Faculty of Health Sciences

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Obesity, renal hyperfiltration and glomerular filtration rate decline in the general population

Results from the Renal Iohexol Clearance Survey

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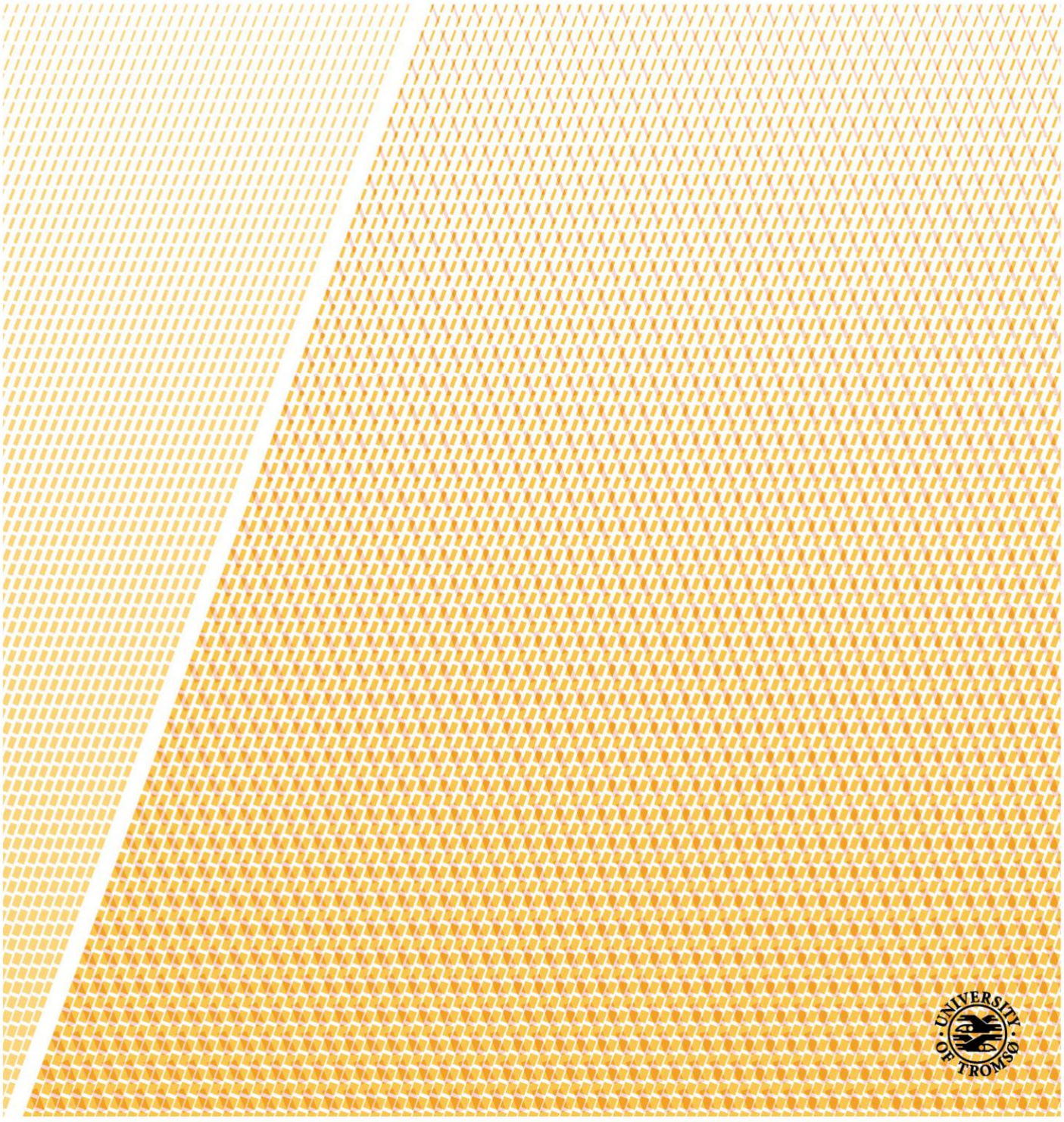

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List of presented papers

This thesis is based on the following papers:

1. Stefansson VTN, Schei J, Jenssen TG, Melsom T, Eriksen BO: Central obesity associates with renal hyperfiltration in the non-diabetic general population: a cross-sectional study. *BMC Nephrology* 2016 Nov 10;17(1):172.
2. Stefansson VTN, Schei J, Solbu MD, Jenssen TG, Melsom T, Eriksen BO: Metabolic syndrome but not obesity measures are risk factors for accelerated age-related glomerular filtration rate decline in the general population. *Kidney International* 2018 May;93(5):1183-1190.
3. Melsom T, Stefansson VTN, Schei J, Solbu MD, Jenssen TG, Wilsgaard T, Eriksen BO: Association of Increasing GFR with Change in Albuminuria in the General Population. *Clinical Journal of American Society of Nephrology* 2016 Dec 7;11(12):2186-2194.

List of abbreviations

ACR	Albumin-creatinine ratio
BMI	Body mass index
BSA	Body surface area
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
ΔACR	Change in albumin-creatinine ratio
ΔGFR	Change in glomerular filtration rate
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
KDIGO	Kidney Disease: Improving Global Outcomes
MetS	Metabolic syndrome
mGFR	Measured glomerular filtration rate
RENIS	The Renal Iohexol Clearance Survey
RENIS-FU	The Renal Iohexol Clearance Survey Follow-Up
RENIS-T6	The Renal Iohexol Clearance Survey in Tromsø 6
RR	Relative risk
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-hip ratio

Summary

Obesity is a well-known risk factor for several severe diseases, including diabetes and cardiovascular disease. The metabolic syndrome is a concept related to obesity which includes additional risk factors for disease: increased waist circumference, high blood pressure, elevated fasting glucose, elevated triglycerides and lowered high-density lipoprotein cholesterol levels.

Both obesity and the metabolic syndrome are known risk factors for chronic kidney disease and end-stage renal disease, but their effect on kidney function before reaching those disease states is less clear. The results from previous studies on these subjects are divergent and inconclusive.

The concept of hyperfiltration, a state of elevated GFR (glomerular filtration rate, a measure of kidney function), may contribute to the inconsistency of research results on the subject. Hyperfiltration is present in diabetes, obesity and hypertension, and is a state of distress which may cause kidney damage in the long term. In the short and medium term, however, it may present as higher or increasing GFR. It may also cause albuminuria, which is an early marker of endothelial damage.

In this thesis, the association between obesity, the metabolic syndrome, changes in GFR and hyperfiltration were explored in the population-based Renal Iohexol Clearance Survey. GFR was measured with an accurate method (iohexol clearance) in 1627 persons in 2007-09 and repeated in 1324 of the same persons in 2013-15. The relationship between changes in GFR and changes in albuminuria was also explored, to further explore the concept of hyperfiltration as an increase in GFR over time.

We found that obesity was associated with hyperfiltration, but not with accelerated GFR decline. Increased albuminuria was associated with increased GFR. The metabolic syndrome was associated with accelerated GFR decline. The results point to hyperfiltration as an

important factor in the relationship between obesity and GFR, and that hyperfiltration is associated with albuminuria.

1 Background

1.1 Obesity

1.1.1 Prevalence

Obesity is a growing problem globally. In large population surveys, body mass index (BMI, defined as body weight in kilos divided by height in metres squared) is the most commonly used measure to define obesity. The World Health Organisation (WHO) criteria classify a person with a BMI ≥25 kg/m^2 as overweight, while a person with a BMI ≥30 kg/m^2 is considered obese. In Norway, the estimated prevalence of overweight and obesity increased from 34.0% and 6.7%, respectively, in 1975 to 58.9% and 23.2%, respectively, in 2014[1]. In 2016, the Center for Disease Control National Center for Health Statistics estimated the prevalence of obesity in the United States to be 39.8%[2]. In the same year, WHO estimated that more than 1.9 billion people worldwide were overweight, of whom more than 650 million people were obese[3].

1.1.2 Obesity as a risk factor

The 2016 Global Burden of Disease Study ranked a high BMI as the second greatest risk factor for global disability-adjusted life years lost in women, and the sixth greatest in men[4]. An estimated 4 million deaths were attributable to high BMI in 2015 globally, and an estimated 120 million disability-adjusted life years lost[5]. The increased mortality in obesity is largely due to the increased risk of cardiovascular disease and diabetes, but obesity also increases the risk of several other diseases and conditions, including certain cancers, sleep apnoea, infertility and venous thromboembolism[5-7]. Compared to persons with a BMI of 20-25 kg/m^2, overweight persons have a more than 1.5 times higher prevalence of cardiovascular

disease and diabetes, while those with a BMI ≥30 kg/m^2 have a more than 3.5 times higher prevalence of diabetes and hypertension[8].

1.1.3 Obesity measurements

Obesity is most commonly measured using BMI. Its origin is with the Belgian scientist Adolphe Quetelet, who first used the formula in 1832[9], but its modern name came from Keys et al. in 1972[10]. BMI is calculated from weight divided by height squared, so by definition it does not account for body shape or composition. However, it has become the leading method for the measurement of obesity in large populations due to its simplicity and almost universal availability.

Several simple body measurement techniques other than the BMI have been proposed and used in population studies. Of these, waist circumference (WC) and its ratio to hip circumference, the waist-to-hip ratio (WHR), are the most commonly used. They capture different aspects of obesity than BMI in that they reflect the placement and distribution of mass in the body rather than the body weight itself. Several studies have pointed to these variables as better predictors of cardiovascular and diabetes risk than BMI[11-14]. However, large meta-analyses have not found clinically significant differences between WC, WHR and BMI in predicting these diseases[15, 16]. WC and WHR are still commonly used in population studies as a supplement to BMI.

BMI, WC and WHR cannot be used to measure the actual amount of fat tissue in the body. Accurate measurements of fat mass include bioelectrical impedance, dual X-ray absorptiometry, computed tomography and magnetic resonance imaging. These methods (with the exception of bioelectrical impedance) allow for distinction between visceral (abdominal) fat, subcutaneous fat, and other contributors to body mass such as muscle and bone. Visceral fat, but not subcutaneous fat, has been associated with an increased risk of

myocardial infarction[17], type 2 diabetes[18], incident chronic kidney disease[19] and metabolic syndrome[20], independent of BMI.

1.1.4 Categorisation

Categorising continuous variables may be useful for the purposes of diagnosis, clinical decision making, public policy and communication to the public. WHO has standardised the categorisation of obesity measurements, as presented in Table 1[3].

The categories of BMI are fairly well rooted in mortality risk. A large meta-analysis found the lowest mortality in the BMI range of 22.5–25.0 kg/m^2, with the excess mortality risk increasing rapidly above a BMI of 30 kg/m^2 [21]. It should be noted that while the BMI categories are often used universally, several studies suggest a different categorisation should be used for Asian population groups due to the higher risk of diabetes at a lower BMI than in European or African populations[22].

The categories of WC are based on a British cohort in a 1995 study by Lean et al.; the cut-off values were chosen based on their ability to identify participants with a high BMI and/or high WHR with high sensitivity and specificity[23]. The lower threshold identified subjects with a BMI ≥25 kg/m^2 and the higher threshold identified subjects with a BMI ≥30 kg/m^2. The same WC cut-off points were used as one of the five criteria of the metabolic syndrome, which will be covered in more detail in the next chapter of this thesis. The origin of the WHR categories appears to be a 1999 WHO consultation on diabetes, in which the WHR cut-off points were suggested as a criterion for the metabolic syndrome[24]. The authors offered no source or explanation for this choice of cut-off points, and neither the WC nor the WHR cut-off points appear to be rooted in epidemiological studies of mortality or morbidity.

Table 1. BMI, waist circumference and waist-hip ratio categories for European, African and Middle Eastern populations according to World Health Organisation and International Diabetes Federation criteria[3].

	World Health Organisation category	Measurement range
Body mass index	Underweight	<18.5 kg/m^2
	Normal	18.5–24.9 kg/m^2
	Overweight	25.0–29.9 kg/m^2
	Obesity	≥30.0 kg/m^2
Waist circumference	Normal	<80 cm (female) <94 cm (male)
	Increased risk of metabolic complications	≥80 cm (female) ≥94 cm (male)
	Severely increased risk of metabolic complications	≥88 cm (female) ≥102 cm (male)
Waist-hip ratio	Normal	<0.85 (female) <0.90 (male)
	Severely increased risk of metabolic complications	≥0.85 (female) ≥0.90 (male)

1.2 Metabolic syndrome

1.2.1 Definition

The concept of metabolic syndrome (MetS) stems from the long-known observation that obesity and a cluster of interrelated risk factors increase the risk for diseases such as diabetes and cardiovascular disease[25]. Many scientists have contributed to our understanding of the relationships between the various risk factors and diseases, but Reaven is often credited for the modern understanding of the syndrome, with insulin resistance as a core concept[26].

The currently used criteria for the syndrome were harmonized in 2009 from different definitions stemming from the WHO and the 2001 National Cholesterol Education Program Adult Treatment Panel III, respectively[27]. The thresholds for criteria are based on the diagnostic criteria for hypertriglyceridaemia (high triglycerides), hypoalphalipoproteinaemia (low high-density lipoprotein cholesterol), and pre-diabetes (high glucose), as well as the blood pressure treatment thresholds in diabetes, and the previously mentioned waist circumference thresholds. There is not yet a consensus on which waist circumference threshold should be used for MetS, and both are often presented in studies of MetS. The criteria are listed in Table 2[27].

Table 2. Criteria for the metabolic syndrome: Three out of 5 criteria must be fulfilled for diagnosis.

Category	Criteria
Abdominal obesity	Waist circumference: In the United States and Europe, two different thresholds are currently in use by researchers: ≥80 cm (female), ≥94 cm (male) (strict definition) ≥88 cm (female), ≥102 cm (male) (less strict definition) Other thresholds may apply to different ethnic groups
Elevated blood pressure	Systolic blood pressure ≥130 mm Hg and/or diastolic blood pressure ≥85 mm Hg, or use of antihypertensive medication
Impaired glucose tolerance	Fasting glucose ≥5.6 mmol/L, or use of anti-diabetic medication
High triglycerides levels	Fasting triglycerides ≥1.7 mmol/L, or use of triglycerides-lowering medication
Low high-density lipoprotein cholesterol levels	Fasting high-density lipoprotein cholesterol < 1.29 mmol/L (female), <1.03 mmol/L (male), or the use of cholesterol-altering medication

1.2.2 Prevalence and relevance

The prevalence of MetS in the United States has increased in tandem with the obesity epidemic, from 22.7% of adults in 1988–94 to 34.2% in 2007-12 in the National Health and Nutrition Examination Surveys, using the less strict definition of MetS[28, 29]. By the same definition, 25.9% of participants in the North Trøndelag Health Study had MetS in 1995–97[30]and 25.5% had MetS in the 2007–8 Tromsø Study[31]. MetS is associated with increased risk of diabetes (relative risk (RR): 3.0)[32], cardiovascular disease (RR: 2.4)[33], chronic kidney disease (estimated glomerular filtration rate <60 ml/min/1.73m^2; RR: 2.5)[34], various cancers[35] and all-cause mortality (RR: 1.6)[33].

1.2.3 Utility and controversy of the metabolic syndrome

Critics of the concept argue that these associations do not yield much value because metabolic syndrome is composed of several well-known risk factors and does not necessarily provide additional risk information beyond its constituent components[36]. Proponents see it as a useful concept to alert and educate patients, healthcare providers and the general public about the high and interrelated risks of insulin resistance, obesity, hypertension and dyslipidaemia which affect a large segment of the population[37].

There has also been debate on whether obesity without MetS may constitute a separate, lower-risk "metabolically healthy" form of obesity, in which the risks normally associated with obesity are absent or greatly reduced[38, 39]. However, obesity appears to increase disease risk significantly even without MetS, although the risk is even higher when MetS is present [40-42].

1.3 Kidney function and albuminuria

1.3.1 Nephron number

The functional unit of the kidney is the nephron. The number of nephrons in humans is set around birth and does not increase afterwards. There is a large variation between individuals in the number of nephrons present in a kidney; estimates vary between 200,000 to more than 2,000,000 per kidney[43]. Low birth weight and family history of end-stage renal disease are risk factors associated with a lower nephron number[43-46]. Adult height and sex are also associated with nephron number and are more easily available to researchers and clinicians than birth weight: tall persons and males generally have higher nephron numbers, though Denic et al. found no sex difference in a multivariable adjusted regression analysis of kidney donors[47]. Height and sex are also associated with birth weight[48], and these three variables may be seen surrogates for the complex interplay between the genetic, nutritional and intrauterine conditions which determine nephron number[43-45, 49, 50]. Low nephron numbers have been associated with hypertension and chronic kidney disease[51, 52]. Nephron numbers decrease gradually with age as nephrons develop sclerosis and cease functioning; donors aged 70-75 years old had 48% fewer nephrons than those aged 18-29 in the study by Denic et al.[47].

1.3.2 The glomerular filtration rate

Kidney function is usually assessed as the glomerular filtration rate (GFR), which is the total volume of blood filtered through all the glomeruli in the nephrons in both kidneys per minute, expressed as ml/min. By tradition, this whole-kidney GFR is adjusted for 1.73 m^2 of body surface area (BSA) to reduce the spread in GFR seen in people of different sizes, although this is not without controversy, as we will see in chapter 1.3.3.

In everyday clinical practice and in most population studies, GFR is estimated using the serum concentration of creatinine or cystatin C and an estimation equation. There are several

equations for estimated GFR (eGFR), but the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation is currently the most commonly used equation for adults[53], and the Schwartz equation is used for children[54]. The Berlin Initiative Study 1 equation has been developed for the elderly,[55] but there is no general agreement about which equation is best for this age group. A unified equation for all ages has been proposed, but has not yet been widely adopted[56].

1.3.3 Critiques of eGFR and body surface area standardisation

All eGFR equations are hampered by the fact that they are estimates based on serum levels of creatinine and/or cystatin C, rather than actual measurements of GFR. Around 10–20% of CKD-EPI eGFR values differ by more than 30% from the measured GFR (mGFR) value, and the absolute differences (in ml/min/1.73m^2) between eGFR and mGFR are larger in the higher ranges of GFR[53, 57]. Additionally, the serum concentrations of both creatinine and cystatin C are known to be influenced by non-GFR-related factors such as muscle mass, cardiovascular risk factors and inflammation, which lead to biased eGFR estimates and contribute to the imprecision of eGFR[58-62].

Another critique points to the traditional standardisation of GFR to a body surface area (BSA) of 1.73m^2. The equation for estimating BSA was created by the Du Bois brothers in 1916[63]. The choice of 1.73 m^2 is based on the average BSA measured in volunteers in 1925[64]; the average BSA today is significantly higher. One strand of the critique rejects the basis for any standardisation at all, because it leads to an underestimation of GFR in obese persons in particular[65-67]. A person who gains weight will have higher GFR due to increased metabolic needs of the heavier body, but does not grow any new nephrons to handle this task, so GFR per nephron (single-nephron GFR, see chapter 1.5) will increase. The corresponding increase in BSA, however, apparently mitigates some of the GFR increase if one adheres to the

tradition of BSA standardisation. Even if the premise of standardisation of GFR is accepted, BSA is a poor choice to improve comparability of GFR across body sizes. Other variables such as extracellular fluid or total body water have been proposed as better alternatives, but have not been widely used in research or clinical practice[68, 69].

1.3.4 Measuring GFR

One way to allay the problems associated with eGFR is measuring GFR precisely with an exogenous marker. The gold standard is continuous infusion of the inert marker inulin, but it is cumbersome and expensive to use in practice. The contrast substrates iohexol and iothalamate are easier to use, and correlate very well with inulin clearance[70-72].

All GFR measurement options require the injection or infusion of the marker followed by one or multiple measurements of the marker concentration in blood or urine. Unfortunately, this causes the methods to be costly and more time-consuming than the serum-based estimates and are thus regarded by many as unfeasible for everyday clinical practice.

mGFR is mostly used in settings where the precise GFR of a patient is central to the decision to treat or not to treat, or when evaluating whether potential kidney donors are eligible to donate a kidney. The Renal Iohexol Clearance Survey (RENIS) study, presented in detail in chapter 3.1, is the only large general population study using repeated GFR measurements over time.

1.3.5 Albuminuria

Albumin is a protein present in the blood, and is normally excreted in very low quantities in the urine in healthy individuals. The presence of elevated albumin levels in urine is termed albuminuria, and is interpreted as a marker of kidney disease and endothelial dysfunction. It is often measured using immunoturbidometric assays, but high albumin levels can also be

detected as proteinuria with a standard dipstick test. Both albumin and creatinine concentrations are measured (in mg and mmol, respectively), and albumin is standardised to creatinine as the albumin/creatinine ratio (ACR), which has a high correlation with the albumin excretion rate[73]. Persistent albuminuria (>3 months of ACR >3 mg/mmol) is sufficient for a chronic kidney disease (CKD) diagnosis independent of GFR[74], and is a marker of increased risk at any CKD stage (see chapter 1.4.1).

