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Quantitative MRI in obesity & reno-cardiovascular function

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The separate contributions of visceral fat and liver fat to CKD-related renal outcomes

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ABSTRACT

Objective

To investigate the separate contributions of liver fat and visceral fat on microalbuminuria and impaired renal function. Second, to examine whether NAFLD is causally related to microalbuminuria and/or impaired renal function.

Methods

In this cross-sectional analysis of the NEO study associations between visceral adipose tissue (VAT), hepatic triglyceride content (HTGC) and risk of microalbuminuria and renal function were studied using logistic regression. Mendelian randomization using GWAS meta-analysis data was performed to estimate the causal effect of NAFLD (*PNPLA3*, *LYPLAL1*, *NCAN*, *GCKR* from) on eGFR ($N_{\max}118,460$), micro-albuminuria ($N_{\max}54,116$), and impaired renal function ($N_{\max}118,147$).

Results

2,023 participants (mean age 55.5 ± 6.0 years, 53% women) were included of which 29% had fatty liver and 2.0% CKD stage ≥ 3 . In joint models, VAT was associated with a two-fold increased risk of microalbuminuria which was mainly driven by the association in women (total population: per SD=55.4 cm² OR=2.02, 95% 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 cm² 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 (but not in men). Liver fat was not associated with microalbuminuria or renal function, which was supported by Mendelian randomization analyses. These findings suggest that 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 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 total body fat 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 for the assessment of the presence of hepatic steatosis rather than direct quantification of hepatic triglyceride content (HTGC) using proton magnetic resonance spectroscopy, which is considered to be the gold standard technique for non-invasive measurement 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 was 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 6671 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/m². In addition, all inhabitants from one 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 hepatic triglyceride content (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, total body fat, smoking, and education. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) 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.

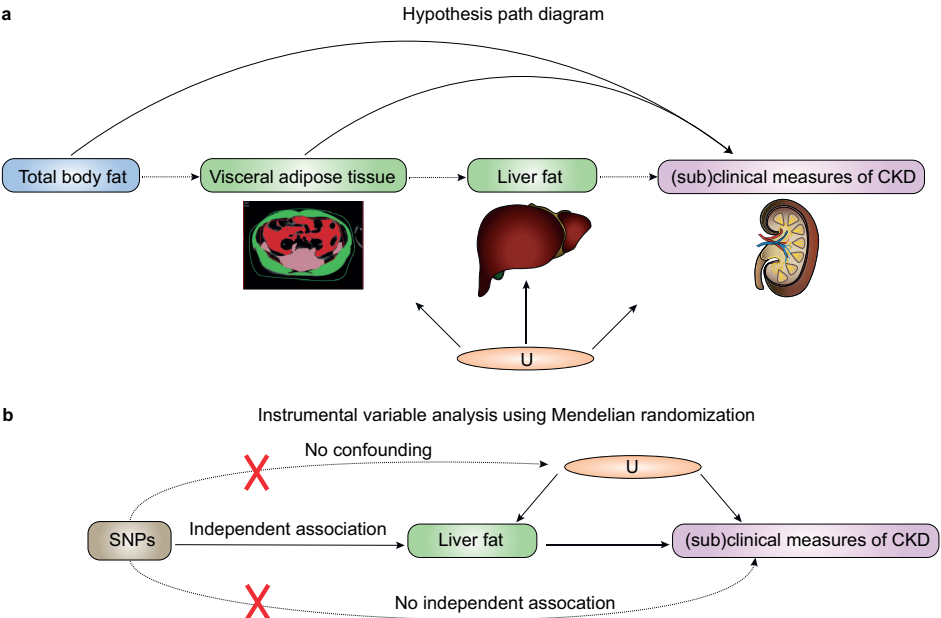


Figure 1. (a) Hypothesis path diagram. Assessed presumed effects of visceral adipose tissue and liver fat on (sub)clinical measures of CKD, and influences of confounders that are either known or unknown (U). (b) Instrumental variable analysis using Mendelian randomization. Assessed presumed effects of the instrumental variable (brown; single nucleotide polymorphism (SNP) genetic variants for non-alcoholic fatty liver disease (NAFLD)) on the exposure (green; liver fat); the instrumental variable does not associate with confounders that are either known or unknown (U).

