UNIVERSITY OF TROMSØ UIT

FACULTY OF HEALTH SCIENCES
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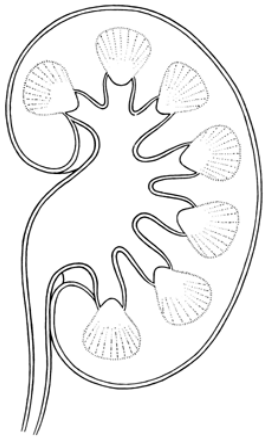
Renal hyperfiltration, impaired fasting glucose and physical exercise in the general population.

A cross-sectional study



TORALF MELSOM

A dissertation for the degree of
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CONTENTS

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The present thesis is based on data from the Tromsø 6 study and the Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6). The RENIS-T6 was conducted at The Unit of Clinical Research, University hospital of North Norway from 2007 to 2009. I have been a PhD student at the Institute of Clinical Medicine (IKM) and I was funded by the Northern Norway Health Authority. These institutions have all contributed to make this work possible.

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ABBREVIATIONS

ACE-i	angiotensin converting enzyme inhibitors
ACR	albumin-creatinine ratio
ARB	angiotensin II-receptor blocker
BMI	body mass index
Ccr	urinary creatinine clearance
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CV	coefficient of variation
CVD	cardiovascular disease
MDRD	Modification of Diet in Renal Disease
eGFR	estimated glomerular filtration rate
eGFRcre	estimated glomerular filtration rate by creatinine
eGFRcys	estimated glomerular filtration rate by cystatin C
ESRD	end-stage renal disease
GFR	glomerular filtration rate
HDL	high-density lipoprotein
IFG	impaired fasting glucose
LDL	low-density lipoprotein
mGFR	measured glomerular filtration rate

NHANES National Health and Nutrition Examination Survey

PREVEND Prevention of Renal and Vascular End-stage Disease

RENIS-T6 Renal Iohexol Clearance Survey in Tromsø 6

LIST OF PRESENTED PAPERS

1. Melsom T, Mathisen UD, Ingebretsen OC, Jenssen TG, Njolstad I, Solbu MD, Toft I, Eriksen BO: Impaired Fasting Glucose Is Associated With Renal Hyperfiltration in the General Population. *Diabetes Care* 34:1546-1551, 2011

2. Melsom T, Mathisen UD, Winther Eilertsen B-A, Ingebretsen OC, Jenssen TG, Njolstad I, Solbu MD, Toft I, Eriksen BO: High-intensity exercise attenuates the association between fasting glucose and renal hyperfiltration. A sex-specific population study. (submitted)

3. Mathisen UD, Melsom T, Ingebretsen OC, Jenssen T, Njolstad I, Solbu MD, Toft I, Eriksen BO: Estimated GFR Associates with Cardiovascular Risk Factors Independently of Measured GFR. *J Am Soc Nephrol* 22:927-937, 2011

SUMMARY

The prevalence of chronic kidney disease (CKD) is linked to the epidemics of obesity, diabetes and prediabetes. Abnormally elevated glomerular filtration rate (GFR), also called hyperfiltration, is common in diabetes and has been found to increase the risk of diabetes-related CKD. Hyperfiltration has also been proposed as an initial step in the development of other types of CKD. However, the causes of hyperfiltration in persons without diabetes have not been definitively determined. Specifically, it is not known whether prediabetes, insulin resistance and physical inactivity are associated with hyperfiltration. In studies of hyperfiltration, GFR must be measured with sufficient accuracy, which is time-consuming and costly. Previous studies of hyperfiltration in the general population are few and limited by the use of estimated GFR, which is imprecise in the upper range of GFRs.

As part of the Tromsø 6 study (2007 / 2008), we measured GFR in 1627 middle-aged people from the general population using an exact method. Subjects with diabetes, cardiovascular disease or known kidney disease were excluded. We found that impaired fasting glucose, but not fasting insulin levels, were associated with hyperfiltration. Leisure-time high-intensity physical exercise was associated with a reduced risk of hyperfiltration in men. In both genders, high-intensity exercise eliminated the positive association between fasting glucose and GFR. The sensitivity and specificity of estimated GFR for defining hyperfiltration was poor when validated against measured GFR. Longitudinal studies using measured GFR are needed to investigate whether hyperfiltration is a risk factor for CKD in the general population.

1. INTRODUCTION

Chronic kidney disease (CKD) is a growing public health problem worldwide. Approximately 10% of the population in most Western and Asian countries has CKD [1;2], a condition that increases the risk of end-stage renal disease, cardiovascular disease and death [3]. The prevalence of CKD is linked to the epidemics of obesity, prediabetes and diabetes [4;5], but the causes of early stage CKD and its underlying pathological mechanism are unclear. Furthermore, little is known about how to prevent CKD at the population level.

To gain knowledge about preventing CKD, it is important to investigate modifiable risk factors for the early stage of CKD. At this stage, renal function, assessed with the glomerular filtration rate (GFR), is normal or even above the normal range. Abnormally elevated GFR, also called hyperfiltration, is thought to be a pathological state that may eventually lead to renal damage. However, hyperfiltration is difficult to investigate with the widely used creatinine-based estimates of GFR (eGFRcre) because these estimates are imprecise and biased, particularly in the normal or upper range. Thus, the causes of hyperfiltration in the general population are largely unknown.

The present study was designed to investigate the relationship between metabolic and cardiovascular risk factors and GFR, measured as iohexol clearance, in a representative sample of the general population. Using a cross-sectional design, this thesis investigates the associations between renal hyperfiltration, fasting glucose, insulin resistance and physical exercise in nondiabetic middle-aged people. In addition, this thesis explores the limitations of using estimated GFR in studies of cardiovascular risk factors and GFR in the normal or upper range.

2. BACKGROUND

2.1 Kidney function and the glomerular filtration rate

The kidney serves several functions, including the excretion of waste products and the regulation of blood pressure, certain hormones and the electrolyte concentration in body fluids. These functions are conducted in the nephrons through filtration, reabsorption and secretion. The total number of nephrons varies among individuals and ranges from 500.000 to 1.5 million in individuals with two kidneys [6]. The filtration process takes place in the glomerulus of each nephron. The rate of filtration, or the glomerular filtration rate (GFR), is the volume of fluid filtered from the renal glomerular capillaries into Bowman's capsule per unit time (ml/min). The GFR is determined by the hydrostatic and oncotic pressure gradients between the capillaries and Bowman's capsule (net ultrafiltration pressure), the surface area available for filtration and the hydraulic conductivity of the glomerular membranes.

The whole-kidney GFR, usually referred to as GFR, is the product of the average filtration rate of each nephron (the single-nephron GFR) and the number of nephrons in both kidneys. The GFR varies considerably among individuals and with age, sex and body size [7;8]. The normal range of GFR according to age and gender is shown in Figure 1 [7]. To compare the GFRs of individuals with different body sizes, GFR is conventionally adjusted for the body surface area (BSA) and expressed as ml/min/1.73 m^2. However, GFR can also be expressed without adjustment for BSA as absolute GFR (ml/min).

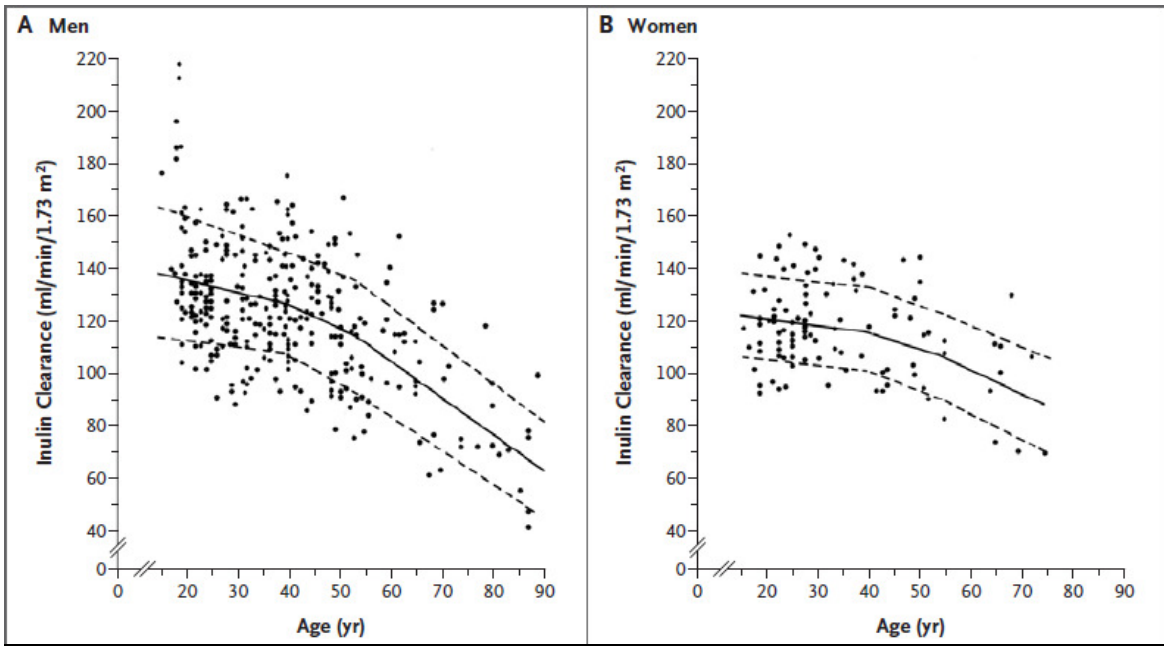


Figure 1. Normal GFR values in men and women, assessed by inulin clearance (adapted from Wesson [7], reprinted in *NEJM* 354;23. 2006 [8]).

2.1.1 GFR measurement with exogenous filtration markers

GFR can be measured by injecting an exogenous filtration marker into the patient and conducting timed blood or urine measurements of the marker. An ideal filtration marker is freely filtered across the basal membrane of the glomeruli and not secreted, metabolized or reabsorbed in the renal tubules or in any other organ. The plasma or urinary clearance of an ideal filtration marker equals the true GFR.

The urinary clearance of inulin is considered the gold standard method of measuring GFR, but it is time-consuming, expensive and complicated [9]. Instead, the plasma- or urinary clearance of alternative filtration markers, including radio-contrast or isotope media such as iohexol, iothalamate and ^{51}Chrom-EDTA, has been widely used. Urinary clearance is cumbersome and prone to measurement error in the urine samples. Plasma clearance is easier to perform and more precise than urinary clearance, given no extrarenal elimination [9]. Plasma clearance can be measured by assessing the plasma concentration of the marker in

several blood samples or in one blood sample (the single-sample method). The single-sample method, in which GFR is calculated using Jacobsson's formula, has been found to correlate very well with the multiple-sample method [10;11] Multiple- and single-sample iohexol clearance also correlate well with inulin or ^{51}Chrom-EDTA clearance (correlation coefficients ranging from 0.91 to 0.99) in different studies [9].

2.1.2 Estimation of GFR by endogenous filtration markers.

GFR measurement is still costly and inconvenient in most clinical settings and in epidemiological studies. Therefore, endogenous markers, such as creatinine or cystatin C, are used to estimate GFR. Creatinine, the most commonly used filtration marker, is a breakdown product of creatine phosphate. Creatinine is freely filtered in the glomeruli, but a small amount is also secreted by the tubules. The generation of creatinine is determined primarily by muscle mass but also by diet. Accordingly, the plasma creatinine level is proportional to muscle mass and varies with age, gender, race and body size. These non-GFR related determinants of creatinine have led to the development of creatinine-based formulas that incorporate age, sex, race and body size to improve GFR estimation. The widely used Modification of Diet in Renal Disease (MDRD) equation was developed on the basis of populations with CKD and has been shown to perform well when GFR < 60 ml/min/1.73 m^2. The equation has lower precision and a larger bias when GFR > 60 ml/min/1.73 m^2, and it has been shown to underestimate GFR at higher levels [12]. In 2009, another creatinine-based equation, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, was developed in a combined population of CKD patients and healthy individuals. This equation has been found to perform somewhat better than the MDRD in the normal range [13]. However, all creatinine-based equations are prone to bias in individuals with reduced muscle mass or atypical body compositions.

Recently, cystatin C has emerged as a promising filtration marker. Cystatin C is a small protein produced by all human cells and is freely filtered, completely reabsorbed and catabolized by the proximal tubular cells. Several cystatin C-based equations (eGFRcys) have been developed. However, the performance of eGFRcys has not been established. Cystatin C levels do not depend on muscle mass; rather, they are affected by other non-GFR factors, such as obesity and inflammation [14;15]. Another problem with eGFRcys is the lack of a standardized method for measuring cystatin C [16].