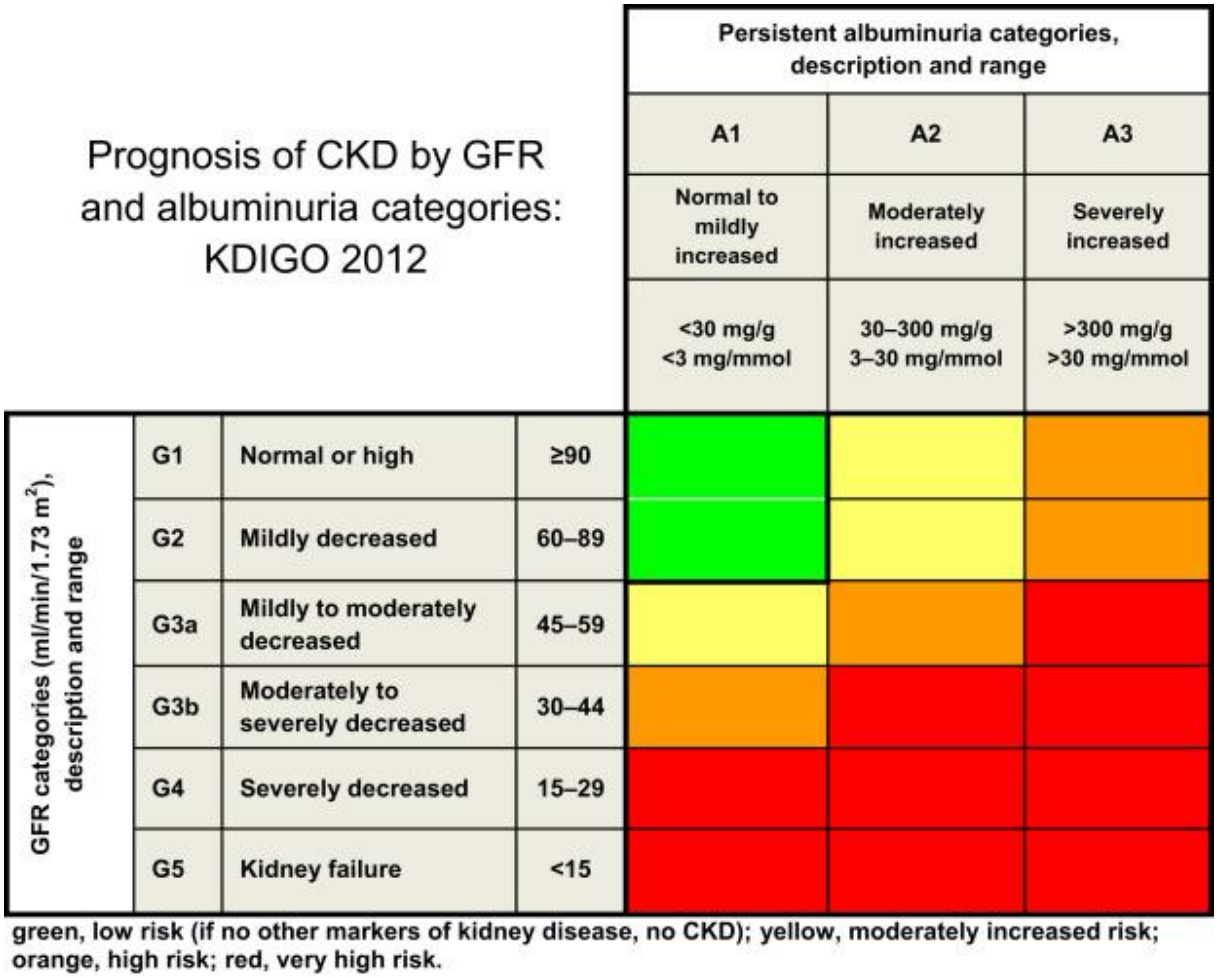
Albuminuria is associated with increased risk of severe CKD, cardiovascular disease and mortality, even at excretions lower than the currently used Kidney Disease: Improving Kidney Outcomes (KDIGO) standard of 3 mg/mmol[75, 76]. Both obesity and MetS are associated with increased albuminuria[77-80]. A recent report from the RENIS cohort found that even trace amounts of albuminuria were associated with more rapid subsequent mGFR decline[81].

1.4 Chronic kidney disease

1.4.1 Definitions

CKD is a prolonged state of reduced kidney function or kidney damage caused by a variety of diseases and risk factors. It was defined by the Kidney Disease Outcomes Quality Initiative study group in 2002 and further refined in 2012 by the KDIGO study group[74, 82]. According to the criteria, CKD is defined by a reduced eGFR and/or increased ACR for more than 3 months. The cause of kidney disease is also formally a part of the definition, but does not seem to play an important role in the practical staging of CKD. The KDIGO classification of CKD is tabulated in Figure 1[74].

Figure 1. Classification (staging) of CKD by estimated GFR and ACR, 2012 KDIGO guidelines, kdigo.org



The KDIGO CKD criteria have been criticised for not taking age into account. GFR declines slowly when people get older, and the prevalence of CKD thus increases rapidly with age, approaching 50% among persons aged over 70[83]. Most elderly persons diagnosed with CKD have an eGFR between 45 and 59 without albuminuria, and their prognosis is good[84]. Others argue that introducing age into the classification would confuse patients and healthcare workers, and that GFR-related drug dosage restrictions should be based on GFR regardless of age[85].

1.4.2 Incidence and prevalence of CKD

End-stage renal disease (ESRD) is the most severe end stage (stage G5) of CKD. The incidence of ESRD in the US rose from a standardised incidence rate (standardised to the age, sex and race distribution of the US in 2011) of 87 per million in 1980 to 357 per million in 2015, however the standardised incidence rate seems to have plateaued in the last 5-10 years[86].

The prevalence of all stages of CKD increased significantly in the United States in the 1980s and 90s, including both the moderate, usually asymptomatic stages (stages G1-3, Figure 1) and ESRD, but has remained fairly stable in the last two decades[86]. ESRD is a serious condition, which requires intrusive and costly renal replacement therapy (dialysis or transplantation). Dialysis has a very high mortality rate of 164 deaths per 1000 patient-years (age-sex-race-standardised), while transplant recipients have a much lower rate (29 per 1000 patient-years). However, standardised death rates for both dialysis and transplant recipients declined by 29% and 40%, respectively, between 2001–16. In 2011–14, an estimated 14.8% of the adult United States population had CKD, including 0.2% with ESRD[86].

Globally, estimated deaths attributable to CKD rose from 937,700 in 2005 to 1,234,900 in 2015 according to the Global Burden of Disease survey[87]. CKD attributable to hypertension was the largest driver of deaths, followed by diabetes.

In Norway, the incidence of ESRD has stabilised, but the prevalence still increases because of better survival among those with ESRD[88]. In 2016, 554 persons (105.8 per million) began renal replacement therapy. The three main causes were vascular/hypertensive (34%), glomerulonephritis (17%) and diabetic nephropathy (16%). This suggests that a significant proportion of ESRD may be preventable because it is rooted in diseases that can be effectively treated or prevented by modifying lifestyle-associated risk factors. The prevalence

of persons in renal replacement therapy in Norway was 4969, or 948.9 per million inhabitants[88].

1.5 Kidney physiology in hyperfiltration and ageing

1.5.1 Hyperfiltration

1.5.1.1 Background

While CKD is defined by a low GFR or kidney damage, a high GFR is not necessarily a sign of health. The theory of hyperfiltration, proposed by Brenner et al. in 1996 based on research from the 1980s and 90s[89], considers GFR at the single-nephron level (GFR per nephron, or whole-kidney GFR divided by nephron number). Brenner et.al demonstrated that rats with a reduced nephron number had high single-nephron GFR (hyperfiltration) and/or higher intraglomerular pressure. This state of hyperfiltration was in turn associated with podocyte damage, mesangial expansion, albuminuria and finally glomerulosclerosis and GFR decline[90]. Nephron loss may further increase the stress in remaining nephrons, causing a vicious cycle. Hyperfiltration has been shown to occur in diabetes[91], but Brenner et al. proposed it as a general mechanism behind many diseases or conditions with nephron loss, including hypertension and obesity[92]. In a recent study, Melsom et al. found that high mGFR adjusted height, sex and age predicted faster subsequent mGFR decline in two cohorts of different ethnic origin: Pima Indians with diabetes and the Norwegian RENIS cohort[112].

1.5.1.2 Mechanisms

The mechanisms of hyperfiltration are not fully understood, but may include several interacting factors. Vascular resistance in the afferent and efferent arterioles regulates single-nephron GFR. The antihypertensive drugs angiotensin converting enzyme inhibitors and angiotensin II receptor blockers both target angiotensin II and cause efferent arteriole vasodilation[93]. They have been shown to reduce GFR in the short term, but to slow GFR

decline in the longer term, possibly because of a reduction of hyperfiltration[94]. A similar pattern of reduced hyperfiltration is seen with the use of sodium-glucose cotransporter 2 inhibitors[95]. These drugs block glucose reabsorption in the proximal tubule, causing the glucose to be excreted in the urine rather than reabsorbed. Because the blocked cotransporter also transports natrium (sodium), natrium reabsorption in the proximal tubule is reduced as well, increasing natrium concentrations in the macula densa. This causes afferent arteriole vasoconstriction by tubuloglomerular feedback, which is mediated by an increase in adenosine. Interestingly, a recent study found increased adenosine concentrations in the urine of patients with type 1 diabetes who were treated with the drug empagliflozin[96]. Other potential mechanisms involved in hyperfiltration include intrarenal nitric oxide signalling and mechanical stress from glomerular hypertension[97-100].

1.5.1.3 Epidemiology

While creatinine-based eGFR is a poor method for hyperfiltration research due to its inaccuracy, large longitudinal population studies using the method have shown increased mortality and morbidity in persons with high creatinine-based eGFR[53]. Traditionally, this has been explained as a falsely elevated eGFR from low serum creatinine being the result of low muscle mass due to wasting from chronic disease, such as cancer or severe emphysema. However, studies that have measured muscle mass and accounted for concurrent disease still found higher mortality in hyperfiltration[101].

Several studies suggest associations between hyperfiltration and many well-known ESRD risk factors, including pre-diabetes and diabetes, hypertension, obesity, albuminuria and smoking[92, 101-108]. Some interventions which reverse these factors, such as treatment of hypertension with losartan, treatment of diabetes with sodium glucose co-transporter 2-

inhibitors and significant weight loss after gastric bypass surgery, result in a GFR decrease which may represent the normalisation of previous hyperfiltration[95, 109-111]

Obesity, MetS and hyperfiltration have only been studied to a limited extent, with divergent results. These conditions are by their nature particularly affected by the imprecision of eGFR because of altered muscle mass in obesity, and low-grade systemic inflammation in obesity and MetS. The customary adjustment for 1.73 m^2 of BSA also distorts the estimation of hyperfiltration in the obese. These factors may explain the divergent results among different study populations[69, 103, 113-121], and are discussed in detail in papers 1 and 2 in this thesis.

1.5.1.4 Definition

There is no consensus on a common definition of hyperfiltration based on whole-kidney GFR. Some have used an arbitrary, round GFR cut-off value[103], while others have used percentiles in their study population[57, 92, 104, 114]. However, a simple absolute or BSA-adjusted whole-kidney GFR cut-off does not adequately consider the varied nephron endowment of individuals, and is less likely to reflect single-nephron GFR. A meta-analysis by Chagnac et al. suggested that a common hyperfiltration definition should at the very least involve an adjustment of GFR for age and gender to at least partially account for nephron numbers[122]. However, the authors did not suggest a definition themselves. A recent study by Chakkera et al. compared different methods to establish hyperfiltration definitions more consistent with single-nephron GFR in kidney donors. The study is discussed in detail in chapter 5.2.4 of this thesis.

1.5.2 The ageing kidney and GFR decline

As mentioned in chapter 1.3.1, the number of nephrons declines gradually with age, as they cease to function and the glomeruli become sclerotic[47, 123-125]. Other changes to the kidneys in old age include increased atherosclerosis in renal vasculature, interstitial fibrosis, tubular

necrosis, an increased number of renal cysts, and a reduction in cortical volume[124]. The nephron loss causes whole-kidney GFR to decline slowly with age, while single-nephron GFR remains fairly constant with age in healthy kidney donors, except in the oldest age group (>75 years old)[126].

In longitudinal studies of whole-kidney GFR, the rate of decline varies greatly. Some risk factors for CKD and ESRD may attenuate GFR decline, or even increase GFR in the short-term, but still increase the risk of ESRD in the long-term[81, 127-130]. This apparent paradox may in part be due to the negative effects of hyperfiltration.

Because no new nephrons are created after birth, an increase in GFR must represent an increase in single-nephron GFR, while a decrease in GFR may be due to a lower single-nephron GFR, a loss of nephrons, or both. This suggests a possible alternative definition of hyperfiltration: a significant increase in whole-kidney GFR over time.

1.6 Obesity as a risk factor for CKD

Obesity is known to increase risk of diabetes, hypertension and cardiovascular disease, and also CKD and ESRD[131-135]. While a large part of the association with CKD is due to the first three factors, obesity may also be associated with CKD and ESRD independent of these intermediaries[39]. In a large meta-analysis, Hsu et al. found a relative risk for ESRD ranging from 1.9 for those with a BMI from 25–29.9 kg/m^2 to 7.1 in subjects with a BMI ≥40 kg/m^2 [134].

However, the relationship between obesity, hyperfiltration and GFR decline in the general population is less clear, with conflicting study results[19, 69, 103, 136, 137]. The inconsistency of the results may be due to a combination of the inherent inaccuracies of eGFR, the misleading BSA correction of GFR, and the nature of hyperfiltration. As we explored in the previous

chapter, hyperfiltration may cause GFR to stabilize or increase in the short term, concealing detrimental effects on the kidney because eGFR appears to be normal. The damage to the kidneys may be reflected in lower eGFR at a much later stage, when preventative efforts may be less effective. A distinct form of kidney damage from severe obesity is obesity-related glomerulopathy, likely related to obesity-related hyperfiltration[138].

In summary, obesity, MetS and CKD are widespread globally, and are major causes of shortened lifespans and decreased quality of life. While obesity and the metabolic syndrome have consistently been shown to increase the risk of ESRD in epidemiological studies with long follow-up periods, their relationship with hyperfiltration and the age-related decline in GFR are not very well understood. This is in large part because of methodological problems caused by the use of eGFR instead of actual GFR measurements.

2 Aims

The primary aim of this thesis was to explore the relationship between obesity, metabolic syndrome, hyperfiltration, and the subsequent GFR decline rate. Since albuminuria is an important early sign of kidney dysfunction, we also examined the association of hyperfiltration (defined as an increase in GFR) with an increase in albuminuria.

3 Methods

3.1 Participants

3.1.1 RENIS-T6

RENIS began in 2007 as a sub-study of the 6th Tromsø study[139]. The purpose of the study was to measure GFR with an accurate method in a large population of fairly healthy participants, representative of the general population. The chosen age group, 50–62 years old, was chosen because many people of that age have risk factors for lifestyle-associated diseases such as diabetes, chronic kidney disease and cardiovascular disease, but are still fairly healthy and have yet to develop those diseases. By studying them during ageing, it is possible to see which factors influence the course of GFR over time.

The 6th Tromsø study invited all citizens of Tromsø 60–62 years of age, and a random sample of 40% of those aged 50-59 years old[140]. This amounted to 5464 people, of whom 3564 (65%) completed both rounds of the main part of the study.

The exclusion criteria for the first round of the RENIS study, named RENIS-T6, were diabetes, any renal disease except urinary tract infections, angina pectoris, myocardial infarction or stroke. Overall, 739 of those who completed the Tromsø study were excluded, leaving 2825 eligible people who were invited to RENIS. Of these, 2107 responded positively, but a further 125 were ultimately excluded because they reconsidered and withdrew, reported allergic reactions to iodine or latex or for other practical reasons. The selection process for the study population of RENIS is also shown in Figure 2.

The predetermined target study population for RENIS-T6, based on power calculations, was 1600. Participants had their appointments scheduled in a random order. When the number of investigations had reached 1632, the study was stopped, leaving the remaining 350 eligible

potential participants uninvited. Five investigations were technical failures, leaving 1627 as the final study population of RENIS-T6. The investigations took place at the Clinical Research Unit at the University Hospital of North Norway between November 2007 and June 2009. All participants provided written informed consent to participate, and the Regional Ethics Committee of Northern Norway approved the study. The study was performed in compliance with the Declaration of Helsinki.

3.1.2 RENIS-FU

The second round of RENIS, RENIS-FU (Follow-Up), invited all participants from RENIS-T6 to repeat the protocol between September 2013 and January 2015, except those who had died (n=23) and 7 individuals who had a possible allergic reaction to iohexol. A total of 1324 participants (83% of those eligible) attended the follow-up. Eighty-eight participants were randomly selected to undergo two GFR measurements with a median (interquartile range) 35 (22–49) days between the measurements, resulting in 3 total measurements for these persons. The extra measurement allowed for the estimation of an intra-individual variation coefficient. A flowchart for the study population selection for each paper is presented in Figure 2.

3.1.3 Study population selection

For the first paper of this thesis, the study population included all participants from RENIS-T6, except those who had previously unknown diabetes (fasting plasma glucose ≥7.0 mmol/L and/or haemoglobin A1c ≥6.5%) and those who lacked waist or hip circumference measurements, leaving 1555 participants. For the second paper, only participants who participated in both RENIS-T6 and RENIS-FU were included (n=1324). The same exclusion criteria as in the first paper were applied, with the additional exclusion of two participants who lacked triglyceride measurements at baseline, leaving 1261 participants as the study population. In the third paper, participants who participated in both RENIS-T6 and RENIS-

FU were included, except for those with previously unknown diabetes and those who had albuminuria (ACR >30 mg/g) at baseline, leaving 1246 persons in the study population.

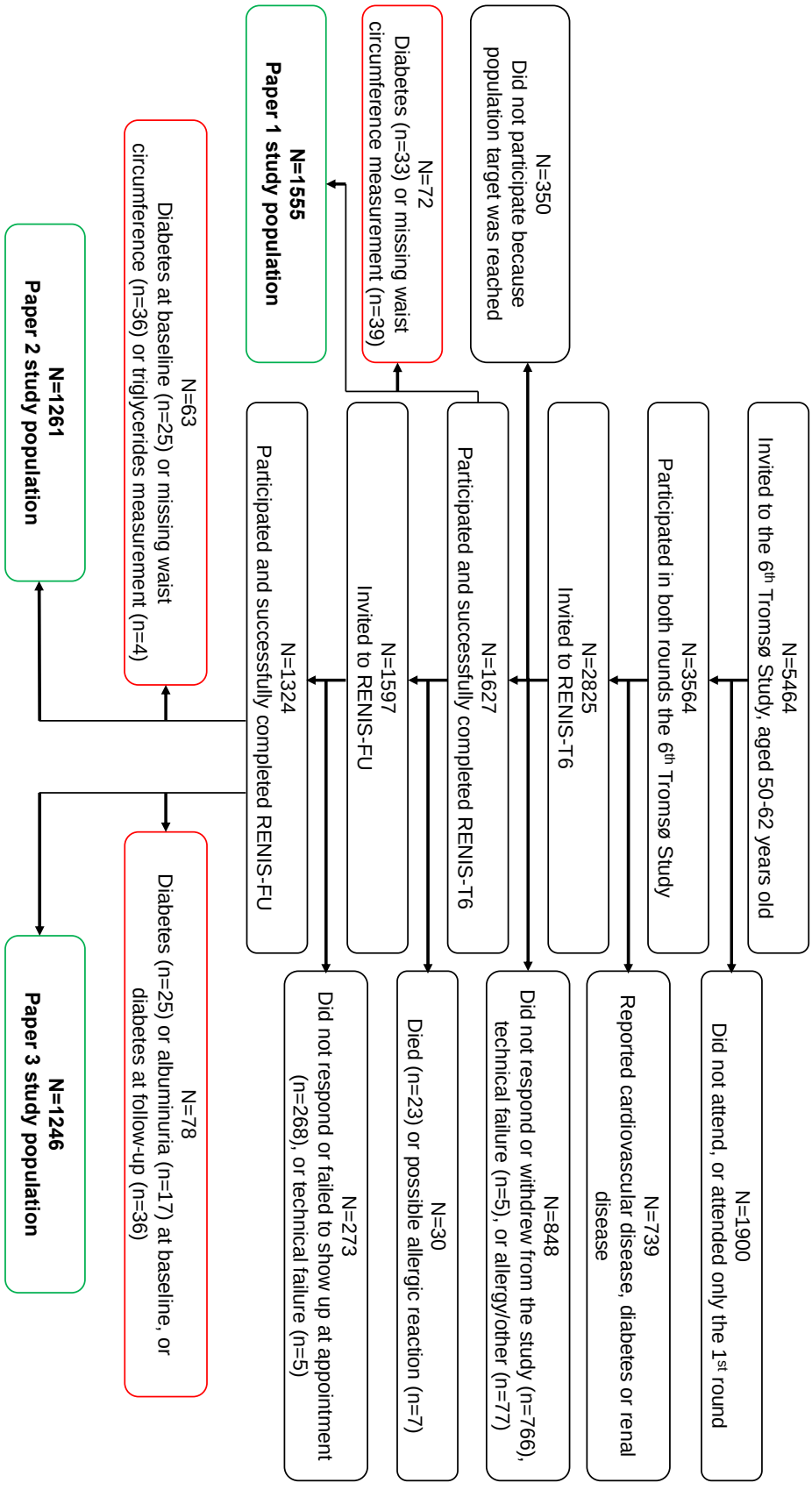


Figure 2. Flowchart presenting the RENIS study population selection.