Data collection

The participants were asked to bring all medication they were using to the study visit. All use of medication 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 grams/day). 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 total body fat (TBF) were assessed using the Tanita bio impedance balance (TBF-310, Tanita International Division, UK).

Laboratory measurements

Fasting blood samples were drawn from the antecubital vein after 5 min rest of the participant. Serum creatinine (mg/dl) was used to calculate the estimated GFR according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 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 LUMC.

Magnetic resonance imaging and spectroscopy

Magnetic resonance Imaging (MRI) was performed on a 1.5 Tesla MRI scanner (Philips Medical Systems, Best, the Netherlands). Visceral fat was imaged using three 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 cm for all three slides using in-house-developed software (MASS, Medis, Leiden, the Netherlands), and the mean VAT areas of the three slides was 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). Hepatic triglyceride content (HTGC) was quantified by proton magnetic resonance spectroscopy (¹H-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 four 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

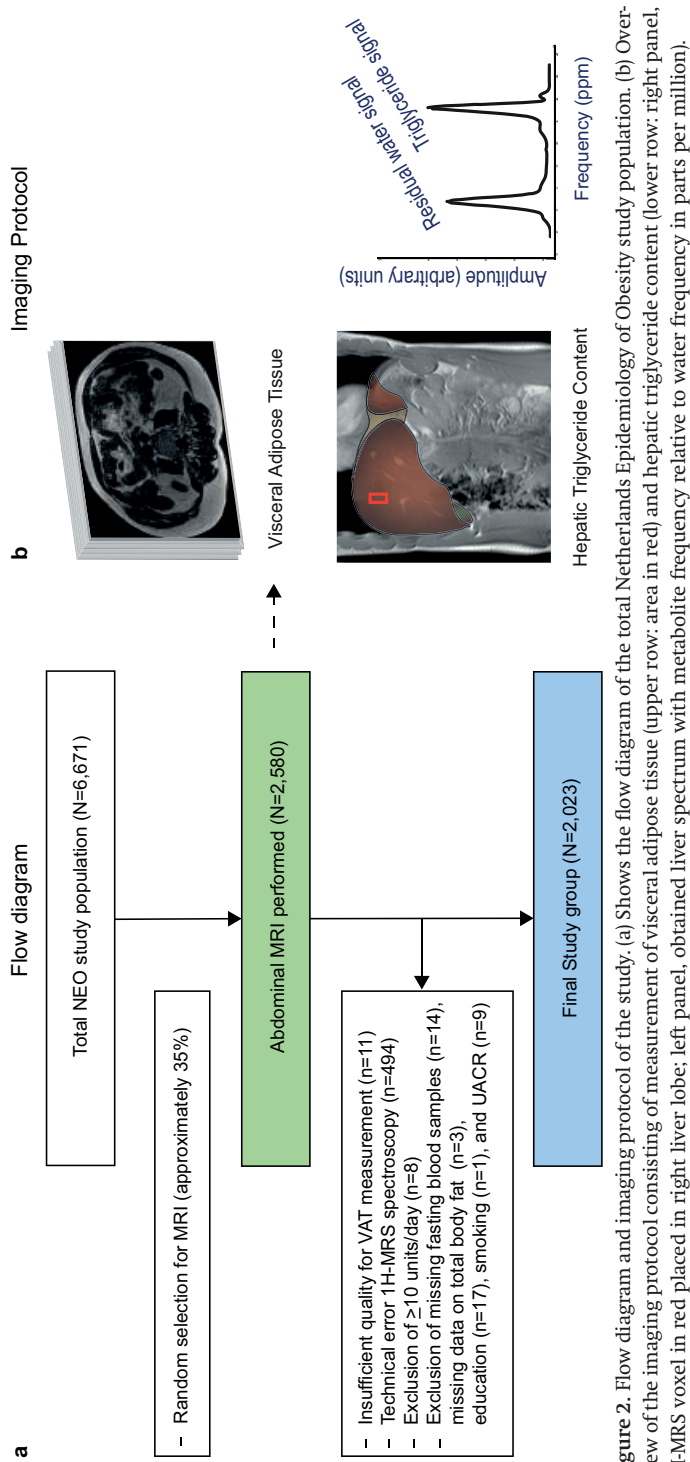


Figure 2. Flow diagram and imaging protocol of the study. (a) Shows the flow diagram of the total Netherlands Epidemiology of Obesity study population. (b) Overview of the imaging protocol consisting of measurement of visceral adipose tissue (upper row: area in red) and hepatic triglyceride content (lower row: right panel, ¹H-MRS voxel in red placed in right liver lobe; left panel, obtained liver spectrum with metabolite frequency relative to water frequency in parts per million).

as (signal amplitude of triglyceride [arbitrary unit]) / (signal amplitude of water [arbitrary unit]) x 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/m² 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/m² 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 one 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 overrepresentation of overweight and obese participants in the NEO study may introduce bias, because of the skewed BMI distribution in the NEO population. Weighting towards 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 (SD), median (25th, 75th percentiles) or as percentage, and stratified by fatty liver, defined as a HTGC as 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 a SD of one. 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). 