2.2 Chronic kidney disease

2.2.1 Definition, prevalence and health burden of CKD

Chronic kidney disease (CKD) is a condition characterized by a GFR below 60 ml/min/1.73 m^2 and/or signs of kidney damage (e.g., albuminuria) lasting for at least three months [17]. CKD is classified into 5 stages according to severity of the disease, in which stage 5 is regarded as renal failure (Table 1).

Table 1.

Stages of chronic kidney disease according to KDOQI-guidelines

Stage	Description	GFR (ml/min/1.73m²)
1	Kidney damage with normal or ↑ GFR	≥90
2	Kidney damage with mild ↓ GFR	60-89
3	Moderate ↓ GFR	30-59
4	Severe ↓ GFR	15-29
5	Kidney failure	<15 (or dialysis)

GFR= glomerular filtration rate

Only a small fraction of patients with CKD progress to end stage renal disease (ESRD). However, the prevalence of ESRD is increasing rapidly worldwide, and this condition is

associated with reduced quality of life, a very high mortality rate and high healthcare costs [18]. Moreover, CKD Stage 1 through 4, which affect approximately 10 % of the population, also increase the risk of cardiovascular disease, especially when GFR < 45 ml/min/1.73 m^2 and/or microalbuminuria (MA) is present [3].

The CKD staging system has been criticized. First, it does not account for the diversity in the etiologies, course and treatment of CKD. Second, the standard and widespread tool for staging CKD, GFR estimation with creatinine (eGFRcre), is imprecise and biased, particularly at levels above 60 ml/min/1.73 m^2. Accordingly, it is difficult to differentiate between CKD Stages 1 and 2. Third, the CKD staging system is based on the GFR level and does not take into account the patients' age and gender. This is problematic because the GFR declines with age and has been found to be lower in women than in men [19] (figure 1). Therefore, the level of GFR corresponding to CKD Stage 3 overlaps with the normal age- and gender-adjusted GFR values [20]. Thus, a substantial fraction of healthy older individuals, particularly in women, are diagnosed as having CKD Stage 3 without any significant disease [20]. Finally, having eGFRcre in the upper range (e.g., eGFRcre > 105 ml/min/1.73 m^2) has been associated with a higher mortality compared to having an eGFRcre of 60 to 90 ml/min/1.73 m^2 [3]. These issues complicate the estimation of real risk and thus the implementation of prophylactic strategies.

2.2.2 Lifestyle-related risk factors for CKD

Diabetes, obesity, smoking and hypertension are considered important risk factors for CKD [4;21-24]. The prevalence of type II diabetes is increasing, and diabetes nephropathy, characterized by persistent albuminuria and a relentless decline in renal function, has become a worldwide epidemic. Recent evidence also indicates that the kidney damage process begins in prediabetes, which is defined as impaired fasting glucose or glucose intolerance [5]. Increased fasting glucose and HbA1c within the nondiabetic range have been associated with

the progression of urinary albumin excretion [25;26]. However, the independent role of prediabetes as a risk factor for CKD needs to be investigated further [27].

Overweight and obesity increase the risk of CKD and ESRD [4;22;28;29], and some studies have found a particularly detrimental effect of abdominal obesity [30;31]. Additionally, insulin resistance, which is related to abdominal obesity [32], has been found to increase the risk of CKD [33;34]. Physical exercise, on the contrary, has been associated with a reduced progression of urinary albumin excretion and a reduced risk of kidney function decline in both diabetics and nondiabetics [26;35-37]. Furthermore, the risk of ESRD or CKD-related death is reduced in physically active nondiabetic individuals, independent of body mass index and blood pressure [24].

Why obesity, insulin resistance, smoking and prediabetes lead to renal damage and why physical exercise may be protective is not known. Advanced glycation end products, adipokines, low-grade inflammation, oxidative stress and direct hemodynamic effects on the renal vasculature could be of importance [38-40]

2.3 Hyperfiltration

2.3.1 The hypothesis of glomerular hyperfiltration as a mechanism in CKD development

Almost 90 years ago, Arataki reported that surgically removing one kidney (unilateral nephrectomy) in a rat led to the enlargement of the remaining kidney [41]. Later, it was found that the reduction of three-fourths or more of the renal mass leads to increased GFR, glomerular enlargement and subsequent injury to the remaining glomeruli [42-44]. Two to four weeks after unilateral nephrectomy in rats, single-nephron GFR increased by approximately 40%, mainly as a result of elevated plasma flow and capillary pressure in the remnant glomeruli because of renal arteriolar vasodilatation [45]. In 1981, Brenner and

Hostetter reported that glomerular pathological findings, such as foot process fusion and mesangial expansion, developed in rats with hyperfiltration [44]. They proposed the hyperfiltration theory and hypothesized that glomerular hyperfiltration and/or the concomitant increased glomerular pressure were in fact maladaptive and represented "a common pathway for renal injury" that eventually leads to glomerulosclerosis in a variety of conditions in which renal mass was reduced to below a critical level [43].

Evidence presented by Brenner and others has also indicated that reducing hyperfiltration and/or glomerular pressure with a low-protein diet or the use of renin-angiotensin system inhibitors protects against renal injury in animals and in humans with diabetes or reduced functional renal mass [43;46;47]. However, later studies have revealed that glomerular hyperfiltration can exist without a concomitant increase in glomerular capillary pressure, possibly because of the close relationship between hyperfiltration and glomerular growth [48]. Increased glomerular capillary surface area can increase single-nephron GFR with constant glomerular capillary pressure. Furthermore, evidence suggests that increased glomerular pressure, rather than hyperfiltration per se, is the most critical determinant of glomerular injury [48]. Nevertheless, experimental studies strongly suggest that glomerular growth and/or its functional counterpart, glomerular hyperfiltration, cause podocyte stress and damage that may lead to glomerulosclerosis [49]. Whether glomerular hyperfiltration causes glomerular growth or vice versa has not been settled [50]. Renal enlargement was found to precede hyperfiltration in a study of diabetes in rats [51].

Diabetes and obesity are associated with an early phase of abnormally elevated whole-kidney GFR, which often precedes CKD in these conditions [40;52]. A marked elevation in whole-kidney GFR within the same subject has been considered to be closely correlated with hyperfiltration at the single nephron level. Accordingly, it has been postulated that prolonged

whole-kidney hyperfiltration may lead to albuminuria, irreversible structural glomerular changes, glomerulosclerosis and, ultimately, to reduced GFR and CKD [53] (Figure 2).

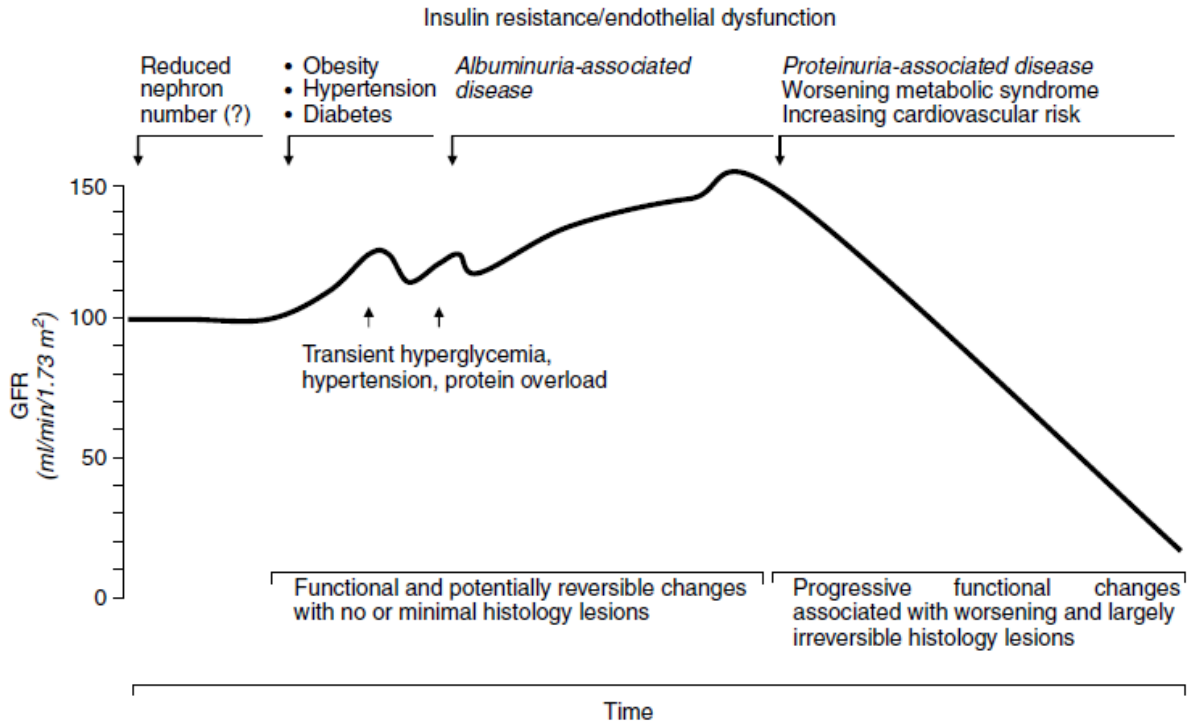


Figure 2. Time course of GFR and albuminuria/proteinuria in a hypothetical subject (adapted from Ruggenenti P., Kidney International 2006).

2.3.2 Renal hyperfiltration in humans

To date, there are no methods for determining single-nephron GFR in humans. However, in situations where a small intraindividual or interindividual variation in the number of nephrons can be assumed, changes in the total GFR can be assumed to reflect changes in the mean single-nephron GFR. Thus, investigators use whole-kidney hyperfiltration, hereafter referred to as renal hyperfiltration, as a proxy for glomerular hyperfiltration. However, there is no consensus on how to define renal hyperfiltration. In diabetes, renal hyperfiltration is often defined as having a GFR greater than the mean GFR + 1.96 x the standard deviation of nondiabetic control subjects [54]. A threshold has also been arbitrarily set at 125 to 140

ml/min/1.73 m^2 in some studies [55;56]. In studies of individuals without diabetes, the threshold has varied from having a GFR in the upper quintile of the total study group to having a GFR above the age- and sex-specific 95^{th} or 97.5^{th} percentile of healthy participants [57-59].

2.3.3 Experimental studies of disturbed glucose metabolism and renal hyperfiltration

In patients with diabetes and in healthy humans, increased GFR has been induced by acute glucose infusion and has resulted in plasma glucose levels between 7.2 and 16.0 mmol/l in different studies [60-62]. However, Mogens et al. found no GFR increase in healthy men after oral glucose intake when plasma glucose levels were raised from 4.4 to 7.6 mmol/l [62]. Whether chronic hyperglycemia in the nondiabetic range (fasting glucose: 5.6 to 6.9 mmol/l) independently influences GFR in humans has not been investigated. In dogs, however, a continuous glucose infusion for 6 days that produced a modest rise in serum glucose (from 6.5 to 7.1 mmol/l) increased GFR significantly [63].

While evidence suggests that glucose per se elevates GFR in type I diabetes, the concomitant rise in insulin levels (in response to hyperglycemia) may play a role in type II diabetes and/or in healthy subjects. Treatment with rosiglitazone, an insulin sensitizing drug, has been shown to blunt hyperfiltration in type II diabetes without a significant change in glucose levels [64]. In isolated, perfused rat kidneys, insulin infusion at physiological levels caused renal vasodilatation and increased the glomerular filtration rate, most likely via a prostaglandin-dependent process [65]. However, not all experimental studies have found that hyperinsulinemia causes an increase in GFR [40]. In rat micropuncture studies, insulin infusion during euglycemic clamping increased the mean single-nephron GFR in normal rats but reduced the GFR in diabetic rats [66].