3.1.4 Participant instructions and body measurements

All subjects showed up at their morning appointment (between 8 and 10 AM) after an overnight fast, having abstained from smoking for 12 hours. They were instructed not to eat unusually large portions of meat or take non-steroidal anti-inflammatory drugs during the last two days before the examination and to drink two glasses of water before arrival. Upon arrival, their height and weight were measured, as were their waist and hip circumferences.

They answered a large questionnaire at home before arrival, which included questions on tobacco and alcohol use, medical history and drug use. A study nurse re-examined the questionnaire with the participants upon arrival, including a thorough review of all current medication usage and medical history to reduce the risk of misclassification

The protocol was the same for both rounds of RENIS, with the exception that in RENIS-T6 the questionnaire, ACR, haemoglobin A1C, waist and hip circumferences and height were measured as part of the 6th Tromsø study, a median (interquartile range) 5.2 (3.0–6.2) months before RENIS-T6.

Body weight was measured to the nearest 0.1 kg on a digital scale. Height was measured with a wall-mounted measuring tape to the nearest centimetre. Waist circumference was measured horizontally over the umbilicus at the point of expiration. Hip circumference was measured around the greatest protrusion of the buttocks.

3.2 Laboratory measurements

3.2.1 Albuminuria measurements

Participants collected fasting morning samples of urine the last two days before the examination, and on the morning of the examination. Albumin and creatinine were measured in fresh (unfrozen) specimen, and the median ACR from the measurements for each

individual was used for the studies. The creatinine concentration was measured using colorimetric methods (Jaffe's reaction), while albumin was measured using the immunoturbidimetric method. Both were done on an ABX Pentra Micro-albumin CP autoanalyser. Although the limit of detection of the assay is given as 4 mg/L in the documentation, the PENTRA instrument in practice detects albumin concentrations down to 1 mg/L. This had consequences for paper 3, see chapter 4.3.1 of this thesis.

3.2.2 Single-sample iohexol clearance measurements

A Teflon catheter was inserted into the antecubital vein, and blood samples were drawn for analyses. Five millilitres of iohexol (Omnipaque, 300 mg I/ml) was injected, the syringe was weighed before and after injection, and the catheter was flushed with 30 ml of isotonic saline. After the iohexol injection, participants were served a light breakfast and were free to walk around or relax at will.

After an individually pre-specified period of time, calculated using the Jacobsson's method based on eGFR from creatinine[141], a new blood sample was taken for iohexol analysis. The exact time from iohexol injection to blood sample extraction was measured using a stopwatch. High performance liquid chromatography was used to measure the iohexol concentration, as described by Nilsson-Ehle[142]. The analytic coefficients of variation were 3.0% in RENIS-T6 and 3.1% in RENIS-FU. The mean coefficient of variation for the intra-individual variation in GFR among the 88 participants who had two GFR measurements in RENIS-FU was 4.2%[127].

3.2.3 Other measurements

Fasting serum glucose, total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride concentrations were measured on a Modular P800 (Roche Diagnostics). The insulin concentration was measured with an enzyme-linked immunosorbent assay kit (DRG

Instruments, Marburg, Germany). The intra- and inter-assay coefficients of variation were 4.7% and 6.3%, respectively. Insulin resistance was expressed by the homeostasis model assessment equation, multiplying fasting glucose (mmol/L) by fasting insulin (mU/L) and dividing the result by 22.5[143]. In RENIS-T6, haemoglobin A1c was measured as part of the 6th Tromsø study using a liquid chromatographic method.

Serum creatinine was measured using an enzymatic assay standardised to the isotope dilution mass spectrometry method (CREA Plus, Roche Diagnostics). Cystatin C was analysed with a particle enhanced turbidimetric immunoassay with reagents from Gentian (Gentian, Moss, Norway) and a Modular E analyser (Roche Diagnostics). The cystatin C measurements were then recalibrated to the international reference standard using a Cobas 8000 (Roche Diagnostics). CKD-EPI equations were used to estimate GFR[53].

Office blood pressure was measured at the study site after two minutes of rest using an automated device (model UA799; A&D, Tokyo, Japan). Daytime ambulatory blood pressure was measured using weighted daytime (10:00–22:00) averages of blood pressure measured at 20-minute intervals. Further details of the blood pressure measurements in RENIS have been described previously[144].

3.3 Statistical methods

3.3.1 Hyperfiltration definition

In paper 1, hyperfiltration was defined using two different approaches. In both cases multiple linear regression models were used, with the natural logarithm (ln) of unadjusted GFR (mL/min) as the dependent variable. In one definition, age, sex and height (all associated with nephron number) were added as independent variables, while in the second, the same variables were added along with body weight.

A participant was defined as having hyperfiltration if the regression residual was greater than the 90th percentile in the distribution of residuals in the regression analyses for the respective hyperfiltration definition. The definitions were exemplified and explained in more detail in the paper. In the third paper, hyperfiltration was defined as an increase in GFR during the follow-up period.

3.3.2 Metabolic syndrome and obesity categorizations

The metabolic syndrome was defined using the previously mentioned harmonised WHO/International Diabetes Federation definition (chapter 1.2.1 and Table 2 in this thesis). The obesity categorisations were also in line with the international standards described in chapter 1.1.4 and Table 1 of this thesis.

3.3.3 Descriptive statistics

The study population characteristics in all papers were presented as the mean (standard deviation) values, median (interquartile range) in cases of skewed data, or numbers (percentages) where appropriate. Differences in characteristics between categories were tested with paired t tests for mean values, Wilcoxon signed rank tests for median values, and McNemar tests for paired dichotomous variables, respectively.

3.3.4 Regression analyses

In the first paper, the main results were analysed with logistic regression models with hyperfiltration (two different definitions) as the dependent variable. In the second paper, the main results were analysed with linear regression models, with change in absolute (unadjusted) mGFR between the RENIS-T6 (baseline) and RENIS-FU (follow-up) measurements as the dependent variable. In the third paper, the main results were analysed in linear regression models with change in ACR between baseline and follow-up as the dependent variable. Logistic regression models were also used with incident albuminuria

(ACR >30 mg/g at follow-up) as a dichotomous dependent variable. The analyses were explained in greater detail in the respective papers. All analyses were performed using STATA MP 14 (www.stata.com).

4 Main results

4.1 Paper 1. Central obesity associates with renal hyperfiltration in the non-diabetic general population: a cross-sectional study

A total of 1555 participants from RENIS-T6 were examined for associations between BMI, WC or WHR and two different definitions of hyperfiltration. The first hyperfiltration definition adjusted for age, sex and height was associated with all three obesity measures in logistic regression models. The associations remained significant after adjustments for potential confounders including the component risk factors of the metabolic syndrome. This definition is an attempt to adjust for some of the main factors known to influence nephron number. When another hyperfiltration definition was used, which adjusted for weight in addition to age, sex and height, only WHR remained significantly associated with hyperfiltration after controlling for confounders. These results suggest that obesity is associated with hyperfiltration in the general non-diabetic population, even when controlling for obesity-associated potential confounding factors. Furthermore, elevated WHR is associated with hyperfiltration even when using a weight-adjusted definition.

4.2 Paper 2. Metabolic syndrome but not obesity measures are risk factors for accelerated age-related glomerular filtration rate decline in the general population

A total of 1261 persons who participated in both RENIS-T6 and RENIS-FU were examined for associations between obesity, MetS and changes in the decline rate of GFR during the mean 5.6 years between the two rounds of RENIS. Obesity, measured with BMI, WC or WHR, was not associated with a statistically significant change in the rate of GFR decline. MetS, however, was associated with a -0.30 ml/min/year faster GFR decline in multivariable adjusted linear regression models. The triglyceride criterion of MetS was the main driver of this result.

4.3 Paper 3. Association of increasing GFR with change in albuminuria in the general population

The relationship between the change in GFR and the simultaneous change in ACR during the mean 5.6 years between RENIS-T6 (baseline) and RENIS-FU (follow-up) was explored. The change in GFR was termed ΔGFR (defined as GFR at follow-up minus GFR at baseline). A positive ΔGFR signified an increase in GFR, and a negative ΔGFR signified a decline in GFR. The same principles applied to changes in ACR (ΔACR).

There was a positive association between ΔGFR and ΔACR in multivariable adjusted linear regression analyses: ΔACR was 8.4% higher per standard deviation of ΔGFR. When participants were split into two groups based on those whose GFR increased during the study period (ΔGFR >0, n=343) and those whose GFR declined (ΔGFR <0, n=903), the group whose GFR increased experienced a 16.3% higher ΔACR (see chapter 4.3.1 for additional analyses of these data). When logistic regression was used to find the odds ratio of incident albuminuria (ACR >30 mg/g at follow-up), those with a higher ΔGFR had an odds ratio of 2.13 for incident albuminuria per standard deviation. The group whose GFR increased during

the study period (ΔGFR >0) had an odds ratio of 4.98 of incident albuminuria compared to those whose GFR declined (ΔGFR <0).

4.3.1 Additional analyses for Paper 3

As mentioned in chapter 3.2.1, urinary albumin in both the Tromsø 6 and RENIS-FU examinations were analysed with the ABX Pentra Micro-albumin CP (Horiba ABX, Montpelier, France). Although the limit of detection of the assay is given as 4 mg/L in the documentation, the PENTRA instrument in practice reports albumin concentrations as low as 1 mg/L. These results were used in paper 3 and in previous publications from the Tromsø Study. In paper 3, all urinary samples with undetectable albumin concentrations (i.e. <1 mg/L) were assigned an ACR of 0.10 mg/mmol, which corresponds to the lowest ACR observed in samples with detectable albumin concentration. Because this is not formally correct, we have now repeated the analyses of the data in paper 3 using two methods: first, we treated all observations of albumin concentrations lower than 4 mg/L as left-censored and repeated the multiple linear regression of ACR in Table 2 of paper 3 using interval regression. Second, because there is no similar procedure for a dichotomous dependent variable, we used multiple imputation to impute the ACR for observations with albumin below 4 mg/L. This was done by extending the previously described RENIS multiple imputation model to include interval regression for the missing ACRs[145]. The 50 imputed datasets were then used to repeat the analyses in Table 3 of paper 3.

For Table 2, the difference in GFR between baseline and follow-up predicted the absolute difference in ACR in all three models ($p<0.05$). When the difference between log-transformed ACR was used as the dependent variable, the results were similar to the original results, but borderline statistically significant in the fully adjusted model ($p=0.05$). However, the dichotomised variable for GFR increase (ΔGFR>0) was not a statistically significant predictor

of ACR increase. For the analyses with multiple logistic regression in Table 3, the results were similar to the original results in all models ($p<0.05$).

5 Discussion

5.1 Methodological considerations

5.1.1 Selection bias

Selection bias occurs when the study population and target population (from which the study subjects are recruited) differ with regard to exposures or outcomes of interest. The RENIS cohort was recruited from participants in the 6th Tromsø Study. In that study, approximately 65% of those invited in the age group 50–62 years old participated. All Tromsø study participants in that age group were invited to RENIS, except those who had reported diabetes or a history of myocardial infarction, stroke or renal disease. The response rate was 75% for those invited to RENIS. Even though these percentages are high by international standards, they nevertheless leave room for significant selection bias.

We know that those who chose not to participate in the Tromsø Study were more likely to be male and in the younger age group (50–55 years old)[140]. We do not know non-participants' motivations, and can only speculate whether their choice not to participate makes them more or less likely to be obese, have metabolic syndrome, albuminuria, or differ from participants in other ways. A study of non-responders in the North Trøndelag Health Study found that the reasons for non-participation differed across age groups, with younger participants more likely to report being hindered by time constraints, while older participants were more likely to report poor physical health as a reason to not participate, or stated that they already

received frequent follow-up from the healthcare system and felt no need for any additional medical examination[146].

We do, however, know more about those who were eligible for participation in RENIS (n=2825), and whether they differed from those who actually participated RENIS-T6. They did not differ substantially, although there was a statistically significant difference of 0.1 years of age and 0.1 kg/m^2 of BMI between the groups[68].

The differences between those who participated in RENIS-T6 but did not participate in RENIS-FU and those who participated in both have been reported previously[127]. Again, the differences were small, except for a higher percentage of smokers among those lost to follow-up. Smokers are at higher risk of poor health, which could potentially be a factor impeding them from participation, but because no survey of non-participants was performed, this remains unclear. Smokers were also more likely to be among the 23 persons who died between the two rounds.

Healthy survival bias is a form of bias in which those who participate in the study show a different association between exposure and an outcome of interest because those most heavily afflicted by the exposure have already died. We excluded subjects with pre-existing diabetes, cardiovascular disease or renal disease to allow focus on GFR progression starting in the normal range. However, this selection may have resulted in a study population which was more robust to the negative effects of obesity: those who were predisposed to cardiovascular disease or diabetes were excluded or may have died at a younger age. This may have resulted in the attenuation of associations between obesity and GFR. The 23 subjects who died between RENIS-T6 and RENIS-FU were more likely to have had underlying risk factors which were not captured by the study's exclusion criteria, such as cancer. Nevertheless, their

number was probably too low to cause significant bias that would have affected the results of this study.

5.1.2 Information bias

Information bias is that resulting from measurement errors. All measurements, whether serum analyses, or weight or circumference measurements, have a margin of error. The RENIS study has taken several measures against measurement errors, including the use of trained staff at the Clinical Research Unit, a detailed study protocol, and high-quality equipment. The iohexol clearance methods in particular were thoroughly and carefully executed, including weighing of the injection syringe before and after injection. Frozen samples from RENIS-T6 were thawed and re-analysed in RENIS-FU to investigate drift between the two rounds, and all GFR variables were adjusted to account for this drift[127]. Any equipment-related measurement errors are likely to be random and unbiased, and more likely to dilute the strength of any associations found than spuriously cause or strengthen non-existent associations. Moreover, the intra-individual coefficient of variation for the GFR measurement (day-to-day variation in GFR which includes biological variation and measurement error) in RENIS FU was 4.2%, which is lower than in most previous studies of measured GFR[71]. This suggests a low level of measurement errors overall.

In the case of categorical variables, information bias is often referred to as misclassification bias. To combat bias resulting from arbitrarily chosen cut-off points for continuous variables, whenever we used categorical values in the papers in this thesis, we also provided analyses with the same variables presented as continuous variables. We also provided results using alternative cut-offs when several cut-offs were in common use in the scientific literature.

5.1.3 Confounding

Confounding occurs when an unknown or hidden variable accounts for all or part of an apparent association between two variables, and the association would have been reduced or nullified in the absence of the confounder. In the papers, we tried to reduce the risk of confounding by including physiologically plausible confounders as independent adjustment variables in the regression analyses. However, there is always a risk that the associations found are influenced by unmeasured or unknown variables rather than those included.

5.1.4 External validity

The study population of RENIS was exclusively middle-aged and anthropologically Caucasian, so generalisations to different populations should be made with caution. Weight distribution and muscle mass are known to diverge somewhat between different ethnic groups, and incidence of kidney disease also differs. However, it is not clear whether these differences are mainly due to environmental or genetic factors[147-149].

5.2 Discussion of the results

5.2.1 The relationship between obesity and hyperfiltration

As presented in chapter 1.5.1 in this thesis, there is currently no established definition of hyperfiltration[57, 122]. Because the theory of hyperfiltration is based on elevated single-nephron GFR, and because whole-kidney GFR is the only variable available for research, it follows that a definition of hyperfiltration should try to account for factors known to associate with nephron number in individuals.

In the absence of kidney biopsies from the individuals in question (in which nephron number and density can be counted and extrapolated) one must rely on imperfect surrogate markers. Kidney biopsy studies have shown five main variables associated with nephron number: age,

height, sex, low birth weight and family history of ESRD in first-degree relatives (parents, children and siblings)[44, 47, 126, 150]. The current practice of adjusting GFR to BSA does thus partially account for nephron numbers, as women generally have lower BSA, and shorter people (who are more likely to have lower birth weight) generally have a lower BSA as well. However, this relationship is distorted in obesity, and there is no indication that obesity is associated with higher nephron numbers. A definition of hyperfiltration based solely on a single cut-off of unadjusted GFR or BSA-adjusted GFR should not be used[57].

In the first paper, we explored two hyperfiltration definitions: the first was based on a cut-off of GFR adjusted for age, sex and height in a regression analysis, while the second was based on the same method but with body weight included as well. The first definition is the one which is theoretically closer to a single-nephron GFR approach, and has been shown to predict high glomerular volume in kidney biopsies in a later publication by Chakkera et al[57]. All of the obesity measures (BMI, WC and WHR) were associated with this hyperfiltration definition, even when the analyses were adjusted for potential confounders that are also associated with obesity and hyperfiltration, such as glucose and hypertension. The associations were quite strong.

The second definition of hyperfiltration, which was adjusted for body weight in addition to age, sex and height, can be useful for distinguishing between the various methods for assessing obesity in relation to hyperfiltration. By adjusting for body weight, the associations with obesity measures reflect whether the measures affect GFR in excess of what would be expected at a given body weight. Only WHR was significantly associated with this definition, which may suggest that a predominantly abdominal fat distribution may be influential in the relationship between obesity and hyperfiltration.

These findings suggest that obesity clearly increases the risk of having hyperfiltration, and the nature of the hyperfiltration definitions would imply a high single-nephron GFR as well, though that cannot be confirmed due to the lack of kidney biopsies in our study.

Study limitations include the lack of gold-standard body fat measurements, and the lack of control measurements in individuals to estimate intra-individual variation in GFR (though the latter was done later as part of RENIS-FU). The choice of cut-off point is by nature arbitrary; however, we did explore alternative cut-offs based on the 95th percentile of residuals rather than the 90th, with similar results.

5.2.2 Obesity, the metabolic syndrome and age-related GFR decline

GFR declines gradually with age, but the rate of decline varies between individuals and may be affected by several factors. Factors that influence the GFR decline rate may either reflect changes in the loss rate of nephrons, changes in single-nephron GFR, or both. In a cross-sectional study of kidney donors, Rule et al. approached single-nephron GFR in an innovative way and found that it was fairly stable across age groups[126]. Coupled with the knowledge that nephron numbers decline over time, this suggests that the decline in GFR in healthy persons is mostly due to the loss of nephron numbers.

We found that obesity, measured with BMI, WC or WHR, did not associate with a change in the rate of GFR decline. However, having MetS at baseline was associated with a significantly steeper decline in GFR. These results do not necessarily suggest that obesity does not affect kidney function, nor that it is not harmful to the kidneys. As we found in the first paper, obesity is associated with hyperfiltration, and hyperfiltration may be harmful to the kidneys in the long-term[112]. The participants who have elevated BMI, WC or WHR may be at various stages of hyperfiltration; some might have increasing GFR (and thus increasing single-nephron GFR, see chapter 5.2.3 and paper 3), others may have plateaued and yet others

may have sufficient nephrons to adequately handle the burden of obesity and may have their GFR decline slowly and naturally due to nephron loss.

Participants with MetS have several risk factors for CKD by definition. The association with steeper GFR decline may reflect that a number of them have an accelerated rate of nephron loss, as a significant lowering of single-nephron GFR is a less likely explanation. We also explored the interaction between MetS and obesity by looking for differences between those with BMI >30 kg/m^2 and MetS compared to those with BMI >30 kg/m^2 and no MetS, but the results were not statistically significant.

Weaknesses of this study again include the lack of gold-standard body fat measurements. The concept of MetS is itself controversial and the components of the syndrome and their cut-off values are somewhat arbitrary. We did, however, explore the components in individual regression analyses both as categorical and continuous variables, and presented the results in the paper.

5.2.3 Increased GFR and increased ACR

As previously mentioned in the Background and Methods chapters, an increase in GFR in an individual indicates an increase in single-nephron GFR, because no new nephrons are created by the body in adulthood. In the third paper we approached this topic using a longitudinal increase in GFR as a marker of hyperfiltration. Participants who experienced such an increase in GFR were associated with a concurrent increase in ACR, and an increased risk of reaching the threshold for the diagnosis of albuminuria. Furthermore, changes in ACR and GFR were linearly associated, i.e. those whose GFR declined only slightly had a higher ACR increase on average than those whose GFR declined more steeply. Those whose GFR increased experienced an even higher ACR increase.

These associations are interesting and may seem counterintuitive apart from the perspective of hyperfiltration. Those whose GFR increased are experiencing an increase in their single-nephron GFR, which may cause maladaptive changes in glomeruli which could lead to shear stress, podocyte damage and, eventually, albuminuria[97].

Some of the same mechanisms may be present in those whose GFR declined only slightly: they too would experience increased single-nephron GFR if their loss of nephrons was greater than the loss of GFR.

Weaknesses of this study include the cross-sectional nature of the analyses, which excludes inferences of causation. Because many risk factors are known to associate with albuminuria, there is great risk of confounding as well. However, we attempted to mitigate this risk by adjusting for several known potential confounders in the regression analyses. The categorization of participants based on whether their GFR increased or decreased will invariably include misclassifications due to intra-individual variability in GFR. However, the intra-individual coefficient of variation was low, and any misclassification would probably dilute the strength of the associations rather than make them misleadingly powerful.

5.2.4 Hyperfiltration and GFR decline

A common topic for all three papers is the theory of hyperfiltration. In chapters 1.3 and 1.5, we explored reasons why it remains hard to find definitive proof for the theory in a clinical setting. In short, it is high single-nephron GFR, not whole-kidney GFR, which is at the heart of the theory, and the number of nephrons in individuals is usually unknown. However, researchers at the Mayo Clinic and Cleveland Clinic have compiled data from kidney donors who underwent both kidney biopsies and abdominal magnetic resonance imaging scans, and have recently published articles highly relevant to this topic[57, 126].

