The separate contributions of visceral fat and liver fat to chronic kidney disease-related renal outcomes

Dekkers, I.A.; Vries, A.P.J. de; Smit, R.A.J.; Rosendaal, F.R.; Rabelink, T.J.; Lamb, H.J.; Mutsert, R. de

The Separate Contributions of Visceral Fat and Liver Fat to Chronic Kidney Disease-Related Renal Outcomes

Ilona A. Dekkers, MD,⋆ Aiko P. J. de Vries, MD, PhD,† Roelof A. J. Smit, MD,‡ Frits R. Rosendaal, MD, PhD,‡ Ton J. Rabelink, MD, PhD,† Hildo J. Lamb, MD, PhD,⋆ and Renée de Mutsert, PhD‡

Objectives: This study aims to investigate the separate contributions of liver fat and visceral fat on microalbuminuria and impaired renal function, and second, to examine whether non-alcoholic fatty liver disease is causally related to microalbuminuria and/or impaired renal function.

Methods: Associations between visceral adipose tissue (VAT), hepatic triglyceride content (HTGC), and risk of microalbuminuria and renal function were studied cross-sectionally in the Netherlands Epidemiology of Obesity study. Mendelian randomization using GWAS meta-analysis data was performed to estimate the causal effect of non-alcoholic fatty liver disease (*PNPLA3*, *LYPLAL1*, *NCAN*, *GCKR*) on eGFR (N_{max} 118,460), microalbuminuria (N_{max} 54,116), and impaired renal function (N_{max} 118,147).

Results: In total, 2,023 participants (mean age 55.5 ± 6.0 years, 53% women) were included of which 29% had fatty liver and 2.0% chronic kidney disease stage ≥3. In joint models, VAT was associated with a 2-fold increased risk of microalbuminuria which was mainly driven by the association in women (total population: per standard deviation [SD] = 55.4 cm^2, odds ratio [OR] 2.02, 95% confidence interval [CI] 1.18-3.47; women: OR 2.83, 95% CI 1.44, 5.56), but HTGC was not (total population: per SD = 7.9%, OR 1.20, 95% CI 0.85, 1.70). No associations were found for VAT and HTGC with eGFR (VAT: per SD = 55.4 cm^2, OR 1.25, 95% CI 0.83, 1.87; HTGC: per SD = 7.9%, OR 0.65, 95% CI 0.42, 0.99). No causal effect of NAFLD on microalbuminuria or impaired renal function was found.

Conclusions: In observational analyses, visceral fat was associated with microalbuminuria in women. Liver fat was not associated with microalbuminuria or renal function, which was supported by Mendelian randomization. Visceral fat might be more important than liver fat in the etiology of microalbuminuria.

Introduction

NON-ALCOHOLIC FATTY LIVER disease (NAFLD) and chronic kidney disease (CKD) have shared pathophysiological mechanisms and increasing evidence suggests that NAFLD is an important risk factor for CKD.[1,2] As decline in renal function often only occurs late in the disease course of CKD, microalbuminuria is of particular interest as an early subclinical marker of endothelial dysfunction, especially in obese individuals who are known to show hyperfiltration in the early phases of CKD.[3] Besides liver fat, also total body fat (TBF) and visceral fat have been implicated as risk factors for microalbuminuria[4] and endothelial dysfunction.[5] It has been postulated that excess visceral fat via increased levels of adipokines and free fatty acids lead to systemic inflammation, and ultimately renal deterioration.[6,7] However, the separate contribution of liver fat on the associations with microalbuminuria and impaired renal function remains unclear as previous studies did not take visceral fat or TBF into account.[8] Furthermore, it has been shown that visceral fat is more strongly associated with cardiometabolic risk factors than liver fat; however, associations with microalbuminuria or renal function were not evaluated.[9] Previous studies have been limited by the use of ultrasonography or computed tomography (CT) for the assessment of the presence of hepatic steatosis rather than direct quantification of hepatic triglyceride content (HTGC) using proton magnetic resonance spectroscopy (^{1}H-MRS), which is considered to be the gold standard technique for non-invasive measurement

⋆*Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands.*

†*Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands.*

‡*Division of Nephrology, Department of Medicine, Leiden University Medical Center, Leiden, The Netherlands.*

Financial Disclosure: The authors declare that they have no relevant financial interests.

Support: The NEO study is supported by the participating Departments, the Division, and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area 'Vascular and Regenerative Medicine.'

Address correspondence to Ilona A. Dekkers, MD, Department of Radiology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands. E-mail: i.a.dekkers@lumc.nl

1051-2276/$36.00

https://doi.org/10.1053/j.jrn.2019.09.002

liver fat.[10] In addition, recent methods such as Mendelian randomization, which offers the ability to infer a causal relationship between a risk factor and a certain disease by using genetic markers as a proxy for a modifiable risk factor,[11] have not yet been applied to study the associations between liver fat and CKD-related renal outcomes such as microalbuminuria and impaired renal function. Our aim is to study the separate contributions of liver fat and visceral fat to microalbuminuria in the general population, and whether NAFLD has a causal effect on microalbuminuria and impaired renal function (Fig. 1).

Methods

Study Population and Study Design

The present study is a cross-sectional analysis of baseline measurements of the Netherlands Epidemiology of Obesity (NEO) study, a population-based, prospective cohort study in 6,671 men and women between 45 and 65 years at baseline.[12] Men and women living in the greater area of Leiden (in the west of the Netherlands) were invited to participate in the study if they were aged between 45 and 65 years and had a self-reported body mass index (BMI) of ≥27 kg/m^2. In addition, all inhabitants from 1 municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. Participants were invited to a baseline visit at the NEO study center after an overnight fast. Prior to this study visit, participants collected a morning spot of urine and completed a general questionnaire at home to report demographic, lifestyle, and clinical information. At the baseline visit, all participants underwent an extensive physical examination including anthropometry and blood sampling. Participants with potential contraindications for magnetic resonance imaging (MRI) (i.e., metallic devices, or claustrophobia) were excluded for additional imaging. Approximately 35% of the participants without potential MRI contraindications were randomly selected for assessment of abdominal visceral adipose tissue (VAT) and HTGC using MRI. Inclusion criteria were a successful measurement of VAT and HTGC. Exclusion criteria were alcohol consumption of ≥10 units per day, and missing data on urine and serum measurements, TBF fat, smoking, and education. The Medical Ethical Committee of the Leiden University Medical Center approved the design of the study and all participants gave their written informed consent. The study was performed according to the ethical standards of the Helsinki Declaration of 1975, as revised in 2013.