2.3.4 Diabetes and renal hyperfiltration

Early type I diabetes is associated with enlarged kidneys and renal hyperfiltration, which are caused by hyperglycemia [67;69]. Moreover, intensive insulin treatment in type I diabetes has been shown to reverse hyperfiltration [68] and decrease kidney size [69;70]. Similarly, at the early stage of type II diabetes, some 40% of patients have an eGFR more than two standard deviations above the mean for the general population [50]. Renal hyperfiltration in type I and II diabetes tends to precede microalbuminuria, whereas the GFR may decline as proteinuria develops [52;71]. A recent meta-analysis of studies that measured GFR in people with type I diabetes found an increased risk of progression from normoalbuminuria to microalbuminuria or overt albuminuria in subjects who had renal hyperfiltration at baseline (OR 2,7 [CI: 1.2-6.1]) [55]. Also, the progression from hyperfiltration to reduced GFR has been shown without an increase in urinary albumin excretion [72]. However, whether hyperfiltration is a cause of diabetic nephropathy is still debated, often with reference to the cystatin C-based 1st Joslin Kidney Study on the natural history of microalbuminuria in type I diabetes [50;73]. In that study, in which 426 normoalbuminuric type I diabetes patients were followed for 15 years, the risk of developing microalbuminuria was not increased in individuals with hyperfiltration, as determined by cystatin C levels.

2.3.5 Obesity and renal hyperfiltration

Several studies show higher absolute GFR measurements in obese individuals compared to their lean counterparts [40;56]. Some of these studies demonstrate a significant reduction in GFR after weight loss [40]. It has been postulated that higher metabolic demands resulting from increased body weight lead to elevated GFR [48]. The underlying mechanisms and the signals that communicate these metabolic needs to the kidney are unknown.

The elevated absolute GFR in obesity is obscured by the current practice of indexing GFR to body surface area (BSA). This issue was recently illustrated in a study of 301 individuals without diabetes in which GFR was measured with inulin. In this study, overweight (BMI>25) and obesity (BMI>30) were associated with hyperfiltration when it was defined as GFR > 140 ml/min but not when hyperfiltration was defined as GFR > 140 ml/min/1.73 m^2 [56]. Because the nephron number does not increase in individuals who gain weight, increased absolute GFR is generally considered to also represent increased GFR at the single nephron level.

Interestingly, both diabetes and obesity are associated with renal hyperfiltration and glomerular enlargement/growth in an early phase. At a later stage, obesity-related glomerulopathy and diabetic nephropathy share several renal histological findings, including segmental glomerulosclerosis, glomerular basement membrane thickening and foot process fusion [74].

2.3.5 Prediabetes, insulin resistance and renal hyperfiltration

Few clinical studies have investigated the association between prediabetes or insulin resistance and renal hyperfiltration. Impaired glucose tolerance was associated with elevated GFR in a study of Pima Indians [75], but these findings lost significance when GFR was adjusted for body size. In a study of never-treated hypertensive patients, fasting glucose was associated with higher mGFR, but the authors did not specify whether GFR was adjusted for body surface area (in the multivariable adjusted analysis), and the association between glucose and hyperfiltration (yes/no) was not investigated [76]. In another study of Stage 1 hypertensive subjects in which GFR was assessed via urinary creatinine clearance, plasma glucose was not associated with hyperfiltration [57]. Neither of these studies provided information about insulin levels. Hyperinsulinemia has been shown to correlate with increased GFR and filtration fraction in overweight individuals with mild hypertension in a

study using the hyperinsulinemic euglycemic clamp technique [77]. However, as reviewed by Griffin et al., it is not likely that hyperinsulinemia is a major or direct cause of hyperfiltration in obesity. Alternative mechanisms, such as increased proximal sodium reabsorption through increased sympathetic activity or the activation of the renin-angiotensin system, could be of importance [78]

2.3.6 Physical exercise and renal hyperfiltration

Exercise conveys general protective effects on vascular function in humans and has been associated with the regression of urinary albumin excretion in both diabetics and non-diabetics [26;79;80]. Nevertheless, no experimental or epidemiological studies have addressed the effect of exercise on hyperfiltration. However, exercise training has been shown to influence brachial arterial flow-mediated dilation and to lower resting renal nerve activity in healthy men, factors that could influence GFR [81;82]. Furthermore, exercise reduces oxidative stress and inflammation, which have been associated with hyperfiltration in several experimental studies [83;84].

2.3.7 Epidemiological studies of renal hyperfiltration.

Few population-based studies have addressed the issue of hyperfiltration in nondiabetic individuals. In a study of 1572 young healthy men (mean age: 18 years), hyperfiltration was associated with body mass index (BMI), blood pressure and low HDL cholesterol but not with fasting glucose or insulin levels [85]. However, in this study, the creatinine-based Cockroft-Gault formula was used to estimate GFR, and it is known to overestimate GFR in obese individuals [86]. A limitation of all studies that estimate GFR is the possible bias and poor precision in the normal and upper ranges of eGFR [9]. Bias is of particular concern in studies of metabolic risk factors and creatinine-based eGFR because these risk factors correlate with body composition and muscle mass and therefore with creatinine production.

Whether eGFRcys performs better than eGFRcre in studies of cardiovascular or metabolic risk factors and GFR in the upper range needs to be determined.

In the Prevention of Renal and Vascular End-stage Disease study (PREVEND), a population-based study using 24-h creatinine clearance (Ccr), microalbuminuria [58] and smoking [87] were associated with renal hyperfiltration. Although the latter study did not comment on it, glucose was also associated with renal hyperfiltration (defined as the mean Ccr + 1.96 standard deviation). However, in that study, the laboratory definition of diabetes was fasting glucose ≥ 7.8 mmol/l, which is above the current cut-off limit for diabetes (≥ 7.0 mmol/l). In another PREVEND publication, higher insulin levels were associated with a higher age-specific Ccr in younger individuals but a normal or reduced age-specific Ccr in older individuals [88]. The authors interpreted this finding as a tendency for hyperfiltration at younger ages leading to accelerated renal function loss later in life [88]. However, although urinary Ccr is not influenced by body composition and/or creatinine production, its accuracy is far from optimal and perhaps not better than that of eGFR [9].

Accordingly, the causes of hyperfiltration in the general population are not settled. In particular, it is not known whether prediabetes, hyperinsulinemia, insulin resistance or physical inactivity are associated with hyperfiltration.

2. AIMS OF THE STUDY

Primary aim:

- To investigate whether renal hyperfiltration is associated with glucose metabolism or physical exercise in the nondiabetic general population.

Secondary aim:

- To explore the validity of using GFR estimated from creatinine or cystatin C in epidemiological studies of cardiovascular and chronic kidney disease in the nondiabetic general population.

3. METHODS

3.1 Subjects

The Renal Iohexol-Clearance Survey in Tromsø 6 (RENIS-T6) is a part of the sixth Tromsø study. The Tromsø study is a population-based survey of inhabitants in the municipality of Tromsø, in northern Norway (current population: 65 000). The primary focus of the Tromsø study has been to explore the prevalence of and risk factors for cardiovascular disease. Tromsø 6 was conducted between October 2007 and December 2008. A random and age-stratified sample of 19 762 persons were invited. Among the people invited to participate were 40 % of all inhabitants between the ages of 50 and 59 years and all inhabitants between 60 and 62 years. A total of 12,984 persons were examined, giving an overall attendance rate of 66%.

RENIS-T6 was conducted between November 2007 and June 2009, and invited participants of Tromsø 6 at the age of 50 - 62 years. The 50 - 62-year-old age group was chosen in order

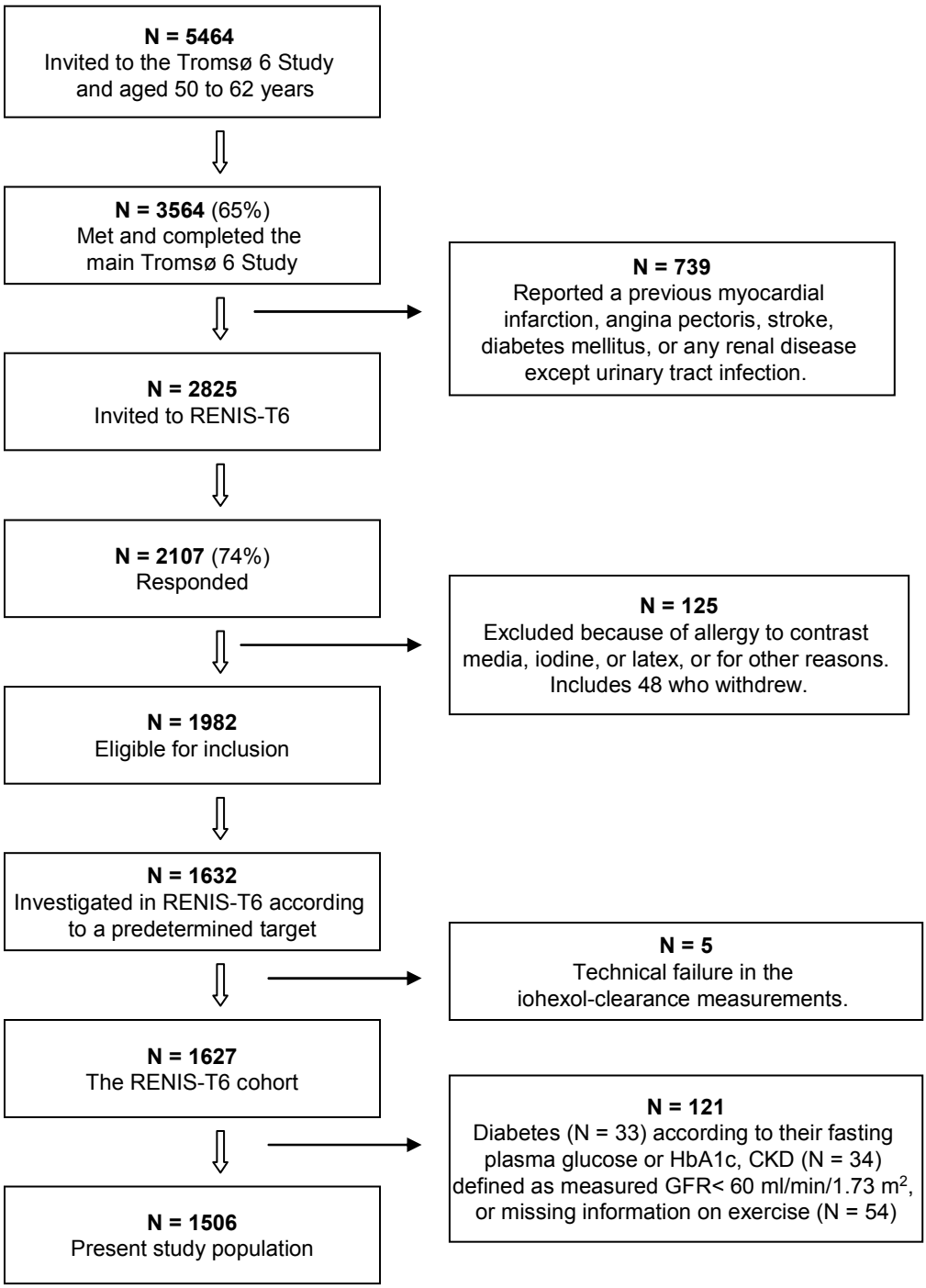
to provide a relatively healthy population with a high attendance rate and a sufficient risk of CKD and cardiovascular disease for a later end-point study. In the age groups of interest, 3564 of 5464 (65%) completed the main part of Tromsø 6, which included a physical examination and a self-administered questionnaire. From this group, we excluded 739 participants who reported previous myocardial infarction, angina pectoris, stroke, diabetes mellitus, or renal disease (Figure 2). The remaining 2825 people were invited to participate in RENIS-T6, and 2107 (75%) responded positively. Twelve people were excluded because of allergies to contrast media, iodine, or latex; 65 were excluded for other reasons (e.g., recent CVD); and 48 did not appear for their appointments. From the remaining 1982 persons, we consecutively included 1632 individuals according to a predetermined target size and stratified by sex and age group. The target sample size was based on the expected effect of GFR on the primary cardiovascular end point of the longitudinal portion of RENIS-T6. Five participants were excluded because their iohexol clearance measurements were technical failures, leaving 1627 persons in the RENIS-T6 cohort.

In paper 1 (n=1560), we excluded 33 participants who had diabetes according to their fasting plasma glucose (≥7.0 mmol/l) or HbA1c level (≥ 6.5%) and 34 with CKD, defined as a measured GFR < 60 ml/min/1.73 m^2.

In paper 2 (n=1506), we excluded another 54 participants who had missed information on the main exercise questions.

In paper 3, the study population (n=1627) consisted of the total RENIS-T6 cohort.

Figure 2. Flowchart of the RENIS-T6 study



3.2 Data collection and measurements

Study participants met in the morning at the Clinical Research Unit at the University Hospital of Northern Norway after an overnight fast. The participants had been instructed to

refrain from tobacco smoking for the previous 12 hours and to drink two glasses of water before arrival. After arrival, the participants completed a questionnaire about their current medications. Body weight and height were measured. A Teflon catheter was placed in an antecubital vein, and fasting plasma samples were drawn for biochemical analyses.

