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Nephrin is produced in visceral adipose tissue in humans and associates with insulin resistance

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A B S T R A C T

Aims: Nephrinuria and podocinuria have been proposed as early markers of podocytopathy. We explored whether nephrin and podocin are expressed in subcutaneous (SAT) and visceral adipose tissue (VAT), and report their determinants.

Methods: Forty-one subjects with obesity (OS, age: 51 [41–57] yrs, M/F: 6/34) and twenty-nine lean controls (HC, age: 43 [35–51] yrs, M/F: 9/20) were studied. During metabolic bariatric surgery (MBS) VAT and SAT samples were collected. Only SAT samples were collected from HC. VAT samples from lean surgical patients served as an additional comparison group. Nephrin and podocin mRNA and protein expression were assessed in VAT/SAT, and nephrin and podocin levels were measured in plasma and urine.

Results: Podocin was not expressed in SAT or VAT. Nephrin mRNA was detected in VAT but not SAT, and nephrin protein expression was higher in OS. In plasma, low levels of nephrin were detected, which were directly associated with insulin sensitivity. Urinary nephrin exhibited an inverse association with insulin sensitivity, which persisted two years after MBS.

Conclusions: Nephrin is ectopically produced in VAT. Circulating and urinary nephrin show opposite associations with insulin sensitivity, compatible with early nephrin loss into the urine and compensatory regulation of VAT derived nephrin. These findings warrant further investigation.

1. Introduction

Incidence and prevalence of obesity and type 2 diabetes (T2D) are dramatically rising over the next decades. According to the World Health Organization (WHO), in 2022, over 890 million adults were living with obesity, and this number is estimated to further increase by 2030, with approximately one billion people expected to be affected [1]. In fact, the global burden of such epidemiologic emergency resides in the increased prevalence of chronic complications linked to such diseases; among these, chronic kidney disease (CKD), expected to involve one in ten humans worldwide [2], and to become the fifth leading cause of death by 2040 [3].

Obesity is recognized as a low-grade inflammatory state accompanied by reduced levels of anti-inflammatory adipokines [4]. The increased body mass, in its initial stage, requires an increased blood perfusion through all organs, including the kidneys, thus generating a hyperfiltration phase that leads to glomeruli enlargement and decreased podocyte density [5]. The process of kidney damage in subjects with

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obesity is, however, quite complex and partially different from kidney involvement during the course of T2D, and includes the accumulation of free fatty acids (FFA) in mesangial cells, podocytes, and tubular epithelial cells, increasing local inflammation and fibrosis [5], promoting cell senescence [6] and influencing renal haemodynamics [7]. In non-diabetic, obese individuals, metabolic surgery reduces 24 h albuminuria and blood urea nitrogen while an effect on GFR decline has not been demonstrated [8,9].

On these premises, it is important to establish strategies that can prevent progressive kidney damage associated with obesity and other chronic non-transmissible diseases. Identifying early biomarkers of tissue damage and studying their physiology and pathophysiology is crucial to halting disease progression in a timely manner.

In recent years, nephrin has emerged as a promising marker of early kidney injury. This large podocyte protein is a key structural component of the slit diaphragm, and its altered expression or localization disrupts filtration barrier integrity, leading to proteinuria and progressive renal dysfunction [10,11]. Nephrinuria is detectable before albuminuria [12] and has been reported across several kidney disease models, including diabetes, lupus, and pre-eclampsia [13–15], although its relevance in obesity-related kidney impairment remains poorly defined. Podocin, another podocyte-specific protein that interacts with nephrin at the slit diaphragm, has a less clearly characterized functional role [16].

Given the close relationship between obesity and CKD, we analysed VAT and SAT samples to determine whether nephrin or podocin are produced in human adipose tissue. Moreover, we measured their circulating and urinary levels and assessed their metabolic predictors.

2. Subjects and Methods

2.1. Participants and study design

The study cohort included forty-one subjects with obesity (OS; BMI 40.0 [36.8–43.3] kg/m^2, age: 51 [41–57] yrs, men/women: 6/34), with or without T2D, consecutively recruited in the Division of Digestive Surgery of Turku University Hospital in the years 2019–2020 and candidates to metabolic bariatric surgery (MBS). Twenty-nine sex-matched healthy, lean subjects (healthy controls, HC; BMI 23.1 [21.5–24.3] kg/m^2, age: 43 [35–51] yrs, men/women: 9/20) recruited on a volunteer basis via advertisements at the local newspapers served as controls. Inclusion and exclusion criteria for the study participants have been previously reported [17,18].

All participants underwent a screening visit to collect information regarding medical history and current therapies. Blood pressure was measured three times with OMRON 711 automatic blood pressure monitor (Omron Corporate, Kyoto, Japan) in subjects seated for at least 10 min, and the average of the last two measurements was recorded. Fasting blood and spot urine samples were collected and a standard (75-g) 2 h OGTT with frequent blood sampling (every 30 min) for measurement of plasma glucose, insulin and C peptide was performed. According to current ADA criteria [19], 9 subjects had T2D; 3 of them were treated only with metformin, 2 received a combined therapy (metformin + GLP-1 receptor agonist) and 4 were on a combination of metformin and SGLT-2 inhibitors. Sixteen subjects with OS had hypertension, and most were treated with a renin-angiotensin-aldosterone system (RAAS) inhibitor. Subjects underwent thorough metabolic evaluation, as described below. About 3 months after screening, OS participants underwent MBS and were re-studied 18–24 months after the surgery procedure. At the follow-up visit, body weight and blood pressure were recorded, and fasting blood and spot urine samples were collected. The study flowchart is shown in Supp. Fig. 1. The study protocol was approved by the Ethics Committee of the Hospital District of Southwestern Finland (ETMK Dnro: 52/1801/2β18, ClinicalTrials.gov: NCT04343469); participants signed a written informed consent. Due to the complexity of the study, not all subjects underwent every measurement. Supp. Fig. 2 presents an intersection plot illustrating the availability of data across participants. *Post-MBS follow-up visit* Twenty-four OS individuals were re-evaluated approximately 21 months after bariatric surgery. At this visit, participants provided plasma and urine samples, underwent a standard OGTT, and completed a full set of anthropometric measurements.