Denic et al. estimated single-nephron GFR by extrapolating the density of nephrons in biopsy samples to the volume of the kidneys in the accompanying magnetic resonance imaging scan[126]. Kidney donors are a selected group of volunteers screened for health, but obesity and some cases of mild hypertension are not contraindications for donation. Interestingly, the factors associated with higher single-nephron GFR were obesity and elevated ACR, as well as a family history of ESRD (and a stature of >190 cm).

Chakkara et al. used the same data to examine different definitions and cut-off points for hyperfiltration and compare them to glomerular volume (a structural biopsy finding indicative of glomerular hyperfiltration)[57]. They found that eGFR was inadequate for predicting glomerular volume. mGFR adjusted for age (but not corrected for BSA) had the highest correlation with glomerular volume but was also positively correlated with nephron number. The positive correlation with nephron number resulted in the disproportional representation of young, male donors in the hyperfiltration category. GFR adjusted for height, sex and age was almost equally correlated to the glomerular volume, but was not associated with nephron number. This conforms very well to the RENIS publications that explored different versions of this hyperfiltration definition and various risk factors for kidney and cardiovascular disease[105, 107, 151, 152], including paper 1 of this thesis.

Denic et al. also looked for associations between single-nephron GFR and the age of donors, and found that single-nephron GFR remained remarkably stable across age groups, with the exception of donors older than 75 years[126]. In other words, GFR decline with increasing age is due to the loss of nephrons, not lower single-nephron GFR. In biopsies, glomerular volume varies greatly within a single kidney, which may represent a mechanism for nephron loss over time: some nephrons in a kidney may face hyperfiltration, while others are spared[153]. Increasing the proportion of nephrons with hyperfiltration in a kidney (in obesity, for

example) might accelerate this ageing process. Melsom et al. found that higher baseline age-sex-height-adjusted mGFR was associated with more rapid subsequent GFR decline, which may support this hypothesis[112].

The loss of nephrons with age even in healthy individuals may suggest that the process of nephron loss is natural, and that the accompanying loss of GFR should not be treated as a disease, as the current definition of CKD would imply[84]. However, the loss of nephrons due to ageing may still represent a loss of "renal reserve", or less resilience to kidney-damaging incidents such as severe dehydration or nephrotoxic substances, which may cause acute damage to a large number of nephrons. Acceleration of the age-related GFR decline rate may thus be a symptom of a low nephron reserve[154].

6 Conclusions and perspectives

We conclude that obesity was associated with hyperfiltration, but was not associated with steeper GFR decline during the following 5.6 years. Those who had MetS, however, had a significantly steeper GFR decline. The participants whose GFR increased in the study period had an increased risk of incident albuminuria. Changes in ACR and GFR correlated positively, and those with increased GFR experienced greater increases in ACR.

The results demonstrate a complex relationship between obesity, MetS and GFR. Precise measurement of obesity with gold-standard methods would add validity to the findings, but were not available for the papers in this thesis. Overall, the findings can be interpreted as supportive of the theory of hyperfiltration, which has been extensively discussed in this thesis.

However, the results should be cautiously interpreted. The study cohort was, by design, generally quite healthy at the beginning of this study, and the papers presented in the thesis

shine a light on hyperfiltration and the age-related GFR decline, not CKD or ESRD. Hyperfiltration has been most extensively described in diabetes, but all RENIS participants were non-diabetic. The results underline the value and necessity of using mGFR rather than eGFR when studying hyperfiltration and age-related GFR decline in the normal and high ranges of GFR.

A longer follow-up period with additional GFR measurements and endpoint (disease or death) analyses to shed further light on the relationship between mGFR, ageing and disease are definitely warranted. Further research into the mechanisms responsible for the effect of obesity on kidney function are welcome, including the potential effects of fat tissue as an endocrine organ, or the role of inflammation in obesity and kidney disease. Studies with precise measurements of fat mass and kidney function would increase confidence in the assumption that it is indeed the fat mass in obesity that is the culprit, and allow the more precise differentiation between fat mass distributions. Studies incorporating several of these features are now being planned as an extension of RENIS. Longitudinal studies of mGFR in different populations would be very welcome to confirm or dispute the findings of our studies.

Paper 1

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Central obesity associates with renal hyperfiltration in the non-diabetic general population: a cross-sectional study

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Abstract

Background: Obesity is a risk factor for end-stage renal disease. Renal hyperfiltration, defined as an abnormally high glomerular filtration rate (GFR), is a link in the causal chain between diabetes and chronic kidney disease. Whether obesity is associated with hyperfiltration in the non-diabetic general population, remains unresolved due to a lack of consensus regarding the definition of hyperfiltration and the limited precision of high-range GFR estimations with creatinine and/or cystatin C.

Methods: 1555 middle-aged participants without diabetes, renal or cardiovascular disease were enrolled from the general population in the Renal Iohexol Clearance Survey from the 6th Tromsø Study (RENIS-T6) between 2007 and 2009. Obesity was assessed using the body mass index (BMI), waist circumference (WC) and the waist-hip ratio (WHR). GFR was measured by iohexol clearance. Dichotomous variables for hyperfiltration were based on two alternative definitions using unadjusted GFR (mL/min) above the 90th percentile. The 90th percentile was age-, sex- and height-specific in one definition and age-, sex-, height- and weight-specific in the other.

Results: In multivariable adjusted logistic regression models, only WHR was consistently associated with hyperfiltration based on both definitions. For the definition based on the age-, sex-, height- and weight-specific 90th percentile, the association with the WHR (odds ratios (95 % confidence intervals)) for hyperfiltration was 1.48 (1.08–2.02) per 0.10 WHR increase.

Conclusions: Central obesity is associated with hyperfiltration in the general population. The WHR may serve as a better indicator of the renal effects of obesity than BMI or WC.

Keywords: Body mass index, Chronic kidney disease, Glomerular filtration rate, Glomerular hyperfiltration, Waist circumference, Waist-hip ratio

Background

The prevalence of obesity, defined as a body mass index (BMI) ≥ 30 kg/m^2, has increased rapidly in high-income nations over the last few decades and is steadily growing in many lower-income countries as well [1]. Obesity is a well-known risk factor for cardiovascular disease, hypertension and diabetes [2, 3]. These diseases are, in turn, well-established risk factors for chronic kidney disease (CKD) and end-stage renal disease (ESRD) [4–7]. However, there is also evidence of a direct causal connection between obesity and ESRD, independent of hypertension and diabetes [8, 9].

Renal hyperfiltration (RHF), or an abnormally high glomerular filtration rate (GFR), has been postulated to represent an early stage in the development of CKD [10], most clearly observed in diabetic nephropathy [11]. RHF is also associated with several established CKD risk factors, including hypertension [12, 13] and smoking [14, 15]. A large longitudinal study by Park et al. of 43,503 Korean health screening participants found that a RHF definition based on eGFR was associated with all-cause mortality, even when adjusted for age, sex, muscle mass, diabetes and hypertension [16]. Although several studies have been

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conducted on the relationship between obesity and RHF [17–25], it remains unclear whether these two conditions are also associated in the general non-diabetic population. The most important reason has been that there is currently no consensus on the definition of the term "hyperfiltration". Most investigators who defined RHF in their studies used a single GFR cut-off point and adjusted their definition for no other variable than body surface area (BSA) [26]. Although there is no generally accepted way of defining RHF, it has been suggested that the definition should use age and sex-specific cut-offs and also some measure of correction for body size [26, 27].

Another methodological problem has been that previous epidemiological studies used GFR estimates based on creatinine and cystatin C, rather than GFR measurements [21–25]. Estimated GFR is inaccurate for high-range GFR [28–30] and can be confounded by associations with non-GFR-related factors [31, 32]. Studies on obesity and RHF using measured GFR (mGFR) have been limited by small sample sizes [17–19] and the lack of adjustment for confounding variables [20].

In the Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6), we measured GFR with iohexol clearance in 1627 middle-aged subjects from the general population. The aim of the present study was to examine the relationship between obesity and two alternative definitions of RHF.

Methods

Subjects

RENIS-T6 was conducted from 2007 to 2009 as a sub-study of the sixth Tromsø Study (Tromsø 6). The RENIS-T6 cohort consisted of a representative sample of 1627 persons from the general population of Tromsø who were between 50 to 62 years of age and without self-reported diabetes mellitus, cardiovascular or kidney disease (Fig. 1); the cohort has previously been described in detail [28].

Subjects were excluded from the present study if they had previously undiagnosed diabetes mellitus (hemoglobin A1c ≥ 6.5 % and/or fasting plasma glucose ≥ 7.0 mmol/L) or if they lacked waist or hip circumference measurements.

Smoking status was ascertained as part of a detailed questionnaire in the Tromsø 6 study. Previous smokers were grouped with non-smokers for the purposes of this study. Medication use was ascertained separately in the RENIS-T6 study. Antihypertensive medication use was categorized into six categories: beta-blockers, calcium channel blockers, diuretics, angiotensin converting enzyme-inhibitors, angiotensin-II receptor blockers, and other antihypertensive medications.

Iohexol-clearance measurements

Examination of the subjects started between 08:00 and 10:00 AM. All subjects had fasted and abstained from smoking since midnight, and they were instructed not to use non-steroid anti-inflammatory drugs or eat large

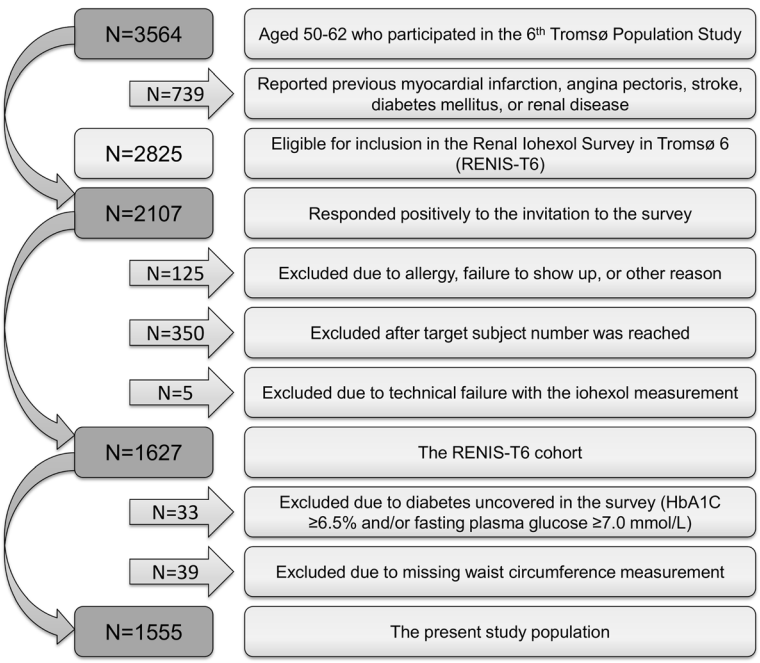

Fig. 1 Inclusion of subjects in the RENIS-T6 cohort and the present study population

quantities of meat during the preceding 48 h. Subjects were instructed to drink two to three glasses of water before arrival. A Teflon catheter was placed in an antecubital vein and was flushed with 30 mL of isotonic saline. Five milliliters of iohexol (Omnipaque, 300 mg/mL; Amersham Health) was injected, and the syringe was weighed before and after injection. GFR was measured as the single-sample plasma clearance of iohexol, a method that has been validated against gold standard methods [33], and analyzed using high-precision liquid chromatography as described by Nilsson-Ehle [34]. The analytical variation coefficient for the study period was 3.0 %. Jacobsson's method was used to calculate the GFR [35]. Further procedural details have been described previously [28].

Laboratory measurements

Glucose, low-density and high-density lipoprotein cholesterol, and triglycerides were measured on a Modular P800 (Roche Diagnostics, Basel, Switzerland). The insulin concentration was measured with an enzyme-linked immunosorbent assay kit (DRG Instruments, Marburg, Germany), with intra- and interassay coefficients of variation of 4.7 % and 6.3 %, respectively. Insulin resistance was expressed by the homeostasis model assessment (HOMA-IR), calculated by multiplying fasting glucose (mmol/L) by fasting insulin (mU/L) and dividing the result by 22.5 [36].

Blood pressure measurement

Office blood pressure (BP) was measured at the study site using an automated device (model UA799; A&D, Tokyo, Japan) after 2 min of rest. Daytime ambulatory BP was measured using weighted daytime (10:00–22:00) averages of BP measured at 20-min intervals. Further details of the BP measurements have been described previously [37].

Body measurements

Waist and hip circumferences, along with height, were measured as part of the main Tromsø 6 study at a median (interquartile range) of 5.2 (3.0–6.2) months before the RENIS-T6 investigations. Body weight was measured in the RENIS-T6 study to the nearest 0.1 kg on a SECA digital scale (SECA, Hamburg, Germany). The same weight scale was used for all subjects and was calibrated just before the study began. Height was measured to the nearest centimeter with a wall-mounted measuring tape. BMI was defined as height in meters divided by weight in kilograms squared. Waist and hip circumferences were measured horizontally over the umbilicus after exhalation and at the greatest protrusion of the buttocks, respectively. The WHR was calculated as the waist circumference divided by the hip circumference.

Subjects were classified into overweight and obesity categories based on cut-off values used by the World Health Organization and the International Diabetes Federation for European populations. BMI classes of 18.5–24.9, 25.0–29.9 and ≥ 30.0 define normal weight, overweight and obesity, respectively. WC categories of > 94 cm for men and > 80 cm for women represent "increased risk of metabolic complications", while a WC of > 102 cm for men or > 88 cm for women, or a WHR of ≥ 0.90 for men or ≥ 0.85 for women represents "substantially increased risk" [38].

There were only four subjects with BMI <18.5, these were grouped with the normal BMI (18.5–24.9) group for the purposes of this study. Fifty-seven subjects had BMI between 35.0 and 39.9, and 5 subjects had BMI ≥ 40.0, these were included in the BMI ≥ 30.0 group.

Definitions of hyperfiltration

The dichotomous variables for hyperfiltration were defined as unadjusted (absolute) GFR (mL/min) above the 90th percentile. We used two alternative definitions where the 90th percentile was either age-/sex- and height-specific (RHF_{Height}) or age-/sex-/height and weight-specific ($RHF_{Weight/height}$) (Table 1).

In both cases, the respective 90th percentiles were calculated from multiple linear regression models, with the natural logarithm (ln) of unadjusted GFR (mL/min) as the dependent variable. For RHF_{Height}, sex, ln(age) and ln(height) were used as independent variables, and for $RHF_{Weight/height}$ ln(body weight) was added (Additional file 1: Table S1). A subject was defined as having RHF_{Height} or $RHF_{Weight/height}$ if her regression residual was greater than the 90th percentile in the distribution of residuals in the regression analyses for the respective RHF definition (Table 1). This implies that the GFR cut-off for RHF for each individual depended on sex, age and height (RHF_{Height}) or sex, age, height and body weight ($RHF_{Weight/height}$). As an illustration, the GFR cut-off points for RHF in a male and female study participant with average measurements of age, height and weight are shown in Additional file 1: Table S2.

Table 1 Alternative definitions of renal hyperfiltration based on different adjustment variables in multiple linear regression

RHF definition	Dependent variable	Independent variables	Definition of dichotomous RHF variable
RHF_{Height}	Logarithm of absolute GFR (in mL/min)	Sex and logarithms of height and age	Residual > 90th percentile
$RHF_{Weight/height}$	Logarithm of absolute GFR (in mL/min)	Sex and logarithms of weight, height and age	Residual > 90th percentile

In both definitions, renal hyperfiltration was defined as residual > 90th percentile in multiple linear regression analysis with the independent variables listed above

RHF Renal hyperfiltration, *GFR* Glomerular filtration rate

Statistical analysis

The characteristics of the study population were tabulated as the mean (standard deviation) or median (interquartile range) for variables with skewed distributions. Pearson's χ^2 test, Welch's *t*-test and the Mann-Whitney *U* test were used to calculate p-values for differences between the WHR groups, classified by the World Health Organization cut-off for WHR.

Separate multiple logistic regression analyses were performed with each of the two alternative RHF variables

Table 2 Characteristics of the study population classified by World Health Organization waist-hip ratio cut-off point

	Normal waist-hip ratio[a]		Increased waist-hip ratio[b]		*P*-value
Subjects	432	27.8 %	1123	72.2 %	
Male gender	142	32.9 %	618	55.0 %	<0.001
Age	58.1	54.1–61.2	58.8	55.0–61.6	0.01
Waist-hip-ratio	0.824	0.046	0.941	0.055	
Waist circumference (cm)	83.5	7.6	99.3	9.7	<0.001
Body Mass Index (kg/m^2)	24.6	3.0	28.3	3.8	<0.001
Height (cm)	168.9	8.5	171.3	8.8	<0.001
Weight (kg)	70.2	10.8	83.1	13.8	<0.001
Daily smokers	89	20.6 %	222	19.8 %	0.71
Daytime ambulatory systolic BP (mmHg)	126.1	12.6	131.5	13.0	<0.001
Daytime ambulatory diastolic BP (mmHg)	79.9	8.6	82.9	8.6	<0.001
Nighttime ambulatory systolic BP (mmHg)	108.5	12.2	111.9	12.2	<0.001
Nighttime ambulatory diastolic BP (mmHg)	64.9	8.6	67.0	8.4	<0.001
Office systolic BP (mmHg)	123.3	17.1	131.8	17.1	<0.001
Office diastolic BP (mmHg)	79.9	10.0	84.7	9.3	<0.001
Hypertension[c]	95	22.0 %	437	38.9 %	<0.001
ACE-inhibitor use	6	1.3 %	22	2.0 %	0.45
Angiotensin II-receptor blocker use	13	3.0 %	116	10.3 %	<0.001
Calcium-channel blocker use	7	1.6 %	71	6.3 %	<0.001
Beta-blocker use	7	1.6 %	60	5.3 %	0.001
Diuretica use	17	3.9 %	119	10.6 %	<0.001
Other anti-hypertensive medicine use	0	-	1	<0.1 %	0.54
Fasting glucose (mmol/L)	5.13	0.44	5.39	0.48	<0.001
Fasting insulin (mIU/L)	6.50	4.37–8.69	9.47	6.90–13.65	<0.001
HOMA-IR	1.47	0.98–2.01	2.30	1.60–3.37	<0.001
HbA1c (%)	5.46	0.30	5.57	0.34	<0.001
Cholesterol (mmol/L)	5.53	0.89	5.67	0.96	0.008
LDL cholesterol (mmol/L)	3.45	0.83	3.73	0.86	<0.001
HDL cholesterol (mmol/L)	1.75	0.44	1.45	0.39	<0.001
Triglycerides (mmol/L)	0.8	0.6–1.1	1.1	0.8–1.6	<0.001
Cholesterol-lowering drug use	21	4.9 %	79	7.0 %	0.12
Absolute GFR (ml/min)	93.8	16.0	104.0	20.4	<0.001
GFR (ml/min/1.73 m^2)	90.1	13.0	92.0	14.8	0.02
RHFHeight	19	4.4 %	137	12.2 %	<0.001
RHFWeight/height	30	6.9 %	123	11.0 %	0.02

Data represented as number of subjects (percentage), median (interquartile range) or mean (standard deviation)
BP Blood pressure, *ACE* Angiotensin converting enzyme, *HOMA-IR* Homeostatic model assessment of insulin resistance, *LDL* Low density lipoprotein, *HDL* High density lipoprotein, *HbA1c* Hemoglobin A1c, *GFR* Glomerular filtration rate
[a]Female < 0.85, male < 0.90
[b]Female ≥ 0.85, male ≥ 0.90
[c]Office systolic BP ≥140 mmHg, office diastolic BP ≥90 mmHg and/or use of antihypertensive medication

(Table 1) as the dependent dichotomous variable and categorical or continuous indices of obesity as the independent variable. Adjustments were made for age, sex, number of cigarettes smoked daily, ambulatory daytime systolic and diastolic BP and their interaction, and individual categories of antihypertensive medication (Model 1). Mathisen et al. found a statistically significant interaction between these BP variables and GFR in the same study population as the present study [37], which is why this interaction model was included. Model 2 included Model 1 and a dichotomous variable for a metabolically unhealthy lipid profile, defined as high-density lipoprotein cholesterol levels < 1.03 mmol/L in men or < 1.29 mmol/L in women, elevated triglyceride levels of ≥ 1.7 mmol/L, and/or use of lipid-lowering medication. The variables in Model 2 constitute two of the five established criteria used to define metabolic syndrome [39]. Model 3 included Model 1, fasting plasma glucose and insulin levels, and HOMA-IR. Model 4 included all models. Additionally, linear regression analyses using absolute and BSA-adjusted GFR as dependent variables and the same independent variables as above were performed.

Fractional polynomial regression analyses [40] were performed to see whether any obesity variables had non-linear relationships with either RHF variable or with mGFR as a continuous variable, adjusting for the same variables as in Model 4.

Statistical significance was set at $p < 0.05$. Statistical analysis was performed using STATA MP 14.0 software (www.stata.com).

Results

Study population

Thirty-three of the 1627 study subjects in the RENIS-T6 cohort were excluded due to undiagnosed diabetes mellitus. Another 39 subjects were excluded because of missing WC measurements, leaving 1555 subjects eligible for the current study (Fig. 1).

