the
peak BP measured during the systole of the heart, and diastolic BP is the lowest
BP measured during the diastole of the heart. The BP is determined by the cardiac
output and the peripheral resistance, and if either or both of these parameters
increase, a rise in BP will be seen. The conventional way to measure BP is to use an inflatable cuff to occlude the blood flow to an extremity – usually the right or the left brachial artery. BP levels are determined either oscillometrically or by detection of the Korotkoff sounds (Beevers et al. 2001). There are many internal and external factors that affect the BP from moment to moment. Ambulatory blood pressure monitoring (ABPM) has been shown to closely relate to organ damage that accompanies hypertension, and it has allowed the identification of many more phenomena in hypertension as compared with office BP (Mancia & Parati 2000, O’Brien et al. 2001).

2.5.2 Dipping pattern in ABPM

In the general population, a characteristic nocturnal fall in BP has been noted. A large number of studies have shown that in hypertensive subjects in whom the nocturnal BP falls by less than 10% (non-dippers), organ damage is greater than in those in whom it falls by more than 10% (dippers). The dipping pattern can be further evaluated based on the change in the systolic BP (systolic dipping), in the diastolic BP (diastolic dipping) or the mean arterial BP. The risk of organ damage progression, the incidence of CVD, and all-cause mortality are greater among non-dippers (Ben-Dov et al. 2007, Brotman et al. 2008, Cuspidi et al. 2003, Mancia & Parati 2000, Ohkubo et al. 1997, Routledge et al. 2007, Verdecchia et al. 1994). Non-dipping is more prevalent in elderly hypertensive subjects, in obstructive sleep apnoea, both types of diabetes mellitus, insulin resistance and different stages of CKD, independently of the mean 24 hour systolic BP. Also salt-sensitivity, sympathetic nerve over-activity, low levels of physical activity, endothelial dysfunction and antihypertensive medications are also associated with loss of diurnal variation of the ABPM. (Davidson et al. 2005, Farmer et al. 1997, Kobrin et al. 1984, Lee et al. 2005, Li Kam Wa et al. 1997, Lurbe et al. 2001, Spallone et al. 2001, Suzuki et al. 1996, Thompson & Pickering 2006.)

2.5.3 Non-dipping in CKD

Non-dipping is common among renal patients, at all CKD stages (Agarwal & Andersen 2005, Farmer et al. 1997, Li Kam Wa et al. 1997, Rahman et al. 2005). In renal patients, non-dipping was found in from 48 to 82% of HD patients, 78% of PD patients and in 53% of conservatively treated patients with CKD stage 2–5, and in 75% of patients who had undergone renal transplantation (Farmer et al.
whereas non-dipping was found in 30% of patients with essential hypertension and normal renal function (Farmer et al. 1997). In HD patients, non-dipping was independently associated with the left ventricular mass index (Rahman et al. 2005). In children with CKD stage 2–4 non-dipping is also common (Mitsnefes et al. 2003). It has been reported that the prevalence of non-dipping increases as the renal function deteriorates in adults and children with CRI (Agarwal & Andersen 2005, Farmer et al. 1997, Mitsnefes et al. 2003). It is still not clear whether non-dipping is an early phenomenon in mild renal dysfunction.

2.5.4 Cardiovascular and renal risks of non-dipping in CKD

There are little data on the effect of non-dipping on the CVD risk in patients with CKD. In a study on conservatively treated CRI patients, non-dipping was associated with increased cardiovascular risk, but not when adjusted for other risk factors (Agarwal & Andersen 2006). In HD patients, the night/day ratio in systolic BP proved to be a significant predictor of all-cause and cardiovascular mortality also in multivariate models (Tripepi et al. 2005). In another study on HD patients, non-dipping was correlated with a high incidence of cardiovascular events and mortality (Liu et al. 2003). The association between ABPM and cardiovascular risk among renal patients has recently been reviewed by Agarwal (2007), Palatini (2008) and Thompson & Pickering (2006).

Systemic hypertension is a well-known risk factor for the deterioration of renal function (Sarnak et al. 2005), but less is known of the impact of the dipping-pattern on renal function in low-risk subjects without any pre-existing renal disease. Non-dipping has been shown to result in a faster decline in renal function among patients with CRI (Timio et al. 1995) or type 2 diabetes (with CKD stage 1) (Knudsen et al. 2009), but also in subjects with minimally deteriorated renal function, although in that study most of the patients had hypertension and many had diabetes or prior atherosclerotic disease (Davidson et al. 2006). In patients with type 1 diabetes and who displayed a normal circadian variation in BP, the progression from normoalbuminuria to microalbuminuria was less likely (Lurbe et al. 2002, Poulsen et al. 1994). In a study on patients with type 1 diabetes, non-dipping status was associated with more extensive renal morphological changes (Torbjörnsdotter et al. 2004). Non-dipping may increase the risk of deterioration of the renal function through many mechanisms. Non-dipping was associated with increased proteinuria in subjects with normotension or hypertension (Soylu
et al. 2009, Tsioufis et al. 2002) and in patients with CKD (stages 1–5 not on dialysis) (Agarwal & Andersen 2005, Agarwal & Light 2009, Poulsen et al. 1997). It is noteworthy in this respect that proteinuria is a risk factor for impaired renal function. Non-dipping also associates with increased sympathetic nerve activity (Sherwood et al. 2002), which in turn diminishes the renal sodium excretion and decreases the GFR by evoking renal vasoconstriction (DiBona 2002).

2.6 Carotid intima-media thickness (cIMT)

2.6.1 cIMT as a risk factor for cardiovascular disease

The arterial wall consists of three layers: intima, media and adventitia. Atherosclerotic changes in the arterial wall can be divided into six types (Table 5). There are many non-invasive markers to assess arterial wall disturbances, including arterial wall thickening and stiffening, endothelial dysfunction and coronary artery calcification (O’Rourke & Hashimoto 2007, Simon et al. 1997).

Carotid intima-media thickness (cIMT) is increasingly used as a surrogate marker of early atherosclerosis, and in a recent review it was shown that cIMT is a strong predictor of future vascular events such as myocardial infarction and stroke (Lorenz et al. 2007). In addition, the presence and area of carotid plaques are strong predictors of CVD (Ebrahim et al. 1999, Johnsen et al. 2007).

Table 5. The types of atherosclerotic lesions in the arterial wall.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Very initial changes, increase in the number of intimal macrophages, macrophages filled with lipid droplets (foam cells)</td>
</tr>
<tr>
<td>II</td>
<td>Fatty streak lesions, layers of foam cells and lipid droplets within intimal smooth muscle cells</td>
</tr>
<tr>
<td>III</td>
<td>Intermediate lesions, Type II changes and accumulation of extracellular lipid droplets and particles that disrupt the coherence of intimal smooth muscle cells</td>
</tr>
<tr>
<td>IV</td>
<td>Dense extracellular lipid accumulation (lipid core) in the intima, atheroma. May be called fibrous plaque. May develop fissures or haematoma.</td>
</tr>
<tr>
<td>V</td>
<td>Prominent fibrous connective tissue as part of a lipid core lesion (fibroatheroma or Type Va lesion), calcification may be present (Type Vb lesion). May develop fissures or haematoma and/or thrombus. May be called fibrous plaque.</td>
</tr>
<tr>
<td>VI</td>
<td>Type V or VI lesions with disruptions of the lesion surface, haematoma or haemorrhage, and thrombotic deposits.</td>
</tr>
</tbody>
</table>

Adapted from Stary et al. 1994 and Stary et al. 1995.
2.6.2 Measuring cIMT with ultrasound

The cIMT is easily, safely, reliably and inexpensively measured with B-mode ultrasound, and the predictive value of this test is increased when cIMT is measured at multiple extracranial carotid sites (Simon et al. 2002). The cIMT can be measured both from the near-wall and the far-wall of the carotid artery. In the far wall, it is usually easy to detect the media-adventitia interface, and this measurement location has been suggested as being more accurate (Wikstrand & Wendelhag 1994, Wong et al. 1993). The normal values of cIMT are dependent on the methodology used for its measurement, and they should be considered taking into account gender and the range of age. The current data indicate that a value of cIMT at or above 1 mm is associated with a significantly increased CVD risk in any age group (Simon et al. 2002).

2.6.3 Association between cIMT and renal function

The cIMT has been found to be increased in subjects with impaired renal function (Ishizaka et al. 2007, Ishizaka et al. 2008, Kawamoto et al. 2008a, Lisowska et al. 2007, Skalska et al. 2007, Zhang et al. 2007, Zoungas et al. 2000), though contrasting results have also been published (Bui et al. 2009). However, these studies have mostly concentrated on elderly subjects or patients with pre-existing CVD, hypertension, diabetes or advanced CRI. There are little data on this association in the middle-aged general population.

2.6.4 Association between cIMT and cardiovascular disease in CKD

The association between cIMT and CVD has to some extent been studied in renal patients. In patients on HD, the cIMT has been found to be a strong predictor of cardiovascular or all-cause mortality (Blacher et al. 2001, Kato et al. 2003, Nishizawa et al. 2003), and in patients with moderate to severe CRI (not on dialysis), cIMT has been claimed to be able to predict CVD (Szeto et al. 2007). In high-risk subjects with diagnosed CHD and impaired renal function, cIMT was also independently associated with the severity of CHD (Lisowska et al. 2009).
3 Aims of the study

The present study was designed to investigate the relationships between renal function and the risk factors of atherosclerosis. The association between renal function and LDL metabolism and the effect of leptin on the lipid profile was studied in patients with CRI (CKD stage 3–5). Furthermore, the effects of mild impairments in the renal function on BP regulation and on carotid atherosclerosis were studied in middle-aged subjects. This thesis specifically addressed the following questions:

1. Is LDL apolipoprotein B metabolism related to the severity of renal insufficiency? (Study I)
2. Is leptin associated with lipid metabolism in CRI? (Study II)
3. Is the dipping pattern associated with renal function in a middle-aged population sample? (Study III)
4. Is mild renal impairment as assessed by eGFR associated with cIMT in a middle-aged population sample? (Study IV)
4  Methods

4.1  Study subjects

The CKD patients aged 18–70 years who had visited the outpatient clinic of the Department of Internal Medicine of the Oulu University Hospital between January 1993 and November 1996 and who had reduced GFR (measured with $^{99}$Tc-DTPA, normal level over 75 mL/min/1.73 m$^2$) (Huttunen et al. 1982) together with either elevated serum creatinine (more than 115 µmol/L) or elevated serum urea (more than 8 mmol/L) concentrations were identified. Subjects with the nephrotic syndrome (defined as proteinuria of 3 g/day or more in conjunction with serum albumin levels of less than 35 g/L and with oedema), FH, liver disease, untreated thyroid disease or alcohol abuse were excluded. Eligible patients (N = 149, mean GFR 40 mL/min/1.73 m$^2$ with a range of 10 - 74 mL/min/1.73 m$^2$) were sent a letter inviting them to participate in the study. 109 (73%) of them expressed initial interest, and these patients were later contacted personally to give them detailed information about the study protocol. Of these 109 patients, 28 were not available for the studies (mean GFR 41 mL/min/1.73 m$^2$ with a range of 11 - 70 mL/min/1.73 m$^2$) and one patient was found to have FH and was therefore excluded. Four patients, who initially enrolled in the study procedure, withdrew before its completion. In one patient, the outcome of the laboratory measurements was contradictory and he was excluded from the analyses. Patients with BMI higher than 30 kg/m$^2$ (N = 18) were excluded from the analyses in Study I, as obesity may interfere with LDL catabolism (Kesäniemi et al. 1985). In two patients, the leptin concentrations were extremely high and did not meet the acceptance criteria given by the assay manufacturer.