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 towards percentages increase: $(\exp(\beta) - 1) \cdot 100$ with 95% confidence intervals per standard deviation of VAT and HTGC. In addition, we performed logistic regression analyses and calculated odds ratios (ORs) with 95% confidence intervals of microalbuminuria or impaired renal function (<60 ml/min/1.73m²). 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 total body fat, for the study of specific effects of abdominal fat it is important to adjust the associations for total body fat (24). Therefore, all models were additionally adjusted for total body fat. 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, Texas, USA), version 12.0.

Selection of genetic instruments and Mendelian randomization approach

Genetic instrumental variables for NAFLD were selected from the largest GWAS meta-analysis on computed tomography (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, four were also significantly associated with histological evidence for NAFLD. These four 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 per 1.73 m², 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 four 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. While 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 (IVW) linear regression of 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 effect on the outcome per doubling of the odds for histologically proven NAFLD in the population, by multiplying the causal effect estimate from the IVW-regression by 0.693 (i.e. log_e 2). As *GCKR* likely has pleiotropic effects on glucose metabolism, we also calculated the causal effect estimates without excluding *GCKR* as a sensitivity analysis. Mendelian Randomization analyses were performed in R version 3.4.3, Vienna, Austria, 2016] using the TwoSample MR R-package.

Table 1. Characteristics of participants stratified by liver fat (n=2,023)

Characteristic	HTGC≤5.56 (71%)	HTGC>5.56 (29%)
Age (years)	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
Glucose lowering therapy (%)	1	5
Antihypertensives (%)	15	31
ACE inhibitors/Angiotensin-II antagonists (%)	8	19
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/1.73m ²)	86.1 (10.8)	86.6 (14.8)
eGFR >90 mL/min/1.73m ²	42	43
eGFR 60-90 mL/min/1.73m ²	56	56
eGFR <60 mL/min/1.73m 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

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). Sex hormone use by woman*; contraceptive pill or hormone replacement therapy. Microalbuminuria was defined as UACR ≥2.5 mg/mmol for men and ≥3.5 mg/mmol for woman. ACE, angiotensin-converting-enzyme; eGFR, estimated Glomerular Filtration Rate according to the CKD-epi formula; HTGC, hepatic triglyceride content; UAE, urinary albumin excretion; UACR, urinary albumin creatinine ratio.

Table 2. Differences in UACR with 95% confidence intervals and eGFR per SD of visceral fat and liver fat