The analysis of the study population showed several statistically significant associations between study variables and WHR categories (Table 2). A substantially higher percentage of males than females were obese according to the cut-off values. Subjects with a high WHR were, on average, older, had a higher absolute and BSA-adjusted GFR, higher BP, worse lipid and glucose profiles, and were more likely to use lipid- or BP-reducing drugs. There was a clear relationship between a greater WHR and higher GFR (Fig. 2). The vast majority of the population was overweight or obese (Fig. 3).

Hyperfiltration and obesity

The RHF definitions (Table 1) resulted in overlap, with 115 hyperfiltrating subjects having RHF by both definitions. Forty-one subjects had only RHF_{Height}, while 38 had only $RHF_{Weight/height}$.

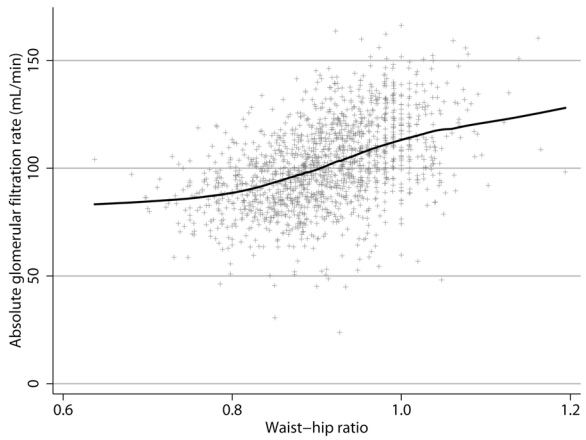

Fig. 2 Scatterplot with locally weighted scatterplot smoothing (LOWESS) showing the relationship between the waist-hip ratio and glomerular filtration rate

In the logistic regression analyses, there was a statistically significant association between RHF_{Height} and all obesity variables, categorical and continuous, except for the intermediate WC category, even in the fully adjusted Model 4 (Table 3). This relationship remained significant when body weight was added to the regression analyses as an independent variable (Additional file 1: Table S3).

With $RHF_{Weight/height}$, these relationships changed. Only the WHR as a continuous variable was consistently associated with $RHF_{Weight/height}$ across all the models ($p < 0.05$). In Model 1, the odds ratio (confidence interval) for $RHF_{Weight/height}$ was 1.66 (1.24–2.21) for each 0.10 increase in the WHR. The association was attenuated, but remained significant, when metabolic risk factors were added as independent variables in Models 2, 3 and 4 (Table 3).

Linear regression analyses with absolute and BSA-adjusted GFR as dependent variables and the same independent variables as above showed significant positive relationships between body size variables and absolute GFR, but no statistically significant relationship with BSA-adjusted GFR (Additional file 1: Table S4).

Interaction analyses were performed on the obesity variables and sex as well as the obesity variables and the dichotomous variable for an unhealthy lipid profile (defined in Model 2); but no statistically significant interactions were found. No statistically significant non-linear relationship was found between any obesity variables and the RHF variables or mGFR when analyzed in fractional polynomial regression models.

Discussion

In this study of non-diabetic, middle-aged subjects from the general population, higher WHR, but not BMI or

Categories	Body mass index (BMI)	Waist circumference (WC)	Waist-hip ratio (WHR)
Normal	18.5-24.9 kg/m^2	♂ ≤94 cm ♀ ≤80 cm	♂ <0.90 ♀ <0.85
Overweight	25.0-29.9 kg/m^2	♂ 95-102 cm ♀ 81-88 cm	
Obese	≥30.0 kg/m^2	♂ >102 cm ♀ >88 cm	♂ ≥0.90 ♀ ≥0.85

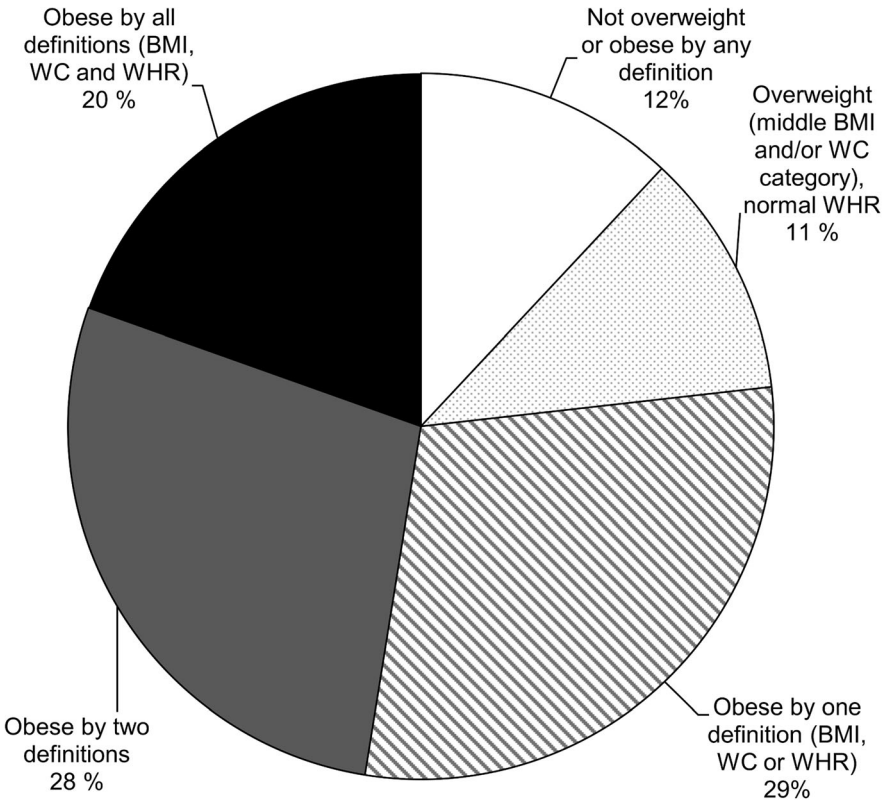

Fig. 3 Distribution of obesity in the RENIS-T6 cohort, by WHO categories for body mass index (BMI), waist circumference (WC) and the waist-hip ratio (WHR)

WC, was consistently associated with RHF, regardless of the RHF definition used. This finding suggests that excessive abdominal fat stores, as opposed to excess body weight distributed more evenly in the body, may potentially be more harmful to kidney function.

Most previous RHF studies with mGFR have found a positive relationship between BMI and RHF that disappears upon the adjustment of GFR to BSA [17–20]. The indexing of GFR to 1.73 m^2 of BSA may be problematic in itself, particularly in the abnormal body sizes encountered when studying obese subjects [41]. Kwakernaak et al. found that the WHR predicted a lower BSA-adjusted mGFR when adjusted for BMI, age, sex and BP [18]. However, the sample size was small and consisted of kidney donors and volunteers, who may not be representative of the general population. Pinto-Sietsma et al. made a similar finding of higher WHR associated with lower GFR in a larger population, but the result was based on GFR estimated by creatinine clearance [22].

The hypothesis of hyperfiltration as a precursor to overt CKD, originally proposed by Brenner, is based on hyperfiltration in individual glomeruli [10]. Because it is not possible to measure single-nephron GFR directly in living humans, an indirect measure of hyperfiltration based on whole-kidney GFR must be used in epidemiological studies. Whole-kidney GFR is a function of single-nephron GFR and the total number of nephrons. Nephron numbers vary by gender and birth weight and decrease with age [42], and adult height has been shown

Table 3 Odds ratios for renal hyperfiltration using alternative renal hyperfiltration definitions and variable models

	Model 1			Model 2			Model 3			Model 4		
	OR	CI	*P*	OR	CI	*P*	OR	CI	*P*	OR	CI	*P*
RHF_{Height}												
BMI < 25 kg/m^2	1.00	(ref)		1.00	(ref)		1.00	(ref)		1.00	(ref)	
BMI 25–30 kg/m^2	2.54	(1.45–4.47)	0.001	2.40	(1.36–4.24)	0.002	2.27	(1.28–4.05)	0.005	2.22	(1.24–3.95)	0.007
BMI > 30 kg/m^2	8.03	(4.50–14.33)	<0.001	7.19	(3.99–12.94)	<0.001	6.11	(3.27–11.44)	<0.001	5.85	(3.12–10.99)	<0.001
BMI per 5 kg/m^2[a]	2.66	(2.13–3.32)	<0.001	2.54	(2.02–3.19)	<0.001	2.40	(1.87–3.09)	<0.001	2.35	(1.83–3.03)	<0.001
WC < 80/94 cm	1.00	(ref)		1.00	(ref)		1.00	(ref)		1.00	(ref)	
WC 80–88/94–102 cm	1.89	(0.93–3.85)	0.08	1.82	(0.89–3.72)	0.10	1.63	(0.79–3.35)	0.18	1.62	(0.79–3.32)	0.19
WC > 88/102 cm	4.96	(2.59–9.49)	<0.001	4.48	(2.32–8.62)	<0.001	3.64	(1.86–7.14)	<0.001	3.52	(1.79–6.91)	<0.001
WC per 10 cm[a]	1.99	(1.68–2.35)	<0.001	1.92	(1.62–2.27)	<0.001	1.80	(1.50–2.17)	<0.001	1.78	(1.47–2.14)	<0.001
WHR < 0,85/0,90	1.00	(ref)		1.00	(ref)		1.00	(ref)		1.00	(ref)	
WHR > 0,85/0,90	2.91	(1.75–4.83)	<0.001	2.66	(1.59–4.43)	<0.001	2.24	(1.33–3.78)	0.002	2.17	(1.28–3.66)	0.004
WHR per 0.10[a]	2.67	(1.98–3.60)	<0.001	2.49	(1.84–3.37)	<0.001	2.20	(1.60–3.02)	<0.001	2.14	(1.55–2.94)	<0.001
$RHF_{Weight/height}$												
BMI < 25 kg/m^2	1.00	(ref)		1.00	(ref)		1.00	(ref)		1.00	(ref)	
BMI 25–30 kg/m^2	0.84	(0.55–1.28)	0.42	0.79	(0.52–1.21)	0.28	0.72	(0.47–1.12)	0.14	0.70	(0.45–1.09)	0.11
BMI > 30 kg/m^2	1.17	(0.72–1.90)	0.53	1.04	(0.63–1.71)	0.88	0.84	(0.49–1.45)	0.53	0.80	(0.46–1.39)	0.43
BMI per 5 kg/m^2[a]	1.14	(0.92–1.42)	0.24	1.08	(0.86–1.36)	0.50	0.97	(0.75–1.26)	0.82	0.95	(0.73–1.23)	0.70
WC < 80/94 cm	1.00	(ref)		1.00	(ref)		1.00	(ref)		1.00	(ref)	
WC 80–88/94–102 cm	1.23	(0.72–2.10)	0.44	1.20	(0.70–2.04)	0.50	1.12	(0.65–1.92)	0.69	1.11	(0.65–1.91)	0.70
WC > 88/102 cm	1.56	(0.95–2.56)	0.08	1.44	(0.87–2.38)	0.16	1.29	(0.76–2.19)	0.35	1.25	(0.73–2.13)	0.41
WC per 10 cm[a]	1.21	(1.03–1.42)	0.02	1.17	(0.99–1.38)	0.07	1.11	(0.92–1.33)	0.28	1.09	(0.91–1.31)	0.35
WHR < 0,85/0,90	1.00	(ref)		1.00	(ref)		1.00	(ref)		1.00	(ref)	
WHR > 0,85/0,90	1.66	(1.07–2.55)	0.02	1.57	(1.01–2.42)	0.04	1.45	(0.93–2.27)	0.10	1.42	(0.90–2.22)	0.13
WHR per 0.10[a]	1.66	(1.24–2.21)	<0.001	1.59	(1.18–2.13)	0.002	1.51	(1.11–2.06)	0.009	1.48	(1.08–2.02)	0.01

RHF Renal hyperfiltration, *OR* Odds ratio, *CI* Confidence interval, *BMI* Body mass index, *WC* Waist circumference, *WHR* Waist-hip ratio
Model 1: Adjustment for age, sex, number of cigarettes smoked daily, ambulatory daytime systolic and diastolic BP (and their interaction), and individual categories of antihypertensive medication
Model 2: Model 1 and a dichotomous variable for a metabolically unhealthy lipid profile, defined as HDL-cholesterol levels < 1.03 mmol/L in men or < 1.29 mmol/L in women, elevated triglyceride levels of ≥ 1.7 mmol/L, and/or use of lipid-lowering medication
Model 3: Model 1 plus fasting plasma glucose and insulin levels, and HOMA-IR
Model 4: All models combined
[a]continuous variable

to correlate with birth weight [43]. Thus, gender, height and age were included in both the RHF definitions (Table 1). RHF_{Height} used the age-, sex- and height-specific 90th percentile, and because an individual's normal body weight is correlated with height, it provides an indirect adjustment for a theoretical "normal" body size. RHF_{Height} is thus defined as excessive GFR relative to the mean GFR for a person with "normal" body weight. Because GFR increases with increasing body weight and increasing metabolic needs [44], it follows that RHF_{Height} is associated with measures of obesity, as shown in Table 3. However, when body weight was added as an independent variable to the same RHF_{Height} logistic regression models as in Table 3, the results were attenuated but remained essentially similar (Additional file 1: Table S3), indicating that an obese figure is associated with hyperfiltration independently of the effect of weight itself.

Another way to correct for interindividual variation in weight is to include weight in the definition of hyperfiltration, as in $RHF_{Weight/height}$. $RHF_{Weight/height}$ accordingly defines hyperfiltration as excessive GFR relative to the mean GFR for persons with a given height and weight, whether obese or not. This definition may underestimate hyperfiltration in obese subjects, and $RHF_{Weight/height}$ can be viewed as more conservative than RHF_{Height}. The association of WHR with hyperfiltration even when using $RHF_{Weight/height}$ is a strong indicator that central obesity also entails hyperfiltration at the glomerular level.

The merits of different body size measurement methods in the context of epidemiological research and risk

estimates for disease have been debated, as have the merits of various cut-off points [38]. BMI has become the dominant measure of obesity, partly due to its well-established association with several obesity-related diseases and partly due to the near-universal availability of height and weight as variables in both large population studies and general clinical practice. WHR, which measures body fat distribution rather than absolute body size, has been shown to be at least equal to, and often better than, BMI as a predictor for obesity-related disease including CKD [22, 45–47].

The mechanisms of the adverse renal effect of abdominal adiposity are not fully understood, but some effects are known. The most severe and well-established mediators are increased risks of diabetes mellitus, hypertension and dyslipidemia [48–50]. The effects of metabolic risk factors can be observed in our results, with a gradual attenuation of the odds ratio for RHF when variables for an unhealthy lipid profile and insulin resistance were included in the regression analyses.

Additionally, some other mechanisms are known, including dysfunction in the renin-angiotensin-aldosterone system, increased tubular sodium reabsorption, and the effects of obesity-related hormones and cytokines such as leptin, adiponectin and Tumor Necrosis Factor-α [48–50].

Weight loss interventions, especially bariatric surgery, have been shown to reduce GFR in hyperfiltrating obese subjects [51]. However, most studies of such interventions have been small, and few studies have been published on long-term effects beyond the first 2 years after the interventions. A recent study by Zingerman et al. suggested a possible reversal of RHF in obese patients using acetazolamide, although the study did not include a placebo arm [52].

The strength of the present study lies in the measurement of GFR with a gold-standard method in a large, representative, mostly healthy cohort in an age group susceptible to early stages of chronic diseases. To our knowledge, this is the largest cohort from the general population that has been studied using precise GFR measurements. The exclusion of subjects with diabetes, cardiovascular disease and renal disease from the study population allowed us to focus on the preliminary stages of potential future CKD with less confounding from these high-risk patient groups. These groups would have been more likely to have passed the transient stage of hyperfiltration into a state of normal-range GFR, perhaps accompanied by slight albuminuria.

There are several limitations to this study. First, it was a cross-sectional study and thus could not prove causation, only correlation. Second, the study population was exclusively Caucasian and middle-aged, which may limit the transferability of findings to other population groups. Furthermore, while GFR was measured with a gold standard method, obesity was measured indirectly with anthropometric data, and not directly with gold standard dual energy X-ray absorptiometry, computed tomography or magnetic resonance imaging methods. Glucose and HbA1c were only measured once to exclude diabetes, while regular clinical practice requires two measurements for the diagnosis.

Conclusions

We conclude that the WHR is associated with RHF, independently of other risk factors and even using $RHF_{Weight/height}$, a conservative, body size-adjusted RHF definition. Longitudinal studies are needed to explore whether RHF predicts future non-diabetic CKD. Further studies on whether the WHR predicts CKD better than other obesity measurements are also warranted.

Additional file

Additional file 1: Table S1. Regression models for the alternative renal hyperfiltration definitions. **Table S2.** mGFR cut-off points for renal hyperfiltration in male and female subjects from the study cohort with average height, weight and age. **Table S3.** Odds ratio for RHF_{height}, with body weight added as an independent variable. **Table S4.** Multiple linear regression with measured GFR and continuous obesity variables. (XLSX 22 kb)

Abbreviations

BMI: Body mass index; BP: Blood pressure; BSA: Body surface area; CKD: Chronic kidney disease; ESRD: End-stage renal disease; GFR: Glomerular filtration rate; HOMA-IR: Homeostatic model assessment of insulin resistance; mGFR: Measured glomerular filtration rate; RENIS-T6: Renal Iohexol Survey in Tromsø 6; RHF: Renal hyperfiltration; WC: Waist circumference; WHR: Waist-hip ratio

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Availability of data and materials

The authors prefer not to disclose the full dataset to the public domain due to study participants' privacy concerns.

Authors' contributions

Research idea and study design: VTNS, BOE, TM; data acquisition: BOE, TM; data analysis/interpretation: VTNS, BOE, TM, JS, TGJ; statistical analysis: VTNS, BOE, TM; supervision or mentorship: BOE, TM, TGJ; critical review VTNS, BOE, TM, JS, TGJ. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All participants provided informed, written consent, and the regional ethics committee of northern Norway approved the study, the reference number is REK NORD 89/2006. The study was in compliance with the WMA Declaration of Helsinki.

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Paper 2

Metabolic syndrome but not obesity measures are risk factors for accelerated age-related glomerular filtration rate decline in the general population

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Rapid age-related glomerular filtration rate (GFR) decline increases the risk of end-stage renal disease, and a low GFR increases the risk of mortality and cardiovascular disease. High body mass index and the metabolic syndrome are well-known risk factors for patients with advanced chronic kidney disease, but their role in accelerating age-related GFR decline independent of cardiovascular disease, hypertension and diabetes is not adequately understood. We studied body mass index, waist circumference, waist-hip ratio and metabolic syndrome as risk factors for accelerated GFR decline in 1261 middle-aged people representative of the general population without diabetes, cardiovascular disease or kidney disease. GFR was measured as iohexol clearance at baseline and repeated after a median of 5.6 years. Metabolic syndrome was defined as fulfilling three out of five criteria, based on waist circumference, blood pressure, glucose, high-density lipoprotein cholesterol and triglycerides. The mean GFR decline rate was 0.95 ml/min/year. Neither the body mass index, waist circumference nor waist-hip ratio predicted statistically significant changes in age-related GFR decline, but individuals with baseline metabolic syndrome had a significant mean of 0.30 ml/min/year faster decline than individuals without metabolic syndrome in a multivariable adjusted linear regression model. This association was mainly driven by the triglyceride criterion of metabolic syndrome, which was associated with a significant 0.36 ml/min/year faster decline when analyzed separately. Results differed significantly when GFR was estimated using creatinine and/or cystatin C. Thus, metabolic syndrome, but not the body mass index, waist circumference or waist-hip ratio, is an independent risk factor for accelerated age-related GFR decline in the general population.

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Age-related kidney function decline is an integral part of aging.[1] Consequently, the prevalence of chronic kidney disease (CKD), defined as glomerular filtration rate (GFR) < 60 ml/min per 1.73 m^2, increases with age and reaches almost 50% at 70 years.[2] Additionally, the prevalence of the most severe form of CKD, end-stage renal disease, increases almost exponentially with age and leads to an impaired quality of life, high mortality, and high health care costs.[3–5]

Obesity, the unhealthy accumulation of excess fat mass usually assessed with anthropometric measurements such as body mass index (BMI), waist circumference (WC), or the waist-hip ratio (WHR), is an established risk factor for CKD and end-stage renal disease.[6,7] The rising prevalence of obesity worldwide is alarming,[8] but this prevalence also offers potential for prevention. A high BMI increases the CKD risk mainly due to the associated risk of diabetes, cardiovascular disease (CVD), and hypertension.[7,9] Whether increased BMI also increases the CKD risk by accelerating age-related GFR decline independent of these conditions is less clear. Several studies have attempted to identify whether BMI or WC affects the rate of GFR decline in people without preexisting CKD, but these studies have not reached a firm conclusion.[10–16]

The most important reason for these divergent results may be that they used estimated instead of measured GFR. Estimated GFR (eGFR) based on serum cystatin C or creatinine is imprecise for normal and high levels of GFR and is biased by non-GFR-related factors.[17–20]

Another obstacle may be that obesity is a heterogeneous condition. Recent reports have studied people with so-called metabolically healthy obesity, defined as a BMI ≥ 30 kg/m^2 without the metabolic syndrome (MS), and compared their risk of kidney disease with the risk in metabolically unhealthy obese people (obesity with MS), but the results of these studies have also been divergent.[21–24] Furthermore, the normalization to body surface area incorporated in eGFR has been criticized and is especially problematic in studies of obesity.[25,26]

Obesity and the components of MS are potentially modifiable conditions. Understanding the factors that affect the rate of GFR decline may enable targeted risk factor interventions in susceptible individuals.[27] Therefore, deeper knowledge of the relationship between these risk factors and the GFR decline rate is of great clinical interest.