2.2. Euglycemic hyperinsulinemic clamp

Metabolic studies were performed at the facilities of the Turku PET Centre, Turku, Finland. Subjects with T2D were advised to suspend their medications one week prior the day of the study. After an overnight fast (10–12 h), two catheters were inserted in the antecubital veins of each arm, one for the administration of glucose and insulin, and the other for arterialized blood sampling. Subjects were instructed to void their bladders; then, the euglycemic clamp was started as previously described [20]. Briefly, a primed-continuous insulin infusion was given at a rate of 40 mU·m^{-2}·min^{-1}, followed by a variable 20% dextrose infusion, to maintain plasma glucose levels steady at 5 mmol/L. Blood sampling was done every 5, 30, and 60 min for the determination of plasma glucose, insulin, and free fatty acids, respectively. The experiment was concluded after 150 min.

2.3. Visceral and subcutaneous adipose tissue volume measurement

A subset of subjects (27 OS and 21 HC) underwent whole-body magnetic resonance imaging (MRI) (Supp. Fig. 2). In brief, quantification of abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) compartments was performed using SliceOmatic v4.3 (Tomovision, Montreal, QC, Canada), as previously described [21]. Abdominal adipose tissues were segmented from the level of the abdominal surface of the diaphragm to the pelvic brim, approximately spanning L1/L2 to L5/S1 [22]. The SAT compartment was defined as the adipose tissue located between the skin and the outermost boundary of the abdominal muscle wall [23]. VAT was defined as the adipose tissue enclosed within the inner border of the abdominal wall, comprising both intraperitoneal (*e.g.,* omental and mesenteric) and extraperitoneal (intraabdominal and intrapelvic) fat depots [24]. Fat volumes (cm^3) were converted to mass (kg) using a standard adipose tissue density of 0.9196 kg/L [22].

2.4. Nephrin and podocin determination in urine and plasma

Nephrin and podocin in biological fluids were measured using commercial ELISA kits. In urine they were quantified with SEA937Hu and SEA938Hu respectively, (Cloud-Clone Corp. Katy, Tx-USA); the assay sensitivity was less than 0.059 ng/ml for nephrin and 0.29 ng/ml for podocin; the inter-assay coefficient of variation (CV) was <12% and intra-assay CV <10% for both. In plasma samples, nephrin were measured with human Nephrin (NPHS1) Elisa Kit HUEB0905 (Assay-Genie, Dublin, Ireland); the assay sensitivity was 0.078 ng/ml; the inter-assay CV was 8.2% and intra-assay CV was 5.4%. Assays were run in duplicate.

2.5. Urine mRNA isolation

mRNA was isolated from frozen urine samples of a subgroup of 10 subjects using Urine Cell-Free Circulating RNA Purification Kits (cat.56900, Norgen Biotek, ON, Canada). After an equilibration period at room temperature, samples were centrifuged to remove cryoprecipitates, and 2 ml of thawed urine were processed following the manufacturer's instructions. Due to the low quantity of isolated RNA, the spectrophotometric quantification of nucleic acids was not reliable; therefore, the same volume of total RNA (8 μl) was used for the reverse transcription (RT), realized with PrimeScript RT Master mix (RR036A Takara Bio Inc., Shiga, Japan).

2.6. Fresh frozen VAT/SAT samples

During bariatric surgery, omental VAT and SAT biopsies were obtained from OS. Abdominal SAT samples were obtained from HC. Omental VAT samples of healthy controls collected in a prior study conducted in Turku PET Centre during operation to treat a benign condition (e.g. hernia surgery, rectopexy, fundoplication) were also analysed. Samples were rinsed with saline (0.9% NaCl) to eliminate residual blood and vascular tissue, sectioned into small fragments, rapidly frozen in liquid nitrogen, and subsequently stored at −80°C.

2.7. Adipose tissue mRNA extraction

Total RNA was extracted from 100-150 mg of VAT or SAT with RNasy lipid tissue mini kit (cat. 74804, Qiagen Hilden, Germany); after evaluation of RNA quantity and quality by spectrophotometry (NanoDrop 2000c, Thermo Fisher Scientific), 500 ng of RNA was reverse transcribed using PrimeScript RT Master mix (RR036A Takara Bio Inc., Shiga, Japan).

2.8. Gene expression

mRNA expression in urine and VAT was assessed in duplicate by QuantStudio3 real time instrument (Thermo Fisher Scientific) according to the standard fast procedure for the following TaqMan Gene Expression Assays (Thermo Fisher Scientific): nephrin: Hs00190446_m1; podocin Hs00387817_m1. The amount of the target gene, normalized respect to Actin gene (Hs01060665_g1), is given as $2^{-\Delta\Delta Ct}$, where Ct is the threshold cycle.

