Clinical aspects of the relation between diabetes mellitus and kidney disease: From hyperfiltration to dialysis

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CHAPTER 2

The association of glucose metabolism and kidney function in middle-aged adults

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Abstract

Background

Previous clinical studies showed that various measures of glucose metabolism are associated with risk of chronic kidney disease in different populations, but results were not consistent in all aspects. In the present study we assessed measures of glucose metabolism and their association with kidney function in a population-based study. Furthermore, we studied glomerular hyperfiltration and micro-albuminuria because these are considered the earliest appearances of impaired kidney function in diabetes mellitus (DM).

Methods

The Netherlands Epidemiology of Obesity (NEO) study is a population-based cohort study of middle-aged men and women. We categorized the study population according to glycaemic levels into normoglycemia (reference group), pre-DM, known DM, and newly diagnosed DM. Outcome variables were serum creatinine, estimated glomerular filtration rate (eGFR), glomerular hyperfiltration and micro-albuminuria. We examined the association between measures of glucose metabolism (fasting glucose, HbA1c, fasting insulin, glucose area under the curve (AUC), insulin AUC, HOMA-IR, HOMA-B, Disposition index) and measures of kidney function. We performed linear regression for continuous outcomes and logistic regression analyses for binary outcomes (hyperfiltration or micro-albuminuria) adjusted for age, sex, BMI, blood pressure, smoking, antihypertensive agents and cardiovascular disease.

Results

Of the total population (N= 6338), 55 % participants were classified as normoglycemic (reference), 35 % as pre-diabetes, 7 % as diabetes mellitus, and 4 % as newly diagnosed diabetes mellitus. Clinically relevant chronic kidney disease (CKD-EPI <= 60 ml/min/ 1.73 m^2) was more frequent in pre-DM (3.5%), diagnosed DM (6.2%), and newly diagnosed DM (7.0%) compared to the reference group (1.2%). Presence of micro-albuminuria was more frequent in pre-DM (3.3%), diagnosed DM (6.3%), newly diagnosed DM (8.6%) than in normoglycemia (1.6 %). Compared to the reference group, diagnosed and newly diagnosed DM was associated with a slightly higher eGFR (+ 2.1 ml/min/ 1.73 m^2 (95% CI -0.2, 4.4) and + 2.7 ml/min/ 1.73 m^2 (95% CI -0.3, 5.7)) respectively. A 1% higher HbA1c was associated with an increased odds of

hyperfiltration: odds ratio 1.41 (95 % CI 1.06, 1.88). Higher levels of fasting plasma glucose, AUC glucose, and HOMA-B, were associated hyperfiltration. Fasting insulin, AUC insulin and HOMA-IR were not associated with hyperfiltration. Per mmol/l higher fasting glucose concentrations the odds ratio of micro-albuminuria was 1.21 (95 % CI 1.04, 1.42).

Conclusion

Both fasting and post-prandial glucose and HOMA-B, but not insulin resistance, were associated with glomerular hyperfiltration, while fasting glucose was also associated with micro-albuminuria. This implies that hyperinsulinemia as a measure of insulin resistance, is not associated with a first increase in eGFR (hyperfiltration) but is associated with a decline in eGFR.

Introduction

The global increase in obesity is a leading cause of the increased prevalence of pre-diabetes and type 2 diabetes mellitus (DM). 40% of patients with obesity has accompanying pre-diabetes[1], defined as a fasting glucose level of 100 to 125 mg/dL (5.6-6.9 mmol/L), or 2 hour plasma glucose of 140–199 mg/dL (7.8–11.0 mmol/L) after 75 mg oral glucose tolerance test, or HbA1c level of 5.7% to 6.4% (39-47 mmol/mol), according to the American Diabetes Association criteria[2]. Pre- diabetes is a clinically relevant condition as about 45-50 % of patients will develop type 2 DM within 10 years[3,4], and pre-diabetes is associated with a higher risk of macrovascular and microvascular complications, such as nephropathy[5-8]. Importantly, the risk of chronic kidney disease (CKD) is directly related to glucose levels: in US patients with normoglycemia the prevalence of CKD is 3 %, in patients with pre-diabetes 9 % and in patients with (un)diagnosed DM 14 %[9].