Determinant	Percent difference (95% CI) UACR		
	Total population	Men	Women
VAT (SD=55.4cm ²)			
Crude	-4 (-8, 0)	3 (-2, 8)	7 (0, 15)
Multivariate	1 (-3, 6)	0 (-4, 5)	4 (-3, 11)
+ TBF	5 (-1, 12)	2 (-5, 8)	13 (1, 27)
+ HTGC	5 (-1, 12)	1 (-6, 8)	14 (1, 28)
HTGC (SD=7.9%)			
Crude	0 (-4, 4)	3 (-3, 8)	3 (-3, 9)
Multivariate	1 (-3, 5)	2 (-3, 7)	1 (-5, 7)
+ TBF	3 (-2, 7)	2 (-3, 8)	2 (-4, 9)
+ VAT	2 (-3, 6)	2 (-3, 8)	0 (-7, 6)
Determinant	Difference (95% CI) per ml/min/1.73m ² lower eGFR		
	Total population	Men	Women
VAT (SD=55.4cm ²)			
Crude	0.52 (-0.12, 1.17)	0.33 (-0.58, 1.24)	1.25 (0.12, 2.39)
Multivariate	0.17 (-0.56, 0.90)	0.18 (-0.76, -1.12)	0.30 (-0.89, 1.49)
+ TBF	0.30 (-0.65, 1.24)	0.76 (-0.39, 1.92)	-0.12 (-1.79, 1.55)
+ 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)
Multivariate	-0.42 (-1.03, 0.19)	-0.72 (-1.48, 0.04)	-0.11 (-1.03, 0.81)
+ TBF	-0.49 (-1.14, 0.16)	-0.65 (-1.46, 0.15)	-0.29 (-1.26, 0.68)
+ VAT	-0.60 (-1.29, 0.09)	-0.84 (-1.69, 0.01)	-0.31 (-1.31, 0.70)

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 percentages increase or decrease in UACR (mg/mmol) or as difference in lower eGFR (ml/min/1.73m²) per standard deviation in visceral adipose tissue (VAT) or hepatic triglyceride content (HTGC). Multivariate analysis was adjusted for age, sex, ethnicity, education, tobacco smoking, alcohol consumption, fasting state during ¹H-MRS, and in women additionally adjusted for current use of estrogens and menopausal state.

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, ¹H-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 total body fat

Table 3. Odds ratio's with 95% confidence intervals for risk of microalbuminuria and impaired renal function per SD change in visceral fat and liver fat

Determinant	OR (95% CI) for microalbuminuria		
	Total population	Men	Women
VAT (SD=55.4cm ²)			
Crude	1.54 (1.19, 1.99)	1.76 (1.32, 2.34)	1.87 (1.31, 2.65)
Multivariate	1.75 (1.34, 2.30)	1.66 (1.19, 2.33)	1.92 (1.19, 3.09)
+ TBF	2.16 (1.29, 3.61)	1.71 (0.95, 3.08)	3.03 (1.51, 6.08)
+ 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)
Multivariate	1.36 (1.05, 1.76)	1.36 (0.96, 1.93)	1.32 (0.98, 1.77)
+ TBF	1.34 (1.02, 1.77)	1.26 (0.83, 1.93)	1.35 (0.96, 1.89)
+ VAT	1.20 (0.85, 1.70)	1.17 (0.69, 1.98)	1.13 (0.76, 1.69)
Determinant	OR (95% CI) for impaired renal function		
	Total population	Men	Women
VAT (SD=55.4cm ²)			
Crude	1.02 (0.75, 1.37)	0.80 (0.40, 1.59)	1.52 (1.09, 2.13)
Multivariate	1.00 (0.64, 1.56)	0.69 (0.32, 1.49)	1.40 (0.84, 2.34)
+ TBF	1.10 (0.75, 1.63)	1.10 (0.63, 1.90)	1.35 (0.74, 2.47)
+ 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)
Multivariate	0.71 (0.45, 1.12)	0.23 (0.06, 0.88)	0.92 (0.64, 1.32)
+ TBF	0.70 (0.47, 1.04)	0.39 (0.17, 0.89)	0.83 (0.58, 1.19)
+ VAT	0.65 (0.42, 0.99)	0.36 (0.14, 0.94)	0.74 (0.50, 1.09)

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 visceral adipose tissue (VAT) or hepatic triglyceride content (HTGC). Multivariate analysis was adjusted for age, sex, ethnicity, education, tobacco smoking, alcohol consumption, fasting state during ¹H-MRS, and in women additionally adjusted for current use of estrogens and menopausal state. 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/1.73m².