2.9. Digital PCR quantification

Since podocin was not detected in urine and VAT, absolute quantification of podocin mRNA was also performed in 10 urine and 15 VAT samples by using the Absolute Q Digital PCR system (Thermo Fisher Scientific) following the manufacturer's protocol (Supp. Fig. 3a and b). In brief, reactions were carried out in a final volume of 9 μl per well, using the same TaqMan assay above reported (Thermo Fisher Scientific) specific for the target gene podocin and actin. The results were generated by the QuantStudio™ Absolute Q™ Digital PCR Software v6.3.0 and expressed as copies/μl reaction

2.10. Western Blot analysis

Total proteins were extracted from 100 to 200 mg of VAT tissue using NP40 Lysis Buffer (FNN0021, Invitrogen-ThermoFisher MA-USA) added with Protease inhibitor cocktail (P2714, Sigma-Merck, Darmstadt-Germany). Briefly, 30 ug of protein extract was diluted in SDS-PAGE buffer (sodium dodecyl sulphate–polyacrylamide gel electrophoresis), heated at 100°C for 5 min, and separated on Any kD Mini-Protean TGX gels (Bio-Rad Laboratories, Italy). After transferring the samples to a polyvinylidene difluoride (PVDF) membrane (BioRad), it was treated with a blocking solution (3% milk in TTBS) and incubated overnight with the primary antibody Nephrin (PA5-20330 Invitrogen) and GAPDH (MAB374 Millipore). A final step with specific secondary HRP-conjugated antibodies enables bands detection by an enzymatic chemiluminescence reaction (Clarity Western ECL, BioRad). Protein signals were acquired on ChemiDoc Instrument (Biorad), and band intensity was evaluated using ImageJ v1.52 software; each sample value was normalized by the intensity of GAPDH. Data were expressed as arbitrary units of Optical Density (OD).

2.11. Calculations

Insulin-stimulated glucose disposal (M value) was used as a measure of whole-body insulin sensitivity, as previously described [25]. Oral glucose insulin sensitivity was calculated from the OGTT data as previously described [26]. Estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [27].

2.12. Biochemical analyses

Plasma glucose was determined in duplicate by glucose oxidase method (Analox GM9, Analox Instruments, London, UK). Serum insulin was measured by an automatized electro-chemiluminescence immunoassay (Cobas e601, Roche Diagnostics GmbH, Mannheim, Germany). Serum steady-state free fatty acids (ssFFA) were measured using an enzymatic assay (ACS-ACOD, Wako Chemicals GmbH, Neuss, Germany) on a Cobas c702 automatic analyzer (Roche Diagnostics GmbH). Serum adiponectin, leptin, and TNFα were measured using HADCYMAG-61 K panel (Merck/Millipore).

2.13. Statistical analysis

Continuous variables are summarized as mean ± SD, or as median [interquartile range]. The normality of distribution was assessed using the Shapiro-Wilk test; variables that were not normally distributed were square root or logarithmically transformed before analysis, as appropriate [28]. Log- or square root-transformed data are shown in the correlation analyses, whereas raw data are presented in the tables for readability. Between groups comparisons were performed by *t*-test or Mann-Whitney *U* test, as appropriate. Correlations between biomarkers, clinical parameters and circulating metabolites were performed using either Pearson's correlation coefficient, or Spearman *rho*, as appropriate. For multivariate analyses, linear regression was performed with BMI, continuous covariates, and categorical factors entered as predictors. Regression coefficients (β) together with their 95% confidence interval are reported. Because of the small age difference between groups, we performed a sensitivity analysis matching participants by age. In addition, given the relatively small number of individuals with T2D, we conducted a second sensitivity analysis excluding these subjects. Finally, because several participants had plasma nephrin concentrations below the limit of detection (LOD), values below the LOD were imputed as LOD/2 for sensitivity analyses. These imputation-based results are presented in the Supplement, whereas all primary analyses in the main text are based on the actual measured data. A $p < 0.05$ was considered significant. Analyses were performed using JMP version 13.0 (SAS Institute, Cary, NC, USA). Images were created using ggplot package on *R* Studio [29].

3. Results

3.1. Baseline

The anthropometric and biochemical characteristics of the study participants are shown in Table 1. In the HC group, 9 of the 20 participants were men, whereas in the OS group only 6 of 34 participants were men ($p = 0.17$). OS exhibited reduced insulin sensitivity, as reflected by lower M values, OGIS, and higher HOMA-IR. In contrast, there were no differences in kidney function as from eGFR (97 ± 15 *vs* 97 ± 12 ml/min/1.73 m^2, in OS and HC, respectively) and albuminuria, within the normal range in both groups. As expected, OS had a higher percentage of total fat mass and expanded VAT and SAT compared with HC. They also displayed a pro-inflammatory profile, characterized by elevated CRP and a trend toward higher circulating cytokine levels, although only TNFα reached statistical significance. As expected, leptin concentrations were higher and plasma adiponectin levels were lower in OS group.

3.2. SAT/VAT analyses

Quantitatively relevant nephrin expression in VAT was found, while

Table 1
Anthropometric and biochemical characteristics of the study participants.

	HC	OS		*p* value
		Before MBS	After MBS	
Age (years)	43 [35–51]	51 [41–57]	53 [42–59]	0.04
Sex (M/F)	9/20	7/34	5/19	0.17
BMI (kg/m^2)	23.1 [21.5–24.3]	40.0 [36.8–43.3]	28.5 [26.8–32.6]	<0.0001
Fat-free-mass (kg)	46.0 [40.7–51.1]	53.0 [48.7–61.7]	49.4 [45.4–57.4]	0.0004
NGT/IGT&IFG/T2D	24/4/0	16/16/9	18/6/0*	0.0003
HbA_{1c} (%)	5.3 [5.1–5.4]	5.4 [5.2–5.7]	5.3 [5.0–5.5]*	0.002
HbA_{1c} (mmol/mol)	34 [32–36]	36 [33–39]	34 [32–37]*	0.002
Systolic BP (mmHg)	121 ± 11	138 ± 15	132 ± 17*	<0.0001
Diastolic BP (mmHg)	77 ± 8	85 ± 9	78 ± 10*	0.0008
Creatinine (mg/dl)	0.84 ± 0.12	0.78 ± 0.15	0.70 ± 0.14*	0.02
eGFR (ml/min/1.73 m^2)	97 ± 12	97 ± 15	102 ± 13*	0.79
M value (μmol/Kg/min)	42.9 [32.1–54.3]	14.2 [9.8–20.7]	–	<0.0001
OGIS (ml/min/m^2)	422 ± 44	342 ± 57	447 ± 69*	<0.0001
HOMA-IR	1.8 [0.8–1.6]	3.5 [2.3–5.4]	1.3 [0.9–1.8]	<0.0001
CRP (mg/dl)	0.4 [0.2–0.8]	2.8 [2.0–4.8]	0.5 [0.3–1.3]*	<0.0001
Leptin (pg/ml)	273 [165–485]	1859 n	–	<0.0001
TNF-α (pg/ml)	1.3 [1.1–1.6]	1.7 [1.3–2.3]	–	0.02
Adiponectin (ng/ml)	55.2 [28.5–83.0]	51.5 [35.8–63.1]	–	0.74
SAT (L)	4.6 [3.6–5.4]	15.8 [11.0–19.9]	10.1 [8.4–11.8]*	<0.0001
VAT (L)	1.3 [0.9–3.7]	4.8 [3.6–7.1]	2.4 [1.8–3.7]*	<0.0001
ACR (mg/mmol)	0.4 ± 0.1	0.7 ± 0.7	0.8 ± 0.9	0.03
Hypertension (n)	0	16	8	<0.0001
Use of RAAS inhibitor (n)	0	14	7	0.0002

