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The impact of adipose tissue dysfunction on cardiovascular and renal disease, volume II

Edited by
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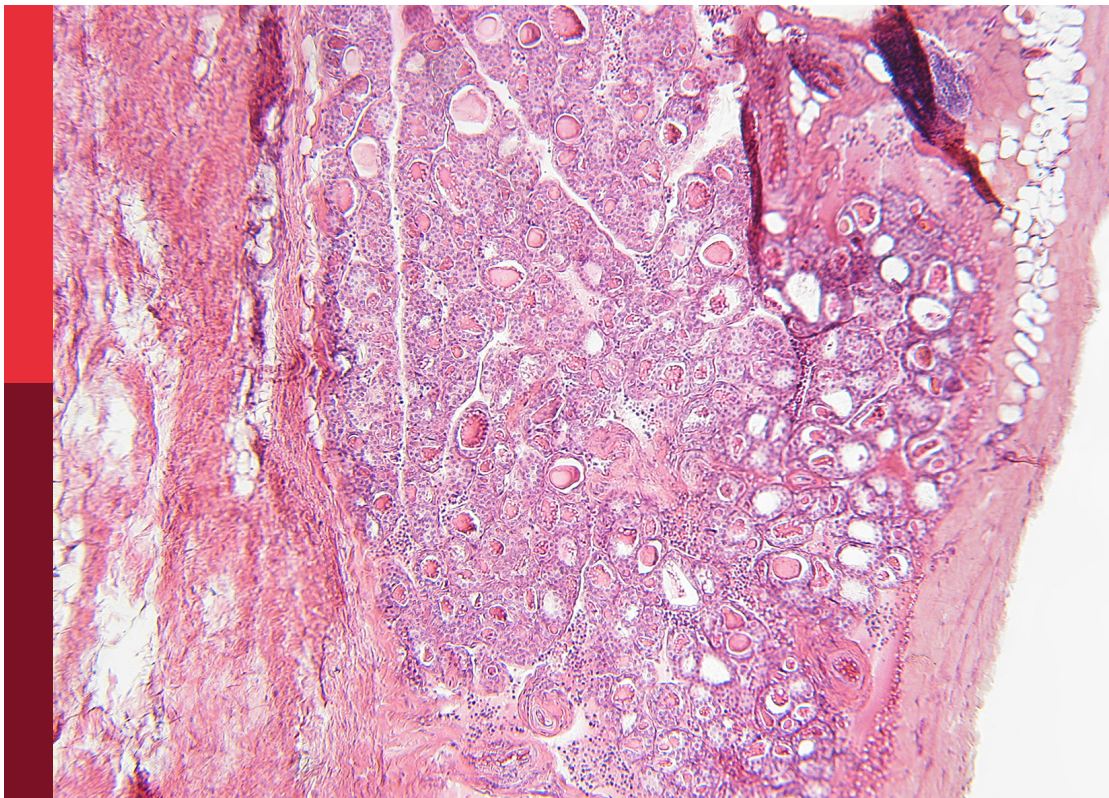

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The impact of adipose tissue dysfunction on cardiovascular and renal disease, volume II

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Editorial: The impact of adipose tissue dysfunction on cardiovascular and renal disease, Volume II

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cardiovascular disease, chronic kidney disease, diabetic kidney disease, adipose, perivascular adipose tissue, epicardial adipose tissue, adipocytes, obesity

Editorial on the Research Topic
The impact of adipose tissue dysfunction on cardiovascular and renal disease, Volume II

Obesity is a global health problem that affects millions of people and increases the risk of various chronic diseases, such as cardiovascular disease (CVD) and chronic kidney disease (CKD). Adipose tissue is a heterogeneous tissue that regulates metabolism, inflammation and immunity. It also mediates the harmful effects of obesity on health. The location and characteristics of adipose tissue determine its positive or negative impact on different organs and systems (1). Excess adipose tissue mass has been traditionally associated with metabolic dysfunction, but recent evidence suggests that adipose tissue quality and distribution are more critical for metabolic health than quantity (2). In addition to the well-known visceral and subcutaneous fat depots, other adipose tissues around blood vessels, such as perivascular, perirenal and epicardial fat, have emerged as novel contributors to CVD and CKD pathogenesis (3–5). These adipose tissues have unique features and dysfunctions in obesity and metabolic syndrome that may cause vascular and renal damage. However, the molecular mechanisms that mediate the communication between these adipose tissues and the cardiovascular and renal systems are still unclear and need further research. This Research Topic showcases a collection of 7 original research and 4 review articles that span from basic to clinical research, providing new insights into the pathophysiology of adipose dysfunction in CVD and CKD.

Obesity predisposes to various comorbidities. However, the risk of developing these comorbidities differs among obese individuals, depending on fat distribution in different body regions. Liu et al. examined the role of central fat distribution and comorbidity in 4899 obese participants from the NHANES database. They found that more than half had at least one comorbidity, and that central fat distribution varied by sex and age. They also

showed that higher android fat ratio, visceral fat ratio and visceral to subcutaneous fat ratio were associated with increased risk of comorbidity in both men and women, while higher gynoid fat ratio and subcutaneous fat ratio were associated with decreased risk of comorbidity. These results suggest that central fat distribution is strongly related to comorbidity in obese individuals.

Perivascular adipose tissue (PVAT) is a fat tissue that wraps around blood vessels and can produce various factors influencing vascular tone, inflammation, and remodeling. One of the mechanisms that may control PVAT function is mechanotransduction, which is how cells sense and respond to mechanical forces. PIEZO1 is a mechanosensitive ion channel protein found in various cell types, including adipocytes. Rendon et al. found that pressure or stretch can activate PIEZO1 in PVAT preadipocytes, affecting multiple cellular processes, such as proliferation, differentiation, migration, and metabolism. These data suggest that PIEZO1 activation in PVAT reduces adipogenesis and lipogenesis and may be an adaptive response to hypertension.

Another fat depot that can modulate CVD is epicardial adipose tissue (EAT), which surrounds the heart and can have both beneficial and detrimental effects on cardiac function and metabolism. EAT can exert cardioprotective and metabolic effects, but it can also induce inflammation and metabolic dysfunction that exacerbate CVD. Li et al. provided a comprehensive overview of the role of EAT in CVD and discussed the methods for EAT quantification and the potential strategies for EAT manipulation. A similar review by Willar et al. summarized how aging and obesity increase EAT size and how EAT is linked to atrial fibrillation and its complications. They proposed that EAT may interact with the cardiac autonomic nervous system and the atrial cardiomyocytes to modulate atrial electrophysiology and arrhythmogenesis. In an original study, Gruzdeva et al. investigated the association of adipocytokines in different fat depots with cardiovascular risk factors in patients with coronary artery disease or valve disease. They reported that low levels of ADIPOQ expression and high levels of interleukin-6 in EAT may increase the risk of atherosclerosis and CAD progression, especially in combination with other risk factors. More research is needed to elucidate the mechanisms and implications of EAT in CVD and to develop effective interventions for EAT modulation.

CKD is another chronic disease that is associated with obesity-related adverse events. Besides the adipose-CVD axis, Arabi et al. provided a comprehensive update on the mechanisms and clinical implications of the adipose-renal axis in CKD. They described how obesity predisposes to CKD and how CKD alters adipose tissue function and exacerbates insulin resistance, creating a feedback loop between the kidney and the fat tissue. This highlights the role of cellular senescence in both adipose tissue and CKD.

Upper-body subcutaneous fat is also a unique fat depot that presents an extra risk for metabolic disorders, estimated by neck circumference and neck-to-height ratio (NHR). He et al. reported that patients with diabetic kidney disease (DKD) had higher neck circumference and neck-to-height ratio (NHR) than those without DKD. They also showed that higher NHR was related to lower estimated glomerular filtration rate (eGFR) and higher albumin-to-creatinine ratio. They concluded that NHR was a risk factor for DKD in this population and suggested that measuring NHR could help identify patients at risk of DKD.

In addition to NHR, other indices that reflect obesity, such as body mass index (BMI) and body roundness index (BRI), also have implications for CKD outcomes, especially in kidney transplant recipients. A cohort study conducted by Bellini et al. studied 396 kidney transplant recipients with different BMI classes and followed them for about 6 years. They found that the recipient's BMI did not affect the patient's survival, but it did affect graft survival and function. They concluded that obesity was a risk factor for graft failure and suggested that BMI should be considered when selecting kidney transplant candidates. Similarly, BRI, which is a measure of body roundness based on height and waist and hip circumferences, also showed a negative impact on kidney function in a large Chinese population. Zhang et al. conducted a cross-sectional study of 36,784 Chinese adults aged over 40 years and measured their BRI and eGFR. They reported that higher BRI was associated with low eGFR and this association was stronger in subgroups of older people, women, smokers, and those with diabetes or hypertension. They concluded that BRI was a positive risk factor for kidney disease in the Chinese population and recommended BRI as a screening tool to identify kidney disease complications.

Another specific type of kidney disease that is influenced by obesity is IgA nephropathy, which is characterized by the accumulation of IgA antibodies in the kidney. Wang et al. examined 1054 IgA nephropathy patients and compared their outcomes according to their body weight status. They found that obese IgA nephropathy patients had impaired kidney function, more metabolic disturbances and unhealthy behaviors than non-obese IgA nephropathy patients. They concluded that obesity is a risk factor for IgA nephropathy patients when coexisting with hypertension.

Obesity is also associated with increased inflammation, which can worsen kidney damage and accelerate CKD progression. One of the inflammatory mediators that has been implicated in CKD is chemerin, a chemokine that initiates the early immune response. A meta-analysis by Behnoush et al. compared chemerin levels between CKD patients and healthy controls using 8 high-quality studies with 875 participants. They reported that chemerin levels were significantly higher in CKD patients, especially those on hemodialysis, suggesting more inflammation. They inferred that chemerin could be a potential biomarker for CKD and recommended further research to investigate its clinical and pathophysiological role in CKD.

Overall, the articles presented in this Research Topic highlight the crucial role of various adipose tissue dysfunction in CVD and CKD development and progression, and raise a timely question of whether manipulating specific adipose tissue depots may offer a novel target for more effective strategies to prevent and/or treat CVD and CKD.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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PIEZO1 mechanoreceptor activation reduces adipogenesis in perivascular adipose tissue preadipocytes

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During hypertension, vascular remodeling allows the blood vessel to withstand mechanical forces induced by high blood pressure (BP). This process is well characterized in the media and intima layers of the vessel but not in the perivascular adipose tissue (PVAT). In PVAT, there is evidence for fibrosis development during hypertension; however, PVAT remodeling is poorly understood. In non-PVAT depots, mechanical forces can affect adipogenesis and lipogenic stages in preadipocytes. In tissues exposed to high magnitudes of pressure like bone, the activation of the mechanosensor PIEZO1 induces differentiation of progenitor cells towards osteogenic lineages. PVAT's anatomical location continuously exposes it to forces generated by blood flow that could affect adipogenesis in normotensive and hypertensive states. In this study, we hypothesize that activation of PIEZO1 reduces adipogenesis in PVAT preadipocytes. The hypothesis was tested using pharmacological and mechanical activation of PIEZO1. Thoracic aorta PVAT (APVAT) was collected from 10-wk old male SD rats (n=15) to harvest preadipocytes that were differentiated to adipocytes in the presence of the PIEZO1 agonist Yoda1 (10 μM). Mechanical stretch was applied with the FlexCell System at 12% elongation, half-sine at 1 Hz simultaneously during the 4 d of adipogenesis (MS+, mechanical force applied; MS-, no mechanical force used). Yoda1 reduced adipogenesis by 33% compared with CON and, as expected, increased cytoplasmic Ca2+ flux. MS+ reduced adipogenesis efficiency compared with MS-. When *Piezo1* expression was blocked with siRNA [si*Piezo1*; NC=non-coding siRNA], the anti-adipogenic effect of Yoda1 was reversed in si*Piezo1* cells but not in NC; in contrast, si*Piezo1* did not alter the inhibitory effect of MS+ on adipogenesis. These data demonstrate that PIEZO1 activation in PVAT reduces adipogenesis and lipogenesis and provides initial evidence for an adaptive response to excessive mechanical forces in PVAT during hypertension.

KEYWORDS

perivascular adipose tissue (PVAT), hypertension, PIEZO1, preadipocytes, adipogenesis

Introduction

The perivascular adipose tissue (PVAT) is an integral part of blood vessels. As such, changes in its structure and function are part of the pathogenesis of hypertension and other cardiovascular diseases (1, 2). Like other adipose tissues (AT) depots, PVAT is composed of adipocytes and a stromal vascular fraction containing immune, vascular, endothelial, neural, and stem cells of different lineages (3). PVAT is present in most blood vessels except the brain (4–6). The importance of the PVAT on vascular function is related in part to its capacity to modulate the contractile function of the vasculature. In addition, many of the vasoactive factors secreted by PVAT are adipokines (e.g., adiponectin). Thus, maintaining a healthy population of adipocytes is essential to support adequate vascular function.

PVATs are unique among AT depots as they are exposed to continuous and synchronous mechanical forces exerted by blood flow, including tensile stress and cyclic strain (reviewed by Hayashi and Naiki (7)). During hypertension, as blood pressure rises, these mechanical forces induce changes in the physiology of cellular components of the vascular tunicas intima, media, and adventitia. This process is known as vascular remodeling and includes alterations such as cellular hypertrophy and hyperplasia and collagen deposition in different tunicas (8–10). The direct effects of this process on the vasculature include reduced vessels' elasticity and compliance and increased stiffness (11, 12). For example, the thoracic aorta in hypertensive patients has lower elasticity, increased vascular thickness, and fibrosis of its anatomical layers compared to normotensive patients (13, 14). The effects of mechanical forces on the adipogenic and lipogenic capacity of PVAT preadipocytes are currently unknown. However, there are reports on cellular responses to mechanical stimuli in cell lines and stromal vascular fraction (SVF)-derived preadipocytes harvested from non-PVAT depots. In the 3T3-L1 fibroblast, equibiaxial stretching above 9% promotes lipogenesis, but its effects on adipogenesis are not described. In the human adipogenic cell line SGBS, compressive force inhibits adipogenesis by suppressing the expression of its master regulator PPARγ. Reports from primary cell lines are scarce and do not include pharmacological and mechanical stimulation of mechanoreceptors (15–17).

Recent studies demonstrate that adipocytes express mechanoreceptors and, therefore, could sense mechanical forces through these specialized proteins (18). Among these, PIEZO1 is present in murine subcutaneous and visceral fats (18, 19) and aortic and mesenteric PVAT (20). This mechanosensor is a transmembrane protein capable of transforming a physical stimulus into a chemical signal. PIEZO1 is a non-selective Na+, K+, and Ca+ permeable channel (21–26). This mechanoreceptor is essential for regulating sensation, touch, and blood pressure (27). PIEZO1 appears to play a role in the differentiation process of resident progenitor cells such as neural stem cells and human mesenchymal stem cells (28, 29). In visceral AT, Offermanns and colleagues showed that during high-fat diet-induced adiposity, the enlargement of lipid droplets in non-PVAT adipocytes generates a stretch force that activates PIEZO1, indicating the capacity of these cells to sense mechanical forces (18). However, it is unknown if PVAT preadipocytes express *Piezo1*, how PIEZO1 activation alters PVAT adipogenic and lipogenic responses, and if PIEZO1 activity could be a mechanism triggering PVAT remodeling during cardiovascular diseases.

This study evaluated the role of the mechanosensor PIEZO1 on PVAT adipogenic processes. We hypothesized that activation of PIEZO1 in PVAT preadipocytes limits their adipogenic capacity. We identified that PVAT preadipocytes express the gene encoding and the protein PIEZO1. Pharmacological and mechanical activation of PIEZO1 reduced adipogenesis efficiency. Our data demonstrate that in PVAT, PIEZO1 activation regulates adipogenesis, possibly linking hypertension with the loss of adipocyte PVAT populations.

Materials and methods

Animals

Male Sprague-Dawley rats of 8-10 weeks (Charles River Laboratories, Inc., Portage, MI, RRID: SCR_003792) were housed in a temperature-controlled room at 22°C, with 12:12-h light-dark cycles and environmental enrichment using standard cages. Animals were fed a regular chow diet with distilled water ad libitum. All animal procedures were approved by the MSU Institutional Animal Care and Use Committee and followed the "Guide for the Care and Use of Laboratory Animals," 8th edition (30). Rats were anesthetized with an intraperitoneal injection of 60-80 mg/kg of pentobarbital. Deep anesthesia was verified by lack of paw pinch and eye-blink reflexes, and death was assured by pneumothorax.

Preadipocyte isolation and culture

The thoracic aorta, including its perivascular adipose tissue (APVAT), was dissected and then immersed in Krebs-Ringer Bicarbonate Buffer (KRBB) containing NaCl 135 mM; KCl 5 mM; MgSO4 1 mM; KH2PO4 0.4 mM; Glucose 5.5 mM; HEPES 20 mM (pH 7.4) (Teknova, Cat N° H1030) and supplemented with 100 units/mL of penicillin; 100 µg/mL of streptomycin, 0.25 µg/mL of Amphotericin B and 50µg/mL of Gentamicin. PVAT preadipocytes were isolated as previously described (5, 31). The APVAT was separated from the thoracic aorta under a dissection stereoscope, and ~50mg fragments were minced in 1-3 mm pieces and then digested for 1 h at 37°C in a rotisserie incubator using 0.5mg/mL of Liberase™ TL (Roche diagnostics,

Cat N° 5401020001) dissolved in Hanks' balanced salt solution supplemented with 4% BSA (Fisher, Cat N° BP9706-100) and 10 mM HEPES. Digested material was filtered through 70 µm cell strainers (Corning, Cat N° 22363548) and then centrifuged at 37°C for 5 min at 300 x g to remove the buoyant cells (adipocytes) from the stromal vascular fraction (SVF) containing preadipocytes. Pellets were resuspended in RBC lysis buffer 1X (Biolegend, Cat N° 420301), incubated at room temperature for 5 min, and then centrifuged at 37°C for 5 min at 300 x g; pellets were resuspended in MesenPRO RS™ (ThermoFisher, Cat N° 12746012) with 2% Fetal Bovine Serum (FBS) (Corning, Cat N° 35-016-CV) and plated in T25 flasks (Sigma, Cat N° SIAL0639) and cultured at 37°C with 5% CO2. Cells were expanded as previously described using preadipocyte medium (PAM) containing 10% FBS, Dulbecco's Modified Eagle's Medium/F12, 44.05 mM sodium bicarbonate (Corning, Cat N° 61-065-RO), 100 µmM ascorbic acid, (Sigma-Aldrich, Cat N° A4544-100G), 33 µM biotin (Sigma-Aldrich, B4501-1G), 17 µM pantothenate (Sigma-Aldrich, Cat N° P5155-100G), 1% L-glutamine (Gibco, Cat N° 25030-081), 1 µg/mL amphotericin (Sigma-Aldrich, Cat N° A-2942) 10 µg/mL ampicillin (Sigma-Aldrich, Cat N° A0166-5G) and 20mM HEPES with replacement every 2 days (32).

Immunohistochemistry

To assess the expression of PIEZO1, preadipocytes and tissue sections were processed for immunohistochemistry as described with some modifications (5). First, preadipocytes cells were seeded on Ibidi µ-slide 8 well (Ibidi GmbH, Cat N° 80822) and incubated overnight to allow cell adherence; after washing with PBS were fixed with 4% of paraformaldehyde in PBS. On the other hand, tissue sections from the thoracic aorta that included PVAT were harvested, kidney samples were used as a positive control for PIEZO1 expression. Tissues were fixed in 4% formalin, embedded in paraffin, and then sectioned into 4–5 µm by the Michigan State University Investigative Histopathology Laboratory. Slides with tissues and cells were incubated in a species-specific serum (1.5% goat serum in PBS-TX; Vector Laboratories, Burlingame, Canada) and then incubated overnight with PIEZO1 primary antibody (1:600, Alomone, Cat N° APC-087, RRID : AB_2756743). The next day, samples were washed with PBS and incubated with AlexaFluor 488 goat anti Rabbit (ThermoFisher, Cat N° A11008, RRID: AB_143165) for 30 mins at room temperature. A second wash was performed, and cells were counterstained with VectaShield® HardSet™ with DAPI (Vector Laboratories, Cat N° H-1500-10). Images were captured with a Nikon Digital Sight DS-Qil camera in a Nikon Eclipse Ti inverted microscope at an x20 magnification with a Lumencore LED light source and Nikon NIS Elements BR 3.00 software. Each photograph is a combination of DAPI (Nuclei marker) and FITC (PIEZO1) channels and standardized for true fluorescence based on the control FITC for that specific tissue section that was then embedded into the image and carried through all image analysis.

Adipogenesis experiments

Preadipocytes were seeded in six well-plates and induced to differentiate into adipocytes in standard adipogenic media alone, as previously described by our group (31), in the presence of the PIEZO1 agonist Yoda1 10 µM (Tocris, Cat N° 5586), a concentration reported in different cell types (18, 29, 33–36). Adipogenesis was evaluated using Bodipy 493/503 (ThermoFisher Cat N° D3922), a neutral lipid staining, and the nuclear stain NucSpot® Live 650 (Biotium, Cat N° 40082) and reported as Bodipy fluorescence intensity/nuclei count using long-term live-cell imaging IncuCyte® S3 system. Cultured cells were imaged every 6 hours during 4 days in culture. Quantification of cell images after 4 days was performed using IncuCyte ZOOM™ software.

Viability and apoptosis assay

Viability and apoptosis assays were performed according to the manufacturer's specifications. Briefly, viability was determined in preadipocytes exposed to 10 µM Yoda1 (Tocris, Cat N° 5586) in adipogenic media for 4 days. Negative control of dead cells was made with 0.1% of saponin (Alfa Aesar, Cat N° J63209) in 1X PBS for 10 mins before collection time, all conditions were incubated at room temperature with Calcein AM (Biotium, Cat N° 30002) during 45 mins. Fluorescence was measured in a microplate reader (BioTek, Synergy H1M). Apoptosis was calculated detecting Caspase 3/7 activity (Biotium, Cat N° 10403), preadipocytes were exposed to different concentrations of Yoda1 during 4 days of adipogenic induction, and with 0.2 µg/mL of Doxorubicin HCl (TCI, Cat N° D4193) used as a positive control of apoptosis, fluorescence is reported as relative fluorescence units (RFU).

RNA isolation and purification

RNA was extracted using the Maxwell® RSC simplyRNA cells kit (AS1390, Promega, Madison, WI) as reported previously (32). Cultured cells were homogenized in 1-Thioglycerol/ Homogenization Solution (Maxwell® Cat N° Z305H) and vortexed for 15 sec. The cell lysate was mixed with 200 µL lysis buffer (Maxwell® Cat N° MC501C) and vortexed for 15 sec. The 400 µL cell lysates were transferred into the sample well of the Maxwell® RSC Cartridge. 5 µL of DNase I solution were added in well #4 of the Maxwell® RSC Cartridge to eliminate

genomic DNA. The RNA was automated extracted in Maxwell® RSC Instrument (Promega, Madison, WI, USA) following the manufacturer protocol. The purity, concentration, and integrity of mRNA were evaluated using a NanoDrop One© spectrophotometer (Thermo Scientific, Cat N° 840274200). All samples had a 260:280 nm ratio between 1.9 and 2.1 and the RNA integrity number > 7.

cDNA synthesis and qualitative PCR

Reverse transcription was performed with 500 ng of RNA in 20 µL of reaction volume containing 4 µL of qScript cDNA SuperMix (95048-500, Quantabio, Beverly Hills, CA, USA). The reverse transcription conditions were 5 minutes at 25°C, 30 minutes at 42°C, and 5 minutes at 85°C; cDNA was stored at -20°C.

Transcriptional studies were performed using the the Quan Studio 7 Flex System (Applied Biosystems, MA, USA) and the high-throughput qPCR instrument Wafergen Smartchip (Takara Bio, Mountain View, CA, USA) conducted with Taqman or SYBR gene expression primers commercially available or designed from murine sequences and synthesized (IDT Technologies (Coralville, IA). For PCR data in Figure 1 samples were assayed in duplicate. Each 20 µL of PCR reaction contained 1X of PerfeCTa Fast Mix II (Quantabio, Cat N° 95119-012), Taqman assays (Suppl Table 1) were used at 1X, and 4 ng/µL of sample cDNA. For all other experiments, the samples were assayed in duplicate. Each 20 µL of PCR reaction contained 1X of PerfeCTa Fast Mix II (Quantabio, Cat N° 95119-012), Taqman assays (Suppl Table 1) were used at 1X, and 4 ng/µL of sample cDNA. For all other experiments, the samples were assayed in duplicate; each 100 nL PCR reaction contained 1X of LightCycler 480 SYBR Green Master Mix (Roche), 200 nM of primer assays, and 1.5 ng/µL of sample cDNA. A non-reverse-transcriptase control and no-template control examined the DNA contamination and primer-dimer formation in the assay reaction. Primer sequences are reported in Suppl Table 1. The cycling conditions for Taqman assays included: initial enzyme activation at 95°C for 20 sec, 40 cycles of denaturation at 95 °C for 1 sec, and annealing/extension at 60 °C for 20 sec. For SYBR green assays, the cycler conditions included: initial enzyme activation at 95°C for 10 min, 45 cycles of denaturation at 95°C for 10 sec, and annealing/extension at 60°C for 60 sec, followed by a melting curve analysis of 65-95°C with 0.5°C increments 5 sec per step. The housekeeping genes with the lowest pairwise variation value were selected, including, Eif3k (eukaryotic translation initiation factor 3 subunit k), Rps29 (ribosomal protein s29), and B2m (Beta-2-microglobulin). The expression of target genes was normalized against the geometric mean of selected housekeeping genes as described by (37).

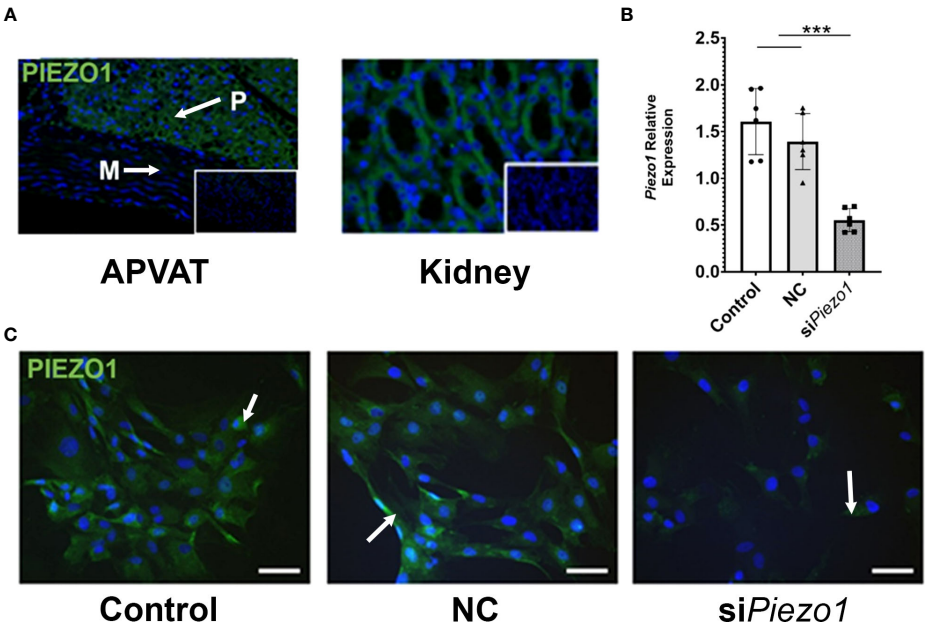

FIGURE 1
PVAT and preadipocytes express PIEZO1. **(A)** PIEZO1 staining of thoracic aorta PVAT and kidney. White arrows indicate positive cells. DAPI is the nuclear stain. Inserts are sections without primary antibody. P=PVAT, M=Media vascular layer. Images are representative of n=4. **(B)** Piezo1 expression in APVAT preadipocytes treated with *Piezo1* siRNA (si*Piezo1*), non-coding si*Piezo1* (NC), and non-treated (Control). **(C)** PIEZO1 staining of cells in B are representative of n=6. Scale bars = 50 µm. Bars are means ± SEM, Bars with *** (P<0.001) are different relative to the expression of *Actb*.

Short interference RNA

To assess the effects of PIEZO1 activation on adipogenesis, we inhibited the expression of the protein by using short interference RNA (siRNA) following a human preadipocyte protocol with some modifications (38). In brief, 21.000/cm2 preadipocytes were plated at 70% confluency. Cells were incubated overnight in αMEM (Corning, Cat N° 50-010-PB), 5% FBS, Hiperfect transfection reagent (Qiagen, Cat N° 301704), and 40 nM of three combined siRNA sequences targeting *Piezo1*, a non-coding siRNA (NC), or a siRNA targeting *Hprt* (Suppl Table 1). All sequences were designed by IDT Technologies (Coralville, IA). Control without the addition of transfection reagent was also included. After incubation, the medium was replaced with preadipocyte media, and cells were allowed to proliferate for 72 h. The fluorescence dye TYE 563 was used to optimize the concentration of sequence and transfection reagent.

Calcium influx experiments

Cellular calcium trafficking was determined using the indicator Fluo-4 AM Ester (Biotium Cat ° 50018). Cells were washed with Krebs-Ringer-HEPES-Glucose Buffer (KRH-glc) that includes 25mM of glucose and then loaded with 5 μM of Fluo-4 AM in KRH-glc + 0.5% bovine serum albumin (BSA) for 30 mins at room temperature protected from light. Two washes removed extracellular fluorophore with KRH-glc + 0.5%BSA, then measured all the conditions using the same buffer. To evaluate the kinetics of calcium, Yoda1 (10μM; 5586, Tocris, Bristol, UK) was added after the basal reading (0 min), DMSO, and 5μM of Ionomycin (Biotium Cat N° 59007) were used as negative and positive control respectively. The fluorescence intensity excited at 488 nm and emitted at 526 nm is proportional to the cytosolic free calcium concentrations, using microplate reader samples were measured in triplicates (BioTek, Synergy Cat N° H1M).

Mechanical stretch experiments

Preadipocytes cells were seeded in collagen type 1-coated Bioflex 6-well plates (Flexcell International, Cat N° BF-3001C) at 40,000 cells/cm^2. Cells were transfected with siRNA and NC sequences as described above; after they reached full confluency 3 d post-transfection were induced to differentiate into adipocytes as previously described. Mechanical stretching was applied simultaneously using the FX-6000 Tension System. This computer-driven instrument creates strain conditions with vacuum pressure to deform the cells on the flexible, matrix-bonded surface of the Bioflex plates. The mechanical stress (MS+) was set up at a rate of 1 Hz with 12% elongation in a half-sine pattern for 4 d; cells without mechanical stretch (MS-) were used as control. Samples were analyzed for gene expression and adipogenesis efficiency using the Spectrum Image Cytometry System. Briefly, cells were washed with PBS 1X and detached with Trypsin 0.05%, centrifugated two times at 300 x g for 5 mins, and then resuspended in 40μl of dye master mix (2X ViaStain Far Red (Nexcelom Cat N° CS1-V0010-1) + 10μM of Bodipy 493/503 in PBS 1X). Images in triplicate were obtained in Spectrum 5 software and analyzed using ImageJ software, and adipogenesis efficiency was determined as Bodipy fluorescence count/nuclei count.

Statistical analysis

Data were analyzed by one- or two-way ANOVA using JMP (SAS, Cary, NC), SAS 9.4 (SAS Inst, Cary, NC), and GraphPad Software (GraphPad, San Diego, CA). Proc Mixed program was used, and *post hoc* comparisons were performed using Tukey's adjustments test. Residuals of the model were checked for normal distribution—random effect of the rat within the treatment and mechanical stretch. Statistical significance was set at $P \leq 0.05$.

Declaration and ethical statements

All animal procedures were approved by the MSU Institutional Animal Care and Use Committee and followed the Guide for the Care and Use of Laboratory Animals," 8th edition (30).

Results

Preadipocytes from PVAT express PIEZO1

The PVAT surrounding the thoracic aorta (APVAT) expresses the protein PIEZO1 (Figure 1A). To validate the specificity of the PIEZO1 antibody, we used targeted gene knockdown with siRNA in APVAT preadipocytes. *Piezo1* expression was reduced by 70% in cells treated with the siRNA targeting its transcription (si*Piezo1*) compared with those exposed to non-coding siRNA (NC, Figure 1B). In addition, preadipocytes treated with si*Piezo1* showed a reduced signal intensity of PIEZO1 compared to untreated cells and NC (Figure 1C).

PIEZO1 activation in PVAT preadipocytes promotes calcium influx

Treating APVAT preadipocytes with the selective agonist Yoda1 increased Ca2+ flux into the cells treated with NC sequence measured with Fluo-4 AM, a molecule that exhibits fluorescence upon Ca2+ binding. Preadipocytes treated with si*Piezo1* were unresponsive to Yoda1 (Figure 2).

PIEZO1 activation reduces adipogenesis in PVAT preadipocytes

To evaluate the effect of PIEZO1 activation on APVAT preadipocytes, we treated cells during the first 4 days of adipogenesis induction with the PIEZO1-specific activator Yoda1. Yoda1 at different concentrations during adipogenesis did not affect the viability as determined by Calcein AM a fluorescent molecule emitted by live cells, or induce Caspase 3/7 activity, an enzyme stimulated during apoptosis (Supplementary Figure 1). After 4 days of culture in adipogenic media, cells treated with Yoda1 had lower adipogenic efficiency than cells cultured in adipogenic media alone and this was reflected as a reduction in lipid droplet formation (Figures 3A, B). Preadipocytes exposed to Yoda1 had reduced expression of critical adipogenic genes such as *Pparg*, *Plin1*, *Adipoq*, and *Fabp4* compared with cells in adipogenic media alone (Figure 3C).

A subset of APVAT preadipocytes were treated with si*Piezo1* or NC; this allowed us to reduce the expression of *Piezo1* during 7 days of culture (Figure 3C). Silencing the mechanoreceptor abrogated the anti-adipogenic effect of Yoda1, which was reflected in a higher adipogenesis efficiency compared to NC cells treated with Yoda1 (Figures 3A, B). si*Piezo1* treated cells cultured in media with Yoda1 had similar expression of adipogenic genes (*Pparg*, *Plin1*, *Adipoq*, and *Fabp4*) to cells treated with NC alone in adipogenic media, while *Fgf9* expression was not increased by Yoda1 when si*Piezo1* was used (Figure 3C).

Cyclic mechanical stretch impairs the adipogenic potential of APVAT preadipocytes regardless of *Piezo1* expression

The next step was to evaluate the effect of direct mechanical stimulation on APVAT preadipocytes adipogenesis. Cells pre-treated with siRNA (si*Piezo1*+) and NC (si*Piezo1*-) sequences were exposed to 4 days of cyclic stretch (MS+) simultaneously during adipogenesis induction (Adipogenic media). Compared to MS-, MS+ reduced adipogenic efficiency in cells cultured in adipogenic media pretreated with NC or si*Piezo1*, and this was reflected as fewer lipid droplets within each cell. A similar response was observed when PIEZO1 was pharmacologically activated with Yoda1 in NC and MS- conditions (Figures 4A, B). Yoda1 and MS+ had an additive effect in the reduction of adipogenesis in NC-treated cells. However, si*Piezo1* inhibited the impact of Yoda1 only in MS- cells. si*Piezo1* did not obliterate the effects of mechanical stretching on adipogenesis of cells exposed to Yoda1 and MS+ (Figures 4A, B).

Pharmacological and mechanical activation of PIEZO1 altered the expression of some adipogenic and lipogenic gene networks in APVAT. The activation of PIEZO1 by mechanical or pharmacological conditions did not change the expression of mechanoreceptors *Piezo1* or *Piezo2* (Table 1). Yoda1 suppressed the lipogenesis-related genes *Dgat1* and *Agpat2* which encode rate-limiting enzymes of triacylglycerol synthesis in adipocytes. Genes associated with extracellular matrix proteins such as *Col6a1* had reduced expression in mechanically stimulated

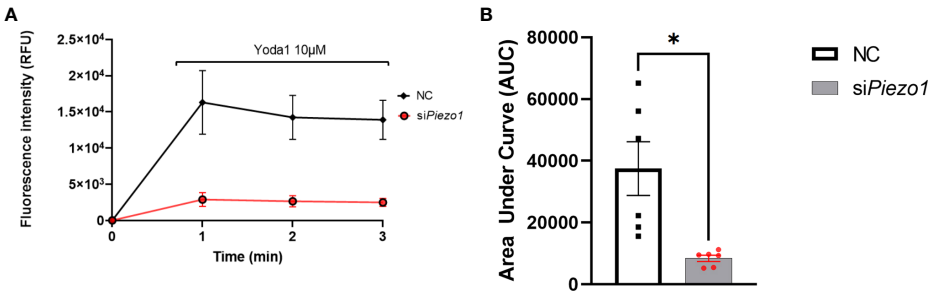

FIGURE 2
PIEZO1 activation enhances calcium influx in PVAT preadipocytes. **(A)** Fluo-4-loaded preadipocytes treated with non-coding (NC) and si*Piezo1* (siRNA *Piezo1*), the activity of Fluo-4 AM was measured in fluorescence intensity after exposure to 10 μM of Yoda1 starting before min 1. **(B)** Area Under the Curve of fluorescence units of Fluo-4 AM in si*Piezo1* treated cells and NC preadipocytes depicted in **(A)** *P<0.05. representative of n=6.

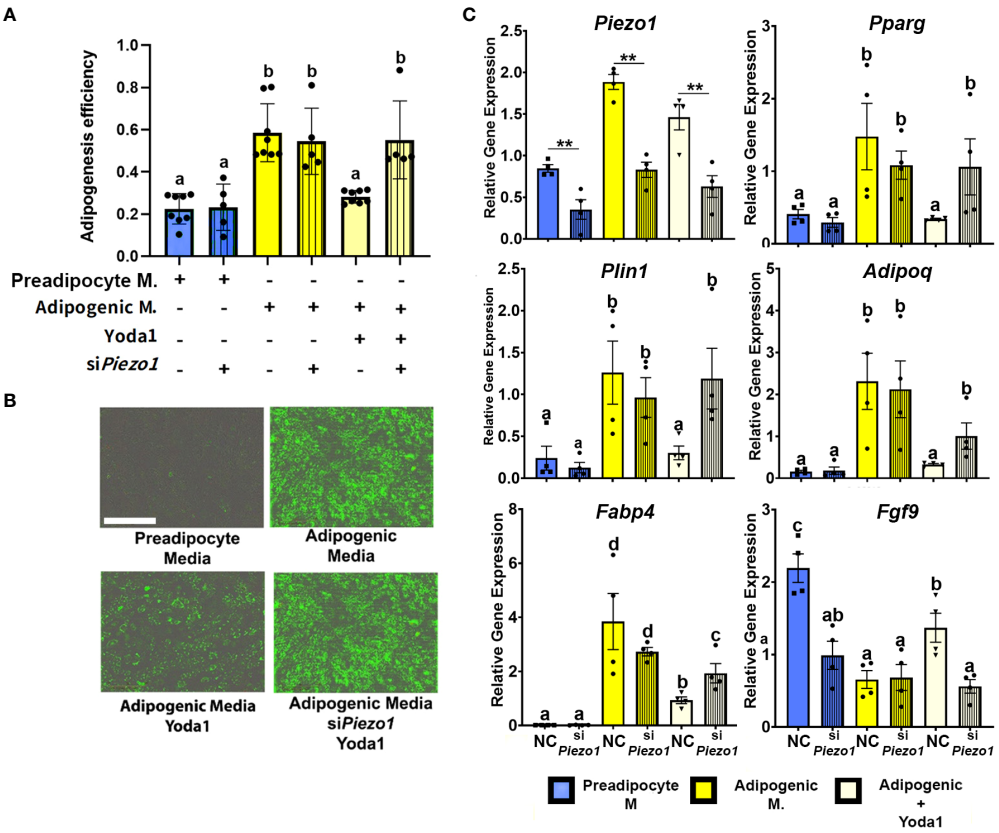

FIGURE 3
Activation of PIEZO1 reduces adipogenesis in APVAT. Preadipocytes were incubated in preadipocyte or adipogenic media (M.) in the presence of the *Piezo1* activator Yoda1. A subset of cells was treated with siRNA targeting Piezo1 (si*Piezo1*) or non-coding (NC) siRNA. **(A)** Adipogenesis efficiency as calculated by the IncuCyte zoom software (N° of cells with at least one lipid droplet over total # of cells in each well), columns with strips indicate (siPiezo1) n=5-8. **(B)** Representative images of cultured cells. Triglycerides, in green, were stained with Bodipy. Scale bar = 200 microns. **(C)** Expression of Piezo1, adipogenic genes (Pparg, Plin1, Adipoq, and Fabp4) and Fgf9. Values are relative mRNA abundance after normalization with the reference gene Rps29. Bars are means ± SEM; bars with different letters a, b, c, d ($P<0.05$) or ** ($P<0.01$) differ. Images in **(B, C)** are representative of n=4.

cells, while *Col1a1*, and *Fn1*, were not altered by either chemical (Yoda1) or mechanical activation (MS+) of PIEZO1 (Table 1).

As for fibroblast growth factors, MS+ increased *Fgf10*, a gene essential for cell proliferation and tissue repair, while *Fgfr2*, a receptor of the same family, was downregulated. Other genes from this network, *Fgf2*, *Fgfr1*, or *Fgfr3*, were not affected by Yoda1 or MS. In addition, *Wnt16*, a member of the WNT/β catenin pathway, was upregulated by Yoda1 exposure (Table 1).

Discussion

A healthy PVAT is a source of vasoactive molecules, including adiponectin and nitric oxide. During hypertension, PVAT inflammation impairs its capacity to secrete these products (8, 14). A possible mechanism is the reduction of adipocyte populations. Results from the present study suggest that these changes in the cellular populations of PVAT may be related to a decline in the adipogenic potential of preadipocytes driven by the activity of the stretch-activated ion channel PIEZO1. Physical forces generated by blood flow may enhance the calcium influx in preadipocytes through PIEZO1 and ultimately reduce adipogenesis.

APVAT preadipocytes mechanosense through PIEZO1

Our results indicate that the PVAT of the thoracic aorta is a vascular layer with mechanosensory capacity. First, preadipocytes isolated from APVAT express the mechanotransducer PIEZO1 abundantly, a result that concurs with the evidence found by Miron et al. in PVAT depots (20). Other tissues subjected to frequent mechanical activity, such as lungs, skin, or kidneys, also express PIEZO1 (39). The APVAT in rodents is mainly composed of brown adipocytes (BAT).

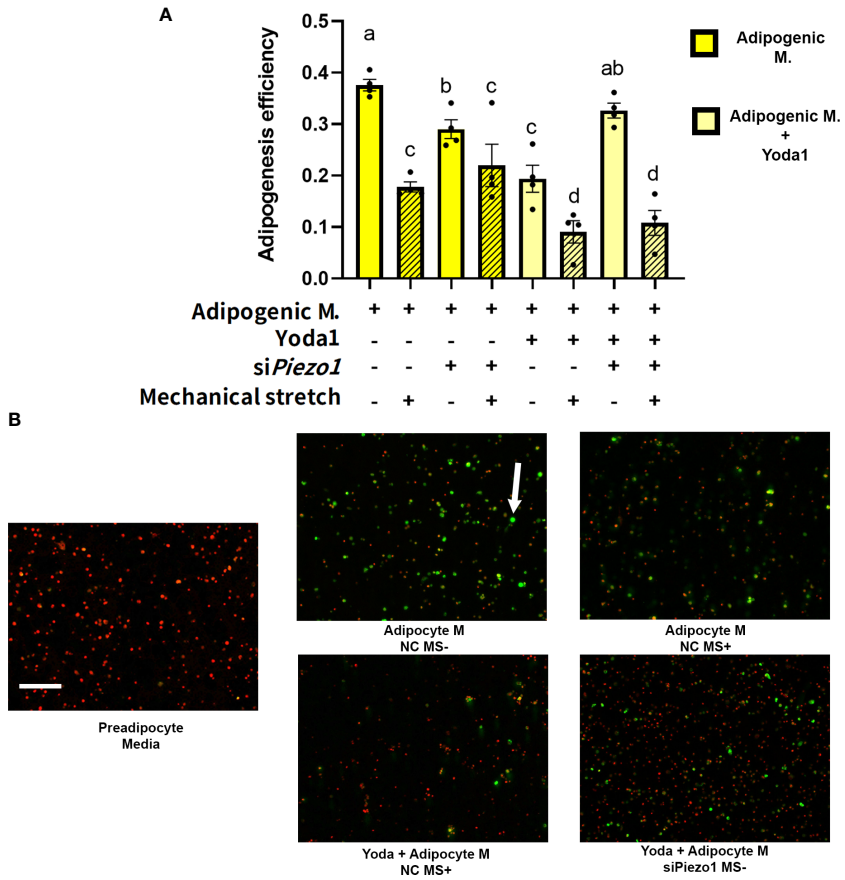

FIGURE 4
Mechanical stretching affects adipogenesis efficiency. Preadipocytes previously exposed to siPiezo1 (si*Piezo1*+) and NC control (si*Piezo1*-) were induced to become adipocytes in the presence of Yoda 1 (10μM: Yoda1+, 0μM: Yoda1-) and 12% of cyclic elongation (MS+) or 0% (MS-) for 4 days. **(A)** Adipogenesis efficiency calculated by ImageJ (N° cells with at least one lipid droplet over total # of cells per image) analyzed by triplicates. Columns with strips indicate mechanical stretch (MS+). Significant differences are indicated by letters a, b, c, and d ($P < 0.05$). Bars are means ± SEM. **(B)** Representative images of cultured cells: Triglycerides indicated by the arrow were stained with Bodipy and nuclei with ViaStain Far Red. Images in B are representative of n=4, scale bar = 200 microns.

Previous reports describe high expression levels of the PIEZO1 mechanosensor in BAT (20, 40, 41). In addition, preadipocytes cultured under adipogenic conditions maintain their *Piezo1* expression. These results are consistent with previous evidence of the expression of PIEZO1 in preadipocytes, adipocytes, and stem/progenitor cells (18, 19, 28, 29, 33).

PIEZO1 activation increases intracellular Ca2+ reducing APVAT adipogenic potential

We demonstrated that PIEZO1 activation reduced adipogenesis in APVAT preadipocytes. The chemical agonist Yoda1 was used in this study to determine the role of PIEZO1 since it is highly specific for this mechanosensor (34), avoiding activation of PIEZO2 channels (42, 43). As expected, the expression of *Piezo1* was essential to enhance Ca2+ influx in preadipocytes (Figure 2). This observation coincides with those in dental pulp stem cells (DP-MSC) (33), adipocytes (18), and endometrial epithelial cells (35), where Ca^{2+} fluxes shifts are essential to induce mechanoreceptor responses.

The influx of calcium *via* PIEZO1 could alter several functions within the cell, including proliferation, homeostasis, apoptosis, and differentiation (44–46). PIEZO1 activation during early adipogenesis (1 to 4 days post-induction) did not affect cell integrity but impaired differentiation of PVAT preadipocytes. We confirmed these responses using Yoda1 as a pharmacological PIEZO1 agonist and siRNA targeting PIEZO1. The latter reduced *Piezo1* by 70%, a reduction similar to those reported in dental pulp (33) and bone marrow progenitors (29). The Ca2+ flux-mediated anti-adipogenic response to PIEZO1 activation observed in this study is consistent with observations in adipocyte progenitor cells derived from different species and

TABLE 1 The effect of mechanical and simultaneous pharmacological activation of PIEZO1 on the expression of genes related to mechanoreceptors, adipogenic, lipogenic, fibrogenic, extracellular matrix, and WNT/B catenin pathway.