3.2.1 Clinical variables and assessment of physical exercise

As part of Tromsø 6, all participants had completed a self-administered questionnaire regarding smoking status, physical exercise, and health status, including current or previous diabetes, cardiovascular disease or renal disease (appendix 1). We used three questions addressing the frequency, intensity, and duration of leisure-time physical exercise to assess the level of physical exercise (table 1, paper 2, and appendix 1). The information from the question on general physical activity (during leisure) or during working hours was not used in this study.

3.2.2 Ambulatory and conventional blood pressure measurements

Conventional blood pressure was measured 3 times at 2 minute intervals using an automatic device (A&D Model UA-799; Tokyo, Japan) after two minutes of rest in a seated position. The average of the last two readings was used. Ambulatory blood pressure was measured after the iohexol clearance measurement and through the next day. An appropriate cuff size was used. More details on the ambulatory blood pressure measurement can be found in the Methods section of paper 2.

3.2.3 Measurement of iohexol clearance

The plasma clearance of iohexol was measured using a single-sample method. Five milliliters of iohexol (Omnipaque, 300 mgI/mL, Amersham Health, London, U.K.) were injected through the venous catheter, and the syringe was weighed before and after injection. The venous catheter was flushed with 30 mL of isotonic saline. The participants were then

allowed to have a light breakfast and to walk around until the iohexol blood sample was drawn from the same catheter. The optimal time for measuring iohexol concentration after injection was calculated using Jacobsson`s method, and based on the GFR estimated from creatinine [89]. The exact time between the iohexol injection and sampling for each person was measured in minutes, using a stopwatch. The serum iohexol concentration was measured using high-performance liquid chromatography, as described by Nilsson-Ehle [90]. The iohexol concentration was calculated using the area under the largest iohexol peak and comparing it with suitable external standards of iohexol. The analytical coefficient of variation during the study period was 3.0%.

The GFR was calculated using the formula described by Jacobsson, in which a small correction is made for nonimmediate mixing and for nonuniform distribution of the tracer [89]. Extracellular volume (distribution volume) was estimated using Granerus` equation [91]. The extrarenal iohexol clearance was ignored, in accordance with previous studies. Further details about the iohexol clearance measurement in RENIS-T6 have been published elsewhere [92].

3.2.4 Biochemical analyses

Creatinine analyses were performed with an enzymatic method that was standardized against isotope dilution mass spectroscopy (CREA Plus, Roche Diagnostics, GmbH, Mannheim, Germany). Cystatin C was measured using a particle-enhanced turbidimetric immunoassay method (Gentian, Moss, Norway). The serum glucose, triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol concentrations were measured on a Modular P800 (Roche Diagnostics) the same day.

Samples for insulin measurement were immediately frozen at –80˚C**.** Later, the samples were thawed once, and insulin was measured with an ELISA kit (DRG Instruments, Marburg, Germany). The intra- and interassay coefficients of variation were 4.7 % and 6.3

%, respectively. HbA_{1c} was measured with a liquid chromatographic method in the main part of Tromsø 6.

Urinary albumin excretion and urinary creatinine were measured in the main part of Tromsø 6. Three samples of first-void morning spot urine were collected on separate days. The urine samples were tested with a dipstick and analyzed unfrozen on the same day for albumin and creatinine using commercial kits, as described in a previous study [26]. Albuminuria was reported as the albumin/creatinine ratio (ACR, mg/mmol). Urine creatinine was measured using colorimetric methods (Jaffe's reaction) with an autoanalyzer (ABX PENTRA, Horiba ABX, Montpellier, France). The urine albumin concentration was measured using the immunoturbidimetric method and an ABX PENTRA autoanalyzer (Horiba ABX, Montpellier, France). For each subject, the ACR was determined for each of the 3 separate urine samples, and the mean of all 3 was used in the analyses.

3.2.5 Definition of hyperfiltration and other variables

Renal hyperfiltration was defined as an absolute mGFR (in mL/min) above the 90th percentile after adjusting for gender, age, weight, height, and the use of angiotensin converting enzyme inhibitors (ACE-i) or angiotensin receptor blockers (ARB). Accordingly, hyperfiltrators were determined by selecting all subjects who were above the 90th percentile in the distribution of residuals from a multiple linear regression analysis in which we used the logarithm of absolute GFR as a dependent variable and gender, ACE-i or ARB use, and the logarithms of age, weight, and height as independent variables.

Impaired fasting glucose (IFG) was defined as a fasting plasma glucose between 5.6 and 6.9 mmol/l according to the American Diabetes Association criteria. Insulin resistance was expressed by the homeostasis model assessment (HOMA-IR), which was calculated by multiplying fasting glucose (mmol/L) by fasting insulin (mU/L) and dividing the result by 22.5 [93]. The participants were categorized as being current smokers or nonsmokers (former

smokers were included in the nonsmoker group). Physical exercise was categorized according to the frequency, intensity and duration of exercise (paper 2). In paper 3, physical activity was dichotomized into active (those who perform hard physical activity for > 1 hours per week (with sweating or breathlessness) and/or light physical activity for > 3 hours per week (without sweating or breathlessness)) or inactive (the remaining participants).

To estimate GFR from creatinine, we used the recalibrated four-variable MDRD equation ($eGFR_{MDRD}$) [94] and the CKD-EPI equation ($eGFR_{CKD\text{-}EPI}$) [13] (table 1, paper 2). Among several existing cystatin C-based equations, we used Rule's cystatin C-based equation of 2006 (eGFRcys)[95] because this equation performed well compared with other cystatin C equations validated against measured GFR in the general population (RENIS-T6) [92].

3.3 Statistical analyses

The age- and sex-adjusted means, geometric means and median values of the population characteristics are presented according to fasting glucose group (paper 1) or physical exercise level (paper 2). In paper 3, we divided participants into gender- and method-specific quintiles for mGFR and eGFR. Differences across groups were tested by analysis of covariance (ANCOVA) or linear regression for mean values, quantile regression for median values and multiple logistic regression for dichotomous variables.

The association between mGFR (in ml/min/1.73 m^2) and fasting glucose, HbA1c, fasting insulin, HOMA-IR (paper 1) and cardiovascular risk factors (paper 3) were assessed by multiple linear regression analysis, adjusting for known or possible determinants of GFR (see paper 1 and 3 for details on adjustments). In paper 1, the same multiple linear regression models were repeated with GFR expressed in ml/min (i.e., absolute GFR, not indexed for body surface area). To reflect the multiplicative relationship between variables, we also repeated regression analyses when GFR, age, weight and height were transformed to natural

logarithms. Possible nonlinear relationships were explored with generalized additive models using smoothing with cubic splines [96].

The associations between hyperfiltration (yes/no) and fasting glucose, HbA1c, fasting insulin, HOMA-IR and exercise categories were assessed with multiple logistic regression, adjusting for the same variables that were used in the linear regression analyses.

We tested for interactions between age, gender and all of the other independent variables in both the linear and logistic regression analyses. We also tested for a possible interaction between fasting glucose, fasting insulin and physical exercise in their effect on mGFR and hyperfiltration (yes/no; described in detail in paper 2).

In paper 2, we repeated all analyses when the absolute GFR in mL/min was adjusted to 40 liters of total body water (GFR40, instead of indexing by 1.73 m^2 of body surface area), as recently suggested in another RENIS-T6 publication [97]. In addition, we defined hyperfiltration as a GFR40 above the 90^{th} percentile after adjusting for age, ACE-I and ARB only.

The sensitivity and specificity for detecting individuals with hyperfiltration (based on measured GFR) was calculated for the Rules cystatin C equation and the creatinine-based $eGFR_{CKD\text{-}EPI}$ equation. In these analyses, eGFRcys and $eGFR_{CKD\text{-}EPI}$ were used to define hyperfiltrators using the same method described for mGFR, by selecting individuals with eGFR (in ml/min/m^2) > 90^{th} percentile adjusted for age, sex, weight, height, and the use of ACE-i or ARB.

To study whether eGFR was associated with non-GFR related CVD risk factors, we performed a multiple linear regression with eGFR as the dependent variable, CVD risk factors as independent variables, and mGFR added as an adjusting variable. The analysis was repeated with errors-in-variables regression to account for the reduced reliability of mGFR [98].

4. MAIN RESULTS

4.1 Paper 1. Impaired fasting glucose is associated with renal hyperfiltration in the general population

A total of 311 (40%) males and 141 (18%) females had impaired fasting glucose (IFG). After adjusting for age and sex, individuals with IFG had higher BMIs, fasting insulin levels, and blood pressure but not higher albumin-creatinine ratio (ACR) compared with those with normal fasting glucose levels ($P < 0.05$). Measured GFR, but not creatinine- or cystatin C-based estimates of GFR, was higher in those with IFG ($P = 0.002$).

Fasting glucose, HbA_1c, fasting insulin, and HOMA-IR were all positively associated with mGFR in separate multiple linear regression models adjusted for age, gender, weight, height, diastolic blood pressure, current smoking, and ACEi or ARB use ($P<0.05$) (table 3, paper 1) The effect estimates of insulin and HOMAR-IR were not significant when fasting glucose was included in the models. A multivariable-adjusted, non-linear association between fasting glucose and mGFR was observed in a generalized additive model. GFR increased with higher glucose levels, with a steeper slope beginning at fasting glucose > 5.6 mmol/L (figure 1, paper 1).

The mean GFRs for female (n = 79) and male (n = 77) hyperfiltrators were 110.1 (range: 98.7 – 138.6) and 118.2 (range: 107.5 – 137.3) ml/min/1.73 m^2, respectively.

The multivariable-adjusted odds ratio of hyperfiltration per one unit increase in fasting glucose, HbA_{1c} and HOMA-IR and for having IFG were 1.97 (95% CI, 1.36 - 2.85), 2.23 (95% CI, 1.30 - 3.86), 1.14 (95% CI, 1.01 - 1.28) and 1,56 (1.07 – 2.25), respectively. Fasting insulin level was not associated with hyperfiltration, and the significance of HOMA-IR disappeared when fasting glucose was included in the model.

4.2 Paper 2: Renal hyperfiltration – Sex-specific effects of high-intensity exercise and fasting glucose

Eight hundred seventy-seven (58.3%) of the participants exercised at least two times a week (table 2, paper 2). The frequency and intensity of exercise were correlated (r = 0.30, $P < 0.001$). After adjusting for age and sex, more intensive exercise was associated with a lower percentage of smoking ($P < 0.001$), lower HDL cholesterol ($P < 0.05$), lower ambulatory heart rate ($P < 0.001$), and lower eGFRcre ($P < 0.05$). Measured GFR or eGFRcys, however, did not differ between exercise levels.

High-intensity exercise reduced the adjusted odds ratio of hyperfiltration in men (0.5; $P < 0.01$) but not in women. The effect of exercise intensity on hyperfiltration in men remained similar and significant after adjustment for exercise frequency and duration (data not shown). Exercise frequency tended to reduce the odds of hyperfiltration in women, but the linear trend was not statistically significant ($P = 0.2$).

In the multiple linear regression analysis, high-intensity exercise eliminated the positive association between fasting glucose and GFR in both sexes. A one-unit increase in glucose was associated with 7.3 and 6.2 ml/min/1.73 m^2 higher GFR in men and women, respectively ($P < 0.001$), but only in individuals who never exercised or exercised with low intensity (interaction, $P < 0.001$). Similar but attenuated results were found for eGFRcys but not for eGFRcre (shown for $eGFR_{CKD\text{-}EPI}$, table 5, paper 2).

The same pattern of effect modification by exercise was found for the association between glucose and hyperfiltration (table 6, paper 2). However, the interaction between exercise category and glucose on hyperfiltration did not reach statistical significance when all of the subjects were analyzed using the same model ($P = 0.25$). Exercise did not modify the association between insulin resistance and GFR or hyperfiltration (not shown), but it tended

to modify the association between HbA1c and GFR/hyperfiltration in a way similar to that found for fasting glucose (not significant).

We obtained essentially the same results when GFR was normalized by total body water (GFR40) and when hyperfiltration was defined as GFR40 above the 90th percentile and adjusted for age, ACE-I and ARB (not shown).