P-value refers to group comparisons between OS and HC. *indicates significant differences for paired analyses for the patients who had data before and after surgery.
Abbreviations: ACR: albumin to creatinine ratio; BMI, body mass index; CRP: C-reactive protein; NGT: normal glucose tolerance; IGT: impaired glucose tolerance; T2D: type 2 diabetes; eGFR: estimated glomerular filtration rate; OGIS: oral glucose insulin sensitivity; RAAS: renin-angiotensin-aldosterone system; SAT: subcutaneous adipose tissue; TNFα: tumour necrosis factor-alpha; VAT: visceral adipose tissue.

nephrin mRNA was undetectable in SAT samples (data not shown). Podocin mRNA expression was also investigated in VAT samples, but it was not detectable.

To address whether nephrin mRNA expression occurs in VAT only in obesity, we also analysed VAT samples collected in a prior study from 14 lean surgical patients. We found that nephrin mRNA was expressed in VAT samples from lean surgical patients and was expressed at higher levels than in OS (2.87 [1.75–4.65] *vs* 1.39 [0.89–2.49] arbitrary units (AU) respectively, $p = 0.004$) (Fig. 1a). Across all subjects, VAT nephrin mRNA expression was inversely associated with BMI, even when adjusting for sex (Fig. 1b, Supp. Table 1). On the contrary, there was no association between VAT nephrin mRNA expression and the degree of insulin sensitivity, or with eGFR. VAT nephrin mRNA expression did not differ among sexes, but when accounting for BMI women exhibited higher VAT nephrin mRNA expression compared to men (Supp. Table 1).

We also detected nephrin protein expression in VAT, which was significantly higher in OS compared to lean surgical patients (0.41 [0.25–0.49] *vs* 0.22 [0.15–0.34] AU respectively, $p = 0.026$) (Fig. 1c). Sex was not related with VAT nephrin protein levels in either univariate or BMI-adjusted analysis; however, there was an interaction effect between sex and BMI, indicating a stronger effect of BMI in men (Supp. Table 1).

3.3. Urine and plasma measurements

Podocin was not detectable in urine samples with gold standard techniques (Suppl Fig. 3a and b). On the contrary, nephrin, even in a small amount, was found in urine, and its levels were significantly higher in OS compared to HC (0.39 [0.28–0.52] *vs* 0.22 [0.14–0.32] ng/ml respectively, $p = 0.0003$). Interestingly, urinary nephrin levels were directly associated with BMI (Fig. 2a). Urinary nephrin levels were also directly associated with plasma glucose and plasma insulin, and were inversely associated with insulin sensitivity (Fig. 2b,c and Fig. 3a). Although urinary nephrin showed several univariate associations with metabolic parameters (Fig. 3a), only its association with the M value remained statistically significant after adjustment for BMI (Fig. 3b).

With respect to renal function, urinary nephrin levels were inversely associated with eGFR (Fig. 2d); whereas no relationship was observed between urinary nephrin levels and the albumin to creatinine ratio (ACR). In a regression model adjusting for age and insulin sensitivity (M value), both the square-root-transformed M value (β = -0.09, 95% C.I. −0.13 to −0.05, $p < 0.0001$) and eGFR (β = -0.001, 95% C.I. −0.016 to −0.003, $p = 0.004$) independently predicted urinary nephrin concentrations.

Urinary nephrin levels were also inversely associated with VAT nephrin mRNA expression (Fig. 4a). In a regression model predicting urinary nephrin levels VAT nephrin mRNA was the only significant predictor after adjustment for BMI (β = -0.33, 95% C.I. −0.62 to −0.04, p value = 0.029).

Finally, given the evidence of ectopic nephrin production, we examined whether nephrin is also detectable in the circulation. Plasma samples from a subset of the original cohort (17 OS and 18 HC) were analysed. Plasma nephrin concentrations were below the detection limit in 10 of 17 OS participants and in 4 of 18 HC participants, with OS showing lower values overall, although the difference did not reach statistical significance 0.09 [0.05–0.11] *vs* 0.18 [0.09–0.23] ng/ml, in OS and HC respectively, ($p = 0.057$). The clinical characteristics of individuals with detectable versus undetectable plasma nephrin are presented in Supp. Table 2. Participants with undetectable plasma nephrin had a higher BMI, and exhibited significantly elevated VAT nephrin mRNA expression.

Among the 21 participants with detectable plasma nephrin, circulating nephrin levels were directly associated with insulin sensitivity (M value (Figs. 3a and 4b), whereas no relationship was observed with BMI (Fig. 3a). Plasma nephrin also showed no association with urinary nephrin levels ($p = 0.18$). Using all data, with plasma nephrin values below the LOD imputed as LOD/2, the association between plasma nephrin and insulin sensitivity was unaltered. In this analysis, an inverse association between plasma nephrin and BMI also became evident (Supplementary Material).