Finally, the results of 57 patients were available for Study I (mean eGFR 32 mL/min/1.73 m$^2$ with a range of 7–62 mL/min/1.73 m$^2$) and the results of 73 patients were available for Study II (mean eGFR 33 mL/min/1.73 m$^2$). Among the 57 subjects in Study I, the renal diagnosis was biopsy-proven in 22 patients, confirmed clinically in 25 patients and by radiological evidence in 8 patients whereas in the other patients the renal diagnosis remained uncertain. Thus, it can be concluded that the diagnosis was reasonably or completely ascertained in 43 (75%) patients. Among the 73 subjects in Study II, the renal diagnosis was biopsy-proven in 30 cases, determined clinically in 30 patients, and with radiological evidence in 10 patients. Thus, in 56 patients (77%) the renal
diagnosis was reasonably or completely ascertained. The patients were tested, examined and interviewed (concerning past and current medical history, smoking habits, alcohol consumption and medication) between May 1995 and May 1999 in the research laboratory of the Department of Internal Medicine. None of the patients were receiving lipid lowering drugs at the time of the studies. The patients were consuming their habitual diet, which was not changed during the studies. All of the patients were receiving conservative treatment (not on dialysis or transplantation).

The 10 control subjects for the LDL turnover study (Study I) were healthy subjects with a mean age of 50 years (range 24 to 64 years). The control group was chosen to match the renal group with respect to age and gender distribution, and they were participants of two previously described studies (Hörkkö et al. 1995, Karjalainen et al. 2000).

The population sample consisted of the healthy cohort of The Oulu Project Elucidating Risk of Atherosclerosis (OPERA), which is a population-based epidemiological case-control study addressing the risk factors and disease endpoints of atherosclerotic CVD (Rantala et al. 1999). In the OPERA study, the hypertensive cohort consisted of 300 men and 300 women living in the city of Oulu, whose age on September 1, 1990, was between 40 and 59 years and who, according to the register of the Social Insurance Institution, were entitled to a special refund (higher reimbursement class) for antihypertensive medication endorsed later than August 1980. The subjects were randomly selected by the Social Insurance Institution in 1990. To ensure an adequate recruitment of younger subjects, the randomization was age-stratified, i.e. for each year of birth (1931 to 1950), 5 hypertensive men and 15 hypertensive women were selected. For each hypertensive subject, an age- and sex-matched control subject living in Oulu was randomly selected from the social insurance register (encompassing all inhabitants) excluding subjects entitled to the special refund for hypertension medication. The subjects visited the research laboratory of the Department of Internal Medicine between January 1991 and March 1993. The examinations took place in the morning after an overnight fast, and included blood sample collections, physical examinations and interviews. The women were studied one year later than the men. The final size of the control cohort was 526 subjects.

For Study II, a control group to match the renal patients was randomly chosen from the control cohort of the OPERA study, to achieve a comparable gender distribution and mean age. A total of 73 controls were chosen, but in five of them, the leptin concentrations were missing, so the final control group for Study II
consisted of 68 subjects. None of these control subjects used lipid-lowering drugs, but three subjects were taking acetylsalicylic acid and six subjects were on antihypertensive medication (four on beta blockers, two on calcium channel blockers). Four subjects were receiving hormonal replacement therapy.

All study subjects provided their informed consent for the investigations (the renal patients in writing) and the studies were approved by the Oulu University Ethical Committee. The investigations were carried out according to the principles of the Helsinki Declaration.

### 4.1.1 Main characteristics of the study subjects in Study I and Study II

The clinical characteristics of the renal patients and the control subjects in Studies I and II are given in Table 6. The renal diagnoses, use of selected medications and smoking habits of the renal study patients are presented in Table 7. In the CRI patients in Study I, the range of the serum creatinine concentration was 102–637 µmol/L, and for the eGFR, the range was 7–62 mL/min/1.73 m², and for the serum urea concentration 4.7–29.5 mmol/L (mean 15.5, SD 6.4 mmol/L). The difference between the genders was significant for the mean eGFR (26 mL/min/1.73 m² in females and 36 mL/min/1.73 m² in males, P = 0.012). The mean duration of diabetes was 26 years. The mean age of the control subjects was comparable to that of the CRI patients (P = 0.167).

In Study II, the mean urea concentration was 16.3 (6.4) mmol/L in the female CRI patients and 14.4 (5.6) mmol/L in the males. The distribution of the renal diagnoses was significantly different between the genders, with more males suffering from glomerulonephritis. The prevalence of patients with diabetes was comparable between the genders. In the CRI group, 14% of the males and 3% of the females were current smokers, whereas in the control group 34% of the males and 27% of the females were current smokers. The difference in smoking habits was statistically significant between the CRI patients and the control group (P = 0.006) and between the female study groups (P = 0.038).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Study I</th>
<th></th>
<th>Study II</th>
<th></th>
<th>Study III</th>
<th></th>
<th>Study IV</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRI patients</td>
<td>Controls</td>
<td>CRI patients</td>
<td>Controls</td>
<td>CRI patients</td>
<td>Controls</td>
<td>CRI patients</td>
<td>Controls</td>
</tr>
<tr>
<td>Number</td>
<td>57</td>
<td>10</td>
<td>73</td>
<td>68</td>
<td>460</td>
<td>505</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender, F/M</td>
<td>21/36</td>
<td>4/6</td>
<td>30/43</td>
<td>30/38</td>
<td>234/226</td>
<td>258/247</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56 (12)</td>
<td>50 (14)</td>
<td>57 (11)</td>
<td>57 (5)</td>
<td>52 (6)</td>
<td>52 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 (2.6)</td>
<td>N.D.</td>
<td>26.6 (4.7)</td>
<td>27.4 (4.5)</td>
<td>26.4 (4.0)</td>
<td>26.4 (4.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>228 (124)</td>
<td>N.D.</td>
<td>217 (114)*</td>
<td>74 (10)</td>
<td>80 (13)</td>
<td>80 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR</td>
<td>32 (15)</td>
<td>N.D.</td>
<td>33 (14)*</td>
<td>90 (16)</td>
<td>83 (16)</td>
<td>84 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>152 (21)</td>
<td>N.D.</td>
<td>151 (20)</td>
<td>148 (20)</td>
<td>142 (21)</td>
<td>141 (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>87 (9)</td>
<td>N.D.</td>
<td>88 (10)</td>
<td>88 (10)</td>
<td>85 (12)</td>
<td>85 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>88 (11)</td>
<td>N.D.</td>
<td>93 (14)</td>
<td>90 (12)</td>
<td>87 (12)</td>
<td>87 (21)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers present mean and in parentheses SD. N = 55 for waist circumference and N = 56 for diastolic BP in CRI patients in Study I. Abbreviations: N.D., no data available. * indicates statistically significant difference (P < 0.05) between the CRI patients and the appropriate control group. ¹ eGFR is expressed as mL/min/1.73 m².
Table 7. The diagnoses, use of selected medications, and smoking habits in the CRI patients in Studies I and II.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study I</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>11 (19%)</td>
<td>18 (25%)</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>13 (23%)</td>
<td>15 (21%)</td>
</tr>
<tr>
<td>ADPKD</td>
<td>6 (11%)</td>
<td>8 (11%)</td>
</tr>
<tr>
<td>Other</td>
<td>27 (47%)</td>
<td>32 (44%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>14 (25%)</td>
<td>19 (26%)</td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>54 (95%)</td>
<td>69 (95%)</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>11 (19%)</td>
<td>14 (19%)</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>11 (19%)</td>
<td>11 (15%)</td>
</tr>
<tr>
<td>Lipid lowering drugs</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Smoking habits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>5 (9%)</td>
<td>7 (10%)</td>
</tr>
<tr>
<td>Previous</td>
<td>21 (37%)</td>
<td>26 (36%)</td>
</tr>
<tr>
<td>Never</td>
<td>31 (54%)</td>
<td>40 (55%)</td>
</tr>
</tbody>
</table>

Numbers indicate prevalence (percentage). Abbreviations: ADPKD, autosomal dominant polycystic kidney disease. Diagnosis Other includes tubulo-interstitial nephropathy, atherosclerosis or nephrosclerosis, renal dysgenesis, reflux nephropathy, medullary cystic disease and renal failure of unknown origin.

4.1.2 Main characteristics of the study subjects in Study III and Study IV

In Study III, the study subjects (N = 460) consisted of the entire control cohort of the OPERA study for whom creatinine measurements and ABPM were available. In Study IV, the study subjects (N = 505) consisted of the entire control cohort of the OPERA study except that subjects with lipid lowering drugs, missing creatinine values or missing cIMT measurements were excluded. The baseline and the clinical characteristics of the study subjects in Studies III and IV are presented in Tables 6 and 8.
Table 8. The diagnoses, use of selected medications, and smoking habits in the study subjects in Studies III and IV.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>460</td>
<td>505</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>20 (4%)</td>
<td>20 (4%)</td>
</tr>
<tr>
<td>IHD</td>
<td>30 (7%)</td>
<td>27 (5%)</td>
</tr>
<tr>
<td>Stroke</td>
<td>7 (2%)</td>
<td>6 (1%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7 (2%)</td>
<td>5 (1%)</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>52 (11%)</td>
<td>50 (10%)</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>17 (4%)</td>
<td>15 (3%)</td>
</tr>
<tr>
<td>Lipid lowering drugs</td>
<td>10 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>HRT</td>
<td>57 (12%)</td>
<td>56 (11%)</td>
</tr>
<tr>
<td>Smoking habits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>145 (32%)</td>
<td>169 (33%)</td>
</tr>
<tr>
<td>Previous</td>
<td>94 (20%)</td>
<td>97 (19%)</td>
</tr>
<tr>
<td>Never</td>
<td>221 (48%)</td>
<td>239 (47%)</td>
</tr>
</tbody>
</table>

Numbers indicate prevalence (percentage). Abbreviations: HRT, hormone replacement therapy; IHD, ischemic heart disease. Diabetes is assessed as self-reported diabetes.