Gene network	Gene		(MS-)		(MS+)		*P* values		
			Adipogenic M	Yoda1	Adipogenic M	Yoda1	MS	Tx	MS*Tx
Mechanoreceptors	*Piezo1*[1]	Estimate	1.95	1.73	1.73	1.47	NS[5]	NS	NS
		LCI[2]	1.70	1.48	1.48	1.23			
		UCI[3]	2.20	1.98	1.97	1.72			
	Piezo2	Estimate	2.05	0.34	0.45	1.82	NS	NS	NS
		LCI	0.75	-0.85	-0.74	0.62			
		UCI	3.36	1.53	1.64	3.01			
Lipogenic	Agpat2	Estimate	14.84[a]	6.61[b]	10.97[a]	5.51[b]	NS	<.0001	NS
		LCI	10.54	2.30	6.67	1.20			
		UCI	19.15	10.92	15.28	9.82			
	Dgat1	Estimate	3.66[a]	2.44[b]	2.75[a]	1.62[b]	NS	<.0001	NS
		LCI	2.77	1.55	1.87	0.73			
		UCI	4.54	3.32	3.64	2.50			
Extracellular matrix	Col1a1	Estimate	0.24	0.24	0.25	0.36	NS	NS	NS
		LCI	0.02	0.01	0.03	0.14			
		UCI	0.46	0.46	0.47	0.58			
	Col6a1	Estimate	1.12[a]	1.51[a]	0.88[b]	0.92[b]	<0.05	NS	NS
		LCI	0.76	1.15	0.52	0.56			
		UCI	1.48	1.87	1.23	1.28			
	Fn1	Estimate	0.66	0.66	0.65	0.74	NS	NS	NS
		LCI	0.32	0.32	0.31	0.40			
		UCI	1.00	1.00	0.99	1.08			
Fibroblastic	Fgf10	Estimate	1.45[a]	1.32[a]	1.70[b]	1.81[b]	<0.05	NS	NS
		LCI	0.69	0.55	0.94	1.05			
		UCI	2.21	2.08	2.47	2.57			
	Fgf2	Estimate	0.63	0.77	0.66	0.72	NS	NS	NS
		LCI	0.20	0.34	0.23	0.29			
		UCI	1.06	1.20	1.09	1.16			
	Fgfr1	Estimate	1.43	1.47	1.45	1.48	NS	NS	NS
		LCI	1.06	1.10	1.09	1.11			
		UCI	1.79	1.83	1.82	1.84			
	Fgfr2	Estimate	2.06[a]	1.54[a]	1.37[b]	1.32[b]	<0.01	NS	NS
		LCI	1.67	1.15	0.99	0.94			
		UCI	2.44	1.93	1.76	1.71			
	Fgfr3	Estimate	1.95	2.28	1.63	1.65	NS	NS	NS
		LCI	1.03	1.36	0.71	0.73			
		UCI	2.88	3.21	2.56	2.58			
WNT/β catenin	Wnt16	Estimate	3.88[b]	10.99[a]	5.98[b]	12.61[a]	NS	<0.05	NS
		LCI	-4.70	2.41	-1.86	4.77			
		UCI	12.45	19.57	13.83	20.45			

PVAT preadipocytes (n=6) exposed to 0 (**MS** -) or 12% elongation (**MS** +) in different media conditions (**Tx**), adipogenic media, and adipogenic media + 10μM Yoda1 (Yoda) for 4 d.
[a-c] Fold changes without a common superscript within a row represent differences determined by Tukey adjustments of ΔCt values.
Fold change = $2^{(-\Delta\Delta Ct)}$; $\Delta\Delta Ct = \Delta Ct_{calibrator\ sample} - \Delta Ct_{target\ sample}$.
[1]Gene expression values were calculated from LSM differences of the ΔCt values (ΔΔCt) normalized to the mean of Eif3k, Rps9, and B2m housekeeping genes.
[2]**LCI**= Lower confidence interval 95%.
[3]**UCI**= Upper confidence interval 95%.
[4]**MS**= Mechanical stretching, **Tx**= treatment.
[5]**NS** = P > 0.10.

AT sites. Murine and human non-PVAT progenitors had reduced adipogenic potency when exposed to agents that increased intracellular calcium (47, 48) or when the activity of calcium-dependent proteins such as calcineurin, a protein essential for muscle renewal and cardiac hypertrophy development, was increased (49, 50). PIEZO1 activation appears to impair the adipogenic program by reducing the expression of *Pparg*, encoding PPARγ, the master key regulator of adipogenesis which reaches maximum expression during days 3-4 of the adipogenesis process (51). Possibly related to lower PPARγ activity, PIEZO1 activation also reduced the expression of genes relevant for maintaining adipocytes' phenotype and function, such as *Plin1*, a protein that coats lipid droplets (52); *Adipoq*, a factor highly secreted by adipose tissue that increases PPARγ ligand activity (53); *Agpat2* and *Dgat1*, rate-limiting enzymes for triglyceride synthesis, and *Fabp4* a fatty acid-binding protein (54).

PIEZO1 activates anti-adipogenic APVAT pathways

In the present study, pharmacological activation of PIEZO1 with Yoda1 elicited expression of *Fgf9* in PVAT preadipocytes. This gene has been identified as a potent inhibitor of adipogenesis and the browning of white adipocytes (55, 56). In addition, *Fgf9* stimulates the proliferation of vascular smooth muscle, epithelial, and colorectal cancer cells (57). The mechanism for this proliferation response is mediated through the activation of the WNT/β catenin pathway (58, 59). Miyazaki et al. demonstrated that pharmacological or mechanical activation of PIEZO1 in dental stem cells upregulates the *Wnt16* member of the Wnt/β catenin family (36). The WNT pathway promotes myogenic differentiation while suppressing adipogenesis (60–64). Remarkably, in the present study, *Wnt16* was upregulated during PIEZO1 activation in APVAT preadipocytes. This may be an alternate mechanism for adipogenesis suppression in APVAT preadipocytes; however, more studies are required to determine if PIEZO1 and *Wnt16* activation can promote myofibrogenesis of these cells during hypertension.

Mechanical stimulation reduces APVAT adipogenic potential independent of *Piezo1* expression

To our knowledge, this is the first report on the impact of mechanical forces on adipogenesis and lipogenesis in APVAT. Preadipocyte exposure to cyclic mechanical strain during the early stages of adipogenesis reduces adipogenic efficiency, a response that was not affected even when *Piezo1* was silent. This finding suggests that other mechanosensors act parallel with PIEZO1 during mechanosensation in APVAT. The mechanotransduction process can involve different mediators, including mechanosensors and structural proteins. Among mechanosensors, *Piezo2*, *Trpv4*, *Tmem16*, and *Panx1* were identified in APVAT and other PVAT previously; these proteins could complement PIEZO1 mechanosensing activity (20) however, the impact of their activity on adipogenesis remains to be elucidated. Among structural proteins, integrins are a family of transmembrane proteins that can suppress adipogenic genes such as *C/ebpα* and *Ppparg* and promote the expression of anti-adipogenic pathways, including Runx2, β-catenin, and SMAD proteins. Remarkably, integrins can also be activated by Ca2+ influx, a direct response to PIEZO1 activation in APVAT preadipocytes (65). Our results highlight the need for studies evaluating the interaction among PIEZO1, other mechanoreceptors, and structural proteins and their impact on APVAT adipogenesis and function.

Limitations

Our study provides evidence for the effect of PIEZO1 activation on APVAT preadipocyte adipogenesis. However, given the differences among PVAT sites (e.g., mesenteric PVAT, abdominal aorta PVAT) on adipocyte phenotype and the characteristics of mechanical forces acting on other sites, results from the present study cannot be extrapolated directly to other PVAT tissues. Second, the cyclic mechanical strain frequencies used in the current experiments are lower than those occurring *in vivo*, given their rapid heart rates (66). Thus, future research is needed to establish the direct effect of mechanical force frequency on APVAT. To date the role of PIEZO1 *in vivo* remains elusive, while global deletions lead to embryonic lethality, tissue-specific gain or loss of function demonstrate specific responses depending on the type of cell involved. Therefore, future studies that include targeted deletion of this mechanosensor in preadipocytes and other PVAT cells that take into account sex differences in normotensive and hypertensive animals are required to determine the impact of PIEZO1 on cardiovascular diseases.

Conclusion

Results from the present study demonstrate that pharmacological activation of the mechanosensory protein PIEZO1 reduces adipogenesis on aortic PVAT preadipocytes. At the same time, mechanical stimulation can activate other routes than PIEZO1 that may suppress adipogenesis. Pharmacological activation of PIEZO1 activation mediated transient calcium influx through the membrane leading to a repression of adipogenic essential genes, which reduces the adipogenic potential. Reduced hyperplasia of adipocytes may

diminish the synthesis of vasoactive chemokines, which explains that the progression of hypertension has been associated with loss of anti-contractile response of PVAT (67).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Michigan State University Institutional Animal Care and Use Committee.

Author contributions

CR, GAC, and SW conception and design of the study; CR, JT, EF, and MC performed experiments; CR, GAC, and MC analyzed data; CR, GAC and MC interpreted results of experiments; CR and GAC prepared figures; CR drafted the manuscript; GAC, EF, JT, MC, and SW edited and revised manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.995499/full#supplementary-material

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Characteristics of adipocytokine expression by local fat depots of the heart: Relationship with the main risk factors for cardio-vascular diseases

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In our study we investigated the relationships between adipocytokines in adipose tissue (AT) and cardiovascular disease (CVD) risk factors; (2) Methods: fat tissue biopsies were obtained from 134 patients with stable CAD undergoing coronary artery bypass grafting and 120 patients undergoing aortic or mitral valve replacement. Adipocytes were isolated from subcutaneous (SAT), epicardial (EAT), and perivascular AT (PVAT) samples, and cultured for 24 h, after which gene expression of adipocytokines in the culture medium was determined; (3) Results: men showed reduced ADIPOQ expression in EAT and PVAT, LEP expression in PVAT, and LEPR expression in SAT and PVAT compared to women. Men also exhibited higher SAT and lower PVAT IL6 than women. Meanwhile, dyslipidemia associated with decreased ADIPOQ expression in EAT and PVAT, LEPR in EAT, and IL6 in PVAT. Arterial hypertension (AH) associated with low EAT and PVAT ADIPOQ, and high EAT LEP, SAT, as well as PVAT LEPR, and IL6 in SAT and EAT. ADIPOQ expression decreased with increased AH duration over 20 years against an increased LEP background in ATs. Smoking increased ADIPOQ expression in all ATs and increased LEP in SAT and EAT, however, decreased LEPR in PVAT. Patients 51–59 years old exhibited the highest EAT and PVAT LEP, IL-6, and LEPR expression compared to other age groups; (4) Conclusions: decreased EAT ADIPOQ expression against an increased pro-inflammatory IL6 background may increase atherogenesis and contribute to CAD progression in combination with risk factors including male sex, dyslipidemia, and AH.

KEYWORDS

adiponectin, leptin, leptin receptor, IL-6, adipose tissue, risk factors cardiovascular diseases

Introduction

Morbidity and mortality from cardiovascular diseases (CVD) remain prevalent in many countries, despite ongoing prophylaxis and the introduction of new treatment methods (1). The epicardial adipose tissue (EAT) and perivascular (PVAT) are important in the pathogenesis of atherosclerosis as they are located in close proximity to the myo-cardium and coronary arteries and function as active endocrine organs, being able to synthesize and produce adipocytokines. In patients with elevated cardiovascular risk, higher pro-inflammatory adipocytokine levels are observed in EAT than subcutaneous adipose tissue (SAT) (2), and the EAT of patients with severe coronary artery disease (CAD) expresses less adiponectin (3). Moreover, EAT thickness correlates with metabolic risk factors and contributes to coronary artery atherosclerotic plaque development (4).

Adiponectin, the main protein secreted by adipocytes, exhibits cardioprotective, an-ti-diabetic, anti-atherogenic, and anti-inflammatory effects, unlike other adipokines (5). Low adiponectin levels are associated with arterial hypertension (AH), obesity, insulin resistance, type 2 diabetes mellitus, and myocardial infarction (MI) (6). Conversely, leptin has pro-inflammatory and prothrombotic effects (7), whereas pro-inflammatory inter-leukin 6 (IL-6) can elicit hypertrophy-inducing effects and is an independent predictor of CAD vessel disease (8). Recently, an increasing body of evidence has demonstrated that the expression of adipocytokines differ depending on the location of the fat depot.

Although assessing cardiovascular risk based on traditional risk factors has high prognostic value, identifying new parameters can significantly improve the stratification model for patients with cardiovascular disease. Various factors, including pro-inflammatory markers, that have demonstrated potential association with athero-genesis, are produced by AT (9), however, data regarding the association between age, sex, and other parameters, with adiponectin, leptin, and IL-6 levels in the various local fat depots, remain limited and are often contradictory. Accordingly, determining the factors that impact the course of CAD, as well as the associated prognosis, remains critical (10). Moreover, as atherosclerosis constitutes a multifactorial disease influenced by both un-modifiable (e.g., sex, age) and modifiable (smoking, dyslipidemia) factors, clarification of the pathogenetic relationships between adipocytokines and CVD risk factors is necessary. Toward this end, we evaluated the expression of adiponectin, leptin, its soluble receptor (sOB-R), and IL-6 in EAT, PVAT, SAT, and their relationships with the main CVD risk factors.

Materials and methods

Study design and patients

This study was performed at the Federal State Budgetary Institution's Research In-stitute for Complex Issues of Cardiovascular Diseases. We examined 134 patients with a median age of 65.6 (49.3; 70.3) years with CAD who underwent elective coronary artery bypass grafting (CABG) and 120 patients median aged 60.47 (45.2; 63.2) years with aortic or mitral valve replacement. Exclusion criteria included: 1) > 75 years of age; 2) clinical conditions including MI, type 1 or type 2 diabetes mellitus, anemia, autoimmune diseases, liver or kidney failure, infectious or inflammatory diseases, and oncological diseases.

Cardiovascular risk factor assessment

Traditional cardiovascular risk factors and patient treatment were recorded. AH was defined as systolic blood pressure > 140 mm Hg Art., diastolic blood pressure > 90 mm Hg. Dyslipidemia was defined as a previously detected increase in total serum cholesterol (> 200 mg/dl), triglycerides (>200 mg/dl), or low-density lipoprotein (LDL) cholesterol (> 150 mg/dl) for at least 1 year or use of lipid-lowering drugs. Smoking was classified as current or former smokers; current smoking status was defined as at least one cigarette daily over the last year.

Sample collection and evaluation

SAT, EAT, and PVAT biopsies (3 to 5 g) were obtained during aortocoronary bypass surgery and aortic or mitral valve replacement. SAT samples were obtained from the subcutaneous tissue of the lower angle of the mediastinal wound. EAT was sourced from its largest source, from the right heart (right atrium and ventricle) and PVAT were ob-tained from the area of the right coronary artery. Adipocytes were isolated from adipose tissues under sterile conditions in a laminar flow hood (BOV-001-AMS MZMO, Millerovo, Russia), as previously described (11). Adipocytes were counted in a Goryaev chamber. Cell viability was evaluated according to the method described by Suga et al. (12). Adipocytes (20×10^5) were seeded into a 24-well plate (Greiner Bio One International GmbH, Kremsmünster, Austria), and the volume in each well was adjusted to 1 mL with culture medium, as previously described (11). Cells were incubation for 24 h at 37 ± 1°C in an atmosphere of 5% CO2 and 10% oxygen. The adipocytes were then immediately processed for RNA extraction to determine adipocytokine gene expression.

RNA extraction

Total RNA was isolated from adipocytes using the commercial RNeasy® Plus Uni-versal Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions with slight modifications, as described previously

(13). The quantity and quality of puri-fied RNA were assessed using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific) by measuring the light absorbance at 280 nm, 260 nm, and 230 nm and calcu-lating the 260/280 (A260/280) and 260/230 (A260/230) ratios. The integrity of the RNA was determined by electrophoresis in agarose gel, followed by visualization using the Gel Doc™ XR+ System (Bio-Rad, Hercules, CA, USA). Extracted RNA was stored at –70°C.

cDNA synthesis

Single-stranded cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) on a VeritiTM 96-Well Thermal Cycler (Applied Biosystems). Reverse transcription was performed using the program suggested by the manufacturer. The quantity and quality of synthesized cDNA were assessed using a NanoDrop 2000 Spectrophotometer. Samples were stored at –20°C.

Gene expression determination

Expression of adiponectin (ADIPOQ), leptin (LEP), soluble leptin receptor (LEPR) and IL6 genes was evaluated by quantitative real-time polymerase chain reaction (qPCR) using TaqManTM Gene Expression Assays (ADIPOQ Hs00605917_m1, LEP Hs00174877_m1, LEPR Hs00174497_m1, IL6 Hs00174131_m1, Applied Biosystems, USA) on a ViiA 7 Real-Time PCR System (Applied Biosystems). Each 20 µL reaction mix contained 10 µL of TaqManTM Gene Expression Master Mix (Applied Biosystems), 1 µL of TaqManTM Gene Expression Assay (Applied Biosystems), and 9 µL of cDNA template comprising 100 ng of cDNA + nuclease-free water). Samples were amplified under the following thermal cycling conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 sec at 95°C and 1 min at 60°C. As a negative control, 20 µL of reaction mix with no cDNA template was used. For each sample and negative control, three technical replicates were prepared.

The results were normalized using reference genes HPRT1, GAPDH, and B2M. Test gene expression was calculated using the Pfaffl method and expressed on a logarithmic (log10) scale as a multiple change relative to the control samples (14).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (La Jolla, CA, USA) and Statistica version 9.1 (Dell Software, Inc., Round Rock, TX, USA). The Kolmogorov–Smirnov test was used to verify normal distribution of data. For non-normally distributed variables, data were presented as median (Me) and 25th and 75th quartiles (Q1; Q3). Comparison of two independent groups was carried out using the nonparametric Mann-Whitney test. Differences between three groups were compared using one-way analysis of variance (ANOVA) for continuous variables. Categorical variables are ex-pressed as percentages and compared using chi-squared test or Fisher's exact test. P values < 0.05 were considered statistically significant.

Results

Adipocytokine gene expression in the culture medium of adipocytes collected from the adipose tissue of patients with coronary artery disease and heart defects

Analysis of the clinical and anamnestic characteristics revealed that 75% of the sub-jects were men, AH was observed in 90.5% of all patients, angina pectoris in 97.63%, family history of CAD in 59.5%, previous MI in 67.86%, history of cerebrovascular acci-dent/transient ischemic attack in 7.14%, and 69.0% of patients smoked (Table 1). Patients received standard therapy with antiplatelet agents, beta-blockers, ACE inhibitors, and HMG-CoA reductase inhibitors.

Moreover, ADIPOQ expression in CAD patients was lower in EAT compared to SAT ($p = 0.038$) and PVAT ($p = 0.027$), while that of leptin was higher in EAT compared to SAT and PVAT ($p = 0.003$ and $p = 0.002$, respectively). Similarly, LEPR was more highly ex-pressed in EAT ($p = 0.001$) and PVAT (0.0003) compared to SAT, with that in the PVAT higher than in the EAT ($p = 0.028$). In addition, EAT was characterized by the highest IL-6 expression in comparison with the of SAT and PVAT samples ($p = 0.001$ and $p = 0.025$, respectively). Similar expression patterns were observed in the tissues collected from patients with heart defects. However, patients with defects were characterized by a higher ADIPOQ expression in EAT ($p = 0.031$), lower LEP expression in EAT ($p = 0.004$) and PVAT ($p = 0.008$), lower LEPR expression in the PVAT ($p = 0.022$), as well as lower IL-6 expression in EAT ($p = 0.0002$) and PVAT ($p = 0.003$) compared to patients with CAD.

Adipocytokine gene expression in the adipocytes collected from adipose tissue based on the sex of coronary artery disease patients

Women exhibited higher ADIPOQ expression in EAT (2.5-fold, $p = 0.001$) and PVAT (2.8-fold, $p = 0.002$) compared to men, whereas expression in SAT did not differ between sexes (Figure 1).

TABLE 1 Clinical and medical history of patients with coronary heart disease.

Parameter	Patients with CAD, n=134	Patients with aortic or mitral valve replacement, n=120	P
Males	80 (60)	58 (48.3)	0.053
Age, years	65.6 (49.3; 70.3)	60.47 (45.2; 63.2)	0.061
Body mass index, kg/m^2	29.57 (25.19; 33.22)	26,59 (23.44;28.31)	0.075
Overweight	45 (33.58)	7 (5.8)	0.011
Arterial hypertension	75 (56)	28 (23.3)	0.014
Dyslipidemia	57 (42.5)	12 (10)	0.009
Smoking	64 (47.8)	14 (11.7)	0.017
Family history of coronary artery disease	79 (58.9)	42 (35)	0.049
Angina prior to MI	118 (88.1)	0	
Previous MI	91 (67.9)	0	
History of cerebrovascular accident/transient ischemic attack	10 (7.5)	0	
Atherosclerosis of other pools	21 (15.7)	0	
No angina	4 (3)	120 (100)	0.001
Angina I FC	0	0	
Angina II FC	62 (46.3)	0	
Angina III FC	68 (50.7)	0	
Angina IV FC	0	0	
CHF I FC	16 (11.9)	16 (13.3)	0.066
CHF II FC	11 (8.2)	54 (45)	0.003
CHF III FC	5 (3.7)	38 (31.6)	0.001
CHF IV FC	0	0	
Atherosclerosis of the 1st coronary artery	12 (9)	0	
Atherosclerosis of the 2st coronary artery	8 (6)	0	
Atherosclerosis of three or more coronary artery	114 (85.1)	0	
Ejection fraction, %	51.4 (43.7; 57.2)	53.1 (45.0;59.2)	0.048
C-reactive protein before surgery	2.79 (2.11;3.38)	3.02 (2.81;3.40)	0.302
	Treatment strategy/group of drugs		
Aspirin	131 (97.8)	0	
Clopidogrel	21 (15.6)	0	
Warfarin	0	100 (83.3)	
β-blockers	131 (97.8)	109 (90.8)	0.177
Angiotensin-converting enzyme	101 (75.4)	91 (75.8)	0.525
Statins	134 (100)	89 (74.2)	0.061
Calcium channel blocker	103 (76.9)	86 (71.7)	0.143
Nitrates	11 (8.2)	10 (8.3)	0.673
Diuretics	102 (76.1)	118 (98.3)	0.055

Data are presented in n (%) or Me (Q1; Q3). CAD, coronary artery disease; Me, median; Q1, first quartile; Q3, last quartile; MI, myocardial infarction; CHF, chronic heart failure; FC, functional class.

Women also showed higher LEP mRNA expression in PVAT (1.4-fold, p = 0.013), while that in SAT and EAT did not significantly differ. In men, LEPR expression was lower in SAT (1.5-fold, p = 0.033) and PVAT (1.3-fold, p = 0.042), but higher in EAT (1.3-fold, p = 0.013). Also, the IL6 mRNA expression in men was significantly higher in SAT (3-fold, p = 0.003), however, was 2-fold lower in PVAT (p = 0.01), whereas no dif-ference was observed between sexes in EAT.

Adipocytokine gene expression in the adipocytes collected from adipose tissue based on the age of coronary artery patients

Evaluation according to patient age (≤ 50 years; younger, 51–59; mid-age, and ≥ 60; older) revealed maximum SAT ADIPOQ expression in the younger group, which was

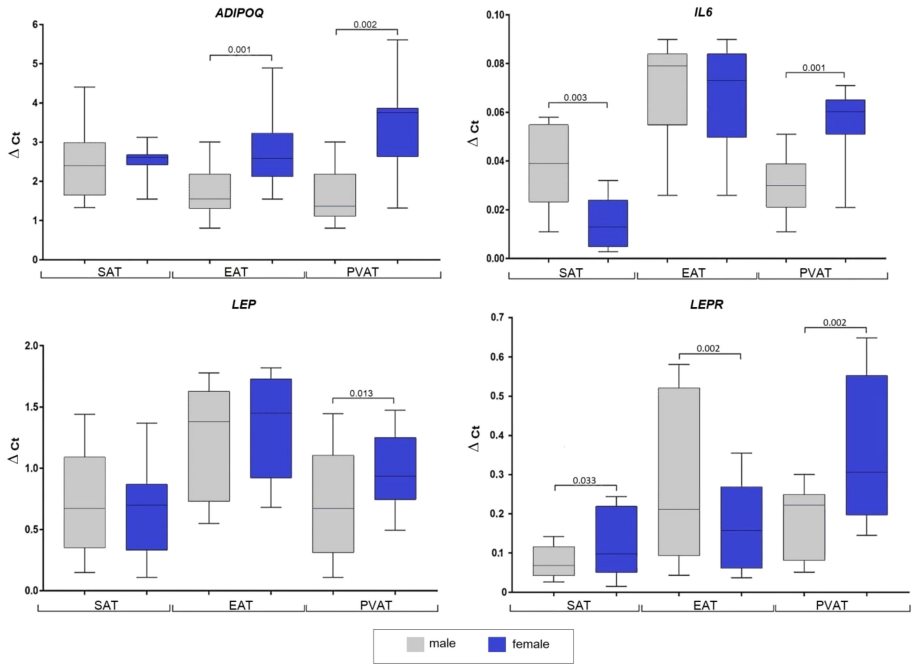

FIGURE 1
Adipocytokine genes expression in the subcutaneous, epicardial, and perivascular adipocytes based on the sex of patients with coronary artery disease. Data are presented in Me (Q1; Q3). SAT, subcutaneous adipose tissue; EAT, epicardial adipose tissue; PVAT, perivascular adipose tissue; p, level of statistical significance; sOB-R, soluble leptin receptor; IL6, interleukin 6.

equivalent to twice that observed in the mid- age (p = 0.021) and older (p = 0.013) groups (Figure 2).

In EAT, the highest ADIPOQ mRNA expression was observed in the older group, which was 1.7-fold that in the younger (p = 0.011) and mid-age (p = 0.003) groups. Lowest PVAT ADIPOQ expression was observed in the younger group which was approximately 1.4-fold lower than that in the mid-age (p = 0.002) and older (p = 0.014) groups.

The mid-age group exhibited decreased LEP in SAT (1.2-fold) compared to the younger (p = 0.002) and older (p = 0.023) groups, however, increased LEP levels were observed in EAT and PVAT of mid-age patients compared to the younger (2-fold, p = 0.013; 1.6-fold, p = 0.004, respectively) and older (1.5-fold, p = 0.003; 2.2-fold, p = 0.001, respectively) groups.

The older group exhibited the highest LEPR expression in SAT (3-fold, p = 0.001 vs. younger; 2.3-fold, p = 0.031 vs mid-age), whereas the mid-age group showed highest ex-pression in EAT and PVAT (1.6-fold, p = 0.003 and 1.8-fold, p = 0.012 vs younger; 1.1-fold, p = 0.024 and 1.2-fold, p = 0.035 vs older).

SAT IL-6 expression was higher in the older group (4-fold, p = 0.011 vs younger; 2-fold, p = 0.023 vs mid-age). Meanwhile, the mid-age individuals exhibited the highest IL6 expression in EAT (2.2-fold, p = 0.013 vs younger; 2.05-fold, p = 0.004 vs older) and PVAT (2.3-fold, p = 0. 011; 2.2-fold, p = 0.014 times, respectively) (Figure 2).

Adipocytokine gene expression in the adipocytes collected from adipose tissue based on the presence of dyslipidemia in coronary artery disease patients

In patients with dyslipidemia, ADIPOQ mRNA was decreased in EAT (2.7-fold, p = 0.021) and PVAT (3.6-fold, p = 0.033), however, did not differ in SAT (Figure 3).

Meanwhile, LEP expression was not correlated with dyslipidemia. Conversely, dyslipidemia was associated with a 2.1-fold (p = 0.014) decrease in LEPR mRNA in EAT and 2.3-fold decrease in IL6 in PVAT (p = 0.023). Alternatively, IL-6 did not differ based on dyslipidemia status in SAT and EAT.

Adipocytokine gene expression in the adipocytes collected from adipose tissue depending based on arterial hypertension in coronary artery disease patients

AH in patients with CAD exhibited decreased ADIPOQ expression in EAT (2-fold, p = 0.004) and PVAT (1.8-fold, p =

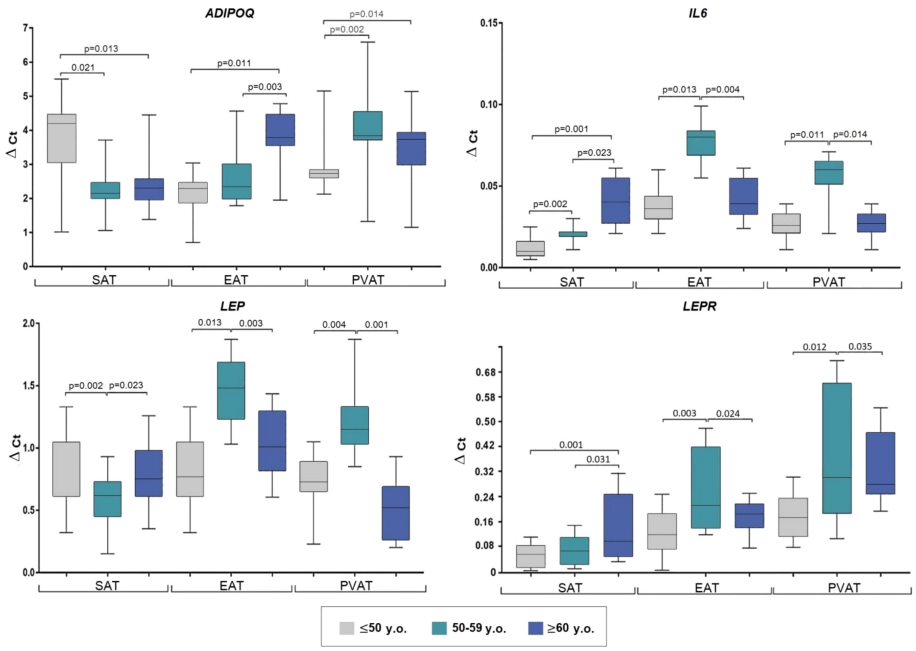

FIGURE 2
Adipocytokine gene expression in the adipocytes collected from adipose tissue based on the age of coronary artery patients. Data are presented in Me (Q1; Q3). SAT, subcutaneous adipose tissue; EAT, epicardial adipose tissue; PVAT, perivascular adipose tissue; p, level of statistical significance; sOB-R, soluble leptin receptor; IL6, interleukin 6.

0.021) coordinated with increased LEP mRNA expression in EAT (1.7-fold, p = 0.001), LEPR in SAT (3-fold, p = 0.003), and PVAT (1.7-fold, p = 0.001), but not in EAT. AH was also found to correlate with increased IL6 expression in SAT (8-fold, p = 0.013) and EAT (10.4-fold, p = 0.001) (Figure 4).

AH duration also proved important for adipocytokine expression dynamics. Spe-cifically, in patients with AH for less than 10 years, LEPR was increased in SAT, whereas it was increased in the EAT of patients with AH for 11–19 years. Meanwhile, an AH du-ration of more than 20 years was associated with decreased ADIPOQ expression owing to LEP increases in all ATs, along with decreased IL6 in SAT, which was increased in PVAT (Figure 5).

Adipocytokine gene expression in the adipocytes collected from adipose tissue based on smoking status in coronary artery disease patients

Smokers with CAD showed increased ADIPOQ expression in SAT (1.9-fold, p = 0.012), EAT (1.7-fold, p = 0.003), and PVAT (1.5-fold, p = 0.033), as well as increased LEP expression in SAT (1.6-fold, p = 0.024) and EAT (1.8-fold, p = 0.003). However, LEPR ex-pression was decreased (1.3- fold, p = 0.001) only in PVAT of smokers. No associations were found for smokers with CAD and IL6 expression (Figure 6).

Discussion

Sex-based differences

The results of this study revealed sexual dimorphism in the expression of adipo-cytokines in local fat depots, with primary EAT and PVAT localization. Specifically, men with CAD had lower ADIPOQ expression in EAT and PVAT, and lower LEP expression in PVAT compared to women. These results agreed with previously obtained data on lower LEP expression in the SAT of men relative to women (15). Our results also show a decrease in ADIPOQ and LEP expression in the EAT of male patients with CAD, while no difference was observed in the SAT (16). However, currently, a unanimous opinion has not been reached regarding the relationship between sex and the level of ADIPOQ in the PVAT. Thus, some researchers believe that sex affects both ADIPOQ expression and secretion, while others note differences only in the level of adiponectin secretion (17).

The revealed differences in adipokine expression may be due to the influence of sex hormones. For example, androgens, including testosterone, can cause dysfunction of AT through repression of

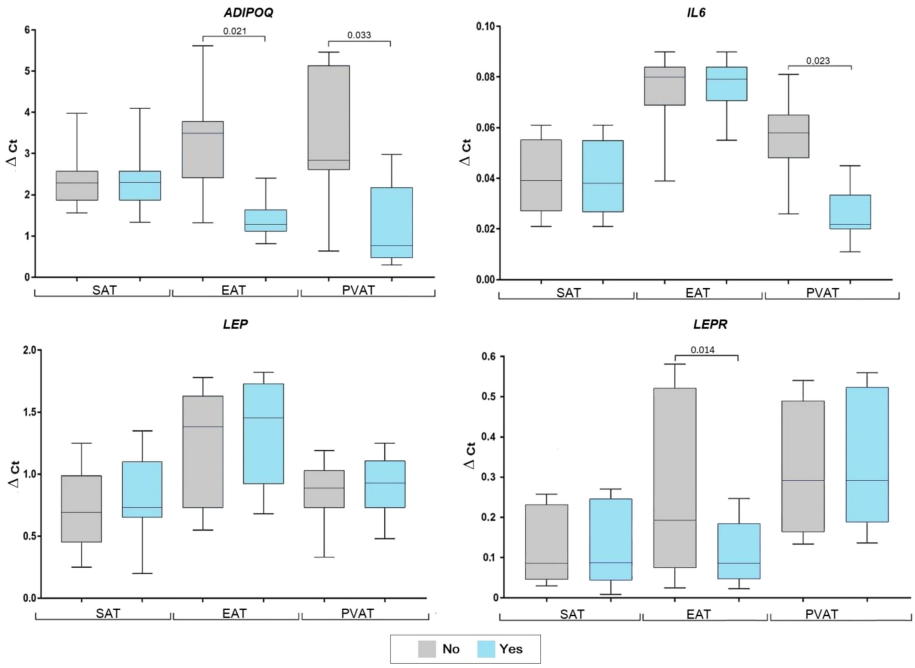

FIGURE 3
Adipocytokine gene expression in the adipocytes collected from adipose tissue based on the presence of dyslipidemia in coronary artery disease patients. Data are presented in Me (Q1; Q3). SAT, subcutaneous adipose tissue; EAT, epicardial adipose tissue; PVAT, perivascular adipose tissue; p, level of sta-tistical significance; sOB-R, soluble leptin receptor; IL6, interleukin 6.

ADIPOQ and LEP mRNA transcription *via* blocking of RNA polymerase and formation of a transcriptional complex (18). Moreover, Machinal-Quélin, F. at al. investigated the effects of *in vitro* 24-hour exposure to androgens and estrogens on LEP expression in the SAT of men and women. In men, only high concentration di-hydrotestosterone (100 nM) caused a decrease in the level of LEP expression, while in women 17-estradiol (10–100 nM) increased the expression of LEP. The authors suggested that the sexual dimorphism of LEP expression in humans is due to estrogen recep-tor-dependent stimulation of LEP expression in the AT by estrogens and estrogen pre-cursors in women (19). Further, a portion of testosterone becomes converted to estrogen through aromatization. McTernan P.G. at al. showed that the level of LEP expression in the adipocytes of women did not change in the presence of testosterone due to the low expression of aromatase in human adipocytes compared to pre-adipocytes (20).

When determining the level of IL6 expression, taking into account patient sex, men were found to have increased IL6 in SAT and decreased expression in PVAT, whereas no differences were observed in the EAT, compared to women. These results are consistent with our previous study, which demonstrated an increase of IL-6 expression in SAT during cardiac surgery (21).

Age

It was hypothesized that the expression of adipocytokines changes with age, which is inextricably linked to CVD risk, an increase in the number and degree of coronary arteries, and the incidence of CAD. Our results demonstrate a clear association between age and adipocytokine mRNA levels. For instance, patients aged 50–59 were characterized by a low level of ADIPOQ in EAT, as well as high levels of LEP and IL-6 in EAT and PVAT. These expression patterns agree with the generally accepted opinion regarding increased pro-inflammatory activity in AT with age and, thus highlights the vulnerability of this patient population. The increase in IL-6 expression is likely caused by AT aging, which represents the main source of this cytokine. This was demonstrated in an *in vitro* study that treated visceral AT of C57BL/6 mice with lipopolysaccharides and found that IL-6 production was significantly higher in adipocyte cultures of mice aged 24 months com-pared to young mice (4 months). The authors also showed that IL-6 overproduction is regulated by the autocrine/paracrine action of IL-1β, which initiates inflammatory pro-cesses in old age (22).

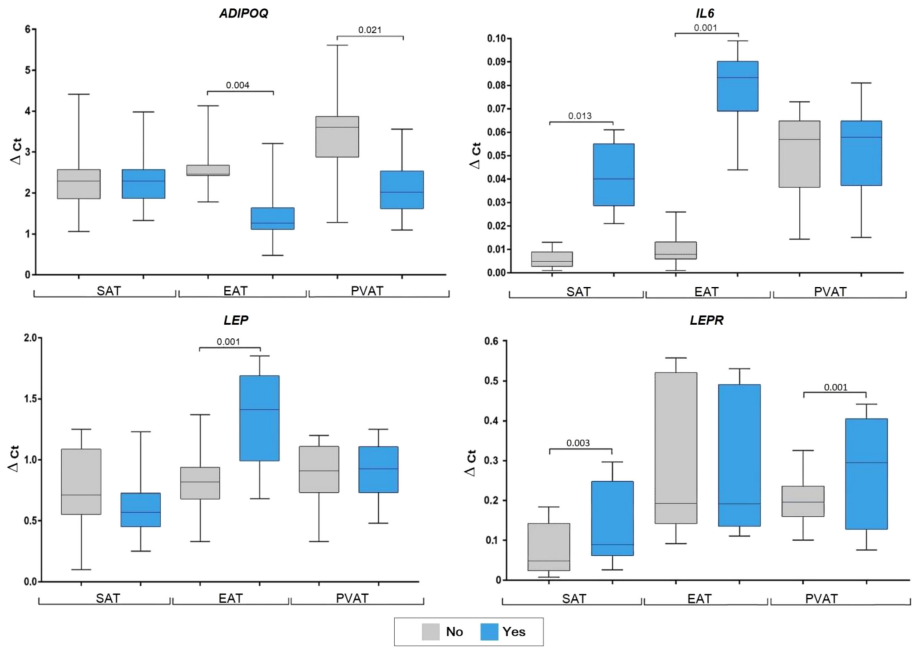

FIGURE 4
Adipocytokine gene expression in the adipocytes collected from adipose tissue based on arterial hypertension in coronary artery disease patients. Data are presented in Me (Q1; Q3). SAT, subcutaneous adipose tissue; EAT, epicardial adipose tissue; PVAT, perivascular adipose tissue; AH, arterial hypertension; p; level of statistical significance; sOB-R, soluble leptin receptor; IL6, in-terleukin 6.

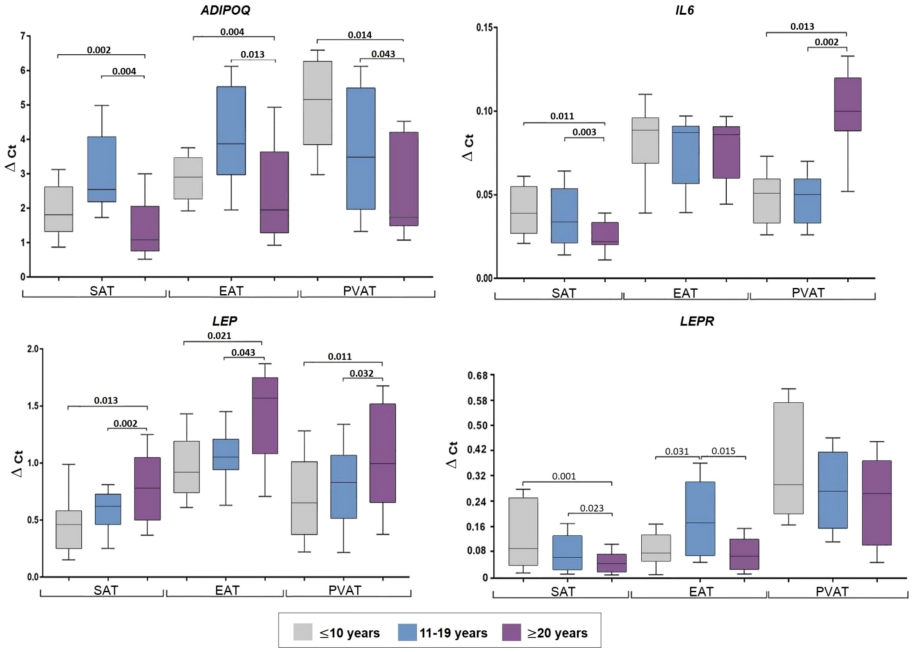

FIGURE 5
Adipocytokine genes expression in the subcutaneous, epicardial, and perivascular adipocytes based on duration of arterial hypertension in patients with coronary artery disease. Data are presented in Me (Q1; Q3). SAT, subcutaneous adipose tissue; EAT, epicardial adipose tissue; PVAT, perivascular adipose tissue; p, level of sta-tistical significance; sOB-R, soluble leptin receptor; IL6, interleukin 6.

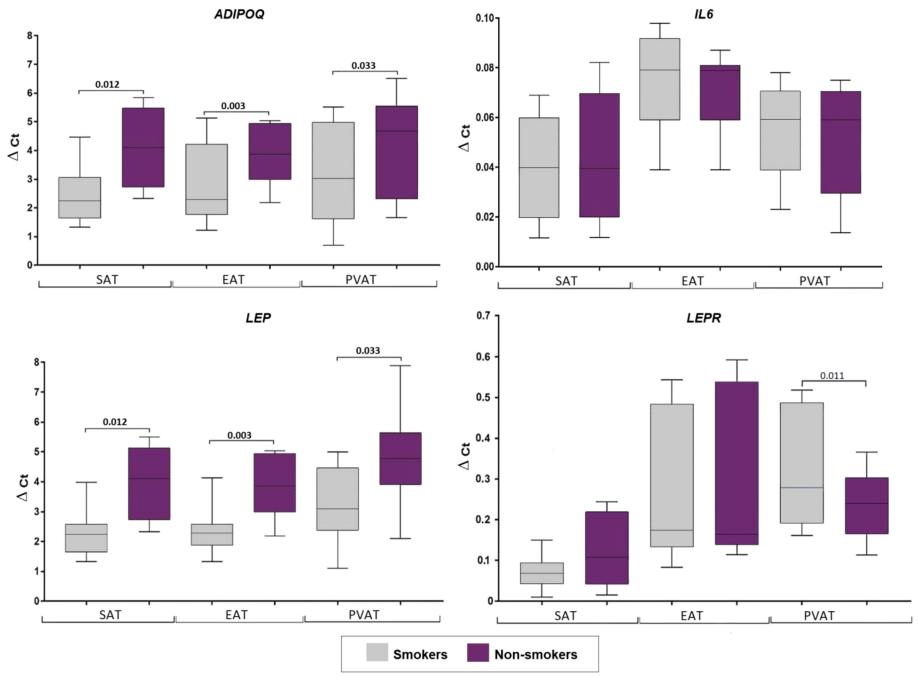

FIGURE 6
Adipocytokine gene expression in the culture medium of adipocytes collected from adipose tissue based on smoking status in coronary artery disease patients. Data are presented in Me (Q1; Q3). SAT, subcutaneous adi-pose tissue; EAT, epicardial adipose tissue; PVAT, perivascular adipose tissue; p, level of statistical significance; sOB-R, soluble leptin receptor; IL6, interleukin 6.

Dyslipidemia

Age-related AT dysfunction is believed to be associated with dyslipidemia, metabolic dysfunction, and mild chronic systemic inflammation, which affect the quality and duration of life (23). In the current study, the presence of dyslipidemia in CAD patients correlated with decreased ADIPOQ expression in EAT and PVAT. Similarly, EAT LEPR expression was lower in individuals with dyslipidemia, as was PVAT IL6 expression.

However, it was previously shown that ADIPOQ expression increases in the PVAT of men with CAD and a BMI above 30 kg/m2 compared to patients with a lower BMI (24), which, according to the authors, is reflective of the "obesity paradox." Adiponectin affects the accumulation of LDL-C in the vascular wall, inhibiting its oxidation, as well as the transformation of macrophages into foam cells, and proliferation of smooth muscle cell neointima, while stimulating expression of the cholesterol ABCA1 ATP-binding transporter in the liver, thereby enhancing the biogenesis and reverse transport of HDL cholesterol exhibiting antiatherogenic properties (25). The observed decrease in ADIPOQ expression within patients with dyslipidemia, therefore, indicates negation of the above protective effects and contributes to the progression of atherosclerosis and vascular damage.

Furthermore, the decreased IL6 expression observed in the PVAT of CAD patients may result in increased accumulation of lipids in adipocytes, causing their hypertrophy. IL-6 is known to inhibit the expression of lipoprotein lipase (LPL), the most abundant of which is the cells of AT, heart and skeletal muscles. Normally, LPL is exported from ad-ipocytes to the endothelial lining of AT capillaries, where it cleaves the triglycerides of chylomicrons and VLDL, thereby regulating the concentration of triglycerides (26).

Arterial hypertension

Analysis of adipocytokine expression based on the presence of AH demonstrated a decrease in ADIPOQ expression in EAT against the background of increased LEP and IL-6 expression. Moreover, the presence of hypertension for more than 20 years was found to be associated with a decrease in the level of ADIPOQ and increase in LEP within all AT types. Our results are consistent with those of a previous study that reported reduced expression of ADIPOQ and its receptors (AdipoR1 and AdipoR2) within the perivascular adipocytes of mice with angiotensin II-induced hypertension (27). Similarly, Teijeira-Fernandez et al. reported a decrease in ADIPOQ expression in the EAT of AH patients. The

authors concluded that of ADIPOQ expression in EAT may be associated with AH status regardless of CAD or other concomitant diseases, which confirms the hypothesis regarding the effect of EAT on CVD (28).

In hypertension, the expression of LEP increases in EAT, which, given the possible proliferative effect of leptin and the effect on vascular permeability, may contribute to the progression of this disease. Research by Nepomuceno at al. demonstrated the presence of a direct correlation between LEP expression and blood pressure (29). The simultaneous decrease of ADIPOQ expression in the EAT and PVAT may also have an unfavorable affect as this adipokine attenuates vascular damage in hypertension.

Smoking

When determining if smoking affects the expression of adipocytokines in CAD pa-tients, it was found that smoking is associated with an increase in ADIPOQ (in all types of AT), and LEP expression (in SAT and EAT), however, does not impact IL-6 expression. Similarly, a previous study sought to examine the effect of tobacco smoke *in vitro* and *in vivo* on the intracellular and extracellular distribution of adiponectin and its high mo-lecular weight form. Results showed that the total secretion of adiponectin was sup-pressed, while administration of tobacco smoke extract to mice reduced the adiponectin concentration in culture medium and the plasma of wild-type mice against the back-ground of its intracellular accumulation in cultured adipocytes. They further reported an enhancement of the adiponectin-retaining chaperone ERp44, localized in the endoplasmic reticulum, as well as suppression of the adiponectin secretion factor DsbA-L, following exposure to tobacco smoke. These results can help explain hypoadiponectinemia and the increased risk of developing T2DM in smokers due to its intracellular delay in the AT when exposed to tobacco smoke (30).