Postulated mechanisms by which pre-diabetes results in chronic kidney disease are an increase in glomerular hyperfiltration, vascular permeability and/or endothelial dysfunction and inflammation[10]. Previous studies showed that different measures of glucose metabolism (fasting glucose, HbA1c, fasting insulin, area under blood concentration curve (AUC), insulin AUC, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), HOMA of β-cell function (HOMA-B) and Disposition index) are associated with risk of chronic kidney disease. However, these studies were not in all aspects consistent. Some studies showed that elevated post-prandial measures of glucose metabolism might contribute more to chronic kidney disease than elevated fasting measures of glucose metabolism[11-16], while other studies showed the opposite[17-19]. However, none of the studies assessed so many different measures of glucose metabolism and their association with kidney function in a single large cohort. Moreover, these studies did not assess glomerular hyperfiltration as well as micro-albuminuria although this is considered the earliest appearances of impaired kidney function in DM and has been linked with an increased risk of diabetic nephropathy[17,20]. To date, it is unknown which measures of glucose metabolism are associated with both glomerular hyperfiltration as well as micro-albuminuria.

In the present study we examined the association between normoglycemia, pre-DM, DM, and newly diagnosed DM with chronic kidney disease, and markers thereof, among a Dutch cohort of middle-aged adults. Furthermore, we examined the

association between measures of glucose metabolism and earliest appearances of impaired kidney function, micro-albuminuria and glomerular hyperfiltration.

Methods

2.1 Study design and study population

The NEO study is a population-based, prospective cohort study designed to investigate pathways that lead to obesity-related diseases. The NEO study includes 6671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. The study design and population is described in detail elsewhere[21]. The present study is a cross-sectional analysis of the baseline measurements of the NEO study .

In short, men and women living in the greater area of Leiden (in the West of the Netherlands) were invited by letters sent by general practitioners and municipalities and by local advertisements. They were invited to respond if they were aged between 45 and 65 years and had a self-reported BMI of 27 kg/m^2 or higher. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing a for a reference distribution of BMI in the general population.

After consecutive exclusion of participants with missing data on diabetes medication (N=64), fasting or post-prandial glucose (N= 245), fasting or post-prandial insulin (N=13), and HbA1c concentrations (N=11), 6338 participants were included in the present analyses.

The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study. All participants gave their written informed consent.

2.2 Data collection

Participants were invited to a baseline visit at NEO study centre of the LUMC after an overnight fast. Prior to this study visit, participants collected urine (morning spot) and completed a general questionnaire at home to report demographic, lifestyle and clinical information. On the questionnaire, participants reported ethnicity by self-identification in eight categories which we grouped into Caucasian (reference) and

other. Tobacco smoking was reported in the three categories currently, formerly, and never smoking (reference). Highest level of education was reported in 10 categories according to the Dutch education system and grouped into high (including higher vocational school, university, and post-graduate education) versus low education (reference). Participants reported their medical history of diabetes and cardiovascular diseases. Pre-existing cardiovascular disease was defined as myocardial infarction, angina, congestive heart failure, stroke, or peripheral vascular disease. In addition, all use of medication in the month preceding the study visit was recorded. Family history of diabetes was reported as having any parent or sibling with diabetes or without DM (reference). Body weight was measured without shoes and one kilogram was subtracted from the body weight. BMI was calculated by dividing the weight in kilograms by the height in meters squared. Brachial blood pressure was measured in a seated position on the right arm using a validated automatic oscillometric device (OMRON, Model M10-IT, Omron Health Care Inc, IL, USA). Blood pressure was measured three times with 5 min rest between consecutive measurements. The mean systolic, diastolic and mean arterial ([(2 _ diastolic blood pressure) + systolic blood pressure]/3) blood pressure were calculated.

2.3. Fasting glucose, insulin measurements and mixed meal test

Fasting blood samples were drawn from the antecubal vein after the participant had rested for 5 min. Within five minutes after the first blood sample, participants drank a liquid mixed meal. This meal (total 400 mL) contained 600 kCal, with 16 percent of energy (En%) derived from protein, 50 En% carbohydrates, and 34 En% fat. Subsequent blood samples were drawn 30 and 150 min after ingestion of the mixed meal. Fasting and post-prandial glucose and insulin concentrations were measured and from these Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), HOMA of β-cell function (HOMA-B) and Disposition index were calculated. In Supplementary Information S1, a detailed overview of laboratory methods and calculations is given.

We categorized the study population in four different groups, according to glycemic levels at baseline.

1. Normoglycemia (reference), defined as a fasting glucose level of < 5.6 mmol/l and a HbA1c level of < 5.7% (39 mmol/mol)

2. Pre-diabetes (pre-DM), defined as a fasting glucose level of 100 to 125 mg/dL (5.6-6.9 mmol/L) or HbA1c level of 5.7% to 6.4% (39-47 mmol/mol)

3. Diagnosed diabetes mellitus, defined as self- reported diabetes mellitus or use of glucose lowering medication.

4. Newly diagnosed diabetes mellitus, defined as no self-reported diabetes mellitus, no use of glucose lowering medication, but diabetes mellitus according to the ADA criteria with a fasting glucose level ≥ 126 mg/dL (≥7.0 mmol/L) or plasma glucose level ≥ 200 mg/dL (≥ 11.1 mmol/l) after mixed meal test or HbA1c level ≥ 6.5% (48 mmol/mol)[2].