4.2 Methods

4.2.1 Anthropometric measurements and other clinical methods

All examinations were made in the morning hours after an overnight fast. At the visit, the anthropometric measurements (weight, height, waist) were carried out by trained study nurses (for the CRI patients, the study physician measured the waist circumference). The office BP was measured with an automatic oscillometric recorder (Dinamap ® model 18465X, Criticon Ltd., Ascot, UK). The subjects were seated for at least 5 minutes, after which the BP was measured three times at one-minute intervals. The mean of the second and the third measurement was used in the analyses. The methods for the anthropometric and clinical studies are listed in Table 9. The interviews were conducted using standard forms, and special attention was paid to the past medical history, symptoms of dyspnoea and chest pain, current medication, family history of cardiovascular and other diseases, smoking habits, alcohol consumption and physical activity. Three physicians with special competence in internal medicine
interviewed and examined all the OPERA study subjects and the CRI patients were interviewed and examined by two physicians.

Table 9. The methods for anthropometric and clinical studies.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>Weight (kg) divided by height squared (m²)</td>
<td></td>
</tr>
<tr>
<td>Waist circumference</td>
<td>Measured to the nearest 0.5 cm with a tape measure midway between the lower rib margin and the iliac crest in light expirium</td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>In the sitting position from the right arm with an oscillometric device after an overnight fast and after a 10 to 15-minute rest</td>
<td>American Society of Hypertension (1992)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>Total amounts of absolute alcohol consumed in a week (g/week) were calculated</td>
<td>Khavari &amp; Farber (1978)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Past and current smoking habits</td>
<td></td>
</tr>
<tr>
<td>Pack-years</td>
<td>1 pack-year = 20 cigarettes smoked/day in one year</td>
<td></td>
</tr>
</tbody>
</table>

4.2.2 Laboratory methods

All of the laboratory test samples were obtained after an overnight fast. Venous blood was drawn into EDTA tubes. Plasma was separated by centrifugation at 2000–2600 rpm for 10 min and kept at 4 °C until further analyses, most of which were done within two days after the blood samples had been drawn. If not, plasma was stored at −20…−70 °C prior to subsequent analyses.

Methods to estimate renal function

Serum urea and creatinine concentrations were determined at the Central Laboratory of the Oulu University Hospital. Serum creatinine was determined with a method based on the Jaffe reaction. Estimated GFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula (Studies I to IV):

\[
eGFR \ (\text{mL} / \text{min} / 1.73 \text{m}^2) = 186 \times (\text{serum creatinine in mg/dL}) - 1.154 \times (\text{Age}) - 0.203 \times (0.742 \text{ if female}).
\]

Creatinine clearance was calculated by using the Cockcroft-Gault equation:
Creatinine clearance (mL/min/1.73 m²) =
\[
\frac{(140 - \text{Age}) \times \text{Weight} \times 88.4}{(\text{serum creatinine in mg/dL}) \times 72} \times 0.85 \text{ if female}
\]
adjusted for body surface area (CrclBSA) (Study IV) (Levey et al. 2003).

**Lipids and lipoproteins**

Total plasma cholesterol and TG concentrations were determined by enzymatic colorimetric methods (kits from Boehringer Diagnostica, Mannheim, Germany, catalogue nos 236691 and 701912, respectively) using a Kone Specific Analyser (Kone Specific, Selective Chemistry Analyser, Kone Instruments, Espoo, Finland). VLDL, IDL and LDL cholesterol were isolated by repeated ultracentrifugations according to their densities as described in the Lipid Research Clinics Program Manual of Laboratory Operations (1974). To compensate for the lipoprotein losses in the LDL and HDL fractions during the sequential ultracentrifugations, the VLDL fraction was first isolated from a separate simultaneous plasma sample as described above and HDL cholesterol was determined from the remaining infranatant (VLDL-infranatant) after precipitation of LDL with heparin-manganese chloride (Bachorik & Albers 1986). The LDL cholesterol concentration was calculated by subtracting the HDL cholesterol and the IDL cholesterol ( ultracentrifugally isolated) from the total cholesterol content of the VLDL-infranatant. The lipid and protein concentrations in the LDL and HDL fractions were then corrected with the ratio of the LDL (or HDL) cholesterol concentration measured with heparin-manganese precipitation to the LDL (or HDL) cholesterol concentration which had been determined after repeated ultracentrifugations. The protein contents of the lipoprotein fractions were measured by the method of Lowry et al. (1951). The plasma apolipoprotein B was measured using isopropanol precipitation (Egusa et al. 1983). Chol/HDL was determined as the ratio of total to HDL cholesterol.

**Clearance and production of LDL apo B**

In the LDL turnover study, 100 ml of fasting blood was drawn for the isolation of LDL, and the LDL protein was labelled with \(^{125}\text{I}\) by use of the iodine monochloride method of McFarlane (McFarlane 1958) as modified by Bilheimer et al. (1975). Radiolabeled LDL was injected in the morning on the day after iodination. Blood samples were collected at 0, 15, and 30 minutes and at 1, 2 and
3 hours, and thereafter three times a week for 14 days after the injection. The radioactivity of the total plasma was measured in each sample. The LDL FCR was calculated from the plasma decay curves as previously described (Langer et al. 1972, Matthews 1957, Nosslin 1964). The LDL apolipoprotein B production rates were calculated from the LDL FCRs, pool volumes and apolipoprotein B concentrations and expressed as milligrams of LDL apo B produced per day normalized for body weight in kilograms.

**Determination of leptin and the QUICK Index**

Plasma leptin concentrations were measured using a commercial double-antibody RIA (Human Leptin RIA Kit; Linco Research, Ind., St. Charles, Mo, USA). The intra- and interassay coefficients of variation for the test were 3.4–8.3% and 3.0–6.2%, respectively. Leptin/BMI was calculated as the ratio of the leptin concentration to BMI. The QUICKI (Quantitative insulin sensitivity check index) was calculated as

\[ \text{QUICKI} = \frac{1}{\log(\text{fasting insulin}) + \log(\text{fasting glucose})} \]

(Katz et al. 2000).

**4.2.3 ABPM**

ABP was recorded using the fully automatic SpaceLabs 90207 oscillometric unit (SpaceLabs Inc., Redmond, Washington, USA), which was set to take a measurement every 15 minutes from 4:00 AM till midnight and every 20 minutes from midnight till 4:00 AM. The accuracy and reproducibility of the BP readings obtained with this device have been validated previously to fulfil the criteria of the British Hypertension Society and the US Association for the Advancement of Medical Instrumentation (O’Brien et al. 1993). The correct positioning of the cuff in each case was ensured by similarity of the means (difference < 5 mmHg) between four SpaceLabs BP measurements and four auscultatory readings using a Y-connector. The subjects were asked to relax their arm during the measurement. Values of systolic BP < 70 or > 250 mmHg, diastolic BP < 40 or > 150, and heart rate < 40 or > 150 beats per minute were automatically excluded from the analyses. Fewer than 3% of the BP readings were rejected as artefacts on the basis of these criteria. A reduction in the systolic BP values from day-time to night-time of less than 10% was considered to represent a non-dipping pattern.
4.2.4 Ultrasonic imaging

The IMT of the carotid arteries was measured by a trained radiologist without knowledge of the clinical data. This measurement was conducted within 6–12 months after the first visit to the research unit. IMT, defined as the distance between the lumen-intima interface and the media-adventitia interface, was measured with a duplex ultrasound system with 7.5MHz scanning frequency in the B-mode, pulsed Doppler mode and colour mode (Toshiba SSA-270A, Toshiba Corp., Tokyo, Japan). The ultrasonographic assessment was performed with the subject in the supine position with his head turned away from the sonographer at an angle of 45°. Each scan of the common carotid artery (CCA) began just above the clavicle and moved past the bifurcation enlargement (BIF) and along both the internal carotid artery (ICA) and the external branches as far distally as possible. The whole scanning procedure was recorded on a Super-VHS videocassette recorder (Panasonic AG-7330, Matsuhita Electric Industrial Co., Ltd., Osaka, Japan). The measurements were assessed about one year later from the video image on the monitor of the ultrasound device using its electronic callipers.

The cIMT was measured at five points on each side: ICA about 10mm distal from the BIF, BIF, and three locations of CCA, (proximal, middle and distal) with about 10–15 mm intervals, the most cranial measuring point being about 10mm proximal of the bifurcation (Figure 1). In all, 20 sites were measured but only 10 far-wall measurements were used in these analyses since the near-wall measurements may be difficult to perform accurately (Wikstrand & Wendelhag 1994). The thickest point of the IMT was measured at each site, avoiding the sites of plaque. The mean cIMT was defined as the mean of ICA, BIF, and the 3 highest CCA measurements.
Data were analyzed using the software package SPSS for Windows (16.0). Results for the continuous variables are presented as mean +/- standard deviation (S.D). The genders were analysed separately if there was a significant interaction between the gender and the independent variable as a predictor of the outcome variable.