Moreover, the observed results regarding LEP expression agree with those of a pre-vious study that reported the effect of nicotine on the expression and secretion of leptin *in vitro*. They found that LEP expression did not differ significantly during the first 6 h of incubation with nicotine in cultured 3T3-L1 mouse adipocytes and AT explants from healthy women who underwent mammoplasty surgery. Meanwhile, LEP expression in 3T3-L1 mouse cells increased in the first hour and subsequently decreased by 45% after a 6-hour incubation with 0.5 μg/ml nicotine. However, low dose nicotine (0.05 μg/ml) did not affect LEP expression in 3T3-L1 cells. The observed change in LEP expression in cul-tured cells incubated with nicotine, and subsequent sharp decrease in plasma leptin concentration when smoking cigarettes suggested that the decrease in plasma leptin concentration in smokers is not associated with the direct effect of nicotine on LEP ex-pression and secretion, but rather with indirect exposure to catecholamines (31).

Study limitations

Certain limitations were noted in this study. First, it was a single-centered study and, second, the sample size was small.

Data availability statement

Data cannot be provided upon request due to the fact that the Local Ethics Committee cannot approve the transfer of any data on the patients participating in scientific and clinical researches which was conducted on the basis of the NII KPSSZ, since this is contrary to ethical standards and Federal Law of the Russian Federation "On Personal Data" No. 152-FZ of July 27, 2006.

Ethics statement

The study protocol was approved by the institutional Local Ethics Committee, and was performed in ac-cordance with the World Medical Association's Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, 2000 edition, and the "GCP Principles in the Russian Federation", approved by the Russian Ministry of Health. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization, OG; data curation, OG, formal analysis YD, EB; investigation YD, EB, MS; methodology, OG, YD, administration, OB; resources, KK; supervision, OB; validation, OG; writing—original draft preparation, OG, YD, EB; writ-ing—review and editing, OG, YD. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Neck-to-height ratio is positively associated with diabetic kidney disease in Chinese patients with type 2 diabetes mellitus

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Introduction: The aim of this study was to investigate the associations of neck circumference (NC) and neck-to-height (NHR) with diabetic kidney disease (DKD) in Chinese patients with type 2 diabetes mellitus (T2DM).

Materials and methods: A total of 2,615 patients with prevalent T2DM were enrolled. NHR was calculated through NC (cm) divided by height (cm), and prevalent DKD was defined as the urinary albumin-to-creatinine ratio (UACR) ≥ 30 mg/g or the estimated glomerular filtration rate (eGFR) < 60 ml/min per 1.73 m^2 in the absence of other primary kidney diseases.

Results: The levels of NC and NHR were higher in DKD patients compared with non-DKD patients (38.22 vs. 37.71, P = 0.003; 0.232 vs. 0.227, P < 0.001, respectively). After full adjustments, individuals at the highest tertile of NHR had higher odds of DKD than those at the lowest tertile (multivariate-adjusted OR = 1.63, 95% CI: 1.22, 2.18), but this association was not pronounced with NC (multivariate-adjusted OR = 1.24, 95% CI: 0.87, 1.76). Individuals at the highest tertile of NHR had lower eGFR (β = -4.64, 95% CI: -6.55, -2.74) and higher UACR levels (β = 0.27, 95% CI: 0.10, 0.45) than those at the lowest tertile. The adverse association between NHR and prevalent DKD remained statistically significant among most of the subgroups analyzed and no interaction effects were observed.

Conclusion: The increase in NHR was adversely and independently associated with DKD in this Chinese T2DM population.

KEYWORDS

upper-body subcutaneous fat, neck-to-height ratio, neck circumference, diabetic kidney disease, interactive analysis

1 Introduction

Diabetic kidney disease (DKD), as one of the most common chronic complications of diabetes, is developed in about 20 - 40% of patients with diabetes (1). Patients with DKD are more likely to progress to end-stage renal disease (ESRD), as well as have a higher risk of cardiovascular diseases (CVD) and all-cause mortality (2, 3). It is vital to discover potential markers to identify patients at a higher risk of DKD.

Obesity is proved to be an important risk factor for kidney damage. Adipose tissue releases a mass of signaling molecules, including inflammatory and hormonal factors, which are critical for inter-organ crosstalk. The communication between adipocytes and the kidney, known as the adipo-renal axis is critical for normal kidney function and the effective response of the kidney to injury (4, 5). Meanwhile, plenty of anthropometric indices of obesity, such as body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), and the Chinese visceral adiposity index (CVAI) have already been reported to be related to DKD (6–8).

Upper-body subcutaneous fat, a unique fat depot independent of generalized and central adiposity, could present extra risk for metabolic disorders (9, 10). Evidence to date have suggested that upper-body subcutaneous fat could always be estimated by neck circumference (NC) (11), which as a simple anthropometric index is not affected by clothing or feeding. However, NC as a regional obesity indicator, could not take the overall body fat distribution fully into account. Neck-to-height ratio (NHR), adjusted for the discrepancies in NC attributable to different heights, shows its advantage in reflecting the whole body fat distribution based on height. And accumulating evidence from clinical studies supported that NHR was a better index for the assessment of upper-body subcutaneous fat than NC in patients with metabolic disorders (12–14). Of note, population-based studies that focused on the relationship between upper-body subcutaneous fat and kidney damage are limited. In populations without diabetes, clinical findings suggested that NC was associated with indicators of kidney dysfunction (15–17). Only a Chinese study targeted subjects with diabetes showed that NC was positively associated with the prevalence of DKD (8). Furthermore, there has been no population-based studies to investigate the association between NHR and renal damage.

Therefore, the goal of our study was to explore the associations of NC and NHR with DKD in patients with type 2 diabetes mellitus (T2DM).

2 Materials and methods

2.1 Study subjects

A total of 3267 adults were enrolled from National Metabolic Management Center (MMC) (18, 19) in the First Affiliated Hospital of Wenzhou Medical University from January 2017 to May 2021. 3067 subjects were diagnosed with T2DM according to the diagnostic criteria of World Health Organization (WHO) (20). The exclusion criteria were as followed: 1) patients without data of NC, height measurements, serum creatinine or the urinary albumin–to–creatinine ratio (UACR); 2) patients with acute or chronic nephritis, IgA nephropathy, or other primary kidney diseases, or had kidney space-occupying surgeries before. Finally, 2615 patients were included in the present study. An overview of the patients selected was presented in Figure 1.

The study was approved by the Ethics Committee in Clinical Research of the First Affiliated Hospital of Wenzhou Medical University (No: KY2021-173), and all participants have been given written informed consent.

2.2 Data collection

Including systolic blood pressure (SBP), diastolic blood pressure (DBP), weight, height, WC and NC were measured by trained staff according to standard protocols. Body weight and standing height were measured accurate to the 0.1 kg and 0.1 cm without shoes or heavy clothes. WC was measured at the midpoint between the lowest rib and the iliac crest. NC was measured with the upper border of a flexible tape placed below the laryngeal prominence and circled vertically to the long axis of the neck (9). BMI was calculated through body weight (kg) divided by the square of height (m^2). NHR was calculated through NC (cm) divided by height (cm).

Biochemical indicators, including fasting plasma glucose (FBG), glycosylated hemoglobin A1c (HbA1c), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and uric acid (UA) were assayed through venous blood samples obtained in the morning after an overnight fast (≥ 8h). Non-HDL-c was calculated through TC minus HDL-C. Serum creatinine (Cr), urinary albumin and urinary creatinine were measured with an automatic biochemical analyzer (Beckmann AU 5800). UACR was ratios of urinary albumin to urinary creatinine, the estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (21).

Diabetes duration, lifestyle factors including education attainment, current smoking and drinking and medication history were all obtained by standardized questionnaires.

2.3 Definition of variables

DKD was defined as UACR ≥ 30 mg/g or eGFR < 60 ml/min per 1.73 m^2, meanwhile in the absence of other primary kidney diseases as suggested by the ADA recommendations (1).

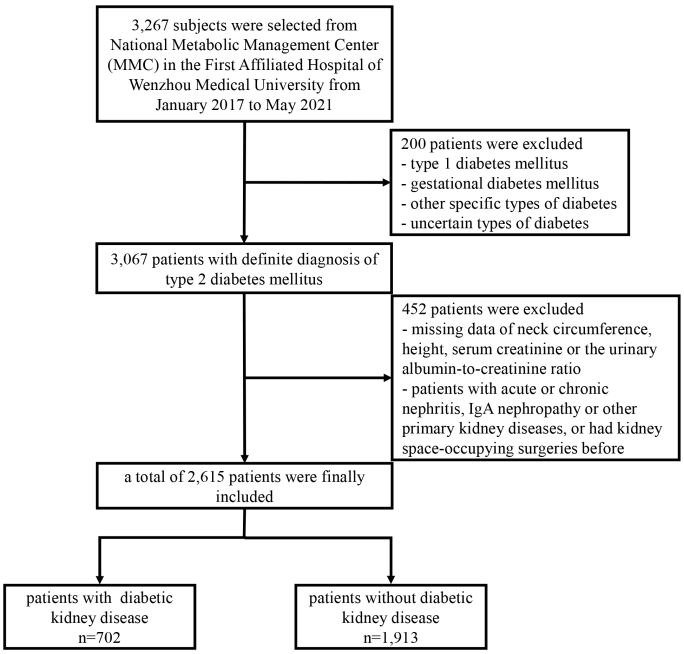

FIGURE 1
Flowchart of study participants included.

Overweight/general obesity was defined as BMI ≥ 24 kg/m^2, and central obesity was defined as WC ≥ 90 cm for men, ≥ 85 cm for women, all according to the Guideline for the Prevention and Treatment of Type 2 Diabetes Mellitus in China (22). Hypertension was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, or undergoing antihypertensive medication currently (23). Dyslipidemia was defined as TG ≥ 2.3 mmol/L, or TC ≥ 6.2 mmol/L, or HDL-c < 1.0 mmol/L, or LDL-c ≥ 4.1 mmol/L, or non-HDL-c ≥ 4.9 mmol/L, suggested by the 2016 Chinese Guidelines for the Management of Dyslipidemia in Adults (24).

2.4 Statistical analysis

All statistical analyses were performed by SPSS version 26.0 software (IBM Corporation). Data were displayed as means ± standard deviation or as median (interquartile range) for continuous variables, numbers and percentage for categorical variables. Discrepancies between subjects with and without DKD were analyzed using Student's t test for normally distributed continuous variables, Mann-Whitney U test for abnormally distributed continuous variables, and chi-square test for categorical variables. Multivariable logistic regression models were applied to investigate the relationship between DKD and the tertiles of NC and NHR, odds ratios (ORs) and 95% confidence intervals (CIs) were provided. In addition, multivariable linear regression models were used for eGFR and log-transformed UACR (LnUACR) in relation to the tertiles of NHR. Subgroup analyses were conducted to test the potential interactions between NHR and the other cardiometabolic factors on DKD. For regression models that mentioned above: age and sex were adjusted for in model 1; age, sex, diabetes duration, smoking and drinking status, SBP, TC, FBG were adjusted for in model 2; age, sex, diabetes duration, smoking and drinking status, SBP, TC, FBG, BMI, WC, antidiabetic agents currently and antihypertensive agents currently were adjusted for in model 3. All P values were two-sided and considered statistically significant when < 0.05.

3 Results

3.1 Baseline characteristics

There were 702 (26.85%) patients with DKD and 1913 (73.15%) patients without DKD enrolled in the present study. NC and NHR levels were significantly higher in patients with DKD compared to those without (38.22 vs. 37.71, P = 0.003; 0.232 vs. 0.227, P < 0.001). In contrast to patients without DKD, those with DKD were older, less educated, and had longer diabetes duration, higher proportions of women and non-smokers, as well as had higher levels of SBP, DBP, BMI, WC,

FBG, TG, TC, Cr, UACR, UA, current antidiabetic agents, current antihypertensive agents usage and lower height, eGFR (all $P < 0.05$). There were no statistical differences in current drinking status, HbA1c, HDL-c or LDL-c between the two groups (Table 1).

3.2 Associations of NC and NHR with prevalent DKD

As shown in Table 2, after full adjustments for age, sex, diabetes duration, smoking and drinking status, SBP, TC, FBG,

TABLE 1 Baseline characteristics of study participants based on DKD status.

	Total	DKD+	DKD-	P Value
Participants (n, %)	2615	702, 26.85	1913, 73.15	/
Socio-demographics factors				
Age (years)	50.96 ± 10.95	53.29 ± 10.43	50.10 ± 11.02	<0.001
Female (n, %)	829, 31.70	267, 38.03	562, 29.38	<0.001
Education attainment: high school or above (n, %)	633, 24.61	116, 16.89	517, 27.43	<0.001
Diabetes duration (month)	68.0 (12.0, 135.0)	109.5 (41.0, 165.0)	60.0 (4.0, 126.0)	<0.001
Lifestyle risk factors				
Current smoking (n, %)	874, 33.44	202, 28.77	672, 35.15	0.002
Current drinking (n, %)	1142, 43.67	297, 42.31	845, 44.17	0.394
Anthropometric parameters				
SBP (mmHg)	127.26 ± 18.72	134.78 ± 20.77	124.51 ± 17.11	<0.001
DBP (mmHg)	75.42 ± 11.00	77.95 ± 12.00	74.49 ± 10.46	<0.001
BMI (kg/m^2)	24.67 ± 3.49	25.28 ± 3.71	24.45 ± 3.38	<0.001
WC (cm)	88.98 ± 9.59	90.39 ± 9.97	88.46 ± 9.39	<0.001
NC (cm)	37.85 ± 3.78	38.22 ± 4.07	37.71 ± 3.66	0.003
Height (cm)	164.7 ± 8.4	163.2 ± 8.5	165.3 ± 8.3	<0.001
NHR	0.228 (0.217, 0.241)	0.232 (0.221, 0.245)	0.227 (0.215, 0.239)	<0.001
Biochemical indexes				
FBG (mmol/L)	7.7 (6.1, 9.7)	8.1 (6.2, 10.4)	7.5 (6.0, 9.5)	<0.001
HbA1c (%)	10.09 ± 2.41	10.12 ± 2.35	10.08 ± 2.43	0.660
TG (mmol/L)	1.55 (1.07, 2.35)	1.81 (1.24, 2.70)	1.45 (1.02, 2.23)	<0.001
TC (mmol/L)	4.83 (4.04, 5.68)	4.97 (4.10, 5.92)	4.78 (4.02, 5.60)	0.001
HDL-c (mmol/L)	0.99 (0.84, 1.16)	0.98 (0.84, 1.14)	0.99 (0.84, 1.17)	0.229
LDL-c (mmol/L)	2.71 ± 0.90	2.69 ± 0.94	2.71 ± 0.89	0.550
Cr (μmol/L)	62.0 (52.0, 73.0)	65.0 (51.0, 83.0)	62.0 (52.0, 71.0)	<0.001
eGFR (ml/min per 1.73 m^2)	104.16 ± 18.44	95.91 ± 25.16	107.18 ± 14.13	<0.001
UACR (mg/g)	11.10 (5.90, 33.00)	107.10 (48.48, 337.50)	7.80 (5.00, 13.00)	<0.001
UA (μmol/L)	316.0 (261.0, 379.0)	329.0 (266.0, 401.5)	312.0 (260.0, 372.0)	<0.001
Antidiabetic agents currently(n, %)	1775, 67.98	543, 77.57	1232, 64.47	<0.001
Antihypertensive agents currently (n, %)	747, 28.61	312, 44.51	435, 22.77	<0.001

The data were displayed as means ± standard deviation or as median (interquartile range) for continuous variables, or numbers and percentage for categorical variables. DKD diabetic kidney disease, SBP systolic blood pressure, DBP diastolic blood pressure, BMI body mass index, WC waist circumference, NC neck circumference, NHR neck-to-height ratio, FBG fasting plasma glucose, HbA1c glycosylated hemoglobin A1c, TG triglyceride, TC total cholesterol, HDL-c high-density lipoprotein cholesterol, LDL-c low-density lipoprotein cholesterol, Cr serum creatinine, UACR urinary albumin-to-creatinine ratio, UA uric acid

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TABLE 2 Associations of NC and NHR with the prevalence of DKD in patients with T2DM.

	Case/Participants (%)	Model 1			Model 2			Model 3		
		OR	95% CI	P Value	OR	95% CI	P Value	OR	95% CI	P Value
NC										
T_1	174/687 (25.33)	1.00			1.00			1.00		
T_2	227/866 (26.21)	1.45	1.13-1.88	0.004	1.29	0.98-1.69	0.071	1.17	0.88-1.56	0.290
T_3	301/1062 (28.34)	1.98	1.50-2.61	<0.001	1.48	1.10-1.99	0.010	1.24	0.87-1.76	0.232
NHR										
T_1	173/877 (19.73)	1.00			1.00			1.00		
T_2	242/864 (28.01)	1.70	1.35-2.13	<0.001	1.48	1.16-1.88	0.002	1.43	1.11-1.85	0.006
T_3	287/874 (32.84)	2.21	1.76-2.78	<0.001	1.77	1.39-2.25	<0.001	1.63	1.22-2.18	0.001

Model 1: age, sex; Model 2: Model 1 + diabetes duration, smoking and drinking status, SBP, TC, FBG; Model 3: Model 2 + BMI, WC, antidiabetic agents currently, antihypertensive agents currently. NC neck circumference, NHR neck-to-height ratio, DKD diabetic kidney disease, T2DM type 2 diabetes mellitus, OR odds ratio, CI confidence interval, SBP systolic blood pressure, TC total cholesterol, FBG fasting plasma glucose, BMI body mass index, WC waist circumference

BMI, WC, antidiabetic agents currently and antihypertensive agents currently, the highest tertile of NC was not associated with prevalent DKD compared to the lowest tertile of NC (OR = 1.24, 95% CI: 0.87, 1.76). However, patients at the highest tertile of NHR were 1.63 times more likely to have DKD (OR = 1.63, 95% CI: 1.22, 2.18) than those at the lowest tertile of NHR in the same full-adjusted model.

The secondary analyses were further performed to explore the associations of NHR with levels of eGFR and UACR. Compared with the lowest one, the highest tertile of NHR was significantly associated with lower eGFR level (β = -4.64, 95% CI: -6.55, -2.74) and higher LnUACR level (β = 0.27, 95% CI: 0.10, 0.45) after full adjustments (Table 3).

3.3 Subgroup analysis

Interaction effects were analyzed in strata of sex, age, diabetes duration, overweight/general obesity, central obesity, hypertension, and dyslipidemia after total adjustments. As presented in Table 4, participants at the highest tertile of NHR remained at a higher risk of DKD than those at the lowest tertile among most of the strata analyzed, except in those with older age, shorter diabetes duration, central obesity, and in those without overweight/general obesity or dyslipidemia. No interactive effects were observed in any of these strata (all *P* for interaction > 0.05)

4 Discussion

The current study was the first population-based epidemiological study to investigate the associations of NC and NHR, as indicators of upper-body subcutaneous fat, with prevalent DKD in Chinese population with T2DM. Our major finding indicated that NHR, instead of NC, was positively associated with the presence of DKD in patients with T2DM, independent of cardiometabolic risk factors. The increase in NHR was also related to a decrease in eGFR and an increase in UACR levels. Such discoveries suggested that NHR might be a potential indicator for identifying patients at a higher risk of DKD.

Studies about the relationship between NC and kidney dysfunction were limited and inconsistent. A research based on the general Chinese adults found the negative association between NC and eGFR (16). Similarly, a Korean community-based study also showed that eGFR was decreased in subjects with higher NC (17). However, a study including 177 patients with high cardiometabolic risk indicated that larger NC was related to the higher eGFR level (15). The disagreements with these studies might due to different health conditions of populations enrolled and distinct influencing factors considered. A Chinese research hold by Wan et al. showed that NC was positively associated with prevalent DKD, but without further adjusted for WC (8). WC, a proxy for central obesity, was found to be closely related to microalbuminuria and renal damage in patients with diabetes (25–27). Thereby, in order to testify the independent effect of NC on DKD, the impact of WC should be considered. Of note, our study found that the connection between NC and DKD disappeared with adjustments for WC. Moreover, in Xue et al.'s study (16), the negative association of NC with eGFR no longer existed in subjects with diabetes, which suggested that the relationship between NC and eGFR might be concealed by the strongly harmful effect of hyperglycemia on renal function. These results indicated that NC might be unstable and inaccurate to reveal kidney damage in patients with diabetes.

TABLE 3 Associations between NHR and eGFR/LnUACR level in patients with T2DM.

NHR	Case/Participants (%)	eGFR[a]			LnUACR[b]		
		β	95% CI	P Value	β	95% CI	P Value
T_1	173/877 (19.73)	1.00			1.00		
T_2	242/864 (28.01)	-2.19	-3.84- (-0.55)	0.009	0.07	-0.08-0.22	0.381
T_3	287/874 (32.84)	-4.64	-6.55- (-2.74)	<0.001	0.27	0.10-0.45	0.002

[a]The model was adjusted for sex, diabetes duration, smoking and drinking status, SBP, TC, FBG, BMI, WC, antidiabetic agents currently and antihypertensive agents currently.
[b]The model was adjusted for age, sex, diabetes duration, smoking and drinking status, SBP, TC, FBG, BMI, WC, antidiabetic agents currently and antihypertensive agents currently.
NHR neck-to-height ratio, eGFR the estimated glomerular filtration rate, LnUACR log-transformed urinary albumin-to-creatinine ratio, T2DM type 2 diabetes mellitus, CI confidence interval, SBP systolic blood pressure, TC total cholesterol, FBG fasting plasma glucose, BMI body mass index, WC waist circumference

TABLE 4 Subgroup analyses on the association of NHR with the prevalence of DKD in T2DM patients.

Subgroup	Case/Subjects (%)	OR	95% CI	P Value	P for interaction
Total	702/2615 (26.85)	1.63	1.22-2.18	0.001	
Sex					0.241
Male	435/1786 (24.36)	1.58	1.08-2.31	0.019	
Female	267/829 (32.21)	1.62	1.02-2.58	0.041	
Age					0.557
< 65 years	598/2348 (25.47)	1.62	1.18-2.20	0.003	
≥ 65 years	104/267 (38.95)	1.93	0.86-4.37	0.112	
Diabetes duration (median)					0.473
< 68 months	257/1289 (19.94)	1.52	0.97-2.37	0.066	
≥ 68 months	439/1292 (33.98)	1.75	1.19-2.57	0.005	
Overweight/ general obesity					0.562
No	257/1149 (22.37)	1.56	0.95-2.56	0.082	
Yes	445/1466 (30.35)	1.58	1.05-2.38	0.027	
Central obesity					0.577
No	292/1239 (23.57)	2.40	1.54-3.75	<0.001	
Yes	409/1371 (29.83)	1.31	0.87-1.97	0.196	
Hypertension					0.414
No	193/1345 (14.35)	1.65	1.02-2.67	0.043	
Yes	509/1270 (40.08)	1.59	1.10-2.30	0.013	
Dyslipidemia					0.266
No	198/898 (22.05)	1.37	0.81-2.30	0.237	
Yes	504/1717 (29.35)	1.91	1.34-2.72	<0.001	

Above analyses were adjusted for age, sex, diabetes duration, smoking and drinking status, SBP, TC, FBG, BMI, WC, antidiabetic agents currently and antihypertensive agents currently.
NHR neck-to-height ratio, DKD diabetic kidney disease, T2DM type 2 diabetes mellitus, OR odds ratio, CI confidence interval, SBP systolic blood pressure, TC total cholesterol, FBG fasting plasma glucose, BMI body mass index, WC waist circumference.

Likewise, several other studies have showed that in contrast to NC, NHR was more closely related to metabolic disorders, such as arterial stiffness, liver stiffness, obstructive sleep apnea syndrome (OSAS) and metabolic syndrome (MetS) (12, 13, 28–30). A community-based study demonstrated that the increase in NHR, rather than NC was related to higher brachial-ankle pulse wave velocity (baPWV) (12). An Indian study revealed that NC and NHR were both great indicators for MetS, but as to

cardiovascular risk prediction, NHR was more plausible (29). It's conceivable that NHR is more reliable than NC to represent for upper-body subcutaneous fat, since it considers the effect of height on whole body fat distribution. However, no studies to date have been done to explore the relationship of NHR with kidney dysfunction in any population. Our study based on Chinese patients with T2DM discovered that NHR was positively associated with prevalent DKD after full adjustments. What's more, none of the interaction effects of cardiometabolic risk factors, which were all found to be closely related to DKD (1, 7, 31, 32), on the association between NHR and DKD were observed in our study. It indicated that the influence of NHR on DKD was not interfered by these cardiometabolic risk factors and further revealed the relative independence and stability of NHR in its relationship with DKD. Additionally, in consideration of BMI and WC as indicators for generalized and central fat accumulations respectively, our findings might indirectly prove that upper-body subcutaneous fat accumulation, represented by NHR, was indeed the unique fat site, which could confer extra metabolic risks beyond generalized and central obesity (33, 34).

The mechanisms for the association between excessive upper-body subcutaneous fat and the increasing risk of DKD remained unclear. Firstly, upper-body subcutaneous fat releases the majority of free fatty acids (FFAs) (35, 36), which could lead to the endothelial dysfunction and motivate the production of reactive oxygen species (37, 38), thereby having pathogenic effects on kidney, especially on tubulointerstitium (39) and podocytes (40–42). Secondly, larger upper-body subcutaneous fat is closely related to the increasing risk of IR (10, 43). While insulin sensitivity of the glomerular podocytes is vital for normal renal function (44), and previous studies have discovered that IR did propel the development of DKD (45, 46). Thirdly, patients with larger upper-body subcutaneous fat are more likely to have OSAS (47), the latter would accelerate the progress of DKD and other diabetic microvascular complications *via* promoting oxidative and nitrosative stress (48–50). At last, Mangge et al. proposed that nuchal fat accumulation, by secreting inflammatory cytokines and adipokines (51), might accelerate cell turnover and mitochondrial activity, thus result in telomeres damage and shortening (52). Shorter pieces of telomeres lead to senescent cells and ultimately influence phenotypes and functions of organs (53). Previous studies discovered that patients with shorter telomere length developed increased microalbuminuria, reduced eGFR and impaired kidney function (54–56). Telomere shortening may be another cause for the renal damage due to excessive upper-body subcutaneous fat.

There were several limitations in our study. Firstly, it was a cross-sectional study, causality between NHR and DKD cannot be established. Secondly, our study participants were from a single center and the great majority of them were hospitalized for relative poor glycemic control, it's generalizability should be verified by involving outpatients or community patients in the future. Thirdly, direct adipose tissue measurements, such as CT or MRI are required to verify the authenticity of NHR. Cohort studies with larger and multicentric samples should also be done.

5 Conclusion

The present study demonstrated that the higher levels of NHR was significantly associated with the higher presence of DKD in Chinese patients with T2DM, independent of cardiometabolic risk factors. NHR might be a potential indicator for screening renal dysfunction in patients with T2DM, which needs more prospective studies to be confirmed in the future.

Data availability statement

The datasets are available from the corresponding author on reasonable request. Requests to access these datasets should be directed to X-JG, guxuejiang@wmu.edu.cn.

Ethics statement

The study involving human participants were reviewed and approved by the Ethics Committee in Clinical Research of the First Affiliated Hospital of Wenzhou Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Z-YH has made substantial contributions to the draft of the manuscript, and analysis of data. Z-YH, XG, L-JD, Y-QL, JL, and L-YP have made acquisition of data. XH, X-XZ, L-JY, BY, X-JG, and X-LL have been involved in revising the manuscript. X-JG and X-LL have given final approval of the version to be published. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Obesity-related kidney disease: Beyond hypertension and insulin-resistance

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Chronic kidney disease (CKD) causes considerable morbidity, mortality, and health expenditures worldwide. Obesity is a significant risk factor for CKD development, partially explained by the high prevalence of diabetes mellitus and hypertension in obese patients. However, adipocytes also possess potent endocrine functions, secreting a myriad of cytokines and adipokines that contribute to insulin resistance and induce a chronic low-grade inflammatory state thereby damaging the kidney. CKD development itself is associated with various metabolic alterations that exacerbate adipose tissue dysfunction and insulin resistance. This adipose-renal axis is a major focus of current research, given the rising incidence of CKD and obesity. Cellular senescence is a biologic hallmark of aging, and age is another significant risk factor for obesity and CKD. An elevated senescent cell burden in adipose tissue predicts renal dysfunction in animal models, and senotherapies may alleviate these phenotypes. In this review, we discuss the direct mechanisms by which adipose tissue contributes to CKD development, emphasizing the potential clinical importance of such pathways in augmenting the care of CKD.

KEYWORDS

chronic kidney disease, obesity, cellular senescence, chronic inflammation, adipokines, senotherapies

1 Introduction

Obesity is a contributing risk factor of 20-25% of chronic kidney disease (CKD) cases worldwide (1). As per the 2011-2014 National Health and Nutrition Examination Survey, 44.1% of CKD patients in the United States of America (USA) were obese (2). The number of end-stage kidney disease (ESKD) kidney transplant recipients who were obese

also grew by 44% from 1999—2009 (3). Diabetes and hypertension—the two most common causes of CKD worldwide—frequently accompany obesity and are often put forward as the major causes of obesity-related CKD. However, obesity is a risk factor CKD-related disability and mortality after adjusting for diabetes and hypertension (4, 5). Othman et al. demonstrated that non-diabetic obese patients were more likely to undergo CKD progression than non-obese subjects (6). These results suggest an independent mechanism by which obesity damages the kidney.

Although lifestyle changes, such as weight loss, are essential for managing obesity, most patients fail to achieve adequate or sustained weight loss (7). Recent clinical trials report that the glucagon-like-peptide-1 (GLP-1) receptor agonist semaglutide and the GLP and gastric inhibitory peptide (GIP) receptor agonist tirzepatide induce significant weight loss in obese patients; high dose terzepatide (10-15 mg weekly) achieved > 20% reductions in body weight, resembling that achieved after bariatric surgeries (8–12). Sattar et al. concluded that GLP-1 receptor agonists slowed decline in estimated glomerular filtration rate (eGFR), ameliorated progression to ESKD, and reduced kidney disease-related mortality (13). Bariatric surgeries are an option for morbidly obese patients who cannot lose weight and are refractory to anti-obesity medications. Bariatric surgery reduces systemic inflammation, proteinuria, and glomerular hyperfiltration in obese CKD patients (14, 15). Bariatric surgery also decreases the 5-year risk of mortality by 79% in obese pre-dialysis CKD patients (16). Such data demonstrate that decreasing adiposity betters various indices of kidney function and mitigates CKD development and progression.

The management of obesity-related CKD is still in its infancy, and evidence-based guidelines are yet to be established (1). Improvements in risk stratification and management protocols are urgently needed to improve the care of obese-related CKD. While diabetes and hypertension are significant contributors to obesity-related CKD, recent decades of research have shown that adipocytes are potent endocrine cells, releasing adipokines which exert direct pathologic effects on the kidney (17). Adipokines also indirectly damage the kidney by contributing to the development of insulin resistance and hypertension (18).

Alternatively, CKD induces several endocrine and immunologic dysregulations in adipose tissue. Identifying the key players of this adipose-renal axis may have clinical practice-changing implications, given the strong links between obesity and CKD and their paralleled rise in prevalence. Identifying mediators of adipose tissue-induced kidney disease is essential in improving risk prediction models of CKD in obese patients and identifying targets for pharmacotherapies. This review discusses the adipose tissue-derived mediators of CKD and translational research on how such mechanisms are targeted.

2 Adipose tissue inflammation in obesity and chronic kidney disease

Chronic low-grade inflammation is a biological hallmark of aging—termed inflammaging (19). Obesity promotes inflammaging, explaining why obese individuals experience age-related chronic disease prematurely (20, 21). Conversely, limiting fat development or inducing adipose tissue depletion extends health and life span (22). Both obesity and aging impair adipogenesis, the process by which adipocyte progenitors differentiate into functional, insulin-responsive adipocytes (23). Consequently, adipose tissue cannot buffer circulating lipids, which then deposit ectopically in other organs, such as the liver, skeletal muscle, and kidney, causing lipotoxicity. Lipotoxicity impairs insulin signaling in the kidney, liver, and skeletal muscle, causing insulin resistance (24).

Individual adipocytes hypertrophy in response to impaired adipogenesis (25). Hypertrophic adipocytes promote adipose tissue inflammation by producing tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (26). These proinflammatory cytokines are critical to the onset of insulin resistance; mice lacking TNF-α have lower circulating free fatty-acids and are protected from insulin resistance (27). Hypertrophic adipocytes also produce macrophage chemoattractant protein-1 (MCP-1), recruiting adipose tissue macrophages (ATMs) (28). ATMs constitute less than 10% of the total cell population of adipose tissue in lean individuals and mice but increase disproportionately in obesity to make up 40-50% of the adipose tissue cellular compartment (28). Indeed, increased ATM recruitment is histologically evident, revealing ATMs surrounding dead or dying adipocytes, forming crown-like structures and engulfing lipid droplets (29). ATMs in obesity are polarized towards a proinflammatory M1 phenotype, elaborating proinflammatory cytokines such as TNF-α (30). Therefore, hypertrophic adipocytes and M1-polarized ATMs actively contribute to adipose tissue inflammation and insulin resistance. In agreement with these findings, knocking out MCP-1 or its receptor attenuates macrophage infiltration into adipose tissue and reduces insulin resistance (31). Pharmacologically polarizing ATMs towards an M2 phenotype also reduces adipose tissue inflammation in high-fat diet (HFD) obese mouse models (32, 33).

The array of cytokines and signaling molecules released by adipose tissue renders them capable of modulating the inflammatory and immunologic phenotypes of various organs, including the kidney. In this light, adipose tissue inflammation exerts detrimental effects on renal function. Plasma TNF-α and IL-6 are elevated in obese pateints and are associated with CKD incidence and severity independent of diabetes (34, 35). Weight loss or bariatric surgery normalizes these proinflammatory cytokines and reduces glomerular hyperfiltration (14). IL-1β is

another pro-inflammatory cytokine elevated in obesity. Importantly, patients with sustained IL-1β elevations post-bariatric surgery experienced no improvement in hyperfiltration (36).

Adipose tissue fibrosis is another important mediator of adipose tissue inflammation in obesity (37). In this regard, ATMs secrete matrix metalloproteinase-14 (MMP-14) to induce extracellular matrix remodeling by activating MMP-2 and MMP-9 (38). Furthermore, certain MMPs impair adipogenesis in obesity (38). In support of the contribution of MMPs to adipose tissue inflammation, MMP-12-deficient mice fed a high-fat diet (HFD) showed better insulin sensitivity and adipogenesis and an anti-inflammatory M2 ATM phenotype compared to wild-type mice fed an HFD (39). MMP-12 depletion also attenuated glomerular inflammation and renal fibrosis (39), indicating that changes in the inflammatory and immune phenotypes of adipose tissue affect the kidney. Along this line, pharmacologically polarizing ATMs to an M2 phenotype has renoprotective effects by preventing glomerular and mesangial expansion and fibrosis (32, 33).

Hypoxia contributes to adipose tissue inflammation and fibrosis. Rapid adipocyte hypertrophy in obesity outgrows its blood supply, resulting in hypoxia, cell death, and inflammation (40). Adipocyte tissue hypoxia activates hypoxia-inducible factor-1α (HIF-1α). HIF-1α does not elicit pro-angiogenic responses in adipose tissue but rather pro-fibrotic and pro-inflammatory transcriptional programs, leading to fibrosis and inflammation (37, 41). Inhibiting HIF-1α *via* PX-478 or introducing a dominant negative mutation prevents these fibrotic and inflammatory responses, even under a high-fat challenge (42). Hypoxic conditions in visceral adipose tissue downregulate the insulin receptor, which is reversible if oxygen supply is restored. Hypoxia-related insulin insensitivity in adipose tissue is mediated by micro-RNA 128, which destabilizes mRNA encoding the insulin receptor (43).

Therefore, adipose tissue inflammation in obesity is multi-factorial and drives renal dysfunction. This adipose-renal crosstalk is bidirectional. CKD reduces subcutaneous fat volume with a redistribution of fat to visceral depots and ectopic lipid deposition in skeletal muscle and the liver with consequent lipotoxicity (44). Ectopic lipid deposition also occurs in the kidneys in CKD, increasing renal inflammation (44). A recent study observed that exposing adipose tissue to uremic serum activates NFκB and HIF-1α, which drive adipose tissue inflammation. Indeed, adipose tissue sampled from dialysis patients also exhibits higher inflammatory markers (45), suggesting that it may be a source of the chronic low-grade inflammation observed in CKD patients in a manner unrelated to excess adiposity (46). CKD promotes macrophage infiltration into adipose tissue and consequent inflammation, leading to glucose intolerance and insulin resistance (44, 47, 48). Martos-Rus et al. recently demonstrated significantly higher ATM density in the adipose tissue of ESKD subjects than BMI-matched controls (49). ATM recruitment in CKD may be IL-6-dependent, as IL-6-KO mice showed reduced ATM densities comparable to wild-type mice. Uremic serum also directly activates ATMs to a pro-inflammatory M1 phenotype (49). Lastly, uremia alters the adipokine profile of adipocytes. Incubating human adipocytes with uremic serum increases leptin secretion and lipolysis while decreasing perilipin mRNA transcripts—perilipin promotes fat storage as triglycerides in adipose tissue (50–52). Urea accumulation in CKD also increases oxidative stress in adipose tissue, leading to the production of adipokines resistin and retinol-binding protein-4, which contribute to insulin resistance (48).

3 Adipokine alterations in obesity and effects on the kidney

Adipocytes produce various adipokines, enabling them to modulate the function of remote organs, such as the kidney. Below we discuss the most studied adipokines, leptin and adiponectin, and how alterations in these adipokines contribute to obesity-related CKD (Figure 1). Conversely, CKD also changes serum leptin and adiponectin levels, which may contribute to CKD stage progression and systemic complications.

3.1 Leptin

In conditions of nutrient excess, adipocytes produce leptin to modulate CNS activity, promote satiety, and increase energy expenditure. Obesity is associated with hyperleptinemia and leptin resistance (53, 54). Indeed, leptin levels are 5-10x higher in obese patients compared to non-obese individuals (55, 56). Since the kidney is the primary organ responsible for leptin clearance (57), CKD is also associated with hyperleptinemia, the degree of which correlates with the CKD stage (18, 58, 59). Park et al. recently demonstrated a significant correlation between elevated serum leptin levels and the risk of CKD in men after adjusting for eGFR and age (60). Such associations are even more evident in females, probably owing to sex-specific differences in circulating leptin levels (61).

Numerous studies have shown leptin to induce glomerulosclerosis and hypertension, both risk factors to CKD (62). The short form of the leptin receptor (Lep-Ra) is the predominant leptin receptor expressed in the kidney compared to the long form (Lep-Rb). Glomerulosclerosis and renal fibrosis in obese mice have been linked to Lep-Rb-dependent JAK2-STAT signaling in renal mesangial cells (63). Leptin promotes TGFβ-1 release and type IV collagen and fibronectin production in the glomerulus, leading to proteinuria and glomerulosclerosis (62). This effect was initially found to be mediated *via* adenosine

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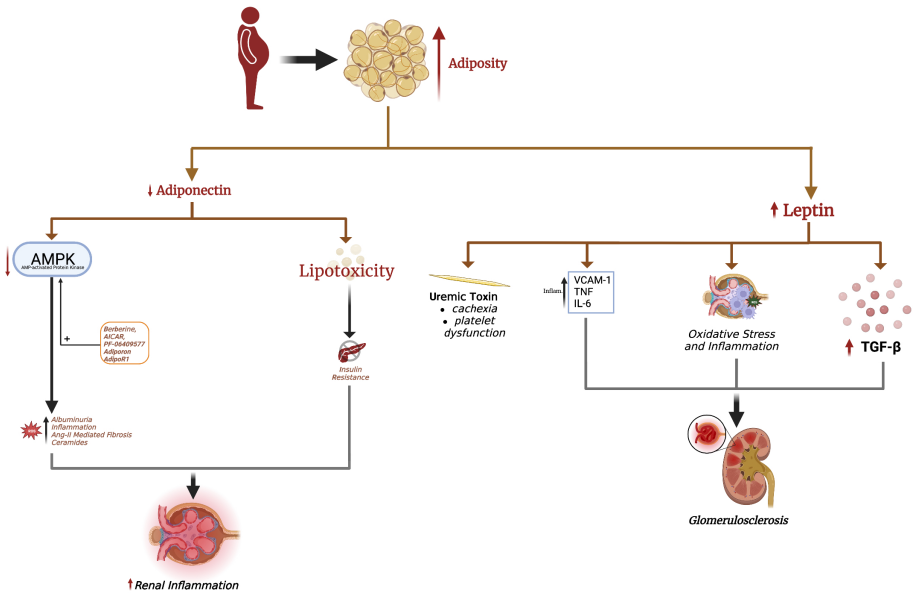

FIGURE 1
Excess adiposity increases serum leptin and decreases adiponectin. Hyperleptinemia promotes renal fibrosis, leading to glomerulosclerosis seen in obesity-induced glomerulopathy. Leptin is also considered a uremic toxin, contributing to uremic cachexia and platelet dysfunction. The decrease in renal adiponectin effect in obesity is causal in the development of albuminuria. Loss of adiponectin effect in obesity also increases renal oxidative stress, fibrosis, and inflammation. Adiponectin-mediated AMPK activation is responsible for many of adiponectin's renoprotective effects, with a decrease in AMPK implicated in albuminuria and renal injury. The adiponectin receptor agonist AdipoRon and direct AMPK activators AICAR, berberine, and PF-06409577 are pharmacological strategies to increase renal AMPK levels and mitigate obesity-related kidney disease.

monophosphate-activated protein kinase (AMPK) activation (64), which paradoxically inhibits TGFβ-1 and protects against renal fibrosis in several mouse models (65). This discrepancy suggested that leptin-mediated fibrosis may additionally involve other signaling pathways in the kidney. Indeed, activation of the p38/MAPK signaling pathway is involved in leptin-mediated renal fibrosis (64). Leptin also induces endothelial dysfunction (ED) by upregulating vascular adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) through AKT/GSK3β and Wnt/β-catenin signaling pathways, promoting renal inflammation and vascular remodeling (66, 67). Lastly, leptin promotes oxidative stress in renal tubular epithelial cells and stimulates monocytes to release IL-6 and TNF-α, promoting renal inflammation (68, 69).

Leptin is also considered a uremic toxin, contributing to many CKD complications including cachexia, protein-energy wasting (PEW), insulin resistance, hypertension, cardiovascular disease, and bone pathologies (70). PEW in CKD features anorexia, increased energy expenditure, decreased protein stores and muscle mass, and weight loss. Leptin suppresses food intake and increases energy expenditure through binding mineralocortin-4 receptors (MC4-R) in the hypothalamus, leading to uremic cachexia (71–73). Inhibiting MC4-R improves cachexia and reduces skeletal muscle wasting in preclinical models, but this needs further investigation in humans (73).

Leptin increases the risk of cardiovascular disease, the most common cause of mortality in CKD patients. Leptin induces hypertension by increasing sympathetic outflow, decreasing nitric oxide (NO) production, and increasing endothelin-1 in endothelial cells (74–76). Leptin promotes atherogenesis through endothelial cells, macrophages, and smooth muscle cells *via* the Lep-Rb receptor, which is reviewed elsewhere (77). Similarly, leptin binding to the Lep-Rb receptor on platelets enhances ADP signaling to induce platelet aggregation, which may cause the platelet dysfunction characteristic of uremia (78, 79). Lastly, hyperleptinemia decreases glucose-stimulated insulin release from pancreatic β-cells and impairs insulin signaling in hepatocytes (80, 81), although it should be noted that normal leptin levels enhance insulin release (80, 81).

Therefore, obesity-associated hyperleptinemia may contribute to renal pathology and CKD, mainly by causing secondary glomerulosclerosis. Furthermore, CKD-associated hyperleptinemia may contribute to numerous CKD complications. In agreement with these findings, leptin-deficient mice are significantly protected against albuminuria, glomerular crescent formation, macrophage infiltration, and glomerular thrombosis (82). Inhibiting leptin using specific

antibodies or antagonists also substantially reduces blood pressure in mice with diet-induced obesity (83) and alleviates cachexia in CKD mice (84). Weight loss, either through lifestyle interventions, pharmacotherapies, or bariatric surgeries, is associated with significant decreases in leptin levels (85–89), but whether leptin normalization after weight loss directly improves renal function remains to be investigated.

3.2 Adiponectin

Adiponectin secretion is decreased in obesity, promoting the development of obesity-related chronic complications. The development of adiponectin-KO animal models have allowed causal relationships to be drawn between adiponectin deficiency and several aspects of the metabolic syndrome. For example, adiponectin-KO mice develop hepatic steatosis, which is attenuated by transfecting the adiponectin gene (90, 91). In skeletal muscle, adiponectin stimulates beta-oxidation and reduces lipid deposition and consequent lipotoxicity (92). Furthermore, adiponectin inhibits lipolysis and stimulates triglyceride storage in subcutaneous adipose tissue. Adiponectin, therefore, promotes fat storage in AT and increases insulin sensitivity, with its decrease in obesity a causal factor in insulin resistance, lipotoxicity, and metabolic syndrome manifestations (92).

The renoprotective effects of adiponectin are well-documented (18). Two adiponectin receptor isoforms, ADIPOR1 and ADIPOR2, are expressed in the kidney. Stimulation of ADIPOR1 and ADIPOR2 activate AMPK and peroxisome-proliferator activated receptor-α (PPAR-α), respectively, which attenuate renal inflammation, fibrosis, glomerulosclerosis, podocyte effacement, and albuminuria (17). A rise in intracellular AMPK by ADIPOR1 activation in podocytes inhibits NADPH oxidase and reduces permeability to albumin (93). In this light, non-obese non-diabetic mice who are adiponectin-deficient still develop effacement of podocyte foot processes and albuminuria due to increased oxidative stress (93, 94). In mesangial cells, adiponectin increases AMPK to attenuate angiotensin-II-induced TGF-β1 production, decreasing renal fibrosis (95).

Adiponectin also exerts anti-inflammatory effects on the kidney. For example, MCP-1 binds to its cognate CCR2 receptor to promote macrophage infiltration into the kidneys and renal inflammation (96). Adiponectin-deficient CKD mice develop significant albuminuria, tubulointerstitial fibrosis, and inflammation characterized by high MCP-1, TNF-α, NADPH oxidase, and VCAM-1 upregulation (97). Adiponectin administration *via* an adenovirus vector significantly reduces albuminuria, tubulointerstitial fibrosis, glomerular hypertrophy, and inflammation by lowering TNF-α, NADPH oxidase, and VCAM1 (97). Adiponectin has also been shown to directly stimulate IL-10 production by macrophages and decrease IL-6 and TNF-α, suggesting polarization to an M2 phenotype (98).

Lastly, ceramides are a group of sphingolipids implicated in renal disease. Serum levels of several ceramides are independent risk factors for CKD development and stage progression (99), as well as insulin resistance and lipotoxicity (100). Ceramides also act at several levels of the insulin signal transduction pathway to impair insulin signaling. Notably, both ADIPOR1 and ADIPOR2 possess intrinsic basal ceramidase activity, which is enhanced by adiponectin binding (101). Elevated ceramidase activity by ADIPOR1 and ADIPOR2 overexpression increases insulin sensitivity and glucose utilization while opposing hepatic steatosis (102). Ceramidase metabolizes ceramides into sphingosine-1-phosphate, which has anti-apoptotic effects and may even induce proliferation (103). These studies indicate that the pleiotropic metabolic, anti-apoptotic, and insulin-sensitizing effects of adiponectin may at least partly involve amplifying receptor-associated ceramidase activity.