2.4 Renal function and albuminuria

Serum creatinine (mg/dL) was used to calculate estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula[22].

We defined glomerular hyperfiltration as an eGFR >90th percentile[17]. Urinary albumin was measured in an early-morning single urine void using an immunoturbidimetric assay and creatinine using a Jaffe kinetic compensated method between 1 September 2008 and 30 November 2010 and an enzymatic assay (IDMS calibrated against SRM 967) from 1 December 2010 until the end of the inclusion period. Because urinary creatinine concentrations are not affected by pseudochromogens they are exchangeable using either a Jaffe or an enzymatic method[23]. Micro-albuminuria was defined as urinary albumin/creatinine ratio of >= 2.5 mg/mmol in men and of >= 3.5 mg/mmol in women[22].

2.5 Statistical analyses

In the NEO study, individuals with a BMI > 27 kg/m^2 are overrepresented due to the sampling frame applied. To correctly represent baseline associations in the general population[24], adjustments for the oversampling of individuals with a BMI ≥ 27 kg/m^2 were made by weighting all participants towards the BMI distribution of participants from the Leiderdorp municipality[25], whose BMI distribution was similar to the BMI distribution of the general Dutch population[26]. All results were based on weighted analyses. As a consequence, all results apply to a population-based study without

oversampling of individuals with a BMI ≥ 27 kg/m^2. Because of the weighted analyses, percentages and proportions are given instead of absolute numbers of participants. Baseline characteristics are therefore expressed as mean (SD), or percentage, stratified by groups of glucose metabolism.

Our first study aim was to examine the association between categorized glucose levels (normoglycemia, pre-DM, DM and newly diagnosed DM) and chronic kidney disease, and markers thereof. Outcome variables studied were serum creatinine, eGFR (CKD-EPI), glomerular hyperfiltration and urinary albumin-creatinine ratio (UACR). A linear regression analysis was performed to calculate age- and sex-adjusted mean differences with 95% confidence intervals (95% CIs) in levels of serum creatinine, eGFR and albuminuria for the defined categories of glucose metabolism. The second study aim was to examine the associations between different measurements of glucose metabolism, (fasting glucose, HbA1c, fasting insulin, glucose AUC, insulin AUC, HOMA-IR, HOMA-B, Disposition index) and measures of kidney function. For this analysis we excluded 444 participants because of protocol violation during mixed meal test. We performed linear regression for continuous outcomes and logistic regression analyses for binary outcomes (hyperfiltration or micro-albuminuria). Participants who used oral glucose lowering medication and/or insulin (N=335) were excluded from the analyses where measures of glucose metabolism were used as independent variable. Analyses were adjusted for potential confounding due to age, sex, BMI, blood pressure, and smoking. In a sensitivity analysis we additionally adjusted for antihypertensive agents**,** heart failure, cerebrovascular accident and myocardial infarction, though for these variables, it may difficult to judge whether they act as a confounding factor or whether they are mediators for the glucose kidney function association. Analyses were performed using STATA (Statacorp, College Station, Texas, USA, version 14.0)

Results

3.1 Characteristics according to different glycemic categories

Of the total participants (N= 6338), 54.6 % participants were classified as normoglycemic (reference), 34.8 % as pre-diabetes, 6.9 % as diabetes mellitus and 3.8 % as newly diagnosed diabetes mellitus, shown in table 1. From the patients with known diabetes mellitus (DM), 58.9 % used oral glucose lowering medication, 4.6 % used insulin therapy,13.6 % patients used a combination of insulin and oral glucose lowering medication and 23.0 % used no glucose lowering medication. Mean age was highest in participants with diagnosed DM (59 years). BMI was lowest in participants with normoglycemia (BMI 25.4 kg/m^2) and highest in participants with diagnosed DM (30.8 kg/m^2). Presence of clinically relevant CKD, defined as eGFR <= 60 ml/min/ 1.73 m^2, was higher in participants with pre-DM (3.5 %), DM (6.2 %) and newly diagnosed DM (7.0 %) than in the reference group (1.2 %). Also, presence of micro-albuminuria was higher in participants with pre-DM (3.3 %), DM (6.3 %), newly diagnosed DM (8.6 %) than in the reference group (1.6 %). More participants with pre-DM used angiotensin converting enzyme inhibitors or angiotensin receptor blockers and statins compared with the participants with normoglycemia. Fasting and post-prandial measures of glucose metabolism according to glycemic catagories are provided in table 2.