To compare the means of the variables measured, Student’s $t$-test or analysis of variance (ANOVA) were used. When appropriate, non-parametric tests (Mann-Whitney or Kruskal-Wallis) were used. Post hoc tests were performed using Bonferroni correction for multiple comparisons. A $\chi^2$-test was carried out to assess the frequency differences in the sex ratio. In case of a skewed distribution, natural logarithmic transformation was performed as appropriate. Pearson’s or Spearman’s correlation coefficients were calculated to reveal any associations between the variables. Age and BMI were controlled for when examining the association between leptin and lipoproteins and lipids. Stepwise linear regression analyses were used to explain the variation in LDL FCR (Study I) and various lipoprotein and lipids (Study II). The effects of the confounding factors on dependent variables were controlled for by adding them into the multivariate models (age, smoking and gender). $R^2$ and standardised regression coefficients ($\beta$) were shown to demonstrate the effects of the independent variables on the
dependent variables. In Study III, a general linear model and a logistic regression model (with stepwise method) were created to study the association between the dipping status and the variation in eGFR. The association between renal function and risk of non-dipping status was studied with a general linear model with adjustments for gender, age, BMI or QUICKI (as BMI and QUICKI were significantly correlated), and smoking status. A general linear model as an analysis of covariance was used to explain the association between cIMT and renal function in Study IV with the traditional atherosclerotic risk factors (age, sex when appropriate, LDL cholesterol, systolic BP, alcohol consumption and pack years) as continuous covariates and smoking status as a fixed factor together with the renal function as presented in tertiles. In that study the patients were divided into three groups based on the eGFR, genders separately. In the male study population, the eGFR was between 58 and 82 mL/min/1.73 m² in Tertile I, between 82 and 93 mL/min/1.73 m² in Tertile II and between 93 and 175 mL/min/1.73 m² in Tertile III. In females, the corresponding eGFR values were between 43 and 71 mL/min/1.73 m² in Tertile I, between 71 and 82 mL/min/1.73 m² in Tertile II and between 82 and 122 mL/min/1.73 m² in Tertile III. Two-sided P-values less than 0.05 were regarded as significant.
5 Results

5.1 LDL apolipoprotein B metabolism in CRI (Study I)

5.1.1 Plasma lipids and lipoproteins, clearance and production rates and concentrations of LDL apo B

The plasma lipid and lipoprotein concentrations, the clearance and production rates as well as the concentrations of LDL apo B in the CRI patients and controls are shown in Table 10. The CRI patients displayed increased concentrations of VLDL and IDL cholesterol and of TGs, whereas the concentration of HDL was decreased as compared with the control subjects. Overall, the mean LDL FCR did not differ significantly between the two groups. However, LDL FCR varied widely in the renal study group, and a total of 25 out of the 57 CRI patients exhibited LDL FCR values less than the minimum of the control subjects.
Table 10. Plasma lipids, lipoproteins, fractional catabolic rates, production rates and concentrations of LDL apo B in CRI patients and their controls in Study I.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CRI patients (N = 57)</th>
<th>Controls (N = 10)</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Range</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.72 (1.17)</td>
<td>3.62–8.79</td>
<td>5.33 (0.63)</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.71 (0.43)</td>
<td>0.11–2.12</td>
<td>0.38 (0.25)</td>
</tr>
<tr>
<td>IDL cholesterol (mmol/L)</td>
<td>0.29 (0.15)</td>
<td>0.07–0.73</td>
<td>0.15 (0.07)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.09 (0.89)</td>
<td>1.64–5.47</td>
<td>3.05 (0.71)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.21 (0.40)</td>
<td>0.57–2.48</td>
<td>1.56 (0.22)</td>
</tr>
<tr>
<td>Total TG (mmol/L)</td>
<td>1.82 (0.90)</td>
<td>0.62–5.81</td>
<td>1.03 (0.25)</td>
</tr>
<tr>
<td>LDL FCR (pools/day)</td>
<td>0.34 (0.09)</td>
<td>0.13–0.56</td>
<td>0.36 (0.04)</td>
</tr>
<tr>
<td>LDL Apo B (mg/dL)</td>
<td>76.6 (20.8)</td>
<td>40.0–147.5</td>
<td>72.1 (18.4)</td>
</tr>
<tr>
<td>LDL Apo B production rate (mg/kg/day)</td>
<td>11.8 (4.4)</td>
<td>5.4–23.7</td>
<td>12.4 (1.9)</td>
</tr>
</tbody>
</table>

* P indicates significance of difference between study groups. Printed with permission from Oxford University Press (Article I).
5.1.2 Correlations and predictors of LDL FCR

In the renal patients, the LDL FCR was negatively correlated with the total cholesterol \((r = -0.422, P = 0.001)\) and the LDL cholesterol \((r = -0.578, P < 0.001)\), but not with age, BMI or gender. Furthermore, among the renal patients, the LDL FCR was positively correlated with the eGFR \((r = 0.340, P = 0.010)\) and negatively with the serum creatinine concentration \((r = -0.313, P = 0.018)\).

To further assess the association between LDL FCR and renal function, the CRI patients were divided into three groups based on their eGFR values (Table 11) corresponding to CKD staging (Table 1). The CKD stage 3 group included one patient with eGFR of 62 mL/min/1.73 m\(^2\). The difference in the mean LDL FCR was statistically significant between the three CKD groups \((P = 0.006)\) and between the CKD stage 5 group and the CKD stage 3 group \((P = 0.005)\) (Figure 2).

Table 11. LDL apo B fractional catabolic rates, production rates and concentrations in 57 patients with CRI stratified by the CKD stage and in 10 control subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CRI patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CKD Stage 5</td>
<td>CKD Stage 4</td>
</tr>
<tr>
<td>Number</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>LDL FCR (pools/day)</td>
<td>0.27 (0.06)</td>
<td>0.33 (0.09)</td>
</tr>
<tr>
<td>LDL apo B production rate (mg/kg/day)</td>
<td>9.8 (1.6)</td>
<td>11.1 (4.8)</td>
</tr>
<tr>
<td>LDL apo B concentration (mg/dL)</td>
<td>77.8 (13.5)</td>
<td>75.5 (26.7)</td>
</tr>
<tr>
<td>Estimated GFR (ml/min/1.73 m(^2))</td>
<td>11 (3)</td>
<td>21 (4)</td>
</tr>
</tbody>
</table>

The CRI patients were divided into three groups based on CKD staging (Table 1). Values are mean (SD).

\(^a\) \(P = 0.008\) for the difference in mean LDL FCR between the four groups. N.D. indicates no data.
Fig. 2. LDL FCR in CRI patients divided into three CKD stage groups (Table 1) and in control subjects. P values refer to the statistical significance of the difference between the indicated group and the CKD stage 5 group.

A multiple linear regression model was constructed in an attempt to explain the variation of LDL FCR among the CRI patients. This revealed that the LDL cholesterol concentration and the eGFR together explained 47% of the total variation (adjusted R Square, $P < 0.001$). In contrast, age, gender and BMI had no significant effect. The eGFR alone explained 13% of the total variation ($P = 0.004$). Furthermore, in the analysis of covariance with the inclusion of gender, presence of diabetes, age, BMI, LDL cholesterol concentration and eGFR, the LDL cholesterol concentration and the eGFR remained independently associated with the LDL FCR ($P < 0.001$ and $P = 0.004$, respectively). It is noteworthy that the presence of diabetes was not significantly associated with LDL FCR, and after exclusion of the patients with diabetes from the analysis, equal results were obtained ($P < 0.001$ and $P = 0.019$ for the associations between the LDL FCR and the LDL cholesterol concentration and between the LDL FCR and the eGFR, respectively).
5.1.3 Correlations and predictors of LDL apo B production rate and concentration

The mean values of the LDL apo B production rates and concentrations did not differ significantly between the CRI and the control group (Table 10). Among the CRI patients, the LDL apo B production rate correlated significantly with the LDL apo B concentration \((r = 0.510, P < 0.001)\) and with the eGFR \((r = 0.264, P = 0.047)\) but not with the LDL cholesterol concentration. When the CRI patients were divided into groups based on the CKD stages, there was a trend towards a reduced LDL apo B production rate with decreasing eGFR, but this did not reach statistical significance (Table 11). The LDL apo B concentration correlated significantly with the total cholesterol \((r = 0.541, P < 0.001)\) and the LDL cholesterol \((r = 0.513, P < 0.001)\) but not with gender, age, BMI or degree of renal dysfunction.

In linear regression analysis, the LDL apo B concentration, age and eGFR together explained 26% of the variation in the LDL apo B production rate \((P < 0.001)\) in the CRI patients, whereas in the analysis of covariance the eGFR was not significantly associated with the LDL apo B production rate. In linear regression analysis, the LDL apo B concentration was best predicted by the LDL cholesterol concentration (adjusted R Square 32%, \(P < 0.001\)), whereas gender, age, BMI, presence of diabetes or eGFR were not significant predictors in this model.

5.2 Leptin and lipoproteins in CRI (Study II)

5.2.1 Concentrations of leptin, lipids and lipoproteins

Tables 12 and 13 present the leptin, lipid and lipoprotein concentrations of the study subjects.
Table 12. Plasma leptin, lipid and lipoprotein concentrations in the CRI patients and control subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CRI patients</th>
<th>Controls</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>73</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>24.0 (37.1)</td>
<td>9.0 (8.5)</td>
<td>0.008</td>
</tr>
<tr>
<td>Leptin/BMI</td>
<td>0.80 (1.03)</td>
<td>0.31 (0.24)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.85 (1.19)</td>
<td>5.69 (0.96)</td>
<td>0.396</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.09 (0.87)</td>
<td>3.60 (0.87)</td>
<td>0.001</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.84 (0.55)</td>
<td>0.35 (0.28)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.18 (0.40)</td>
<td>1.39 (0.36)</td>
<td>0.002</td>
</tr>
<tr>
<td>Chol/HDL</td>
<td>5.37 (1.64)</td>
<td>4.37 (1.30)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total TG (mmol/L)</td>
<td>2.10 (1.09)</td>
<td>1.39 (0.66)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). * P value relates to the difference between the CRI patients and the control subjects.

The concentrations of leptin were significantly higher in the CRI patients of both genders compared with the control subjects and in the females compared to the males in both groups. BMI, a factor known to influence the leptin concentrations, did not differ significantly between the study groups or between the genders. The leptin/BMI ratio was significantly higher in the CRI patients than in the control subjects and higher in females than in males in both study groups.

Table 13. Clinical characteristics and plasma leptin concentrations in the CRI patients and control subjects stratified by gender.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRI</td>
<td>Control</td>
</tr>
<tr>
<td>Number</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57 (13)</td>
<td>58 (6) b</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1 (4.8)</td>
<td>28.4 (5.7)</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>40.4 (42.9) x b</td>
<td>15.2 (9.6) b</td>
</tr>
<tr>
<td>Leptin/BMI</td>
<td>1.37 (1.21) x b</td>
<td>0.51 (0.23) b</td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). x P < 0.05 for difference between women with CRI and control women; y P < 0.05 for difference between women and men with CRI, or between control women and control men, as appropriate; z P < 0.05 for difference between men with CRI and control men; only statistically significant differences noted.

5.2.2 Association between leptin and lipid metabolism

In CRI patients, leptin correlated positively with total and HDL cholesterol as well as with TGs (Table 14). In the control group, a positive correlation was
detected between leptin and HDL cholesterol and an inverse correlation between leptin and VLDL cholesterol and the Chol/HDL ratio. The leptin/BMI ratio displayed very similar correlations as leptin with lipoprotein and lipid variables in both bivariate and partial correlation analyses. There were two exceptions; the correlation between the leptin/BMI ratio and the Chol/HDL ratio in the control group, which was significant also in the bivariate correlation ($r = -0.241, P = 0.048$) and the correlation between the leptin/BMI ratio and total TG in the CRI group, which was insignificant in the bivariate correlation analysis ($r = 0.225, P = 0.055$). The renal diagnosis was not associated with leptin, leptin/BMI ratio, lipid or lipoprotein concentrations in a general linear model adjusted for age, BMI, eGFR and gender.