Data Collection

The participants were asked to bring all medications they were using to the study visit. All use of medications in the month preceding the study visit was recorded by research nurses. On the questionnaire, participants reported ethnicity by self-identification, tobacco smoking, highest level of education, and alcohol consumption using a food frequency questionnaire (in g/d). In women, we grouped use of contraceptives and hormone replacement therapy into current, past, and never (reference) use of estrogens. Menopausal state was categorized in pre- and postmenopausal state (reference) according to information on oophorectomy, hysterectomy, and self-reported state of menopause in the questionnaire. Body weight and percent TBF were assessed using the Tanita bioimpedance balance (TBF-310; Tanita International Division, UK).

Laboratory Measurements

Fasting blood samples were drawn from the antecubital vein after 5-minute rest of the participant. Serum creatinine (mg/dL) was used to calculate the estimated glomerular filtration rate (eGFR) according to the Chronic Kidney Disease Epidemiology Collaboration formula.[13] Urinary albumin-creatinine ratio (UACR) was derived from a first morning void. Microalbuminuria was defined as UACR ≥2.5 mg/mmol in men and ≥3.5 mg/mmol in women. All laboratory analyses were performed in the central clinical chemistry laboratory of the Leiden University Medical Center.

Magnetic Resonance Imaging and Spectroscopy

MRI was performed on a 1.5 T MRI scanner (Philips Medical Systems, Best, the Netherlands). Visceral fat was imaged using 3 transverse turbo spin echo slices at the level of the fifth lumbar vertebra (repetition time 300 ms; echo time 20 ms; flip angle 90°; slice thickness 10 mm, slice gap 2 mm). VAT was quantified by converting the number of pixels to square centimeter for all 3 slides using in-house-developed software (MASS; Medis, Leiden, the Netherlands), and the mean VAT areas of the 3 slides were calculated and used in the analyses. Cross-sectional images at the level of the fifth lumbar vertebra are highly correlated to total volumes and thus validly represent total VAT.[14,15] HTGC was quantified by ^{1}H-MRS of the liver using the point-resolved spectroscopy sequence.[16] An 8 mL voxel was positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Spectra were obtained with an echo time of 26 ms and a repetition time of 3,000 ms, and 64 averages were collected with water suppression. Data points were collected using a 1,000 Hz spectral line. Without changing any parameters, spectra without water suppression, with a repetition time of 10 seconds, and with 4 averages were obtained as an internal reference. Spectral data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium; http://www.jmrui.eu).[17,18] HTGC relative to water was calculated as (signal amplitude of triglyceride [arbitrary unit])/(signal amplitude of water [arbitrary unit]) × 100%. A short overview of the imaging protocol is visualized in Figure 2B.

Statistical Analyses

In the NEO study, individuals with a BMI of 27 kg/m^2 or higher were oversampled. First, inhabitants of Leiden and its surroundings between 45 and 65 years of age and with a self-reported BMI of 27 kg/m^2 or higher were invited to participate in the NEO study. In addition, we included a reference population. To that extent, all inhabitants between 45 and 65 years living in 1 municipality, Leiderdorp, were asked to participate irrespective of their BMI. This resulted in an additional sample of 1,671 participants with a BMI distribution that was similar to the BMI distribution of the general Dutch population.[19] If inference is made on the general population, the over-representation of overweight and obese participants in the NEO study may introduce bias, because of the skewed BMI distribution in the NEO population. Weighting toward the BMI distribution of the general population may solve this problem.[20] Using the BMI distribution of the reference population, we calculated weight factors for the NEO study, resulting in a higher weight factor for participants with a lower BMI.[21] Use of sampling weights yields results that apply to a population-based study without oversampling of individuals with a high BMI.[22] Data are presented as mean (standard deviation [SD]), median (25th, 75th percentiles), or as percentage, and stratified by fatty liver, defined as an HTGC of 5.56%.[10] We calculated population-based Z-scores and standardized the values of visceral fat and liver fat to a mean of zero with an SD of 1. Population-based Z-scores are a widely used method for analyzing anthropometric data, as the calculated Z-scores are likely to be normally distributed and thus allow for the use of analysis methods that assume normality such as regression.[23] In addition, the use of SDs in our models allows us to compare the strength of the associations of, e.g., 1 SD of visceral fat with microalbuminuria, with 1 SD of liver fat with microalbuminuria. With linear regression analysis we examined associations between visceral fat and liver fat (determinants), and eGFR and UACR (outcome variables). Because of skewed distributions we used the natural logarithm of UACR in the regression analyses. For interpretation of the results, we back-transformed the regression coefficients toward percentages increase: (exp(beta) − 1) × 100 with 95% confidence intervals (CIs) per standard deviation of VAT and HTGC. In addition, we performed logistic regression analyses and calculated odds ratios (ORs) with 95% CIs of microalbuminuria or impaired renal function (<60 mL/min/1.73 m^2). Crude associations were adjusted for age, sex, ethnicity, education, tobacco smoking, alcohol consumption, fasting state during MRI, and in women additionally for current use of estrogens and menopausal state. Since abdominal fat is strongly related with TBF, for the study of specific effects of abdominal fat it is important