Table 1. Characteristics of the study population of the Netherlands Epidemiology of Obesity study*.

	Normoglycemia (reference)	Pre-DM	DM	Newly diagnosed DM
Proportion of participants (%)	54.6	34.8	6.9	3.8
Age (y)	54.9 (5.4)	57.4 (6.4)	58.5 (7.3)	57.6 (6.8)
Sex (% men)	38	57	53	64
BMI (kg/m2)	25.4 (3.5)	27.8 (5.2)	30.8 (8.0)	29.7 (7.0)
Blood pressure (mmHg)				
systolic	128.1 (15.0)	134.2(19.3)	135.7 (23.5)	137.2 (21.1)
diastolic	82.3 (9.1)	84.7 (11.9)	84.8 (13.4)	88.1 (12.8)
Tobacco smoking (% never)	41	35	25	29
Ethnicity (% Caucasian)	95	95	90	94
Waist circumference (cm)	89.0 (11.0)	97.5 (14.3)	106.2 (19.1)	103.3 (17.1)
Hip circumference (cm)	102.3 (7.5)	106.0 (10.5)	109.8 (17.1)	108.7 (13.6)
Use of ACEi/ARB (% yes)	10	18	52	23
Statin use (% yes)	6	13	71	19
Family history of diabetes (% yes)	25	34	57	41
Comorbidity				
Heart failure (%)	0.4	0.4	1.3	0.8
Cerebrovascular disease (%)	1.4	2.4	7.8	1.0
Cardiovascular disease* (%)	1.2	2.0	6.7	4.0

	Normoglycemia (reference)	Pre-DM	DM	Newly diagnosed DM
Biomarkers				
Serum creatinine (umol/ll)	75.6 (12.0)	79.3 (18.1)	75.7 (25.1)	78.3 (19.6)
CKD-EPI (ml/min/1.73 m2)	86.6 (10.7)	85.0 (14.7)	86.9 (20.7)	86.7 (18.7)
Urine (morning spot)				
albumin/ creat ratio (mg/mmol)	0.7 (2.5)	1.1 (6.8)	2.6 (21.5)	2.0 (17.4)
micro- albuminuria (% yes)	1.6	3.3	6.3	8.6
Total cholesterol (mmol/L)	5.7 (0.9)	5.8(1.2)	4.7 (1.4)	5.7 (1.6)
HDL (mmol/L)	1.6 (0.4)	1.5 (0.5)	1.3 (0.5)	1.3 (0.5)
LDL (mmol/L)	3.5 (0.8)	3.7 (1.1)	2.7 (1.2)	3.5 (1.4)
Triglycerides (mmol/L)	1.1 (0.7)	1.5 (1.1)	1.6 (1.4)	1.8 (1.4)
ALT (U/l)	24 (11)	28 (15)	31 (22)	34 (23)
AST (U/l)	24 (8)	25 (9)	26 (14)	27 (15)
Hb (mmol/l)	8.6 (0.9)	8.9 (1.0)	8.7 (1.0)	9.3(0.8)
CRP (mg/L)	1.9 (2.4)	2.4 (3.6)	3.0 (5.1)	3.6 (4.7)

* Results were weighted toward the BMI distribution of the general population (n= 6338). Data are expressed as %, mean (standard deviation).

DM; diabetes mellitus, ACEi; angiotensin converting enzyme inhibitor, ARB; angiotensin receptor blocker, HDL; high density lipoprotein, LDL; low density lipoprotein. Cardiovascular disease was defined as myocardial infarction.

Table 2. Glucose metabolism in participants with normoglycemia, pre-DM, diagnosed DM and newly diagnosed DM*.

	Normoglycemia DM	Pre-DM	Diagnosed DM	Newly diagnosed
Fasting glucose (mmol/l)	5.1 (0.3)	6.0 (0.4)	8.0 (3.0)	7.9 (2.7)
HbA1c (%)	5.2 (0.2)	5.4 (0.3)	6.7 (1.6)	6.2 (1.5)
Fasting insulin (mU/l)	8.0 (4.9)	12.0 (8.4)	17.7 (28.7)	18.5 (23.6)
Glucose *AUC*	5.5 (0.8)	6.8 (1.1)	10.6 (4.5)	9.8 (3.9)
Insulin *AUC*	44.0 (23.5)	57.9 (38.0)	55.6 (49.3)	72.7 (58.4)
HOMA-IR	1.8 (1.1)	3.2 (2.3)	6.6 (13.7)	6.4 (8.4)
HOMA-B	104.0 (62.2)	98.3 (66.9)	91.0 (124.8)	92.8 (111.9)
Disposition index	51.8 (38.4)	30.6 (11.7)	14.1 (11.8)	16.6 (12.9

* Results were weighted toward the BMI distribution of the general population (N= 5894) 444 participants were excluded because of protocol violation during mixed meal test. Data are shown as mean (standard deviation).