4.3 Paper 3. Estimated GFR is associated with cardiovascular risk factors independently of GFR

In the RENIS-T6 cohort (N=1627), the mean mGFR was 87.8 ml/min/m^2 in women and 95.7 ml/min/m^2 in men. The mean eGFRcre, however, was slightly lower in women, and the mean eGFRcys was 2.2 ml/min/m^2 higher in women than men (table 1, paper 3). In the multivariable-adjusted regression model, mGFR was positively associated with current smoking (β=2,87, $P < 0.001$) and negatively associated with diastolic blood pressure (β = -0.94, $P < 0.05$; per standard deviation increase). BMI and HDL cholesterol were associated with higher mGFR in men but not in women (P for gender interaction < 0.05).

Several CVD risk factors influenced eGFR after adjusting for mGFR (table 4, paper 3). Current smoking was associated with a 3.9 ml/min/1.73 m^2 higher GFR_{MDRD}, a 1.6 ml/min/1.73 m^2 higher $GFR_{CKD\text{-}EPI}$ and a -8.9 ml/min/1.73 m^2 lower eGFRcys after adjusting for all of the independent variables and mGFR ($P < 0.001$). More risk factors influenced eGFRcys than eGFRcre after adjusting for mGFR. Smoking, BMI, triglycerides and HDL cholesterol were associated with eGFRcys in the fully adjusted model ($P < 0.001$). The results from errors-in-measurements regression were similar. Analyses with generalized additive models revealed nonlinear effects of BMI on eGFRcre and of triglycerides on eGFRcys after adjusting for mGFR ($P < 0.05$; shown in Figure 1, Paper 3).

In analyses of ten-year cardiovascular risk scores (estimated with the Framingham equation) across quintiles of mGFR and eGFR, lower eGFRcys was associated with a higher cardiovascular risk score in both sexes (*P* for trend <0.001). However, the cardiovascular risk score was not different across mGFR and eGFRcre quintiles for men, and it increased across mGFR and eGFRcre quintiles for women (*P* for trend < 0.05). After adjustment for smoking, the difference in cardiovascular risk score was no longer significant across eGFRcre and mGFR quintiles in women, but it was still significant for eGFRcys in both sexes.

4.4 Additional analysis

In the logistic regression analyses performed in paper 1, we found that current smoking was independently associated with renal hyperfiltration (odds ratio: 2.12; $P < 0.001$; not presented in paper 1).

The sensitivity and specificity for defining hyperfiltration with $eGFR_{CKD\text{-}EPI}$ were 0.36 (95% CI, 0.28 – 0.44) and 0.93 (CI, 0.91 – 0.94); for eGFRcys, they were 0.30 (CI, 0.23 – 0.38) and 0.92 (CI, 0.91 – 0.94).

In agreement with the linear and logistic regression analyses that used mGFR as a dependent continuous or dichotomous variable (tables 2 and 3, paper 1), we found a similar but attenuated association between fasting glucose and eGFRcys (β= 2.75.ml/min/m2, $P < 0.05$) and between fasting glucose and hyperfiltration based on the eGFRcys (OR = 1.5, $P < 0.05$). There were no associations between fasting glucose and eGFRcre or hyperfiltration defined according to the eGFRcre. Current smoking was associated with an increased odds ratio of hyperfiltration defined by eGFRcre (for $eGFR_{CKD\text{-}EPI;}$ OR = 1.8, $P < 0.05$) but a decreased odds ratio of hyperfiltration defined by eGFRcys (OR = 0.35, $P < 0.001$).

5. GENERAL DISCUSSION

5.1 Methodological discussion

5.1.1 Design

The RENIS-T6 was designed as a prospective cohort study. This thesis is based on cross-sectional investigations of baseline data from the RENIS-T6. The information and data were recorded at a single point in time or within few months. Thus, as in other cross-sectional studies, no definite conclusions about cause and effect relationships can be drawn.

5.1.2 Bias

Bias is defined as the result of a systematic error in the design or conduct of the study [99]. A study with little bias is considered valid. Bias can be divided into two main categories: Selection bias and information bias [100].

5.1.2.1 Selection bias

Selection bias occurs when there are "different probabilities of being included in the study sample according to the presence of the exposure or outcome of interest" [100]. In the current study, individuals were first invited to participate in the main Tromsø 6 study and thereafter to the RENIS-T6. The attendance rate for the main Tromsø 6 study was 66%, and the response rate in RENIS-T6 was 77%, which is high compared to similar studies in other countries. However, some selection bias may still be present. For example, having enough time or energy to participate in a study or being concerned about one's own health could affect lifestyle-related risk factors (e.g., diet and exercise) and the probability of participating in a study. To assess the magnitude of selection bias, one should compare the characteristics of those who participate and those who were eligible to participate and did not. We do not have data on nonattendees of Tromsø 6, but we have information about the study

characteristics of all subjects who were eligible for RENIS-T6. There were only small differences between those included in the study (n = 1621) and all eligible persons (n = 2825) [92].

Another possible bias in cross-sectional studies is healthy survival bias. Survival bias in our study could exist if, for example, smokers with high GFRs were oversampled because smokers with low GFRs had higher mortality and thus had died. However, because we excluded all persons with known CKD, mGFR < 60 ml/min/1.73 m^2, CVD or diabetes, this type of bias is less likely.

Incident-prevalence bias may exist if the exposure of interest affects the duration of the outcome. In our study, this could be a problem when examining the risk of hyperfiltration. We found a small and borderline significant increased odds ratio of hyperfiltration by insulin resistance (assessed using the HOMA index). Let us assume that both fasting glucose and insulin resistance cause hyperfiltration to the same degree but that hyperfiltration in the presence of insulin resistance has a shorter duration before progressing to proteinuria and decreased GFR (e.g., because of concomitant hypertension or endothelial dysfunction). The point prevalence of hyperfiltration would therefore be higher during hyperglycemia than during insulin resistance. Similarly, there could also be other short-term risk factors for hyperfiltration that are difficult to assess in a cross-sectional study.

5.1.2.2 Information Bias

Information bias occurs if definitions of study variables are inadequate or if data collection is flawed. This may result in systematic errors and data that, on average, differ from the truth [99].

Some exposure variables in the current study were obtained with a questionnaire. Subjective interpretation of questions (e.g., in the reporting of smoking or physical exercise) is possible. Such errors could lead to misclassification, which also could differ between subgroups, e.g.,

between men and women. This factor could explain the sex disparity in the effect of high-intensity exercise that we found in our study. Nevertheless, it is unlikely that such misclassification is substantial because intensive exercise tends to be better reported than less-intensive exercise [101;102].

Another issue is the possibility that errors in the reporting of exercise could differ according to glycemic status or other health-related factors that also affect GFR. In this case, a differential misclassification may occur, which could have biased the estimates in either direction. However, the exercise questions in the current study were recently found to have good reproducibility and acceptable validity in studies that assessed physical fitness by measuring maximal oxygen consumption (VO_{2max}) [103;104]. However, one of these studies included only men. Additionally, the questionnaire had higher validity for assessing more intense physical activity than overall energy expenditure [103]. This could explain why the effect of physical exercise in the current study was intensity dependent. On the other hand, several studies have found that the intensity of exercise is crucial for improving fitness and endothelial function and thus cardiovascular outcome [79;104;105].

Information bias because of stress from participating in the study is also a possibility. Mental stress triggers the sympathetic nervous system, which again could influence the level of fasting insulin or fasting glucose [106]. Furthermore, stress causes vasoconstriction, and likely also causes vasoconstriction in the kidney, and could thereby affect GFR, which was measured over a short period of time [107]. Therefore, stress could bias the level of the risk factor and the dependent variable (hyperfiltration) and lead to differential misclassification. However, regarding the results in paper 2, exercise did not alter the renal vascular response to mental stress in a study of healthy humans [108].

5.1.1.3 Temporal bias

In cross sectional studies, no cause-and-effect relationship can be firmly established. Temporal bias occurs when inferences about a temporal sequence of cause and effect are made erroneously [100]. For example, the main hypothesis put forward in the current thesis is that elevated fasting glucose causes hyperfiltration. Instead, the opposite (that elevated GFR causes hyperglycemia) is also possible. However, although the kidneys play a role in endogenous glucose production [109], this direction of causality is less biological plausible. Furthermore, experimental evidence supports that an infusion of glucose in humans causes the GFR to rise [60;61].

5.1.3 Definition of renal hyperfiltration

There is no consensus on how to define hyperfiltration. In line with previous studies, we chose an arbitrary level of GFR as a cut-off for hyperfiltration (adjusted GFR > 90^{th} percentile) (see discussion in paper 1), thus transforming a continuous variable into a dichotomous variable. Our results may have been influenced by the chosen cut-off level. However, these results remained essentially the same when we repeated the analyses with a stricter definition of hyperfiltration (adjusted GFR > 95^{th} percentile).

Because the nephron number varies significantly among individuals, increased whole-kidney GFRs could represent increased nephron endowment as well as elevated mean single nephron GFR. It seems unlikely that our finding of hyperfiltration in the setting of impaired fasting glucose is caused by a higher nephron number in these individuals. The opposite would be more likely, as low birth weight has been associated with reduced nephron endowment and with later development of prediabetes or the metabolic syndrome [110].

To increase the probability of detecting single nephron hyperfiltration, we adjusted our definition of hyperfiltration for factors assumed to influence the number of nephrons (age, sex, and height). In both genders, GFR decreases after the age of 30 to 40 years, mainly

because of a decrease in the number of functioning nephrons [111]. Some, but not all studies have found the number of nephrons to be lower in females than in males [6;112;113]. Lower birth weight is associated with decreased nephron numbers and is strongly associated with lower adult height [114]. Although we are not aware of any studies that have investigated the association between height and nephron number, it seems reasonable to adjust for height. Accordingly, a nonadjusted threshold for renal hyperfiltration would mask single-nephron hyperfiltration in persons who are older, and possibly in women and in persons with a short stature.

Absolute GFR correlates positively with body weight and decreases after weight reduction [40;115]. However, the nephron number does not increase in individuals who gain weight, but nephron size and the single-nephron GFR do, according to animal experiments [48]. In humans, a similar elevation in the single-nephron GFR with weight gain would be masked by the current practice of indexing GFR according to body surface area (BSA). Thus, if the purpose is to estimate the association between body weight and hyperfiltration, GFR should be treated as an absolute value without adjusting for BSA, as others have recommended [56;116]. On the other hand, because body weight, age and sex may be associated with both glucose metabolism and GFR, these factors could confound the association between, e.g., hyperglycemia and hyperfiltration. For this reason, we have chosen to correct the definition of renal hyperfiltration not only for age, gender and height but also for body weight.

ACE-i and ARB use influence GFR and may be associated with fasting glucose levels [117]. Therefore, we also adjusted for these variables in the definition of hyperfiltration. However, the logistic regression analysis without this adjustment showed results that were essentially similar to those in table 3, paper 1.

5.1.4 External validity

The external validity of a study expresses the generalizability of its results to other populations. The participants in the Tromsø 6 study were mainly Caucasians, and the study's results are not necessarily applicable to other ethnicities. The associations between CVD risk factors and eGFR or mGFR may be different in other ethnic groups. However, there is no reason to assume that the main results of papers 1 and 2 are not applicable to Africans or Asians. Impaired glucose metabolism has recently been associated with elevated mGFR in a small study of Africans and with elevated eGFR in Japanese subjects [59;118]. Furthermore, the risk of CKD in diabetes is high worldwide, which indicates that hyperglycemia has a similar detrimental effect on kidney function in all ethnicities. A protective effect of physical exercise on early kidney decline has been found in both African Americans and Caucasians [35].

The participants in our study were 50 to 62 years old. Thus, our results cannot automatically be generalized to other age groups. However, we did not find an interaction between age and any of the independent risk factors in this restricted age group. Still, the associations between fasting glucose or insulin levels and GFR could be different in younger versus older individuals. This difference was indicated in a report from the PREVEND study, which included participants ranging in age from 28 to 75 years. In this population, higher insulin levels were associated with a tendency toward increased Ccr in younger individuals but normal or reduced Ccr in older individuals [88]. Indeed, hyperfiltration is hypothesized to cause a steeper age-related decline in GFR. However, insulin levels were not associated with hyperfiltration assessed by eGFR in a study of young healthy men [85]. Our finding of a positive association between plasma glucose and GFR was also reported in experimental studies in young healthy people and in both young and old patients with diabetes [67;119].

Furthermore, the association between impaired fasting glucose and hyperfiltration was recently found in a Japanese population aged between 20 and 89 years old [59].