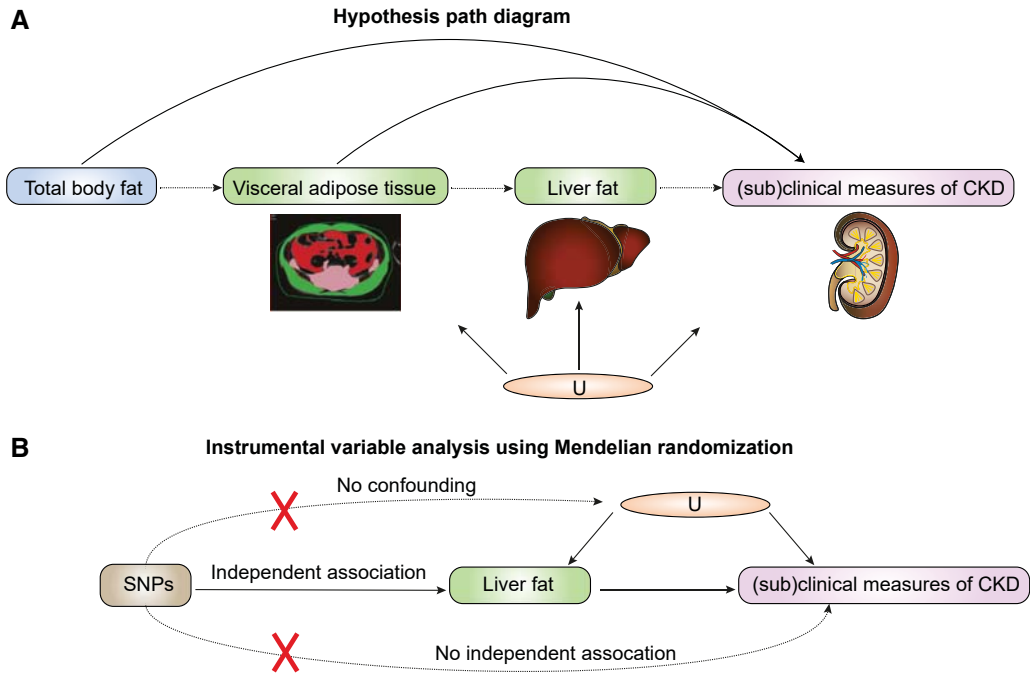

Figure 1. (A) Hypothesis path diagram. Assessed presumed effects of visceral adipose tissue and liver fat on (sub)clinical measures of CKD, and influences of confounders that are either known or unknown (U). (B) Instrumental variable analysis using Mendelian randomization. Assessed presumed effects of the instrumental variable (brown; SNP genetic variants for NAFLD) on exposure (green; liver fat); the instrumental variable does not associate with confounders that are either known or unknown (U). CKD, chronic kidney disease; NAFLD, non-alcoholic fatty liver disease; SNP, single nucleotide polymorphism. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

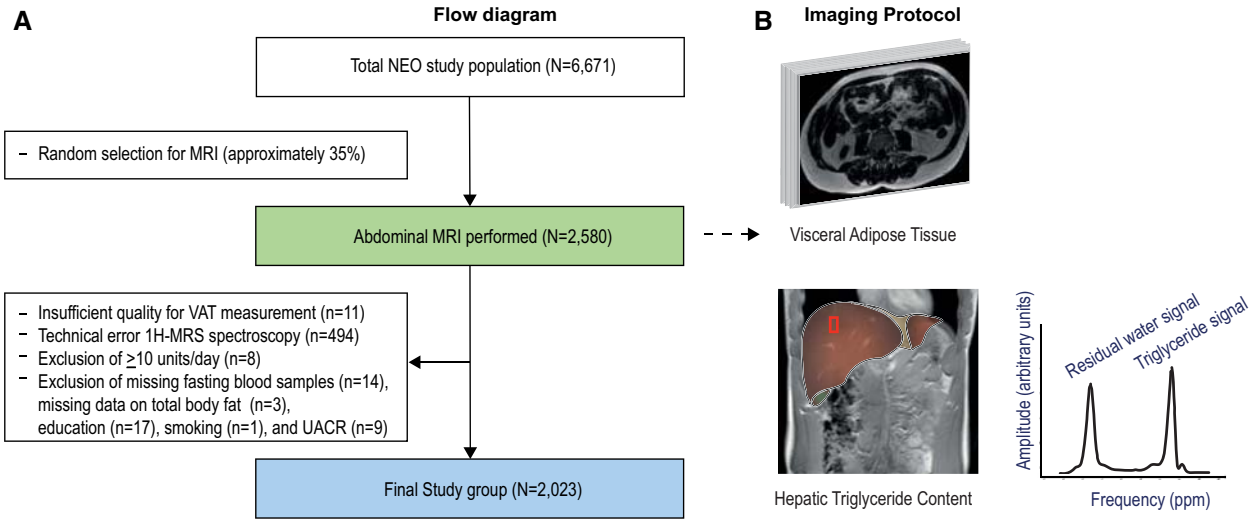

Figure 2. Flow diagram and imaging protocol of the study. (A) Flow diagram of the total Netherlands Epidemiology of Obesity study population. (B) Overview of the imaging protocol consisting of measurement of visceral adipose tissue (upper row: area in red) and hepatic triglyceride content (lower row: right panel, ^{1}H-MRS voxel in red placed in the right liver lobe; left panel, obtained liver spectrum with metabolite frequency relative to water frequency in parts per million). ^{1}H-MRS, proton magnetic resonance spectroscopy; MRI, magnetic resonance imaging; UACR, urinary albumin-creatinine ratio; VAT, visceral adipose tissue. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

to adjust the associations for TBF.[24] Therefore, all models were additionally adjusted for TBF. To examine the separate contributions of VAT and HTGC we performed joint models and simultaneously included VAT and HTGC into the models. To investigate whether associations were different between men and women, we repeated all analyses separately for men and women. The above-mentioned analyses were performed with STATA Statistical Software (StataCorp, College Station, TX), version 12.0.

Selection of Genetic Instruments and Mendelian Randomization Approach

Genetic instrumental variables for NAFLD were selected from the largest genome-wide association study (GWAS) meta-analysis on CT measured hepatic steatosis in individuals of European ancestry to date.[25] Of the 46 variants showing suggestive statistical evidence of association with CT hepatic steatosis, 4 were also significantly associated with histological evidence for NAFLD. These 4 variants were selected as genetic proxies for NAFLD, and were each located in the following genes; *PNPLA3* (rs738409), *LYPLAL1* (rs12137855), *NCAN* (rs2228603), and *GCKR* (rs780094). These variants explained 2.4, 0.2, 0.8, and 0.2 of the variation in CT hepatic steatosis, respectively.[25] We subsequently extracted information on the association between these variants and (sub)clinical measures of CKD from summary-level datasets from published European-descent GWAS meta-analyses on eGFR (sex- and age-adjusted residuals of eGFR based on serum creatinine, in up to 118,460 non-diabetic individuals),[26] microalbuminuria (defined as UACR ≥2.5 mg/mmol in men and ≥3.5 mg/mmol in women, using sex-specific residuals, in up to 54,116 individuals),[27] and impaired renal function (defined as eGFR <60 mL/min/1.73 m^2, in up to 118,147 individuals, analyses adjusted for sex and age).[26] Specifically, we extracted per-allele beta estimates as well as accompanying standard errors for the 4 instruments from datasets publicly available on the CKDgen consortium website (http://ckdgen.imbi.uni-freiburg.de/). The studies contributing to these GWAS meta-analyses had also adjusted for study-specific covariates including study center, principal components of ancestry, and family-based studies accounted for relatedness. Although we could not precisely determine the sample overlap between the GWAS on NAFLD and those on microalbuminuria and impaired renal function, this may have been up to 13% for CKD, 14% for eGFR, and 18% for microalbuminuria, all with respect to the larger dataset.