3.2 Associations between different glycemic catagories and measures of kidney function

Table 3 shows the adjusted mean difference in eGFR between the four glycaemic categories. eGFR was similar in participants with normoglycemia and pre-DM. Diagnosed and newly diagnosed DM was associated with a higher eGFR, respectively + 2.1 ml/min/ 1.73 m^2 (95% CI -0.2, 4.4) and + 2.7 ml/min/ 1.73 m^2 (95% CI -0.3, 5.7). Pre-DM and (newly) diagnosed DM were associated with increased micro-albuminuria (table 4). The odds ratio for micro-albuminuria was 1.6 (95% CI 0.9, 2.7) in participants with pre-DM and 2.8 (95% CI 1.5, 5.4) in participants with newly diagnosed DM compared with normoglycemia.

Table 3. Mean difference CKD-EPI in patients with pre-DM, diagnosed DM, newly diagnosed DM, compared with normoglycemia*.

	Normoglycemia (reference)	Pre-diabetes	Diagnosed DM	Newly diagnosed DM
Model a	86.3	0.0 (-0.9, 1.0)	2.1 (0.0, 4.2)	2.6 (-0.3, 5.5)
Model b	86.3	0.1 (-0.9, 1.1)	2.2 (-0.1, 4.4)	2.7 (-0.2, 5.7)
Model c	86.3	-0.1 (-1.1, 0.9)	2.2 (-0.0, 4.5)	2.7 (-0.3, 5.6)
Model d	86.3	-0.1 (-1.1, 0.9)	2.1 (-0.2, 4.4)	2.7 (-0.3, 5.7)

* Results were weighted toward the BMI distribution of the general population (N= 5879). 444 participants were excluded because of protocol violation during mixed meal test.

- Model a adjusted for age and sex
- Model b additionally adjusted for BMI
- Model c additionally adjusted blood pressure, smoking, antihypertensive agents
- Model d additionally adjusted heart failure, cerebrovascular accident and myocardial infarction

Table 4. Odds ratio's of micro- albuminuria in patients with pre-DM, diagnosed DM, newly diagnosed DM compared to normoglycemia*.

	Normoglycemia (reference)	Pre-diabetes	Diagnosed DM	Newly diagnosed DM
Model a	1.0	2.2 (1.3, 3.6)	3.8 (2.2, 6.8)	5.3 (2.8, 10.3)
Model b	1.0	1.7 (1.0, 2.8)	2.1 (1.3, 3.7)	3.4 (1.8, 6.5)
Model c	1.0	1.6 (0.9, 2.6)	1.6 (1.0, 2.8))	2.8 (1.5, 5.4)
Model d	1.0	1.5 (0.9, 2.6))	1.6 (0.9, 2.7)	2.8 (1.5, 5.4)

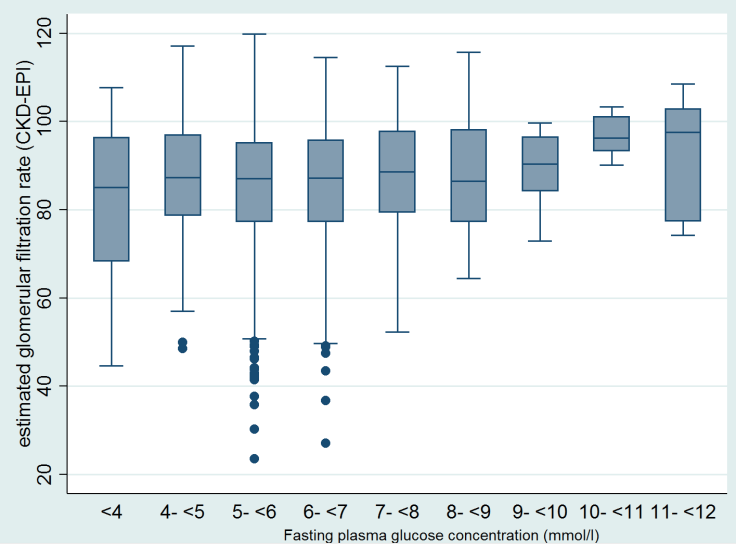
* Results were weighted toward the BMI distribution of the general population (N=5870). 444 participants were excluded because of protocol violation during mixed meal test.