Neither plasma nor urinary nephrin was related to hypertension or the use of RAAS inhibitors in univariate analyses or in regression models adjusted for BMI (data not shown).

3.4. Post MBS assessment

Twenty-four OS were re-studied approximately 21 months after MBS. These individuals had lost an average of 11 BMI units; as expected, serum creatinine decreased ($p < 0.0001$) and eGFR increased ($p = 0.005$). Urinary nephrin levels showed only a small, non-significant reduction following weight loss (0.46 [0.31–0.57] *vs* 0.41 [0.25–0.57] ng/ml pre *vs* post, $p = 0.40$) and were no longer associated with eGFR ($p = 0.33$). Fig. 4d and e illustrate individual changes in urinary nephrin and the correlation between pre and-post surgery values; although MBS

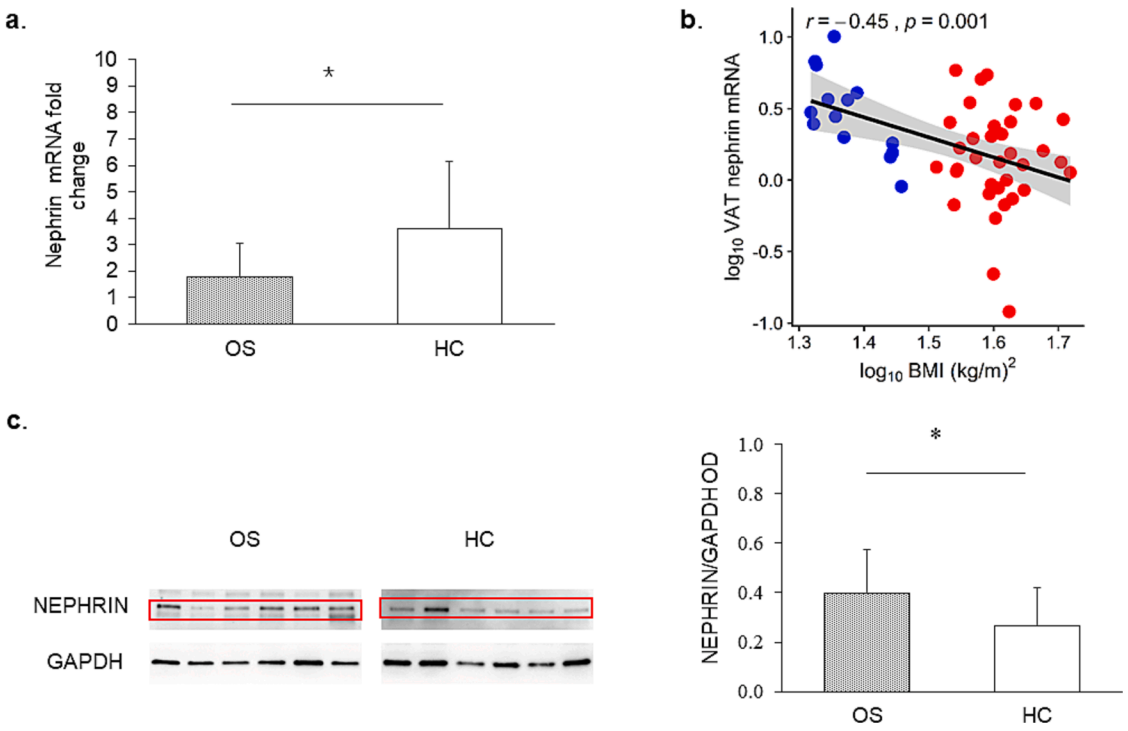

Fig. 1. VAT nephrin mRNA expression in patients with obesity (OS) and lean controls (HC) (Fig. 1a) and its correlation with BMI (Fig. 1b). Nephrin protein expression in VAT of OS and HC: representative blots (left) and densitometric analysis of all sujects (OS n = 32, HC n = 12) (Fig. 1c). Red circles: OS, blue circles: HC.