Subsequently, to estimate the causal effect of NAFLD on (sub)clinical measures of CKD, we performed an inverse-variance weighted linear regression of single nucleotide polymorphism (SNP) outcome associations on SNP exposure associations, with the intercept constrained to zero.[28] The SNP exposure estimates were defined as the per-allele regression coefficient for presence of histological evidence for non-alcoholic steatohepatitis (log-odds).[25] We rescaled the causal effect estimates such that they represent the effect on the outcome per doubling of the odds for histologically proven NAFLD in the population, by multiplying the causal effect estimate from the inverse-variance weighted regression by 0.693 (i.e., $\log_e 2$). As the glucokinase regulator gene (*GCKR*) likely has pleiotropic effects on glucose metabolism, we

also calculated the causal effect estimates without excluding the instrument for *GCKR* as a sensitivity analysis. Mendelian randomization analyses were performed in R (version 3.4.3, Vienna, Austria. 2016] using the TwoSampleMR R-package.

Results

Baseline Characteristics

Of the 6,671 included participants, 2,580 participants without potential contraindications for MRI were randomly selected to undergo abdominal imaging. In 11 participants, the images were of insufficient quality for the quantification of VAT. In another 494 participants, ^{1}H-MRS of the liver was not available owing to technical errors, exceeded scan time, or claustrophobia. In addition, we excluded 8 participants who reported to consume more than 10 units of alcohol per day. Finally, we consecutively excluded participants with missing fasting blood samples (n = 14), and missing data for TBF (n = 3), smoking (n = 1), UACR (n = 9), and education (n = 17) (see flow diagram, Fig. 2A). After these exclusions, 2,023 participants (1,052 men and 971 women) were included in the present analysis with a mean (SD) age of 55.5 (6.0) years (range 44-66) and 53% were women. For the total population, mean BMI was 25.9 (4.0) kg/m^2, and mean eGFR was 86.2 (12.2) mL/min/1.73 m^2. Microalbuminuria was present in 1.9% (UACR ≥2.5 mg/mmol in men and ≥3.5 mg/mmol in women) and 0.1% of the total study sample had UACR levels ≥25 mg/mmol in men and ≥35 mg/mmol in women. About 1.5% of the total study population had both diabetes and microalbuminuria. Mildly to moderately impaired renal function (eGFR ≤60 mL/min/1.73 m^2) was present in 2.0% of the study population. The baseline characteristics of these participants are shown in Table 1, and flow diagram and scan protocol are shown in Figure 2.

Associations Between Visceral Fat, Liver Fat, Microalbuminuria, and Impaired Renal Function

Results of linear regression analyses showing the differences in UACR and eGFR per SD in visceral fat and liver fat are provided in Table 2.

In crude analysis, no associations were found for both VAT (per SD = 55.4 cm^2, −4% difference, 95% CI −8, 0) and HTGC (per SD = 7.9%, 0% difference, 95% CI −4, 4) with UACR in the total population. After adjustment for confounding factors including HTGC, the association for VAT and UACR was +5% difference per SD in VAT (95% CI −1, 12). The associations between HTGC and UACR was 2% difference in adjusted analysis per SD in HTGC (95% CI −3, 6). In the sex-stratified adjusted analysis, VAT was associated with UACR in women only (per SD 14% difference, 95% CI 1, 28).

Regarding renal function, in crude analysis VAT was significantly associated with lower eGFR in women (per SD = 1.25 mL/min/1.73 m^2, 95% CI 0.12, 2.39), but not in men (per SD = 0.33 mL/min/1.73 m^2, 95% CI −0.58, 1.24). No associations were found between HTGC and renal function for both men (per SD = −0.65 mL/min/1.73 m^2, 95% CI −1.41, 0.10) and women (per SD = 0.25 mL/min/1.73 m^2, 95% CI −0.64, 1.14) in unadjusted analyses. In adjusted analyses, neither VAT (per SD = 0.52 mL/min/1.73 m^2, 95% CI −0.46, 1.50) nor HTGC (per SD = −0.60 mL/min/1.73 m^2, 95% I −1.29, 0.09) were associated with renal function (Table 2).

Results of logistic regression analyses for the associations between visceral fat and liver fat with the risk of microalbuminuria and impaired renal function are provided in Table 3. In crude analysis, per SD change in VAT the OR for microalbuminuria was 1.54 (95% CI 1.19, 1.99) and 1.34 (95% CI 1.05, 1.72) per SD in HTGC. In adjusted models, VAT was associated with a 2-fold increased risk of microalbuminuria (OR 2.02, 95% CI 1.18, 3.47), whereas for HTGC the OR of microalbuminuria per SD change was 1.20 (95% CI 0.85, 1.70). In sex-stratified analysis, VAT was associated with microalbuminuria in women (OR 2.83, 95% CI 1.44, 5.56), but not in men, and HTGC was not associated with microalbuminuria in either sex (Table 3). For renal function, no associations were found for both VAT and HTGC with the risk of impaired renal function in crude analysis and adjusted analysis. In sex-stratified adjusted models, per SD in HTGC the OR of impaired renal function was 0.36 (95% CI 0.14, 0.94) in men, and 0.74 (95% CI 0.50, 1.09) in women.

Mendelian Randomization Analyses

Using publicly available data of 4 genetic instruments for histological evidence of NAFLD, only the lead variant for GCKR (rs780094) showed statistically significant per-allele effects on impaired renal function and presence of microalbuminuria (Table 4). However, combining the genetic instruments, we did not observe any evidence of a causal effect of histologically proven NAFLD on any of the CKD-related renal outcomes (Table 4), also after excluding the instrument for *GCKR* (data not shown).

Discussion

In our observational analyses, visceral fat was associated with microalbuminuria in women (but not in men), although an association with impaired renal function was not found. Liver fat was not associated with microalbuminuria or renal function in either sex, which was supported by our Mendelian randomization analyses showing no evidence for a causal relationship between liver fat and microalbuminuria or impaired renal function.

Table 1. Characteristics of Participants of the NEO study (n = 2,023) Stratified by Hepatic Triglyceride Content