- Model a adjusted for age and sex
- Model b additionally adjusted for BMI
- Model c additionally adjusted blood pressure, smoking, antihypertensive agents
- Model d additionally adjusted heart failure, cerebrovascular accident and myocardial infarction

3.3 Associations of different measures of glucose metabolism, hyperfiltration and micro-albuminuria in the total study population.

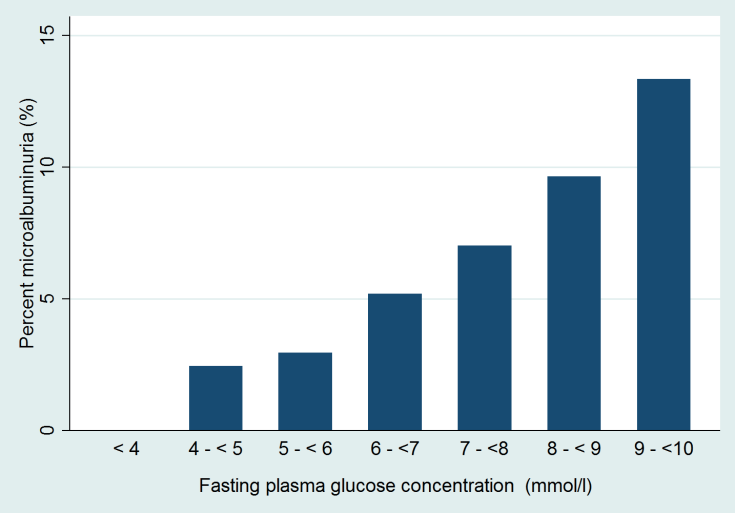
Higher fasting glucose concentrations were associated with higher eGFR (figure 1A). Micro-albuminuria was associated with higher fasting glucose levels: micro-albuminuria was not seen in fasting glucose levels < 4 mmol/L, while in participants with fasting glucose levels between 9 and below 10 mmol/L the percentage was almost 14 % (figure 1B).

Figure 1A. Association between fasting plasma glucose and eGFR.



Results were weighted toward the BMI distribution of the general population (N=6003). Patients using glucose lowering drugs were excluded.

Figure 1B. Association between fasting plasma glucose and micro-albuminuria.



* Results were weighted toward the BMI distribution of the general population (N=6003). Patients using glucose lowering drugs were excluded. Micro-albuminuria was defined as urinary albumin/creatinine ratio of >= 2.5 mg/mmol in men and of >= 3.5 mg/mmol in women.

We examined the associations between different measures of glucose metabolism, hyperfiltration and micro-albuminuria (see table 5). A 1% higher HbA1c level was associated with an odds ratio for hyperfiltration of 1.41 (95 % CI 1.06, 1.88). Also, higher levels of fasting plasma glucose, AUC glucose, and HOMA-B were associated with increased odds ratio's of hyperfiltration (table 5). Hyperinsulinemia, as a measure of insulin resistance, was not associated with hyperfiltration. Data were similar if we included participants who used oral glucose lowering medication and/or insulin (data not shown). Both fasting plasma glucose and HbA1c were associated with micro-albuminuria (table 5).

Table 5. Association of different measures of glucose metabolism with hyperfiltration and micro-albuminuria.

Measures of glucose metabolism	Odds ratio (95 % CI) for hyperfiltration	Odds ratio (95 % CI) for micro-albuminuria
Fasting glucose		
- per mmol/L (18 mg/dL)	1.27 (1.11, 1.46)	1.21 (1.04, 1.42)
HbA1c		
- per % unit	1.41 (1.05, 1.87)	1.36 (1.00, 1.86)
Fasting insulin		
- per mU/l	0.99 (0.97, 1.01)	1.01 (1.00, 1.03)
AUC glucose		
- per unit	1.22 (1.11, 1.34)	1.11 (0.97, 1.26)
AUC insulin		
- per unit	1.00 (1.00, 1.01)	1.00 (1.00, 1.01)
HOMA-IR		
- per unit	1.00 (0.94, 1.05)	1.05 (0.97, 1.14)
HOMA-1B		
- per unit	1.00 (0.99, 1.00)	1.00 (1.00, 1.00)
IDI-isi		
- per unit	1.00 (1.00, 1.01)	1.00 (0.99, 1.01)

* Results were weighted toward the BMI distribution of the general population (N=5572). Patients using glucose lowering drugs were excluded. Logistic regression analysis with hyperfiltration and micro-albuminuria as the dependent variable. All models were adjusted for age, sex, weight, diastolic and systolic BP smoking, and the use of ACE inhibitors or ARB.