In the present study, we aimed to investigate whether BMI, WC, or WHR at baseline were associated with changes in the subsequent age-related decline rate of measured GFR (mGFR) using iohexol clearance. We also examined whether MS and its individual components are related to the decline rate.

RESULTS

Population characteristics

This study was a follow-up to the Renal Iohexol-Clearance Survey in Tromsø 6 (RENIS-T6), which included 1627 people representative of the general population without baseline self-reported CKD, CVD or diabetes. One thousand three hundred and twenty-four subjects (81%) had a second GFR measurement after a median (interquartile range [IQR]) observation period of 5.6 (IQR: 5.2–6.0) years. Among those who had 2 measurements, 25 had diabetes at baseline (defined as fasting plasma glucose ≥ 7.0 mmol/l, or glycosylated hemoglobin ≥ 6.5%, or both); 36 had a missing WC measurement; and 2 had missing triglyceride values at baseline. These people were excluded, resulting in a study population of 1261 people (Figure 1). As previously reported, there were only small differences in the characteristics of the included participants compared with the 19% who were lost to follow-up.[28]

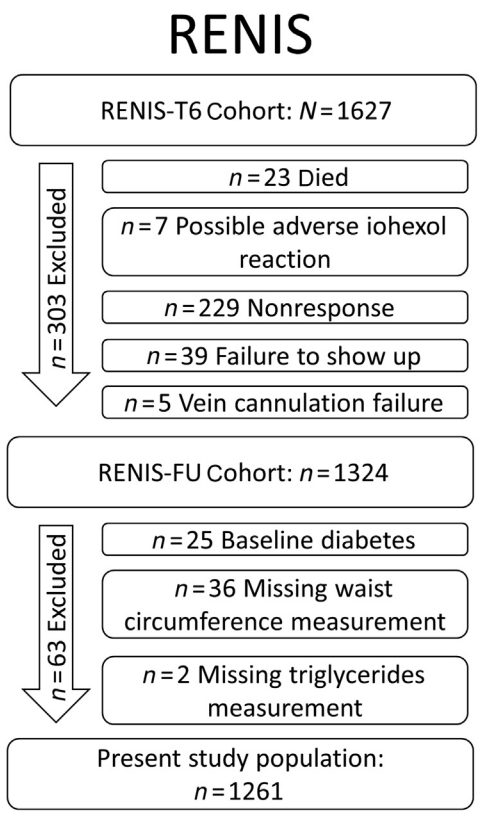

Figure 1 | Flowchart depicting the selection of subjects in the Renal Iohexol-Clearance Survey (RENIS) cohort for the current study. RENIS-FU, Renal Iohexol-Clearance Survey Follow-Up; RENIS-T6, Renal Iohexol-Clearance Survey in Tromsø 6.

The mean ± SD age at baseline was 58.0 ± 3.9 years, mean BMI was 27.1 ± 3.8 kg/m^2, and mean mGFR was 103.6 ± 19.6 ml/min among subjects who had 2 GFR measurements. Three hundred eighty-two subjects (30%) had MS, fulfilling at least 3 of the following 5 criteria: WC > 94 cm in men or > 80 cm in women; fasting plasma glucose ≥ 5.6 mmol/l; systolic blood pressure ≥ 130 mm Hg, diastolic blood pressure ≥ 85 mm Hg, or use of antihypertensive medication, or a combination of these; triglycerides ≥ 1.7 mmol/l or the use of triglyceride-altering drugs; HDL cholesterol levels < 1.03 mmol/l in men or < 1.29 mmol/l in women or the use of HDL-altering drugs.[29] Most of the baseline characteristics differed between the groups when the population was stratified by MS status (Table 1), including the mean baseline mGFR, which was 10.2 ml/min higher in the MS group ($P < 0.001$). Twenty-five subjects had mGFR < 60 ml/min per 1.73 m^2 at baseline, increasing to 33 at follow-up (Supplementary Table S1).

BMI, WC, WHR, and the GFR decline rate

The unadjusted mean ± SD mGFR decline rate was 0.95 ± 2.25 ml/min per year. In separate multivariable adjusted linear regression models analyses, there was no statistically significant linear relationship among the age-related mean mGFR decline rate and BMI, WC, or WHR (Table 2).

In the same models, we examined whether mean mGFR decline was associated with any of the constituent components of MS when analyzed as continuous variables. Higher HDL cholesterol was linearly associated with a 0.58 ml/min/per year faster mean mGFR decline per mmol/l in the fully adjusted model with BMI as an independent variable ($P = 0.002$) (not shown), and there were very similar results in the analyses with WC and WHR. There was no statistically significant linear or nonlinear relationship in generalized additive models among the mean mGFR decline rate and glucose, blood pressure, HDL, or triglycerides.

There was a statistically significant nonlinear relationship between the mean mGFR decline rate and BMI in the fully adjusted model, but the relationship lost its statistical significance when 1 subject with a very high BMI and steep mGFR decline was removed from the dataset. No nonlinear relationship was found among the age-related mean mGFR decline and WC, WHR, or body weight.

The metabolic syndrome

The dichotomous variable MS (yes or no) was included as an independent variable in 3 new models. The new models were based on the previously used models, but they excluded variables that overlapped with the components of MS. In the fully adjusted model, subjects with MS had a mean (95% confidence interval [CI]) 0.30 (95% CI: 0.02–0.58) ml/min per year faster decline ($P = 0.03$) than those without the

Table 1 | Baseline characteristics for subjects with and without the metabolic syndrome

Variable	No metabolic syndrome	Metabolic syndrome	*P* value
Number of subjects	879 (69.7)	382 (30.3)	
Male	388 (44.1)	235 (61.5)	<0.001
Age (yr)	58.5 (54.5–61.4)	59.1 (55.0–61.5)	0.16
mGFR (ml/min)	100.5 ± 19.0	110.7 ± 19.2	<0.001
mGFR (ml/min per 1.73 m^2)	92.9 ± 14.3	95.1 ± 14.1	0.01
mGFR decline rate (ml/min per yr)	0.82 ± 2.21	1.23 ± 2.33	0.004
mGFR decline rate (ml/min per 1.73 m^2/yr)	0.74 ± 2.04	1.03 ± 1.96	0.02
mGFR decline >3 ml/min per 1.73 m^2/yr	82 (9.3)	47 (12.3)	0.11
Height (cm)	170.1 ± 8.6	172.5 ± 8.5	<0.001
Weight (kg)	75.8 ± 12.8	87.9 ± 12.6	<0.001
Body mass index (kg/m^2)	26.1 ± 3.5	29.5 ± 3.5	<0.001
Body mass index ≥30 kg/m^2	105 (11.9)	155 (40.6)	<0.001
Waist circumference (cm)	91.6 ± 10.4	102.0 ± 9.5	<0.001
Waist-hip ratio	0.89 ± 0.07	0.95 ± 0.06	<0.001
Daily smoker	164 (18.7)	66 (17.3)	0.56
Fasting plasma glucose (mmol/l)	5.2 ± 0.4	5.7 ± 0.4	<0.001
Hemoglobin A1c (%)	5.5 ± 0.3	5.6 ± 0.3	<0.001
LDL cholesterol (mmol/l)	3.6 ± 0.8	3.9 ± 0.9	<0.001
HDL cholesterol (mmol/l)	1.6 ± 0.4	1.3 ± 0.3	<0.001
Fasting triglycerides (mmol/l)	0.9 (0.7–1.2)	1.6 (1.1–2.0)	<0.001
Cholesterol-lowering medication use	48 (5.5)	31 (8.1)	0.07
Urinary albumin-creatinine ratio	0.20 (0.10–0.51)	0.25 (0.10–0.61)	0.01
Heart rate (beats/min)	65.4 ± 9.6	68.5 ± 9.8	<0.001
Hypertension[a]	271 (30.8)	253 (66.2)	<0.001
Systolic blood pressure (mm Hg)	125.7 ± 16.5	136.8 ± 16.6	<0.001
Diastolic blood pressure (mm Hg)	81.3 ± 9.4	87.8 ± 8.9	<0.001
ACE inhibitor use	13 (1.5)	13 (3.4)	0.03
Calcium blocker use	31 (3.5)	29 (7.6)	0.002
ARB use	40 (4.6)	62 (16.2)	<0.001
Beta blocker use	22 (2.5)	25 (6.5)	<0.001
Diuretic use	50 (5.7)	53 (13.9)	<0.001
Fulfilled metabolic syndrome criterion			
Blood pressure criterion	402 (45.7)	336 (88.0)	<0.001
Triglyceride criterion	28 (3.2)	181 (47.4)	<0.001
HDL cholesterol criterion	43 (4.9)	144 (37.7)	<0.001
Glucose criterion	105 (11.9)	258 (67.5)	<0.001
Waist circumference criterion	624 (71.0)	376 (98.4)	<0.001

ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; mGFR, measured glomerular filtration rate. Results are expressed as *n* (%), mean ± SD, or median (IQR).
[a]Systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, and/or use of antihypertensive medication.

syndrome (Table 3). Sensitivity analyses were performed using higher cut-offs for the waist criterion, >102 cm in men or >88 cm in women (resulting in 384 fewer subjects fulfilling the criterion, and 78 fewer subjects classified as having the MS).[29] This diluted the association between MS and GFR decline, with a mean 0.14 (95% CI: −0.16 to 0.44) ml/min per year faster decline in MS subjects ($P = 0.35$) in fully adjusted models.

The analyses were repeated using the individual components of MS as dichotomous independent variables. The triglycerides component of MS was the only statistically significant individual component variable ($P = 0.04$ in the fully adjusted models) (Table 3). A scatter plot with a locally weighted scatterplot smoothing line of the unadjusted relationship between triglycerides and the mGFR decline rate is shown in Supplementary Figure S1.

The same anthropometric and MS models were then used in logistic regression analyses with mGFR decline > 3 ml/min per 1.73 m^2/yr as a dichotomous outcome variable. Not BMI, WC, WHR, or MS was associated with an increased risk of the dichotomous variable rapid mGFR decline in the fully adjusted models (Tables 2 and 3, respectively).

Analyses using eGFR

The analyses were repeated using eGFR based on the Chronic Kidney Disease Epidemiology research group (CKD-EPI) equations for creatinine, cystatin C, and both together.[17] There were no statistically significant associations between mean eGFR decline or the dichotomous rapid eGFR decline variable, and BMI, WC, or WHR in the fully adjusted models (Supplementary Tables S2 and S3). However, MS was associated with accelerated mean creatinine-based eGFR decline ($P = 0.007$), but not mean cystatin C-based decline ($P = 0.38$), in fully adjusted models. Paradoxically, the dichotomous variable cystatin C-based eGFR decline > 3 ml/min per 1.73 m^2/yr was associated with MS ($P = 0.003$ in fully adjusted models), while the same dichotomous creatinine-based variable was not ($P = 0.39$). The use of the CKD-EPI eGFR equation incorporating both cystatin C and creatinine yielded statistically nonsignificant results

Table 2 | Analyses of baseline anthropometric variables and changes in mGFR decline

Linear regression model analyses of anthropometric variables and change in mean yearly absolute mGFR decline (ml/min per yr)									
	Model 1			Model 2			Model 3		
Anthropometric variable	Coef.	95% CI	*P* value	Coef.	95% CI	*P* value	Coef.	95% CI	*P* value
Body mass index[a]	−0.09	(−0.22 to 0.03)	0.15	−0.08	(−0.24 to 0.07)	0.27	−0.08	(−0.23 to 0.07)	0.28
Waist circumference[a]	−0.13	(−0.26 to 0.01)	0.08	−0.12	(−0.28 to 0.05)	0.16	−0.12	(−0.28 to 0.05)	0.16
Waist-hip ratio[a]	−0.05	(−0.20 to 0.10)	0.48	−0.01	(−0.18 to 0.16)	0.89	−0.01	(−0.18 to 0.16)	0.90
Logistic regression analyses of anthropometric variables and risk of rapid yearly mGFR decline (>3 ml/min per 1.73 m^2/yr)									
	Model 1			Model 2			Model 3		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Body mass index[a]	1.10	(0.91–1.32)	0.32	1.04	(0.83–1.28)	0.75	1.03	(0.83–1.28)	0.79
Waist circumference[a]	1.13	(0.93–1.38)	0.22	1.08	(0.85–1.36)	0.54	1.07	(0.85–1.36)	0.55
Waist-hip ratio[a]	1.18	(0.95–1.48)	0.14	1.13	(0.87–1.46)	0.37	1.12	(0.87–1.45)	0.39

CI, confidence interval; Coef., coefficient (ml/min per yr); mGFR, measured glomerular filtration rate; OR, odds ratio.
Model 1: Adjusted for baseline height, sex, and age. For rapid decline, the model is not adjusted for height.
Model 2: Model 1 + adjusted for baseline smoking, triglycerides, high-density and low-density lipoprotein cholesterol, cholesterol-lowering medication use, systolic and diastolic blood pressure, antihypertensive medication use, heart rate, fasting glucose, and nonsteroid anti-inflammatory drug use.
Model 3: Model 2 + baseline urinary albumin-creatinine ratio.
[a]Each obesity variable is expressed per SD and was analyzed in a separate regression model.

intermediary to the results using either biomarker separately. The discrepancies between mGFR and eGFR decline are further elucidated in Supplementary Figure S2, including a scatterplot of the correlations between mGFR and eGFR decline and a Venn diagram of the rapid decline variables.

Interaction analyses

There was no statistically significant interaction among participants' sex and BMI, WC, WHR, MS, or any of its components, for the relationship with the mean mGFR decline. We found no statistically significant interaction between BMI categorized as <30.0 and ≥30.0 kg/m^2 and MS, for the relationship with the mean mGFR decline ($P = 0.12$ in the fully adjusted model). Finally, we tested pairwise interactions between the dichotomous waist criterion and the other criteria of MS, and we found no statistically significant interactions in the fully adjusted models.

DISCUSSION

In this middle-aged nondiabetic cohort from the general population, BMI, WC, and WHR did not predict changes in the age-related GFR decline. This suggests that increased body fat alone is not an important determinant of age-related GFR decline independent of CVD, diabetes, obesity-related glomerulopathy, or other proposed mechanisms in a healthy population. However, MS was independently associated with a 0.30 ml/min per year faster mean mGFR decline rate in the multivariable adjusted models including the urinary albumin-creatinine ratio (ACR). This result was mainly driven by the triglyceride criterion of MS (Table 3). When using a less stringent WC criterion, as suggested by the American Heart Association and the National Heart, Lung, and Blood Institute, the association between MS and GFR decline was diluted and not statistically significant.

Table 3 | Regression analyses of baseline metabolic syndrome, its components, and changes in mGFR decline

Linear regression model analyses of baseline metabolic syndrome, its components, and change in mean yearly absolute mGFR decline (ml/min per yr)									
	Model 1			Model 2			Model 3		
Variable	Coef.	95% CI	*P* value	Coef.	95% CI	*P* value	Coef.	95% CI	*P* value
Metabolic syndrome[a]	−0.39	(−0.66 to −0.11)	0.006	−0.32	(−0.60 to −0.04)	0.03	−0.30	(−0.58 to −0.02)	0.03
Waist circumference criterion[a]	−0.24	(−0.56 to 0.07)	0.13	−0.18	(−0.50 to 0.14)	0.27	−0.17	(−0.49 to 0.15)	0.29
Glucose criterion[a]	-−0.10	(−0.38 to 0.18)	0.48	−0.06	(−0.33 to 0.21)	0.67	−0.06	(−0.35 to 0.22)	0.66
Blood pressure criterion[a]	−0.10	(−0.36 to 0.16)	0.47	−0.03	(−0.30 to 0.23)	0.80	−0.03	(−0.29 to 0.24)	0.84
HDL cholesterol criterion[a]	0.12	(−0.23 to 0.47)	0.49	0.21	(−0.14 to 0.56)	0.25	0.22	(−0.13 to 0.57)	0.23
Triglycerides criterion[a]	−0.44	(−0.77 to −0.10)	0.01	−0.38	(−0.73 to −0.03)	0.03	−0.36	(−0.71 to −0.01)	0.04
Logistic regression analyses of baseline metabolic syndrome and the risk of rapid yearly mGFR decline (>3 ml/min per 1.73 m^2/yr)									
	Model 1			Model 2			Model 3		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Metabolic syndrome	1.34	(0.91–1.98)	0.14	1.32	(0.89–1.98)	0.17	1.27	(0.84–1.91)	0.25

CI, confidence interval; Coef., coefficient (ml/min per yr); HDL, high-density lipoprotein; mGFR, measured glomerular filtration rate; OR, odds ratio.
Model 1: Adjusted for baseline height, sex, and age. For rapid decline, the model is not adjusted for height.
Model 2: Model 1 + baseline smoking, low-density lipoprotein cholesterol, cholesterol-lowering medication use, resting heart rate, and nonsteroid anti-inflammatory drug use.
Model 3: Model 2 + baseline urinary albumin-creatinine ratio.
[a]Each variable was analyzed in a separate linear regression model.

To the best of our knowledge, all previous population-based longitudinal studies of obesity and GFR decline used eGFR to assess kidney function. Five previous longitudinal studies reported on associations between increased BMI or WC, and mean eGFR decline.[10–14] Only 1 of the 5 studies found a statistically significant association in their fully adjusted models. Halbesma *et al.*[10] reported an association between a higher WC and slower mean eGFR decline in the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study; however, this association was only in male subjects, and it was for a model that did not include possible confounding variables, such as smoking and triglyceride levels.

Several studies investigated the association between BMI or WC and the risk of more rapid annual decline in eGFR as a dichotomous outcome, although variously defined.[12–16] They all reported an association between either BMI or WC and either dichotomous rapid creatinine- or cystatin C-based eGFR decline variable, but some reported mixed results with negative findings as well.[13–15] We found no statistically significant association among BMI, WC, or WHR and the dichotomous rapid mGFR or eGFR decline variables, defined as >3 ml/min per 1.73 m^2/yr, in the fully adjusted models. We did find an association between MS and the dichotomous rapid cystatin C-based eGFR decline, but there was no association with the dichotomous rapid mGFR decline variable in the fully adjusted models (Supplementary Table S3, Table 3).

Creatinine- and cystatin C-based GFR estimates are known to associate with non-GFR-related factors, and eGFR decline may occur due to changes in those factors independently of real changes in GFR. Indeed, we found a significant association between BMI and faster cystatin C-based mean eGFR decline (Supplementary Table S2), while the mean mGFR decline was not associated with BMI. Previous studies, except the study by Malkina *et al.*[13] in the MESA cohort, included diabetic subjects who may have different GFR trajectories than nondiabetic subjects.

Our finding of the lack of a statistically significant association between the body fat variables and changes in mGFR decline may also reflect a transient state of hyperfiltration in which GFR rises or remains high through a state of nephron overload.[30] We have previously reported an association between obesity and hyperfiltration in the RENIS-T6 baseline cohort;[31] the lack of a more rapid mGFR decline might represent subjects remaining in this state of hyperfiltration for a prolonged period of time.

To the best of our knowledge, no previous study has examined the relationship between MS and changes in age-related GFR decline. We found that the association between MS and an accelerated age-related mGFR decline was mainly driven by the triglyceride criterion of MS. High triglyceride levels are known to be associated with an increased risk of CKD,[9,32] and may contribute to kidney dysfunction through its proinflammatory and atherogenic effects; furthermore, triglyceride levels can act as a marker for insulin resistance.[33,34] Our finding suggests triglyceride-lowering interventions could be a possible strategy for slowing age-related GFR decline, but further confirmation in other studies would be necessary. We have previously reported the lack of a relationship between baseline blood pressure and the GFR change rate.[35] The other constituent components of MS were not significantly associated with changes in the mGFR decline; instead, higher HDL cholesterol, when analyzed as a continuous variable, was associated with an accelerated mGFR decline.

HDL cholesterol is known to be inversely associated with obesity, the risk of CKD, and CVD. Therefore, our finding is surprising and seemingly counterintuitive. However, a U-shaped relationship between HDL cholesterol levels and risk of CKD has been described before, with both low and high levels being associated with increased risk.[36] Importantly, our cohort consists of fairly healthy people without diabetes or CKD, and the association between lipid abnormalities and age-related mGFR decline need not mirror the associations among lipid abnormalities, CKD, and CVD.

Several recent studies have examined differences in the risk of incident CKD between obese people (BMI $\geq$ 30 kg/m^2) with and without MS, who were labeled "metabolically unhealthy obesity" and "metabolically healthy obesity," respectively.[21–24] Some did not find any increased risk of eGFR-defined CKD in people with metabolically unhealthy obesity compared with metabolically healthy obese people,[21,22] while others found that a residual increased risk of CKD remains.[23,24] We found no statistically significant difference in mGFR decline between people with metabolically healthy and unhealthy obesity in our study population, but the power of the test of interaction between BMI and MS may have been insufficient to detect smaller differences in the mGFR decline rate between the subgroups.

The strength of this longitudinal study lies in the use of 2 measurements of GFR with a gold-standard method in a large cohort covering an age group susceptible to early stages of chronic diseases. To the best of our knowledge, this is the largest cohort from the general population that has been studied using precise GFR measurements. We excluded people with self-reported CVD, renal disease, and those with self-reported or lab-revealed diabetes at baseline to focus on age-related GFR decline in relatively healthy individuals.