Characteristic	HTGC ≤ 5.56 (71%)	HTGC > 5.56 (29%)
Age (y)	55 (5)	57 (7)
Sex (% women)	59	38
Menopausal state (% postmenopausal)	54	72
Ethnicity (% white)	96	96
Current smoker (%)	15	12
Former smoker (%)	42	54
Body mass index (kg/m^2)		
Men	25.6 (2.4)	28.2 (4.3)
Women	24.4 (3.3)	28.8 (6.3)
Waist circumference (cm)		
Men	94.3 (7.6)	102.5 (12.2)
Women	82.9 (9.4)	95.8 (15.5)
Total body fat (%)		
Men	22.8 (4.0)	27.4 (6.7)
Women	34.8 (5.4)	41.2 (7.0)
Visceral adipose tissue (cm^2)		
Men	95.1 (39.9)	144.3 (71.7)
Women	55.4 (28.8)	109.1 (65.8)
Hepatic triglyceride content (%)		
Men	2.4 (1.54, 3.51)	11.0 (7.23, 17.11)
Women	1.5 (1.02, 2.40)	11.0 (7.07, 19.00)
Alcohol consumption (g/d)	12.8 (11.4)	18.4 (23.4)
Hypertension (%)	31	47
Cardiovascular disease (%)	4	6
Type 2 diabetes (%)	2	11
Use of glucose-lowering therapy (%)	1	5
Use of antihypertensives (%)	15	31
Use of ACE inhibitors/angiotensin-II antagonists (%)	8	19
Use of statins (%)	6	15
Current use of sex hormones* (%)	11	2
Serum creatinine (μmol/L)	76.6 (12.8)	78.7 (17.0)
eGFR CKD-epi (mL/min/1.73 m^2)	86.1 (10.8)	86.6 (14.8)
eGFR >90 mL/min/1.73 m^2	42	43
eGFR 60-90 mL/min/1.73 m^2	56	56
eGFR <60 mL/min/1.73 m^2	2	1
UAE (mg/L)	3.55 (2.99, 4.52)	3.78 (2.99, 5.11)
UACR (mmol/mg)	0.43 (0.30, 0.66)	0.42 (0.28, 0.66)
Microalbuminuria (%)	1	3

ACE, angiotensin-converting enzyme; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate according to the CKD-Epidemiology Collaboration formula; HTGC, hepatic triglyceride content; UACR, urinary albumin-creatinine ratio; UAE, urinary albumin excretion.

Results were based on analyses weighted toward the BMI distribution of the general population (n = 2,023; 1,052 men, 971 women). Continuous variables are expressed as %, means (standard deviation), or medians (25th, 75th percentile). Microalbuminuria was defined as UACR ≥2.5 mg/mmol for men and ≥3.5 mg/mmol for woman.

*Sex hormone use by woman: contraceptive pill or hormone replacement therapy.

Our findings suggest that visceral fat is more important in the etiology of microalbuminuria than liver fat and supports the hypothesis that microalbuminuria might be a manifestation of visceral adiposity.[4] Possible mechanisms linking visceral adiposity to microalbuminuria and renal dysfunction include higher levels of adipokines, leptin, and resistin.[29,30] In addition, the free fatty acid flux of visceral fat and liver fat possibly predisposes to insulin resistance and microalbuminuria.[31] The combined Framingham Offspring and the multi-detector computed-tomography cohort previously investigated the association of subcutaneous adipose tissue (SAT) to VAT ratio on prevalent CKD. In this combined cohort neither VAT nor SAT (assessed by CT) was independently associated with CKD when using eGFR based on the creatinine-based Modification of Diet in Renal Disease (MDRD) equation.[32] However, in a subsequent study VAT was associated with microalbuminuria in men, whereas SAT was more associated with microalbuminuria in women.[4] In our study, the association between VAT and microalbuminuria was mainly driven by the association in women, suggesting a possible sex difference in the association of visceral fat with microalbuminuria. This is supported by previous research showing stronger

Table 2. Differences in UACR With 95% Confidence Intervals and eGFR per SD of Visceral Fat and Liver Fat

	Percent Difference (95% CI) UACR		
Determinant	Total Population	Men	Women
VAT (SD = 55.4 cm^2)			
Crude	−4 (−8, 0)	3 (−2, 8)	7 (0, 15)
Model 1	1 (−3, 6)	0 (−4, 5)	4 (−3, 11)
Model 2: Model 1 +TBF	5 (−1, 12)	2 (−5, 8)	13 (1, 27)
Model 3: Model 2 +HTGC	5 (−1, 12)	1 (−6, 8)	14 (1, 28)
HTGC (SD = 7.9%)			
Crude	0 (−4, 4)	3 (−3, 8)	3 (−3, 9)
Model 1	1 (−3, 5)	2 (−3, 7)	1 (−5, 7)
Model 2: Model 1 +TBF	3 (−2, 7)	2 (−3, 8)	2 (−4, 9)
Model 3: Model 2 +VAT	2 (−3, 6)	2 (−3, 8)	0 (−7, 6)
	Difference (95% CI) per mL/min/1.73 m^2 Lower eGFR		
Determinant	Total Population	Men	Women
VAT (SD = 55.4 cm^2)			
Crude	0.52 (−0.12, 1.17)	0.33 (−0.58, 1.24)	1.25 (0.12, 2.39)
Model 1	0.17 (−0.56, 0.90)	0.18 (−0.76, −1.12)	0.30 (−0.89, 1.49)
Model 2: Model 1 +TBF	0.30 (−0.65, 1.24)	0.76 (−0.39, 1.92)	−0.12 (−1.79, 1.55)
Model 3: Model 2 +HTGC	0.52 (−0.46, 1.50)	0.99 (−0.18, 2.15)	0.06 (−1.67, 1.79)
HTGC (SD = 7.9%)			
Crude	−0.19 (−0.79, 0.41)	−0.65 (−1.41, 0.10)	0.25 (−0.64, 1.14)
Model 1	−0.42 (−1.03, 0.19)	−0.72 (−1.48, 0.04)	−0.11 (−1.03, 0.81)
Model 2: Model 1 +TBF	−0.49 (−1.14, 0.16)	−0.65 (−1.46, 0.15)	−0.29 (−1.26, 0.68)
Model 3: Model 2 +VAT	−0.60 (−1.29, 0.09)	−0.84 (−1.69, 0.01)	−0.31 (−1.31, 0.70)

^{1}H-MRS, proton magnetic resonance spectroscopy; BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; HTGC, hepatic triglyceride content; SD, standard deviation; TBF, total body fat; UACR, urinary albumin-creatinine ratio; VAT, visceral adipose tissue.

Results were based on analyses weighted toward the BMI distribution of the general population (n = 2,023; 1,052 men, 971 women). Results were derived from beta coefficients (β) with 95% confidence intervals from linear regression analyses and are expressed as percentage increase or decrease in UACR (mg/mmol) or as difference in lower eGFR (mL/min/1.73 m^2) per standard deviation in VAT or HTGC. Model 1 was adjusted for age, sex, ethnicity, education, tobacco smoking, alcohol consumption, fasting state during ^{1}H-MRS, and in women additionally adjusted for current use of estrogens and menopausal state; Model 2 included the covariates of Model 1 plus additional adjustment for TBF; Model 3 included the covariates of Model 2 plus additional adjustment for either VAT or HTGC.

relationship between visceral fat and cardiometabolic outcomes in women than in men.[27,33,34] Although the underlying pathophysiology of this difference is still not yet fully understood and merits further study, the smaller amount of visceral fat depot in women and the influence of sex hormones on the regulation of adipose tissue distribution and function[35] are likely involved. In men the results for VAT and microalbuminuria were not statistically significant, and might be due to the lack of statistical power considering the limited amount of participants with microalbuminuria. This indicates the need for larger studies to further elucidate the association between VAT and microalbuminuria in men.