Discussion

In this large cross-sectional study of middle-aged men and women we observed a relatively low proportion of patients with chronic kidney disease. Furthermore we found that both fasting and post-prandial glucose and HOMA-B, but not insulin resistance, were associated with glomerular hyperfiltration, while fasting glucose was also associated with micro-albuminuria.

As expected, our study showed that patients with chronic kidney disease had higher glucose levels compared to patients without CKD. Also, we demonstrated that the proportion of patients with kidney disease in this Dutch study is much lower than in previous studies[9,13]. This relatively low proportion of patients with chronic kidney disease might be explained by a relatively good metabolic control in our study as

illustrated by a good glycemic, blood pressure and lipid control. Furthermore, it might also be related to different study populations. First, in a US study[9], many patients had no insurance (8.9 %-19.4 %) while in the Netherlands all patients have basic insurance and patients with an insurance have better health care outcomes[27]. Second, mean BMI is higher in the United States than in the Netherlands and BMI is a strong risk factor of chronic kidney disease[28]. Third, almost all our participants had a non-hispanic caucasian ethnicity, which might contribute to the lower prevalence of CKD, as the prevalence of CKD in a non-caucasian population is higher[29].

Previous cohort studies showed conflicting results whether pre-DM is associated with chronic kidney disease. Some studies showed a positive association between pre-diabetes and chronic kidney disease, as in a higher prevalence of glomerular hyperfiltration and albuminuria compared to participants without DM [11,30,31]. Other longitudinal studies showed that pre-diabetes was not an independent risk factor for chronic kidney disease[13,32]. However, information on micro-albuminuria was not available in these studies, meaning that this early sign of kidney damage may had been missed. We showed in participants with pre-DM that eGFR was similar to participants with normoglycemia, but the odds of micro-albuminuria was 1.7 (95 % CI 1.0, 2.8) times higher. However, after adjustment for vascular risk factors the odds ratio was attenuated (OR 1.5 (95 % CI 0.9, 2.7) suggest that concomitant vascular disease risk factors explain most of the increased odds of development of micro-albuminuria in pre-DM.

The observed higher eGFR in patients with (newly) diagnosed DM is in line with other studies [11,33,34]. In our study, patients with diagnosed DM, although not recently diagnosed, had a relatively "mild" diabetes mellitus. This was reflected by good glycemic control, a small number of patients with insulin use and a low prevalence of diabetic complications. These characteristics might explain the observed higher eGFR, reflecting glomerular hyperfiltration, instead of a decline in eGFR.

Glomerular hyperfiltration is considered as an early manifestation of diabetic kidney nephropathy and may contribute to nephropathy progression and GFR decline[20,35,36]. However a recent study in patients with type 1DM showed that early hyperfiltration was not associated with a higher long-term risk of decreased GFR, defined as an eGFR < 60 ml/min per 1.73 m^2[37]. A potential limitation of that study was that hyperfiltration was assessed at baseline (median 4 years after diagnosis)

and not during long term follow up (median 28 years). Furthermore, glucose control was not optimal (mean HbA1c 8.8 %). A reason for the negative result of that study, which showed that hyperfiltration was not associated with a higher long-term risk of decreased GFR, might be that glomerular hyperfiltration is (partially) reversible after improvement of glucose control.

In our study, we evaluated different measures of glucose metabolism, and showed that both fasting and post-prandial glucose levels and HOMA-B were associated with glomerular hyperfiltration, while only fasting glucose was associated with micro-albuminuria. Our results showed no association between insulin resistance with glomerular hyperfiltration and micro-albuminuria. These results are in line with another study which reported that impaired fasting glucose was associated with glomerular hyperfiltration, whereas hyperinsulinemia was not[17]. Our results add to the hypothesis that hyperinsulinemia as a measure of insulin resistance, is not associated with a first increase in eGFR (hyperfiltration) but is associated with a decline in eGFR. This might be explained by direct kidney damage, by mechanisms such as inflammatory cytokines and lipotoxicity[38-40]. Other studies reported that insulin resistance in a non-diabetic population contributes to progressive CKD[41,42, but hyperfiltration was not assessed in these studies. Our study also showed that hyperinsulinemia was not associated with micro-albuminuria. This is line with another study[43] but in contrast with other reports[44,45]. However, these studies were performed in a different study population (mainly non-caucasian) and furthermore no adjustments were made for the use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers.