Serum adiponectin is lower in obese patients compared to lean individuals. ADIPOR1 and ADIPOR2 expression is also reduced in the kidneys of obese and diabetic mice (104). Therefore, kidneys from obese mice and humans showed reduced AMPK levels (105). Treatment with 5-aminoimidazole-4-carboxamide-1-β-D-furanoside (AICAR), which enhances adiponectin-mediated AMPK signaling, increases AMPK levels in obese kidneys and reduces mesangial expansion and albuminuria (106). The antioxidant resveratrol also restores ADIPOR expression in the kidney and increases AMPK activation in diabetic mice, associated with reductions in albuminuria, oxidative stress, and inflammation (104). The molecule berberine enhances adiponectin signaling through AMPK to ameliorate renal pathology in diabetic mice (107). In animal models of diabetic nephropathy and obesity, the AMPK agonist PF-06409577 and adiponectin receptor agonist AdipoRon reduce proteinuria, inflammation, and renal fibrosis (108–110). These results suggest that targeting adiponectin receptors or AMPK directly may be beneficial in obesity- and diabetes-related kidney disease.

Despite adiponectin having numerous renoprotective effects, adiponectin levels are paradoxically increased in CKD and are positively correlated with albuminuria, CKD stage, and mortality, independent of body mass index (BMI) (58, 111). Adiponectin also predicts adverse cardiovascular outcomes in CKD patients (112). Unlike leptin, higher adiponectin levels in CKD cannot be simply explained by decreased renal clearance because the liver clears the high-molecular-weight form of adiponectin (113). Therefore, why adiponectin is elevated in CKD and is predictive of disease severity remains investigational.

Tian et al. induced CKD in non-obese mouse models with deoxycorticosterone acetate-salt (DOCA) and angiotensin II infusion (114). Transgenic adiponectin-overexpressing CKD mice showed significantly lower albuminuria, glomerular and interstitial fibrosis, and attenuated effacement of podocyte foot

processes. Markers of tubular injury and inflammation were also lower in the transgenic models (114). These results are contrary to the unfavorable prognostic effect attributed to adiponectin in CKD patients. Yang et al. demonstrated that elevated adiponectin levels were associated with the presence of bone marrow-derived fibroblasts in kidneys with unilateral ureteral obstruction and ischemia/reperfusion injury (115). Adiponectin-deficient mice showed reduced renal fibroblast and M2 pro-fibrotic macrophage infiltration. The same study also showed adiponectin to activate AMPK on bone-marrow-derived monocytes, thereby increasing α-smooth muscle antigen (α-SMA) and production of extracellular matrix proteins. Therefore, the Yang et al. study suggested inhibiting the adiponectin/AMPK axis may ameliorate fibrotic renal disease (115). Similarly, Perri et al. reported that administration of lipopolysaccharide (LPS) induces adiponectin production by renal tubular epithelial cells to cause renal fibrosis (116). Numerous other studies have demonstrated the production of adiponectin by the kidney itself (117, 118). However, how kidney-derived adiponectin contributes to circulating adiponectin levels and any potential functional differences are not yet known.

PPAR-α is also known to exert renoprotective effects. Boor et al. demonstrated PPAR-α expression in the renal tubular epithelium but not the interstitium. PPAR-α levels decreased after fibrosis induction through unilateral ureteral obstruction and 5/6 nephrectomy (119). Treatment with the PPAR-α agonist BAY PP1 significantly increased PPAR-α expression, correlated with a reduction in tubulointerstitial fibrosis, inhibition of interstitial fibroblasts, lower TGF-β1 levels, and slowed down the progression of renal dysfunction. Therefore, PPAR-α in tubular epithelial cells attenuates fibrosis upon renal injury (119).

4 Cellular senescence in obesity and CKD

Cellular senescence was initially described by Hayflick and Moorhead when they observed that fibroblasts stop dividing after a set number of cell divisions (120). This cell cycle arrest was due to telomere attrition. The list of senescence-inducing stimuli has exponentially grown, but most culminate in DNA damage or oncogene activation, which activate senescence-inducing pathways. Hence, senescence is defined as an irreversible growth arrest upon the cell's exposure to DNA-damaging or mitogenic stimuli. Senescence is characterized by numerous structural, biochemical, and metabolic alterations: a flattened and enlarged cellular morphology, decreased nuclear Lamin B1 expression, increased p53, $p16^{INK4a}$ and/or $p21^{CIP1}$ expression, elevated mitochondrial ROS production, elevated senescence-associated lysosomal β-galactosidase (SA-β gal) activity, apoptosis resistance *via* upregulation of senescence-associated anti-apoptotic pathways (SCAPs), and elaboration of a senescence-associated secretory phenotype (SASP) (121). The transient induction of senescence is considered physiological and critical to embryogenesis, wound healing, and tumor suppression. However, the chronic accumulation of senescent cells is implicated in the pathogenesis of numerous age-related disorders, including osteoporosis, obesity, stroke, neurodegenerative diseases, CKD, cancer, myocardial infarction and the geriatric syndromes (frailty, sarcopenia, and mild cognitive impairment) (122). Cellular senescence is indeed considered a biological hallmark of aging.

4.1 Cellular senescence in adipose tissue

Senescence plays a crucial role in propagating age-related diseases (123). Senescent cells accumulate in most tissues and organs with aging, including in adipose tissue. Importantly, obesity increases the senescent cell burden in adipose tissue. The p53-dependent DNA damage response is the main inducer of senescence in adipose tissue (124). A study showed that DNA polymerase-η *KO* mice (to increase DNA damage) accumulate senescent cells in adipose tissue (125). SREBP1—a transcription factor involved in regulating the expression of genes encoding proteins involved in lipid metabolism—was recently found to also facilitate DNA repair in adipocytes (126). Deletion of SREBP1 increased DNA damage and accelerated senescence in adipocytes, followed by adipose tissue inflammation and consequent insulin resistance (126). Mice with senescent cell accumulation in adipose tissue are more prone to obesity and adipose tissue inflammation, even with a standard chow diet (125).

Oxidative stress-induced senescence in adipose tissue is linked to higher leptin, IL-6, and TNF-α production in the SASP, suggesting that adipocyte senescence may be causal in obesity-related chronic inflammation (124, 127). Depleting senescent cells in adipose tissue improves glucose homeostasis and insulin resistance (discussed below). Activin A is another SASP component which disrupts insulin signaling by decreasing the expression of insulin-dependent transcription factors including PPARγ and CCAAT-enhancer-binding protein α (C/EBPα) (128). These observations suggest that senescence in adipose tissue results in the production of cytokines and chemokines, leading to adipose tissue inflammation and insulin resistance. Adipose tissue is composed of many different cell types, each exhibiting varying susceptibility to senescence. The discussion herein focuses on the major cell types comprising adipose tissue and the causes and consequences of senescence induction in these cell types (Figure 2).

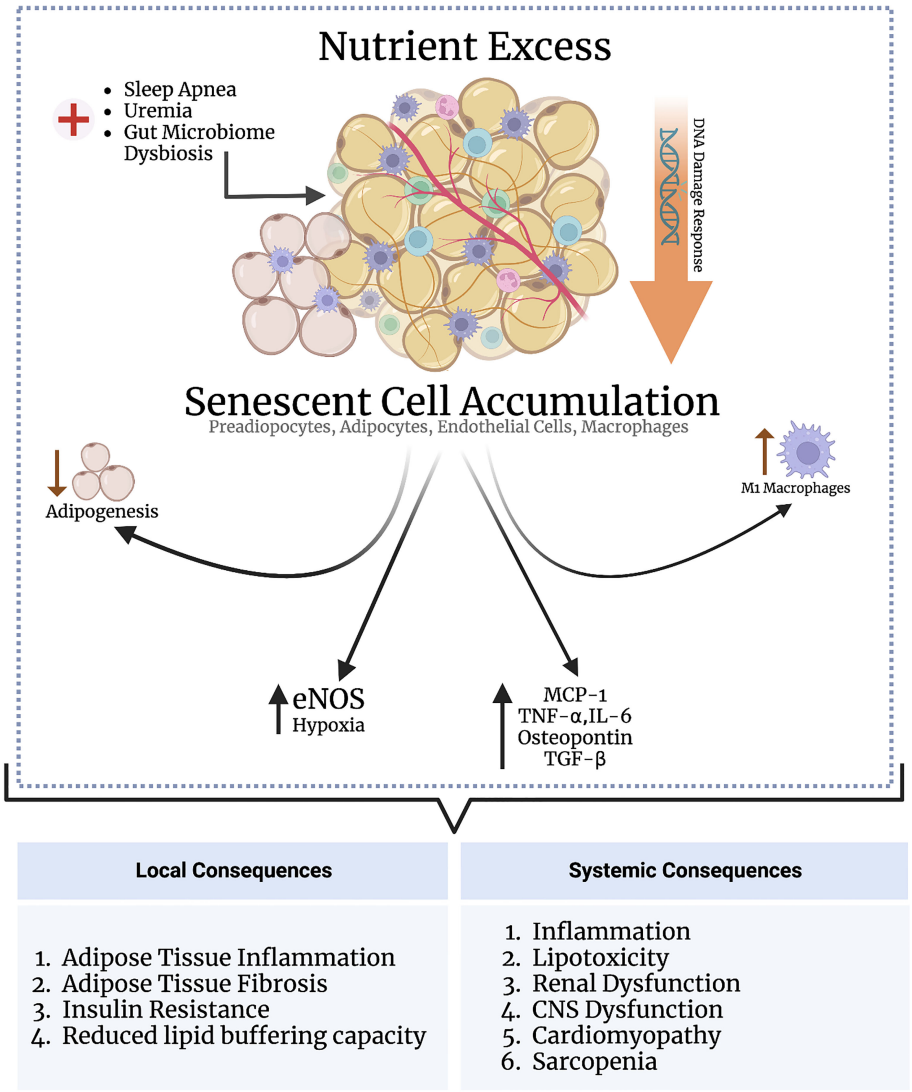

FIGURE 2
Nutrient excess triggers senescence in adipose tissue through DNA damage response signaling. Various other conditions, such as sleep apnea, uremia, and gut microbiome dysbiosis, may directly indce senescence in the adipose tissue independent of nutrient status. The adipose tissue is populated by various subsets of cells, including preadipocytes, adipocytes, endothelial cells, and macrophages. Senescence induction in preadipocytes impairs adipogenesis; senescence in macrophages and adipocytes enhances adipose tissue inflammation; and senescence in andothelial cells promotes adipose tissue hypoxia, inflammation, and fibrosis. An increased senescent cell burden in adipose tissue exerts several systemic consequences through the SASP as part of the inflammaging process, including cardiomyopathy, cognitive dysfunction, and renal impairment.

4.1.1 *Preadipocytes*

Preadipocytes are adipocyte precursors are responsible for fat cell turnover by replacing dead and dying cells—i.e., adipogenesis. Both irradiation and telomere attrition induce senescence in preadipocytes, thereby impairing adipogenesis. Impaired adipogenesis in obesity in turn drives hypertrophic expansion of the subcutaneous fat compartment, increasing adipose tissue inflammation and metabolic dysfunction (129). Senescent preadipocytes also secrete a proinflammatory SASP, driving adipose tissue macrophage infiltration and inflammation. Accordingly, eliminating senescent preadipocytes in obese mice reduces adipose tissue inflammation and improves insulin sensitivity (130). These results indicate that senescence in preadipocytes contributes to adipose tissue inflammation and insulin resistance by impairing adipogenesis.

4.1.2 *Endothelial Cells*

The vasculature of adipose tissue is not fenestrated. Transport across the vasculature into the adipose tissue interstitium is mediated by ECs, which express key transport proteins such as CD36 and fatty acid binding proteins (FABPs) that transport fatty acids between adipose tissue and blood (131). Importantly, PPARγ activation in ECs enhances transendothelial lipid transport and fat storage in adipose tissue. Free fatty acids activate PPARγ. Additionally, activated endothelial cells release PPARγ ligands that activate PPARγ in adipocytes (132). Adipose tissue storage of fats, therefore, depends on normal endothelial cell function, and depleting PPARγ in endothelial cells leads to systemic hyperlipidemia (133). ECs also produce factors that regulate adipose tissue blood flow, such as NO and angiogenic factors that increase adipose vascularity.

Adipose tissue EC senescence can be detrimental to normal adipose tissue function. In this context, ECs of HFD-mice undergo numerous p53-dependent gene expression alterations, including endothelial nitric oxide synthase (eNOS) downregulation, a change associated with insulin resistance (134). However, this study did not explore if p53 expression was associated with other senescence-related cellular alterations. Along similar lines, Barinda et al. showed that senescent ECs release a SASP that propagates senescence in mature adipocytes in a paracrine manner, associated with downregulation of the insulin receptor on mature adipocytes and, consequently, reduced insulin sensitivity (135). Cellular senescence also reduces PPARγ activation, decreasing the ability of endothelial cells to transport fatty acids, and the fat storage capacity of adipocytes (136). Lastly, senescent ECs isolated from visceral adipose tissue of obese individuals show higher expression of hypoxia-related genes and elaborate a proinflammatory SASP (136). Therefore, EC senescence may impair the lipid-buffering capacity of adipocytes by reducing PPARγ and cause adipose tissue hypoxia and inflammation.

4.1.3 *Mature adipocytes*

Mature adipocytes are not expected to enter the cell cycle and divide; they respond to obesity by hypertrophying rather than dividing. However, Li et al. recently demonstrated that mature adipocytes can enter the cell cycle and increase in cell number in response to obesity and hyperinsulinemia (137). Chronic hyperinsulinemia induces premature adipocyte senescence, which release a SASP comprising MCP-1, TNF-α and IL-6 that drives adipose tissue inflammation (125, 137). Mature adipocyte senescence in obesity occurs before adipose tissue inflammation and insulin resistance, suggesting a causal relationship between adipocyte senescence and these phenotypes (138).

4.1.4 *Macrophages*

Senescence is associated with adipose tissue inflammation *via* the SASP. Although ATMs play a key role in adipose tissue inflammation and consequent insulin resistance, there is a paucity of data on whether they can senesce and what consequences this may have on the inflammatory phenotype of adipose tissue. An elevated senescent cell burden in adipose tissue with SASP expression drives ATM recruitment and polarization into a proinflammatory M1 phenotype (139). Notably, depleting macrophages attenuates adipose tissue inflammation and fibrosis, with improvements in glucose tolerance parameters, indicating that macrophage infiltration contributes to senescence-induced adipose tissue dysfunction. However, whether ATMs themselves senesce was not determined in this study (140). A recent study demonstrated that senescent macrophages accumulate in the visceral adipose tissue derived from obese subjects who underwent bariatric surgery, and their numbers correlated with BMI, insulin resistance and degree of hyperinsulinemia (141). Importantly, both senescent adipocytes and macrophages elaborated a pro-inflammatory SASP, which prompted the authors to suggest that premature adipose tissue senescence in obesity contributes to inflammaging, possibly explaining why obese individuals develop age-related disease prematurely (141).

4.1.5 *Systemic*

HFD mouse models accumulate senescent cells in multiple organ systems, which is associated with functional impairment. HFD mice show senescent cell accumulation in the liver and hepatic steatosis (142). In the brain of HFD mice, senescent cells accumulate near the lateral ventricle, which is associated with anxiety and gliosis (143). Kidneys of HFD mice also reveal a higher senescent cell burden, associated with renal dysfunction (144). Sawaki et al. demonstrated that aging adipose tissue releases osteopontin and TGF-β—in the SASP—which stimulate cardiac fibroblasts and drive myocardial fibrosis (145). Removing visceral adipose tissue in these mice reduced cardiac fibroblast activation, increased fibroblast senescence, and ameliorated myocardial fibrosis (145). Although this study did not directly examine senescence in visceral adipose tissue, Khan et al. reported that an elevated senescent cell burden in adipose tissue is associated myocardial fibrosis. p53-KO mice or removing senescent adipose tissue mitigated myocardial fibrosis (146). To correlate these findings clinically, myocardial fibrosis is a known mediator of obesity-associated cardiomyopathy; clinical studies are needed to explore whether targeting senescence may be beneficial in ameliorating this condition (145).

4.2 Senescence in CKD

Numerous studies report a higher senescent cell burden in diseased and aged kidneys. The regenerative potential of the kidney after injuries diminishes with aging and CKD. Cellular senescence is believed to impair regenerative mechanisms in aged and diseased kidneys, leading to maladaptive repair and fibrosis.

Proximal tubular epithelial cells are particularly affected by senescence. Biopsies of transplanted kidneys and those with glomerular diseases stain positive for senescence markers $p16^{INK4a}$ and $p21^{CIP1}$ in the proximal tubular epithelial cells (147, 148). Telomere attrition, elevated SA-β gal, $p16^{INK4a}$, and $p21^{CIP1}$ expression have also been directly correlated with IgA nephropathy progression (149). Baker et al. showed that senescent cells accumulate in aging kidneys in INK-ATTAC transgenic mice. An elevated renal senescent cell burden was associated with glomerulosclerosis, which was attenuated by depleting senescent cells in INK-ATTAC mice (150). Braun et al. demonstrated that transplanted kidneys in wild-type mice exhibit elevated senescence markers and incrementally undergo atrophy and fibrosis (151). By contrast, transplanted kidneys in $p16^{INK4A}$-KO mice show less atrophy and fibrosis after ischemia-reperfusion injury (151). Transplanting kidneys from senescence-depleted mice consistently show better longevity and proliferation of tubular epithelial cells. An elevated senescent cell burden may therefore contribute to long-term allograft kidney deterioration and CKD development in humans (151).

In vivo mouse models of aged and irradiated kidneys demonstrate elevated senescence markers, reduced proliferative repair after injury, and produce TGF-β as a part of their SASP to induce fibrosis. Using the senolytic ABT-263 to eliminate senescent proximal tubular epithelial cells improves these parameters (152). A recent study demonstrated renal tubular epithelial cell senescence – evidenced by higher $p16^{INK4a}$, p19, and $p21^{CIP1}$ expression – secondary to chronic ischemia from renal artery stenosis in mice and humans. A dasatinib and quercetin senolytic combination alleviated renal dysfunction in these mice, suggesting a causal relationship between chronic ischemia, cellular senescence, and kidney damage (153). Diabetic nephropathy is the major cause of CKD worldwide and a significant contributor to obesity-related kidney disease. Biopsies of kidneys from patients with type 2 diabetic nephropathy show elevated senescence markers SA-β gal and $p16^{INK4a}$ in proximal tubular epithelial cells, mesangial cells, podocytes, and endothelial cells (154). WT diabetic kidney disease mouse models develop proteinuria and glomerular hypertrophy, and both effects are attenuated in $p21^{CIP1}$-KO mice (155). Dasatinib and quercetin combination also reduce AKI to CKD transition in murine models of cisplatin and radiation-induced kidney injury (156). Senescent markers SA-β gal, $p16^{INK4A}$, and $p21^{CIP1}$ are also elevated in the kidneys of rats and cats with CKD (157, 158). These data show cellular senescence underpins various renal pathologies that lead to CKD, and senolytics could mitigate this progression.

CKD is considered a systemic premature aging phenotype, known as uremia-associated aging (159). Uremic patients can develop age-related conditions, including osteoporosis, sarcopenia, frailty, impaired wound healing, infections, insulin resistance, cognitive dysfunction, hypogonadism, and vascular aging (160–163). Hence, certain uremic toxins expectedly accelerate biological aging hallmarks, including cellular senescence, to precipitate a premature aging phenotype (164). Uremia-induced senescenced was first demonstrated in the aortas of hypertensive rats, where indoxyl sulfate-related cellular senescence was correlated with aortic wall calcification and thickness, a sign of vascular aging (165). Uremia-induced senescence is mediated by oxidative stress and consequent DNA damage response signaling and upregulation of $p21^{CIP1}$ and p53 (166). A recent review by Huang et al. summarized the mechanisms behind CKD-induced senescence (167). The uremic toxins indoxyl sulfate and *p*-cresyl sulfate induce senescence in mesenchymal stem cells, evidenced by elevated $p21^{CIP1}$ expression (168, 169). A normocytic normochromic anemia is common in CKD pateints, and is mainly thought to be due to low erythropoietin production by the kidney. Mas-Oodi et al. recently demonstrated that indoxyl sulfate induced senescence in CD34+ hematopoietic stem cells, thereby arresting their proliferation and reducing erythropoiesis (170). Indoxyl sulfate also induces senescence in renal proximal tubular epithelial cells in CKD through ROS-dependent p53 expression (171). Senescent proximal tubular cells display a proinflammatory and profibrotic protein signature, with elevations in NFκB and TGF-β production, possibly contributing to further declines in renal function (171, 172).

P-cresyl sulfate activates NADPH oxidase and induces oxidative stress in mouse 3T3-L1 adipocytes. Exposure to p-cresyl increases TNF-α and IL-6 production by 3T3-L1 adipocytes and increases ATM infiltration, suggesting that this uremic toxin is a mediator of CKD-induced adipose tissue inflammation (172, 173). In agreement with these findings, Koppe et al. reported that administering p-cresyl sulfate to normal mice for 4 weeks triggered lipotoxicity and insulin resistance (174). Although senescence markers were not evaluated in these studies, it is conceivable that the intracellular accumulation of *p*-cresyl sulfate may induce adipocyte senescence, since oxidative stress is the major pathway of adipose tissue senescence. In the context of the adipose-renal axis, these studies suggest that the accumulation of uremic toxins in CKD trigger senescence in adipose tissue, amplifying the inflammaging seen in CKD pateints. Further studies are needed to determine which uremic toxins induce adipose tissue senescence, the mechanisms involved, which subpopulations of cells in adipose tissue are affected, the

important SASP components by which uremia-induced senescent adipose tissue exerts pathologic systemic effects, and whether senolytic or other senescent-targeting strategies are effective in ameliorating uremia-induced senescence. Another pathway to consider in the adipose-renal axis in CKD is the gut microbiome. CKD alters the symbiotic relationship between the intestinal microbiome and the body (i.e., gut microbiome dysbiosis), leading to the fermentation of macronutrients and production of various uremic toxins, including indoxyl sulfate, *p*-cresyl sulfate and others. CKD-related impairment of the intestinal epithelial barrier allows for the spillover of these toxins into the bloodstream, which drive systemic oxidative stress and inflammation (175, 176). Whether and how certain dietary modifications lifestyle interventions such as exercise, restore host-enterobiome symbiosis and alleviate senescence in the context of the adipose-renal axis is an important topic for future studies to address.

4.3 Senotherapies in obesity and kidney disease

Targeting senescent cells pharmacologically can alleviate numerous age-related diseases. Baker et al. initially demonstrated that depleting senescent cells prevents the development of age-related changes in adipose tissue, skeletal muscle, and eyes of INK-ATTAC transgenic mice (150). Numerous strategies have emerged to deplete senescent cells or mitigate their harmful effects. Briefly, senolytic drugs inhibit SCAPs characteristic of senescent cells, allowing for the selective depletion of senescent cells. Dasatinib, quercetin, and fisetin are the most studied senolytics in preclinical animal models thus far, and numerous clinical trials testing their efficacy in age-related disorders are underway (177). Senomorphic drugs inhibit various SASP components without inducing senescent cell death. Most senomorphics target transcriptional regulators of the SASP, including ATM, p38 MAPK, JAK/STAT, NFκB, and mTOR pathways. Other strategies are also emerging, recently reviewed by Zhang et al. (178, 179). Many preclinical studies have shown senolytics to alleviate aging phenotypes, including cancer, chemotherapy- and radiation-induced premature aging, diabetes, osteoarthritis, neurodegeneration, glaucoma, age-related macular degeneration, idiopathic pulmonary fibrosis, heart failure, and CKD (180).

The impact of senescent cell depletion has also been investigated in obesity and kidney disease. Palmar et al. cleared senescent cells either by senolytic combination dasatinib+quercetin or by selective depletion of $p16^{INK4a}$-expressing cells and observed reduced adipose tissue inflammation and improved glucose tolerance and insulin sensitivity (181). Since DNA damage is the major inducer of senescence in adipose tissue, interventions such as exercise, N-acetylcysteine, and senolytics that reduce oxidative stress in adipose tissue decrease adipose tissue SC burden and attenuate ATM infiltration and adipose tissue inflammation (125). Another study showed that senescent cell clearance in adipose tissue of HFD obese mice by dasatinib+quercetin and navitoclax improved insulin sensitivity and increased plasma adiponectin levels (182). Consistent with higher adiponectin levels, senescent cell clearance anormalizes microalbuminuria and podocyte barrier integrity (181). An HFD increases senescent cell burden in mouse kidneys—detected by $p16^{INK4a}$, p19, and p53 expression and SASP upregulation—linked to renal fibrosis and functional impairment (144). Quercetin administration reduced senescent cell burden in the kidney, attenuated renal fibrosis, increased renal cortical oxygenation, and lowered plasma creatinine levels (144).

These findings suggest that depleting adipose tissue-resident senescent cells by senolytics restores adipogenesis, reduces adipocyte hypertrophy, improves glucose tolerance and insulin sensitivity, reduces macrophage infiltration into adipose tissue, and increases adiponectin secretion.

5 Sleep apnea, obesity and CKD

Obstructive sleep apnea (OSA) is a globally prevalent disorder increasing in incidence. OSA is characterized by collapse of the upper airway during sleep, causing arousal with or without oxygen desaturation, leading to sleep fragmentation and daytime sleepiness. Obesity is a strong risk factor for OSA: OSA affects 40% of moderately obese (BMI >30 kg/m^2) and 90% of severely obese patients (BMI >40 kg/m^2) (183). A 10% increase in bodyweight increases the Apnea-Hypopnea Index (AHI) by 32%, whereas a 10% decrease in body weight decreases the AHI by 26% (184). Obesity increases pharyngeal collapsibility by reducing upper airway diameter and lung volume, predisposing to collapse and consequent OSA (185).

OSA patients are at a significantly higher risk of stroke, myocardial infarction, arrhythmias, insulin resistance and diabetes, heart failure, pulmonary hypertension, and CKD. The pathogenic hallmark of OSA is chronic intermittent hypoxia (CIH), which exerts direct pathologic effects in multiple organs. Although the kidney receives 25% of the cardiac output, blood flow to the renal medulla is tightly regulated to maintain the interstitial medullary osmotic gradient which facilitates water reabsorption. The renal medulla is, therefore, highly vulnerable to ischemic injury. CIH leads to significant tubulointerstitial damage by increasing oxidative stress and inflammation (186). In agreement with these findings, treatment with lipoic acid, an antioxidant, ameliorates hypoxia-related renal injury by decreasing oxidative stress, renal cell apoptosis, and tubular injury (187). CIH also activates interstitial fibroblasts and induces renal tubular epithelial cells to undergo an epithelial-to-mesenchymal transition by upregulation of HIFs, leading to

renal fibrosis (188–191). Nocturnal hypoxia in OSA patients over-activates the sympathetic and renin-angiotensin-aldosterone systems, associated with long-term renal impairment (192–194). These experimental models explain clinical studies showing that OSA contributes to CKD development and progression. For example, a cross-sectional analyzing over 7700 subjects with OSA for CKD revealed that, in additional to traditional CKD risk factors, lower nocturnal oxygen saturation was associated with CKD, with a 2% rise in CKD probability for every 1 unit drop in oxygen saturation (195). Furthermore, studies following OSA patients longitudinally have revealed that nocturnal hypoxia is independently associated with steeper declines in eGFR, cardiovascular mortality, and all-cause mortality (196–200).

Continuous positive airway pressure (CPAP) therapy is the mainstay of treating OSA and resolves CIH. CPAP significantly reduces snoring and daytime sleepiness and improves the quality of life in OSA patients. CPAP significantly decreases renal sympathetic and RAAS activity and blood pressure, improves renal hemodynamics, slows the rate of eGFR decline, and reduces microalbuminuria in patients with severe OSA (201–204). However, data suggest that CPAP may be ineffective at improving renal function in moderate nocturnal hypoxia and men (205, 206). Furthermore, CPAP is ineffective at reducing the incidence of a composite clinical end point of cardiovascular mortality, myocardial infarction, stroke, transient ischemic attack, and heart failure in patients with moderate-to-severe OSA (207, 208). Varying degrees of compliance to treatment among patient groups may partly be responsible for these discrepant findings. Nevertheless, such data highlight the need for elucidating the pathogenesis of OSA-related kidney disease. No pharmacological treatments are currently available for OSA. Identifying mediators of the systemic organ dysfunction caused by OSA may reveal pathways that may be clinically beneficial to target and supplement CPAP therapy to enhance the long-term outcomes of these patients.

In this regard, OSA may alter patients' adipokine profiles. OSA patients have significantly reduced adiponectin compared to non-OSA patients, regardless of sex, age, or BMI (209, 210). Low serum adiponectin levels are associated with decreased cystatin C urinary excretion in male OSA patients (192). Ding et al. demonstrated that CIH in rats increased oxidative stress and markers of apoptosis in the kidney compared to normal controls (211). Treating CIH rats with adiponectin reduces oxidative stress and renal cell apoptosis (211). Similar results have been observed in cardiomyocytes, neurons, and pulmonary cells (212–214). Improving sleep quality in OSA patients with cardiovascular disease either by CPAP, nocturnal supplemental oxygen, or sleep hygiene education significantly increased serum adiponectin levels and improved glucose tolerance parameters (215). Therefore, low serum adiponectin in OSA may contribute to their higher risk of systemic complications including renal impairment and insulin resistance.

OSA also increases leptin levels with consequent leptin resistance (216). Li et al. concluded that leptin is significantly higher in OSA than non-OSA patients and correlates with a higher AHI (217). Obesity, a frequent comorbidity of OSA, causes hyperleptinemia and leptin resistance. Leptin is key in stabilizing upper airway muscles and stimulating CNS respiratory drive (218–221). Therefore, it is conceivable that leptin resistance may contribute to the higher risk of OSA in obese individuals. Administering leptin intranasally to obese mice alleviates OSA independent of body weight reduction (222). OSA also increases leptin levels and causes leptin resistance through CIH. A recent study reported that CIH for 96 days in rats significantly increased leptin levels (223). Furthermore, while leptin injections into normoxic controls reduced food intake, no such effect was observed in the CIH animals, indicating leptin resistance (223). High levels of leptin drive oxidative stress and chronic inflammation that underlie the long-term cardiovascular complications of OSA (224).

Aging is a significant risk factor for many complications seen in OSA, suggesting that OSA may accelerate aging at the cellular level and precipitate a premature aging phenotype (225–228). Sleep deprivation activates a DNA damage response in peripheral blood mononuclear cells of older adult humans, with consequent increases in $p16^{INK4a}$ expression and elaboration of a SASP (229). Sleep fragmentation also induces senescence in the aorta of adult male C57BL/6J mice (230), possibly related to a pro-oxidant response in the vascular endothelium induced by CIH that accelerates vascular aging (231). Indeed, CIH induces a state of systemic chronic low-grade inflammation through NFκB activation, which can induce senescence (232–234). Lee et al. recently demonstrated CIH in elderly mice to increase lung oxidative stress, inflammation, and fibrosis (235). Many of the pro-inflammatory cytokines measured—such as TNF and IL-6 —are SASP components, although the lungs of these mice were not examined for senescence markers. Polonis et al. reported that OSA-related CIH induces senescence in human preadipocytes—expressing $p16^{INK4a}$, SA-β gal, and γH2AX —through a ROS-dependent pathway (236). The subcutaneous abdominal adipose tissue of OSA patients also demonstrated higher $p16^{INK4a}$ and γH2AX than non-OSA individuals. Importantly, treatment with statins, aspirin and/or a RAS inhibitor significantly reduced senescent cell burden *in vitro* and *in vivo* (236). A recent study by Khan et al. showed that two weeks of CIH increased senescence in the visceral white adipose tissue of C57BL6 male mice through a DNA damage response (146). Adipose tissue senescence was accompanied by increased adipose tissue fibrosis, macrophage infiltration, and inflammation. Furthermore, CIH was associated with the upregulation of pro-fibrotic genes in the myocardium and consequent myocardial fibrosis (146). A major finding of this study was that p53-KO mice (i.e., a defect in a key senescence-inducing pathway) did not develop myocardial

fibrosis, and resection of senescent adipose tissue also prevented myocardial fibrosis (146).

In summary, the findings above indicate that CIH, which is a hallmark of OSA, induces senescence in adipose tissue and several other organs, contributing to systemic functional impairment. For future research, it will be important to ascertain whether the available senolytics reduce senescent cell burden in OSA and whether this ameliorates OSA severity and prevents complications. OSA-related senescence has also not been investigated in the kidney but is likely since renal artery stenosis, which, similar to CIH, leads to chronic ischemia that induces senescence in the renal tubular epithelium and causes renal dysfunction (153). It is also important to note that CKD itself can dysregulate sleep and is a risk factor for OSA development and/or progression through a variety of mechanisms (237–240). These observations suggest the existence of a positive feedback loop, whereby OSA, obesity, and CKD all worsen systemic oxidative stress, inflammation, and senescence in multiple organs.

6 Conclusion and perspectives

The increasing prevalence of obesity and kidney disease necessitates a better understanding of the mechanisms behind obesity-induced kidney disease. Although weight loss and lifestyle interventions represent the primary modality of treating obesity, peripheral treatments based on normalizing the adipokine profile or reducing senescent burden in obesity could better clinical outcomes in these patients.

We described the most studied adipokines implicated in obesity-induced kidney disease, but a myriad of other adipokines —including resistin, visfatin, angiotensinogen, and lipocalin— have also been studied in this context. Multiple studies have demonstrated the existence of an adipose-renal axis, whereby obesity-derived cytokines and adipokines damage the kidney, and CKD-related metabolic dysregulation accelerates adipose tissue aging and dysfunction. This axis is influenced by senescent cell burden and the presence of sleep apnea, both of which can amplify inflammation in obesity and CKD. Gut microbiome dysbiosis is another pathway to consider in the adipose-renal axis in obesity and CKD (241–243).

How cellular senescence plays into the adipose-renal crosstalk is largely unexplored in both laboratory and clinical studies but is likely since senescent cells accumulate with age, and obesity-related senescence in adipose tissue and other organs is well-established. Investigating senescence in different CKD complications could also reveal novel biomarkers and targets for pharmacologic intervention. Senolytic and senomorphic drugs could have potential clinical practice-changing implications in treating multiple conditions, including obesity and CKD. Still, their efficacy and, more importantly, safety profile remains to be shown in ongoing clinical trials.

Author contributions

TA, AS, BS, NA, HS, HA, ANS, AR, and ZA participated in the drafting of the manuscript. TA, AS, and ZA conceptualized and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Chemerin levels in chronic kidney disease: A systematic review and meta-analysis

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Introduction: Chemerin as an inflammatory biomarker has gained attention in its biomarker capability. Several studies measured its levels in chronic kidney disease (CKD), as one of the common non-communicable causes of mortality and morbidity. Hence, this systematic review and meta-analysis aimed to investigate this association.

Methods: PubMed, Scopus, Embase, and the Web of Science databases were systematically searched for studies investigating chemerin levels in any CKD stage (including end-stage renal disease patients undergoing hemodialysis (HD)) and comparing it with healthy controls. Random effect meta-analysis was performed to calculate the standardized mean difference (SMD) and 95% confidence interval (CI).

Results: A total of eight studies were included, comprised of 875 individuals, with a mean age of 56.92 ± 11.78 years. All studies had high quality based on the New Castle-Ottawa Scale (NOS). Meta-analysis revealed significantly higher levels of chemerin in CKD patients compared to healthy controls (SMD 2.15, 95% CI 0.83-3.48, p-value<0.01). Additionally, HD patients had statistically higher levels of chemerin than controls (SMD 2.10, 95% CI 0.58-3.62, p-value=0.01). In meta-regression, publication year accounted for 23.50% and 24.17% of heterogeneity for these analyses, respectively.

Conclusion: Chemerin can be potentially used as a biomarker in CKD patients, which can suggest the inflammatory pathways for the disease. Further research is warranted for the assessment of its clinical applications and enlightening its role in the pathophysiology of CKD.

KEYWORDS

chemerin, chronic kidney disease, renal disease, systematic review, meta-analysis

1 Introduction

Chronic kidney disease (CKD) is a significant contributor to noncommunicable disease morbidity and mortality. More than 10% of the adult population had markers for renal disease, according to large-scale, nationally representative screening programs carried out in the 2000s (1). This chronic disease is characterized by a permanent serious impairment of kidney function and a reduced glomerular filtration rate (GFR) for at least three months, which results in a loss of the kidneys' normal ability to remove toxins from the body. Renal injury markers, such as urinary and hematological changes, can be used to detect this impairment (2). Biomarkers can be used as tools for screening, diagnosing, and monitoring diseases as well as evaluating the response to therapeutic interventions (3).

Chemerin was initially identified as a chemokine found in the inflammatory fluids of cancer and rheumatoid arthritis patients (4). It is also known by the names tazarotene-induced gene 2 (TIG2) and retinoic acid responder 2 (RARRES2). Chemerin and its receptor CMKLR1 appear to control insulin sensitivity, adipocyte differentiation, and glucose and lipid balance (5, 6). Adipose tissue, liver, platelets, placenta, and to a lesser degree, other tissues such as the kidneys have all been discovered to express it. The connection between chemerin and renal function has also drawn more attention recently (7). This marker is thought to influence the beginning and development of the local inflammatory state. The inflammatory cells that have been triggered release the enzymes that convert the circulating pro-chemerin to chemerin. Other immune cells are drawn to the area of inflammation by this, which strengthens their adherence (8).

Some studies reported that kidney function is inversely related to circulating chemerin in CKD patients. Blaszak et al. expressed that the mean serum chemerin level in stages 3 and 4 of CKD was 70% higher than the control group and according to a study conducted by Rutkowski et al., the serum chemerin concentration decreased to values observed in control subjects after successful kidney transplantation (6, 8). In another study, Sarhat et al. found that the concentration of chemerin was significantly lower in patients with renal failure, compared to controls (2).

To combine recent data of investigations on chemerin in recent years, addressing the link of this unique marker with CKD, a thorough evaluation, and meta-analysis of original research publications were undertaken in this study.

2 Methods

2.1 Search strategy

In November 2022, a comprehensive search of worldwide web databases, including PubMed, SCOPUS, Web of Science, and Embase, was conducted. The keywords utilized were "chemerin" AND "chronic renal disease", in addition to other pertinent keywords, which were explained in detail in Supplementary Table 1. The search was performed without any restrictions or filters.

2.2 Inclusion and exclusion criteria

The inclusion criteria were: 1) studies assessing chemerin levels in patients with CKD and controls and 2) studies evaluating chemerin levels in patients with CKD undergoing dialysis (hemodialysis (HD)) and controls. We excluded: 1) studies without a control group; 2) studies without exact concentration of chemerin levels, after emailing the corresponding author; 3) non-English articles; and 4) letters, commentaries, case reports, conference abstracts, and reviews.

2.3 Screening

Two reviewers (AHB and PB) individually reviewed titles and abstracts for relevant articles based on inclusion and exclusion criteria, after eliminating duplicates from the initial search. Then, the entire texts of included papers were evaluated, and in cases of disagreement, a discussion with the third reviewer (AK) finalized the conclusion. Lastly, the references to the included papers were investigated.

2.4 Data extraction

Using a data extraction sheet, two independent reviewers (AHB and PS) extracted the following information from each study: 1) First author's name, publication year, and country of conduct; 2) the demographic characteristics of the cases (sample size, mean age, and gender distribution in each CKD and control group); 3) plasma and/or serum chemerin concentration in each group; 4) chemerin gene polymorphism alleles for each study. In situations where precise data regarding the concentration of chemerin were unavailable, we contacted the corresponding author of the investigations.

2.5 Quality assessment

The "Newcastle-Ottawa Quality Assessment Scale" (NOS) for observational studies was used for the quality assessment of included studies (9). Two authors did the quality evaluation individually. In the event of a dispute, a third author settled the issue. The NOS contains three primary classifications of bias: selection, comparability, and outcome. Studies with quality scores of 9-10, 7-8, 5-6, and less than 5 were deemed "very good," "good," "satisfactory," and "unsatisfactory," respectively.

2.6 Statistical analysis

Random-effect meta-analysis was used to determine the standardized mean difference (SMD) and 95% confidence interval (CI) of chemerin concentrations in CKD and control groups. The p-values <0.05 were used as the cutoff for statistical significance.

Where median and interquartile range or median and range were presented in the studies, they were transformed to mean and standard

deviations (SDs) following the methods recommended by Luo et al. and Wan et al. (10, 11). In addition, means and standard deviations were combined where necessary, as suggested by the Cochrane handbook (12).

To calculate the heterogeneity, we utilized Higgins' I-square test based on Cochrane's Q. The heterogeneity thresholds for low, moderate, and high heterogeneity were 25%, 26-75%, and 75%, respectively. Due to the high heterogeneity among studies, random-effect meta-analysis (restricted maximum likelihood (REML)) was employed. We performed a sensitivity analysis by omitting each study and examining the effect on the total effect size. To identify potential outliers, Galbraith plots were also used and analyzed. To identify the source of heterogeneity, meta-regression of the sample size, mean age, female percentage, and publication year was also performed. In addition to Egger's and Begg's statistical tests, publication bias was evaluated using a visual examination of funnel plots (13, 14).

3 Results

3.1 Literature search and baseline characteristics of included studies

Our search included 381 records from PubMed (n = 74), SCOPUS (n = 117), Web of Science (n = 84), and Embase (n = 106). After removing 178 duplicates, 156 records were excluded based on title and abstract screening. Assessment of full-texts resulted in excluding 39 articles due to not reporting chemerin levels, not assessing chemerin levels in CKD, review articles, and conference abstracts. Details of the identification of studies are illustrated in Figure 1.

Eight studies with 875 participants were included in our study that measured serum levels of chemerin in CKD patients and controls (2, 6, 8, 15–19) (Table 1). The mean age of participants was 56.92 ± 11.78 years and 58.6% were male. Studies were conducted in Poland (6, 8), Egypt (15, 19), Iraq (2, 16), China (17), and Germany (18) between 2009 and 2022. All studies had good quality based on the NOS system, while two of them had very good quality due to high comparability among studies groups (15, 19) (Supplementary Table 2).

3.2 Meta-analysis of chemerin levels in all-stage-CKD patients and controls

Pooling of the eight studies comparing CKD in any stage and healthy controls revealed significantly higher chemerin levels in CKD patients (SMD [95% CI]: 2.15 [0.83, 3.48], p-value <0.01, Figure 2). However, this was associated with high heterogeneity (I^2: 98.29%).

3.3 Meta-analysis of chemerin levels in HD patients and controls

Assessment of chemerin levels in HD patients in comparison with controls was done in eight of the studies. Random-effect meta-analysis showed statistically higher chemerin blood concentrations in HD cases (SMD [95% CI]: 2.10 [0.58, 3.62], p-value: 0.01, Figure 3). Heterogeneity was also high in this analysis (I^2: 98.49%).

3.4 Meta-analysis of chemerin levels in non-HD CKD patients and controls

Three of the studies reported chemerin levels in CKD patients who were not undergoing HD and compared it with healthy controls. Blaszak et al. (8) and El-Khashab et al. (15) investigated stage 3 and 4 CKD patients, while Salama et al. (19) reported levels in CKD patients on conservative treatment. Meta-analysis of comparison with healthy controls resulted in significantly higher levels of chemerin (SMD [95% CI]: 2.55 [1.73, 3.36], p-value<0.01, I^2: 76.63%, Figure 4).

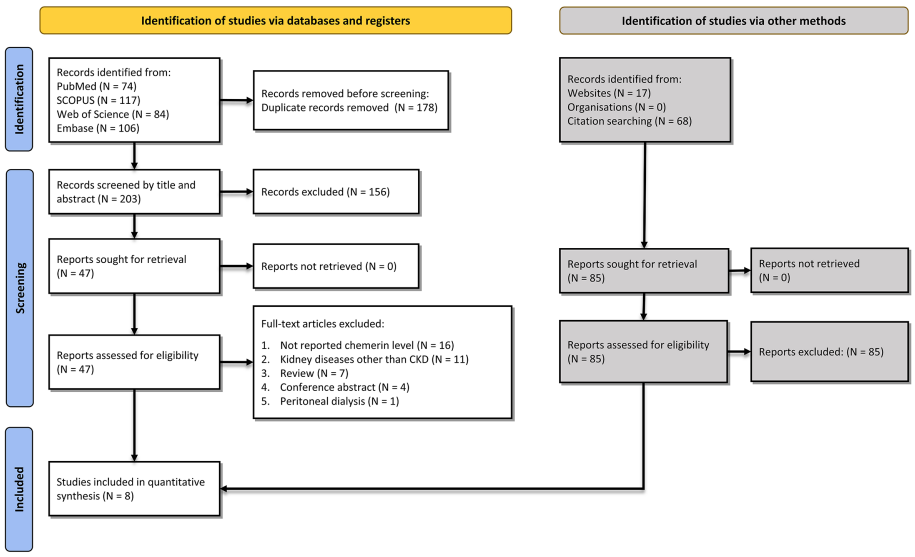

FIGURE 1
Flow diagram summarizing the selection of eligible studies based on the PRISMA guidelines.

TABLE 1 Characteristics of studies evaluating the relation between chemerin levels and chronic kidney disease.

Author	Year	Location	Specimen	Population	N Total	Age (years)	Male (%)	Findings
Blaszak et al.	2015	Poland	Serum	CKD stages 3&4), HD, KT, Control	187	67.5 ± 12.1	60.96	The mean serum chemerin level in the CKD group (stages 3&4) was 70% higher than in controls (122.9 ± 33.7 vs. 72.6 ± 20.7 ng/mL; p<0.001). In addition, no statistical difference was observed between HD patients and CKD ones (115.7 ± 17.6 vs. 122.9 ± 33.7 ng/mL; n.s.). The mean Chemerin levels significantly decreased after HD (115.7 ± 17.6 vs. 101.5 ± 16.4 ng/mL; p<0.001). Chemerin levels after HD were significantly higher than those with KT (101.5 ± 16.4 vs. 74.8 ± 16.0 ng/mL; p<0.001). There was also no significant difference between KT patients and controls (74.8 ± 16.0 vs. 72.6 ± 20.7 ng/mL; n.s.).
El-Khashab et al.	2019	Egypt	Serum	CKD stages 3&4, ESRD (HD), Control	80	49.5 ± 13.9	67.5	The mean chemerin level was significantly higher in CKD patients compared to the healthy controls (p<0.001).
Fahad et al.	2020	Iraq	Serum	HD with and without DM, T2DM, Control	120	54.0 ± 10.1	45.83	The mean serum chemerin was significantly higher in HD patients with DM (230.13 ± 78.26 ng/ml), followed by HD patients without DM (221.90 ± 65.17 ng/ml) compared with controls (110 ± 20.42 ng/ml). Also, the mean of serum chemerin significantly increased in DM patients (212.29 ± 70.88 ng/ml) when compared with the control.
Liu et al.	2022	China	Serum	CRF (GFR ≤ 60), Control	148	59.2 ± 8.1	66.89	Compared with the healthy group, the expression level of chemerin in the observation group was decreased, and the difference was statistically significant (p<0.001). Also, serum levels of chemerin in the death group were significantly higher than those in the survival group(p<0.001, 145.41 ± 18.75 vs 98.52 ± 14.92). The ROC-AUC analysis for the prediction of mortality resulted in an AUC of 0.775 (95% CI: [0.614-0.872]).
Pfau et al.	2009	Germany	Serum	CKD (GFR<50), Control	120	65.0 ± 17.5	51.67	Median circulating chemerin was more than two-fold higher in CKD patients (542.2 ± 98.1 µg/l) compared with control patients (254.3 ± 88.7 µg/l) (p<0.001). There was no significant difference between males and females or patients with or without T2DM.
Rutkowski et al.	2011	Poland	Serum	ESRD, Control	46	47.3 ± 13.6	60.86	Patients before KT had significantly higher chemerin levels compared to healthy controls. After KT, the chemerin levels were reduced to the range of healthy controls. In 76% of the patients, a decrease in serum chemerin concentration was observed; however, the percentage of the changes differed from 13% to 66% (p<0.05).
Salama et al.	2016	Egypt	Serum	Pre-HD, HD, Control	78	48.6 ± 8.6	NR	Compared with the control participants, pre-dialysis patients and patients on HD had significantly higher chemerin levels (p=0.001 and p<0.001). In addition, the chemerin level in patients on HD was higher than that in pre-dialysis patients (p<0.001).
Sarhat et al.	2018	Iraq	Serum	CRF (pre- and post-HD), Control	96	43.9 ± 7.6	57.29	CRF patients had significantly lower chemerin levels compared to controls. In addition, post-HD patients had increased levels of chemerin.