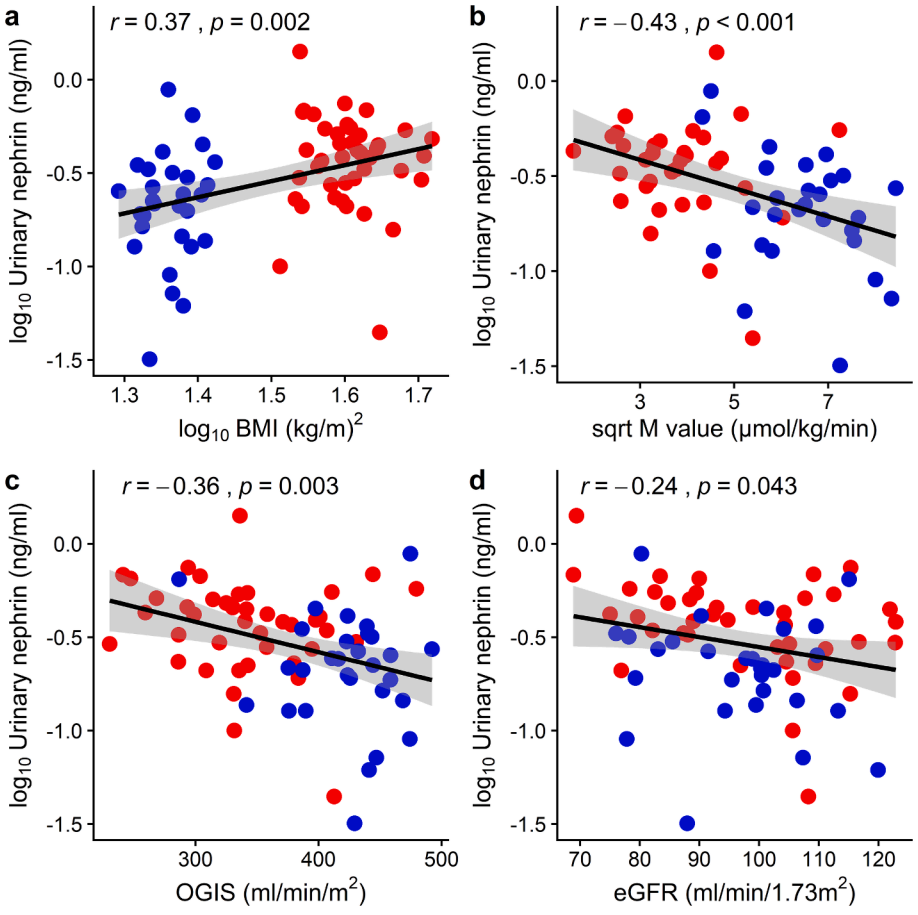

Fig. 2. Urinary nephrin correlations with anthropometric (Fig. 2a), metabolic parameters (Fig. 2b-c) and eGFR (Fig. 2d) are reported . Red circles: OS, blue circles: HC.

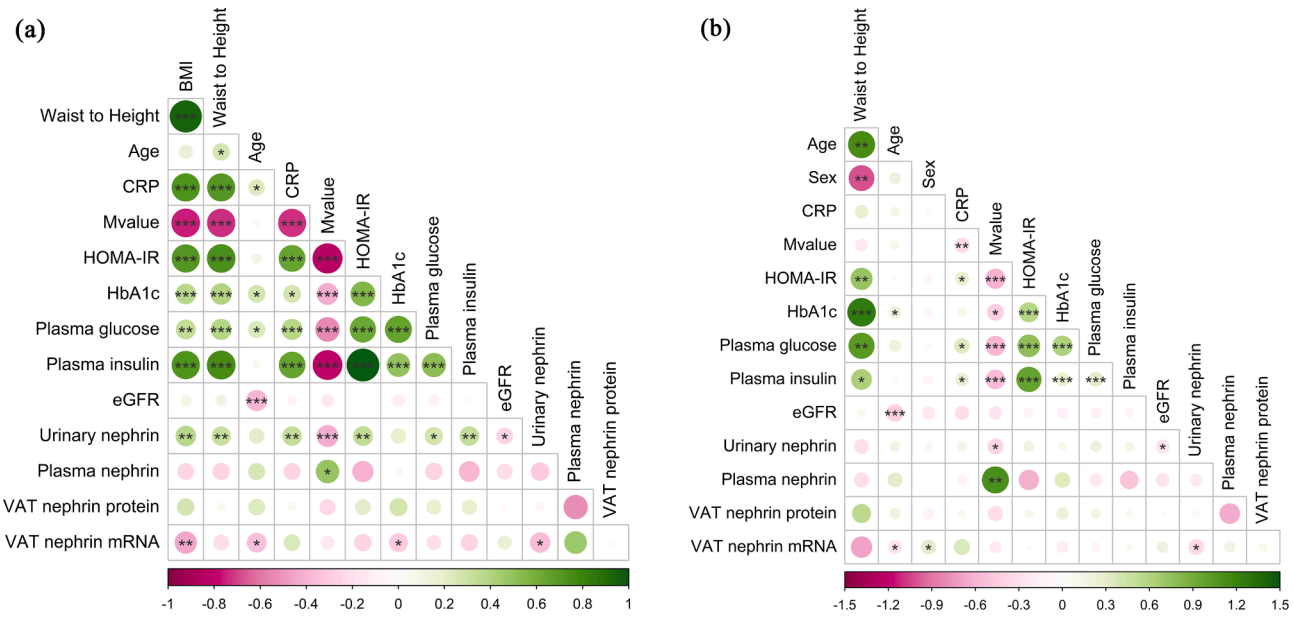

Fig. 3. Correlation matrices between all measured nephrin parameters and clinical variables are shown in Fig. 3a, and BMI-adjusted regression analyses of the same parameters are shown in Fig. 3b. All available data were reported, including samples from surgical HC. Because surgical HC did not undergo a euglycemic clamp or an OGTT, HOMA-IR is also shown. Variables were square-root or logarithmically transformed before analysis, as appropriate. The colour scale and circle size represent the strength of the correlation (Fig. 3a) or of the standardized regression coefficient (Fig. 3b). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