We are not aware of any other reports on the modifying effects of exercise on the association between glucose and GFR. However, a protective effect of exercise on the risk of CKD or GFR decline has been indicated in two population-based studies with a wider age range than RENIS-T6 and in a study of individuals older than those included in RENIS-T6 [23;24;37].

In paper 3, we found that eGFR was dependent on non-GFR-related factors. Similar results have been found in studies with wider age ranges; thus, we believe that the main results of paper 3 can be generalized to other age groups.

5.1.5 Confounding

The term confounding refers to a situation in which a noncausal association between a given exposure and an outcome is observed as a result of the influence of a third variable. This confounding variable is 1) causally associated with the outcome, 2) noncausally or causally associated with the exposure and 3) is not an intermediate variable in the causal pathway between exposure and outcome [100].

In the current study, several healthy characteristics may be linked to GFR in addition to fasting glucose or physical exercise. Indeed, HDL cholesterol, triglycerides and current smoking were associated with glycemic status (table 1, paper 1), exercise (table 3, paper 2) and GFR (paper 3). However, the main results in papers 1 and 2 remained essentially the same after adjusting for known and possible determinants of GFR, including age, sex, weight, height, smoking, blood pressure, BMI, HDL cholesterol, triglycerides, insulin, ACR and ACE-i or ARB use. In paper 2, we also adjusted for ambulatory BP and heart rate. Nevertheless, there is always the possibility of residual confounding.

Albuminuria is a putative confounder in our study. However, the results in papers 1 and 2 remained essentially the same after adjusting for ACR (not shown). In paper 3, we used

different models of adjustments in which the fully adjusted model included traditional cardiovascular risk factors but not albuminuria or fasting glucose (see table 4 for details). These variables may have modified the associations between CVD risk factors and eGFR or mGFR. However, even if this should be the case, it does not alter the conclusion that eGFR is influenced by non-GFR-dependent factors.

5.1.6 Single-sample iohexol clearance.

GFR has day-to-day variability caused by measurement error and intraindividual biological variability. This variability introduces "noise" that makes it more difficult to detect true between-individual variations. Several precautions were taken to reduce measurement errors in RENIS-T6. The GFR measurements were performed in a fasting state at the same time of the day for all subjects. We also standardized the procedure and used trained personnel who followed a strict protocol.

The intraindividual coefficient of variation (CV) of GFR measurements varies according to the GFR level and the measurement method [9]. The CV for repeated measurements of plasma iohexol clearance (multiple sample) in the same individual has been reported to be between 5 and 6 % in the near-normal range of GFR [120;121], which was lower than the CVs for cystatin C, creatinine clearance or eGFRcre [121]. The single-sample iohexol clearance method has a performance comparable to the multi-sample method for either iohexol or ^{51}Cr-EDTA, particularly at GFR levels above 60 ml/min/m^2 [10;122].

The most important source of error in the single-sample iohexol clearance compared with the multiple-samples method is the formula that estimates the distribution volume of iohexol. This estimate is calculated according to weight and gender and is not very accurate. However, the imprecision in the estimate can be reduced by finding the optimal time point for the iohexol sample [89]. Another potential source of error and bias for the iohexol measurement in our study was that we used the same Teflon catheter to inject iohexol and to

collect serum samples for measuring the iohexol concentration. A small fraction of the injected iohexol could become entrapped in the plastic wall of the catheter, thereby creating a measurement error in the iohexol analysis sample. To minimize the contamination of the catheter, we flushed the catheter with 30 ml isotonic saline. This procedure has been evaluated (in 14 adult patients with GFR> 40 ml/min) and found to produce GFR results equal to those obtained from using two separate Teflon catheters [122].

Ideally, we should have repeated the iohexol clearance method in a subsample of the RENIS-T6 and validated the iohexol measurement with another gold standard. However, the single-sample iohexol clearance method has been validated in other studies [9]. Furthermore, a true validation procedure would have had to be performed in another hospital to avoid the same "in-hospital" bias in the alternative gold standard method. This would have been expensive and inconvenient for the participant, and it was not feasible in this study. The age- and sex-specific mean GFR values in our study were comparable to previously published data, as summarized by Hallan et al. [123]. Thus, any bias in the GFR measurements was most likely small.

The iohexol measurement was performed with the HPLC method, which has been shown to have better reproducibility than the X-ray fluorescence method used in some other studies [9]. The interassay CV (analytical precision) of the HPLC analysis was 3.0%.

5.1.7 Assessment of insulin resistance

In our study, insulin resistance was assessed with the HOMA-IR and not measured with the gold standard, the euglycemic clamp method. The reproducibility of HOMA-IR estimates has been shown to be low, partially because of the pulsatility of insulin secretion [93]. However, the HOMA-IR correlates well with values obtained with the euglycemic clamp technique ($R = 0.88$, $p < 0.0001$) and remains the preferred method for use in large epidemiological studies [93;124]. Nevertheless, suboptimal precision and reproducibility in

the insulin measurement and HOMA-IR may have diluted our regression estimates towards null.

5.1.8 Hemolysis

In our study, 180 (11%) serum samples had some degree of hemolysis due to problems with the blood sampling technique. The proportion of samples with hemolysis is close to that reported in other studies [125;126]. Hemolysis in the blood sample significantly influenced the mean insulin levels but not the mean GFR or fasting glucose levels. There was no change in regression estimates after excluding individuals with hemolysis in the serum samples.

5.2 Discussion of the main results

5.2.1 Fasting glucose and renal hyperfiltration

We found that fasting glucose and impaired fasting glucose (IFG) was associated with hyperfiltration in a nondiabetic middle-aged population, independent of possible confounders such as age, gender, BMI, blood pressure and plasma lipids. Furthermore, we found a similar effect of HbA1c on GFR. This result indicates that the association between glucose and GFR is not related to acute hyperglycemia secondary to stress during GFR measurement but is rather an effect of chronically elevated glucose on GFR. To the best of our knowledge, no previous studies have found an independent association between IFG or prediabetes and GFR. However, in agreement with our results, a recent study of 99140 persons who underwent health checkups in Japan observed that IFG was associated with hyperfiltration assessed with eGFRcre [59]. In agreement with our nonlinear effect of fasting glucose, they found a higher odds ratio of hyperfiltration when IFG was defined as fasting glucose > 6.1 mmol/l compared to >5.6 mmol/l.

The exact mechanism of hyperfiltration in hyperglycemia is unknown. Vasodilatation of the afferent arteriole has been found in several experimental studies of diabetes, but vasoconstriction of the efferent arteriole with subsequent increased glomerular pressure has also been reported [64;127]. Recent studies indicate a key role of increased sodium reabsorption through sodium-glucose cotransport in the proximal renal tubulus [128;129], which decreases the delivery of sodium to the distal tubulus and could thereby increase GFR through the tubuloglomerular feedback system [130]. Hyperfiltration during hyperglycemia could also be related to nitric oxide, oxidative stress, vascular inflammation and activation of the renin-angiotensin system [129].

We found that IFG was present in 40% of middle-aged men and in 18% of middle-aged women, which is in agreement with other studies [131]. The effect of IFG on the risk of CKD has not been fully elucidated. Fox et al. found the IFG increases the risk of CKD but not after adjustment for cardiovascular risk factors [27]. However, in that study, CKD was defined solely as a creatinine-based estimated GFR < 59 ml/min/1.73 m^2 for woman and < 64 ml/min/1.73 m^2 for men. In two other studies, elevated fasting glucose or HbA1c predicted increased urinary albumin excretion in the general population when individuals with diabetes were excluded [25;26]. Higher levels of plasma glucose was also found to predict decline in GFR estimated by creatinine on three separate occasions during 6 years in a study of the general population [132].

In our study, having IFG was not associated with increased urinary albumin excretion (table 1, paper 1). It is possible that the relatively healthy participants in the present study were examined in an early phase of renal dysfunction, before an increase in albumin excretion has occurred, and that hyperfiltration still is a link between IFG and albuminuria. Potentially, hyperfiltration could also predispose to renal function decline without causing albuminuria, as recently found in a study of type II diabetes patients [72].

Finally, it is possible that hyperfiltration during hyperglycemia, but with the absence of other renal risk factors, may not be a major risk factor for albuminuria or decreased GFR. This suggestion is in accordance with the so-called multi-hit hypothesis of renal injury, where additional CKD risk factors are needed to cause kidney injury [133]. Low birth weight has been proposed as a congenital or "first hit" CKD risk factor because it is associated with reduced number of nephrons at birth. Congenital nephron deficit coexists with renal and glomerular hypertrophy, which indicate the presence of single nephron hyperfiltration already during the first months of life [110;134]. Low birth weight is also associated with impaired glucose tolerance and hypertension in adulthood, which add to the burden of renal risk [110]. Individuals with low birth weight have been found to have a 70% increased risk for developing CKD, defined as albuminuria, reduced GFR or end-stage renal disease [135]. The only prospective study of hyperfiltration in nondiabetics included subjects with Stage 1 hypertension. In these subjects, hyperfiltration at baseline increased the hazard risk of albuminuria (HR 4.0 (CI, 2.1-9.2)) after 7.8 years [57].

5.2.2 Insulin resistance and renal hyperfiltration

Fasting insulin levels or insulin resistance assessed by HOMA-IR were not associated with hyperfiltration in our study when glucose was included in the regression models (table 2, paper 1). Previous population-based studies of insulin and GFR are scarce, and none has measured GFR with an exact method. Two cross-sectional studies of the general population found a negative association between insulin levels and creatinine-based estimates of GFR [136;137]. In contrast to our study, these two studies included people with CKD. The PREVEND study found that hyperinsulinemia was associated with a tendency towards hyperfiltration in young individuals but not in the elderly, even after adjustment for fasting glucose [88]. Our results indicated that plasma glucose, but not hyperinsulinemia or insulin resistance, was independently related to hyperfiltration in a middle-aged population.

Theoretically, insulin resistance may have been linked with a shorter period of hyperfiltration at a younger age, before the later reduction of GFR to normal levels or to reduced GFR at an older age.

5.2.3 Smoking and renal hyperfiltration

Current smoking was associated with mGFR (in linear regression; table 3, paper 3) and with the odds of hyperfiltration in the current study (see Section 4.4). Similar results have been found in previous studies in which renal function was assessed as eGFR. In these studies, confounding by lower muscle mass in smokers was suggested as a possible explanation. In our study, GFR was measured in a fasting state, including abstinence from tobacco. Accordingly, our findings may have been affected by a withdrawal phenomenon. Indeed, experimental studies have found reduced GFR immediately after smoking or the administration of nicotine [138;139]. However, in the PREVEND study, smoking was associated with hyperfiltration, as assessed by 24-hour urinary Ccr [87]. Moreover, a recent longitudinal study of 10118 middle-aged Japanese men found that current smokers had a 1.32-times higher risk of developing glomerular hyperfiltration and a 1.51-times higher risk of proteinuria than nonsmokers during a 6-year follow-up period [140]. In light of these studies and our findings, there is now solid evidence of an association between smoking and hyperfiltration.

5.2.4 Leisure-time exercise and renal hyperfiltration

We found that leisure-time high-intensity exercise reduced the odds ratio for hyperfiltration in men. In women, a non-significant trend of reduced risk of hyperfiltration was found for exercise frequency. The association between exercise and renal hyperfiltration has not been investigated before. Some studies reported the cross-sectional association between physical activity and eGFR, with divergent results [141] [142]. In the third National Health and

Nutrition Examination Survey (NHANES III), frequency of physical activity was associated with a lower eGFRcre, while the activity calculated as metabolic equivalents was associated with an increased eGFRcre [141]. In that study, GFR was estimated using the Cockcroft-Gault equation and was not adjusted to body surface area. Differences in body size of physically active versus inactive participants may therefore have confounded the results. Increased muscle mass in physically active subjects may also bias the results in studies of eGFRcre and exercise. In the Cardiovascular Health Study, a higher baseline eGFRcys, but not a higher eGFRcre, was found in physically active versus inactive older adults [37]. In this study, high-intensity exercise, but not moderate- or low-intensity exercise, reduced the hazard ratio for rapid kidney function decline (eGFRcys) during 7 years of follow-up. In an Australian study of the general population, where they did not differentiate between high and low-intensive exercise, physical activity was not associated with the incidence of CKD based on eGFRcre or albuminuria. Our findings of reduced odds of hyperfiltration by high-intensity exercise offers a possible explanation for why exercise, particularly intensive exercise, is found to be a protective risk factor for urinary albumin excretion, renal function decline or CKD in some epidemiological studies [23;24;26;37]

We do not have a good explanation for the gender disparity found in our study. Men and women may have interpreted the exercise questions in the questionnaire differently. On the other hand, gender-specific risk factors for early renal dysfunction have been observed previously. In the PREVEND study, Verhave et al. found a stronger association between cardiovascular risk factors and urinary albumin excretion in men than in women [143]. Similarly, results from the fourth Tromsø study showed that initiating strenuous physical activity $\geq$ 1 hour per week reduced the risk of increased albumin excretion in men but not in women [26]. In another report from the Tromsø study, physical activity at baseline predicted an increase in eGFRcre for women, but not for men, after 7 years [36]. However, in a large

cross-sectional study from the HUNT Study, Hallan et al. did not observe any gender differences in the odds of CKD by exercise, although the confidence intervals did not exclude a gender interaction [23]. Longitudinal studies on exercise and the risk of CKD are needed and should include the exercise intensity level and a gender-specific analysis.