Table 14. Coefficients for correlations ($r$) and partial correlations ($r^2$, age and BMI controlled for) between the plasma leptin concentrations and different variables in 73 CRI patients and 68 control subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CRI patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.345 **</td>
<td>0.143</td>
</tr>
<tr>
<td></td>
<td>$r^2$ 0.243 *</td>
<td>0.006</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.171</td>
<td>- 0.031</td>
</tr>
<tr>
<td></td>
<td>$r^2$ 0.135</td>
<td>- 0.153</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>0.187</td>
<td>- 0.117</td>
</tr>
<tr>
<td></td>
<td>$r^2$ - 0.025</td>
<td>- 0.248 *</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.026</td>
<td>0.311 *</td>
</tr>
<tr>
<td></td>
<td>$r^2$ 0.287 *</td>
<td>0.470 ***</td>
</tr>
<tr>
<td>Chol/HDL</td>
<td>0.126</td>
<td>- 0.199</td>
</tr>
<tr>
<td></td>
<td>$r^2$ - 0.166</td>
<td>- 0.390 **</td>
</tr>
<tr>
<td>Total TG</td>
<td>0.242 *</td>
<td>- 0.002</td>
</tr>
<tr>
<td></td>
<td>$r^2$ 0.003</td>
<td>- 0.195</td>
</tr>
</tbody>
</table>

* indicates significance of correlation of less than 0.05; ** significance of correlation less than 0.01; *** significance of correlation less than 0.001.

5.2.3 Ability of leptin or leptin/BMI to predict lipid and lipoprotein concentrations

In order to assess the independent relationships between the leptin concentration and the lipid and lipoprotein concentrations, BMI and waist, linear regression analyses were performed with logarithms of age and leptin or leptin/BMI, smoking and gender as the tested variables. In the CRI patients, the leptin levels were positively and independently associated with the variation in the total
cholesterol ($\beta = 0.300, P = 0.010$) and TG ($\beta = 0.480, P = 0.001$), whereas in the control group, leptin was positively associated with the Chol/HDL ratio ($\beta = 0.430, P = 0.024$). The leptin/BMI ratio was not able to predict any of the variations in the lipid or lipoprotein concentrations in the control group, whereas in the CRI group, this ratio was associated with the studied lipoprotein variables in a similar way as the association observed with leptin.

5.3 Renal function and dipping pattern (Study III)

5.3.1 Main characteristics of dippers and non-dippers

The main characteristics of the dippers and the non-dippers are presented in Table 15. Non-dippers accounted for 19% of the study population, and they were on average older and had higher BMI values, higher total TG and insulin concentrations, lower HDL cholesterol concentration and lower QUICKI, i.e. they had a less favourable cardiovascular risk profile. The office BP values did not differ between the dippers and the non-dippers, whereas the day-time systolic and diastolic BP values in the ABPM were significantly lower among the non-dippers. The use of alcohol did not differ between the groups. With respect to the medical history, cerebrovascular disease was more common among the non-dippers (4 subjects, 5%) as compared to dippers (3 subjects, 1%) ($P = 0.020$), but the differences were not significant with respect to diabetes or CHD.
Table 15. The main characteristics of the study subjects presented by the dipping status.

| Variable                      | Dippers (N=374) | Non-dippers (N=86) | P *  
|-------------------------------|----------------|-------------------|-----
| Gender (Females/Males)       | 188/186        | 46/40             | 0.590 |
| Age (years)                  | 51 (6)         | 53 (6)            | 0.024 |
| BMI (kg/m²)                  | 26.2 (3.9)     | 27.5 (4.3)        | 0.004 |
| Waist (cm)                   | 87 (12)        | 89 (13)           | 0.432 |
| Creatinine (µmol/L)          | 80 (13)        | 83 (13)           | 0.050 |
| eGFR (ml/min/1.73 m²)        | 84 (16)        | 79 (16)           | 0.005 |
| Total cholesterol (mmol/L)   | 5.63 (1.01)    | 5.58 (1.27)       | 0.118 |
| LDL cholesterol (mmol/L)     | 3.50 (0.91)    | 3.70 (1.17)       | 0.130 |
| HDL cholesterol (mmol/L)     | 1.41 (0.39)    | 1.31 (0.35)       | 0.046 |
| Total TG (mmol/L)            | 1.32 (0.69)    | 1.61 (0.94)       | 0.004 |
| QUICKI                       | 0.64 (0.11)    | 0.61 (0.10)       | 0.036 |
| Office systolic BP (mmHg)    | 142 (22)       | 141 (20)          | 0.968 |
| Office diastolic BP (mmHg)   | 85 (12)        | 85 (12)           | 0.828 |
| Office heart rate (beats/min)| 74 (13)        | 71 (13)           | 0.066 |
| ABPM systolic BP Day (mmHg)  | 133 (14)       | 130 (14)          | 0.040 |
| ABPM systolic BP Night (mmHg)| 111 (12)       | 122 (13)          | < 0.001 |
| ABPM diastolic BP Day (mmHg) | 84 (9)         | 82 (9)            | 0.024 |
| ABPM diastolic BP Night (mmHg)| 67 (8)        | 73 (9)            | < 0.001 |
| Current smokers (Yes/No)     | 120/254        | 25/61             | 0.861 |
| Pack-years                   | 12 (14)        | 14 (16)           | 0.571 |

Data are presented as prevalence or mean (SD). * P values refer to the difference between the dippers and the non-dippers. Printed with permission from Nature Publishing Group (Article III).

5.3.2 Effect of dipping pattern on renal function

The baseline characteristics of the study subjects as presented by the dipping status are shown in Table 15. The mean creatinine concentration was higher and the mean eGFR was significantly lower in the non-dipping group, and in a general linear model adjusted for age, BMI and gender, dipping status remained significantly associated with eGFR (P = 0.032). Furthermore, in linear regression analysis, dipping status was a significant predictor of the variation in eGFR (P = 0.031), in a similar manner as gender and age.
5.3.3 Ability of renal function to predict dipping status

The study subjects were divided into tertiles according to their eGFR values. The proportion of non-dippers was significantly different between the eGFR tertiles with a trend towards increasing prevalence with decreasing eGFR as shown in Table 16, which also displays the eGFR values for each tertile.

Table 16. Distributions of dippers and non-dippers across renal function tertiles.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range of eGFR in the tertile</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>43–74</td>
<td>75–88</td>
</tr>
<tr>
<td>Dipper</td>
<td>116</td>
<td>122</td>
</tr>
<tr>
<td>Non-dipper</td>
<td>37 (32%)</td>
<td>32 (26%)</td>
</tr>
<tr>
<td>Estimated GFR</td>
<td>67 (6)</td>
<td>81 (4)</td>
</tr>
</tbody>
</table>

Data are given as range, prevalence (percentage, when appropriate), or mean (SD). P = 0.010 for the difference of distribution of dipping pattern between renal function tertiles. eGFR is expressed as mL/min/1.73 m².

A logistic regression model was created to analyse the independency of the association between renal function (expressed as eGFR tertiles) and dipping status. The subjects in the lowest and in the middle eGFR tertiles had an increased risk of being non-dippers as compared to subjects in the highest eGFR tertile in a model adjusted for age, BMI, gender, and smoking status as seen in Figure 3. The use of the QUICKI instead of BMI in the analysis produced a similar result.
Fig. 3. Odds Ratios (OR) for non-dipping pattern in ABPM in relation the eGFR tertiles. Logistic regression analysis adjusted for age, gender, BMI and smoking status. Numbers represent OR, and in parentheses are the 95% confidence intervals.

Finally, those subjects with self-reported diabetes mellitus, diagnosed CHD or cerebrovascular disease and those with any antihypertensive or lipid-lowering drug treatment were excluded from the analyses. Nonetheless, the association between renal function and the risk of non-dipping status remained also in this subset (N = 387) of healthy middle-aged subjects (OR 2.22 (95% confidence interval (CI) 1.03 to 4.80)) for the lowest eGFR tertile versus the highest eGFR tertile, though for subjects in the middle eGFR tertile, the association was no longer statistically significant (OR 1.99 (95% CI 0.962 to 4.13)).

5.4 Renal function and cIMT (Study IV)

5.4.1 Main characteristics of the study subjects stratified by gender

There were multiple differences between the genders pointing towards an increased amount of risk factors for CVD in the male subjects as seen in Table 17.
Table 17. The main characteristics of the study subjects stratified by gender.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>247</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>51 (6)</td>
<td>52 (6)</td>
<td>0.090</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.5 (3.5)</td>
<td>26.3 (4.5)</td>
<td>0.467</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>94 (10)</td>
<td>81 (11)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>146 (20)</td>
<td>137 (21)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>88 (11)</td>
<td>81 (12)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pack-years</td>
<td>17 (15)</td>
<td>8 (12)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Alcohol consumption (g/wk)</td>
<td>88.5 (98.8)</td>
<td>22.8 (35.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.76 (1.08)</td>
<td>5.52 (1.03)</td>
<td>0.011</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.71 (0.95)</td>
<td>3.31 (0.91)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.23 (0.30)</td>
<td>1.56 (0.38)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.46 (0.32)</td>
<td>0.30 (0.27)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.55 (0.79)</td>
<td>1.17 (0.61)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>85 (12)</td>
<td>74 (11)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>eGFR</td>
<td>90 (17)</td>
<td>78 (14)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CrclBSA</td>
<td>93 (18)</td>
<td>85 (16)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>cIMT (mm)</td>
<td>0.92 (0.21)</td>
<td>0.82 (0.12)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The data are given as number or mean (SD). CrclBSA, creatinine clearance adjusted for body surface area. The units for eGFR and CrclBSA are mL/min/1.73 m². * P value relates to the difference between males and females.

The range for serum creatinine values was 47–125 µmol/L for males and 49–118 µmol/L for females; and the range for eGFR was 56–175 ml/min/1.73 m² for males and 43–122 ml/min/1.73 m² for females. There were a total of 19 subjects (4%) (2 males and 17 females) with eGFR values less than 60 ml/min/1.73 m². Also the mean cIMT, a marker of carotid atherosclerosis, was significantly increased in males compared with females as shown in Table 17.