Data are presented as mean± standard deviation or percentage. NR, not reported; CI, confidence interval; HD, hemodialysis; CKD, chronic kidney disease; KT, kidney transplantation; ESRD, end-stage renal disease; DM, diabetes mellitus; T2DM, type-2 diabetes mellitus; GFR, glomerular filtration rate; CRF, chronic renal failure; AUC, area under the receiver operating characteristic curve.

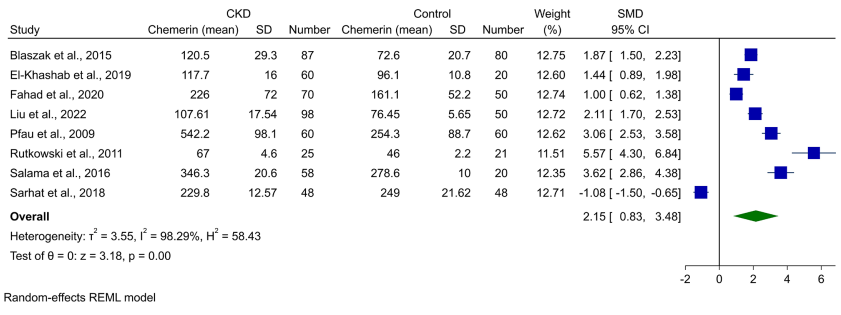

FIGURE 2
Forest plot for meta-analysis of chemerin levels in chronic kidney disease patients compared to controls.

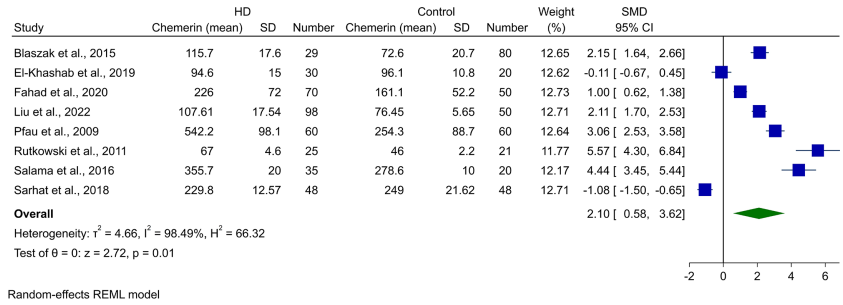

FIGURE 3
Forest plot for meta-analysis of chemerin levels in hemodialysis patients compared to controls.

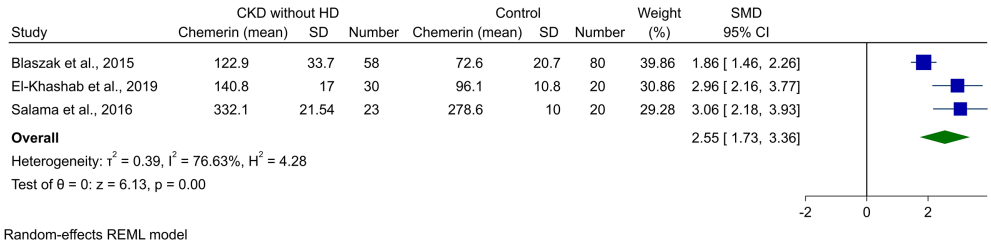

FIGURE 4
Forest plot for meta-analysis of chemerin levels in chronic kidney disease patients without hemodialysis compared to controls.

3.5 Publication bias

Publication bias was assessed for CKD vs. control and HD vs. control meta-analyses. While there was no apparent symmetry for the former, the latter showed an asymmetry in the funnel plot (Supplementary Figures 1, 2). Begg's statistical test could not reveal any sign of publication bias in either CKD vs. controls (p-value: 0.173), or HD vs. controls (p-value: 0.265); however, Egger's test showed a significant publication bias for both of them (p-value: 0.002 and 0.001, respectively).

3.6 Meta-regression analysis

Meta-regression was performed on possible modifiers, including sample size, mean age, male percentage, and publication year. Univariable meta-regression showed no significant relationship between any of these and two of the main analyses. Additionally, publication year contributed to 44.19% and 24.17% of the observed heterogeneity in CKD vs. control and HD vs. control meta-analyses, respectively (Table 2). Bubble plots for all mentioned meta-regressions are illustrated in Supplementary Figures 3-10.

3.7 Outlier's detection and sensitivity analysis

Galbraith plots for both CKD/HD vs. control analyses were designed and investigated. No outlier study was identified in any of the two analyses (Supplementary Figures 11, 12). In addition, sensitivity analysis was performed by removing each of the studies and assessing its impact on overall results. Similarly, none of the studies had a significant effect on the overall pooled result.

4 Discussion

CKD is a global health burden that is strongly associated with a decreased quality of life and premature mortality. It is usually defined using the serum creatinine concentration to estimate GFR as an index of kidney function (20). However, commonly used formulas to estimate kidney function based on creatinine alone have severe shortcomings, with an accuracy of less than 65%, according to estimates (21). These failures point to the need for additional markers of kidney function to augment current clinical tools. An example of a successful marker in diagnosing CKD is cystatin C, which is independent of patient muscle mass and has been used successfully alone and together with creatinine in new equations for estimating GFR that outperformed traditionally used equations (22, 23). We investigated whether chemerin could play a similar role.

The observed elevation in chemerin levels can reasonably be thought to be a result of either an increase in chemerin production or a decrease in its excretion. Chemerin is expressed throughout the body, but its production is believed to be dominated by the liver, with adrenal and pancreatic glands as additional significant sources (24). Comparatively, the expression of chemerin in the kidney is less than 5% of that in the liver (25). Alternatively, adipose tissue is thought to be the main source of chemerin in the body (26), which has led to the

TABLE 2 Meta-regression of CKD/Controls and HD/Controls meta-analyses.

Moderator	No. of subjects		Meta-regression				R^2 Analog (proportion of variance explained)
	Case	Control	Slope	95% CI		*p-value*	
CKD vs. Control							
Sample Size	506	349	-0.018	-0.055	0.018	0.317	0%
Age (mean, years)	506	349	-0.024	-0.151	0.200	0.785	0%
Male Percentage	448	329	0.034	-0.183	0.251	0.758	0%
Publication year	506	349	-0.254	-0.534	0.026	0.075	23.50%
HD vs. Control							
Sample Size	395	349	-0.015	-0.060	0.029	0.495	0%
Age (mean, years)	395	349	0.036	-0.163	0.236	0.720	0%
Male Percentage	360	329	0.000	-0.237	0.236	0.997	0%
Publication year	395	349	-0.290	-0.608	0.027	0.073	24.17%

CI, confidence interval; CKD, chronic kidney disease; HD, hemodialysis.

investigation of chemerin expression in subcutaneous adipose tissue in CKD patients in one of our included studies (8). The results showed no change in tissue expression, despite increased chemerin levels. While this result shows that subcutaneous chemerin expression is not the source of increased chemerin levels in CKD patients, it does not necessarily establish that an increase in chemerin expression throughout the body, e.g., by visceral adipose tissue, might not be the cause of the observed increase in chemerin. Additional studies are required to investigate chemerin expression in the main chemerin-producing tissues, including the liver, endocrine glands, and kidneys in patients with CKD.

The second mechanism by which the increased chemerin levels might be explained is the reduced excretion of chemerin due to impaired kidney function. This is the main mechanism by which creatinine and cystatin C are increased in kidney dysfunction as well (22). Chemerin, in its main form, is a 143 residue polypeptide weighing 16kDa, which lies within the range associated with decreased clearance in kidneys with impaired function (7). Elevated levels of adipokines, including leptin, adiponectin, tumor necrosis factor-α (TNF-α), interleukin-6, resistin, visfatin, and angiotensinogen, with similar molecular shapes and weights to chemerin, have been observed in kidney dysfunction (27). Decreased renal function may increase serum adipokine levels by reducing renal elimination or degradation. A possible source of evidence for this mechanism is the significant negative association between GFR and chemerin levels in patients with ESRD as well as the restoration of chemerin levels after successful kidney transplantation (6). Stronger evidence is required to validate this hypothesis, most obviously by measuring urine chemerin levels to calculate chemerin clearance in CKD patients. This approach was considered in (8) but was not reported owing to measurement problems.

In addition to the unknown precise mechanism of increased chemerin levels in CKD, its role in the pathophysiology of renal failure is unclear. We have discussed reports of meaningful associations between chemerin levels and the degree and risk of kidney dysfunction. However, it is unclear whether this observation is simply a byproduct of reduced kidney function due to other means or if chemerin is, in fact, an active pathological agent. Adipokines, in general, seem to have both protective and degenerative effects on kidney function, with all but adiponectin causing CKD progression by mediating endothelial dysfunction, inflammation, fibrosis, and oxidative stress (27). Chemerin specifically produces its function by binding one of three receptors: chemerin receptor 1 (chem1), chemerin receptor 2 (chem2), or chemokine receptor-like 2 (CCRL2). Chem1, the most common chemerin receptor, is a Gi/Go protein of the G protein family, which inhibits the production of cAMP and promotes the production of IP3, calcium influx, and activation of phospholipase C, PI3 kinase, and MAPK pathways (28). Chem1 is highly expressed in the kidneys, much more so than chemerin itself, suggesting high sensitivity to chemerin produced elsewhere (24). This receptor has been shown to mediate the proinflammatory function of chemerin owing to its expression on macrophages and dendritic cells. The proinflammatory function of chemerin has additionally been confirmed in arthritis and psoriasis, firmly establishing the proinflammatory activity of chemerin (29). Inflammation, along with the modulation of endothelial damage characteristic of adipokines, has been recently confirmed in the kidneys of patients and rats with diabetic nephropathy (DN), where the expression of chemerin, chem1, and inflammatory factors was significantly increased in DN, implicating chemerin as an active agent in the pathophysiology of glomerular endothelial cell inflammation (30). Additional investigations are needed to shed light on the role that the chemerin/chem1 axis plays in causing kidney injury.

The observed association between chemerin and kidney disease is of importance in two general axes (1): as a result of kidney damage, itself either caused by reduced renal clearance or increased production, and (2) as a cause of kidney damage, through a variety of proinflammatory, endothelial, and oxidative mechanisms. These two axes, in turn, implicate chemerin both as a marker for CKD diagnosis, as well as a possible target for the treatment of a variety of kidney diseases.

The strengths of this study include precise compliance with PRISMA protocols, including a comprehensive search of relevant databases and the independent screening of search results by two

reviewers. The measured outcomes are mostly identical and directly comparable. The major limitation of this analysis was the relevant differences among included studies' populations and the small sample size of them, resulting in a limitation in the generalizability of the findings. Secondly, few subgroup analyses were performed, which introduces a possible source of bias. Third, we calculated mean and SDs from median and IQRs with the methods suggested by Luo et al. and Wan et al. (10, 11), which despite being used before, may add some limitations to the analyses. Finally, the lack of additional direct prognostic indices limits the scope of our reasoning regarding the appropriateness of chemerin as a prognostic marker for CKD.

5 Conclusion

Evidence from recent studies suggests that the cytokine chemerin is consistently elevated in patients diagnosed with CKD and in patients undergoing HD compared to healthy controls. This evidence can be used to justify the inclusion of chemerin as one of several possible biomarkers for future models used to classify patients into CKD vs. normal as well as to predict the future course of their disease. Evidence from additional studies is needed to solidify and confirm this observed association as well as to specify pitfalls, including specific comorbidities or covariates, that cause the observed association to fail to replicate.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

AHB: Writing - original draft/Conceptualization/Formal analysis/Visualization, PS, PB, and NA: Writing - original draft/Review and Editing, Data curation, AK: Supervision/Conceptualization/Writing - Review and Editing, SP: Writing - Review and Editing. All authors contributed to the article and approved the submitted version

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1120774/full#supplementary-material

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The relationship between fat distribution in central region and comorbidities in obese people: Based on NHANES 2011–2018

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Background: Central obesity is closely related to comorbidity, while the relationship between fat accumulation pattern and abnormal distribution in different parts of the central region of obese people and comorbidity is not clear. This study aimed to explore the relationship between fat distribution in central region and comorbidity among obese participants.

Methods: We used observational data of NHANES 2011–2018 to identify 12 obesity-related comorbidities in 7 categories based on questionnaire responses from participants. Fat distribution is expressed by fat ratio, including Android, Gynoid, visceral, subcutaneous, visceral/subcutaneous (V/S), and total abdominal fat ratio. Logistic regression analysis were utilized to elucidate the association between fat distribution and comorbidity.

Results: The comorbidity rate was about 54.1% among 4899 obese participants (weighted 60,180,984, 41.35 ± 11.16 years, 57.5% female). There were differences in fat distribution across the sexes and ages. Among men, Android fat ratio (OR, 4.21, 95% CI, 1.54–11.50, P_{trend}=0.007), visceral fat ratio (OR, 2.16, 95% CI, 1.42–3.29, P_{trend}<0.001) and V/S (OR, 2.07, 95% CI, 1.43–2.99, P_{trend}<0.001) were independent risk factors for comorbidity. Among these, there was a "J" shape correlation between Android fat ratio and comorbidity risk, while visceral fat ratio and V/S exhibited linear relationships with comorbidity risk. The Gynoid fat ratio (OR, 0.87, 95%CI, 0.80–0.95, P_{trend}=0.001) and subcutaneous fat ratio (OR, 0.81, 95%CI, 0.67–0.98, P_{trend}=0.016) both performed a protective role in the risk of comorbidity. In women, Android fat ratio (OR, 4.65, 95% CI, 2.11–10.24, P_{trend}=0.020), visceral fat ratio (OR, 1.83, 95% CI, 1.31–2.56, P_{trend}=0.001), and V/S (OR, 1.80, 95% CI, 1.32–2.45, P_{trend}=0.020) were also independent risk factors for comorbidity, with a dose-response relationship similar to that of men. Only the Gynoid fat ratio (OR, 0.93, 95% CI, 0.87–0.99, P_{trend}=0.016) had a protective effect on female comorbidity. This association was also seen in obese participants of

different age groups, comorbidity numbers, and comorbidity types, although it was more statistically significant in older, complex comorbidity, cardiovascular, cerebrovascular, and metabolic diseases.

Conclusions: In the obese population, there were strong correlation between fat distribution in central region and comorbidity, which was affected by sex, age, number of comorbidities, and type of comorbidity.

KEYWORDS

obesity, fat distribution, comorbidity, public health, NHANES

Introduction

Obesity is becoming more widespread all over the world, according to Global Burden of Disease Group research (1). Wang et al. predicted that by 2030, the total medical cost of obesity will double to 860.7-956.9 billion US dollars, accounting for about 16–18% of the total medical costs in the United States (2). It can threaten the health of people of any age, including induced cardiovascular disease (3), metabolic disease (4), liver disease (5), cancer (6), joint disease (7), and other comorbidities. However, these comorbidity studies focus mostly on the elderly and children, with little attention to adults aged 20–59 years, who account for the majority of the population distribution (8). This might be due to the low comorbidity rate of this age group. However, we must be aware that once young and middle-aged people are accompanied by these chronic diseases, they will be permanently and profoundly affected, and their quality of life and survival time may be significantly reduced. Therefore, we include this prevalent yet distinct category in this study.

Although obesity is closely related to a variety of comorbidities, the advent of the "obesity paradox" in recent years has broken everyone's traditional understanding. Obesity based on body mass index (BMI) alone does not seem to well explain the protective effects of overweight and obesity in cardiovascular and cerebrovascular diseases (CCVD), cancer, and other diseases (9, 10), while individualized research on obesity types, body composition, lean and fat distribution have become increasingly valuable (11, 12).

We know that fat in obese people is often centrally accumulated, which is reflected in visceral fat, abdominal subcutaneous fat, hip fat and other regional fat. Previous studies have shown that the excessive distribution of Android fat and trunk fat may have a deleterious impact on subclinical right ventricular function, while the peripheral fat distribution may have a positive impact (13).

As far as we know, although some studies have conducted separate researches on waist hip ratio or visceral fat, no large-scale study has been conducted to explain the relationship between fat distribution in central region and comorbidities, even the number of comorbidities in obese patients of different ages and sexes. Therefore, this study aimed to explore the relationship between fat distribution and comorbidities such as CCVD, metabolic diseases (MD), respiratory diseases (RD), cancer, liver diseases, renal diseases, and joint diseases in obese adults aged 20-59 by analyzing the population in National Health and Nutrition Examination Survey (NHANES) database from 2011 to 2018, to provide us with a better scientific understanding of obesity and fat distribution in central region.

Methods

Participants and study design

The population of this study was sourced from the NHANES database—a large cross-sectional survey conducted by the National Center for Health Statistics—to investigate the health and nutritional status of the population in the United States (14–17). Its research design is complex and exquisite. The principal sample design consisted of multiyear, stratified, clustered 4-stage samples (18). According to the over-sampling standard, researchers over-sampled some subgroups of people and gave them corresponding weights so as to improve the accuracy and reliability of the overall data so that it can represent the demographic characteristics of the entire United States (19). On the official website of NHANES, we referred to the detailed survey contents, survey operations, and data-use methods (20). Personal information was mainly collected through personal interviews and mobile examination center, and all participants provided their signed informed consent (18).

In the present study, we analyzed 39,156 participants from the NHANES during 2011–2018, excluding the following patients: (1) participants aged <20 years, >59 years, and pregnant; (2) lack of data information that can be used to evaluate obesity (BMI and waist circumference); (3) non-obese participants; (4) lack of fat mass data; (5) lack of baseline data (such as income, marital status, smoking, and drinking); (6) lack of comorbidity information. Finally, we included 4,899 obese participants (60,180,984 participants after weighting). The screening process is depicted in Figure S1.

Exposure variables and definitions

In this study, all participants were examined by dual-energy X-ray absorptiometry (DXA) to determine the fat mass, which is the most widely accepted method of measuring body composition (21). The fat distribution in the central region includes Android, Gynoid, visceral,

subcutaneous, visceral/subcutaneous (V/S), and total abdominal fat ratio (See the Supplementary materials for the definition of these areas). The fat distribution was described by the ratio (%), that is, the fat mass of each part/total fat mass × 100%. Obesity was defined as BMI ≥30 or waist circumference (wc) ≥88 cm in women or wc ≥102 cm in men (22).

Outcome

Our primary study outcome was the comorbidity risk among obese participants. We obtained whether the patient also had other diseases from the medical conditions file in the NHANES questionnaire section. We included 12 obesity-related diseases in 7 categories reported previously, which included CCVD (such as hypertension, coronary heart disease, heart failure, and stroke), MD (diabetes and gout), RD (asthma, chronic bronchitis), liver disease, renal disease, and cancer and joint diseases. Among these, simple comorbidity is defined as <4 comorbidities, while the participants with ≥4 diseases were defined as complex comorbidities (23).

Statistical analyses

Considered the complex survey design of NHANES, all statistical analysis was based on sample weight, stratification, and clustering. Continuous variables were expressed by means ± standard deviation (SD), and categorical variables were expressed by percentage (%). Continuous variables were compared with the Student's *t*-test or non-parametric test, and the categorical variables were compared with the Rao-Scott Chi-square test. Considering the large difference in the distribution of fat between men and women, we classified the study participants into 2 groups of men and women and applied logistic regression analysis to clarify the relationship between the distribution of fat in different portions and the risk of comorbidity. For continuous variables that did not conform to the normal distribution, we conducted a natural logarithm transformation and also described these variables in the form of sex-specific quintiles. The cutoff value was calculated from the ROC curve. In addition, we used the variance inflation factor (VIF) to detect multicollinearity among covariates. VIF >10 was considered to indicate multicollinearity.

In order to clarify the correlation between fat distribution and comorbidity risk among different subgroups, we analyzed the age subgroups (<45 and ≥45 years), comorbidity number subgroups (simple comorbidity and complex comorbidity), and comorbidity-type subgroups. In order to test the robustness of the results, we performed a sensitivity analysis, adjusted the age subgroups to <40 and ≥ 40 years old, and then performed a logistic regression analysis to clarify the relationship between fat distribution and comorbidity. Two-sided $P < 0.05$ was considered to indicate statistical significance, and all statistical analyses were performed by the R software (Version 4.1.2).

Result

Characteristics of study participants

The mean age (SD) of these 4899 obese participants was 41.35 ± 11.16 years. The majority of them were female, with about 2950 participants. We found significant differences in total fat mass (32.84 ± 8.94 kg vs 36.56 ± 10.52 kg) and fat distribution between men and women ($P < 0.001$), including Android, Gynoid, visceral, subcutaneous, and abdominal fat ratio and V/S (Table 1).

TABLE 1 Clinical characteristics and body measurements of study participants in NHANES 2011-2018.

	Male (n=1950)	Female (n=2949)	P value
Weighted sample size, No. (%)	25,553,771 (42.5)	34,627,213 (57.5)	
Age (mean)	41.37 (11.12)	41.34 (11.19)	0.941
Race, No. (%)			<0.001
Mexican American	358 (12.7)	528 (11.0)	
Other Hispanic	214 (7.9)	340 (7.3)	
Non-Hispanic White	760 (63.2)	1043 (61.9)	
Non-Hispanic Black	387 (9.4)	717 (13.1)	
Other Race	231 (6.8)	321 (6.7)	
Education, No. (%)			<0.001
Less than high school	359 (12.4)	533 (12.5)	
High school or equivalent	523 (27.2)	617 (20.4)	
College or above	1068 (60.3)	1799 (67.1)	
Marital, No. (%)			<0.001
Married	1091 (58.4)	1413 (53.1)	
Separated	218 (11.6)	586 (18.3)	

(Continued)

TABLE 1 Continued

	Male (n=1950)	Female (n=2949)	P value
Never married	641 (30.0)	950 (28.7)	
Ratio of family income to poverty, No. (%)			<0.001
0-1.0	445 (15.1)	848 (21.2)	
1.1-3.0	806 (36.0)	1161 (35.1)	
>3.0	699 (48.9)	940 (43.7)	
Medical insurance, No. (%)			0.019
No	543 (21.3)	680 (17.8)	
Yes	1407 (78.7)	2269 (82.2)	
Alcohol drinking, No. (%)			<0.001
No	312 (13.9)	954 (24.6)	
Yes	1638 (86.1)	1995 (75.4)	
Smoke, No. (%)			<0.001
No	1014 (52.6)	1927 (61.4)	
Yes	936 (47.4)	1022 (38.6)	
BMI (mean)	33.29 (4.70)	32.48 (6.41)	<0.001
Arm circumference (mean)	37.59 (3.54)	34.72 (4.56)	<0.001
Waist (mean)	112.32 (10.94)	104.73 (13.36)	<0.001
Total fat mass (kg, mean)	32.84 (8.94)	36.56 (10.52)	<0.001
Android fat ratio (mean)	10.34 (1.27)	8.52 (1.31)	<0.001
Gynoid fat ratio (mean)	15.48 (1.76)	17.11 (2.11)	<0.001
Visceral fat ratio (mean)	2.22 (0.76)	1.65 (0.60)	<0.001
Subcutaneous fat ratio (mean)	5.69 (0.66)	6.48 (0.83)	<0.001
Visceral to Subcutaneous fat ratio (mean)	0.40 (0.15)	0.26 (0.10)	<0.001
Abdominal fat ratio (mean)	8.06 (0.91)	8.27 (1.06)	<0.001
Comorbidity, No. (%)	1074 (53.6)	1640 (54.4)	0.626

The majority of patients had comorbidity (54.1%), and there was no significant difference in the comorbidity rates between men and women (P = 0.626). The comorbidity rate of men was about 53.6%, while women had about 54.4%. The comorbidity types of the two groups were similar, including hypertension (men, 32.6%, women, 26.5%), arthritis (men, 14.9%, women, 20.9%) and asthma (men, 12.8%, women, 19%).

Relationship between fat distribution and comorbidity risk in different sexes

After adjusting for age, race, education level, marital status, income, medical insurance, alcohol drinking, smoking, BMI, wc, and arm circumference, the Android fat ratio (OR, 4.21, 95% CI, 1.54–11.50, P_{trend}=0.007), visceral fat ratio (OR, 2.16, 95% CI, 1.42–3.29, P_{trend}<0.001), and V/S (OR, 2.07, 95% CI, 1.43–2.99, P_{trend}<0.001) were independent risk factors for comorbidity in men. The Android fat ratio is "J" type related to comorbidity risk, while the visceral fat ratio and V/S were very significantly linear type related to comorbidity risk, that is, compared to Quintile 1, the OR values of Quintiles 2, 3, 4, and 5 exhibited progressive growth. Simultaneously, the Gynoid fat ratio (OR, 0.87, 95%CI, 0.80–0.95, P_{trend}=0.001) and the subcutaneous fat ratio (OR, 0.81, 95%CI, 0.67–0.98, P_{trend}=0.016) played a protective role in the risk of comorbidity, and this trend was still visible after dividing by the cutoff value. After dividing by sex-specific quintiles, we discovered a significant linear inversely dose-response relationship between Gynoid fat ratio, subcutaneous fat ratio, and comorbidity risk, implying that their protective effects were accumulated as fat ratio increases (Table 2).

We also adjusted for covariates among women. The results showed that as continuous variables, Android fat ratio (OR, 4.65, 95% CI, 2.11–10.24, P_{trend}=0.020), visceral fat ratio (OR, 1.83, 95% CI, 1.31–2.56, P_{trend}=0.001), and V/S (OR, 1.80, 95% CI, 1.32–2.45,

TABLE 2 Odds ratio (95%CI) of comorbidity risk with different fat distribution in male obese people.

		Android fat(%)		Gynoid fat(%)		Visceral fat(%)	
		OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value
As continuous (per SD)		4.21 (1.54, 11.50)	0.006	0.87 (0.80, 0.95)	0.002	2.16 (1.42, 3.29)	0.001
By cut-off	Low	Ref		Ref		Ref	
	High	1.58 (1.20, 2.05)	0.001	0.46 (0.33,0.62)	<0.001	1.67 (1.20,2.31)	0.003
Quintile	Q1	Ref		Ref		Ref	
	Q2	0.75 (0.50, 1.12)	0.154	0.44 (0.30, 0.64)	<0.001	1.39 (0.92, 2.10)	0.112
	Q3	0.97 (0.69, 1.36)	0.856	0.33 (0.21, 0.50)	<0.001	1.62 (1.02, 2.57)	0.042
	Q4	1.05 (0.69, 1.62)	0.805	0.37 (0.25, 0.56)	<0.001	2.05 (1.31, 3.19)	0.002
	Q5	1.53 (1.01, 2.33)	0.047	0.32 (0.20, 0.50)	<0.001	2.35 (1.44, 3.84)	0.001
	Ptrend		0.007		0.001		<0.001
		Subcutaneous fat(%)		V/S		Abdominal fat(%)	
As continuous (per SD)		0.81 (0.67, 0.98)	0.032	2.07 (1.43, 2.99)	<0.001	1.13 (0.96,1.32)	0.13
By cut-off	Low	Ref		Ref		Ref	
	High	0.60 (0.47,0.77)	<0.001	2.10 (1.62,2.72)	<0.001	1.37 (1.05,1.79)	0.022
Quintile	Q1	Ref		Ref		Ref	
	Q2	0.89 (0.61, 1.31)	0.553	1.53 (1.12, 2.10)	0.008	0.70 (0.49, 1.02)	0.062
	Q3	0.80 (0.55, 1.17)	0.249	2.04 (1.38, 3.02)	0.001	0.90 (0.58, 1.40)	0.634
	Q4	0.61 (0.39, 0.96)	0.033	2.42 (1.66, 3.54)	<0.001	1.06 (0.68, 1.67)	0.779
	Q5	0.66 (0.42, 1.05)	0.078	3.92 (2.54, 6.07)	<0.001	1.00 (0.61, 1.64)	0.996
	Ptrend		0.016		<0.001		0.298

Adjusted for Age, Race, Education, Marital status, Ratio of family income to poverty, Medical insurance, Smoke, Alcohol, BMI, Waist, Arm circumference.

P_{trend}=0.020) were also independent risk factors for comorbidity. And their dose-response association showed the same trend as in males. Unlike the male results, however, only the Gynoid fat ratio (OR, 0.93, 95% CI, 0.87–0.99, P_{trend}=0.016) played a protective role (Table 3). Although there was a protective trend in the subcutaneous fat ratio (OR, 0.69, 95% CI, 0.31–1.53), it was not statistically significant (P = 0.350, P_{trend}=0.208).

Relationship between fat distribution and comorbidity risk stratified by age

We separated men and women into two groups(<45 and≥45 years old) to explore the differences in fat distribution and comorbidity across age groups. We found variations in all fat ratio among participants of two groups, regardless of sex (Figure S2). Further logistic regression analysis showed that (Tables S1, S2; Figure 1), in contrast to the results of the total male population, the Android fat ratio (OR, 2.77, 95%CI, 0.87–8.80, P = 0.082) and the Gynoid fat ratio (OR, 0.91, 95%CI, 0.83–1.00, P = 0.056) of men with <45 were not statistically significant with the risk of comorbidity. But in men with ≥45, Android fat ratio (OR, 7.24, 95%CI, 1.25-41.49, P = 0.020) and Gynoid fat ratio (OR, 0.78, 95%CI, 0.68–0.90, P = 0.001) were significantly associated with comorbidity risk, and this trend also existed in women (Tables S3, S4; Figure 2)

Relationship between fat distribution and risk of complex comorbidity

We reclassified the patients according to the number of comorbidities. Complex comorbidities were defined as four or more comorbidities. Based on this, we studied the relationship between fat distribution as a continuous variable and various degrees of comorbidity (Figures S3, S4). The forest plot in Figures 3, 4 clearly showed that, with the emergence of complex comorbidity, Android fat ratio, visceral fat ratio, and V/S have significantly increased the risk of comorbidity, while the protective effect of Gynoid fat ratio and the subcutaneous fat ratio on the risk of comorbidity had also increased.

Relationship between fat distribution and risk of different types of comorbidities

We reclassified the patients according to their comorbidity types, mainly including CCVD, MD, RD, liver disease, renal disease, cancer, and joint disease, and studied the relationship between fat distribution and different types of comorbidities. As continuous and categorical variables, the quintile OR value of CCVD comorbidity risk of Android fat ratio, visceral fat ratio, and V/S showed a significant increase. Similarly, the quintiles OR value of Gynoid fat ratio and

TABLE 3 Odds ratio (95%CI) of comorbidity risk with different fat distribution in female obese people.

		Android fat (%)		Gynoid fat (%)		Visceral fat(%)	
		OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value
As continuous (per SD)		4.65 (2.11, 10.24)	<0.001	0.93 (0.87, 0.99)	0.022	1.83 (1.31, 2.56)	0.001
By cut-off	Low	Ref		Ref		Ref	
	High	1.37 (1.09, 1.73)	0.009	0.64 (0.49, 0.83)	0.001	1.71 (1.31, 2.22)	<0.001
Quintile	Q1	Ref		Ref		Ref	
	Q2	0.91 (0.65, 1.28)	0.586	0.57 (0.39, 0.83)	0.004	1.08 (0.79, 1.50)	0.617
	Q3	1.16 (0.82, 1.65)	0.401	0.46 (0.32, 0.66)	<0.001	1.03 (0.77, 1.38)	0.815
	Q4	1.11 (0.77, 1.60)	0.565	0.46 (0.32, 0.67)	<0.001	1.41 (1.01, 1.97)	0.044
	Q5	1.55 (1.04, 2.31)	0.030	0.55 (0.37, 0.82)	0.004	2.18 (1.35, 3.50)	0.002
	P_{trend}		0.020		0.016		0.001
		Subcutaneous fat(%)		V/S		Abdominal fat(%)	
As continuous (per SD)		0.69 (0.31, 1.53)	0.35	1.80 (1.32, 2.45)	<0.001	1.04 (0.94, 1.16)	0.398
By cut-off	Low	Ref		Ref		Ref	
	High	0.85 (0.68, 1.06)	0.136	2.13 (1.67, 2.74)	<0.001	1.26 (0.99, 1.60)	0.061
Quintile	Q1	Ref		Ref		Ref	
	Q2	0.81 (0.59, 1.10)	0.169	1.23 (0.91, 1.67)	0.173	0.87 (0.66, 1.16)	0.331
	Q3	0.92 (0.66, 1.28)	0.605	1.36 (1.03, 1.79)	0.032	1.01 (0.75, 1.38)	0.926
	Q4	0.72 (0.52, 0.98)	0.036	1.72 (1.20, 2.46)	0.004	1.05 (0.76, 1.45)	0.755
	Q5	0.92 (0.68, 1.25)	0.578	3.55 (2.30, 5.49)	<0.001	1.06 (0.76, 1.46)	0.739
	P_{trend}		0.208		<0.001		0.371

Adjusted for Age, Race, Education, Marital status, Ratio of family income to poverty, Medical insurance, Smoke, Alcohol, BMI, Waist, Arm circumference.

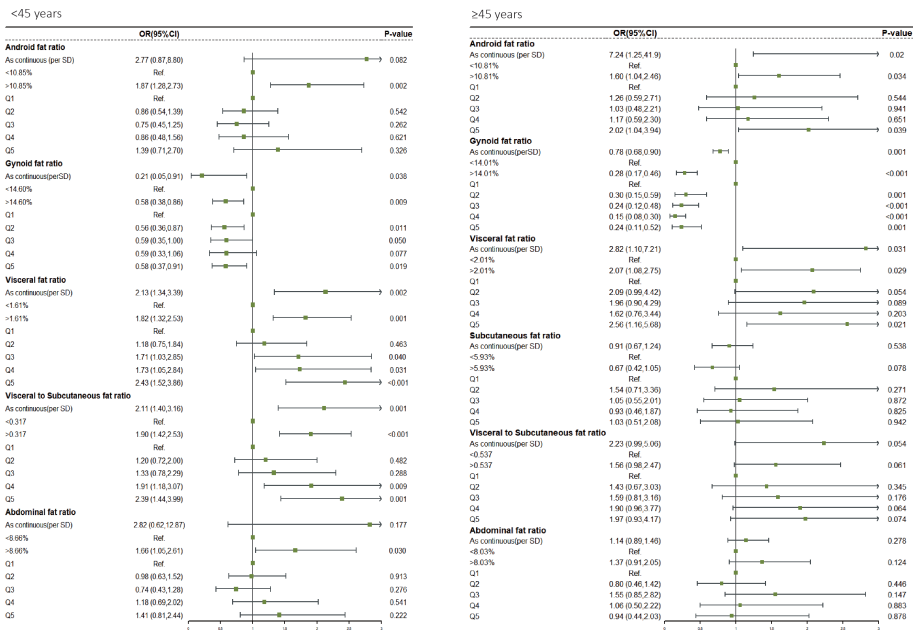

FIGURE 1
Comorbidity risk odds ratio (95% CI) of different fat distribution in obese male.

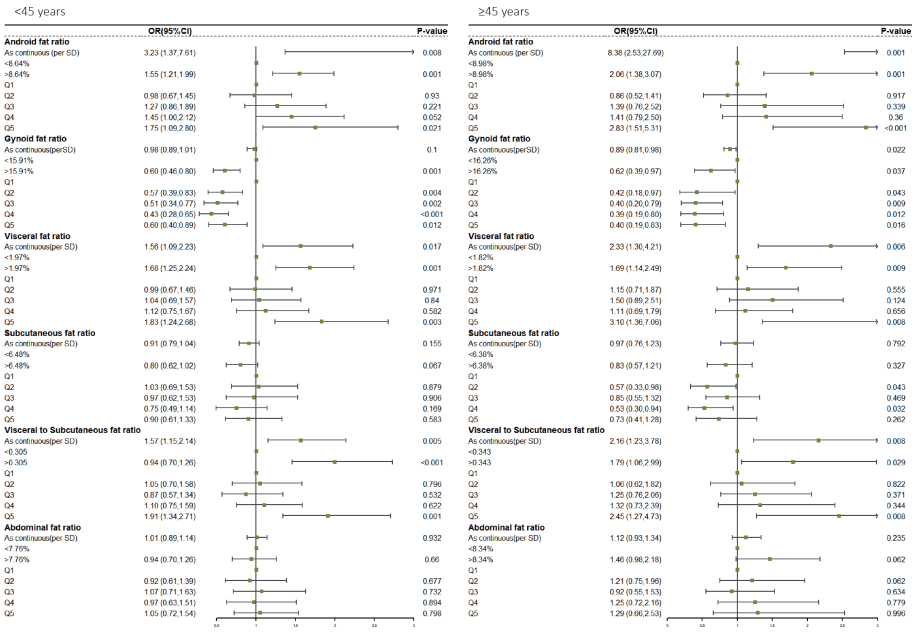

FIGURE 2
Comorbidity risk odds ratio (95% CI) of different fat distribution in obese female.

subcutaneous fat ratio, and CCVD comorbidity risk showed a decreasing trend, indicating that its protective effect was gradually increasing (Figure S5). Except for subcutaneous fat ratio, Android fat ratio, Gynoid fat ratio, visceral fat ratio, and V/S were the same in MD as they were in CCVD (Figure S6). However, when fat distribution was associated with the risk of RD, liver disease, renal disease, cancer, and joint disease, these relationships were less regular (Figures S7–S11).

Sensitive analysis

In addition, we also conducted some sensitivity analyses and discovered that the results were stable. To overcome the bias caused by age grouping, we reset the age boundary and investigated the role of fat distribution in obese participants at the age of 40. Android fat ratio (OR, 2.60, 95% CI, 0.56–12.12, P = 0.219) and Gynoid fat ratio (OR, 0.96, 95% CI, 0.86–1.08, P = 0.501) were not significantly associated with comorbidity among male obese adults aged <40 years (Figure S12). Conversely, the risk effect of Android fat ratio (OR, 5.30, 95% CI, 1.33–21.13, P = 0.019) and the protective effect of Gynoid fat ratio (OR, 0.80, 95% CI, 0.71–0.91, P = 0.006) were obvious for male obese people aged ≥40 years. In women, the results were similar (Figure S13). Considering the impact of menstrual status on female fat distribution and some disease risks (CCVD), the results of subgroups of people with menstrual status by age showed that the comorbidity risk of postmenopausal (older) participants seemed more likely to be affected by abnormal fat distribution (Figure S14). In addition, in order to avoid the impact of estrogen use, we made additional adjustments to the estrogen use of participants with complete estrogen use information, and the results were still stable (Table S5).

Discussion

We analyzed 60 million obese individuals aged 20–59 years in this large-scale prospective study that can represent the majority of the US population. The results showed that even the central regional fat was highly heterogeneous, with different fat distributions having distinct consequences on comorbidity risk. In obese patients, the Android fat ratio, visceral fat ratio, and V/S were all independent risk factors for comorbidity. The Gynoid fat ratio accumulation provided protection. Furthermore, in men, the accumulation of abdominal subcutaneous fat performed a protective role in the risk of comorbidity. However, the change in total abdominal fat had no discernable effect on the incidence of comorbidity. Further subgroup analysis showed that the effects of fat distribution were more strongly correlated with comorbidity risk in older participants, as well as complex comorbidity, CCVD, and MD.

Fat distribution and clinical characteristics

This study initially investigated the differences in fat distribution among obese participants of different sexes and ages. To begin, males had greater Android fat, visceral fat, and V/S compared to women, but less Gynoid fat and subcutaneous fat, which may be related to hormone levels, eating habits, living habits, and genetic differences (24). Second, this difference was mirrored in fat function. We also discovered that in men, both visceral fat ratio and subcutaneous fat ratio were strongly linked with comorbidity risk, but in women, only visceral fat ratio was significantly associated with comorbidity risk. This result was completely consistent with the results of Mutsert et al. (25). As a result, in women, just variations in visceral fat may need to be assessed for stratification

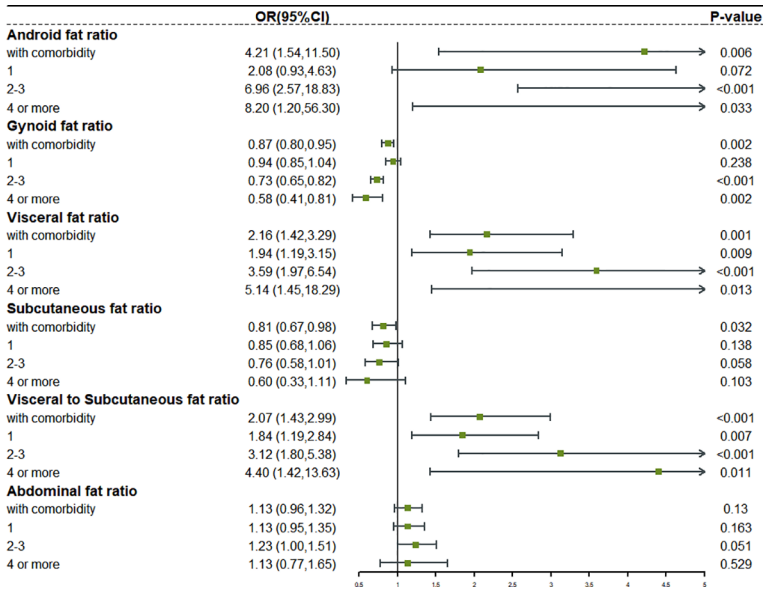

FIGURE 3
The relationship between different fat distribution and different degrees of comorbidity in obese male.

of comorbidity risk, but in men, the potential effects of subcutaneous fat may need to be additionally assessed. Second, age was an important reason for the differences in fat distribution among participants. With advancing age, Android fat, visceral fat, and abdominal fat increased, but Gynoid fat and subcutaneous fat decreased. This also coincided with previous research results. Aging promotes fat redistribution, that is, loss of subcutaneous fat and growth of visceral fat, and hormonal imbalance can also invert the distribution of Android and Gynoid fat (26). In terms of fat function, older participants were more susceptible to fat than younger participants, which was consistent with previous studies. Preis et al. also found a stronger correlation between fat distribution and metabolic diseases in older participants (27).

Fat distribution and complex comorbidity

For obese participants, complex comorbidities are a difficult public health prevention target (28). Although some studies have noted the relationship between fat distribution or obesity degree and various comorbidities, for example, a recent study by Mika et al. found a dose-response relationship between obese individuals' BMI and complex comorbidities, obese participants exhibited a 5-fold greater risk of simple comorbidity and a 12-fold increased risk of complex comorbidity compared to healthy weight participants (23). However, few studies have elucidated the relationship between fat distribution and complex comorbidity. When compared to people with simple comorbidity, the fat distribution of participants with complex comorbidity was more closely related to comorbidity risk, and this trend was not affected by sex. The results of this study were unprecedented because it effectively filled the deficiency in previous studies that relied solely on BMI to determine the risk of complex comorbidity.

Fat distribution and comorbidity type

A large number of studies have shown a strong correlation between obesity and various types of comorbidities, with the most widely reported comorbidities being cardiovascular, metabolic, and respiratory diseases (29–31). Albert et al. showed that obesity can cause a variety of hemodynamic changes, which may lead to cardiac morphological changes and ventricular dysfunction (32). Although this theory has been widely confirmed, we cannot ignore the latest research on the obesity paradox in cardiovascular disease. The mortality of patients with any kind of heart failure has decreased as BMI has increased (33). This contradictory phenomenon prompts us to focus our research on body composition. We found that increasing the Android fat ratio, visceral fat ratio, and V/S would increase the risk of CCVD and even the mortality of special causes. However, the Gynoid fat ratio and subcutaneous fat ratio played considerable protective roles. This conclusion has not been explored in depth before and it may be a reasonable explanation for the "obesity paradox". The increase in BMI will not benefit all obese people. Participants will not benefit from a rise in BMI induced by Android and visceral fat. Only the increase in BMI caused by Gynoid and subcutaneous fat may achieve the effect of the "obesity paradox". Similarly, while obesity is associated with the occurrence of MD such as diabetes and gout (34, 35), the risk of MD caused by fat in different regions was not the same.

The most reasonable explanation for this phenomenon is fat heterogeneity. The Android fat and visceral fat are composed of white adipose tissues (WAT), which contribute to metabolism and chronic inflammation *in vivo*, while triglycerides accumulation in WAT cells in obese people triggers WAT cells remodeling, proliferation, and hypertrophy. The ERK and p38 MAPK pathways are activated by adipocytokines secretion, resulting in increased CCL2 expression in adipocytes. This in turn triggers pro-inflammatory macrophage

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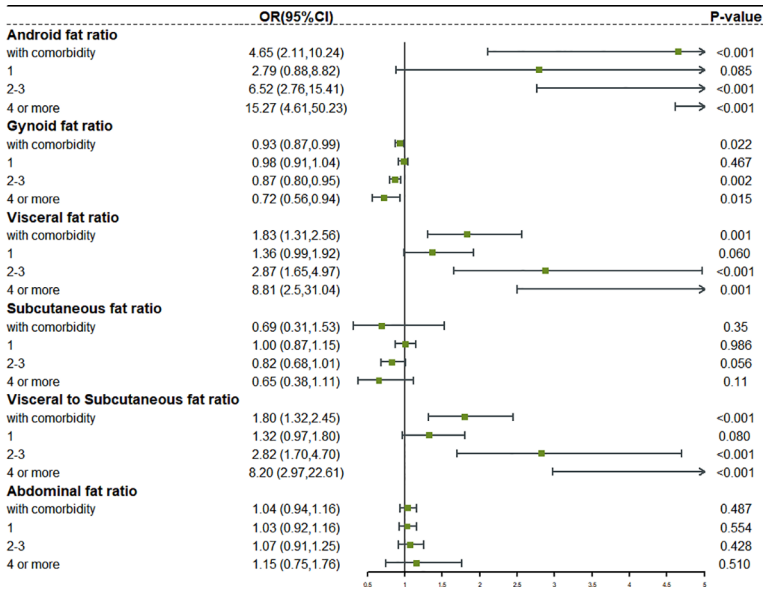

FIGURE 4
The relationship between different fat distribution and different degrees of comorbidity in obese female.

aggregation, Treg cell reduction, and IL-6 and TNF-α secretion increases, leading to systemic inflammation, insulin resistance, oxidative stress, and a series of metabolic reactions (36, 37). As for Gynoid fat, estrogen induction increases the anti-lipolytic α2-adrenergic receptors in the gluteal-femoral subcutaneous fat depot, causing fat to accumulate in the Gynoid area; hence, Gynoid fat distribution is closely related to estrogen levels (38). Estrogen has been widely recognized as an important factor in regulating obesity and metabolic balance in the body (39). Tran's study showed that estrogen-regulated multiple calcium-dependent activities in cardiovascular tissues *via* influencing calcium signaling mechanism components (40). Alternatively, by activating eNOS and increasing NO production, as well as activating cardioprotective signaling cascades including Akt and MAP kinases, cardiac and endothelial cells are protected against apoptosis and necrosis, alleviating pathological myocardial hypertrophy (41). Estrogen also plays an important role in metabolic pathways. Animal experiments have shown that estrogen can increase insulin content and glucose-stimulated insulin secretion in isolated mouse islets, and maintain glucose homeostasis, while its deficiency will disturb oxidative stress and endoplasmic reticulum function, resulting in a complete disorder of insulin function and *in vivo* metabolism. Therefore, the accumulation of Gynoid fat caused by increased estrogen significantly reduces the risk of comorbidity in obese people.