Only middle-aged white people participated in the study, which limits the generalizability of the findings to other population groups. Because the use of mGFR is costlier than eGFR, our cohort was smaller than in eGFR-based studies. This limits our ability to study GFR decline in subgroups. Also, a larger cohort would have increased the power of the statistical tests, especially for the dichotomous outcome. We used BMI, and WHR as anthropometric approximations of body fat in place of gold-standard computed tomography, dual X-ray absorptiometry, or magnetic resonance imaging methods, which is another study limitation. However, Madero *et al.*[37] recently found no significant difference among these methods and BMI or WC when determining the risk of eGFR decline and CKD. Finally, because our study had an observational design, inferences regarding causality should not be made.

We conclude that MS is associated with an accelerated age-related mGFR decline during 5.6 years of follow-up. We found no statistically significant association among the mean age-related mGFR decline and baseline BMI, WC, or WHR. Additional GFR measurements over a longer observation period are required to study obesity and any long-term, nonlinear trajectories in the GFR decline.

MATERIALS AND METHODS

Participants

RENIS-T6 was a substudy of the population-based sixth Tromsø Study in the municipality of Tromsø, Norway, conducted from 2007 to 2009. The study included a representative sample of 1627 people, ages 50 to 62 years, from the general population without self-reported CVD, kidney disease, or diabetes. A follow-up study, RENIS-FU, was conducted from 2013 to 2015; 1324 of the subjects in the cohort (81%) participated with a median (IQR) follow-up of 5.6 years (IQR: 5.2–6.0). The studies have previously been described in detail.[28,38]

We included all subjects who participated in both rounds, except the following: people with diabetes at baseline, defined as fasting glucose $\geq$ 7.0 mmol/l, or glycosylated hemoglobin $\geq$ 6.5%, or both, and people with missing measurements for any of the study variables.

All subjects provided written informed consent to participate, and the Regional Ethics Committee of Northern Norway approved the study. The study was performed in compliance with the Declaration of Helsinki.

Data collection and measurements

RENIS-T6 and RENIS-FU were conducted with a standardized procedure and specifically trained clinical staff responsible for measurements. Details have been previously described.[28]

Body weight was measured in the RENIS-T6 study to the nearest 0.1 kg on a SECA digital scale (SECA, Hamburg, Germany). The same weight scale was used for all subjects and was calibrated just before the study began. Height was measured to the nearest centimeter with a wall-mounted measuring tape. BMI was defined as the height in meters divided by weight in kilograms squared. The waist and hip circumferences were measured horizontally over the umbilicus after exhalation and at the greatest protrusion of the buttocks, respectively. The WHR was calculated as the WC divided by the hip circumference.

GFR was measured using single-sample plasma clearance of iohexol, which has previously been described in detail.[35,38] The serum iohexol (Omnipaque, 300 mg/ml, Amersham Health, London, UK) concentration was measured by high-performance liquid chromatography, previously described by Nilsson-Ehle.[39] GFR was calculated by Jacobsson's method.[40] The analytical coefficient of variation was 3.0% in RENIS-T6 and 3.1% in RENIS-FU, and the mean coefficient of variation (95% CI) for the intra-individual variation in GFR was 4.2% (3.4%–4.9%).[28]

Study subjects collected 3 samples of their first-void morning spot urine on the 3 days preceding the GFR measurements. The urinary creatinine and albumin concentrations were measured in unfrozen urine using an ABX PENTRA autoanalyzer and kits from ABX Diagnostics (Horiba ABX SAS, Montpellier, France). The urinary ACR was calculated for each urine specimen, and the median ACR from the 3 samples was used. The ACR was set at 0.1 mg/mmol in samples with no detectable urinary albumin concentration, which corresponded to the lowest ACR in samples with detectable albumin.

Serum samples for fasting glucose, triglycerides, and cholesterol levels were measured on a Modular P800 (Roche Diagnostics, Mannheim, Germany) on the day of the subject's appointment. Serum creatinine was analyzed with an enzymatic assay standardized to the isotope dilution mass spectrometry method (CREA Plus, Roche Diagnostics). Cystatin C was measured using a particle-enhanced turbidimetric immunoassay with reagents from Gentian (Gentian, Moss, Norway) and a Modular E analyzer (Roche Diagnostics). The cystatin C measurements were recalibrated to the international reference standard using a Cobas 8000 (Roche Diagnostics). Further details have previously been provided.[19] eGFR was calculated using the CKD-EPI equations.[17]

Blood pressure and resting heart rate were measured 3 times in a seated position after 2 minutes of rest using an automated device (model UA799; A&D, Tokyo, Japan). The average of the second and third measurements was used in the analyses. Subjects with a conventional systolic blood pressure $\geq$ 140 mm Hg, diastolic blood pressure $\geq$ 90 mm Hg, or taking antihypertensive medications were categorized as having hypertension.

Statistical analyses

The study population characteristics are presented as the mean $\pm$ SD, median (IQR), or total number (percentage), and the participants were categorized into 2 groups according to the criteria for MS. MS was defined as the presence of at least 3 of the following 5 criteria: WC $>$ 94 cm in men or $>$ 80 cm in women; fasting plasma glucose $\geq$ 5.6 mmol/l; systolic blood pressure $\geq$ 130 mm Hg, diastolic blood pressure $\geq$ 85 mm Hg, or use of antihypertensive medication, or a combination of these; triglycerides $\geq$ 1.7 mmol/l or the use of triglyceride-altering drugs; HDL cholesterol levels $<$ 1.03 mmol/l in men or $<$ 1.29 mmol/l in women or the use of HDL-altering drugs.[29] No subjects used antidiabetic medication, nor medications that altered the HDL cholesterol or triglycerides levels. The *P* values for differences between the 2 groups were calculated using Pearson chi-squared test, Welch unequal variances *t* test, or Mann-Whitney *U* test as appropriate. Sensitivity analyses were done using an alternative WC criterion with cut-off values of $>$102 cm in men or $>$88 cm in women, which are the cut-offs used by the American Heart Association and the National Heart, Lung, and Blood Institute.[29]

The GFR decline rates (both mGFR and eGFR) were defined as the difference between GFR at baseline and follow-up, divided by observation time in years. Associations between predictors and the decline rates were tested using multivariable adjusted linear regression models. We analyzed the associations among the GFR decline rates and BMI, WC, WHR, MS, and components of MS, in separate models. Nonlinear associations were analyzed using generalized additive models.[41]

Rapid GFR decline (both mGFR and eGFR) was defined as $>$3 ml/min per 1.73 m^2/yr; this cut-off was chosen because it has been used in previous studies.[14,16,42]

We adjusted for the following baseline variables: model 1: age, sex, and height; model 2: model 1 + number of cigarettes smoked daily, systolic and diastolic blood pressure, heart rate, individual dichotomous variables for the use of angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, beta blockers, diuretics, cholesterol-lowering drugs and nonsteroid anti-inflammatory drugs, fasting glucose, triglycerides, HDL and low-density lipoprotein cholesterol; and model 3: model 2 + the ACR. For the MS analyses and analyses of its components, the variables already included in the MS criteria were removed from the analyses.

Statistical analysis was performed with Stata MP 14.2 (Stata Corp., College Station, TX; www.stata.com), except generalized additive models which were analyzed with the gam-procedure in R 3.3 (www.r-project.org).

DISCLOSURE

The RENIS-T6 and RENIS-FU projects were funded by the Northern Norway Regional Health Authority. RENIS-FU was also supported by a grant from Boehringer-Ingelheim. The funding source had no role in the study design or conduct.

All the authors declared no competing interests. The results presented in this paper have not been published in whole or part, except in abstract format at the 2016 ASN Kidney Week in Chicago, IL, USA.

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SUPPLEMENTARY MATERIAL

Figure S1. The glomerular filtration rate (GFR) change plotted against fasting serum triglycerides ($n = 1261$). The blue line is an unadjusted locally weighted scatterplot smoothing fit to the data. The vertical red line indicates a triglyceride value of 1.7 mmol/l, the threshold for the triglycerides criterion of the definition of the metabolic syndrome. The upper panel shows the whole cohort. The lower panel is a magnification of the area for GFR change rates between −1.5 and 0.5 ml/min per year.

Figure S2. Scatter plots with annual change in measured glomerular filtration rate (mGFR) versus annual changes in estimated glomerular filtration rate (eGFR) based on creatinine, cystatin C, or both. The red line indicates perfect correlation. Also, a Venn diagram details the number of subjects with rapid measured, or estimated GFR decline (>3 ml/min per 1.73 m^2/yr), or both, and the overlap between these categorical variables.

Table S1. The metabolic syndrome and glomerular filtration rate (GFR) <60 ml/min per 1.73 m^2 at follow-up.

Table S2. Linear regression model analyses of anthropometric variables, metabolic syndrome (MS), and change in yearly mean estimated glomerular filtration rate (eGFR) decline.

Table S3. Logistic regression analyses of anthropometric variables, metabolic syndrome (MS), and odds ratio of rapid estimated glomerular filtration rate (eGFR) decline (>3 ml/min per 1.73 m^2/yr).

Supplementary material is linked to the online version of the paper at www.kidney-international.org.

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Paper 3

Association of Increasing GFR with Change in Albuminuria in the General Population

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Abstract

Background and objectives Hyperfiltration at the single-nephron level has been proposed as an early stage of kidney dysfunction of different origins. Evidence supporting this hypothesis in humans is lacking, because there is no method of measuring single-nephron GFR in humans. However, increased whole-kidney GFR in the same individual implies an increased single–nephron GFR, because the number of nephrons does not increase with age. We hypothesized that an increase in GFR would be associated with an increased albumin-to-creatinine ratio in a cohort of the general population.

Design, setting, participants, & measurements We measured GFR by iohexol clearance at baseline in 2007–2009 and follow-up after 5.6 years in a representative sample of 1246 persons (aged 50–62 years) who were nondiabetic from the general population of Tromso, northern Norway. Participants were without cardiovascular disease, kidney disease, or diabetes at baseline. We investigated the association between change in GFR and change in albumin-to-creatinine ratio. Increased GFR was defined as a positive change in GFR (change in GFR>0 ml/min) from baseline to follow-up. An albumin-to-creatinine ratio >30 mg/g was classified as albuminuria.

Results Change in GFR was positively associated with a change in albumin-to-creatinine ratio in the entire cohort in the multiple linear regression. The albumin-to-creatinine ratio$_{\text{follow-up}}$-to-albumin-to-creatinine ratio$_{\text{baseline}}$ ratio increased by 8.0% (95% confidence interval, 1.4 to 15.0) per SD increase in change in GFR. When participants with increased GFR (n=343) were compared with those with a reduced GFR (n=903), the ratio increased by 16.3% (95% confidence interval, 1.1 to 33.7). The multivariable adjusted odds ratio for incident albuminuria (n=14) was 4.98 (95% confidence interval, 1.49 to 16.13) for those with an increased GFR (yes/no).

Conclusions Increasing GFR is associated with an increase in albumin-to-creatinine ratio and incident albuminuria in the general nondiabetic population. These findings support single-nephron hyperfiltration as a risk factor for albuminuria in the general population.

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Introduction

The global death rates from CKD increased by 37% from 1990 to 2013 (1). Reduced eGFRs below 60 ml/min per 1.73 m^2 and even small increments in urinary albumin excretion are independent risk factors for cardiovascular disease (CVD) and all-cause mortality (2). Recent cohort studies of the general population have found that elevated and increasing eGFRs also predict CVD and death (3,4). This apparent increased risk associated with a high or increasing eGFR has been explained by confounding because of muscle wasting and thus, lower serum creatinine levels in individuals with chronic illness. However, an abnormally high GFR or glomerular hyperfiltration may be a pathologic state in response to metabolic disturbances and a cause of albuminuria (5,6).

This hypothesis is on the basis of experimental studies in animals that show that hyperfiltration at the single-nephron level is a risk factor for albuminuria and subsequent glomerulosclerosis (5). Because single-nephron GFRs cannot be measured in humans, investigators have used elevated whole–kidney GFR as a proxy for single-nephron hyperfiltration. Whole-kidney GFR increases in a large proportion of patients with diabetes before albuminuria develops (7,8). Recently, we reported that having prediabetes predicted both an increased whole–kidney GFR and an increased albumin-to-creatinine ratio (ACR) at follow-up in a longitudinal study of the general population (9). Several other CKD risk factors, such as hypertension, obesity, and smoking, have been associated with elevated whole–kidney GFR in cross-sectional studies (10–15). However, whether hyperfiltration is a risk factor for albuminuria in the general population remains unclear. The primary reason for this uncertainty may be that assessing hyperfiltration defined as the elevated whole–kidney GFR in a cross-sectional design may not represent hyperfiltration at the single-nephron level, because there is a large variation in nephron number between individuals ranging from 200,000 to 1,800,000 (16). However, an increase in the whole-kidney GFR of the same individual implies an increased

single–nephron GFR as well as hyperfiltration in all or at least a large proportion of the nephrons, because the number of nephrons does not increase with age. Accordingly, we tested the hypothesis that an increase in GFR from baseline to follow-up would be associated with increases in the ACR and albuminuria in a cohort representative of the general population.

Because the eGFRs on the basis of serum creatinine and cystatin C are imprecise and biased in the normal GFR range (15,17–19), we measured GFR using iohexol clearance in the Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6) Study at baseline and after a median of 5.6 years of follow-up (the Renal Iohexol Clearance Survey in Tromsø 6 Follow-Up [RENIS-FU] Study).

Materials and Methods

Study Participants

The RENIS-T6 Study was conducted from 2007 to 2009 as a substudy of the population–based sixth Tromsø Study in the municipality of Tromso, northern Norway (20). The RENIS-T6 Study included a representative sample of 1627 persons aged 50–62 years old from the general white population without self–reported kidney disease, CVD, or diabetes (Figure 1).

In the RENIS-FU Study, we invited all 1627 participants from the RENIS-T6 Study, with the exceptions of seven persons who had a possible adverse reaction to iohexol in the RENIS-T6 Study and 23 persons who had died during the follow-up period (Figure 1). In total, 1324 (83%) participants were examined with an updated GFR measurement between September of 2013 and January of 2015. In this study, we excluded participants who had diabetes (fasting glucose ≥7.0 mmol/L [126 mg/dl], hemoglobin A1c [HbA1c] ≥6.5%, or the use of antidiabetic medication; n=25) or albuminuria (ACR>30 mg/g) at baseline (n=17). Finally, we excluded 36 participants who had diabetes at follow-up (Figure 1).

The Regional Ethics Committee of Northern Norway approved the study, and all participants provided written informed consent.

Data

The RENIS-T6 Study and the RENIS-FU Study were conducted at the Clinical Research Unit at the University Hospital of Northern Norway with a standardized procedure, and the same staff members were responsible for all

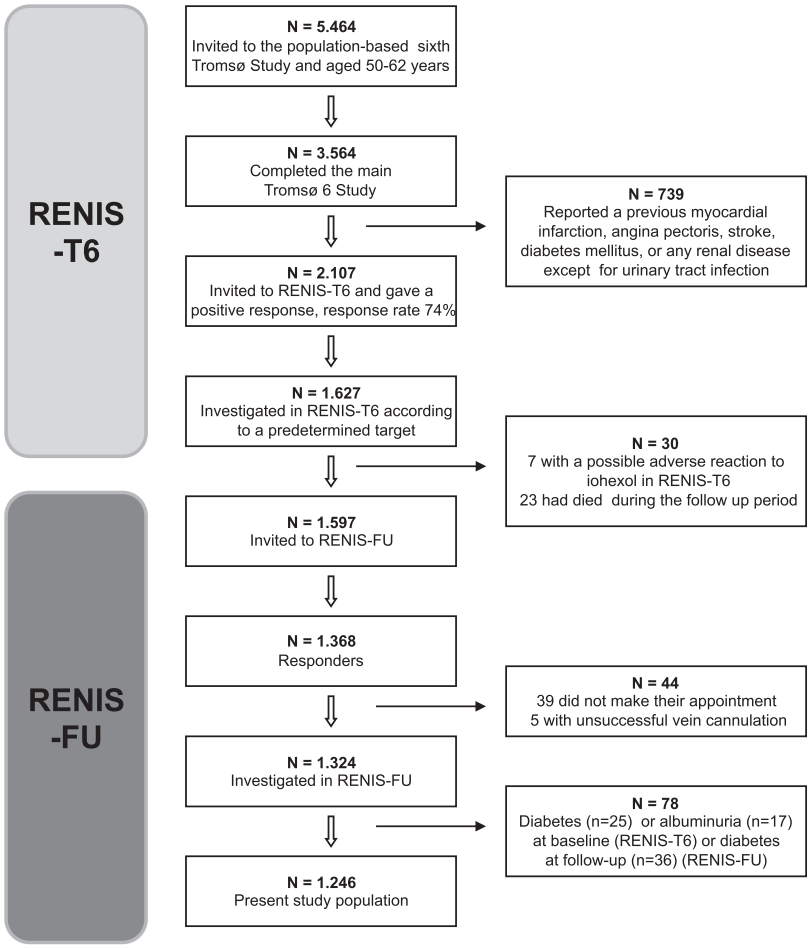

Figure 1. | **Inclusion of participants.** The Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6) Study and the Renal Iohexol Clearance Survey in Tromsø 6 Follow-Up (RENIS-FU) Study.

measurements. The participants met with study staff between 8:00 a.m. and 10:00 a.m. after an overnight fast, including abstinence from tobacco. Participants with intercurrent disease (*e.g.*, respiratory or urinary infection) had their appointments rescheduled.

Both visits included a health questionnaire with questions on alcohol and tobacco use and all current medications. Regular alcohol use was categorized as consuming alcohol more than once a week (yes/no), and current smoking was categorized as daily tobacco use (yes/no).

Measurements

The GFR was measured using single–sample plasma clearance of iohexol as described in detail elsewhere (9,20). The participants were instructed to avoid large meals with meat and nonsteroidal anti–inflammatory drugs 2 days before the investigation. The serum iohexol (300 mg/ml; Omnipaque; Amersham Health, London, United Kingdom) concentration was measured using HPLC as described by Nilsson-Ehle (21). The analytic coefficients of variation during the study period were 3.0% in the RENIS-T6 Study and 3.1% in the RENIS-FU Study. The GFR was calculated as described by Jacobsson (22). In the RENIS-FU Study, we obtained a repeated GFR measurement after 2 weeks and within 2 months from a random sample of 86 participants. The mean coefficient of variation for the intraindividual variation in GFR was 4.2% (95% confidence interval, 3.4% to 4.9%) as recently reported (23).

Three samples of first void morning spot urine were collected on separate days at baseline and follow-up. Urinary albumin excretion and urinary creatinine were measured in unfrozen urine (24). The ACR in milligrams per millimole was calculated for each urine specimen, and the median ACR value was used in the analyses. Albuminuria was defined as an ACR>30 mg/g according to Kidney Disease Improving Global Outcomes 2012 (25).

Serum fasting lipids, fasting glucose, and HbA1c were analyzed as previously reported (26). Ambulatory BP recordings began after the baseline GFR measurement and continued for 24 hours (12).

Statistical Analyses

The study population characteristics are presented as the mean (SD) values, medians (interquartile ranges [IQRs]) in cases of skewed data, or numbers (percentages). Differences in characteristics between baseline and follow-up variables were tested with paired *t* tests for mean values, Wilcoxon signed rank tests for median values, and McNemar tests for paired dichotomous variables. The differences between participants in the follow-up investigation and persons lost to follow-up were tested with two–sample *t* tests, Wilcoxon rank sum tests, two-sample tests of proportions, or Fisher exact test as appropriate.

The 14 missing values in ambulatory BP were replaced by the office BP values.

The change in GFR (ΔGFR) from baseline to follow-up was calculated for each person ($\Delta GFR = GFR_{follow\text{-}up} - GFR_{baseline}$ in milliliters per minute; not indexed by body surface area). We defined an increased GFR within the

Table 1. Population characteristics at baseline and follow-up in the Renal Iohexol Clearance Survey in Tromsø 6 Follow-Up Study

Characteristics	Baseline	Follow-up	*P* Value
N (%)	1246	1246	
Men, *n* (%)	620 (49.8)	620 (49.8)	
Age, yr	57.9 (3.9)	63.5 (4.0)	
Height, cm	170.9 (8.6)	170.7 (8.7)	<0.001
Body weight, kg	79.1 (13.6)	78.9 (13.9)	0.33
Body mass index, kg/m^2	27.0 (3.7)	27.0 (3.9)	0.45
Current smoker, *n* (%)	225 (18.2)	162 (13.1)	0.01
Use of alcohol more than once a week, *n* (%)	358 (28.7)	420 (33.7)	<0.001
LDL cholesterol, mg/dl	141.3 (32.8)	138.6 (34.8)	0.01
HDL cholesterol, mg/dl	59.9 (17.8)	63.3 (18.2)	<0.001
Fasting glucose, mg/dl	95.4 (8.3)	98.0 (8.8)	<0.001
Hemoglobin A1c, %	5.51 (0.33)	5.59 (0.29)	<0.001
Fasting triglycerides, mg/dl	88.5 (61.9–123.9)	88.5 (71.0–115.0)	0.09
Urinary albumin-to-creatinine ratio, mg/g	1.85 (0.89–4.46)	2.96 (0.89–5.02)	<0.001
Conventional systolic BP, mmHg	128.7 (17.3)	130.4 (16.9)	<0.001
Conventional diastolic BP, mmHg	83.2 (9.7)	81.9 (9.2)	<0.001
Ambulatory systolic BP, mmHg	129.5 (13.0)		
Ambulatory diastolic BP, mmHg	82.0 (8.7)		
Antihypertensive medication, *n* (%)	206 (16.6)	373 (29.9)	<0.001
ACE inhibitor	21 (1.7)	40 (3.2)	<0.001
A2 blocker	96 (7.7)	183 (15)	<0.001
Measured GFR,[a] ml/min	103.4 (19.4)	98.1 (19.5)	<0.001
Measured GFR,[a] ml/min per 1.73 m^2	93.6 (14.1)	88.9 (14.2)	<0.001

Estimates are given as the means (SDs), medians (interquartile ranges), or numbers (percentages). Not including the Renal Iohexol Clearance Survey in Tromsø 6 Follow-Up Study participants with diabetes at baseline or follow-up or participants with albuminuria at baseline. ACE, angiotensin-converting enzyme.