5.2.5 Leisure-time exercise, fasting glucose and GFR

In RENIS-T6, fasting glucose was one of the strongest predictors of mGFR and was the strongest predictor of hyperfiltration. However, performing high-intensity exercise eliminated the effect of fasting glucose on mGFR in both genders and attenuated the effect of glucose on hyperfiltration. This novel finding may be of clinical importance. In animals, hyperfiltration combined with hyperglycemia has been shown to induce podocyte stress, podocyte injury, and cell apoptosis [144]. In humans, both hyperfiltration and borderline hyperglycemia have been associated with the progression of urinary albumin excretion and renal function decline [25;55;57;72;132]. However, there are no longitudinal studies on the effect of exercise on renal injury caused by hyperglycemia.

Several beneficial effects of exercise could influence renal hemodynamics and urinary albumin excretion levels. Physical exercise, particularly high-intensity exercise, has been shown to improve endothelial function, decrease inflammation and oxidative stress, and reduce the activation of the renin-angiotensin-aldosterone system and renal sympathetic activity [79;81;82]. In a randomized study of 82 type II diabetes patients with metabolic syndrome, high-intensity exercise, but not low-intensity exercise, reduced urinary albumin excretion levels without any reduction in BMI or blood pressure [80]. In that study, HbA1c, HOMA-IR and inflammatory biomarkers were also reduced, which may have influenced urinary albumin excretion levels either through reduced hyperfiltration and/or some other mechanism, such as endothelial dysfunction. In the present study, metabolic factors such as insulin resistance (HOMA-IR), waist-to-hip ratio, heart rate and triglyceride levels did not

influence the effect of exercise on the association between glucose and mGFR. We did not measure markers of inflammation or oxidative stress. Emerging evidence links inflammation and oxidative stress to kidney injury and particularly to diabetic nephropathy [38]. Inflammation and oxidative stress increases in hyperglycemia and is reduced by exercise [38;79]. Several experimental studies also suggest that inflammation and oxidative stress induce hyperfiltration [83;84].

Not all studies report independent effects of prediabetes and exercise on the development of albuminuria or CKD [27;142]. Our findings indicate that the renal risk of hyperglycemia may differ according to the level of physical exercise. Longitudinal studies should investigate the potential interaction between these variables.

5.2.6 GFR estimated with cystatin C- and creatinine-based formulae

In epidemiological research, GFR is usually estimated based on serum levels of creatinine or cystatin C. Cystatin C has been proposed as a promising marker of GFRs in the normal or upper range in the general population and in diabetic patients [145]. In our study, eGFRcys was not a superior method for the identification of individuals with hyperfiltration compared with eGFRcre (see Section 4.1.1) This result is consistent with results from two recent publications, one from RENIS-T6 and one validation study of eGFR in 448 type 2 diabetes patients, in which eGFRcre was found to perform equal to or better than eGFRcys in the normal range [92] [146]. Our results indicate that both eGFRcre and eGFRcys have low sensitivity for detecting individuals with hyperfiltration. Accordingly, results from hyperfiltration studies that use eGFR should be interpreted with caution.

5.2.7 Cardiovascular risk factors and estimated GFR

In paper 3, we found that estimated GFR was associated with traditional cardiovascular risk factors, even after adjusting for mGFR. eGFRcys was associated with smoking, BMI, HDL-

cholesterol and triglycerides in the fully adjusted model. There were also nonlinear relationships between eGFRcre and BMI and between triglycerides and eGFRcys after adjustment for mGFR. These findings indicate that eGFR, particularly eGFRcys, is influenced by cardiovascular risk factors other than true GFR. These findings are in line with the results from the following two previous studies. In the PREVEND study, Knight et al. found that male gender, current smoking, greater weight, greater height and higher C-reactive protein (CRP) were associated with higher cystatin C levels (lower eGFRcys) after adjustment for creatinine clearance [14]. However, creatinine clearance has limited precision, and the study included people with CVD. Therefore, these findings are not directly comparable to our results. Furthermore, we included BMI rather than height and weight, while Knight et al. did not include serum lipids as continuous variables in their analysis. Stevens et al. found that cystatin C was associated with several factors, including gender, BMI, CRP and hemoglobin, after adjusting for iothalamate clearance [15]. Their study included people with CKD, and the authors did not perform multivariate analyses.

In our study, eGFRcre and mGFR showed a similar pattern of increased cardiovascular risk scores (the Framingham score) across increasing GFR quintiles for women (table 5, paper 3). However, eGFRcys was associated with a marked gradient in the opposite direction, that is, a lower cardiovascular risk score at higher eGFRcys in both sexes. These findings indicate that correct adjustment for confounding by cardiovascular risk factors in survival analyses is more critical for eGFRcys than eGFRcre. Several studies have found that elevated eGFRcre, as an indicator of hyperfiltration, is associated with increased mortality, whereas elevated eGFRcys is not [147;148]. This phenomenon has been explained by a possible confounding from chronic disease with muscle wasting and low creatinine production. Our results indicate that both a false overestimation of eGFRcre and a true elevated GFR (e.g., caused by smoking) could partly explain the increased mortality associated with elevated eGFRcre.

Although the abovementioned longitudinal studies adjusted for smoking and cardiovascular risk factors, the adjustment may have been insufficient due to the gender-specific and non-linear associations between these risk factors and eGFR and mGFR.

Studies of eGFR and CVD risk as well as studies of eGFR or hyperfiltration assessed using eGFR and albuminuria as an outcome could be biased, particularly in cystatin C studies. Obesity, smoking and inflammation (CRP), which influence (increase) cystatin C levels independently of mGFR, have been found to predict albuminuria. As a consequence, the risks of developing albuminuria in studies of hyperfiltration based on eGFRcys could be biased (towards null) if proper adjustment is not performed. The non-GFR related effects of eGFR also represent a potential source of misclassification in studies where eGFR is used to define hyperfiltration (yes/no) without adjusting for these factors. Consequently, a higher proportion of women, a lower proportion of obese patients and a lower proportion of smokers could be falsely classified as having hyperfiltration assessed by cystatin C.

6. CONCLUSIONS AND PERSPECTIVES

We conclude that higher fasting glucose levels, but not higher fasting insulin levels, are associated with hyperfiltration in a general middle-aged population without diabetes. Furthermore, leisure-time high-intensity exercise reduced the odds ratio of hyperfiltration in men and eliminated the positive association between glucose and GFR in both sexes. The sensitivity and specificity of eGFRcre and eGFRcys for defining hyperfiltration is poor. eGFRcre and especially eGFRcys are influenced by non-GFR-related cardiovascular risk factors that may induce bias in longitudinal studies. The significance of hyperfiltration as a risk factor for CKD at the population level remains unknown. Longitudinal, population-based studies of hyperfiltration assessed by measured GFR are needed.

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PAPERS I-III

APPENDIXES

Tromsø undersøkelsen

Skjemaet skal leses optisk. Vennligst bruk blå eller sort penn. Du kan ikke bruke komma, bruk blokkbokstaver.

2007 – 2008 KONFIDENSIELT

HELSE OG SYKDOMMER

1 **Hvordan vurderer du din egen helse sånn i alminnelighet?**

- ☐ Meget god
- ☐ God
- ☐ Verken god eller dårlig
- ☐ Dårlig
- ☐ Meget dårlig

2 **Hvordan synes du at helsen din er sammenlignet med andre på din alder?**

- ☐ Mye bedre
- ☐ Litt bedre
- ☐ Omtrent lik
- ☐ Litt dårligere
- ☐ Mye dårligere

3 **Har du eller har du hatt?**

	Ja	Nei	Alder første gang
Hjerteinfarkt	☐	☐	
Angina pectoris *(hjertekrampe)*	☐	☐	
Hjerneslag/hjerneblødning	☐	☐	
Hjerteflimmer *(atrieflimmer)*	☐	☐	
Høyt blodtrykk	☐	☐	
Beinskjørhet *(osteoporose)*	☐	☐	
Astma	☐	☐	
Kronisk bronkitt/emfysem/KOLS	☐	☐	
Diabetes	☐	☐	
Psykiske plager *(som du har søkt hjelp for)*	☐	☐	
Lavt stoffskifte	☐	☐	
Nyresykdom, unntatt urinveisinfeksjon	☐	☐	
Migrene	☐	☐	

4 **Har du langvarige eller stadig tilbakevendende smerter som har vart i 3 måneder eller mer?**

☐ Ja ☐ Nei

5 **Hvor ofte har du vært plaget av søvnløshet de siste 12 måneder?**

- ☐ Aldri, eller noen få ganger
- ☐ 1-3 ganger i måneden
- ☐ Omtrent 1 gang i uken
- ☐ Mer enn 1 gang i uken

6 **Under finner du en liste over ulike problemer. Har du opplevd noe av dette den siste uken (til og med i dag)?** (Sett ett kryss for hver plage)

	Ikke plaget	Litt plaget	Ganske mye	Veldig mye
Plutselig frykt uten grunn	☐	☐	☐	☐
Føler deg redd eller engstelig	☐	☐	☐	☐
Matthet eller svimmelhet	☐	☐	☐	☐
Føler deg anspent eller oppjaget	☐	☐	☐	☐
Lett for å klandre deg selv	☐	☐	☐	☐
Søvnproblemer	☐	☐	☐	☐
Nedtrykt, tungsindig	☐	☐	☐	☐
Følelse av å være unyttig, lite verd	☐	☐	☐	☐
Følelse av at alt er et slit	☐	☐	☐	☐
Følelse av håpløshet mht. framtida	☐	☐	☐	☐

BRUK AV HELSETJENESTER

7 **Har du i løpet av de siste 12 måneder vært hos:** Hvis Ja; Hvor mange ganger?

	Ja	Nei	Ant ggr
Fastlege/allmennlege	☐	☐	
Psykiater/psykolog	☐	☐	
Legespesialist utenfor sykehus *(utenom fastlege/allmennlege/psykiater)*	☐	☐	
Fysioterapeut	☐	☐	
Kiropraktor	☐	☐	
Annen behandler *(homøopat, akupunktør, fotsoneterapeut, naturmedisiner, håndspålegger, healer, synsk el.l)*	☐	☐	
Tannlege/tannpleier	☐	☐	

8 **Har du i løpet av de siste 12 måneder vært på sykehus?**

	Ja	Nei	Ant ggr
Innlagt på sykehus	☐	☐	
Konsultasjon ved sykehus uten innleggelse;			
Ved psykiatrisk poliklinikk	☐	☐	
Ved annen sykehuspoliklinikk	☐	☐	

9 **Har du gjennomgått noen form for operasjon i løpet av de siste 3 årene?**

☐ Ja ☐ Nei

BRUK AV MEDISINER

10 **Bruker du, eller har du brukt, noen av følgende medisiner?** (Sett ett kryss for hver linje)

	Aldri brukt	Nå	Før	Alder første gang
Medisin mot høyt blodtrykk	☐	☐	☐	
Kolesterolsenkende medisin	☐	☐	☐	
Medisin mot hjertesykdom	☐	☐	☐	
Vanndrivende medisin	☐	☐	☐	
Medisin mot beinskjørhet *(osteoporose)*	☐	☐	☐	
Insulin	☐	☐	☐	
Diabetesmedisin *(tabletter)*	☐	☐	☐	
Stoffskiftemedisinene Thyroxin/levaxin	☐	☐	☐	

11 **Hvor ofte har du i løpet av de siste 4 ukene brukt følgende medisiner?** (Sett ett kryss pr linje)

	Ikke brukt siste 4 uker	Sjeldnere enn hver uke	Hver uke, men ikke daglig	Daglig
Smertestillende på resept	☐	☐	☐	☐
Smertestillende uten resept	☐	☐	☐	☐
Sovemidler	☐	☐	☐	☐
Beroligende medisiner	☐	☐	☐	☐
Medisin mot depresjon	☐	☐	☐	☐

12 **Skriv ned alle medisiner – både de med og uten resept – som du har brukt regelmessig i siste 4 ukers periode.** (Ikke regn med vitaminer, mineraler, urter, naturmedisin, andre kosttilskudd etc.)