Interestingly, the increase in total abdominal fat did not appear to affect the risk of any type of comorbidity in this study, which differs slightly from previous reports indicating total abdominal fat was an independent risk factor for cardiovascular disease (42). Visceral fat is primarily responsible for the risk of total abdominal fat, while subcutaneous fat has a protective effect. Therefore, we specially analyzed the role of V/S in comorbidity. It has been proved that V/S can be used to assess the risk of comorbidity, but merely judging total abdominal fat cannot be very effective in guiding clinical work. Individual evaluation of visceral fat, abdominal subcutaneous fat and V/S is necessary to meet the requirements of precision nutrition and precision medicine.

Strengths and limitations

Obesity has been on the rise in adults since the 1980s, but over the past decade, the prevalence of obesity and severe obesity has continued to increase among young and middle-aged adults aged 20 to 59 years, compared to those aged <20 years and >60 years (43), despite previous studies focusing primarily on adolescents and the elderly. This is the first study to systematically study the fat distribution and comorbidity risk, complex comorbidity, and comorbidity types of obese people aged 20–59 years. Second, the part of our study on complex comorbidities is of great public health significance. Obese individuals aged <50 years had a higher risk of complex comorbidity than older obese participants, and the extremely high complex comorbidity rate imposes a huge socioeconomic burden (23). This study's population is obese people aged 20–59 years, so it may effectively guide public health prevention and control and reduce the complex comorbidity rate of such people, improving their quality of life and survival time. Notwithstanding, our study also had some limitations. First, since this is a cross-sectional study, we could not obtain the dynamic changes in the participants' body composition, which may lead to unclear causality; second, we did not account for diet, exercise, and lifestyle habits, which could confound our results. Finally, changes in menstrual status, hormone treatment and hormone level may affect the distribution and mass of fat, thus affecting the results. However, due to the limitations of NHANES database, we did not adjust these covariants. In future clinical research, we will pay more attention to these aspects.

Conclusions

Taken together, these results have clinical and public health implications, and our study highlights the correlation between fat distribution and comorbidity, which is influenced by sex, age, number of comorbidities, and type of comorbidity. As we age, we should pay more attention to changes in central fat distribution, and people with abnormal fat distribution should be on the lookout for CCVD and MD. Furthermore, because of the strong correlation between abnormal fat distribution and complex comorbidities, it is particularly important to distinguish the fat function of various parts of obese people. This result provides clinical guidance that obesity treatment (such as life intervention, pharmacotherapy and bariatric surgery) should be used with greater caution and precision for young and middle-aged obese people.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by National Center for Health Statistics. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization, H-PS and C-AL; methodology, G-TR, H-LX; software, S-QL, Y-ZG and C-AL; validation, M-MS, TL, and C-AL; investigation, H-PS; resources, C-AL; data curation, C-AL and S-QL; writing—original draft preparation, C-AL; writing—review and editing, C-AL, M-MS, G-TR, LD and H-LX; visualization, QZ, and TL; supervision, C-AL and H-PS; project administration, H-PS. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1114963/full#supplementary-material

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Body mass index affects kidney transplant outcomes: A cohort study over 5 years using a steroid sparing protocol

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Background: There is controversy regarding the suitability of high body mass index (BMI) candidates accessing the transplant waitlist.

Patients and methods: Observational study on consecutive kidney transplant recipients undergoing surgery between January 2014 and March 2016 at our center. Patients were stratified according to BMI. Survival outcomes and graft function were analyzed to investigate the effect of donor's and recipient's demographic characteristics.

Results: 396 kidney transplant recipients: 260 males, mean age 51.8 ± 15.9 years, followed up for a mean time of 5.86 ± 2.29 years. Mean BMI 26.2 ± 5.1. BMI class 1 (20 ≤ BMI ≤ 24.9) n=133, class 2 (25 ≤ BMI ≤ 29.9) n= 155, class 3 (30 ≤ BMI ≤34.9) n=53, class 4 (BMI ≥ 35) n=21, class V (BMI ≤ 19.9) n=34. Patient survival was not significantly different according to the recipient's BMI class (p=0.476); graft survival was affected (p=0.031), as well as graft function up to 2 years post-transplant and at 4 years follow up (p=0.016). At logistic regression the factors independently associated with graft loss were only donor's age (p=0.05) and BMI class of the recipient (p=0.002).

Conclusions: Obesity did not impact on patient's survival but affected graft function and graft loss.

KEYWORDS

obesity, kidney transplant, body mass index, bariatric surgery, equity

Introduction

Obesity represents a major healthcare alert worldwide with a growing incidence in the last decades, accounting more than 1.9 billion individuals aged > 18 years, being overweight (39% of the population), of which over 650 million obese (13%) (1).

High body mass index (BMI) poses critical consideration when selecting candidates for surgery (2). In particular, in view of the limited organ donor pool, there is still controversy

Abbreviations: BMI, body mass index; ESKD, end stage kidney disease; MDRD, Modification of Diet in Renal Disease.

whether end stage kidney disease (ESKD) patients suffering from obesity should be eligible candidates for the waiting list (3), or if given the increased risk of complications (4), mostly wound infections and dehiscence (5), but also delayed graft function and acute rejection (6), they should first lose weight as per a modifiable condition to optimize transplant outcomes (7).

Since kidney transplantation represents the best replacement therapy for ESKD (8), it could be seen as discrimination to not let a patient access this resource only because of his/her BMI status, especially if a living donor has come forward to avoid dialysis for the controversial candidate (9). Even for deceased donor transplantation there is increased life expectancy and quality of life in the literature well described (10), so ethically the decision to decline or delay a position on the transplant waiting list due to BMI alone cannot be taken lightly, but should be evaluated taking into account all the characteristics of the prospective recipient (11).

Additionally, while on the waiting-list, another important consideration must be given to the "obesity paradox" (12), a complex phaenomenon for which higher BMI is associated with improved outcomes and lower BMI with reduced survival. A possible explanation might consist in a better nutrition in general meaning a better immune response against chronic infections or other threatening complications, which are often a cause of death in the lower BMI dialysis population (13). This is also supported by the J-shaped association of dialysis mortality, where the nadir of the curve corresponds to normal BMI patients (14), while the historical unintended weight loss is an independent predictor of death (15).

We have previously demonstrated that overweight and obese patients did not have inferior outcomes at one-year post-transplant (16) and that in the mid-follow up, i.e. 3 years, renal function, but not allograft survival was affected (16). The aim of the present study is to assess patient and graft outcomes of kidney transplant recipients in a steroid-free immunosuppression regimen at 5 years follow up, using BMI as a classifier.

Patients and methods

This is an observational study of a single center kidney transplant recipient cohort who have consecutively undergone surgery between January 2014 and March 2016. Clinical data were prospectively stored in an electronic record. The primary outcomes were death-censored graft loss and patient survival. Graft loss was defined as need for return to chronic dialysis. Secondary outcomes included graft function, expressed as estimated glomerular filtration rate (eGFR) according to the Modification of Diet in Renal Disease (MDRD) (17) equation, measured at 3, 6, 12, 24, 36, 48, 60 and 72 months of follow up, as well as other factors known to be independently associated to graft loss.

Patients were stratified on the basis of their BMI calculated at the time of transplant as weight (in kg) divided by height (in meters) squared. In this way, the entire cohort was divided into 5 weight classes: group 1 = 20 ≤ BMI ≤ 24.9; category 2 = 25 ≤ BMI ≤ 29.9; class 3 = 30 ≤ BMI ≤34.9; group 4 = BMI ≥ 35 and category 5 = BMI ≤ 19.9.

All patients underwent treatment with a steroid-sparing immunosuppressive regimen (7-day course of steroids) with alemtuzumab induction and tacrolimus monotherapy (trough level, 5–8 ng/mL) or interleukin-2 induction with tacrolimus (trough level, 8–12 ng/mL) and mycophenolate mofetil.

The study was performed in accordance to the Declaration of Helsinki principles. The data used were anonymized and did not require patient or public involvement nor affected patient care. The study fell under the category of research through the use of anonymized data of existing databases which, based on the Health Research Authority criteria, does not require proportional or full ethics review and approval.

Statistical analysis

Continuous variables are presented as mean ± standard deviation and compared using one-way ANOVA, ordinal and dichotomous variables with frequency and compared with chi square test. Survival was calculated with Kaplan-Meier estimate and the differences were evaluated with Cox regression. A linear regression model with backwards procedure tested which parameters are acting as independent predictors for graft loss. A generalized linear model of univariate repeated ANOVA with *post hoc* Bonferroni correction was used to determine whether mean eGFR differed statistically significantly among different BMI classes during follow up. Statistical analysis was performed using IBM® SPSS® Statistics version 27. The confidence interval was set to 95%, and p was considered significant at less than 0.05.

Results

396 patients were included in the analysis. Donor's and recipient's demographic characteristics are reported in Table 1. At univariate analysis, only donor's age was related to graft survival (p=0.002).

Mean BMI was 26.2 ± 5.1. Mean follow up was 5.86 ± 2.29 years. Patient survival was not statistically significantly different according to the recipient's BMI class (p=0.476, HR 0.935, C.I. 0.774-1.129), Figure 1. Expanding this further, at 5 years of follow up, mean patient survival was 77.5%, with 78.6%, 75.0%, 76.0%, 77.8%, 87.1% and 77.5% for class I-V respectively.

However, graft survival was instead affected by BMI (p=0.031, HR 1.217, C.I. 1.024-1.448), Figure 2, with a mean survival of 80.3% and with 83.6%, 82.5%, 77.6%, 75.0%, 66.7% for class I-V respectively.

Graft function was also significantly affected by BMI class during follow up. Results are summarized in Table 2, with a mean eGFR of 47.6 ± 19.32, 46.37 ± 18.99, 45.66 ± 17.3, 44.94 ± 17.52, 44.69 ± 17.27, 45.32 ± 17.12, 44.09 ± 18.62, 44.13 ± 18.86 ml/min/1.73m^2 for all the classes at 3, 6, 12, 24, 36, 48, 60 and 72 months of follow up. Figure 3 compares kidney function between the five BMI classes.

In Figure 4 mean eGFR up to 5 years follow up for the different BMI classes is represented. Logistic regression showed that the factors independently associated with graft loss were only donor's age (p=0.05) and BMI class of the recipient (p=0.002).

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TABLE 1 Donor's and recipient's demographic characteristics.

	Characteristic	Overall	Graft survival yes	Graft survival no	OR (CI)	p
Donor's	Age	51,8 ± 15,9	50.55 ± 15.9	56.64 ± 15.2	-	**0.002**
	Donation type (DBD/DCD)	195/81	156/61	39/20	1.43 (0.6- 3.2)	0.387
	CIT (hours)		13.69 ± 0.43	14.94 ± 0.75	–	0.151
	Type of donor •Cadaveric •live donor •simultaneous kidney-pancreas •pancreas after kidney	 258 119 2 17	 201 102 1 15	 57 17 1 2	–	0.148
Recipient's	Age	52,2 ± 12,8	51.77 ± 12.5	53.57 ± 13.8	–	0.268
	Sex (M)	260	215	45	1.46 (0.75-2.83)	0.137
	Ethnicity •Asian •Black •Caucasian •Mixed	 134 39 157 66	 110 28 132 52	 24 11 25 14	 0.73 (0.33-1.62) 0.45 (0.1-2.05) * 1.13 (0.46-2.76)	0.308
	BMI classes: •20 ≤ BMI ≤ 24.9 •25 ≤ BMI ≤ 29.9 •30 ≤ BMI ≤34.9 •BMI ≥ 35 •BMI ≤ 19.9	 133 155 53 21 34	 112 129 42 16 22	 21 26 11 5 12	 * 0.99 (0.54-1.82) 1.29 (0.58-2.86) 1.6 (0.53-4.81) 2.61 (1.14-5.99)	0.084

BMI, body mass index; CIT, cold ischemic time; DBD, donation after brainstem death; DCD, donation after circulatory death. For ethnicity, the comparisons are made between Caucasians and the other ethnicities. *For BMI, the comparisons are made between normal BMI (control group) and the other classes OR and CI are related to normal.

Discussion

The survival benefit for end stage kidney disease following kidney transplantation over long-term dialysis is known (18), and in the present study we demonstrated there is no significant difference in terms of overall survival for patients, when considering BMI as a possible determinant. This poses the important question to allow access to a limited and precious resource, i.e., the organ donor pool, for patients otherwise discriminated only on the basis of weight and height (19). It is very easy to dismiss access for transplantation based on BMI, as an easily measured metric. However, this study shows that with a steroid sparing protocol at five years, overall survival probability is the same, independent of BMI, and thus the expected life after transplantation (20) is not a reason to not list patients based on BMI alone.

On the other side, a high BMI is proved to represent a risk factor for graft failure, and this confirms the necessity of a weight loss strategy ahead of transplantation (21), particularly in view of the incoming lifelong side effects of steroid therapy and immunosuppression (22). This concept is common to a more generalized pre-habilitation strategy and could be potentially translated also to other organs (23), but for kidney in particular, cardiovascular disease prevention and thus obesity treatment appears fundamental, given this represents a major cause of death (24). Pre-transplant diet and exercise should be encouraged

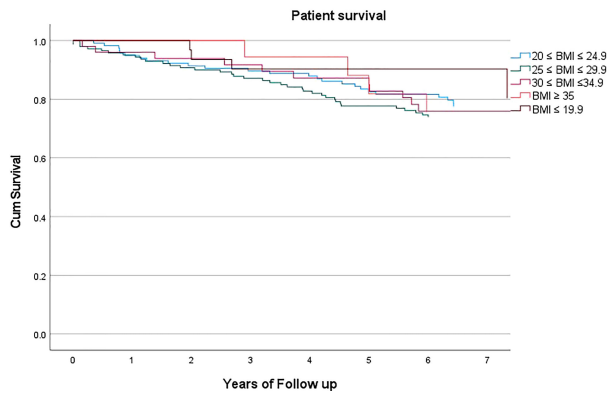

FIGURE 1
Patient survival according to BMI class.

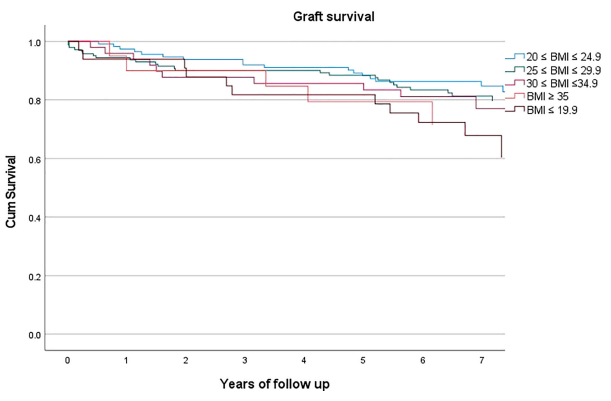

FIGURE 2
Graft survival according to BMI class.

TABLE 2 Mean and standard deviation for kidney function during follow up per BMI category.

BMI category	eGFR 3 months p <0.001	eGFR 6 months p =0.001	eGFR 1 year p =0.026	eGFR 2 years p =0.009	eGFR 3 years p =0.211	eGFR 4 years p =0.016	eGFR 5 years p=0.496	eGFR 6 years p=0.187
* 20 ≤ BMI ≤ 24.9	52.49 ± 17.98	49.72 ± 17.97	47.79 ± 17.03	47.35 ± 17.56	45.27 ± 15.93	45.28 ± 15.25	45.59 ± 17.11	44.62 ± 18.14
25 ≤ BMI ≤ 29.9	44.46 ±17.89	43.17 ± 17.56	44.33 ± 16.29	43.28 ± 16.12	44.93 ± 17.02	46.14 ± 17.13	44.20 ± 18.44	44.87 ± 17.82
30 ≤ BMI ≤34.9	46.35 ± 20.71	46.31 ± 20.1	44.67 ± 18.82	43.44 ± 19.12	42.05 ± 17.98	43.74 ± 18.53	41.54 ± 19.32	42.87 ± 19.55
BMI ≥ 35	33.55 ± 13.91	35.47 ± 14.61	37.11 ± 15.72	38.06 ± 13.65	37.59 ± 15.5	33.53 ± 14.53	37.42 ± 15.31	30.60 ± 14.28
BMI ≤ 19.9	53.21 ± 22.26	53.52 ± 21.82	51.22 ± 17.56	48.65 ± 19.64	48.93 ± 20.55	52.07 ± 19.03	46.56 ± 23.26	47.12 ± 23.79
Total	47.61 ± 19.13	46.34 ± 18.86	45.76 ± 17.15	44.91 ± 17.37	44.65 ± 17.1	45.44 ± 17.06	44.25 ± 18.50	44.18 ± 18.79

Kidney function is expressed as eGFR ml/min/1.73 m^2.

before being actively listed for surgery, in order to improve aerobic and functional capacity, thus especially for frail candidates, as malnourished obese patients are, in order to reach a significant weight loss through a healthier lifestyle, including also diet and physical exercise. The issue for ESKD transplant candidates is yet the necessity of dialysis treatment while waiting for surgery, therefore the above described interventions may require years before becoming effective or could not even become a real option at all, while for the patient every more year spent on dialysis reduces the overall survival in a significant manner (25). Of note, we did not find inferior patient survival when stratifying for BMI classes, therefore the survival benefit kidney transplantation offers could be interpreted as uniform (26).

Bridge interventions, such as bariatric surgery are increasingly being adopted to overcome wait listing barriers and we support their utilization, particularly with regards to sleeve gastrectomy (27), given its lower risk of adverse renal consequences such as hyperoxaluria and nephrolithiasis (28), although there is still controversy on timings and on patient's selection (3, 9). Additionally, potential post-surgical complications could translate into lower wait listing and transplant access for ESKD patients, therefore, there is still concern that bariatric surgery and the following weight loss could cause significant protein malnutrition and frailty, negatively impacting on dialysis patients, with worse waitlist and post-transplant outcomes (29).

From our analysis, there is evidence that graft function deteriorates over time and consequently leads to an increased risk of graft loss for obese individuals, as may other systemic diseases finally leading to end stage kidney function, particularly when recurring after transplantation (30). It is known in fact a direct causal connection between obesity and ESKD, because of an underpinning renal hyperfiltration driven by the excess weight, also known as obesity-related glomerulopathy (31). This syndrome can synergically act with other frequent comorbidities in obese patients, such as hypertension and diabetes, and in fact the latter was more common in higher BMI patients (p=0.0.22) (16). Furthermore, these conditions could worse in the post-transplant period, following immunosuppressive drugs administration, especially steroids (32). Yet, it appears not sustainable to condemn to lifelong dialysis someone only because of their BMI, but it is recommendable to rather intervening on modifiable risk factors for cardiovascular mortality, as for example to avoid steroids (32).

An interesting finding of our analysis is that donor age, and not donor type, affects the incidence of graft loss. Previous work reports

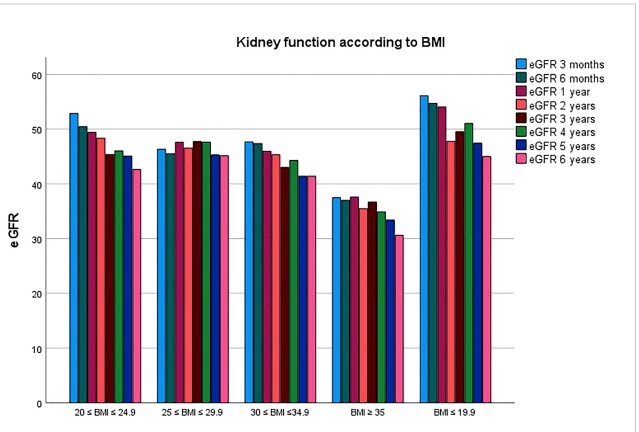

FIGURE 3
Comparison of kidney function between BMI classes.

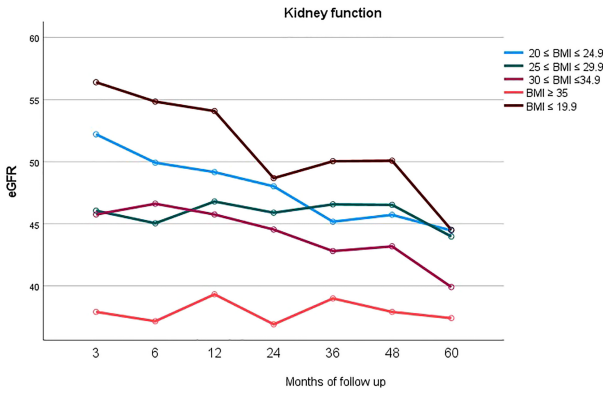

FIGURE 4
Longitudinal representation of mean eGFR up to 5 years follow up for the different BMI classes.

that recipients of grafts from live donors aged < 60 have a 38% lower risk of developing acute rejection compared to those aged > 60 years (33). Since recipients of older grafts are generally also older in age, this leaves to the open debate on immunosuppression in the elderly, in whom, although physiological immunosenescence linked to biological aging is known, other potential contributors, such as the engraftment of older organs, is associated with higher rejection rates, and thus the need for tailored, age-adopted immunosuppression. If we attribute this to the fact that obese patients do suffer of immunosuppression side-effects more because of their intrinsic metabolic condition, it then could be favored the use of younger donors for obese recipients, especially because these candidates appear younger and fitter in general, to be selected for transplantation in view of their important comorbidities.

Finally, another important finding of the present study, is that graft and patient survival for class V (BMI<19.9) parallels those of class IV (BMI > 35). Although caution is warranted, given the sample size, we assumed that the obesity paradox might be the underpinning mechanism, in fact as already mentioned above, obesity in ESKD patients, may play a protective role (12) and could be associated with decreased mortality, particularly when looking at infections. Conversely, the presence of signs of undernutrition, like BMI <19.9, that is often associated to frailty, could lead to a higher susceptibility to serious complications (34), such as sepsis, another major cause of mortality in transplanted patients.

Our study presents some limitations: patients fit for transplantation were selected and many have undergone intensive medical workup to optimize their cardiovascular risk factors. This could have biased in selection for transplantable patients, especially in the high BMI cohort. Also, the use of BMI as a measure for adiposity is imperfect because it does not differentiate between fat and lean body mass, as could girth, for example, although most population variance in obesity is explained by BMI.

In conclusion, the present study suggests that obesity is not an absolute criterion to exclude a patient from the kidney transplant waiting list. Further research is warranted to investigate whether another surrogate marker for obesity could be adopted, and which patients might benefit of an overall bariatric strategy. If the possibility of a living donor comes forward, the transplant should not be postponed, as a survival benefit over dialysis is to be preferred to the risks related to the decision to defer it.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The study was performed in accordance to the Declaration of Helsinki principles. The data used were anonymized and did not require patient or public involvement nor affected patient care. The study fell under the category of research through the use of anonymized data of existing databases which, based on the Health Research Authority criteria, does not require proportional or full ethics review and approval.

Author contributions

Conceptualization, MB and PH; methodology, MB, ED, FC, and PH; validation, MB, FC, and PH; formal analysis, MB and FC; data curation, MB, ED, and PH; writing—original draft preparation, MB; writing—review and editing, MB, FC, and PH. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Epicardial adipocytes in the pathogenesis of atrial fibrillation: An update on basic and translational studies

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Epicardial adipose tissue (EAT) is an endocrine organ containing a host of cell types and undoubtedly serving a multitude of important physiologic functions. Aging and obesity cause hypertrophy of EAT. There is great interest in the possible connection between EAT and cardiovascular disease, in particular, atrial fibrillation (AF). Increased EAT is independently associated with AF and adverse events after AF ablation (e.g., recurrence of AF, and stroke). In general, the amount of EAT correlates with BMI or visceral adiposity. Yet on a molecular level, there are similarities and differences between epicardial and abdominal visceral adipocytes. In comparison to subcutaneous adipose tissue, both depots are enriched in inflammatory cells and chemokines, even in normal conditions. On the other hand, in comparison to visceral fat, epicardial adipocytes have an increased rate of fatty acid release, decreased size, and increased vascularity. Several studies have described an association between fibrosis of EAT and fibrosis of the underlying atrial myocardium. Others have discovered paracrine factors released from EAT that could possibly mediate this association. In addition to the adjacent atrial cardiomyocytes, EAT contains a robust stromal-vascular fraction and surrounds the ganglionic plexi of the cardiac autonomic nervous system (cANS). The importance of the cANS in the pathogenesis of atrial fibrillation is well known, and it is quite likely that there is feedback between EAT and the cANS. This complex interplay may be crucial to the maintenance of normal sinus rhythm or the development of atrial fibrillation. The extent the adipocyte is a microcosm of metabolic health in the individual patient may determine which is the predominant rhythm.

KEYWORDS

epicardial adipose tissue, obesity, atrial fibrillation, inflammation, left atrium, remodeling, adipocyte

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Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia in adults, affecting 37% of patients over the age of 55, hence the importance of risk factor identification and modification (1). Environmental factors including chronic disease, age, and acute triggers have been implicated as risk factors for developing AF. Coronary artery disease (CAD), hypertension, obesity, diabetes, obstructive sleep apnea (2), chronic kidney disease (CKD), and inflammatory diseases increase the risk for AF as do acute triggers such as binge drinking and physical stressors (e.g., infection, surgery, and metabolic derangements). AF has been associated with an increased incidence of stroke, heart failure, dementia, as well as death and globally, AF is associated with an increased risk for mortality and morbidity and accounted for 6 million disability adjusted life years in 2017 (1, 3). Given this impact upon human health there is a great need for interventions to prevent the development of AF and treat prevalent cases. Epicardial adipose tissue (EAT) has garnered significant interest as a direct connection between obesity and cardiovascular disease, and AF in particular. To the extent that EAT may be modified by behavioral, pharmacologic, or even surgical interventions it is an attractive therapeutic target to combat AF. A PUBMED search for "epicardial adipose tissue" returns 1,509 results, with 228 in 2022 alone (Figure 1). Since 2003, commensurate with the publication of landmark studies by Mazurek and Iacobellis, there has been a burgeoning interest in the effects of EAT on cardiovascular pathophysiology (4, 5). There have been numerous recent comprehensive reviews on this topic to which the reader is referred (6–9). Herein we will aim to focus more on the biology of epicardial adipocytes and how this might affect neighboring cardiomyocytes and contribute to the pathogenesis of AF. Our goal is to stimulate the readers interest and perhaps spark new lines of inquiry in this fascinating and important field.

Pathophysiology of atrial fibrillation

The mechanism for developing AF is not clearly understood but likely changes as a patient progresses from paroxysmal AF to long-standing persistent AF. With this progression the impetus transitions from an arrhythmogenic trigger to an electro pathology mediated trigger (3). Paroxysmal AF, an arrhythmia that self-terminates within 7 days, is typically driven by the cardiac muscle sleeve around the pulmonary veins (PVs) in 90% of cases and is associated with rapid focal activity and local reentry. As a patient transitions to persistent AF, requiring pharmacological or electrical cardioversion, the driver evolves into electrical remodeling of ion channels and irreversible structural changes to the atria (10). We hypothesize that any primary effect of epicardial adipocytes would be in the paroxysmal stage. However, to the extent that cross talk between atrial myocytes, epicardial adipocytes, and the cardiac autonomic nervous system (cANS) can occur, adipocytes may play other roles in the persistent/permanent stage of AF, by modulating the cANs afferent signals to the brain and promoting efferent cANs discharge (11).

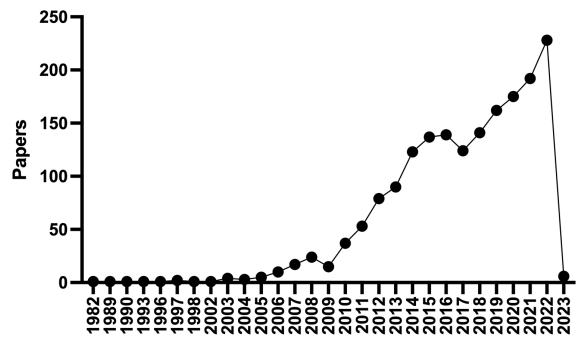

FIGURE 1
PubMed Timeline Results for "Epicardial adipose tissue". There has been a dramatic increase in the number of publications regarding epicardial adipose tissue since 2003.

Molecular changes in atrial myocytes

In terms of the primary changes in the atrial myocardium, electrical remodeling from altered expression and functioning of cardiac ion channels favors the development of functional reentry substrates (10). The molecular mechanism leading to repolarization changes are not clearly understood, but are thought to involve sodium, potassium, and calcium channels. Atrial myocytes in patients with AF show unchanged or slightly reduced sodium current amplitude. Reduced calcium channel density is consistent in patients with AF and may be a determinant of shortening refractoriness and arrhythmogenesis. In addition, the sarcoplasmic reticulum's handling of calcium, which is affected by alterations in ryanodine receptor channels (RyR2), can lead to calcium leak and thus modulation of RyR2, which is a prevalent finding in this patient population. Patients with AF have a more negative resting membrane potential indicating that potassium channels may also play a role in the development of AF. Remodeling of gap junction subunits such as connexin may also be involved in a genetic predisposition to AF (12).

Fibrosis, fibrofatty infiltration, and ganglionic plexi

Structural changes, such as fibrosis, neural/autonomic remodeling, and anatomic features also contribute to the pathogenesis of AF. Fibrosis can separate muscle bundles, replace dead myocytes, and can couple electrically to cardiomyocytes, leading to reentry and ectopic activity. Fibrosis leads to progression from paroxysmal to permanent forms of AF, which in turn creates a positive feedback loop of increased fibrosis. In addition to fibrosis, infiltration of the atrial myocardium by epicardial fat can contribute to atrial conduction abnormalities (13). Autonomic/neural remodeling through vagal discharge, beta-adrenoceptor activation, and atrial sympathetic hyper-

innervation can also contribute to positive feedback loops that promote AF persistence and recurrence. The left and right atria including PVs, LA posterior wall/roof, the ligament of Marshall, as well as the vena cava have features that promote both focal and reentrant triggers (10). The junction of the pulmonary veins and left atrium has a constellation of ganglionic plexi that receive input from both the sympathetic and parasympathetic autonomic nervous system (Figure 2) (11). It is estimated that each plexus contains 200-1000 neurons. AF can be induced by activation of these GPs. On the other hand, ablation of these plexi and the ostia of the pulmonary veins can cure or reduce arrythmia burden in patients with AF (11). The interaction of epicardial adipocytes with the GPs of the cANS has yet to be investigated. However, it is well established that other adipose depots receive important input from the autonomic nervous system, and feed back to the ANS to provide metabolic signals to the CNS (14, 15). Recent studies have shown that macrophages associated with sympathetic neurons (SAMs) help to regulate this interaction. For example, in obesity, SAMs in brown adipose tissue induce expression of solute carrier family 6 member 2 (*SLC6A2*) and monoamine oxidase A(*MAOA*) (2). SLC6A2 is a membrane transporter for norepinephrine (NE) and MAOA is an enzyme responsible for enzymatic degradation of NE. With obesity, the induction of these proteins in SAMS causes a reduction in the sympathetic activation of brown adipose tissue, thus reducing thermogenesis and energy expenditure (2). We hypothesize that macrophages in EAT may mediate interactions with neurons in cardiac GPs in a similar fashion, allowing for changes in metabolism to regulate afferent feedback from the cANs to the brain.

Left atrial size and obesity

On a macroscopic scale, left atrial size, inflammation, and obesity all seem to play important roles in the development of AF and the progression from paroxysmal to permanent subtypes. Patients with paroxysmal AF have smaller LA diameter (4.3 vs 4.8cm) and fewer incidences of LA >5cm as compared to patients with permanent AF (16). This is particularly important as LA enlargement is a significant predictor of stroke in men and death in both sexes (17). Regarding obesity, there is a strong association between obesity (BMI >30) and AF. The Framingham heart study showed that for every 1 unit of increased BMI above 25, there was a 4% increase in AF risk. This conclusion was supported by a large meta-analysis showing that for every 5 unit increase in BMI, AF incidence increased by 29% (18). The mechanism of obesity increasing the incidence of AF may be related to epicardial and abdominal adiposity. A large meta-analysis by Wong showed that epicardial fat, waist circumference, and waist to hip ratio were all associated with a higher incidence of AF. However, the strength of

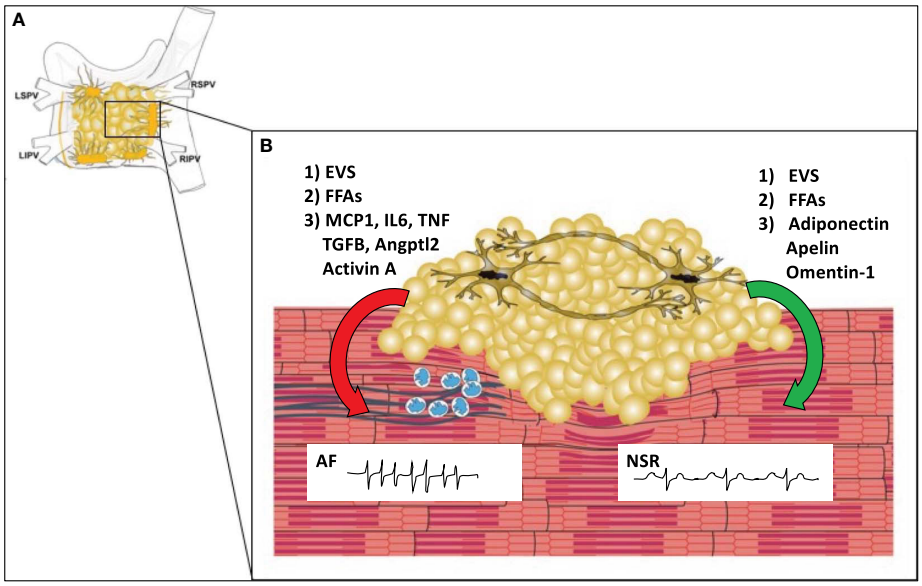

FIGURE 2
The Microenvironment of the Posterior Left Atrial Wall. **(A)** The posterior left atrial wall has variable amounts of EAT, which includes the four major ganglionic plexi and confluence of the pulmonary veins. **(B)** EAT covers portions of the left atrial wall and includes the ganglionic plexi. In obesity (left), EAT may promote infiltration of macrophages (blue) *via* secretion of MCP1 and other pro-inflammatory cytokines. Macrophages and or myofibroblasts then promote atrial fibrosis (blue lines) with stimulation of pro-fibrotic factors such as TGFB, Angptl2, and Activin A. This causes fibrosis of the atrial myocardium which creates a substrate for reentry leading to the development of atrial fibrillation. In normal conditions (right), EAT secretes anti-inflammatory and anti-fibrotic paracrine factors (adiponectin, apelin, omentin-1) that help maintain normal electrophysiologic properties of the atrial myocardium leading to normal sinus rhythm (NSR). In both obese and normal conditions epicardial adipocytes may secrete other factors such as extracellular vesicles (EVS) or free fatty acids (FFAs) which may have protective or adverse effects depending upon the EV contents and particular fatty acid species. MCP1 (macrophage chemoattractant protein-1), IL6 (Interleukin 6), TGFB (transforming growth factor beta), TNF (tumor necrosis factor), Angptl2 (Angiopoietin Like 2), LSPV (left superior pulmonary vein), LIPV (left inferior pulmonary vein), RSPV (right superior pulmonary vein), RIPV (right inferior pulmonary vein).

association was highest for epicardial fat (19). Epicardial fat, given its proximity to the myocardial cells may confer structural remodeling like the substrates described above.

Epicardial adipocytes; the same, but different

It is now well understood that adipose tissue is an active organ vital to human health (20). Any given adipose depot is comprised of a multitude of cell types, and it is easiest to consider it in terms of the adipocyte and stromal vascular fraction (SVF). The SVF is comprised of all the non-adipocyte cells within a certain fat pad. These may include, but are not limited to, fibroblasts, stem cells, neurons, vascular cells, and a broad array of immune cells. Broadly speaking, there are three different types of adipocytes; white, beige and brown (20). Brown adipocytes are adipocytes that generate heat by uncoupling the respiratory chain *via* the protein uncoupling protein 1 (UCP1). Brown adipose is present in babies and in smaller quantities in metabolically healthy adults. It is activated by the sympathetic nervous system in response to cold exposure, by NE binding to the β3 adrenergic receptor. Brown adipocytes express UCP1 constitutively and expression is reduced in chronic thermoneutral conditions (20). In contrast, beige adipocytes are adipocytes within subcutaneous fat that induce UCP1 expression rapidly upon cold exposure. Beige and brown adipocytes generate heat and contribute to metabolic health by increasing energy expenditure and releasing "BATokines" which have salutary effects of peripheral tissues. The amount of brown and beige adipocytes decreases with aging and obesity (20).

In contrast to brown adipocytes, the principal role of white adipocytes is to sequester lipid by lipogenesis or regulating lipolysis. They also secrete bioactive molecules (e.g., extracellular vesicles, lipokines, and adipokines) that target systemic organs. The mean volume of a white adipocyte is proportional to the rate of lipogenesis, the rate of lipolysis, and the nutrient supply/blood flow (21). EAT and visceral adipose are both predominantly white adipose depots and similar in many respects. Generally, EAT volume correlates with visceral adiposity, as measured by echocardiogram and CT. Epicardial adipocytes and adipocytes from visceral fat depots are smaller than subcutaneous adipocytes (22). EAT has a greater concentration of capillaries and increased expression of a broad array of inflammatory markers (21, 22). On the other hand, there are subtle differences between epicardial and adipocytes from fat within the abdominal cavity (21). Ovine epicardial adipocytes have a greater ratio of oleic acid (18:1) to stearic acid (18:0) than peri-renal or omental adipocytes. Although stearoyl-CoA desaturase (SCD) expression was lowest in epicardial fat compared to all other depots, there was a high correlation between SCD expression and oleic acid content in epicardial adipocytes, whereas there was no correlation in visceral adipocytes (21). Like visceral adipose tissue, EAT has a greater expression of inflammatory genes than subcutaneous adipose tissue (22, 23). This is true even in the absence of cardiovascular disease or obesity. In humans with obesity and in animal models of high fat feeding visceral adipose becomes inflamed which leads to dysfunctional adipocyte biology, lipotoxicity, and insulin resistance in remote tissues. This vast field of research (immunometabolism) has ushered in a new era of discovery, investigation, and therapeutic opportunity that is beyond the scope of this review (20, 24). However, whether epicardial adipose tissue responds in the same way to high fat feeding/obesity has yet to be shown. White perivascular adipose tissue surrounding the abdominal aorta in mice becomes severely inflamed with HFD (25). Given the similarities between VAT and EAT, it is reasonable to consider the possibility that epicardial fat does too.

The predominant stimulus for adipose inflammation in response to obesity remains elusive. Adipocyte hypertrophy is likely a primary event, followed by immune cell infiltration, apoptosis, revascularization, and fibrosis (9, 26). In obese conditions macrophages account for 40-50% of cells in visceral adipose tissue (27). There are many different populations of macrophages in adipose tissue. Adipose tissue resident macrophages are present even in lean conditions to help maintain tissue homeostasis. With the onset of obesity and adipocyte hypertrophy, chemokines such as monocyte chemoattractant protein-1 (MCP-1) stimulate infiltration of monocyte derived macrophages (CD11b+, CD11c+, F4/80+). These macrophages are pro-inflammatory and express factors such as TNFα, IL-1β, IL6 and NO (26). Thus, a chronic inflammatory state is established in visceral adipose tissue, leading to failure to effective store TG, lipotoxicity, and subsequent insulin resistance in skeletal muscle and liver. Whether or not these processes occur in EAT has yet to be established.

Basic and translational studies of EAT

For purposes of this section, EAT will be considered to be adjacent to the atrial myocardium of interest unless noted otherwise. It should be mentioned that the amount of EAT adjacent to the right atrium, left atrium and pulmonary veins is highly variable. Studying EAT in small animal models is problematic, because rats and mice do not have epicardial adipose, except after prolonged high fat diet (28). Furthermore, AF is not common in mice except in only a few genetic strains. Therefore, atrial fibrillation and EAT are both usually studied in large animal models (i.e., sheep, dogs, or rabbits), often using the artificial rapid atrial pacing(RAP) (18) model to induce AF.

Li et al. studied the effect of RAP on EAT. 6 weeks of RAP induced AF and lowered the effective refractory periods in the left and right atria (29). This was associated with increased reactive oxygenated species (ROS) production and phosphorylation of NF-κB. Concentrations of inflammatory cytokines such as TNFα, IL6, and TGFβ were increased in the left atrial myocytes and EAT (29). Histology showed atrial and adipose tissue fibrosis, in addition to adipocyte infiltration into the atrial myocardium. PPARγ and Adiponectin expression was reduced in EAT. Metformin reversed these alterations. These findings are analogous to prior studies of perivascular fat in high fat diet fed mice; obese mice had reduced

Adiponectin expression in perivascular fat surrounding the femoral artery (30). Loss of adiponectin resulted in decreased nitric oxide synthase activation and vasoconstriction. With the onset of obesity expression of beneficial paracrine factors in perivascular adipocytes is lost; this may also be true of aging adipocytes. In addition to the loss of beneficial factors, expression of pathologic paracrine factors is increased, for example TNFα, which promotes neointimal hyperplasia (31). Therefore, it seems that like perivascular adipocytes, peri-atrial epicardial adipocytes may signal *via* paracrine mechanisms to the underlying atrial cardiomyocytes. In this case, loss of adiponectin may result in reduced SERCA2a expression and abnormal calcium handling. Many other studies have shown putative adverse effects of factors secreted by EAT (32–35).

Venteclef et al. showed that the EAT secretome stimulated fibrosis in rat atria *in vitro*. This fibrosis was associated with associated with high Activin A concentrations in EAT; in contrast SAT had low Activin A concentrations (32). The fibrotic effects of Activin A *in vitro* were blocked with an antibody targeting activin A.

Nalliah et al. conducted an elegant study of the right atrial appendage of humans without AF who were having heart surgery (13). Greater amounts of EAT around the right atrial appendage correlated with slow conduction, electrogram fractionation, and fibrosis of the underlying atrial myocardium. It was also noted that the gap junction protein connexin-40 migrated laterally in subjects with increased EAT, becoming dissociated from cadherin (13). Conditioned media from sheep EAT altered the electrophysiologic properties of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMS), resulting in a decreased spontaneous beating rate and prolonged field potential duration in comparison to non-conditioned media. Proteomic analysis of murine pericardial fat and inguinal fat was then performed. Pericardial fat was enriched in proteins that regulate cell metabolism (e.g., ATP-citate synthase, alcohol dehydrogenase class 3, Long-chain enoyl-COA hydratase). The top enriched cellular component pathway was "focal adhesion" (GO:0005925). Interestingly, the top two GO biological processes were "fatty acid beta oxidation" (GO:0006635) and "cellular response to interleukin12" (GO:0070671) (13). It should be noted that this proteomic analysis was done using secreted proteins from murine pericardial fat, which is likely quite different from EAT (36).

Abe et al. studied the resected left atrial appendages and associated EAT from 59 consecutive cardiac surgery patients with AF (35). Fibrosis of EAT was associated with left atrial fibrosis. The collagen content of left atrial myocardium correlated with inflammatory proteins in EAT (TNFα, MCP-1, IL6, VEGF, MMP2, and MMP9). Expression of HIF-1α and Angptl2 was associated with inflammation in EAT. In a second study, the same group showed that treatment of rat atria *in vitro* with Angptl2 caused fibrosis; this effect was reversed with an anti-Angptl2 antibody (33). Angptl2 caused an increase in expression of α-smooth muscle actin, TGFβ1, and stimulated phosphorylation of ERK, inhibitor of κBa, and p38 MAPK. The authors concluded that antagonism of Angptl2 in EAT may be a therapeutic option for the prevention of AF (33).

In addition to aging and obesity/insulin resistance, a third mechanism that may contribute to EAT inflammation is hypoxia. Obstructive sleep apnea (2) is a known risk factor for the development of AF and untreated OSA leads to recurrence after AF ablation or cardioversion (37–39). In canine models of OSA induced AF there is evidence of cANS hyperactivity in ganglionic plexi of the left atrium (40). The ganglionic plexi are surrounded by EAT and form a ring around the confluence of the pulmonary veins (Figure 2). Dai et al. studied the effects of chronic OSA on EAT in canines (41). Chronic OSA resulted in a dramatic fibrosis of EAT and the adjacent atrial myocardium. OSA resulted in increased expression of inflammatory markers in EAT, including Activin A, TGFβ1, MMP9, TNFα, and IL-6. Treatment with metoprolol reversed fibrosis and lowered inflammatory marker expression. The authors hypothesized that hypoxia may trigger activation of beta-adrenergic receptors on epicardial adipocytes, and this stimulation was prevented by treatment with the non-selective beta blocker metoprolol. This hypothesis is supported by experiments showing that isoproterenol, a non-selective beta-adrenergic agonist, was previously noted to stimulate IL6 and TNFα production in 3T3L1 adipocytes (42). Furthermore, exposing adipocytes to hypoxia *in vitro* results in decreased adiponectin secretion and increased β1 and β2 adrenergic receptor expression (43).

Wang et al. studied the atria and EAT of subjects with (n=28) and without AF (n=36) having coronary artery bypass surgery (34). They found that YKL-40(CHI3L1) mRNA and protein was significantly higher in the EAT of subjects with AF than of those without. There was no difference in the serum levels of YKL-40. There was a positive association between YKL-40 expression in EAT and the collagen fraction of the atrial myocardium. Obesity was an independent risk factor for YKL-40 expression in EAT (34). YKL-40 is a secreted glycoprotein highly expressed in neutrophils, activated macrophages, and other cell types. YKL-40 may act in fibroblast proliferation and matrix deposition, and it's expression by macrophages in adipose tissue inhibits type I collagen breakdown (44). Interestingly, the expression of YKL-40 is increased in the visceral fat of obese patients and decreases with weight loss (45).

Recently, a provocative study by Shaihov-Taper et al. showed that EAT also releases extracellular vesicles (EVs) (46). EVs are membrane bound vesicles released from all cell types, containing a variety of molecules (e.g. proteins, nucleic acids, and lipids), that can transmit a molecular signal from the releasing cell to a recipient cell (47). EAT from subjects with AF secreted a greater number of EVs than EAT from subjects without AF. The EVs from subjects with AF had higher concentrations of inflammatory and pro-fibrotic cytokines, and lower concentrations of IL-10, VEGF, and sFLT-1 (46). Subsequent proteomic analysis revealed that the EVs from those with AF were related to distinct molecular pathways, including cardiomyopathy, apoptosis, angiogenesis, and fibrosis. Furthermore, these enriched EVs triggered fibrosis, angiogenesis, and facilitated re-entry when co-incubated with mesenchymal, endothelial, and pluripotent stem cells *in vitro* (46).

In summary, fibrosis, and inflammation of EAT is associated with fibrosis of the underlying atrial myocardium. There are many

bioactive factors released from EAT that could potentially cause fibrosis of the atria; this causative effect has yet to be clearly demonstrated (Table 1). Nonetheless, it is clear that EAT is responsive to changes in metabolic health and could signal this change to neighboring and possibly even remote systems.

Implications for treatment and future directions

Weight loss

With the recognition that EAT is associated with increased cardiovascular risk there have been several studies examining non-pharmacologic interventions that can reduce the amount of epicardial fat. In general, epicardial fat is a marker of visceral fat, and weight loss strategies tend to impact both depots. Diet and bariatric surgery both result in a significant reduction in epicardial fat (48). A meta-analysis by Saco-Ledo et al. included 10 studies and 521 subjects (49). They found that endurance training also resulted in a significant reduction in EAT. It seems that any method of weight loss can result in a decrease in epicardial fat. Moderate exercise and weight loss have known effects on reducing AF burden (18). Whether or not this is effect is dependent on a reduction in EAT, or simply a reduction in weight, is unknown. For example, in an echocardiographic study of subjects before and after bariatric surgery, weight loss was associated with a 30% reduction in visceral fat area and a 14% reduction in EAT thickness (50). However, despite these reductions, left atrial function remained impaired and left atrial volume and pressure increased (50). Therefore there may be a threshold of obesity or exposure to EAT beyond which remodeling of the left atrium is irreversible. More studies are needed to determine if weight loss results in a reduction in EAT thickness and whether this translates into clinically meaningful results.