Although our study supports the link between visceral fat and microalbuminuria, our study did not show any associations between VAT or liver fat with renal function, which could be explained by the limited number of participants with an eGFR below 60 mL/min/1.73 m^2 in our sample. Furthermore, it can be argued whether the use of angiotensin-converting enzyme inhibitors/angiotensin-II antagonists may confound the results, additional adjustment for angiotensin-converting enzyme inhibitors/angiotensin-II antagonists did not markedly change the results (Table S1).

In our Mendelian randomization analysis only the lead variant for *GCKR* showed evidence for causal effects of NAFLD on impaired renal function and presence of microalbuminuria. However, the lead variant for *GCKR* is also known to be involved in glucose metabolism and diabetic nephropathy.[36] Our results thus do not support the hypothesis that liver fat by itself is causally related to CKD-related renal outcomes. In contrast, a recent meta-analysis estimated that NAFLD is associated with a nearly 40% increase in the long-term risk of incident CKD,[8] albeit none of the included studies adjusted for VAT nor used gold standard techniques such as ^{1}H-MRS for the measurement of ectopic lipids. A recent small (n = 400) Chinese study evaluating the association between liver fat measured by ^{1}H-MRS, however, did show that NAFLD was independently associated with CKD after adjustment for VAT.[37]

We were not able to perform a Mendelian randomization analysis for VAT, since the most recent GWAS meta-analysis on VAT was unable to identify genome-wide significant

Table 3. Odds Ratios With 95% Confidence Intervals for Risk of Microalbuminuria and Impaired Renal Function per SD Change in Visceral Fat and Liver Fat

	OR (95% CI) for Microalbuminuria		
Determinant	Total Population	Men	Women
VAT (SD = 55.4 cm^2)			
Crude	1.54 (1.19, 1.99)	1.76 (1.32, 2.34)	1.87 (1.31, 2.65)
Model 1	1.75 (1.34, 2.30)	1.66 (1.19, 2.33)	1.92 (1.19, 3.09)
Model 2: Model 1 +TBF	2.16 (1.29, 3.61)	1.71 (0.95, 3.08)	3.03 (1.51, 6.08)
Model 3: Model 2 +HTGC	2.02 (1.18, 3.47)	1.65 (0.88, 3.09)	2.83 (1.44, 5.56)
HTGC (SD = 7.9%)			
Crude	1.34 (1.05, 1.72)	1.46 (1.06, 2.01)	1.30 (0.94, 1.81)
Model 1	1.36 (1.05, 1.76)	1.36 (0.96, 1.93)	1.32 (0.98, 1.77)
Model 2: Model 1 +TBF	1.34 (1.02, 1.77)	1.26 (0.83, 1.93)	1.35 (0.96, 1.89)
Model 3: Model 2 +VAT	1.20 (0.85, 1.70)	1.17 (0.69, 1.98)	1.13 (0.76, 1.69)
	OR (95% CI) for Impaired Renal Function		
Determinant	Total Population	Men	Women
VAT (SD = 55.4 cm^2)			
Crude	1.02 (0.75, 1.37)	0.80 (0.40, 1.59)	1.52 (1.09, 2.13)
Model 1	1.00 (0.64, 1.56)	0.69 (0.32, 1.49)	1.40 (0.84, 2.34)
Model 2: Model 1 +TBF	1.10 (0.75, 1.63)	1.10 (0.63, 1.90)	1.35 (0.74, 2.47)
Model 3: Model 2 +HTGC	1.25 (0.83, 1.87)	1.24 (0.64, 2.38)	1.56 (0.86, 2.84)
HTGC (SD = 7.9%)			
Crude	0.74 (0.52, 1.05)	0.28 (0.11, 0.73)	0.99 (0.77, 1.27)
Model 1	0.71 (0.45, 1.12)	0.23 (0.06, 0.88)	0.92 (0.64, 1.32)
Model 2: Model 1 +TBF	0.70 (0.47, 1.04)	0.39 (0.17, 0.89)	0.83 (0.58, 1.19)
Model 3: Model 2 +VAT	0.65 (0.42, 0.99)	0.36 (0.14, 0.94)	0.74 (0.50, 1.09)

^{1}H-MRS, proton magnetic resonance spectroscopy; BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; HTGC, hepatic triglyceride content; OR, odds ratio; SD, standard deviation; TBF, total body fat; UACR, urinary albumin-creatinine ratio; VAT, visceral adipose tissue.

Results were based on analyses weighted toward the BMI distribution of the general population (n = 2,023; 1,052 men, 971 women). Results were derived from logistic regression analyses and ORs with 95% confidence intervals are expressed per standard deviation in VAT or HTGC. Model 1 was adjusted for age, sex, ethnicity, education, tobacco smoking, alcohol consumption, fasting state during ^{1}H-MRS, and in women additionally adjusted for current use of estrogens and menopausal state; Model 2 included the covariates of Model 1 plus additional adjustment for TBF; Model 3 included the covariates of Model 2 plus additional adjusted for either VAT or HTGC. Microalbuminuria was defined as UACR ≥2.5 mg/mmol for men and ≥3.5 mg/mmol for woman. Impaired renal function was defined as eGFR <60 mL/min/1.73 m^2.

VAT-specific SNPs.[38] Future large-scale GWAS studies are needed to identify the genetic variants of VAT before investigating the causal relationship between visceral fat and CKD.