(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 years) and 53% were women. For the total population, mean BMI was 25.9 (4.0) kg/m², and mean eGFR was 86.2 (12.2) ml/min/1.73m². 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. 1.5% of the total study population had both diabetes and microalbuminuria. Mildly to moderately impaired renal function (eGFR ≤ 60 ml/min/1.73m²) was present in 2.0% of the study population. The baseline

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
eGFR _{crea} (log-transformed)	PNPLA3	rs738409	G	3.24	-0.0016 (-0.0038, 0.0006)	0.14
	LYPLAL1	rs12137855	C	1.21	-0.0004 (-0.0026, 0.0018)	0.71
	NCAN	rs2228603	T	1.9	-0.0020 (-0.0055, 0.0015)	0.27
	GCKR	rs780094	T	1.18	0.0063 (0.0045, 0.0081)	4.8x10⁻¹²
N _{max} 118,460	Causal effect estimator				-0.0004 (-0.005, 0.004)	0.87
Micro-albuminuria (odds ratio)	PNPLA3	rs738409	G	3.24	0.97 (0.93, 1.02)	0.31
	LYPLAL1	rs12137855	C	1.21	0.97 (0.93, 1.02)	0.29
	NCAN	rs2228603	T	1.9	1.05 (0.97, 1.14)	0.23
	GCKR	rs780094	T	1.18	1.09 (1.04, 1.13)	4.9x10⁻⁰⁵
N _{max} 54,116	Causal effect estimator				1.00 (0.93, 1.07)	0.97
Impaired renal function (odds ratio)	PNPLA3	rs738409	G	3.24	1.03 (0.99, 1.07)	0.13
	LYPLAL1	rs12137855	C	1.21	0.99 (0.97, 1.05)	0.57
	NCAN	rs2228603	T	1.9	1.01 (0.95, 1.07)	0.73
	GCKR	rs780094	T	1.18	0.98 (0.95, 1.01)	0.20
N _{max} 118,147	Causal effect estimator				1.01 (0.99, 1.03)	0.21

NAFLD denotes non-alcoholic fatty liver disease; eGFR_{crea}, estimated glomerular filtration rate based on serum creatinine, using sex-and age-adjusted residuals of logarithm; CKD, chronic kidney disease. Data presented as per-allele effect on outcome for odds-for-NAFLD (i.e. histological evidence of NAFLD) increasing allele. Per-allele effect on biopsy-proven NAFLD (odds ratio) as observed by Speliotes et al (23).

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.

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=55.4cm², -4 percent difference, 95% CI -8, 0) and HTGC (per SD=7.9%, 0 percent 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 percent difference per SD in VAT (95% CI -1, 12). The associations between HTGC and UACR was 2 percent 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 percent 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/1.73m², 95% CI 0.12, 2.39), but not in men (per SD 0.33 ml/min/1.73m², 95% CI -0.58, 1.24). No associations were found between HTGC and renal function for both men (per SD -0.65 ml/min/1.73m², 95% CI -1.41, 0.10) and women (per SD 0.25 ml/min/1.73m², 95% CI -0.64, 1.14) in unadjusted analyses. In adjusted analyses, neither VAT (per SD 0.52 ml/min/1.73m², 95% CI -0.46, 1.50) nor HTGC (per SD -0.60 ml/min/1.73m², 95% CI -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 two-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 four 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.

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 MDCT cohort previously investigated the association of SAT/VAT on prevalent CKD, found that neither VAT nor SAT (assessed by computed tomography) was independently associated with CKD, using eGFR based on the creatinine-based MDRD equation (32). However, in a subsequent study VAT was associated with microalbuminuria in men, whereas SAT was more associated with microalbuminuria in women (33). 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 relations between visceral fat and cardiometabolic outcomes in women than in men (34–36). 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, are likely involved (37). In men the results for VAT and microalbuminuria were not statistically significant, and might be due to the lack 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/1.73m² in our sample. Furthermore, it can be argued whether the use of ACE inhibitors/Angiotensin-II antagonists may confound the results, additional adjustment for ACE inhibitors/Angiotensin-II antagonists did not markedly change the results (**Supplementary Table 1**).