Får du ikke plass til alle medisiner, bruk eget ark.

FAMILIE OG VENNER

13 **Hvem bor du sammen med?** (Sett kryss for hvert spørsmål og angi antall)

	Ja	Nei	Antall
Ektefelle/samboer	☐	☐	
Andre personer over 18 år	☐	☐	
Personer under 18 år	☐	☐	

14 **Kryss av for de slektninger som har eller har hatt**

	Foreldre	Barn	Søsken
Hjerteinfarkt	☐	☐	☐
Hjerteinfarkt før fylte 60 år	☐	☐	☐
Angina pectoris *(hjertekrampe)*	☐	☐	☐
Hjerneslag/hjerneblødning	☐	☐	☐
Beinskjørhet *(osteoporose)*	☐	☐	☐
Magesår/tolvfingertarmsår	☐	☐	☐
Astma	☐	☐	☐
Diabetes	☐	☐	☐
Diabetes før fylte 30 år	☐	☐	☐
Psykiske plager	☐	☐	☐
Rusproblemer	☐	☐	☐

15 **Har du nok venner som kan gi deg hjelp når du trenger det?**

☐ Ja ☐ Nei

16 **Har du nok venner som du kan snakke fortrolig med?**

☐ Ja ☐ Nei

17 **Hvor ofte tar du vanligvis del i foreningsvirksomhet som for eksempel syklubb, idrettslag, politiske lag, religiøse eller andre foreninger?**

☐ Aldri, eller noen få ganger i året
☐ 1-2 ganger i måneden
☐ Omtrent 1 gang i uken
☐ Mer enn en gang i uken

ARBEID, TRYGD OG INNTEKT

18 **Hva er din høyeste fullførte utdanning?** (Sett ett kryss)

☐ Grunnskole, framhaldsskole eller folkehøyskole
☐ Yrkesfaglig videregående, yrkesskole eller realskole
☐ Allmennfaglig videregående skole eller gymnas
☐ Høyskole eller universitet, mindre enn 4 år
☐ Høyskole eller universitet, 4 år eller mer

19 **Hva er din hovedaktivitet?** (Sett ett kryss)

☐ Yrkesaktiv heltid
☐ Yrkesaktiv deltid
☐ Arbeidsledig
☐ Hjemmeværende
☐ Pensjonist/trygdet
☐ Student/militærtjeneste

20 **Mottar du noen av følgende ytelser?**

- ☐ Alderstrygd, førtidspensjon (AFP) eller etterlattepensjon
- ☐ Sykepenger (er sykemeldt)
- ☐ Rehabiliterings-/attføringspenger
- ☐ Uføreytelse/pensjon, hel
- ☐ Uføreytelse/pensjon, delvis
- ☐ Dagpenger under arbeidsledighet
- ☐ Overgangstønad
- ☐ Sosialhjelp/-stønad

21 **Hvor høy var husholdningens samlede bruttoinntekt siste år?** Ta med alle inntekter fra arbeid, trygder, sosialhjelp og lignende.

- ☐ Under 125 000 kr
- ☐ 125 000-200 000 kr
- ☐ 201 000-300 000 kr
- ☐ 301 000-400 000 kr
- ☐ 401 000-550 000 kr
- ☐ 551 000-700 000 kr
- ☐ 701 000 -850 000 kr
- ☐ Over 850 000 kr

22 **Arbeider du utendørs minst 25 % av tiden, eller i lokaler med lav temperatur, som for eksempel lager-/industrihaller?**

- ☐ Ja
- ☐ Nei

FYSISK AKTIVITET

23 **Hvis du er i lønnet eller ulønnet arbeid, hvordan vil du beskrive arbeidet ditt?**

- ☐ For det meste stillesittende arbeid *(f.eks. skrivebordsarbeid, montering)*
- ☐ Arbeid som krever at du går mye *(f.eks ekspeditørarbeid, lett industriarbeid, undervisning)*
- ☐ Arbeid der du går og løfter mye *(f.eks postbud, pleier, bygningsarbeider)*
- ☐ Tungt kroppsarbeid

24 **Angi bevegelse og kroppslig anstrengelse i din fritid. Hvis aktiviteten varierer meget f eks mellom sommer og vinter, så ta et gjennomsnitt. Spørsmålet gjelder bare det siste året.** (Sett kryss i den ruta som passer best)

- ☐ Leser, ser på fjernsyn eller annen stillesittende beskjeftigelse
- ☐ Spaserer, sykler eller beveger deg på annen måte minst 4 timer i uken *(her skal du også regne med gang eller sykling til arbeidsstedet, søndagsturer med mer)*
- ☐ Driver mosjonsidrett, tyngre hagearbeid, snømåking e.l. *(merk at aktiviteten skal vare minst 4 timer i uka)*
- ☐ Trener hardt eller driver konkurranseidrett regelmessig og flere ganger i uka

25 **Hvor ofte driver du mosjon?** (Med mosjon mener vi at du f.eks går en tur, går på ski, svømmer eller driver trening/idrett)

- ☐ Aldri
- ☐ Sjeldnere enn en gang i uken
- ☐ En gang i uken
- ☐ 2-3 ganger i uken
- ☐ omtrent hver dag

26 **Hvor hardt mosjonerer du da i gjennomsnitt?**

- ☐ Tar det rolig uten å bli andpusten eller svett.
- ☐ Tar det så hardt at jeg blir andpusten og svett
- ☐ Tar meg nesten helt ut

27 **Hvor lenge holder du på hver gang i gjennomsnitt ?**

- ☐ Mindre enn 15 minutter
- ☐ 15-29 minutter
- ☐ 30 minutter – 1 time
- ☐ Mer enn 1 time

ALKOHOL OG TOBAKK

28 **Hvor ofte drikker du alkohol?**

- ☐ Aldri
- ☐ Månedlig eller sjeldnere
- ☐ 2-4 ganger hver måned
- ☐ 2-3 ganger pr. uke
- ☐ 4 eller flere ganger pr.uke

29 **Hvor mange enheter alkohol** (en øl, et glass vin, eller en drink) **tar du vanligvis når du drikker?**

- ☐ 1-2
- ☐ 3-4
- ☐ 5-6
- ☐ 7-9
- ☐ 10 eller flere

30 **Hvor ofte drikker du 6 eller flere enheter alkohol ved en anledning?**

- ☐ aldri
- ☐ sjeldnere enn månedlig
- ☐ månedlig
- ☐ ukentlig
- ☐ daglig eller nesten daglig

31 **Røyker du av og til, men ikke daglig?**

- ☐ Ja
- ☐ Nei

32 **Har du røykt/røyker du daglig?**

- ☐ Ja, nå
- ☐ Ja, tidligere
- ☐ Aldri

33 **Hvis du har røykt daglig tidligere, hvor lenge er det siden du sluttet?**

Antall år ☐☐

34 **Hvis du røyker daglig nå eller har røykt tidligere: Hvor mange sigaretter røyker eller røykte du vanligvis daglig?**

Antall sigaretter ☐☐

35 **Hvor gammel var du da du begynte å røyke daglig?**

Antall år ☐☐

36 **Hvor mange år til sammen har du røykt daglig?**

Antall år ☐☐

37 **Bruker du, eller har du brukt, snus eller skrå?**

- ☐ Nei, aldri
- ☐ Ja, men jeg har sluttet
- ☐ Ja, av og til
- ☐ Ja, daglig

KOSTHOLD

38 **Spiser du vanligvis frokost hver dag?**

☐ Ja ☐ Nei

39 **Hvor mange enheter frukt og grønnsaker spiser du i gjennomsnitt per dag?** (Med enhet menes f.eks. en frukt, glass juice, potet, porsjon grønnsaker)

Antall enheter ☐☐

40 **Hvor mange ganger i uken spiser du varm middag?**

Antall ☐☐

41 **Hvor ofte spiser du vanligvis disse matvarene?**
(Sett ett kryss pr linje)

	0-1 g pr. mnd	2-3 g pr.mnd	1-3 g pr.uke	4-6 g pr.uke	1-2 g pr. dag
Poteter	☐	☐	☐	☐	☐
Pasta/ris	☐	☐	☐	☐	☐
Kjøtt	☐	☐	☐	☐	☐
Kvernet kjøtt *(pølser, hamburger o.l)*	☐	☐	☐	☐	☐
Mager fisk	☐	☐	☐	☐	☐
Fiskepålegg	☐	☐	☐	☐	☐
Feit fisk *(f.eks.laks, ørret, makrell, sild, kveite,uer)*	☐	☐	☐	☐	☐

42 **Hvor mye drikker du vanligvis av følgende?**
(Sett ett kryss pr. linje)

	Sjelden/ aldri	1-6 glass pr. uke	1 glass pr. dag	2-3 glass pr. dag	4 glass el. mer pr. dag
Melk, kefir, yoghurt	☐	☐	☐	☐	☐
Fruktjuice	☐	☐	☐	☐	☐
Brus/leskedrikker med sukker	☐	☐	☐	☐	☐

43 **Hvor mange kopper kaffe og te drikker du daglig?**
(sett 0 for de typene du ikke drikker daglig)

	Antall kopper
Filterkaffe	☐☐
Kokekaffe/presskanne	☐☐
Annen kaffe	☐☐
Te	☐☐

44 **Hvor ofte spiser du vanligvis fiskelever?**
(For eksempel i mølje)

☐ Sjelden/aldri ☐ 1-3 g i året ☐ 4-6 g i året
☐ 7-12 g i året ☐ Oftere

45 **Bruker du følgende kosttilskudd?**

	Daglig	Iblant	Nei
Tran, trankapsler	☐	☐	☐
Omega 3 kapsler *(fiskeolje,selolje)*	☐	☐	☐
Vitamin og eller mineraltilskudd	☐	☐	☐

SPØRSMÅL TIL KVINNER

46 **Er du gravid nå?**

☐ Ja ☐ Nei ☐ Usikker

47 **Hvor mange barn har du født?**

Antall ☐☐

48 **Hvis du har født, fyll ut for hvert barn: fødselsår og vekt samt hvor mange måneder du ammet.**
(Angi så godt som du kan)

Barn	Fødselsår	Fødselsvekt i gram	Ammet ant.mnd
1	☐☐☐☐	☐☐☐☐	☐☐
2	☐☐☐☐	☐☐☐☐	☐☐
3	☐☐☐☐	☐☐☐☐	☐☐
4	☐☐☐☐	☐☐☐☐	☐☐
5	☐☐☐☐	☐☐☐☐	☐☐
6	☐☐☐☐	☐☐☐☐	☐☐

49 **Har du i forbindelse med svangerskap hatt for høyt blodtrykk?**

☐ Ja ☐ Nei

50 **Hvis Ja, i hvilket svangerskap?**

☐ Første ☐ Senere

51 **Har du i forbindelse med svangerskap hatt protein (eggehvite) i urinen?**

☐ Ja ☐ Nei

52 **Hvis Ja, i hvilket svangerskap?**

☐ Første ☐ Senere

53 **Ble noen av disse barna født mer enn en måned for tidlig (før termin) pga. svangerskapsforgiftning?**

☐ Ja ☐ Nei

54 **Hvis Ja, hvilke(t) barn**

Barn 1	Barn 2	Barn 3	Barn 4	Barn 5	Barn 6
☐	☐	☐	☐	☐	☐

55 **Hvor gammel var du da du fikk menstruasjon første gang?**

Antall år ☐☐

56 **Bruker du for tiden reseptpliktige legemidler som påvirker menstruasjonen?**

P-pille, hormonspiral eller lignende ☐ Ja ☐ Nei
Hormonpreparat for overgangs-alderen ☐ Ja ☐ Nei

VED FRAMMØTE vil du få utfyllende spørsmål om menstruasjon og eventuell bruk av hormoner. Skriv gjerne ned på et papir navn på hormonpreparater du har brukt, og ta det med deg. Du vil også bli spurt om din menstruasjon har opphørt og eventuelt når og hvorfor.

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