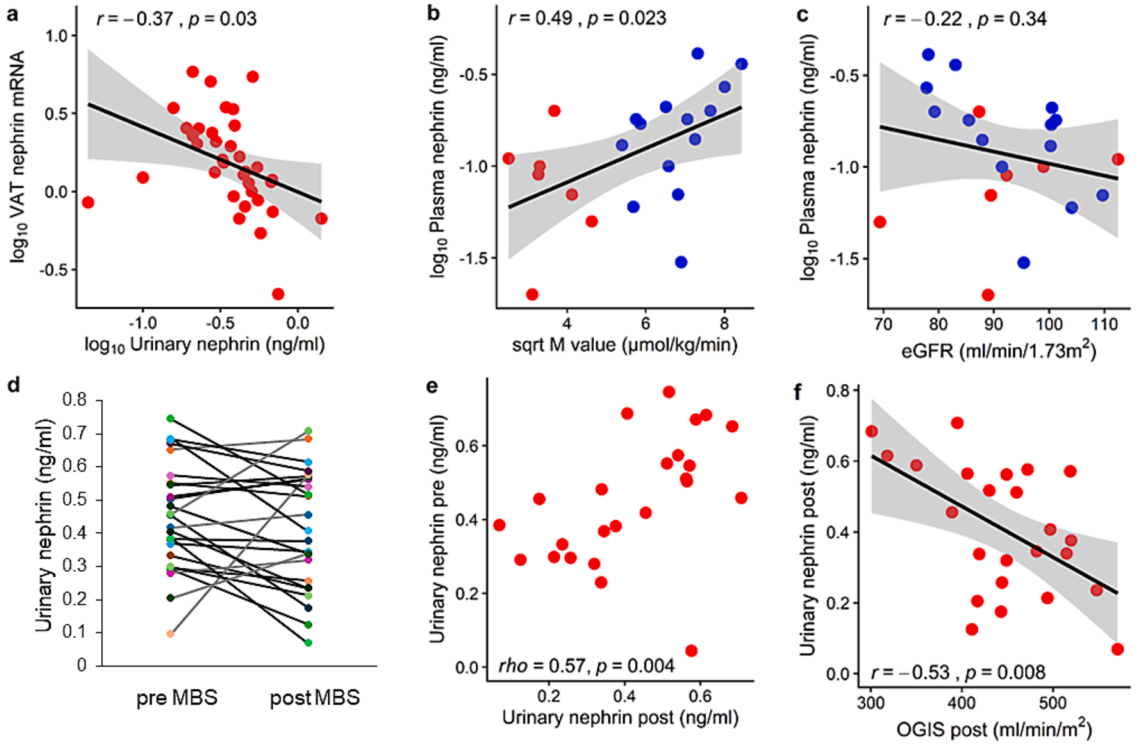

Fig. 4. VAT nephrin mRNA expression was inversely associated with urinary nephrin (Fig. 4a). Plasma nephrin was directly related with the M value (Fig. 4b) but there was no association with eGFR (Fig. 4c). The urinary nephrin variation for each patient before and after MBS (Fig. 4d) and the correlation between pre-post MBS urinary nephrin levels is shown (Fig. 4e). At 2 years follow-up urinary nephrin levels were still inversely related with degree of insulin sensitivity (Fig. 4f). Red circles: OS, blue circles: HC.

did not reduce urinary nephrin levels, a strong direct relationship between pre- and post-surgery values was evident. Of note, also at 2 years follow-up the inverse association between urinary nephrin and insulin sensitivity (indexed by OGIS), remained significant (Fig. 4f).

4. Discussion

In this study, we investigated the hypothesis that markers of early renal damage might be produced in VAT and SAT. Although podocin was not detectable in either depot, we demonstrate for the first time that nephrin is ectopically expressed in human VAT, whereas it is completely absent in SAT. The presence of nephrin mRNA in VAT was accompanied by detectable nephrin protein, with higher levels in individuals with obesity compared with healthy controls.

In obesity, both SAT and VAT expand, but their metabolic implications differ markedly. SAT enlargement is generally considered a more benign form of orthotopic fat accumulation, often associated with a more favourable metabolic profile. In contrast, VAT expansion is strongly linked to adverse cardiometabolic features, including insulin resistance, dyslipidemia, and systemic inflammation [30,31]. Growing evidence also suggests that increased VAT, more than SAT, is associated with worse kidney function, likely through its contribution to chronic inflammation, ectopic fat deposition, and altered adipokine signalling [32,33]. VAT and SAT differ not only in metabolic function but also in their distribution across sexes. Women typically accumulate proportionally less VAT and exhibit a more metabolically favorable adipose phenotype, whereas men show greater visceral expansion and higher VAT associated inflammation [34]. This biological context is relevant to our findings: a significant interaction between sex and BMI was observed for VAT nephrin protein expression, with the direction of this interaction suggesting that BMI exerts a weaker effect on VAT nephrin levels in women, consistent with their lower visceral fat burden and reduced adipose tissue dysfunction. Importantly, despite this interaction at the protein level, the association between BMI and nephrin measures (plasma and urine) was independent of sex. This argues against bias arising from the slightly unequal sex distribution between groups in the present cohort.

Our findings add nephrin to the growing list of bioactive molecules produced by VAT, highlighting a depot-specific expression pattern. Notably, VAT nephrin mRNA expression was strongly and inversely associated with nephrinuria, suggesting a potential negative feedback loop between adipose tissue production and urinary elimination. Although the mechanisms underlying this relationship remain unclear and systemic inflammatory markers were not related with VAT nephrin mRNA expression, the inflammatory milieu of VAT—characterized by elevated TNF-α and IL-6—has been shown to suppress nephrin expression in podocytes [35], and a similar inhibitory effect may occur within adipose tissue. Additional regulatory pathways may involve microRNAs targeting nephrin [36] or altered protein turnover in hypertrophic adipocytes, which could reduce nephrin degradation and promote protein accumulation despite lower mRNA levels [37].

Small amounts of nephrin were detectable in plasma, although approximately one-third of participants had undetectable levels. Interestingly, participants with undetectable plasma nephrin levels had higher VAT nephrin mRNA expression, potentially suggestive of a feedback loop providing more nephrin to plasma and which in turn is lost in the urine.

Insulin resistance emerged as a central determinant of nephrin dynamics. Although nephrinuria correlated with several metabolic markers, most associations were attenuated after adjustment for BMI. However, when insulin sensitivity was accounted for, insulin sensitivity—rather than BMI—emerged as the strongest predictor of nephrinuria, alongside eGFR. The robustness of this relationship was further supported by follow-up data: in the subgroup reassessed two years after metabolic bariatric surgery, nephrinuria remained inversely associated with insulin sensitivity (OGIS) in univariate analysis. The attenuation of this association after BMI adjustment likely reflects the limited sample size at follow-up.

On the contrary, plasma nephrin correlated directly with insulin sensitivity. Thus, plasma and urinary nephrin exhibit opposite associations with insulin sensitivity. One possible explanation is that reduced insulin sensitivity increases podocyte stress, promoting nephrin loss into the urine and thereby lowering circulating nephrin levels. This mechanism could explain the inverse association between nephrinuria and M value together with the positive direct association between plasma nephrin and M value. Moreover, in VAT nephrin protein expression is increased in obesity contributing to the serum pool when needed i.e. when there is insulin resistance and leaking of nephrin in the urine. This hypothesis is consistent with the associations observed but needs to be tested in the future in more detail. Moreover, future prospective studies are needed to clarify the metabolic and renal implications of nephrin, including its potential systemic effects. Beyond studies in healthy individuals, heterozygous carriers of NPHS1 mutations may represent an informative population for further investigation.