[a]GFR was measured by plasma iohexol clearance.

same individual as ΔGFR>0 ml/min. In sensitivity analyses, we defined increased GFR as a ΔGFR greater than the 95th percentile for the intraindividual day to day variation in the GFR measurement. The ACR was log transformed (natural logarithm) because of its skewed distribution, and the change in albumin-to-creatinine ratio (ΔACR) was calculated as log $ACR_{follow\text{-}up}$ − log $ACR_{baseline}$.

We used multiple linear regression to assess the association between ΔGFR and ΔACR and multivariable logistic regression to estimate the odds ratios for incident albuminuria at follow-up. We adjusted for variables that have been associated with both GFR and risk of albuminuria. In model 1, we adjusted for sex and baseline age. Model 2 included the variables in model 1 as well as baseline daytime diastolic ambulatory BP, body mass index, fasting glucose, current smoking, regular use of alcohol, and use of an angiotensin–converting enzyme inhibitor (ACE-i) or angiotensin receptor blocker (ARB). Model 3 included the variables in model 2 and added baseline triglycerides, LDL cholesterol, HDL cholesterol, C-reactive protein, fasting insulin, regular physical exercise (yes/no), and changes in BP, fasting glucose, body weight, smoking habit, and use of antihypertensive medications from baseline to follow-up.

We repeated the logistic regression analyses using exact logistic regression (27). Possible nonlinear associations between ΔGFR and ΔACR were explored using multiple fractional polynomials (28).

Stata software, version 14 (StataCorp., College Station, TX) was used for statistical analysis. Statistical significance was set at $P<0.05$.

Results

Patient Characteristics

In total, 1246 participants who were nondiabetic and without albuminuria at baseline of the RENIS-T6 Study had a follow-up GFR measurement in the RENIS-FU Study after a median (IQR) observation time of 5.6 years (IQR, 5.2–6.0) (Figure 1).

All population characteristics changed from baseline to follow-up, except for body weight and fasting triglycerides (Table 1). The percentage of persons receiving antihypertensive medication increased from 16.6% to 29.9%.

The characteristics of the included participants compared with the 288 lost to follow-up are presented in Supplemental Table 1. The differences were small, except for the percentage of current smokers (18 versus 28; $P<0.01$).

Distribution of ΔACR and ΔGFR

The distributions of ΔACR and ΔGFR are shown in Figure 2. One hundred seventy-six (14.2%) participants had undetectable urinary albumin concentration at both baseline and follow-up, corresponding to the spike at zero in Figure 2. The mean (SD) annual ΔGFR was −0.94 (2.2) ml/min per year. In total, 343 (27.6%) participants (167 women and 176 men) had an increased GFR defined as ΔGFR>0 ml/min from baseline to follow-up.

The Association between ΔGFR and ΔACR

ΔGFR as a continuous variable in the entire study population and ΔGFR dichotomized as an increased GFR (yes/no) were both positively associated with ΔACR in the multiple linear regression (Table 2). There were no significant nonlinear associations between ΔGFR and ΔACR analyzed with multiple fractional polynomials and no statistically significant age or sex interactions. When analyzing ΔACR in relation to the annual rate of GFR change instead of the total change in the study period, the $ACR_{follow\text{-}up}$-to-$ACR_{baseline}$ ratio increased by 8.4% (95% confidence interval, 1.8 to 15.5) per SD change in annual GFR in the fully adjusted model ($P=0.01$). The

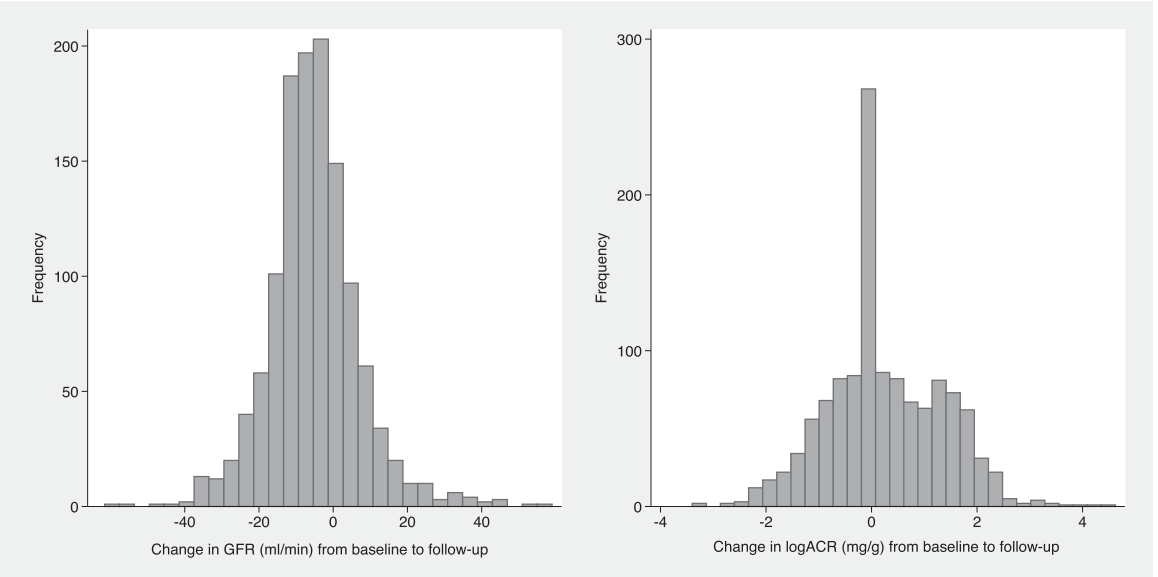

Figure 2. | **Distribution of change in GFR and ACR.** Frequency histogram of change in GFR (ΔGFR) and change in albumin-to-creatinine ratio (ΔACR) from baseline to follow-up. ΔGFR = $GFR_{follow\text{-}up}$ − $GFR_{baseline}$ in milliliters per minute. ΔACR = log $ACR_{follow\text{-}up}$ − log $ACR_{baseline}$.

Table 2. Association of change in albumin-to-creatinine ratio with change in GFR or increased GFR in separate linear regression analyses

Independent Variable	Model 1			Model 2			Model 3		
	ΔeGFR, %[a]	95% CI	*P* Value	ΔeGFR, %[a]	95% CI	*P* Value	ΔeGFR, %[a]	95% CI	*P* Value
ΔGFR, per SD higher	6.8	0.5 to 12.8	0.03	6.9	0.5 to 13.6	0.03	8.0	1.4 to 15.0	0.02
Increased GFR[b], yes/no	14.2	−0.2 to 30.6	0.05	14.6	0.1 to 31.3	0.05	16.3	1.1 to 33.7	0.03

Change in albumin-to-creatinine ratio: log albumin-to-creatinine ratio at follow-up − log albumin-to-creatinine ratio at baseline. Change in GFR (ΔGFR): measured GFR at follow-up − measured GFR at baseline (milliliters per minute). Model 1: adjusted for age and sex. Model 2: the same as model 1 and adjusted for baseline ambulatory daytime diastolic BP, body mass index, fasting glucose, current smoking, regular alcohol consumption, and use of angiotensin–converting enzyme inhibitor or angiotensin receptor blocker. Model 3: the same as model 2 and adjusted for baseline triglycerides, LDL cholesterol, HDL cholesterol, C-reactive protein, physical exercise, fasting insulin, and changes in diastolic BP, fasting glucose, body weight, smoking habits, and antihypertensive medication from baseline to follow-up. ΔeGFR, change in eGFR; 95% CI, 95% confidence interval.

[a]Represents the percentage change in albumin-to-creatinine ratio$_{\text{follow-up}}$-to-albumin-to-creatinine ratio$_{\text{baseline}}$ ratio.

[b]Increased GFR defined as ΔGFR>0 ml/min.

marginal association between ΔACR and annual ΔGFR in this model is shown in Figure 3.

Odds Ratios of Incident Albuminuria

Fourteen persons developed incident albuminuria at follow-up, eight of the 343 persons (2.3%) with an increased GFR (ΔGFR>0 ml/min) developed incident albuminuria at follow-up, and six of the 903 persons (0.7%) with a decreased GFR (ΔGFR≤0 ml/min) developed incident albuminuria at follow-up.

The odds ratios of incident albuminuria are presented in Table 3. Of the independent variables, only ΔGFR, increasing GFR (yes/no), and baseline fasting glucose were associated with new onset albuminuria. Similar results were obtained using exact logistic regression.

The results in Tables 2 and 3 were similar after adjusting for HbA1c instead of fasting glucose and after adjusting for ambulatory systolic instead of diastolic BP.

Sensitivity Analyses

In separate analyses, we excluded individuals with a measured GFR <60 ml/min per 1.73 m^2 (n=27) at baseline to investigate a subgroup without possible acute kidney disease or CKD. We also excluded participants ever on angiotensin blockers (at baseline or follow-up). The association between ΔGFR and albuminuria remained essentially unchanged. All analyses were repeated using a more conservative definition of increased GFR (defined as a >11 ml/min increase, which is above the 95th percentile of the day to day variation in the GFR measurement). Using this definition, the number of persons with an increased GFR was reduced from 343 (27.5%) to 91 (7.3%). The associations with ΔACR and incident albuminuria became stronger, although not significant for ΔACR (Supplemental Table 2). Finally, we obtained similar results when we included the 36 participants with diabetes at follow-up.

Discussion

Hyperfiltration followed by albuminuria has been proposed as a common pathway resulting in CKD (6); however, evidence supporting this hypothesis in persons without diabetes has been lacking. An obstacle to studies on hyperfiltration in humans has been the lack of consensus regarding how to measure hyperfiltration. Most investigators have defined hyperfiltration as an elevated whole–kidney GFR >120–150 ml/min per 1.73 m^2, often without adjusting for age (29). This definition may be poorly correlated with single-nephron hyperfiltration because of the interindividual variation in nephron endowment and because of the fact that the number of functional nephrons decreases with age as a result of age-related glomerulosclerosis (30,31).

We found that an increase in GFR within the same individual was associated with increases in ACR and incident albuminuria in a representative sample of the general middle–aged, nondiabetic population. This finding has important implications, because it supports the hypothesis of hyperfiltration as a common early stage of CKD and because an elevated ACR is a risk factor for CVD and mortality in the general population (2). Although only 14 persons developed incident albuminuria

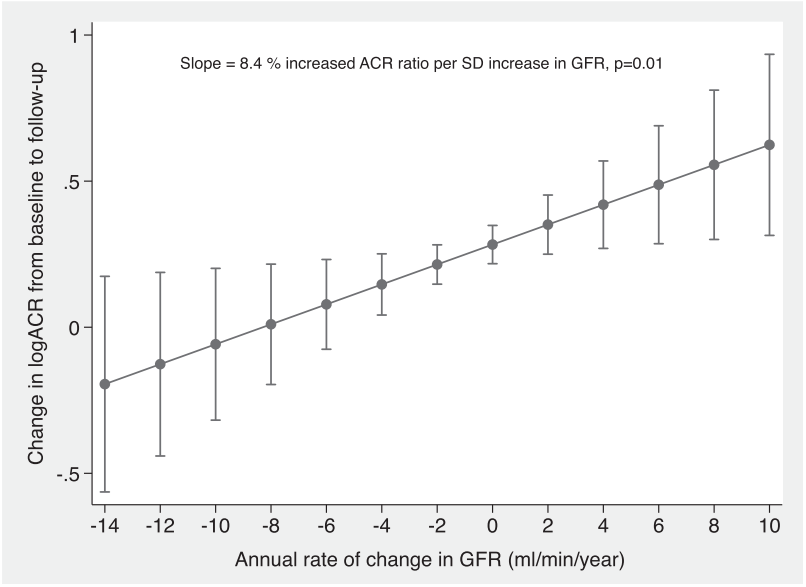

Figure 3. | **Association of annual change rate in GFR with change in albumin-to-creatinine ratio.** The marginal effect of annual GFR change on ΔACR (ΔACR = $\log ACR_{follow\text{-}up} - \log ACR_{baseline}$). Adjusted for sex, baseline age, ambulatory daytime diastolic BP, body mass index, fasting glucose, current smoking, regular alcohol consumption, triglycerides, LDL cholesterol, HDL cholesterol, use of angiotensin–converting enzyme inhibitors or angiotensin receptor blockers, C-reactive protein, fasting insulin, regular physical exercise (yes/no), and changes in BP, fasting glucose, body weight, smoking habit, and use of antihypertensive medication from baseline to follow-up. Vertical lines are 95% confidence intervals. ΔACR, change in albumin-to-creatinine ratio.

in this low-risk population, the association with an increased GFR was strong, with an odds ratio of 4.98 (95% confidence interval, 1.49 to 16.13) for those with an increased GFR in the fully adjusted model ($P<0.01$).

We are not aware of previous longitudinal studies on the risk of albuminuria associated with changes in GFR or hyperfiltration in the general population. However, a cross-sectional study of a general nondiabetic population reported an association between microalbuminuria and hyperfiltration defined as an elevated 24-hour urinary creatinine clearance (CrCl) (32). In a longitudinal study of 534 patients with hypertension, the risk of albuminuria increased across groups defined as persons with stable GFR (CrCl), incident hyperfiltration (CrCl>150 ml/min per 1.73 m^2), persistent hyperfiltration, and regression of hyperfiltration to normofiltration from baseline to follow-up (33). Although partially consistent with our findings, the studies are not comparable because of the different study populations and different methods of measurement of GFR.

Several studies on patients with types 1 and 2 diabetes have investigated the role of hyperfiltration in predicting albuminuria using different cutoff levels for hyperfiltration with or without adjustment for age and sex (8). The results have been inconsistent (8). Notably, the majority of the studies that measured GFR but not those that estimated GFR by creatinine or cystatin C observed an increased risk of developing albuminuria in persons with hyperfiltration (8,34). Recently, Ruggenenti *et al.* (35) reported that persistent hyperfiltration defined as a GFR>120 ml/min per 1.73 m^2 measured by plasma iohexol clearance at baseline and after 6 months but not hyperfiltration at baseline only predicted albuminuria after 4 years of follow-up in patients with type 2 diabetes.

Several other risk factors for CKD, such as obesity, prediabetes, metabolic syndrome, hypertension, and smoking, have been associated with hyperfiltration defined as having an elevated whole–kidney GFR (9,12–15,36–38). These conditions may cause albuminuria in part through mechanisms other than hyperfiltration, thus introducing a spurious association between hyperfiltration and albuminuria. However, our results remained similar after adjusting for these possible confounders, including the use of antihypertensive medication at baseline and follow-up.

Our findings should be interpreted with caution. Rather than being a causal factor, an increasing GFR could be a risk marker of an unmeasured pathologic process that leads to albuminuria, such as endothelial dysfunction or oxidative stress. In addition, hemodynamic effects without any long–term effects on ACR may have caused the observed association between ΔACR and ΔGFR. However, animal models have shown that single-nephron hyperfiltration and the coexisting glomerular hypertrophy induce shear stress and a loss of podocytes, which subsequently lead to albuminuria and glomerulosclerosis (39,40).

In humans, the number of functional glomeruli decreases with normal aging but is not closely correlated with the age-related decline in kidney volume, most likely because of the compensatory hyperfiltration and hypertrophy of the remaining nephrons (41). Indeed, we observed that the GFR increased along with an increasing ACR in a considerable proportion of healthy persons during the 5.6 years of follow-up. Moreover, not only an increased GFR but also increased glomerular volume have been associated with albuminuria in healthy adults, possibly caused by a loss of podocytes (42,43).

Table 3. Odds ratios for incident albuminuria analyzed with multiple logistic regression

Independent Variable	Model 1			Model 2			Model 3		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Increasing GFR,[a] yes/no	3.55	1.21 to 10.35	0.02	4.22	1.41 to 12.67	<0.01	4.98	1.49 to 16.13	0.001
ΔGFR, per SD	1.79	1.21 to 2.67	0.004	1.94	1.23 to 2.79	0.003	2.13	1.38 to 3.32	0.001
Age, per yr	0.96	0.85 to 1.12	0.55	0.98	0.84 to 1.13	0.74	0.97	0.83 to 1.13	0.68
Men	0.87	0.28 to 2.57	0.81	0.51	0.15 to 1.79	0.29	0.41	0.10 to 1.61	0.20
Baseline ambulatory diastolic BP, per SD				1.28	0.72 to 2.27	0.38	1.10	0.57 to 1.95	0.85
Baseline BMI, per SD				1.27	0.75 to 2.16	0.91	1.47	0.80 to 2.70	0.20
Baseline fasting glucose, per SD				2.05	1.17 to 3.60	0.01	2.52	1.31 to 4.84	<0.01
Smoking at baseline, yes/no				2.81	0.81 to 9.72	0.10	2.49	0.52 to 11.89	0.25
Baseline regular alcohol use, yes/no				1.50	0.47 to 4.74	0.50	1.11	0.30 to 4.08	0.90
Baseline triglycerides, per SD							1.00	0.60 to 1.70	>0.99
Baseline LDL cholesterol, per SD							0.94	0.50 to 1.70	0.80
Baseline HDL cholesterol, per SD							0.94	0.36 to 1.90	0.90

Incident albuminuria defined as albumin-to-creatinine ratio >30 mg/g. Model 1: adjusted for age and sex. Model 2: independent variables adjusted for each other and the use of angiotensin–converting enzyme inhibitor or angiotensin receptor blocker at baseline. Model 3: the same as model 2 and adjusted for baseline triglycerides, LDL cholesterol, HDL cholesterol, C-reactive protein, physical exercise, fasting insulin, and changes in diastolic BP, fasting glucose, body weight, smoking status, and use of antihypertensive medication from baseline to follow-up. OR, odds ratio; 95% CI, 95% confidence interval; ΔGFR, measured GFR at follow-up − measured GFR at baseline (milliliters per minute); BMI, body mass index.
[a]Increased GFR from baseline to follow-up (ΔGFR>0 ml/min). Adjusted for the variables below but separately from ΔGFR per SD.

Our results and these morphologic data indicate that an increased GFR in aging individuals represents hyperfiltration, which may be maladaptive over time. Individuals with a reduced nephron number because of reduced nephron endowment or nephron loss by glomerular injury may be more vulnerable to this process, because these individuals exhibit a higher single-nephron GFR, a higher glomerular volume, and a greater risk of kidney failure (44). There is also evidence indicating that treatment that causes an initial drop in GFR, such as ACE-is for hypertension, sodium-glucose cotransport inhibitors in diabetes, and bariatric surgery in obesity (45–47), mediates a long–term renoprotective effect (35,47–49).

Recent population studies have reported an independent association between a longitudinal increase in eGFR and risk of CVD and death (3,4). Whether this is a true association or caused by confounding from lower serum creatinine levels in persons with chronic illness is unknown. However, in a previous study from the RENIS-T6 Study cohort, we reported a cross–sectional independent association between a high GFR (by iohexol clearance) and carotid atherosclerosis and left ventricular hypertrophy (50). The association between increased GFR and incident albuminuria in this study suggests that hyperfiltration may be a marker of increased cardiovascular risk.

This study has limitations. Only middle–aged white individuals participated, which limits the generalizability to other groups. Although we used a longitudinal study design, the analyses were partly cross-sectional, and we cannot exclude reverse causality between changes in GFR and albuminuria. There were few cases of incident albuminuria in this study of relatively healthy individuals. An elevated ACR below this cutoff is a risk factor for CVD and mortality in both high– and low–risk groups, but its role in predicting CKD in the general nondiabetic population is unclear (2). We did not have information regarding possible confounders, like vitamin D levels, changes in protein intake, and the dosage of ACE-i or ARB. However, participants met in the morning after an overnight fast at baseline and follow-up, and we obtained similar results after excluding those ever on an ACE-i or ARB.

The major strength of this study is the GFR measurements. The RENIS-T6 Study is the only longitudinal study with repeated measurements of GFR in a representative sample of the general population. Moreover, the intra-individual variation in the GFR measurement was lower than in most previous studies (51). We investigated the role of increased GFR within the same individuals, which is likely to represent hyperfiltration at the single-nephron level. Urine was collected on 3 separate days at both baseline and follow-up, albumin and creatinine were assessed in unfrozen specimens, and we adjusted for several variables, such as ambulatory BP and changes in antihypertensive medication during follow-up.

An increase in GFR was associated with increasing albuminuria in the general nondiabetic population. These findings support the idea that single-nephron hyperfiltration is a common risk factor for albuminuria, a well known CVD and CKD risk factor. Whether hyperfiltration is a risk factor for subsequent GFR decline, CVD, and mortality should be investigated.

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Disclosures

None.

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