5.4.2 Correlation between renal function and cIMT

The cIMT was weakly but significantly correlated with the serum creatinine (r = 0.186, P < 0.001) and with CrclBSA (r = −0.113, P = 0.011) in the whole population, but not with the eGFR. However, when the genders were tested separately, the cIMT was significantly correlated with the eGFR (r = −0.150, P = 0.019 for males and r = −0.132, P = 0.034 for females). Similar associations were found between CrclBSA and cIMT in men (r = −0.188, P = 0.003) and in women (r = −0.181, P = 0.003).
The subjects were furthermore divided into tertiles based on their eGFR to assess the relationships between renal function and traditional risk factors of CVD. Since there was an evident gender difference in the mean eGFR, gender-specific analyses were performed. Table 18 displays the results for male participants and Table 19 for female participants. The CrCl\textsubscript{BSA} values were 5%, 3% and 2% higher in Tertile I, II and III, respectively, as compared to the eGFR estimated with the MDRD formula in males, with the corresponding values in females being 9%, 11% and 9%. In males, with respect to the traditional risk factors, age and TGs showed significant associations with the eGFR tertile with the highest values in the lowest eGFR tertile, as expected. In females, renal function was significantly associated with total and LDL cholesterol as well as with age.

**Correlation between renal function and cIMT in male study subjects**

Interestingly, the cIMT was significantly associated with the renal function in males (Table 18, Figure 4). The change in mean cIMT was most pronounced between the Tertile I and the Tertile II ($P = 0.004$), but the difference between the Tertile I and the Tertile III was also significant ($P = 0.042$).

**Table 18. Main characteristics of the male study subjects according to tertiles of eGFR.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tertile I</th>
<th>Tertile II</th>
<th>Tertile III</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>82</td>
<td>82</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 (6)</td>
<td>51 (6)</td>
<td>49 (5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>26.9 (3.5)</td>
<td>26.5 (3.5)</td>
<td>26.2 (3.5)</td>
<td>0.433</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>95 (9)</td>
<td>94 (9)</td>
<td>93 (10)</td>
<td>0.385</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>146 (20)</td>
<td>146 (20)</td>
<td>144 (20)</td>
<td>0.674</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>88 (11)</td>
<td>88 (9)</td>
<td>87 (11)</td>
<td>0.816</td>
</tr>
<tr>
<td>Pack-years</td>
<td>17 (15)</td>
<td>17 (15)</td>
<td>16 (14)</td>
<td>0.889</td>
</tr>
<tr>
<td>Alcohol consumption (g/wk)</td>
<td>87.2 (113.6)</td>
<td>91.6 (91.4)</td>
<td>86.6 (91.0)</td>
<td>0.746</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.94 (1.06)</td>
<td>5.72 (0.99)</td>
<td>5.62 (1.17)</td>
<td>0.145</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.89 (0.90)</td>
<td>3.66 (0.87)</td>
<td>3.59 (1.04)</td>
<td>0.105</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.19 (0.28)</td>
<td>1.22 (0.29)</td>
<td>1.26 (0.34)</td>
<td>0.375</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.49 (0.30)</td>
<td>0.46 (0.34)</td>
<td>0.42 (0.33)</td>
<td>0.196</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.65 (0.81)</td>
<td>1.60 (0.83)</td>
<td>1.39 (0.71)</td>
<td>0.034</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>98 (7)</td>
<td>85 (3)</td>
<td>72 (7)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Variable & Tertile I & Tertile II & Tertile III & P * \\
--- & --- & --- & --- & --- \\
eGFR & 75 (6) & 87 (3) & 109 (14) & < 0.001 \\
Range of eGFR & 58–82 & 82–93 & 93–175 & \\
CrCl_BSA & 91 (17) & 104 (17) & 127 (26) & < 0.001 \\
cIMT (mm) & 0.97 (0.23) & 0.88 (0.18) & 0.91 (0.22) & 0.013 \\

The data are presented as number, mean (SD) or range. * P value refers to the difference between the renal function tertiles. The units of eGFR and CrCl_BSA are mL/min/1.73 m².

Fig. 4. The mean values for cIMT in relation to the eGFR tertiles in males. P values refer to the statistical significance of the differences between the designated tertile and tertile I. The P value for the difference between all renal function tertiles was 0.013.

Correlation between cIMT and renal function in female study subjects

In females, renal function was significantly associated with cIMT, and similarly, the difference in mean cIMT was significant between the eGFR tertile I and II (p = 0.041) and the eGFR tertile I and III (P = 0.013) (Table 19, Figure 5).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Tertile I</th>
<th>Tertile II</th>
<th>Tertile III</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>86</td>
<td>86</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 (6)</td>
<td>51 (6)</td>
<td>50 (6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 (3.9)</td>
<td>26.4 (4.4)</td>
<td>26.4 (5.1)</td>
<td>0.674</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>80 (10)</td>
<td>80 (10)</td>
<td>82 (12)</td>
<td>0.610</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>140 (24)</td>
<td>135 (19)</td>
<td>137 (19)</td>
<td>0.292</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83 (11)</td>
<td>80 (13)</td>
<td>81 (11)</td>
<td>0.113</td>
</tr>
<tr>
<td>Pack-years</td>
<td>8 (13)</td>
<td>7 (11)</td>
<td>10 (13)</td>
<td>0.206</td>
</tr>
<tr>
<td>Alcohol consumption (g/wk)</td>
<td>19.9 (32.7)</td>
<td>25.1 (37.4)</td>
<td>23.4 (37.7)</td>
<td>0.604</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.77 (0.92)</td>
<td>5.41 (1.09)</td>
<td>5.39 (1.03)</td>
<td>0.023</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.54 (0.83)</td>
<td>3.24 (0.95)</td>
<td>3.15 (0.91)</td>
<td>0.014</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.59 (0.37)</td>
<td>1.53 (0.41)</td>
<td>1.58 (0.38)</td>
<td>0.817</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.32 (0.22)</td>
<td>0.30 (0.32)</td>
<td>0.30 (0.24)</td>
<td>0.126</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.21 (0.54)</td>
<td>1.17 (0.72)</td>
<td>1.13 (0.54)</td>
<td>0.130</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>86 (8)</td>
<td>75 (3)</td>
<td>62 (5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>eGFR</td>
<td>64 (6)</td>
<td>76 (3)</td>
<td>94 (9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Range of eGFR</td>
<td>43–71</td>
<td>71–82</td>
<td>82–122</td>
<td></td>
</tr>
<tr>
<td>CrClBSA</td>
<td>70 (12)</td>
<td>86 (15)</td>
<td>103 (20)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>cIMT (mm)</td>
<td>0.85 (0.14)</td>
<td>0.81 (0.11)</td>
<td>0.81 (0.11)</td>
<td>0.031</td>
</tr>
</tbody>
</table>

The data are presented as number, mean (SD) or range. * P value refers to the difference between the renal function tertiles. The units of eGFR and CrClBSA are mL/min/1.73 m².
Fig. 5. The mean values for cIMT in relation to the eGFR tertiles in females. P values refer to the statistical significance of the differences between the designated tertile and tertile I. The P value for the difference between all renal function tertiles was 0.031.

5.4.3 Renal function as a predictor of cIMT

General linear models were created to investigate the independent association between cIMT and eGFR (as divided in tertiles) (Table 20). In the whole population and in males, eGFR tertile was significantly associated with cIMT after adjusting for age, BMI and gender (when appropriate). After further adjustments for systolic BP, LDL cholesterol, TG, and smoking status, significant associations were seen between cIMT and several covariates such as gender, age, systolic BP, LDL cholesterol, TG and smoking status in the whole population, and between cIMT and eGFR tertile, age, systolic BP, and LDL cholesterol in the males. In females, the eGFR tertiles were not associated with the cIMT, and in these models, only age, systolic BP and smoking habits were associated with cIMT.
Table 20. Significances of the associations between cIMT and renal function in general linear models.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cIMT</td>
<td>cIMT</td>
<td>cIMT</td>
</tr>
<tr>
<td>Model I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR tertile</td>
<td>0.033</td>
<td>0.033</td>
<td>0.444</td>
</tr>
<tr>
<td>Age</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>0.557</td>
<td>0.288</td>
<td>0.880</td>
</tr>
<tr>
<td>Gender</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR tertile</td>
<td>0.144</td>
<td>0.026</td>
<td>0.550</td>
</tr>
<tr>
<td>Age</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>0.725</td>
<td>0.333</td>
<td>0.992</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>&lt; 0.001</td>
<td>0.026</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TG</td>
<td>0.023</td>
<td>0.338</td>
<td>0.051</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.173</td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.037</td>
<td>0.388</td>
<td>0.024</td>
</tr>
<tr>
<td>Gender</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted R Squared</td>
<td>0.224</td>
<td>0.216</td>
<td>0.150</td>
</tr>
</tbody>
</table>

Subsequently, subjects with pre-existing or suspected CVD or antihypertensive medication or medication for diabetes were excluded from the analyses. This healthy group consisted of 215 males and 221 females, and in these individuals, renal function was associated with cIMT in both males (P = 0.009) and females (P = 0.043). Furthermore, in the general linear model after adjustment for age, BMI, systolic BP, LDL cholesterol concentration, TG and smoking status, the eGFR tertiles remained significantly associated with cIMT (P = 0.041) in males, but not in females (P = 0.740).

The study population included 149 postmenopausal females, and the analyses were repeated with this subset of subjects. In the analysis of covariance, eGFR was associated with cIMT after adjustment for age and BMI (P = 0.037), and this difference persisted even after additional adjustments for systolic BP, LDL cholesterol, TG and smoking status (P = 0.008).

5.4.4 cIMT as a predictor of renal function

In order to study the direction of the association between carotid atherosclerosis and renal function, the patients were divided into gender-specific tertiles
according to their cIMT values. The cIMT tertiles were not significantly associated with the eGFR in univariate or multivariate models (including age, BMI, systolic BP, LDL cholesterol, TG and smoking status).
6 Discussion

6.1 Study population

Studies I and II

The mean GFR of the eligible patients was 40 mL/min/1.73 m² and that of those not available for the studies was 41 mL/min/1.73 m². In the final analyses, the mean eGFR of the patients was 32 mL/min/1.73 m² in Study I and 33 mL/min/1.73 m² in Study II. Therefore there was no selection bias towards healthier patients in either of these studies. Furthermore, the GFR of the studied patients varied considerably, from 8 to 74 mL/min/1.73 m² at the time of sampling, giving a wide variance of renal function. This also made it possible to study the effect of the degree of CRI on many variables. Patients with various renal diagnoses were included in the Studies I and II. The large variation in renal function and in renal diagnoses however means that in clinical terms, the CRI population is rather heterogeneous. In Study I, the analysis was also performed with patients with diabetes excluded, as diabetes itself can modify lipids and lipoproteins (Betteridge 1994). The effect of the particular renal diagnoses, independently of renal function, was taken into account in Study II, where the renal diagnosis did not show any significant associations with either leptin or leptin/BMI in a model adjusted for age, BMI, eGFR and gender. These data are supported by a study in patients on HD, where diabetic and non-diabetic patients had comparable leptin levels (Nakazono et al. 1998).