GLP-1 agonists/SGLT2 inhibitors

Liraglutide and other glucagon-like peptide-1 (GLP-1) receptor agonists are indicated for the treatment of diabetes mellitus. GLP-1 receptor agonists also have weight loss effects. Treatment of diabetic patients with GLP-1 receptor agonists lowers cardiovascular events (51). The mechanism by which these drugs exert their beneficial effects is not clear. Iacobellis et al. found that treatment with liraglutide for 6 months causes a rapid and significant decrease in the thickness of EAT as measured by echocardiogram (52). 95 subjects with DM2 were randomized to metformin or metformin plus liraglutide 1.8 mg SC daily. Subjects in the liraglutide and metformin group had a 36% reduction in their EAT thickness at 6 months. Interestingly, the GLP-1 receptor is expressed in EAT (52). Others have shown similar albeit less dramatic results on EAT thickness with GLP-1 receptor agonists exenatide and dulaglutide (53, 54). Potential mechanisms by which GLP1 receptor agonists work in EAT include but are not limited to, reduction in fat mass, improved differentiation of pre-adipocytes, reduced lipogenesis, or browning of EAT (55).

Sodium-glucose co-transporter 2 inhibitors (SGLT2i) reduce blood glucose and cause weight loss in diabetic patients. This class of drugs has revolutionized the treatment of heart failure and are now recommended as one of the four pillars of goal directed medical therapy (56). Apart from weight loss and the natriuretic effects of SGLT2 inhibitors, they are thought to have salutary effects on myocardial metabolism, including a shift in fuel utilization from free fatty acids to b-hydroxybutyrate (55). However, SGLT2 inhibitors have

TABLE 1 Factors Released from EAT with Possible Paracrine Effects.

Protective Factors	Mechanism	Pathologic Factors	Mechanism
Adiponectin (29)	Downregulated in EAT of subjects with AF	MCP-1, IL6, TNF-α (41, 46)	Profibrotic and proinflammatory
Apelin	May reduce fibrosis by inhibiting TGFβ1 signaling in atrial myofibroblasts (75)	TGF-β1 (76)	Upregulated in the EAT of subjects with AF. Promotes fibrosis and ENdMT.
Omentin (76)	Downregulated in EAT of subjects with AF. May inhibit TGF signaling.	HIF-1α, Angptl2 (35)	Proinflammatory and profibrotic
		MMP2, MMP9 (41)	Remodeling of adipose tissue stroma
		Activin A (32)	Profibrotic
		Resistin (77)	Proinflammatory
		Visfatin	Proinflammatory
		cTGF (78)	Increased in EAT from subjects with AF. Correlates with atrial fibrosis.
		Leptin	
		YKL-40/ CHI3LI (34)	Secreted glycoprotein expressed by activated macrophages, neutrophils, and other cells. Correlates with atrial collagen fraction and increased in EAT of AF subjects.

also been shown to reduce EAT thickness by up 20% (55). It is thought that these compounds may reduce foster adipocyte differentiation and reduce the secretion of pro-inflammatory cytokines in EAT (57). Whether or not treatment with SLGT2 inhibitors or GLP-1 receptor agonists reduce incident AF is unknown.

Statins

Systemic inflammation is a characteristic of chronic obesity. It is known that obese patients are at increased risk of AF and heart failure with preserved ejection fraction. Increased epicardial fat thickness and EAT inflammation may contribute to the development of both AF and HFpEF in obese patients (58). HMG-coa reductase inhibitors or "statins" are known to have anti-inflammatory effects separate from their lipid lowering effects. EAT is known to a greater degree of inflammation on than subcutaneous adipose tissue (SAT). This correlates with a higher average contrast attenuation on CT scan in EAT (-89 HU) than SAT (-129 HU) (59). In a study of 420 women who had serial CT scans to measure coronary artery calcification, the use of statins for one year was found to reduce the attenuation of EAT (-89 HU at baseline vs. -94 HU at follow up, $p<0.001$). There was no change in the attenuation of SAT of the same subjects suggesting that the anti-inflammatory effect was specific to EAT. Furthermore, this effect was independent of changes in EAT volume, total cholesterol, or coronary calcium (59). The same group had previously shown that statin use was also associated with a reduction in EAT volume over time (60). In 145 patients who had serial coronary angiography, atorvastatin showed a greater effect of reducing EAT thickness than simvastatin/ezetimibe(0.47 mm vs. 0.12 mm, $p<0.001$) (61). Others have shown that statin use is associated with lower EAT thickness and decreased inflammation in patient having cardiac surgery (62). Statins were also shown to have an inhibitory effect on a broad array of inflammatory cytokines released from EAT *in vitro* (62). It appears clear that statins demonstrate salutary anti-inflammatory properties that are beneficial in metabolic disorders such as obesity, and these effects may be mediated by a reduction in EAT thickness or inflammation (58).

Ablation

Increased EAT thickness has been associated with recurrence of AF after ablation (63). Larger peri atrial EAT volume is also related to the occurrence of embolic stroke after catheter ablation of AF (64). Some studies have failed to find an association between EAT and AF recurrence after catheter ablation, and it may depend on the stage of AF (paroxysmal vs. persistent) (65). Traditionally AF ablation has used an endocardial approach to isolate the pulmonary veins. Recently, a clinical trial evaluated the efficacy of a hybrid procedure utilizing endocardial and epicardial ablation of the posterior left atrial wall (66). Compared to endocardial ablation alone, those who underwent the hybrid procedure had increased primary effectiveness at 12 months. It is not known whether incidental modification of EAT at the time of this procedure plays is instrumental to its efficacy.

Modulation of ganglionic plexi within EAT

Activation of both the sympathetic and parasympathetic nervous system is thought to play a role in the initiation of atrial fibrillation (67, 68). Stimulation of the pulmonary veins in dogs does not induce AF unless the adjacent ganglionic plexi are also stimulated (69). A clinical trial of botulinum toxin injection into the epicardial fat pad at the time of cardiac surgery showed a reduction in the incidence of both early and late post-operative AF (70). Interestingly, acetylcholine (Ach) has been found to have acute and chronic effects on epicardial adipocytes *in vitro* (71). In comparison to subcutaneous adipocytes, epicardial adipocytes have greater increases in calcium flux in response to Ach. Ach also induces MCP-1 expression in epicardial, but not subcutaneous adipocytes, and Epicardial adipocytes have increased expression of the g protein linked muscarinic receptors (mAChR2, mAchR3) (71). Finally, chronic treatment of cells with Ach caused increased lipid accumulation in both subcutaneous and epicardial adipocytes (71). Therefore it seems that Ach may stimulate inflammation and lipid accumulation in epicardial adipocytes, an effect that cut putatively be inhibited by botulinum toxin or other methods. In dogs, ablation of ganglionic plexi with a neurotoxin (resiniferatoxin) decreased sympathetic and GP activity and reduced AF inducibility. Resiniferatoxin is a transient receptor potential vanilloid 1 (TRPV1) agonist (72). These studies highlight the potential utility of chemical modification of ganglionic plexi as an adjunct to hybrid or surgical procedures.

Browning

"Browning" refers to the possibility of inducing UCP-1 expression in white adipose *via* a pharmacologic or alternative intervention. This would cause increased energy expenditure and insulin sensitivity; a concept with great promise for treatment of metabolic disorders and the induction of weight loss. An additional beneficial aspect of brown adipose tissue is that it is relatively resistant to inflammation induced by high fat diet (25). Two early studies in the field detected increased expression of brown adipose tissue associated genes in EAT (23, 73). However, on a histological basis, EAT appears more like white adipose tissue. Nonetheless, the induction of UCP1 in EAT is an intriguing concept to combat the development of cardiovascular disease associated with EAT (74).

Conclusion

EAT is a unique fat depot with distinct biochemical and metabolic properties. The exact function of EAT in cardiovascular physiology is unknown. The amount of EAT has been shown to correlate with the development of atrial fibrillation and adverse outcomes after atrial fibrillation ablation. As in other fat depots, it is highly likely that EAT interacts with the autonomic nervous system, and this is a topic that merits

further investigation. There are many questions that remain to be answered about this fat depot, but the potential for therapeutic opportunities is intriguing.

Author contributions

BW contributed to writing the manuscript, editing, and approving the final draft. K-VT created the figure, contributed to writing the manuscript, editing, and approving the final draft. TF contributed to writing the manuscript, editing, and approving the final draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Association of obesity with the development of end stage renal disease in IgA nephropathy patients

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Background and aim: Immunoglobulin A nephropathy (IgAN) is the most common primary glomerulonephritis worldwide. We aimed to evaluate whether obesity is a risk factor for IgAN patients.

Methods: A total of 1054 biopsy-proven IgAN patients were analyzed in this retrospective study. Patients were divided into four groups according to their body weight index (BMI) at the period of renal biopsy: underweight group (BMI< 18.5, N=75), normal weight group (18.5≤BMI<24, N=587), overweight group (24≤BMI<28, N=291) and obesity group (28≤BMI, N=101). The endpoint of our study was end stage renal disease (ESRD: eGFR <15 mL/min/1.73 m^2 or having renal replacement treatment). Kaplan-Meier analyses and Cox proportional hazard models were performed to evaluate renal survival. Propensity-score matching (PSM) was performed to get the matched cohort to evaluate the role of obesity in IgAN patients. Besides, the effect modification of obesity and hypertension in IgAN patients was clarified by the synergy index.

Results: IgAN patients complicated with obesity had more severe renal dysfunction at the time of renal biopsy than those with optimal body weight. In addition, patients with obesity tended to have higher risk of metabolic disorders, such as hyperuricemia (64.4% vs 37%, p<0.001), hypertriglyceridemia (71.3% vs 32.5%, p<0.001) and hypercholesterolemia (46.5% vs 35.6%, p=0.036). It was observed that obesity patients had higher rate of unhealthy behaviors, such as smoking (27.7% vs 16.4%, p=0.006) and alcohol drinking (29.7% vs 19.9%, p=0.027). Although obesity was not confirmed as an independent risk factor for IgAN patients, we found that IgAN patients with obesity presented with higher incidence of hypertension, as well as lower event-free renal survival rate (log-rank p < 0.001), especially in patients with 24-h urine protein ≥ 1g (log-rank p =0.002). In addition, the synergy index showed that there was positive interaction between obesity and hypertension in IgAN.

Conclusion: Obesity is an important risk factor for IgAN patients when combined with hypertension. Hypertension appears to be common in obese IgAN patients.

KEYWORDS

IgA nephropathy, obesity, end stage renal disease, renal outcome, renal prognosis

Introduction

The prevalence of overweight and obesity have increasing impact on the quality of life of patients, on health services, and on society (1). Recent studies have suggested that elevated body mass index (BMI) was a significant risk factor for coronary heart disease, stroke (2) and chronic kidney disease (CKD) (3). Immunoglobulin A nephropathy (IgAN) is the most common primary glomerulonephritis globally and represents the leading cause of CKD and renal failure (4). The relationship between obesity and IgAN has not yet been clarified clearly because controversial results have been obtained in previous studies. Several studies have showed that overweight and obesity might be related to severe renal dysfunction and poor prognosis (5–7). However, other studies suggested that high BMI was not a direct risk factor for IgAN (8, 9). Therefore, we performed this study to determine whether obesity plays a role in the progression and prognosis of IgAN in Chinese patients.

Materials and methods

Study design

A total of 1588 renal biopsy proven adult IgAN patients from West China Hospital of Sichuan University between January 2009 and December 2018 were recruited for this retrospective study. Patients with systemic lupus erythematosus, Henoch–Schonlein purpura, chronic liver disease and systemic diseases (including diabetes mellitus) were excluded (31 patients). The other exclusion criteria were as follows (1): insufficient clinical data and missing data during follow up (192 patients) (2), renal biopsy specimens containing <8 glomeruli (284 patients) (3), duration of follow-up <1 year before reaching end stage renal disease (ESRD: eGFR <15 mL/min/1.73 m^2 or having renal replacement treatment) (25 patients), and (4) estimated glomerular filtration rate (eGFR) <15 ml/min/1.73 m^2 at the time of renal biopsy (2 patients). Ultimately, 1054 patients were included (Figure 1). Written informed consent was obtained from each patient involved. This study was approved by the Ethical Committee of West China Hospital of Sichuan University (2019–33).

Clinical data

Patients enrolled in this study were divided into four groups according to their BMI at the period of renal biopsy. BMI is calculated as weight in kilograms divided by the measured height in square meters and expressed in kg/m^2. It remains a good parameter to appreciate excessive weight and the classification is as follows based on WHO (World Health Organization) classification: underweight group (BMI < 18.5), normal weight group (18.5 ≤ BMI < 24), overweight group (24 ≤ BMI < 28), and obesity group (BMI ≥ 28) (10). Demographics (gender, age), clinical data [systolic blood pressure (SBP), diastolic blood pressure (DBP), serum creatinine (Scr), hemoglobin (Hb), serum albumin (Alb), smoking and drinking status, serum total cholesterol (TC), serum triglycerides (TG), uric acid (UA), 24-h urine protein and eGFR] were collected at the time of renal biopsy. Hypertension was defined as blood pressure > 140/90 mmHg or using antihypertensive agents. Mean arterial pressure (MAP) was calculated as DBP + 1/3(SBP-DBP) (11). eGFR was calculated using the CKD-EPI equation (12). Anemia was defined as Hb <120 g/L in males or Hb <110 g/L in females (13). Hyperuricemia was defined as UA >420 μmol/L or >360 μmol/L in males and females (14). Hypercholesterolemia was defined as serum TC ≥ 5.2 mmol/L. Hypertriglyceridemia was defined as serum TG ≥1.7 mmol/L (14). Stages of CKD were based on the Kidney Disease: Improving Global Outcomes (KDIGO) practice guidelines (15).

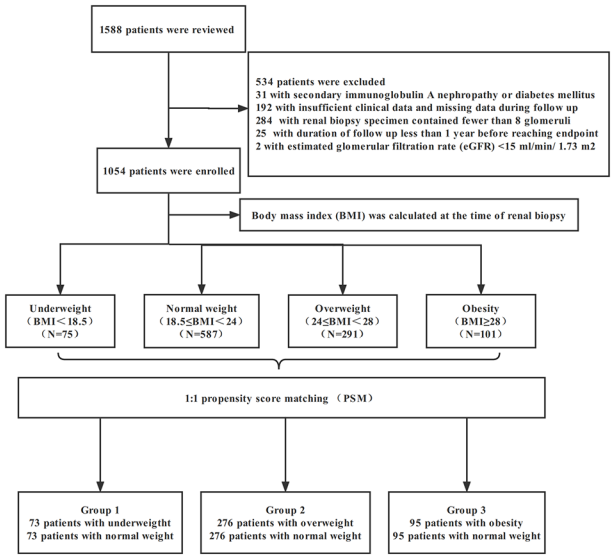

FIGURE 1
The flow chart of excluded patients.

Pathological data

Renal biopsy samples were evaluated by light (HE, PAS, Masson, PASM), immunofluorescence (IgA, IgG, IgM, C3, C4, C1q) and electron microscopy. All the renal pathology reports were evaluated by our hospital experienced pathologists and nephrologists according to the Oxford classification of IgAN (MEST-C): mesangial hypercellularity (M0/M1); endocapillary hypercellularity (E0/E1); segmental glomerulosclerosis (S0/S1); tubular atrophy/interstitial fibrosis (T0/T1/T2) and cellular or fibrocellular crescents (C0/C1/C2) (16). We also performed clinical-pathological discussion for all patients, especially for those with difficulty of diagnosis.

Treatment data

According to different treatment strategies, patients were divided into two groups: supportive treatment, corticosteroids

alone or/and plus immunosuppressant therapy. Patients in the supportive treatment group only received optimized supportive care with a full dose angiotensin-converting-enzyme inhibitor (ACEI) or angiotensin receptor blockers (ARB) to achieve target blood pressure. Patients in the corticosteroids alone or/and plus immunosuppressant therapy group received optimized supportive care along with corticosteroids (0.5 - 1 mg/kg prednisone daily, tapering down within 6 - 8 months) or/and immunosuppressant therapy (cyclophosphamide 2 mg/kg daily for 3 months, or mycophenolate mofetil 1-2 g daily for 6-8 months, or cyclosporine 3–5 mg/kg daily for 6–8 months, or tacrolimus 0.03–0.05 mg/kg daily for 6–8 months) (17).

Endpoint

The main predefined study outcome for the present analysis was the occurrence of end stage renal disease (ESRD), which was defined as eGFR <15 mL/min/1.73 m2 or accepting renal replacement treatment.

Statistical analysis

All the statistical analyses were carried out by using IBM SPSS Statistic software (version 26.0). Continuous variables were expressed as the means ± standard deviations (SD) or median with interquartile range and analyzed with independent samples t-test or nonparametric test for normally and nonnormally distributed variables. Categorical data were analyzed using Chi-square tests and presented as frequencies (percentages). Kaplan-Meier analyses and Cox proportional hazard models were performed to evaluate the risk factors for renal progression and prognosis, and survival curves were compared with the log-rank test. Combining the results of the univariate Cox proportional hazard model and clinical experience, we screened out confounding variables and added them to the different multivariate Cox proportional hazard models. In the adjusted Cox proportional hazards models, gender, age, pathologic lesions (MEST-C), treatment, eGFR and proteinuria were included. Results were expressed as hazard ratios (HR) and 95% confidence intervals (CI). To control the impact of confounding factors among the four groups, we performed propensity-score matching (PSM) according to important clinical and pathologic factors (gender, age, MAP, hypertension, 24-h urine protein, eGFR, treatment, and Oxford MEST-C score). Underweight, overweight and obesity groups were matched to normal weight group with 1:1 nearest neighbor matching without replacement (the caliper width was set at 0.02) to address the marked differences between the groups (9), and the matched cohort was divided into three groups: group1 (normal weight and underweight), group2 (normal weight and overweight) and group3 (normal weight and obesity). Then Kaplan-Meier analyses and Cox proportional hazard models were also conducted to evaluate the risk factors in matched cohort. Besides, the effect modification of obesity and hypertension in IgAN patients was clarified by the synergy index. Statistical significance was considered if p value < 0.05.

Results

Demographic and clinicopathological characteristics

Among 1054 patients, 587 patients (55.7%) were normal weight, 75 patients (7.1%) were underweight, 291 patients (27.6%) were overweight and 101 patients (9.6%) were obesity. The mean follow-up time was 61 ± 29 months, the mean age of all patients was 35 ± 11 years, and male patients accounted for 45.2% of the whole cohort. It was noticed that obesity patients had higher rate of unhealthy behaviors, such as smoking (27.7%, p=0.006) and drinking (29.7%, p=0.027). Regarding clinical features, hypertension was observed in 51.5% of patients in obesity group. Furthermore, increased serum creatinine and urine protein levels were associated with higher BMI, especially in obesity patients (p<0.05). In addition, IgAN patients with obesity tended to have higher rates of hypertriglyceridemia, hypercholesterolemia and hyperuricemia (p<0.05). However, no significant difference was found in pathologic lesions (MEST-C) or medical treatments among the four groups (Table 1).

TABLE 1 Demographic and clinicopathological characteristics of 1054 unmatched IgAN patients at the time of renal biopsy.

Parameters	Normal weight (N=587)	Underweight (N=75)	Overweight (N=291)	Obesity (N=101)
Male (%)	236 (40.2%)	24 (32%)	160 (55%) **	56 (55.4%) **
Age (years)	31 (26,40)	25 (21,29) **	38 (30,46) **	41 (33,47) **
MAP (mmHg)	95(88,104)	90 (84,99) **	99 (90,107) **	102(92,112) **
Hypertension (%)	121 (20.6%)	9 (12%)	107 (36.8%) **	52 (51.5%) **
BMI (kg/m^2)	21.2 (19.8,22.7)	17.6 (17,18.3) **	25.5 (24.8,26.6) **	30(28.5,31.7) **
Smoking (%)	96 (16.4%)	4 (5.3%) *	53 (18.2%)	28 (27.7%) **
Drinking (%)	117 (19.9%)	12 (16%)	69 (23.7%)	30 (29.7%) *

(Continued)

TABLE 1 Continued

Parameters	Normal weight (N=587)	Underweight (N=75)	Overweight (N=291)	Obesity (N=101)
Anemia (%)	81 (13.8%)	53 (29.3%) **	30 (10.3%)	7 (6.9%)
24h-proteinuria (g/d)	1.2 (0.7,2.38)	1.0 (0.5,2.16) *	1.47 (0.9,3) **	2.27 (1,3.74) **
Alb (g/L)	40 (36.2,43.1)	40.9 (37.1,44.5)	40.7 (37.1,43.7) *	40.7 (35.7,43.8)
Hypertriglyceridemia (%)	191 (32.5%)	13 (17.3%) **	171 (58.8%) **	72 (71.3%) **
Hypercholesterolemia (%)	209 (35.6%)	13 (17.3%) **	121 (41.6%)	47 (46.5%) *
Hyperuricemia (%)	217 (37%)	16 (21.3%) **	129 (44.3%) *	65 (64.4%) **
eGFR (mL/min/1.73 m^2)	96 (66,119)	113 (82,130) **	83(61,107) **	81(58,106) **
SCr (umol/L)	80 (64,109)	69(58,96) **	90 (71,114) **	93 (74,119) **
CKD stages				
stage1	327 (55.7%)	54 (72%) *	129 (44.3%) **	44 (43.6%)
stage2	135 (23%)	11 (14.7%)	9 (31.3%)	30 (29.7%)
stage3	110 (18.7%)	7 (9.3%)	65 (22.3%)	24 (23.8%)
stage4	15 (2.6%)	3 (4%)	6 (2.1%)	3 (3%)
Pathology (Oxford classification)				
M1	446 (76%)	53 (70.7%)	203 (69.8%)	75 (74.3%)
E1	21 (3.6%)	5 (6.7%)	12 (4.1%)	7 (6.9%)
S1	360 (61.3%)	39 (52%)	178 (61.2%)	56 (55.4%)
T1/T2	115 (19.6%)	13 (17.3%)	58 (19.9%)	18 (17.8%)
C1/C2	133 (22.7%)	17 (22.7%)	59 (20.3%)	17 (16.8%)
Treatment				
Corticosteroids alone or/and plus immunosuppressant (%)	338 (57.6%)	42 (56%)	175 (60.1%)	63 (62.4%)

MAP, mean arterial pressure; BMI, body mass index; ALB, albumin; eGFR, estimated glomerular filtration rate; SCr, serum creatinine; CKD, chronic kidney disease. M, mesangial proliferation; E, endocapillary proliferation; S, segmental sclerosis; T, tubular atrophy/interstitial fibrosis; C, crescents; IgAN, Immunoglobulin A nephropathy. Other three BMI groups compared with normal weight group, which was used as reference.
*, P value <0.05; **, P value <0.01.

The relationships between BMI and renal outcomes in IgAN patients

In the unmatched cohort, the percentage of patients progressing to ESKD was 11.30 per 1000 person-years in normal group and 17.68 per 1000 person-years in obesity group. But, there was no significant difference in the results of univariate Cox proportional hazard model and Kaplan-Meier survival analysis (Table 2). Then, to investigate the association of BMI with the progression of IgAN by excluding the impact of confounding indicators, we performed PSM to get the matched cohort (Table 3). After PSM significant relationships were determined between different groups, compared with normal weight group, patients with underweight presented with higher proportions of mesangial hypercellularity, overweight patients had higher rate of hypertriglyceridaemia. Notably, patients with obesity had worse clinical features, such as higher proteinuria, anaemia and hyperuricaemia. However, BMI did not have a significant influence on the renal outcome of IgAN patients based on the Kaplan-Meier survival and univariate Cox proportional hazard model (Table 4).

Obesity contributes to risk factors for renal progression and outcomes when combined with hypertension

In the unmatched cohort, compared with normal weight patients, obesity groups were associated with higher risk of hypertension. Thus, we conducted a series of analyses to assess whether hypertension and obesity could have an effect on the renal outcomes in IgAN patients. Correspondingly, the patients who presented with normal weight and non-hypertension were considered as the reference group. The Kaplan-

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TABLE 2 The relationships between BMI and renal outcomes in the unmatched cohort.

Subgroup	ESRD (%)	P value of Cox	Hazard Ratio (95%CI)	P value of KM
Group 1		0.819	1.116 (0.436-2.855)	0.819
Normal weight	35 (6%)			
Underweight	5 (6.7%)			
Group2		0.889	1.043 (0.577-1.886)	0.889
Normal weight	35 (6%)			
Overweight	16 (5.5%)			
Group3		0.173	1.714 (0.789-3.723)	0.168
Normal weight	35 (6%)			
obesity	8 (7.9%)			

P value of Cox and KM were analyzed by Univariate Cox proportional hazard model and Kaplan-Meier survival analysis. In each group, normal weight group was used as reference.

TABLE 3 Demographic and clinicopathological characteristics of matched groups.

Parameters	Group1		Group2		Group3	
	Normal weight (N=73)	Underweight (N=73)	Normal weight (N=276)	Overweight (N=276)	Normal weight (N=95)	Obesity (N=95)
Male (%)	18 (24.7%)	24 (32.9%)	149 (54%)	149 (54%)	55 (57.9%)	51 (53.7%)
Age (years)	26 (21,32)	25 (21,29)	37 (29,43)	38 (29,45.8)	38 (32,48)	40 (32,46)
MAP (mmHg)	91.7 (85.3,94.8)	90 (83.5,98.8)	96.7 (90,107.3)	98.7 (90,106.7)	99.7 (92.3,113)	101.7 (91.7,111.7)
Hypertension (%)	3 (4.1%)	9 (12.3%)	87 (31.5%)	93 (33.7%)	44 (46.3%)	46 (48.4%)
BMI (kg/m^2)	20.7 (19.8,21.8)	17.7 (17,18.3) **	21.6 (20.2,22.7)	25.5 (24.8,26.6) **	21.6 (20.2,23.1)	29.4 (28.5,31.7) **
Smoking (%)	6 (8.2%)	4 (5.5%)	67 (24.3%)	46 (16.7%) **	25 (26.3%)	24 (25.3%)
Drinking (%)	11 (15.1%)	12 (16.4%)	76 (27.5%)	61 (22.1%)	32 (33.7%)	27 (28.4%)
Anemia (%)	13 (17.8%)	21 (28.8%)	41 (14.9%)	29 (10.5%)	17 (17.9%)	7 (7.4%) **
24h-proteinuria (g/d)	0.83 (0.34,1.98)	1 (0.51,2.17)	1.4 (0.7,2.5)	1.5 (0.9,3)	1.7 (0.7,2.7)	2.2 (1,3.7) **
Alb (g/L)	39.8 (37.4,42.8)	40.9 (36.9,44.5)	39.8 (36.3,42.9)	40.7 (37.1,43.5)	39.9 (35,42.6)	40.8 (35.8,43.9)
Hypertriglyceridemia (%)	22 (30.1%)	13 (17.8%)	104 (37.7%)	159 (57.6%) **	40 (42.1%)	68 (71.6%)
Hypercholesterolemia (%)	19 (26%)	13 (17.8%)	104 (37.7%)	117 (42.4%)	41 (43.2%)	44 (46.3%)
Hyperuricemia (%)	19 (26%)	16 (21.9%)	112 (40.6%)	121 (43.8%)	37 (38.9%)	61 (64.2%) **
eGFR (mL/min/1.73 m^2)	114.5 (81.3,125.8)	112.8 (80.8,129.7)	80.1 (54.8, 109.3)	82.8 (60.7, 107.1)	78.2 (51.6,109.6)	84.9 (62.4,106.4)
SCr (umol/L)	67 (57.7,89.5)	69.1 (58,96.5)	91 (71.8,129.4)	90 (71,114)	94.6 (69.9,136.9)	93 (74,115.9)
CKD stages						
Stage 1	51 (69.9%)	53 (72.6%)	112 (40.6%)	124 (44.9%)	39 (41%)	43 (45.3%)
Stage 2	9 (12.3%)	10 (13.7%)	79 (28.6%)	85 (30.8%)	24 (25.3%)	29 (30.5%)
Stage 3	12 (16.4%)	7 (9.6%)	73 (26.5%)	61 (22.1%)	26 (27.4%)	20 (21.1%)
Stage 4	1 (1.4%)	3 (4.1%)	12 (4.3%)	6 (2.2%)	6 (6.3%)	3 (3.1%)

(Continued)

TABLE 3 Continued

Parameters	Group1		Group2		Group3	
	Normal weight (N=73)	Underweight (N=73)	Normal weight (N=276)	Overweight (N=276)	Normal weight (N=95)	Obesity (N=95)
Pathology (Oxford classification)						
M1	65 (89%)	52 (71.2%) **	196 (71%)	196 (71%)	73 (76.8%)	70 (73.7%)
E1	4 (5.5%)	4 (5.5%)	12 (4.3%)	11 (4%)	5 (5.3%)	4 (4.2%)
S1	34 (46.6%)	39 (53.4%)	157 (56.9%)	108 (60.9%)	46 (48.4%)	53 (55.8%)
T1/T2	10 (13.7%)	13 (17.8%)	63 (22.8%)	54 (19.6%)	20 (21.1%)	18 (18.9%)
C1/C2	20 (27.4%)	17 (23.3%)	62 (22.5%)	57 (20.7%)	15 (15.8%)	16 (16.8%)
Treatment						
Corticosteroids alone or/and plus immunosuppressant (%)	48 (65.8%)	41 (56.2%)	168 (60.9%)	169 (61.2%)	62 (65.3%)	58 (61.1%)

MAP, mean arterial pressure; BMI, body mass index; ALB, albumin; eGFR, estimated glomerular filtration rate; SCr, serum creatinine; CKD, chronic kidney disease. M, mesangial proliferation; E, endocapillary proliferation; S, segmental sclerosis; T, tubular atrophy/interstitial fibrosis; C, crescents. Each group was compared with normal weight, which was used as reference.
*, P value <0.05; **, P value <0.01.

TABLE 4 The relationships between BMI and renal outcomes in the matched cohort.

Subgroup	ESRD (%)	P value of Cox	Hazard Ratio (95%CI)	P value of KM
Group 1		0.252	2.345 (0.545-10.093)	0.240
Normal weight	4 (5.5%)			
Underweight	5 (6.8%)			
Group2		0.729	0.894 (0.473-1.689)	0.729
Normal weight	24 (8.7%)			
Overweight	16 (5.8%)			
Group3		0.415	1.486 (0.573-3.853)	0.413
Normal weight	11 (11.6%)			
obesity	8 (8.4%)			

P value of Cox and KM were analyzed by Univariate Cox proportional hazard model and Kaplan-Meier survival analysis. In each group, normal weight group was used as reference.

Meier analysis revealed that IgAN hypertension patients with obesity had the worse renal outcome (Figure 2) Table 5 shows the results of different multivariate Cox proportional hazards models. Obesity and hypertension increased the risk of ESRD, both adjusted and unadjusted for confounding factors. When unadjusted for confounder factors, patients with obesity and hypertension were associated with a 5.2-fold increased risk of ESRD ($p < 0.001$). After adjustment for gender, age, pathologic lesions (MEST-C), treatment, eGFR and proteinuria did not affect the trend (HR = 3.246, 95%CI = 1.207-8.726, $p = 0.02$). Results for the remaining covariates in these multivariate Cox regression analyses are provided in Supplementary Table 1. In addition, risk stratification has long been developed as a strategy to predict patient outcomes and potentially alter or optimize comorbidities and modifiable risk. Clinical risk factors, such as persistent proteinuria or hypertension, appear to generally have greater predictive power for the renal outcome (18). Thus, according to the urine protein of the whole unmatched cohort, patients were divided into 24-h urine protein ≥ 1 g or < 1 g per day. We found that hypertension and obesity could accelerate the progression of IgAN, especially for the patients with 24-h urine protein ≥ 1 g (Figure 3) In addition, the synergy index was calculated to assess the effect modification of obesity and hypertension on the renal outcome of ESRD, and the synergy index was 1.52, indicating that there was positive interaction between obesity and hypertension in IgAN.

Discussion

With changes in lifestyle and dietary habits, the prevalence of metabolism-related diseases such as obesity has increased at a rapid rate in developed and developing countries around the world in recent decades (19).

Previously, high BMI was reported as a significant risk factor for progression and prognosis in IgAN (5, 20), and weight reduction was suggested to decrease proteinuria in overweight patients with IgAN to delay renal dysfunction progression (21). Current epidemiologic evidence indicates that obesity is not a risk factor for IgAN (8, 9). However, these studies were confined by the small sample size of the

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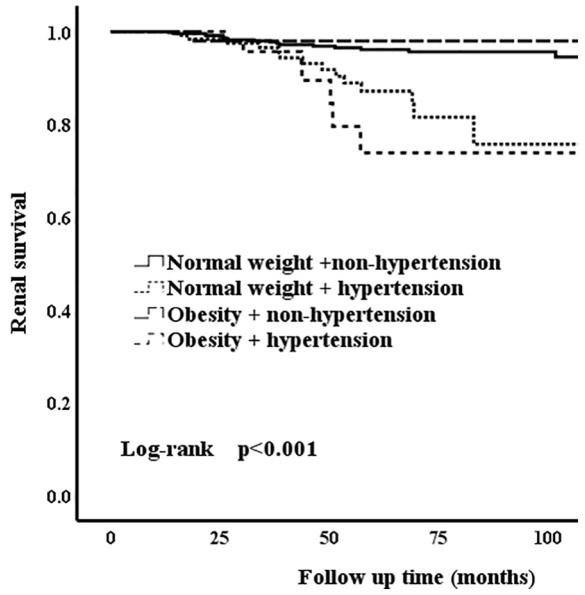

FIGURE 2
Kaplan-Meier analysis of obesity and hypertension for the unmatched cohort.

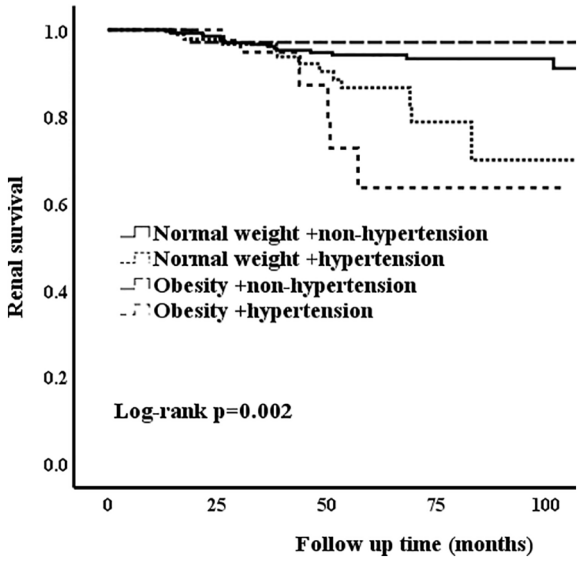

FIGURE 3
Kaplan-Meier analysis of obesity and hypertension for the unmatched patients with 24-h urine protein ≥ 1 g.

population and no or limited adjustments were conducted for other critical risk factors for IgAN patients. Moreover, the treatment was significantly different between obesity and non-obesity patients. In addition, the influence of obesity on IgAN patients may be confused by some confounding factors. Therefore, the association between obesity and IgAN still remains unclear.

Consistent with some previous studies, the main finding of this retrospective study of 1054 patients with IgAN is that being obesity at the time of renal biopsy significantly correlated with more severe clinical features and increased proteinuria and favored the subsequent development of ESRD. In our current study, all the patients were divided into four groups: underweight, normal weight, overweight and obesity. IgAN patients with obesity tended to be old, male and have severe proteinuria and renal dysfunction. Besides, they always had poor healthy lifestyle, smoking and alcohol drinking. More importantly, in our study, obesity patients were noted to have metabolic syndrome, including but not limited to hypertension, hyperuricemia, hypertriglyceridemia and hypercholesterolemia. It was reported that high BMI indirectly accelerated the progression of IgAN by inducing metabolic syndrome. Additionally, hypertension, hypertriglyceridemia and hyperuricemia, which contribute to metabolic syndrome, are strongly related to the development of ESRD (22). As a result, it is worth assessing and paying attention to the risk of obesity.

Obesity can induce afferent arteriole vasodilation and high intraglomerular pressures in order to augment glomerular filtration rate to meet the higher metabolic demands. As an individual gains

TABLE 5 Univariate and multivariate Cox proportional hazard model for the renal outcome in IgAN patients with obesity and hypertension.

Parameter	Unadjusted		Model 1		Model 2		Model 3	
	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value
Normal weight and non-hypertension	1 (Reference)		1 (Reference)		1 (Reference)		1 (Reference)	
Normal weight and hypertension	4.038 (2.004-8.138)	<0.001	4.307 (2.047-9.060)	<0.001	2.270 (1.057-4.873)	0.035	1.612 (0.755-3.441)	0.217
Obesity and non-hypertension	0.609 (0.081-4.568)	0.630	0.549 (0.072-4.170)	0.562	0.644 (0.084-4.964)	0.673	1.195 (0.149-9.568)	0.867
Obesity and hypertension	5.177 (2.128-12.596)	<0.001	5.154 (1.956-13.579)	0.001	3.767 (1.357-10.458)	0.011	3.246 (1.207-8.726)	0.02

Model 1 was adjusted for gender and age.
Model 2 was adjusted for covariates in model 1 plus Oxford M (mesangial hypercellularity), E (endocapillary proliferation), S (segmental glomerulosclerosis), T (tubular atrophy/interstitial fibrosis), and C (crescent) and Corticosteroids alone or/and plus immunosuppressant (yes or no).
Model 3 was adjusted for covariates in model 2 plus eGFR, 24h-proteinuria≥ 1g (yes or no).

weight, the glomerular and glomerular capillary diameter might increase, leading to terminally differentiated podocytes covering a larger glomerular capillary surface and glomerular capillary wall tension increasing. These were associated with an increased risk of proteinuria (23).

There was no significant difference among the four groups regarding the pathological lesions of MEST-C and treatment after renal biopsy. Because obesity-related glomerulopathy (ORG) is characterized by glomerulomegaly in the presence or absence of focal and segmental glomerulosclerosis lesions (24), more obesity IgAN patients are needed to investigate pathologic lesions.

A previous study revealed that hypertension and proteinuria increased the risk of ESRD in IgAN patients (25). However, our results showed that obesity was not a direct and independent risk factor for IgAN, and the combination of obesity and hypertension might be a risk indicator for IgAN. In clinical practice, many patients are exposed to the combined effects of obesity and hypertension. Our study found that such exposure is associated with a higher risk for ESRD. Furthermore, the positive interaction between obesity and hypertension in IgAN patients was verified by our results.

These results indicated that obesity may not be an independent risk factor for poor renal outcome, but to some extent, coexists with hypertension could increase the risk of ESRD in IgAN patients. When obesity coexists with common risk factors, such as hypertension, it may further aggravate the patient's disease progression. Based on our results, we suggest that IgAN patients, especially those with hypertension, should strictly control their weight and prevent obesity, so as to reduce the risk of poor renal prognosis.

The study had three main limitations. First, the body mass index was calculated at the time of renal biopsy and the groups were classified by those data. which may lead to different categorization over time. Besides, it was a retrospective study and only in a single hospital center. As a result, Multi-center studies would be useful to verify the findings of this study. In addition, the mean follow-up time of 61 months was relatively short, especially for IgAN, which is a slowly progressing disease. Though we tried our best to collect all the information retrospectively, a total of 192 patients (12%) with missing data were excluded from the study, which may have affected renal outcome. However, because of their small number, we do not think it would have had a significant impact on our findings. In addition, we are expanding the cohort population and extending the follow-up time, and expect to find more compelling results in the future.

Conclusions

Obesity had an effect on the progression of IgAN when combined with hypertension. In addition, hypertension was common in obesity-IgAN patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Research idea and study design: WQ and SW. Data acquisition: WQ, SW, and LD.Data analysis and interpretation: WQ, SW and JT. Statistical analysis: SW, XZ andAQ. Supervision: WQ. Each author accepted accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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Body roundness index is related to the low estimated glomerular filtration rate in Chinese population: A cross-sectional study

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Background: Kidney disease is related to visceral obesity. As a new indicator of obesity, body roundness index (BRI) has not been fully revealed with kidney disease. This study's objective is to assess the relationship between estimated glomerular filtration rate (eGFR) and BRI among the Chinese population.

Methods: This study enrolled 36,784 members over the age of 40, they were from 7 centers in China by using a random sampling method. BRI was computed using height and waist circumference, eGFR ≤ 90 mL/min/1.73 m^2 was considered to indicate low eGFR. To lessen bias, propensity score matching was employed, multiple logistic regression models were utilized to examine the connection between low eGFR and BRI.

Results: The age, diabetes and coronary heart disease rates, fasting blood glucose, and triglycerides were all greater in participants with low eGFR. The BRI quartile was still positively connected with low eGFR after controlling for confounding variables, according to multivariate logistic regression analysis. (OR [95%CI] Q2:1.052 [1.021-1.091], OR [95%CI] Q3:1.189 [1.062-1.284], OR [95%CI] Q4:1.283 [1.181-1.394], P trend < 0.001). Stratified research revealed that the elders, women, habitual smokers, and those with a history of diabetes or hypertension experienced the connection between BRI level and low eGFR. According to ROC, BRI was able to detect low eGFR more accurately.

Conclusion: Low eGFR in the Chinese community is positively connected with BRI, which has the potential to be used as an effective indicator for screening kidney disease to identify high-risk groups and take appropriate measures to prevent subsequent complications.

KEYWORDS

chronic kidney disease, body roundness index, visceral obesity, cross-sectional study, estimated glomerular filtration rate

1 Background

Global public health is challenged by chronic kidney disease (CKD). The projected global prevalence rate of CKD in 2017 was 9.1%. it has been reported to be closely related to the occurrence of multiple diseases, bringing great economic burden and public health pressure (1). Due to the insidious occurrence and poor prognosis of chronic kidney disease, early detection of renal function decline has been the focus of people's attention. eGFR is currently used to identify renal insufficiency in clinical practice.

Over the past 30 years, the prevalence of obese individuals has significantly risen worldwide (2). Obesity, particularly visceral obesity, has been linked to the beginning and development of CKD (3). The most popular method for determining obesity is body mass index (BMI) (4), but it has drawbacks because it does not account for how a person's fat is distributed. The new obesity measurement index BRI developed by Thomas (5) has higher predictive power for visceral adipose tissue (VAT) percentage than BMI, and has been demonstrated to be linked to metabolic syndrome (6), non-alcoholic fatty liver disease (7) and other diseases are closely related.

Few research, meanwhile, have examined the link between BRI and chronic renal disease. In order to assess the relationship between BRI and low eGFR, we gathered data from 38361 persons in China for this study. The goal of this study was to provide evidence for the early prevention and management of CKD and to identify high-risk patients as early as possible.

Abbreviations: BRI, body roundness index; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; BMI, body mass index; VAT, visceral adipose tissue; WC, waist circumstance; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; TC, total cholesterol; TG, triglyceride; PBG, 2-h postload blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine transferase; AST, aspartate transferase; γ-GGT, gamma-glutamyl transferase; WHR, Waist-to-Hip Ratio; WHtR, waist-to-Height Ratio; UACR, urinary albumin-creatinine ratio; ORs, odds ratios; CI, confidence intervals; SD, standard deviation; DM, diabetes mellitus; CHD, coronary heart disease; AUC, area under the receiver operating characteristic curve.

2 Methods

2.1 Participants and study design

We obtained data from a prospective cohort study of cancer risk assessment in Chinese diabetic patients and non-diabetic people based on the community population (8), it received approval from Shanghai Jiao Tong University School of Medicine's Ruijin Hospital's Clinical Research Ethics Committee (No. 2014-25). Prior to taking the survey, each respondent provided written informed consent, which was carried out in accordance with the Helsinki Declaration. From May to December of 2011, data were gathered.

In seven geographically varied regional centers in China, a total of 47,808 participants over the age of 40 were registered using multi-stage stratified cluster random sampling (Dalian, Wuhan, Lanzhou, Zhengzhou, Luzhou, Shanghai, and Guangzhou). Propensity matching was employed to lessen potential bias, considering the variances in baseline traits between the two groups. Participants having a primary kidney disease diagnosis, a history of cancer, past antihypertensive drug use (angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers), or insufficient data were excluded from the study. In the end, there were 36,784 participants (11,546 men and 25,238 women) (Figure 1).

2.2 Data collection and measurements

Trained investigators used standardized questionnaires to collect basic information about the participants, including demographic information (e.g., sex, age), lifestyle information (e.g., smoking, alcohol consumption), and disease history (e.g., kidney failure and tumors). Weight, height, waist measurement (WC), blood pressure, and other anthropometric measurements are included. Before collecting the measures, the patients were asked to remove their clothing and shoes. The height measurement is accurate to 0.1 cm and is done with a rangefinder. To accurately determine your weight to the nearest 0.1 kg, use a digital standing scale. Three readings of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken with a mercury sphygmomanometer, and the average was taken. Prior to measurement, each subject was instructed to remain still for at least 5

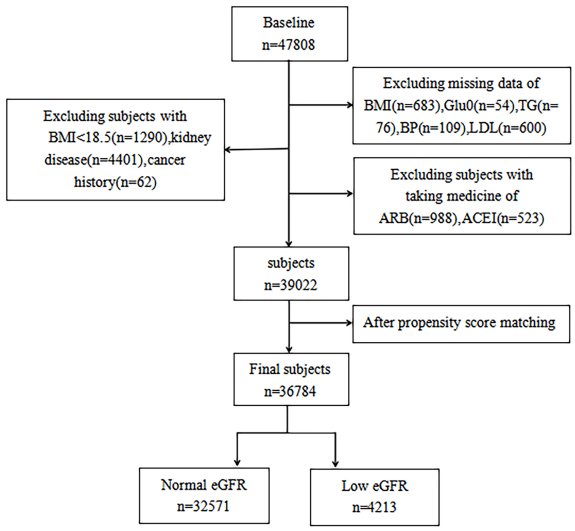

FIGURE 1
Flow chart of study population.

minutes. While trained investigators measured WC, participants had to stand up straight, while dropping their arms naturally, and keeping their feet together. When exhaling, the exact halfway between the iliac crest's topmost border and the bottom of the thorax is 0.1 cm (9).

A fast of 10 hours was followed by the collection of morning urine and fasting blood samples by qualified inspectors. Chemiluminescence immunoassay was used to measure the amounts of urinary albumin and creatinine, fasting blood glucose (FBG), glycated hemoglobin (HbA1c), triglycerides (TG), total cholesterol (TC), two hours postprandial blood glucose (PBG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine transferase (ALT), glutamyl transferase (γ-GGT), and aspartic acid transferase (AST).

2.3 Definition of variables

Weight divided by the square of height yields BMI (kg/m^2). This formula is used to compute BRI: 364.2-365.5*(1-[WC(m)/2π]^2/[0.5*height(m)]^2)½ [5]. Height and WC are both expressed in meters. Waist-to-Hip Ratio (WHR) and waist-to-Height Ratio (WHtR) are, respectively, WC divided by hip circumference and WC divided by height. No smoking, occasional smoking (less than one cigarette per day or seven cigarettes per week), and regular smoking were considered to be the three types of smoking (at least one cigarette per day). No drinking, occasional (less than once a week), and regular drinking were the three categories used to categorize drinking behaviors (at least once a week for more than six months).

Using the Collaborative Equation for the Epidemiology of Chronic Kidney Disease (CKD-EPI eGFR) (10) to estimate the eGFR. Participants were split into two groups: eGFR < 90 mL/min/1.73 m^2 for low eGFR and eGFR ≥ 90 mL/min/1.73 m^2 for normal eGFR. Blood pressure was divided into the normal group (SBP < 120 and DBP < 80), prehypertension group (120 ≤ SBP < 140 and/or 80 ≤ DBP < 90), and hypertension group (SBP ≥ 140 or DBP ≥ 90).