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 (38). 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 ¹H-MRS for the measurement of ectopic lipids. A recent small (n=400) Chinese study evaluating the association between liver fat measured by ¹H-MRS however, did show that NAFLD was independently associated with CKD after adjustment for VAT (39). 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 VAT-specific SNPs (40). 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 hepatic triglyceride content in over two thousand individuals, which is considered the gold standard technique for the non-invasive measurement of liver fat (41). Previous studies showed that the presence of NAFLD was associated with an increased risk of CKD were 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 WC poorly predict the volumes of internal body fat compartments, which could lead to underestimation of associations with chronic disease risks (42).

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-h 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-h urine albumin excretion and UACR is more reliable than urinary albumin excretion for the assessment of microalbuminuria in spot urine samples (43). Furthermore, although the CKD-EPI formula has proven to be more accurate and precise for estimating renal function than the older MDRD formula, it has not been validated ≥ 90 ml/min/1.73m² (13). Also the error of eGFR in the normal to high range of renal function is quite substantial (44). 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 1.5T using conventional non-water-saturated T1 weighted images and ¹H-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 (45). Moreover, recent technical advances have also enabled non-invasive quantification of renal sinus fat volume, pararenal fat, and intra-renal triglyceride content (46,47), which are other potentially important ectopic fat compartments related to the kidney (e.g. fatty kidney) (48). Especially pararenal fat is of great interest considering this is an 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 (49). In addition, future studies are needed investigating 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.

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Supplemental Table 1. Differences in UACR with 95% confidence intervals and eGFR per SD of visceral fat and liver fat with additional adjustment for ACE inhibitors / Angiotensin-II antagonists.

Determinant	Percent difference (95% CI) UACR		
	Total population	Men	Women
VAT (SD=55.4cm ²)			
Crude	-4 (-7, 0)	3 (-2, 8)	7 (0, 15)
Multivariate	1 (-3, 5)	0 (-5, 4)	3 (-4, 11)
+ TBF	4 (-1, 11)	1 (-5, 8)	13 (1, 26)
+ HTGC	4 (-1, 11)	1 (-6, 8)	13 (1, 28)
HTGC (SD=7.9%)			
Crude	0 (-4, 4)	3 (-3, 8)	3 (-3, 9)
Multivariate	1 (-3, 5)	1 (-4, 6)	1 (-5, 7)
+ TBF	2 (-2, 7)	2 (-3, 8)	2 (-4, 9)
+ VAT	1 (-3, 6)	2 (-3, 8)	0 (-7, 6)
Determinant	Difference (95% CI) per ml/min/1.73m ² lower eGFR		
	Total population	Men	Women
VAT (SD=55.4cm ²)			
Crude	0.52 (-0.12, 1.17)	0.33 (-0.58, 1.24)	1.25 (0.12, 2.39)
Multivariate	0.26 (-0.49, 1.00)	0.23 (-0.74, -1.20)	0.43 (-0.79, 1.66)
+ TBF	0.37 (-0.59, 1.32)	0.78 (-0.37, 1.93)	0.04 (-1.68, 1.75)
+ HTGC	0.58 (-0.40, 1.56)	1.00 (-0.17, 2.17)	0.19 (-1.57, 1.95)
HTGC (SD=7.9%)			
Crude	-0.19 (-0.79, 0.41)	-0.65 (-1.41, 0.10)	0.25 (-0.64, 1.14)
Multivariate	-0.37 (-0.99, 0.25)	-0.69 (-1.47, 0.09)	0.04 (-0.99, 0.90)
+ TBF	-0.46 (-1.11, 0.21)	-0.64 (-1.45, 0.17)	-0.23 (-1.23, 0.76)
+ VAT	-0.57 (-1.27, 0.13)	-0.83 (-1.68, 0.02)	-0.27 (-1.30, 0.76)

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 percentages increase or decrease in UACR (mg/mmol) or as difference in lower eGFR (ml/min/1.73m²) per standard deviation in visceral adipose tissue (VAT) or hepatic triglyceride content (HTGC). Multivariate analysis was adjusted for age, sex, ethnicity, education, tobacco smoking, alcohol consumption, fasting state during ¹H-MRS, ACE inhibitors/angiotensin-II antagonists and in women additionally adjusted for current use of estrogens and menopausal state.