Nephrotic patients were excluded from the analyses irrespective of the renal diagnosis as proteinuria itself may interfere with the lipoprotein metabolism through protein loss in the urine which causes a secondary increase in lipoprotein synthesis.

The control group in Study I was comparable with the renal group with respect to mean age (but also with age range) and gender distribution. These control subjects were healthy (free of chronic diseases), but the possible effect of co-morbidity other than renal dysfunction cannot be excluded. Furthermore, the number of subjects was considerably smaller than in the renal group. However, the control group exhibited LDL FCR values that were similar to the values described in other studies and the results can therefore be considered to represent normal values (Kesäniemi & Grundy 1982, Kesäniemi et al. 1985, Langer et al.)
In Study II, each control subject was chosen from the control cohort of the OPERA study in order to achieve comparable mean age and gender distribution. However, as the OPERA study consisted only of middle-aged subjects, the age distribution differs from that of the renal patients. There were 7 CRI patients that were younger and 32 CRI patients that were older than the age range of the control subjects. Therefore, age was included as a co-variate in most of the analyses.

Studies III and IV

The study subjects in Studies III and IV were originally chosen in the OPERA study to represent a control cohort for hypertensive subjects. At the time of the sampling of the OPERA cohort, a total of 7539 subjects (8% of the total population of the city) were entitled to the higher reimbursement for antihypertensive medication in the City of Oulu. Only subjects with severe hypertension met the criteria for the higher reimbursement of the Social Insurance Institution (systolic BP > 180/200 mmHg or diastolic BP > 95/105 mmHg). Therefore the control cohort in the OPERA study included subjects suffering from mild or moderate uncomplicated hypertension, and it did not represent the normotensive part of the population. However, the main characteristics of the control cohort of the OPERA study are comparable to those of the general Finnish population (Vartiainen et al. 1994).

In Study III, the required data were available from 460 subjects, whereas 64 subjects were excluded from the analyses. The gender distribution was comparable in the included and excluded subjects, but there were slight but statistically significant differences in the mean age (51 years for included subjects versus 50 years for excluded subjects, P = 0.023) and the mean eGFR (83 ml/min/1.73 m² in the included subjects versus 88 ml/min/1.73 m² in the excluded subjects, P = 0.007). However, there were no significant differences in BMI, office systolic or diastolic BP, lipoproteins, insulin concentrations or QUICKI.

In Study IV, the analyses were performed both in the eligible group (the main dependable and the co-variates available) and in the healthy group from which the subjects with antihypertensive (10%) or anti-diabetic medication (0.6%) or subjects with pre-existing CVD (6%) were excluded. However, the results in the former and latter groups were very similar making them relevant not only for a general middle-aged population but also for a low-risk general middle-aged population.
6.2 Methodological considerations

The renal function of the study subjects was estimated with GFR equations. The Cockcroft-Gault equation is validated in general populations, but it overestimates GFR in overweight subjects and in subjects with renal insufficiency, and underestimates GFR in the elderly and in subjects with reduced muscle mass or muscle activity. Therefore, the MDRD equation was used in the CRI patients, as generally recommended (Levey et al. 2003). The MDRD equation was used also in Studies III and IV to estimate GFR. In Study IV, the Cockcroft-Gault equation adjusted for body surface area was also calculated although not used in the main analyses. Since the subjects in the Studies III and IV were slightly overweight (mean BMI 26.4 kg/m²), it was decided to use the MDRD equation in this study population. The new equation developed to evaluate GFR in subjects with near-normal kidney function (CKD-EPI) (Levey et al. 2009) was not available at the time of the studies. It is noteworthy that the eGFR values were based on a single measurement of serum creatinine.

The LDL turnover study (Study I) was completed in 57 patients. A kinetic study such as this is strenuous to perform, and the patient number is impressive for this kind of studies. The LDL turnover studies were performed over a long period of time (several years), and this poses the risk of methodological changes. Nevertheless, the trained laboratory staff has remained the same during all of the study years, as have the laboratory technique and the equipment.

In Study II, the sample size was relatively small and thus its statistical power to detect modest associations was limited. Second, insulin concentrations were not measured; previously these have been associated with the leptin concentrations in CRI patients (Stenvinkel et al. 1997). Furthermore, as the study was cross-sectional, it is not possible to ascribe causality between leptin and lipoprotein and lipid concentrations.

When interpreting the results of the present study, it must be borne in mind that the eGFR values were obtained using the MDRD formula, which has not been validated in subjects with normal creatinine values. There is no data available on proteinuria in the subjects that took part in Studies III and IV. Nevertheless, both decreased GFR and microalbuminuria/or albuminuria are
known to be independent risk factors or markers for atherosclerosis (Astor et al. 2008).

In Study III, the dipping pattern was defined by the changes in the systolic BP, whereas non-dipping can also be defined as an inability to produce a reduction in the values of diastolic BP. Importantly, systolic non-dipping has been found to increase the risk of mortality by 30% as compared to normal dipping pattern (Ben-Dov et al. 2007). It is noteworthy that the dipping status was based on a single ABPM.

There are several methods available for the assessment of the presence or degree of atherosclerosis. These include angiograms with or without computerized tomography, ultrasound, pulse wave analysis and cardiac magnetic resonance imaging (O’Rourke & Hashimoto 2007). Abdominal aortic calcification measured from a plain lateral abdominal roentgenograph has been shown a useful prognostic tool to evaluate CVD and all-cause mortality in advanced CRI (Honkanen et al. 2008, Okuno et al. 2007). In the present study, the wall thickness of the carotid arteries was measured using B-mode ultrasound. This method is simple, safe, non-invasive, inexpensive, precise and reproducible. Increased arterial wall thickness has been shown to be closely associated with CVD and to be a powerful predictor of coronary and cerebrovascular complications (Lorenz et al. 2007). Since the study is cross-sectional, it is not possible to determine the causal direction of the association between carotid atherosclerosis and renal function.

Finally, these study subjects were all Caucasians, so the results can not be generalized as such to populations of other ethnic origins.

6.3 LDL apo B metabolism in CRI

In previous studies, the LDL FCR has on average been 0.36 pools/day in normal subjects, 0.34 pools/day in obese subjects and 0.41 pools/day in patients with CHD (Kesäniemi et al. 1985). In heterozygous patients with FH, the mean LDL FCR was low, 0.19 pools/day (Teng et al. 1986). In the present study, several CRI patients displayed LDL FCR values that were even lower than the values observed among FH patients, and 25 patients (44%) exhibited LDL FCRs lower than the minimum of the control subjects. Patients with CKD stage 5 presented with significantly lower LDL FCRs than patients with better although abnormal eGFRs, confirming the results of a previous study in 12 patients with severe CRI (mean GFR 16.4 (SD 11.4) ml/min/1.73 m²), that detected a decreased LDL
catabolism in those patients (Hörkkö et al. 1994b). In the present study, a significant correlation between LDL FCR and the remaining renal function was also observed.

Contrary to the LDL clearance, the LDL apo B production rates were not associated with renal function in the CRI patients although there was a trend toward a decreased LDL apo B production rate in the patients with more severe CRI. Furthermore, LDL apo B production rates were comparable between the CRI group and the control group. A similar finding was seen in a previous study with 12 CRI patients (Hörkkö et al. 1994b).

One possible mechanism to explain the comparable LDL apo B concentration between the CRI patients and the control subjects may be a decreased catabolism of IDL apo B to LDL apo B (Ikewaki et al. 2005), but it is also noteworthy that very little is known about the direct hepatic production of the apo B in CRI. It has been shown that it is synthesis rather than the clearance that is the crucial factor that determines the plasma LDL apo B and LDL cholesterol concentrations in non-FH patients (Kesäniemi & Grundy 1982). In the present study the synthesis of LDL apo B remained comparable in CRI and control subjects.

Why is LDL FCR decreased in CRI? An interesting recent finding emerged from experiments conducted in 5/6 nephrectomized rats. It was noted that hepatic LDL receptor related protein was down-regulated at the level of gene transcription (Kim & Vaziri 2005), which could reflect depressed clearance of chylomicron remnants and IDL. Carbamylation of LDL, which is known to occur in uraemia (Apostolov et al. 2005), can influence LDL clearance (Hörkkö et al. 1992). The reduced LDL clearance could be due to alterations in both the LDL receptor and the LDL particle itself. A longer residence time in the uraemic milieu may also predispose the LDL to atherogenic changes such as oxidation and non-receptor-mediated uptake by macrophages.

The clinical significance of decreased LDL clearance in subjects with normal or even low LDL cholesterol concentrations is unclear. In patients not yet on dialysis, the LDL cholesterol concentrations did not correlate with CVD mortality after adjustments for malnutrition, inflammation and cachexia (Kovesdy et al. 2007), suggesting that in CRI, the LDL cholesterol concentration per se may not be a major risk factor for CVD as in subjects without CRI. However, according to data from the United States Renal Data System, the use of statins has been associated with a reduction in cardiovascular deaths in patients on dialysis (Seliger et al. 2002), and in a meta-analysis of subjects with moderate CRI, statin treatment reduced the cardiovascular risk by 23–25% (Tonelli et al. 2005).
Nevertheless, in the 4D Study in patients with type 2 diabetes on HD there was no advantage seen to atorvastatin treatment (20 mg/day, median follow-up 4 years) as assessed by several parameters i.e. death from cardiac causes, nonfatal myocardial infarction, or stroke (Wanner et al. 2005). In the AURORA study it was concluded that despite marked reductions in the lipoprotein concentrations, rosuvastatin treatment (10 mg/day, median follow-up 3.8 years) had no significant effect on cardiovascular events in HD patients (Fellström et al. 2009). An ongoing study (SHARP) will assess the effects of cholesterol-lowering therapy using a combination of simvastatin and the cholesterol-absorption inhibitor ezetimide in several thousand CRI patients (Baigent & Landry 2003). Thus, it remains to be seen whether an early intervention with statin treatment is effective to prevent CVD in renal patients. Nevertheless, given the recent knowledge that the type and site of atherosclerosis in CRI may differ from that in non-CRI populations (London et al. 2003, McCullough et al. 2008), the negative results in the statin studies obtained so far may not be as unexpected as first thought.

Taken together, the results of the present study show that the LDL clearance is reduced in relation to the severity of CRI, whereas LDL catabolism seems to be significantly reduced only in patients with CKD stage 5.