This study adds new knowledge to existing literature. First, the proportion of patients with kidney disease in this Dutch study cohort with a good metabolic control was relatively low. Second, we showed that insulin resistance was not associated with glomerular hyperfiltration and micro-albuminuria. The strengths of this study are the evaluation of kidney function by eGFR, as well as glomerular hyperfiltration and micro-albuminuria. Micro-albuminuria is a known risk factor for the development of kidney disease in the setting of diabetes [46,47]. Furthermore, the NEO population consisted of a large study population and included an extensive set of measures of glucose metabolism, including a mixed meal test. This made it possible to evaluate the associations of many different measures of glucose metabolism with measures of kidney function in one large patient cohort.

Our study also has a number of limitations that need to be considered. The cross-sectional design limits inferences on causality. Further we did not perform a glucose tolerance test, but a mixed meal test and measured post-prandial glucose levels at 30 and 150 min. A two-hour glucose value was not obtained and therefore impaired glucose tolerance could not be assessed. However, we combined fasting glucose, HbA1c levels, elevated post-prandial levels (≥ 11.1 mmol/l) and used accepted definitions of pre-diabetes[2]. Another limitation is the use of estimated GFR, although the CKD-EPI formula has proven to be more accurate compared with the MDRD formula, it has not been validated above 90 ml/min/1.73m^2 and might underestimate hyperfiltration in obesity[48]. Isotope clearance measurements are the gold standard but are seldom used in large epidemiological studies due to time, high cost and invasiveness. Further there is no consensus about the definition of hyperfiltration. Some studies referred to hyperfiltration as a GFR exceeding the upper limit of normal range (eGFR > 90th percentile) or a measured GFR > 120 ml/min/1.73m^2[17,34,35]. Another limitation is the lack of confirmation of micro-albuminuria in a second urine portion. Although patients delivered a morning urine sample, which reduces the number of false positives, we cannot exclude that some patients had transient micro-albuminuria.

Further follow up studies are needed to investigate whether patients with pre-DM or DM and hyperfiltration are at risk of further progression of kidney disease. If future studies confirm that patients with glomerular hyperfiltration have a high risk of further progression of kidney disease, this will also give the opportunity for early intervention in diminishing renal disease progression, by modulating intraglomerular pressure with sodium-glucose cotransporter-2 inhibitors and renin-angiotensin-aldosteron system inhibitors.

In conclusion, in contrast with other studies, the proportion of patients with kidney disease in this Dutch study population was relatively low, which might be related to good metabolic control. Furthermore, we showed that both fasting and post-prandial glucose and HOMA-B, but not insulin resistance, were associated with glomerular hyperfiltration, while fasting glucose was also associated with micro-albuminuria. This implies that hyperinsulinemia as a measure of insulin resistance, is not associated with a first increase in eGFR (hyperfiltration) but is associated with a decline in eGFR. The results need further confirmation in cohorts with long term follow up.

Supplementary Information S1

Laboratory methods and calculations

Fasting plasma glucose concentrations were determined by enzymatic and colorimetric methods (Roche Modular Analytics P800, Roche Diagnostics, Mannheim, Germany; CV < 5%) and serum insulin concentrations were determined by an immunometric method (Siemens Immulite 2500, Siemens Healthcare Diagnostics, Breda, The Netherlands; CV < 5%). HbA1c concentrations were measured by HPLC boronate affinity chromatography (Primus Ultra, Siemens Healthcare Diagnostics, Breda, The Netherlands; CV < 3%). All analyses were performed in the central clinical chemistry laboratory of the LUMC (4). From measured glucose and insulin concentrations, we calculated Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and HOMA of beta-cell function (HOMA-B) as markers of hepatic insulin resistance and steady-state insulin secretion (5). HOMA-IR was calculated as fasting insulin (μU/ml) * fasting glucose (mmol/l) / 22.5 and HOMA-%B as 20 * fasting insulin (μU/ml) / fasting glucose (mmol/l) − 3.5 (5;6). In addition, we calculated the total area under the curve for overall glucose ($glucose_{AUC}$) and insulin ($insulin_{AUC}$) concentrations by the trapezoidal rule as (15 * fasting concentration + 75 * $concentration_{30min}$ + 60 * $concentration_{150min}$) / 150. The Disposition Index a marker of whole-body beta-cell function that accounts for variations in whole-body insulin sensitivity, was calculated as Insulinogenic Index * Matsuda Insulin Sensitivity Index (7). Insulinogenic Index was calculated as total $insulin_{AUC}$ / total $glucose_{AUC}$ and Matsuda Insulin Sensitivity Index as 10,000/square root [fasting glucose (mg/dl) * fasting insulin (μU/ml)] * [$mean_{glucose0\text{-}150}$ * $mean_{insulin0\text{-}150}$] (7-9)

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