Besides combining observational research with Mendelian randomization analysis, one of the major strengths of the present study is the use of magnetic resonance spectroscopy for the measurement of HTGC in over 2,000 individuals, which is considered the gold standard technique for the non-invasive measurement of liver fat.[39] Previous studies showed that the presence of NAFLD was associated with an increased risk of CKD and limited by the relatively small sample size of biopsy-proven NAFLD and by the suboptimal sensitivity of ultrasound and/or liver enzyme elevations for the detection of NAFLD in population-based studies.[8] Moreover, these studies did not take VAT into account, and anthropometric indices such as BMI and waist circumference poorly predict the volumes of internal body fat compartments, which could lead to under-estimation of associations with chronic disease risks.[40]

There are several limitations that need to be considered. First, as we use data of a population-based cohort, our study consists of relatively healthy participants and the prevalence of microalbuminuria and moderately to severely impaired renal function is very low. Because of these low numbers we decided not to make a distinction between microalbuminuria and macroalbuminuria (UACR levels ≥25 mg/mmol in men and ≥35 mg/mmol in women) as macroalbuminuria was virtually absent in the study sample. Another limitation is the use of first morning urine void samples rather than 24-hour urine collection in the present study. However, previous studies have shown that albuminuria measures derived from first morning voids are a reliable alternative to 24-hour urine albumin excretion and UACR is more reliable than urinary albumin excretion for the assessment of microalbuminuria in spot urine samples.[41] Furthermore, although the Chronic Kidney Disease Epidemiology Collaboration formula has proven to be more accurate and precise for estimating renal function than the older MDRD formula, it has not been validated to be ≥90 mL/min/1.73 m^2.[13] Also the error

Table 4. Per-allele Effect of Genetic Instruments for NAFLD, and Inverse-Variance Weighted Mendelian Randomization Estimators, on Renal Function, Microalbuminuria, and Impaired Renal Function

Outcome	Locus	SNP	Effect Allele	OR for NAFLD	Effect Estimate (95% CI)	*P* Value
$eGFR_{crea}$ (log-transformed)	PNPLA3	rs738409	G	3.24	−0.0016 (−0.0038, 0.0006)	.14
	LYPLAL1	rs12137855	C	1.21	−0.0004 (−0.0026, 0.0018)	.71
	NCAN	rs2228603	T	1.9	−0.0020 (−0.0055, 0.0015)	.27
	GCKR	rs780094	T	1.18	0.0063 (0.0045, 0.0081)	4.8×10^{-12}
N_{max} 118,460	Causal effect estimator				−0.0004 (−0.005, 0.004)	.87
Microalbuminuria (odds ratio)	PNPLA3	rs738409	G	3.24	0.97 (0.93, 1.02)	.31
	LYPLAL1	rs12137855	C	1.21	0.97 (0.93, 1.02)	.29
	NCAN	rs2228603	T	1.9	1.05 (0.97, 1.14)	.23
	GCKR	rs780094	T	1.18	1.09 (1.04, 1.13)	4.9×10^{-5}
N_{max} 54,116	Causal effect estimator				1.00 (0.93, 1.07)	.97
Impaired renal function (odds ratio)	PNPLA3	rs738409	G	3.24	1.03 (0.99, 1.07)	.13
	LYPLAL1	rs12137855	C	1.21	0.99 (0.97, 1.05)	.57
	NCAN	rs2228603	T	1.9	1.01 (0.95, 1.07)	.73
	GCKR	rs780094	T	1.18	0.98 (0.95, 1.01)	.20
N_{max} 118,147	Causal effect estimator				1.01 (0.99, 1.03)	.21

CI, confidence interval; CKD, chronic kidney disease; eGFRcrea, estimated glomerular filtration rate based on serum creatinine, using sex-and age-adjusted residuals of logarithm; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; SNP, single nucleotide polymorphism.

Data presented as per-allele effect on biopsy-proven NAFLD (odds ratio) as observed by Speliotes et al.[25] The causal effect estimates were calculated using inverse-variance weighted regression of SNP outcome on SNP exposure estimates, with the intercept constrained to zero, and can be interpreted as the change in the outcome (or odds ratio for CKD and microalbuminuria) per doubling in the odds of NAFLD in the population.

of eGFR in the normal to high range of renal function is quite substantial.[42] These limitations of eGFR could also be the explanation for the found contradictory association between HTGC and lower risk of impaired renal function in men. In this study, visceral fat and liver fat were quantified at 1.5 T using conventional non-water-saturated T1-weighted images and ^{1}H-MRS, respectively. However, use of advanced multiecho techniques that generate fat-only MR images, and use of high-field strength MRI scanners might have improved measurement accuracy and precision.[43] Moreover, recent technical advances have also enabled non-invasive quantification of renal sinus fat volume, pararenal fat, and intra-renal triglyceride content,[44,45] which are other potentially important ectopic fat compartments related to the kidney (e.g., fatty kidney).[46] Especially pararenal fat is of great interest considering this is a location that in adults consists mainly of dormant brown adipose tissue which could be potentially reactivated into active brown adipose, a potential strategy for combatting obesity and metabolic disease including obesity-related renal disease.[47] In addition, future studies are needed to investigate the potential mechanisms underlying the association between visceral fat and microalbuminuria. Adipocyte-derived hormones and cytokines leading to low-grade inflammatory state may play a role in this.

In conclusion, in observational analyses visceral fat was associated with microalbuminuria in women (but not in men), although an association with impaired renal function was not found. Liver fat was not associated with microalbuminuria or renal function in either sex, which was supported by our Mendelian randomization analyses showing no evidence for a causal relationship between liver fat and microalbuminuria or impaired renal function. Future Mendelian randomization studies are needed to investigate the causality between visceral fat and CKD.

Practical Applications

Our study indicates that visceral fat might be more important in the etiology of obesity-related renal disease rather than liver fat. In addition, Mendelian randomization analysis did not support a causal relation between liver fat and renal outcomes, suggesting that liver fat plays a less important role in the risk of CKD than previously suspected. The findings with regard to visceral fat support the importance of body composition assessment in renal disease.

Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1053/j.jrn.2019.09.002.

References

1. Targher G, Byrne CD. Non-alcoholic fatty liver disease: an emerging driving force in chronic kidney disease. *Nat Rev Nephrol*. 2017;13:297-310.

2. Armstrong MJ, Adams LA, Canbay A, Syn W-K. Extrahepatic complications of nonalcoholic fatty liver disease. *Hepatology*. 2014;59:1174-1197.

3. Palatini P. Glomerular hyperfiltration: a marker of early renal damage in pre-diabetes and pre-hypertension. *Nephrol Dial Transplant*. 2012;27:1708-1714.

4. Foster MC, Hwang S-J, Massaro JM, et al. Association of subcutaneous and visceral adiposity with albuminuria: the Framingham Heart Study. *Obesity (Silver Spring)*. 2011;19:1284-1289.

5. Romero-Corral A, Sert-Kuniyoshi FH, Sierra-Johnson J, et al. Modest visceral fat gain causes endothelial dysfunction in healthy humans. *J Am Coll Cardiol*. 2010;56:662-666.

6. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes.* 2007;56:1010-1013.

7. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature.* 2006;444:875-880.

8. Mantovani A, Zaza G, Byrne CD, et al. Nonalcoholic fatty liver disease increases risk of incident chronic kidney disease: a systematic review and meta-analysis. *Metabolism.* 2018;79:64-76.

9. Liu J, Fox CS, Hickson D, Bidulescu A, Carr JJ, Taylor HA. Fatty liver, abdominal visceral fat, and cardiometabolic risk factors: the Jackson Heart Study. *Arterioscler Thromb Vasc Biol.* 2011;31:2715-2722.

10. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Metab.* 2005;288:E462-E468.

11. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol.* 2003;32:1-22.

12. de Mutsert R, den Heijer M, Rabelink TJ, et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. *Eur J Epidemiol.* 2013;28:513-523.

13. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150:604-612.

14. Schweitzer L, Geisler C, Pourhassan M, et al. What is the best reference site for a single MRI slice to assess whole-body skeletal muscle and adipose tissue volumes in healthy adults? *Am J Clin Nutr.* 2015;102:58-65.

15. Han TS, Kelly IE, Walsh K, Greene RM, Lean ME. Relationship between volumes and areas from single transverse scans of intra-abdominal fat measured by magnetic resonance imaging. *Int J Obes Relat Metab Disord.* 1997;21:1161-1166.

16. Bottomley PA. Spatial localization in NMR spectroscopy in vivo. *Ann N Y Acad Sci.* 1987;508:333-348.

17. Naressi A, Couturier C, Devos JM, et al. Java-based graphical user interface for the MRUI quantitation package. *MAGMA.* 2001;12:141-152.

18. Stefan D, Di Cesare F, Andrasescu A, et al. Quantitation of magnetic resonance spectroscopy signals: the jMRUI software package. *Meas Sci Technol.* 2009;20:104035.

19. Ministerie van, V W S [Dutch Ministry of Health, Welfare and S]. *Hoeveel Mensen Hebben Overgewicht? [How many people are overweight?]* 2013.

20. Korn EL, Graubard BI. Epidemiologic studies utilizing surveys: accounting for the sampling design. *Am J Public Health.* 1991;81:1166-1173.

21. Dekkers IA, de Mutsert R, de Vries APJ, et al. Determinants of impaired renal and vascular function are associated with elevated levels of procoagulant factors in the general population. *J Thromb Haemost.* 2018;16:519-528.

22. Lumley T. Analysis of complex survey samples. *J Stat Softw.* 2004;9:1-19.

23. Gorstein J, Sullivan K, Yip R, et al. Issues in the assessment of nutritional status using anthropometry. *Bull World Health Organ.* 1994;72:273-283.

24. Seidell JC, Bouchard C. Visceral fat in relation to health: is it a major culprit or simply an innocent bystander? *Int J Obes Relat Metab Disord.* 1997;21:626-631.

25. Speliotes EK, Yerges-Armstrong LM, Wu J, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet.* 2011;7:e1001324.

26. Pattaro C, Teumer A, Gorski M, et al. Genetic associations at 53 loci highlight cell types and biological pathways relevant for kidney function. *Nat Commun.* 2016;7:10023.

27. Teumer A, Tin A, Sorice R, et al. Genome-wide association studies identify genetic loci associated with albuminuria in diabetes. *Diabetes.* 2016;65:803-817.

28. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol.* 2013;37:658-665.

29. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev.* 2000;21:697-738.

30. Guerre-Millo M. Adipose tissue hormones. *J Endocrinol Invest.* 2002;25:855-861.

31. Votruba SB, Jensen MD. Regional fat deposition as a factor in FFA metabolism. *Annu Rev Nutr.* 2007;27:149-163.

32. Young JA, Hwang S-J, Sarnak MJ, et al. Association of visceral and subcutaneous adiposity with kidney function. *Clin J Am Soc Nephrol.* 2008;3:1786-1791.

33. de Mutsert R, Gast K, Widya R, et al. Associations of abdominal subcutaneous and visceral fat with insulin resistance and secretion differ between men and women: The Netherlands Epidemiology of Obesity Study. *Metab Syndr Relat Disord.* 2018;16:54-63.

34. Hanley AJG, Wagenknecht LE, Norris JM, et al. Insulin resistance, beta cell dysfunction and visceral adiposity as predictors of incident diabetes: the Insulin Resistance Atherosclerosis Study (IRAS) Family study. *Diabetologia.* 2009;52:2079-2086.

35. Geer EB, Shen W. Gender differences in insulin resistance, body composition, and energy balance. *Gend Med.* 2009;6:60-75.

36. Bonetti S, Trombetta M, Boselli ML, et al. Variants of GCKR affect both β-cell and kidney function in patients with newly diagnosed type 2 diabetes: the Verona newly diagnosed type 2 diabetes study 2. *Diabetes Care.* 2011;34:1205-1210.

37. Pan L-L, Zhang H-J, Huang Z-F, et al. Intrahepatic triglyceride content is independently associated with chronic kidney disease in obese adults: a cross-sectional study. *Metabolism.* 2015;64:1077-1085.

38. Chu AY, Deng X, Fisher VA, et al. Multiethnic genome-wide meta-analysis of ectopic fat depots identifies loci associated with adipocyte development and differentiation. *Nat Genet.* 2017;49:125-130.

39. Bohte AE, van Werven JR, Bipat S, Stoker J. The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. *Eur Radiol.* 2011;21:87-97.

40. Neamat-Allah J, Wald D, Hüsing A, et al. Validation of anthropometric indices of adiposity against whole-body magnetic resonance imaging—a study within the German European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts. *PLoS One.* 2014;9:e91586.

41. Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJL, de Jong PE, Gansevoort R. First morning voids are more reliable than spot urine samples to assess microalbuminuria. *J Am Soc Nephrol.* 2009;20:436-443.

42. Porrini E, Ruggenenti P, Luis-Lima S, et al. Estimated GFR: time for a critical appraisal. *Nat Rev Nephrol.* 2019;15:177-190.

43. Zhou A, Murillo H, Peng Q. Impact of partial volume effects on visceral adipose tissue quantification using MRI. *J Magn Reson Imaging.* 2011;34:1452-1457.

44. Jonker JT, de Heer P, Engelse MA, et al. Metabolic imaging of fatty kidney in diabesity: validation and dietary intervention. *Nephrol Dial Transplant.* 2018;33:224-230.

45. Dekkers IA, de Heer P, Bizino MB, de Vries APJ, Lamb HJ. 1 H-MRS for the assessment of renal triglyceride content in humans at 3T: a primer and reproducibility study. *J Magn Reson Imaging.* 2018;48:507-513.

46. de Vries APJ, Ruggenenti P, Ruan XZ, et al. Fatty kidney: emerging role of ectopic lipid in obesity-related renal disease. *Lancet Diabetes Endocrinol.* 2014;2:417-426.

47. Jespersen N, Feizi A, Andersen E, et al. Heterogeneity in the perirenal region of humans suggests presence of dormant brown adipose tissue that contains brown fat precursor cells. *Mol Metab.* 2019;24:30-43.