Our findings align with experimental data showing that nephrin activates the PI3K/Akt pathway [38], and is required for insulin-stimulated glucose uptake in podocytes through regulation of GLUT4 trafficking [39]. Nephrin expression, in addition to VAT as shown herein, has also been documented in pancreatic β-cells, where it localizes to insulin-containing vesicles [40]. Mice with β-cell-specific nephrin deletion exhibit impaired glucose-stimulated insulin secretion, and children with NPHS1-related congenital nephrotic syndrome display glucose intolerance despite preserved insulin sensitivity [41] indicating that nephrin may stimulate insulin secretion by the pancreas and that higher insulin levels may downregulate nephrin expression as part of a potential feedback loop. Collectively, these observations underscore the broader role of nephrin in insulin and glucose homeostasis that needs to be explored more in depth in the future.

Nephrinuria and podocinuria have traditionally been regarded as podocyte-specific markers, with elevated levels interpreted as an early sign of glomerular injury [13–15]. Consistent with the absence of podocin expression in adipose tissue, podocin was undetectable in plasma or urine from these normoalbuminuric participants. In contrast, our findings might challenge this prevailing paradigm for nephrinuria by demonstrating that nephrin is also produced outside the kidney. This raises the possibility that nephrinuria may not exclusively reflect podocyte stress but could also include contributions from VAT. Consequently, studies using nephrinuria as an early marker of podocyte loss or glomerular dysfunction should consider BMI and adipose tissue phenotype when interpreting results. On the other hand, nephrinuria does not seem to depend on circulating nephrin levels, as there was no significant difference between participants with undetectable plasma nephrin and those with measurable levels (Suppl. Table 2), and the two measurements were not related, whether using observed or imputed plasma nephrin values.

A major strength of this study is the comprehensive metabolic phenotyping performed using state-of-the-art methodologies, including the euglycemic hyperinsulinemic clamp to assess insulin sensitivity, magnetic resonance imaging to quantify the volumes of various adipose tissue depots, and the use of freshly frozen VAT and SAT samples collected during surgery. Additionally, the inclusion of a HC group from whom VAT samples were obtained enhances the comparative robustness of the findings. Tissue samples were treated with standard methodology for gene and protein expression (real time PCR and western blot analysis, respectively), also using more sensitive methodologies (such as Digital PCR) for samples not detectable with standard procedures, making us confident in the efficiency of our work and the reliability of the results. The present study also has limitations. First, in the complete dataset, subjects with obesity were slightly older than healthy controls and included somewhat higher proportion of women. We performed a sensitivity analysis, excluding the four older participants from the group of subjects with obesity. The results remained essentially unchanged. Second, due to the complex study design only twenty-four persons with obesity completed the 2-year follow-up. Third, the number of participants with diabetes was small, which limited our ability to assess potential effects of antidiabetic medications – such as SGLT2 inhibitors or GLP-1 receptor agonists – on nephrin measurements. These

medication-related effects should be evaluated in future studies. Finally, because the study population was restricted to individuals with preserved renal function (eGFR 69–123 ml/min/1.73 m^2), these findings may not be extended to individuals with impaired kidney function. Future studies should evaluate whether the observed associations hold in populations with renal function decline, where nephrin handling and its clinical significance may differ substantially.

In conclusion, nephrin is produced in VAT but not in SAT, and both plasma and urinary nephrin levels are strongly associated with insulin sensitivity. We hypothesize that insulin resistance leads to podocyte damage and nephrin loss in the urine, which is partly compensated by enhanced VAT nephrin protein expression. These findings advance our understanding of the interplay between insulin resistance and renal function and identify nephrin as a potentially important metabolic molecule. Further mechanistic and prospective human studies are needed to elucidate the systemic roles of nephrin and its contribution to metabolic and renal physiology.

5. Author's contribution

ER conceived the study design, collected and analysed the data and wrote the manuscript. MJH, ALR, NT collected and analysed the data. CR, FR performed the in vitro experiments and analysed the data. DM analysed the data. ES collected and analysed the MRI data. PD analysed the MRI data. SK, MH, PS operated the patients and collected the biopsies. CSM interpreted the data, performed statistical analyese and critically revised the text. PN conceived the study design, acquired funding and critically revised the manuscript. AS conceived the study design, acquired funding, and wrote the manuscript. All authors approved the final version of the text. ER had full access at all data and is the guarantor of this work.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Data availability statement

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Eleni Rebelos: Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Chiara Rossi:** Writing – review & editing, Methodology, Formal analysis. **Miikka-Juhani Honka:** Writing – review & editing, Investigation, Formal analysis. **Francesco Raggi:** Writing – review & editing, Formal analysis, Data curation. **Diego Moriconi:** Writing – review & editing, Formal analysis. **Ekaterina Saukko:** Writing – review & editing, Investigation, Data curation. **Prince Dadson:** Writing – review & editing, Formal analysis, Data curation. **Aino Latva-Rasku:** Writing – review & editing, Data curation. **Nelli Tuomola:** Writing – review & editing, Data curation. **Saila Kauhanen:** Writing – review & editing, Data curation. **Mika Helmiö:** Writing – review & editing, Data curation. **Paulina Salminen:** Writing – review & editing, Investigation, Data curation. **Christos S. Mantzoros:** Writing – review & editing, Formal analysis, Data curation. **Pirjo Nuutila:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Anna Solini:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors have nothing to declare in relation to this manuscript. Prof. Anna Solini is a member of the Project Darwin Renal, funded by the Italian Society of Diabetology; this affiliation did not influence her contribution to the present work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2026.113312.

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