6.4 Leptin and lipid metabolism in CRI

The major finding was that leptin as well as leptin/BMI ratios were significantly associated with lipoprotein concentrations in patients with CRI and in healthy control subjects. Hyperleptinaemia is a known feature of CRI, and in concordance with this, the leptin concentrations were significantly higher in the renal patients in comparison with the healthy control subjects. The leptin/BMI ratios were also higher in the renal patients, but they were not associated with the renal function per se in the CRI patients, suggesting that the regulation of leptin remains dependent on BMI, even in advanced CRI.

In previous studies on dialysis patients, leptin levels have revealed a positive correlation with the total and LDL cholesterol as well as the TG (Aguilera et al. 2002, Nakazono et al. 1998). In patients with severe CRI but not yet on dialysis, the results have been conflicting (Heimbürger et al. 1997, Rodríguez-Carmona et al. 2000), and they have been obtained from univariate analyses. Furthermore, the association between leptin and the lipoprotein profile has not been studied in CRI patients suffering from less severe renal insufficiency. The patients of the present study had varying degrees of renal impairment, and in them, leptin did correlate
positively with both total cholesterol and TG and with HDL cholesterol, and in the regression analysis, leptin concentrations were significantly associated with total cholesterol and TG even after adjusting for age, gender and smoking.

The studies that have assessed the association between leptin and clinical CVD have reported conflicting results, and in the renal population there are limited data on the associations between leptin levels and cardiovascular risk. It has also been speculated that leptin has both anti- and pro-atherogenic effects and that it is the balance of these components that determines the final outcome. There are some therapeutical intervention possibilities to modify the leptin concentrations, including lifestyle modification and some drugs, but further studies are needed to clarify their potential benefits (Koh et al. 2008).

In conclusion, the leptin levels displayed significant pro-atherogenic lipoprotein profile associations in both study groups, as did the leptin/BMI in the CRI group. The increased leptin levels in CRI may contribute to the high cardiovascular risk seen in this patient group.

6.5 Dipping pattern and renal function

A significant independent association between renal function and the dipping status was observed in a randomly selected middle-aged population with normal serum creatinine values. There was an increased risk of non-dipping even in those subjects with eGFR values of less than 88 ml/min/1.73 m².

The disappearance of the nocturnal BP decline is common in advanced CRI. Non-dipping was found to be prevalent in 53% to 82% of subjects with CRI (Agarwal & Light 2009, Farmer et al. 1997, Rahman et al. 2005). In subjects without CRI, the proportion of non-dippers has been 30–52% (Agarwal & Light 2009, Farmer et al. 1997). In the present study, the subjects could have been regarded as having normal renal function if the assessment was based on their serum creatinine values, but when GFR estimates were used, many subjects were identified with eGFR values of less than 90 ml/min/1.73 m², which is the lower limit of normal renal function. The prevalence of non-dipping increased from 10% to over 20% when the eGFR values were less than 88 ml/min/1.73 m². Based on this finding, it appears that non-dipping is an early phenomenon in the course of deteriorating renal function.

Many, if not most, of the conditions that have been associated with loss of diurnal variation in ABPM are also present in patients with CRI. Hypertension is an early phenomenon in the progression of renal insufficiency, and has been
shown to occur in CKD stages 1–2 (Gabow et al. 1990). Increased sympathetic nerve activity is known accompany CRI, and it has been associated with reduced renal excretory function (DiBona 2002). Moreover, sympathetic tone has been shown to be increased in hypertensive renal disease even though it does not evoke reduced renal function (Klein et al. 2001). Patients with CRI exhibit glucose intolerance due to an impaired response of target organs to insulin, and in a recently published study in middle-aged subjects, CKD stage 2 was associated with insulin resistance (Onat et al. 2007). Endothelial dysfunction has been shown to be present in all stages of CKD including stage 1, and to worsen in parallel with the reduction in eGFR (Caglar et al. 2008, Endemann & Schiffrin 2004). Patients with sleep apnoea display a high prevalence (31%) of CRI (Iseki et al. 2008). In CRI, there is also a tendency toward retention of sodium (Guyton & Coleman 1999).

Systemic hypertension is a well known risk factor for the deterioration of renal function (Sarnak et al. 2005), but less is known of the impact of the dipping-pattern on the renal function in subjects without pre-existing renal disease. In a recent study, a blunted diurnal BP variation was found to be associated with a deterioration in the renal function also in subjects with a mean baseline eGFR of 81 ml/min/1.73 m², although it has to be emphasized that in that study 87% of the non-dippers were hypertensive and 25% of them had CHD (Davidson et al. 2006). In the present study with randomly selected middle-aged subjects, dipping status was a significant predictor of the variation in eGFR, but due to the cross-sectional design of the study, it is not possible to assess the direction of the causality between the renal function and the dipping status.

It would have been most interesting to have data on the albuminuria of our study subjects. Even slight proteinuria (proteinuria of less than 300 mg/day or urine protein/creatinine ratio of more than 0.22 g/g) has been found to increase the risk of non-dipping (Agarwal & Light 2009) and in patients with CKD stage 2–5 (not on dialysis) nocturnal dipping was associated not only with higher eGFR but also with less extensive proteinuria and a higher serum albumin concentration (Agarwal & Andersen 2005). The independent and joint contribution of reduced renal function and proteinuria on the circadian variation in BP and on cardiovascular outcomes remains to be elucidated in future studies.

Thus, even a mild deterioration in the renal function is associated with an increased prevalence of non-dipping in ABPM in a randomly selected middle-aged population. The loss of diurnal variation of the BP may be one mechanism behind the increased risk of CVD, already seen in patients with mild renal
dysfunction. A non-dipping pattern may also predispose to or worsen an already established renal damage. In subjects that present with a non-dipping pattern on ABPM, renal function should be carefully assessed in order to identify mild renal dysfunction, and it goes without saying, that BP should be treated adequately for 24 hours a day in all subjects.

6.6 Renal function and cIMT

This study showed that reduced renal function is an independent risk factor for increased cIMT in a healthy middle-aged male population even after adjusting for the traditional risk factors. Furthermore, the increase in cIMT was seen already in subjects with very mildly decreased eGFRs.

The cIMT has been increasingly used as a surrogate marker of early atherosclerosis, and it has been shown that cIMT is a strong predictor of future vascular events including myocardial infarction, stroke or cardiovascular or all-cause mortality in both the general population and in CRI patients (Blacher et al. 2001, Kato et al. 2003, Lorenz et al. 2007, Nishizawa et al. 2003, Szeto et al. 2007). In addition, in otherwise high-risk subjects with verified CHD and impaired renal function, cIMT was independently associated with the severity of CHD (Lisowska et al. 2009).

Carotid IMT has been reported to be increased in subjects with impaired renal function (Ishizaka et al. 2007, Ishizaka et al. 2008, Kawamoto et al. 2008a, Skalska et al. 2007, Zhang et al. 2007, Zoungas et al. 2000). However, these previous studies have mostly included elderly subjects or patients with pre-existing CVD, hypertension, diabetes or advanced renal impairment. In high risk patients with angiographically diagnosed coronary artery disease, significant negative correlations were observed between the creatinine based eGFR and cIMT (Lisowska et al. 2007). In elderly subjects, the eGFR was independently and inversely correlated with cIMT value (Kawamoto et al. 2008a). On the contrary, in a large study evaluating middle-aged and elderly subjects, there was no significant association between the cIMT and a reduced renal function assessed with a cystatin C based GFR estimate (Bui et al. 2008). In the present study, only 5% of the subjects had ischemic CHD and 1% of the subjects had a history of a cerebrovascular event. Furthermore, less than 1% of the subjects were using medication for diabetes, and the use of lipid-lowering medication was an exclusion criteria. Nevertheless, as in most of the above-mentioned studies, in the present study, an increased cIMT was observed in low-risk male subjects with
mildly reduced eGFRs and serum creatinine values ranging from 88 to 125 μmol/l.

The cIMT was independently increased in males in the lowest eGFR tertile. This association remained significant even after adjustment for the traditional atherosclerotic risk factors, which were not associated with renal function with the exception of age and the mean TG concentration. In females, total and LDL cholesterol concentrations were significantly associated with eGFR such that the highest cholesterol concentrations were found in the lowest renal function group. In females, the association between renal function and cIMT was neither independent of the traditional risk factors nor significant in the covariate analysis. It is possible that in females, the confounding effects of the major risk factors for atherosclerosis, such as age, total cholesterol or LDL cholesterol, blunt the association between cIMT and renal function. Furthermore, the use of hormonal replacement therapy may also influence this relationship. In the present study, many of the female subjects were using oestrogen or progesterone replacement therapies. In many of the previous studies, the association between cIMT and eGFR has not been reported separately in a gender-dependent manner. In one study, the association between cIMT and eGFR was similar in both genders, but that study consisted of elderly hospitalized subjects with a large percentage of patients burdened with cardiovascular risk factors (Kawamoto et al. 2008a). The present study differs from the previous reports in that the prevalence of hypertension or diabetes was lower in this study population.

Taken together, the results show that in a randomly selected middle-aged male population, and in postmenopausal females, even a mild decrease in renal function is associated with cIMT independently of traditional risk factors. This observation underlines the importance of early detection of subjects with mildly decreased renal function and emphasizes the need for aggressive management of atherosclerotic risk factors in this population. However, it may not be adequate to assess renal function simply by measuring creatinine concentrations alone, but the use of equations to estimate GFR might help in the identification of patients with mild impairment of their renal function and who are at an increased risk of CVD.
7 Conclusions

1. LDL clearance is correlated with renal function in subjects with CRI. LDL catabolism seems to be significantly reduced only in patients with advanced renal insufficiency (CKD stage 5).

2. Leptin displays significant pro-atherogenic lipoprotein profile associations both in patients with CRI and in healthy controls, as does the leptin/BMI ratio in the CRI group. Leptin concentrations and leptin/BMI ratios are significantly higher in patients with CRI than in healthy controls. Thus, the increased leptin concentrations in CRI may contribute to the increased cardiovascular risk seen in this patient group.

3. In a randomly selected middle-aged population, the risk of non-dipping is significantly increased in subjects with very mildly impaired renal function. Non-dipping may reveal subjects with early renal impairment. Hypertensive subjects with CKD should receive appropriate and effective treatment throughout 24 hours a day.

4. In a randomly selected middle-aged male population, and also in postmenopausal females, even a mild decrease in renal function is associated with increased cIMT, independently of traditional cardiovascular risk factors. It is important to recognize subjects with mild renal insufficiency and to treat the atherosclerotic risk factors aggressively also in this population. GFR estimates are useful in identifying these subjects with mild reductions in renal function.
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Original publications


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