2.4 Statistical analysis

Because eligible participants in the two sets of eGFR had different baseline characteristics, propensity score matching was used to minimize bias. All covariates were matched at a ratio of 1:6, with caliper widths equal to 0.05 of the propensity score Logit's standard deviation. Continuous variables were described by mean and standard deviation (SD), or median and (25th percentile, 75th percentile) depending on whether they were regularly distributed. T-tests or Mann-Whitney U tests were used to determine group differences. Categorical variables were expressed as percentages (%), and Chi-square tests were used to compare differences.

To identify the factors that were substantially linked with either the BRI or the eGFR, Spearman correlation tests were run. We utilized logistic regression analysis to obtain the odds ratios (ORs) and 95% confidence intervals (CI) to assess the association between BRI quartile and low eGFR. We split BRI according to quartile cutoff points. The reference group was the bottom quartile. Model 1 was unadjusted. Model 2 adjusted for center, sex, and age. On the basis of Model 2, Model 3 further adjusted for drinking, smoking, diabetes, a history of coronary heart disease (CHD) and diabetes mellitus disease (DM). On the basis of Model 3, Model 4 additionally adjusted for FBG, TG, LDL-C, TC, SBP, and DBP. Make OR (95%CI) calculations for each model.

The study was carried out for gender, age (< 60/≥ 60 years), diabetes history, blood pressure, and smoking habits were stratified and adjusted for many potential confounders in order to examine the relationship between the BRI quartile and low eGFR in more detail. The ROC curve was then displayed, and the 95% CI and area under the receiver operating characteristic curve (AUCs) were reported to more clearly illustrate the predictive value of BRI and several anthropometric factors for low eGFR. SPSS Version 25.0 (IBM, Chicago, IL, USA) was used for data analysis, and MedCalc Version 13.0 was used for ROC analysis (MedCalc Software, Mariakerke, Belgium). In order for the findings to be considered statistically significant, the bilateral P values had to be less than 0.05.

3 Results

3.1 Clinical characteristics of study participants

After matching based on propensity scores, a total of 36,784 participants (11,546 men and 25,238 women) were enrolled in the

study. According to whether or not the participants' eGFR was raised, Table 1 displays the clinical and biochemical characteristics of the subjects, who were divided into two groups. The low eGFR group had lower HDL-C, was older, had a higher prevalence of DM and CHD, and had higher LDL-C, WC, BMI, HbA1c, FBG, PBG, SBP, and DBP values than the normal eGFR group.

3.2 Correlation between BRI quartile and low eGFR

Multiple logistic regression analysis was done to investigate the connection between the BRI quartile and low eGFR (Table 2). Patients with higher BRI levels had a higher likelihood of having low

TABLE 1 Characteristics of the study population by eGFR category.

Variables	Before propensity score matching			After propensity score matching		
	Normal eGFR	Low eGFR	*P*-value	Normal eGFR	Low eGFR	*P*-value
n	39681	4256		32571	4213	
Age,years	57.00 ± 9.06	60.74 ± 10.08	<0.001	57.92 ± 9.00	60.11 ± 10.01	<0.001
Men,%	12301(31.0%)	1128(26.5%)	<0.001	10455(32.1%)	1091(25.9%)	<0.001
BMI,kg/m^2	24.14 ± 3.66	24.67 ± 3.83	<0.001	24.25 ± 3.62	24.63 ± 3.80	<0.001
WC,cm	85.00(78.00,92.00)	87.00(80.00,94.00)	<0.001	85.00(78.00,92.00)	87.00(80.00,94.00)	<0.001
SBP,mmHg	130.00(117.00,144.00)	140.00(124.00,156.00)	<0.001	131.00(118.00,145.00)	140.00(124.00,156.00)	<0.001
DBP,mmHg	77.00(70.00,85.00)	79.00(72.00,88.00)	<0.001	77.00(70.00,85.00)	79.00(72.00,88.00)	<0.001
TC,mmol/L	4.95(4.21,5.71)	5.02(4.29,5.77)	0.006	4.98(4.25,5.73)	5.04(4.31,5.79)	0.004
TG,mmol/L	1.32(0.94,1.90)	1.54(1.08,2.22)	<0.001	1.36(0.98,1.93)	1.56(1.11,2.24)	<0.001
HDL,mmol/L	1.30(1.09,1.53)	1.23(1.04,1.46)	<0.001	1.31(1.10,1.55)	1.25(1.06,1.48)	<0.001
LDL,mmol/L	2.82(2.23,3.43)	2.92(2.34,3.54)	<0.001	2.83(2.24,3.45)	2.94(2.36,3.56)	<0.001
FBG,mmol/L	5.50(5.10,6.10)	5.77(5.20,6.90)	<0.001	5.51(5.11,6.12)	5.77(5.19,6.90)	<0.001
PBG,mmol/L	7.31(6.00,9.48)	8.51(6.60,12.20)	<0.001	7.30(6.00,9.46)	8.51(6.59,12.20)	<0.001
HbA1c,%	5.90 ± 0.30	6.10 ± 0.50	<0.001	5.90 ± 0.30	6.10 ± 0.50	<0.001
AST,U/L	20.00(17.00,24.00)	21.00(17.00,26.00)	<0.001	20.00(17.00,24.00)	21.00(18.00,26.00)	<0.001
GGT,U/L	20.00(14.00,31.00)	22.00(15.00,35.00)	<0.001	20.00(14.00,31.00)	21.00(15.00,35.00)	<0.001
UACR(mg/g)	8.51(5.38,14.16)	47.97(36.35,79.18)	<0.001	8.56(5.40,14.21)	47.89(36.31,79.02)	<0.001
BRI	3.95(3.19,4.81)	4.32(3.42,5.27)	<0.001	3.97(3.20,4.83)	4.33(3.43,5.29)	<0.001
eGFR,mL/min/1.73m^2	116.63(105.62,130.14)	81.60(73.60,86.25)	<0.001	115.78(104.32,128.98)	81.61(73.60,86.26)	<0.001
Smoking habits, %			0.007			0.003
no	34125(86.0%)	3579(84.1%)		28076(86.2%)	3551(84.3%)	
occasionally	913(2.3%)	85(2.0%)		717(2.2%)	89(2.1%)	
usually	4643(11.7%)	592(13.9%)		3778(11.6%)	573(13.6%)	
Current drinker(%)			<0.001			<0.001
no	29880(75.3%)	3170(74.5%)		24591(75.5%)	3143(74.6%)	
occasionally	7222(18.2%)	796(18.7%)		5830(17.9%)	775(18.4%)	
usually	2579(6.5%)	290(6.8%)		2150(6.6%)	295(7.0%)	
Previous DM (%)	4206(10.6%)	775(18.2%)	<0.001	3485(10.7%)	728(18.1%)	<0.001
Previous CHD(%)	2380(6.0%)	272(6.4%)	<0.001	1987(6.1%)	270(6.4%)	<0.001

Data expressed as mean ± SD for continuous variables or median (IQR) for skewed variables and percentage (%) for categorical variables.
BMI, body mass index; WC, waist circumstance; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; FBG, fasting blood glucose; PBG, 2-h postload blood glucose; HbA1c, glycosylated hemoglobin; AST, aspartate transferase; GGT, gamma-glutamyl transferase; UACR, urinary albumin-creatinine ratio; BRI, body roundness index; eGFR, estimated glomerular fifiltration rate; DM, diabetes mellitus; CHD, coronary heart disease.

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TABLE 2 Association between BRI quartiles and eGFR in the total population.

Variables	BRI Quartiles				
	Q1	Q2	Q3	Q4	P-value for trend
Model 1					
OR(95%CI)	1	1.135(1.043-1.235)	1.373(1.266-1.490)	1.985(1.837-2.146)	
P value		0.004**	<0.001***	<0.001***	<0.001
Model 2					
OR(95%CI)	1	1.127(1.036-1.226)	1.359(1.251-1.475)	1.889(1.740-2.052)	
P value		0.005**	<0.001***	<0.001***	<0.001
Model 3					
OR(95%CI)	1	1.096(1.007-1.193)	1.306(1.202-1.420)	1.773(1.631-1.928)	
P value		0.035*	<0.001***	<0.001***	<0.001
Model 4					
OR(95%CI)	1	1.052(1.021-1.091)	1.189(1.062-1.284)	1.283(1.181-1.394)	
P value		0.046*	0.037*	<0.001***	<0.001

*: P-value <0.05; **: P-value <0.01; ***: P-value <0.001.
Model 1: unadjusted; Model 2: adjusted for age, sex, centres; Model 3: further adjusted for smoking habits, drinking habits, CHD history, and DM history based on Model 2; Model 4: additionally adjusted for FBG, TG, LDL, TC, SBP, and DBP based on Model 3; OR, odds ratio; CI, confidential interval; BRI, body roundness index; eGFR, estimated glomerular filtration rate, CHD, coronary heart disease; DM, diabetes mellitus; FBG, fasting blood glucose; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

eGFR in Models 1-3 compared to participants in the BRI first quartile (P < 0.001). Even after further adjusting for FBG, TG, LDL-C, TC, SBP, and DBP, the correlation for Model 4 remained significant (OR [95%CI] Q2 vs Q1 1.052 [1.021-1.091], OR [95% CI] Q3 vs Q1 1.189 [1.062-1.284], OR [95%CI] Q4 vs Q1 1.283 [1.181-1.394], P-value for trend < 0.001).

3.3 The relationship between BRI and low eGFR in stratified analysis

After thoroughly controlling for age, sex, center, drinking and smoking habits, history of CHD, history of DM, FBG, TC, TG, LDL-C, SBP, and DBP, stratified analysis was utilized to further confirm the stability of the connection between BRI and low eGFR in various populations (Table 3). BRI in the fourth quartile in women was significantly linked with low eGFR compared to the first quartile (OR [95%CI] Q4vs Q1 2.427 [1.616-3.645], P-value for trend = 0.032), when gender was taken into account (P-interaction = 0.001). Men's BRI of the third and fourth quantiles was significantly connected with low eGFR when compared to the first quantile (OR [95%CI] Q3vsQ1 1.724[1.122-2.648], OR [95% CI] Q4vsQ1 2.699 [1.484-4.908], P-value for trend = 0.001). When the subjects had no prior history of DM, the third and fourth quantiles of BRI were significantly correlated with low eGFR compared to the first quantile (OR [95%CI] Q3vsQ1 1.108 [1.002-1.278], OR [95%CI] Q4vsQ1 1.227 [1.011-1.346], P-value for trend = 0.008), according to the DM history stratification (P-interaction = 0.056). Participants who had a history of DM showed the similar tendency (OR [95%CI] Q3 vs Q1 1.131 [1.107-1.156] and OR [95%CI] Q4 vs Q1 1.522 [1.138-2.465], P-value for trend < 0.001).

Younger participants in Q3 and Q4 (age < 60 years) were more likely to experience a low eGFR when stratified by age (P-interaction = 0.143), compared with the first quartile of BRI (OR [95%CI] Q3 vs Q1 1.468[1.021-2.135], OR [95%CI] Q4 vs Q1 2.147 [1.097-4.205], P-value for trend = 0.006). However, BRI in the third and fourth quartiles was significantly linked with low eGFR in participants who were older (age ≥ 60 years) (OR [95%CI] Q3 vs Q1 1.404[1.033-2.120], OR [95%CI] Q4 vs Q1 2.563[1.742-3.771], P-value for trend < 0.001).

Blood pressure stratification (P-interaction = 0.024) revealed a significant relationship between BRI and low eGFR in the third and fourth quartiles of the normal blood pressure group (OR [95%CI] Q3 vs Q1 1.496[1.016-3.173], OR [95%CI] Q4 vs Q1 2.854[1.033-4.687], P-value for trend < 0.001). Only BRI in the fourth quartile in the prehypertensive group showed a statistically significant relationship with low eGFR (OR [95%CI] Q4 vs Q1 2.983[1.160-4.718], P-value for trend = 0.026). Low eGFR was significantly linked with BRI in the third and fourth quartiles in the hypertensive group (OR [95%CI] Q3 vs Q1 2.306[1.489-3.572], OR [95%CI] Q4 vs Q1 3.677[1.842-6.697], P-value for trend < 0.001).

Smoking habit stratification revealed a significant correlation between BRI in the third and fourth quartile of nonsmokers and low eGFR (OR [95%CI] Q3 vs Q1 1.132[1.003-2.134], P-interaction = 0.068). There was no statistically significant relationship between BRI and low eGFR among occasional smokers (OR [95%CI] Q4 vs Q1 1.394 [1.054-1.845], P-value for trend = 0.002). BRI was

TABLE 3 Association between BRI quartiles and eGFR in different participants.

Variable	BRI Quartiles					
	Q1 OR(95%CI),P value	Q2 OR(95%CI),P value	Q3 OR(95%CI),P value	Q4 OR(95%CI),P value	P-value for trend	P for interaction
Gender						<0.001
Women	1.0	1.200(0.653-2.205)	1.724(1.122-2.648)*	2.699(1.484-4.908)**	0.001	
Men	1.0	0.911(0.557-1.493)	1.043(0.549-1.982)	2.427(1.616-3.645)**	0.032	
DM history						0.056
No	1.0	0.818(0.643-1.042)	1.108(1.002-1.278)*	1.227(1.011-1.346)*	0.008	
Yes	1.0	0.874(0.8121.304)	1.131(1.107-1.156)**	1.522(1.138-2.465)**	<0.001	
Age, years						0.143
<60	1.0	0.767(0.335-1.755)	1.468(1.021-2.135)*	2.147(1.097-4.205)*	0.006	
≥60	1.0	1.085(0.706-1.669)	1.404(1.033-2.120)*	2.563(1.742-3.771)**	<0.001	
Blood pressure, mmHg						0.024
SBP<120 and DBP<80	1.0	0.858(0.286-2.571)	1.496(1.016-3.173)*	2.854(1.033-4.687)*	<0.001	
120≤SBP<140 and/or 80≤DBP<90	1.0	1.067(0.733-1.554)	1.692(0.865-3.253)	2.983(1.160-4.718)*	0.026	
SBP≥140 or DBP≥90	1.0	1.188(0.778-1.813)	2.306(1.489-3.572)***	3.677(1.842-6.697)***	<0.001	
Smoking habits						0.068
No	1.0	0.877(0.798-0.964)	1.132(1.003-2.134)*	1.394(1.054-1.845)*	0.002	
Occasionally	1.0	0.654(0.360-1.188)	0.931(0.053-1.699)	0.969(0.553-1.169)	0.320	
Usually	1.0	1.187(0.916-1.539)	1.305(1.004-1.697)*	1.583(1.069-2.132)*	0.004	

*: P-value <0.05; **: P-value <0.01; ***: P-value <0.001.
adjusted for age, sex, centres, smoking habits, drinking habits, CHD history, DM history, FBG, TG, LDL, TC, SBP, and DBP.
OR, odds ratio; CI, confidential interval; BRI, body roundness index; eGFR, estimated glomerular filtration rate; CHD, coronary heart disease; DM, diabetes mellitus; FBG, fasting blood glucose; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

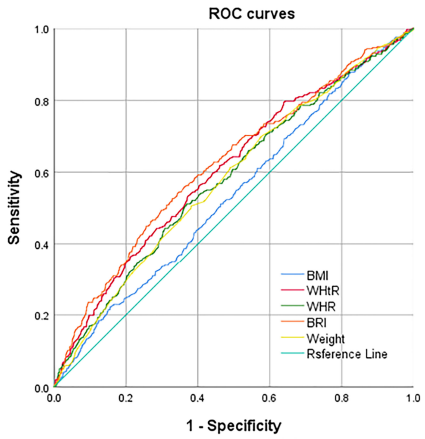
significantly linked with low eGFR in the third and fourth quartiles of frequent smokers (OR [95%CI] Q3 vs Q1 1.305[1.004-1.697], OR [95%CI] Q4 vs Q1 1.583[1.069-2.132], P-value for trend = 0.002).

3.4 Anthropometric indicators and area under ROC of low eGFR

The ROC curves for several anthropometric indicators and the ideal cutoff values as determined by the Youden index are shown in Figure 2. The BRI's predictive performance was significantly higher than BMI, WHtR, and WHR. The BRI's cutoff value was 4.49, and its AUC was the largest (0.667, 95%CI: 0.624, 0.692), sensitivity was 54.3%, and specificity was 65.4%. The optimum cutoff for WHtR is 0.557, AUC was 0.606 (95%CI: 0.505, 0.561), the sensitivity and specificity were 50.5% and 56.1% respectively. The optimum cutoff for WHR is 0.9031, AUC was 0.581 (95%CI: 0.550, 0.612), 56.0% and 61.1%, respectively, were the sensitivity and specificity. The maximum BMI cutoff was 27.77, AUC was 0.536 (95%CI: 0.505, 0.567), Specificity was 84.3%, and sensitivity was 22.3%. When the body weight cutoff value was 46.45 kg, the AUC was 0.580 (95%CI: 0.550, 0.611), sensitivity was 96.1%, and specificity was 0.45%.

4 Discussion

In this investigation, we discovered a strong correlation between rising BRI levels and low eGFR. Importantly, the association was diminished after additional adjustments for drinking and smoking habits, FBG, TC, TG, SBP, DBP, history of CHD, and history of DM, indicating that drinking, smoking habits, abnormal glucose or lipid metabolism, and history of cardiovascular disease may increase the risk of low eGFR in patients. Further stratified analysis revealed that participants with higher BRI were more likely than participants with lower BRI to have low eGFR, particularly those who were older (≥ 60 years), women, and those



Anthropometric Measures	Best thresholds	Sensitivity	Specificity	AUC (95%CI)	*P* for difference in AUC
BRI	4.49	0.543	0.654	0.667(0.624,0.692)	Reference
Weight(kg)	46.45	0.961	0.045	0.580(0.550,0.611)	<0.001
BMI	27.77	0.223	0.843	0.536(0.505,0.567)	<0.001
WHR	0.9031	0.56	0.611	0.581(0.550,0.612)	0.006
WHtR	0.5577	0.542	0.657	0.606(0.505,0.561)	0.039

FIGURE 2
ROC curves of different anthropometric measures for discriminating low eGFR.

with hypertension or DM history, as well as participants who regularly smoked. Additionally, we discovered that BRI had a stronger correlation with the low eGFR than other anthropometric variables as BMI, weight, WHtR, WHR, and had higher predictive power.

The prevalence of overweight and obese adults globally has increased dramatically over the past 30 years, which presents a serious challenge to public health due to the rapid economic development and changes in living patterns. The majority of overweight or obese people are now found in China, where one in five children and approximately half of adults are overweight or obese (11). Obesity can be categorized as systemic, central, or peripheral depending on where the fat deposits are located. However, there is growing interest due to the strong connection between central obesity and numerous disorders, including cardiovascular disease, DM, hypertension, CKD, and metabolic diseases.

The WHO recognizes BMI as an indicator of obesity, but it has limitations, including the inability to distinguish visceral fat from subcutaneous fat, similar to other conventional anthropometric indicators like WC, WHtR, and WHR (12). The new anthropometric index BRI was more accurate in predicting visceral adipose tissue than BMI (5) and WC (13). In DM patients, BRI is a useful marker of visceral fat accumulation (14). The metabolic syndrome (15), hyperuricemia (16), arteriosclerosis (13), and left ventricular hypertrophy (17) have all been demonstrated to be intimately related to BRI.

Obesity is linked to a number of harmful health effects, including a higher risk of CKD. The majority of earlier research, however, concentrated on the relationship between BMI and eGFR. BMI was found to be closely associated with eGFR decline among older adults (18). Martin et al. (19)discovered a similar significant positive correlation between elevated BMI and low eGFR in patients with CKD and obesity in Ireland. In a study of 75,000 participants, John Munkhaugen et al. discovered a significant link between BMI and the risk of kidney disease, with obese people being more likely to develop kidney disease (20).

Few studies have examined the relationship between visceral obesity and CKD, by exploring correlation between WHR or WC and GFR decline (21), or End stage renal disease (22), independent of BMI levels. High visceral adiposity index, a measure of visceral adiposity, has been linked to an increased risk of developing CKD in earlier studies (23). High WC (24) and lipid accumulation product (25) are linked to a quicker loss of GFR. The risk of low eGFR was independently linked with visceral adiposity as measured by BRI in our investigation, which comprised 36,784 Chinese people.

The relationship between BRI and low eGFR is currently unclear, the following theories are put forth: The metabolic demands placed on the kidneys may firstly rise as a result of obesity. Obese people experience compensatory hyperfiltration to satisfy the metabolic demands of weight gain (26). The kidneys are harmed by elevated glomerular pressure, it has been demonstrated that hyperfiltration is a reliable predictor can raise the danger of chronic CKD. Second, obesity's complicated endocrine activity, which is characterized by the production of leptin (27), resistin (28), and adiponectin (29), can also cause direct kidney injury, causing oxidative stress (30), abnormal lipid metabolism (31), renin-angiotensin-aldosterone system activation (32), chronic inflammation (33), and insulin resistance (34). Ectopic lipid accumulation (35), increased fat deposition in the renal sinuses (36), glomerular hypertension, increased glomerular permeability, impairment to the glomerular filtration barrier associated with ultrafiltration (37), GFR decline are the outcomes of these pathophysiological alterations. Third, obesity is a complicated metabolic disorder that can have a variety of adverse effects on several organ systems, including the kidneys (38), DM, and hypertension, both of which are risk factors for CKD and may mediate the effects of obesity on the kidneys, are linked to the development or exacerbation of obesity. Sex hormones may have an impact on how fat is distributed. After menopause, lower estrogen levels in women are substantially linked to obesity and more visceral fat accumulation (39). Age is known to be an independent risk factor for renal impairment (40). Older adults over 60 years of age are more prone to low estimated glomerular filtration than middle-aged.

The study found that patients who were overweight at age 20 had a three-fold increased risk of subsequent new kidney disease, even after adjusting for high blood pressure and diabetes. Excess fat may also increase the risk of DM, high blood pressure and atherosclerosis, which can indirectly lead to CKD (41). Considering that the trend of obesity is getting younger and younger, we have to pay attention to its damage to the multi-organ function of the whole body as well as the challenge to national health and economic pressure. The good news is that CKD brought

on by obesity is largely preventable. Weight-loss therapies consistently reduced blood pressure, glomerular ultrafiltration, and albuminuria in obese CKD patients, according to a meta-analysis (42). Obesity and renal disease can be significantly avoided with adequate nutrition, exercise, and education about the risks associated with being overweight (43). Our study demonstrates the utility of BRI as a trustworthy, user-friendly, and efficient screening method to determine the population's elevated risk of low estimated glomerular filtration in subsequent clinical practice.

This study has the advantage of being the first multi-center, large-sample, cross-sectional clinical analysis to examine the relationship between BRI and low eGFR. To the best of our knowledge. This study does, however, have certain limitations. First of all, In order to pinpoint the precise cause of the low eGFR and BRI, additional prospective cohort studies are needed because this study was cross-sectional. Secondly, the applicability of our findings to other regions and populations may be constrained because the participants we recruited were all from China and around 40 years old. Thirdly, because direct measurement of GFR is cumbersome, we used eGFR instead, but it must be considered that it may be affected by metabolism. Finally, the gold standard for determining the distribution of visceral obesity is considered to be magnetic resonance imaging and computed tomography. however, because of the radiation exposure risk and the time and cost associated with using them, we did not use them in our epidemiological investigations. However, previous studies have shown a strong correlation between BRI and VAT.

5 Conclusion

In this study, we discovered a statistically significant positive connection between rising BRI levels and low eGFR in adults from the Chinese population. A greater BRI level was associated with a higher incidence of low estimated glomerular filtration in women, the elderly, habitual smokers, those with a history of diabetes or hypertension. In comparison to BMI, WHtR, and WC, it has also been demonstrated that BRI may be the best anthropometric marker for predicting low eGFR. BRI may therefore be a helpful clinical indicator of chronic kidney disease in Chinese adults since it is a marker of visceral fat. We propose BRI as a quick and effective screening method to identify persons at high risk of low estimated glomerular filtration and advise them to adjust their lifestyles and engage in regular exercise in light of the association between obesity, particularly visceral fat, and CKD.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by The Committee on Human Research of the Shanghai Jiao Tong University School of Medicine's associated Rui-Jin Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

All authors have read and approved the final manuscript. YZ, WG, and RR analyzed the data and wrote the manuscript. YL and BL were of significant assistance in using and applying SPSS. AW, XT, LY, ZL, GQ, LC, QW, ZG, WW and GN provided advice and assistance, YM contributed by revising the article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The role of epicardial adipose tissue dysfunction in cardiovascular diseases: an overview of pathophysiology, evaluation, and management

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In recent decades, the epicardial adipose tissue (EAT) has been at the forefront of scientific research because of its diverse role in the pathogenesis of cardiovascular diseases (CVDs). EAT lies between the myocardium and the visceral pericardium. The same microcirculation exists both in the epicardial fat and the myocardium. Under physiological circumstances, EAT serves as cushion and protects coronary arteries and myocardium from violent distortion and impact. In addition, EAT acts as an energy lipid source, thermoregulator, and endocrine organ. Under pathological conditions, EAT dysfunction promotes various CVDs progression in several ways. It seems that various secretions of the epicardial fat are responsible for myocardial metabolic disturbances and, finally, leads to CVDs. Therefore, EAT might be an early predictor of CVDs. Furthermore, different non-invasive imaging techniques have been proposed to identify and assess EAT as an important parameter to stratify the CVD risk. We also present the potential therapeutic possibilities aiming at modifying the function of EAT. This paper aims to provide overview of the potential role of EAT in CVDs, discuss different imaging techniques to assess EAT, and provide potential therapeutic options for EAT. Hence, EAT may represent as a potential predictor and a novel therapeutic target for management of CVDs in the future.

KEYWORDS

epicardial adipose tissue, obesity, cardiovascular diseases, cardiac imaging, management

1 Introduction

Cardiovascular disease (CVD) is a considerable health condition that affects millions of individuals all over the world. To date, many risk factors are associated with the increasing incidence of CVDs. Among them, obesity has gained wide scientific interest. Obesity is closely associated with many other cardiovascular disease risk factors such as hypertension, dyslipidemia, metabolic syndrome, and diabetes mellitus. It is well-established that increased adiposity releases plenty of inflammatory cytokines that lead to a low-grade inflammatory microenvironment, endothelial dysfunction, and oxidative stress, and finally results in several CVDs (1, 2). The epicardial adipose tissue (EAT) is the visceral fat that deposits between the visceral pericardium and the myocardium and has direct contact with the myocardium and coronary artery (3). It usually presents as a white adipose tissue, but it also displays brown or beige fat-like features (4). Physiologically, EAT serves as thermoregulator and provides energy to the myocardium. Furthermore, EAT displays as an endocrine organ with metabolic activities and secretes bioactive molecules that affect the heart and coronary arteries *via* paracrine or vasocrine effects (5, 6). In recent years, evidence has shown that EAT is associated with CVDs. Therefore, different non-invasive imaging techniques have been proposed to identify and assess EAT to evaluate the risk of CVDs.

There are mainly three non-invasive imaging techniques that are used to evaluate EAT. First, echocardiography is used to evaluate EAT, which measures two-dimensional EAT thickness. It is an inexpensive, readily available, fairly accurate, and reproducible technique. Cardiac computed tomography (CCT) and cardiac magnetic resonance (CMR) imaging allow for three-dimensional EAT estimation. The former has higher space resolution and reproducibility for fat quantification, but it has limitations of radiation exposure and complex manual segmentation. However, the latter has no radiation exposure, but it is limited by space resolution, reproducibility, and higher cost. CMR is also difficult to perform in obese patients.

In this review, we summarize anatomical, physiological, and pathophysiological characteristics of EAT and focus on the potential role of EAT in CVDs and discuss different imaging techniques to assess EAT. In recent years, several papers have shown that EAT measurement *via* non-invasive imaging techniques serves as an important diagnostic tool to assess cardiovascular risks. Therefore, EAT may be a potential biomarker to monitor CVDs and their complications.

2 Epicardial adipose tissue: anatomy and physiology

2.1 Anatomy

The adipose tissue surrounding the heart can be divided into EAT, pericardial adipose tissue, paracardial adipose tissue, and perivascular adipose tissue (Figure 1). EAT lies between the myocardium and visceral pericardium and is made up of adipocytes, ganglia, nerves, and inflammatory, stromovascular, and immune cell (6). The pericardial adipose tissue (PAT) consists of epicardial and paracardial fat depots (7). The EAT and PAT are the entire pericardial fat. They have different embryological origins but share similar morphological features. EAT is derived from the splanchnopleuric mesoderm, and PAT is derived from the thoracic mesoderm (7).

EAT makes up 20% of the cardiac mass and covers 80% of the cardiac surface under normal physiological condition. It is non-homogeneously distributed around the heart (6, 8). EAT is mostly localized at the cardiac base and apex, in the atrioventricular and interventricular grooves, and around the coronary arteries. It is thicker around the right ventricle than around the left ventricle. In general, EAT can be differentiated into peri-coronary and peri-myocardial EAT. The former is located directly around or on the coronary artery adventitia; the latter is located just over the myocardium and is in direct contact with the myocardium (9). Vascular supply by coronary arteries in EAT forms part of the perivascular adventitia (10). It is thought to play a protective mechanical role against the tension and twist of an arterial pulse (11, 12). The increased EAT might result in cardiac disorders with increased arrhythmogenicity (13). It is hypothesized that EAT increases fatty infiltrates in the proximity of myocytes that leads to structural remodeling and abnormal impulse generation, which contributes to cardiac arrhythmias (13).

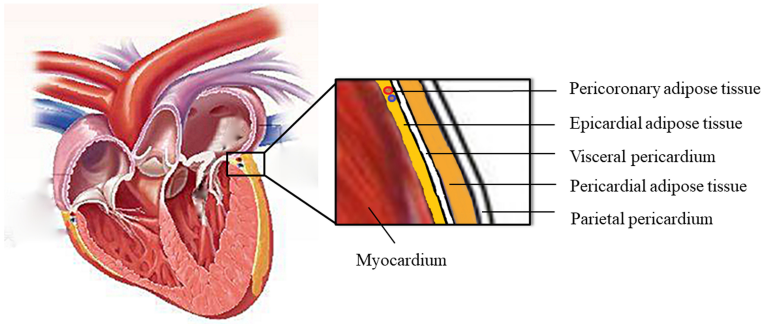

FIGURE 1
The anatomy of epicardial adipose tissue.

2.2 Physiology

2.2.1 Physical protective barrier and energy fat source

The epicardial fat surrounds the coronary arteries and myocardium; hence, EAT is considered to act as a buffering system in normal physiological conditions. It protects the heart and coronary arteries from mechanical deformation and facilitates vascular remodeling (14). In addition, EAT has thermogenic function that protects the heart from hypothermia. The increased thermogenic potential is due to brown adipocytes in EAT. Since EAT is more lipolytic than other adipose tissue depots, it releases abundant free fatty acids (FFAs) during high energy demand period, which is the main source of energy for the myocardium (14–16). In addition, EAT is present close to the myocardium and acts as a buffer to protect the heart from exposure to excessively high FFAs and lipotoxicity (17, 18).

2.2.2 Adipose tissue properties

Based on embryological, histological, and functional aspects, adipose tissue can be divided into two major groups: white and brown adipose tissue. The former has relatively few mitochondria and a single big lipid droplet, while the latter has multiple small lipid droplets and abundant mitochondria (19). EAT is basically a white adipose tissue but also has brown and beige fat-like features. It releases many mediators through expressing thermogenic genes related to brown and beige adipose tissues, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-1 receptor antagonist, IL-6, IL-8, IL-10, C-reactive protein (CRP), and plasminogen active inhibitor 1 (20). These factors may be involved in the communication between EAT and myocardial tissue through endocrine effect because they share the same capillary circulation (19). It is reported that EAT is twice as metabolically active as normal white adipose tissue, which is related to lipolysis and free fatty acid release. In addition, EAT has an increased capacity to release free fatty acids into the blood circulation and decreased glucose consumption compared to other adipose tissues (18). It also alters the bioavailability of adipokines and leads to adipocyte hypertrophy, tissue hypoxia, inflammation, and oxidative stress (7, 21). Brown fat generates heat in response to cold temperature and autonomic nervous system activation. Like brown fat, EAT also protects the myocardium from hypothermia.

2.2.3 Endocrine organ

Besides acting as energy depot, EAT also serves as an endocrine organ that regulates the heart homeostasis. There are two classical interaction mechanisms between the myocardium and the EAT: vasocrine and paracrine. On the one hand, adipokines and FFAs, as vasocrine signaling molecules, are released from EAT that enters the vasa vasorum directly and are transported downstream into the arterial wall. On the other hand, EAT-derived adipokines diffuse in interstitial fluid that cross the vascular wall (adventitia, media, and intima), and finally interact with vasa vasorum, endothelia, and vascular smooth muscle cells of the coronary arteries (18, 22). However, extracellular vesicles, containing cytokines and microRNAs have been confirmed as new communication modes (23). FFAs are the main energy source of the heart. EAT secretes vasoactive products that regulate coronary arterial tone to facilitate the FFA influx. In addition, fatty acid binding protein-4, expressed by EAT, may participate in the intracellular transport of FFAs from EAT into the myocardium (24).

3 Cardiac imaging of EAT

3.1 Echocardiography

The advantages of echocardiography to measure EAT thickness include low cost and more convenient, accessible, and reproducible (Table 1). However, there are few limitations. It is operator dependent. EAT is located in some areas of the heart that cannot be visualized with the ultrasound. In addition, obese patients have poor acoustic window. Although EAT thickness is considered as a useful diagnostic tool, the normal value of EAT is still undetermined. Iacobellis et al. (25, 26) reported a transthoracic echocardiographic method of evaluating EAT thickness on the free wall of the right ventricle from both parasternal long- and short-axis views. They choose the right ventricle to measure EAT because it is considered as the thickest absolute epicardial fat layer (27), and parasternal long- and short-axis views allow the most accurate measurement of EAT on the right ventricle with optimal cursor beam orientation. In addition, they reported an average epicardial fat thickness of 7 mm in men and 6.5 mm in women for standard clinical references (28). Another study that enrolled 459 patients with Grade I and II essential hypertension demonstrated that patients with EAT thickness >7 mm exhibited higher left ventricular mass index, diastolic dysfunction, and increased carotid stiffness and intima-media thickness (29). In addition, Islas et al. reported that acute myocardial infarction patients with EAT >4 mm have worse left ventricular systolic function and have large infarct size. EAT >4 mm is an independent predictor of major adverse cardiovascular events at 5-year follow-up (30).

In addition, Parisi et al. presented a novel method to measure EAT thickness at the level of the fold of Rindfleisch, a pericardial recess where the parietal pericardium does not exert a mechanical compression on visceral fat (31). Moreover, echo-EAT thickness showed a significant correlation with the CMR-EAT thickness, both measured at the Rindfleisch fold. Although echocardiography is convenient and reproducible, it cannot reflect the variability in EAT thickness or EAT volume accurately. Multidetector CT and cardiac MRI can provide a more accurate and volumetric quantification of EAT.

3.2 Cardiac computed tomography

CCT is another imaging modality used to measure EAT. Although CCT has high spatial resolution and provides three-dimensional view of the heart, it is costly and requires radiation exposure (Table 1). Currently, coronary CT angiography (CTA) provides an optimal method that enables the characterization of morphological changes in the pericoronary adipose tissue (PCAT) and simultaneous assessment of coronary atherosclerosis (32). CT attenuation of the adipose tissue reflects morphological derangements of adipocytes that are exposed to the effects of local vascular inflammation. EAT volume and density are considered as

TABLE 1 Comparison among the main imaging techniques for the evaluation of EAT.

Imaging techniques	Echocardiography	Computed tomography imaging	Magnetic resonance imaging
Availability	readily available	not readily available	not readily available
Invasive	non-invasive	minimally invasive	minimally invasive
Cost	low	medium	high
Radiation	no	yes	no
Operator-dependent	yes	no	no
Definition	low	high	medium
Scan time	quick	quick	long
Patient limitation	severely obese	allergic to contrast media	claustrophobia
Attenuation quantification	no	yes	no
EAT thickness assessment	yes	yes	yes
EAT volume assessment	No	yes	yes
Coronary artery calcification	No	yes	no

independent markers of adverse cardiometabolic risk that can be measured by CCT (33, 34). Additionally, increased EAT volume is a predictor of CVDs that include obstructive coronary stenosis, myocardial ischemia, and coronary syndromes (33, 35–37). However, the upper cutoff CT value of normal EAT remains undermined. Dey et al. reviewed literatures that reported different inclusion criteria with CT value varying between 125.0 and 139.4 cm^3 for men and 119.0–125.0 cm^3 for women (38). In addition, EAT density or attenuation has also been associated with CVDs. It is reported that EAT attenuation is associated with coronary artery calcification, acute myocardial infarction, and coronary adverse events (34, 39–41). PCAT is considered as a metric of local vascular inflammation. The widely accepted definition of PCAT by coronary CTA is voxels ranging from −190 to −30 Hounsfield units, with volume of interest that extends to an orthogonal distance equivalent to the diameter of the target vessel (42). Antonopoulos et al. presented a method to detect coronary inflammation by characterizing the changes in PCAT CT attenuation (43). They have demonstrated that the average attenuation of EAT is inversely related to adipogenic gene expression and adipocyte size in a large cohort of patients who have undergone cardiac surgery.

Nowadays, although manual segmentation of EAT quantification is the method of choice, it is operator dependent, time consuming, and not suitable for routine clinical practice. Thus, artificial intelligence that includes machine and deep learning received more attention to obtain fast, automatic, and reliable measures of EAT by CCT.

3.3 Cardiac magnetic resonance

CMR is now considered as the gold standard for measuring visceral adipose tissue (44–46). CMR provides excellent visualization of visceral and parietal pericardia. Cardiac imaging is not affected in patients with excess subcutaneous fat. It enables easy assessment and volumetric quantification of EAT. Although there is no use of radiation and contrast agents, CMR is expensive and time consuming. It is also difficult to perform in patients with claustrophobia (Table 1). Additionally, CMR can differentiate cardiac fat into EAT and paracardial fat.

MRI provides explicit parameters about EAT volume and mass by using the spin-echo sequence technique (2, 47). Manual contouring of EAT area at end-diastole during cardiac cycles provides precise quantification of EAT volume (47). A 3D-Dixon sequence (an electrocardiography triggered and respiratory navigator gated 3D-gradient echo pulse sequence was used for cardiac Dixon imaging) has been shown to be a reliable method for EAT quantification in studies (48). Dixon method separates fat and water signal *via* voxel intensity differences present between in- and opposed-phase MR images (48). Rami Homsi et al. (49) enrolled 34 healthy volunteers (22 men; BMI range, 14–42 kg/m (2); age range, 21–79 years) and measured parameters of pericardial and epicardial adipose volume (PAV, EAV) using a 3D-Dixon-based CMR approach. They found that the average EAV was 77.0± 55.3 ml, and PAV was 158.0 ± 126.4 ml; both were highly correlated. Therefore, they proposed a 3D-Dixon-based method that allows accurate measurement of cardiac fat volume and provides a valuable tool for cardiovascular risk stratification.

Moreover, CMR measures EAV, left ventricular compliance, pulse wave velocity, and other indicators simultaneously, which can evaluate aortic stiffness, myocardial strain, and fibrosis (50, 51). A combined measurement by CMR may support the evaluation of risk and prognosis of CVDs.

4 Epicardial adipose tissue: a new biomarker for cardiovascular disease risk assessment

Evidence strongly supports the role of structural and functional changes of EAT in the pathogenesis of various cardiovascular diseases (Figure 2).

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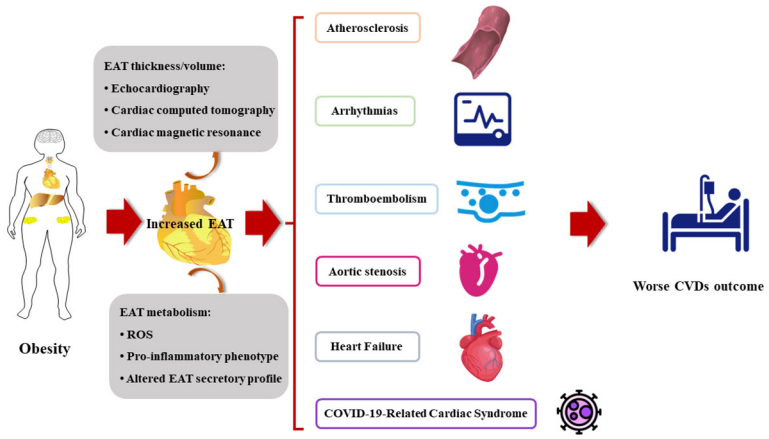

FIGURE 2
The increased EAT contributes to the onset and the poor prognosis of cardiovascular diseases. CVDs, cardiovascular diseases; EAT, epicardial adipose tissue; ROS, reactive oxygen species.

4.1 Atherosclerosis

Atherosclerosis is characterized by deposition of immune cells and cholesterol in the subendothelial space of arteries. As EAT is a metabolically endocrine tissue with abundant proinflammatory cytokines, it is considered to be associated with atherosclerosis. It is reported that EAT leads to CVDs by involving in the mechanism of inflammation, insulin resistance, and oxidative stress. C1q TNF-related protein 3 (CTRP3) is an adipokine with anti-inflammatory and cardioprotective properties. It has also been demonstrated to activate nuclear factor kappa B (NF-κB) signaling and PI3K/Akt/eNOS pathway to attenuate obesity-related inflammation responses and insulin resistance and thus regresses atherosclerosis (52, 53). A study involving 34 patients with elective post-coronary artery by-pass graft (post-CABG) showed that EAT with lower CTRP3 mRNA level is closely associated with coronary atherosclerosis and cardiac dysfunction (54). IL-1β and angiopoietin-like-4 (an inhibitor of lipoprotein lipase secreted by adipose tissue) were highly expressed in EAT patients with coronary artery diseases (55). Adipocyte oxidative stress, characterized by the imbalance of ROS and redox signaling, is related to metabolic CVDs. It has been demonstrated that EAT can produce more ROS compared to subcutaneous adipose tissue because it has higher expression of NADPH components gp91phox and p47 phox (56). The hyperglycemia and insulin resistance accelerate adipocyte oxidative stress (57–59). A recent study showed that patients with severe coronary atherosclerosis, glucose and insulin metabolic disorder, and serum adiponectin reduction are significantly linked with higher oxidative stress in EAT adipocytes (60). In addition, EAT thickness was related with endothelial dysfunction (61), and it was concluded that EAT may predict the early reversible stages of atherosclerosis. The data from different studies showed that increased EAT volume is involved in high-risk coronary plaque formation. In addition, patients with high-risk coronary plaque have quantitatively higher EAT volume (35, 62). A prospective cohort study found that EAT and plasma IL-8 level are associated with elevated coronary artery calcium score, which is an independent predictor of coronary atherosclerosis (63).

EAT functions as an endocrine organ. Recent studies have focused on the signaling molecules released by EAT. A novel mechanism for EAT-induced CVDs is the secretion of exosomes that contain non-coding RNAs, especially microRNAs (miRNAs), which are subsequently absorbed by endothelial cells or cardiomyocytes (64–66). A previous study verified that increased has-miRNA-34a is associated with coronary artery diseases (67). A recent micro- and lncRNA microarrays followed by GO-KEGG functional enrichment analysis demonstrated a sex-dependent unique mi/lncRNAs. They are involved in inflammation, adipogenesis, and cardiomyocyte apoptosis. They are also modified in human epicardial fat in both patients with and without coronary artery disease. Examples include has-miR-320 family, hsa-miR-21, hsa-24-3p, hsa-miR-378, and hsa-miR-33 (68).

4.2 Arrhythmias

Atrial fibrillation (AF), the most common arrhythmia, is the major cause of ischemic stroke, heart failure, and cardiovascular mortality. Atrial electrophysiological and structural remodeling is the underlying mechanism of AF, which is characterized by myocardial fibrosis, and the underlying mechanism is heterogeneous (69). The mechanism of arrhythmias includes adipocyte infiltration, pro-fibrotic, and pro-inflammatory paracrine effects, oxidative stress, and other pathways (69–71). A study in 215 acute embolic stroke patients showed that increased periatrial EAT thickness on the left side is associated with AF (72). In patients with AF who have undergone pulmonary vein

isolation, EAT volume is associated with AF recurrence (73). In another study, most of the persistent AF patients who are not anticoagulated and with increased periatrial EAT thickness were also associated with an increased risk of cardiovascular events (74). Julia and colleagues found that patients with lone AF have larger volume and higher attenuation of EAT compared with patients without cardiac arrhythmias (75). Moreover, non-uniformity of EAT radiomic gray level is the only independent predictor of post-ablation AF recurrence within 12 months follow-up (75).

EAT was found to be significantly higher in the patients with nephrotic syndrome and in patients with ECG showing the atrial depolarization and ventricular repolarization (76). Another study demonstrated that EAT volume exerts reverse relationship with heart rate recovery that indicates the potential adverse effects of EAT on cardiac autonomic function (77). It may result from the pathogenic effect of local inflammatory cytokines secreted from nearby visceral fats. As an endocrine organ, EAT influences adjacent myocardium by secreting a series of bioactive molecules, such as exosomes carrying circular RNAs (circRNAs), and regulates atrial electrical and structural remodeling. Zheng and colleagues identified differently expressed circRNA in EAT *via* RNA sequencing, such as hsa_circRNA_000932 and hsa_circ_0078619, which may work as endogenous RNAs to capture various miRNAs miR-103a-2-5p and miR-199a-5p, and subsequently regulate the expression of cardiovascular disorders-related protein-coding genes (78).

4.3 Aortic stenosis

With the global epidemiological increase in elderly population, AS becomes a challenging disease, representing an important cause of morbidity, hospitalization, and death in aged population. Generally, AS is considered as the result of a complex process, driven by inflammation and involving multifactorial pathological mechanisms promoting valvular calcification and valvular bone deposition (79). Importantly, obesity-related chronic systemic inflammation is associated with a significant increase in the amount of EAT, the cardiac visceral fat, which is considered a transducer of the adverse effects of systemic inflammation and metabolic disorders on the heart (80). As EAT can mediate the deleterious effects of systemic inflammation on the myocardium, it may contribute to the pathogenesis of calcific AS. Parisi et al. hypothesized that EAT may participate in the inflammatory burden of aortic stenosis (81). Mahabadi et al. found that EAT thickness, quantified using transthoracic echocardiography, was significantly associated with severe aortic stenosis, independent of traditional risk factors (82). Moreover, Arangalagea et al. showed that EAT volume was independently associated with LV mass in a prospective cohort of patients with aortic stenosis (83). These results support the hypothesis of a potent proinflammatory activation of EAT in patients with AS and suggest the involvement of cardiac visceral fat in inflammatory and atherogenic phenomena occurring in the AV and promoting its degeneration and calcification (79, 81).

4.4 Thromboembolism

The relationship between AF and thromboembolism is well established. Recently, preliminary investigations demonstrate the possible relationship between EAT and AF-related thromboembolism. In a recent study in AF patients who developed stroke, EAT volume was significantly increased. Hence, total EAT is considered as an independent predictor of higher risk of stroke occurrence after AF diagnosis (84). In addition, EAT thickness was higher in non-valvular AF patients compared to healthy subjects. EAT thickness was also related with the CHA2DS2–VASc score in patients with non-valvular AF (85). A multicenter study in Korea enrolled 3,464 individuals and showed that larger peri-atrial EAT volume was independently associated with post-ablation embolic stroke regardless of AF recurrence and CHA2DS2-VASc score (86). Patients with post-ablation embolic stroke had a greater prevalence of prior thromboembolism, lower creatinine clearance, larger left atrial diameter, frequent AF recurrence, and abundant total and peri-atrial EAT (86).

However, another study showed that EAT thickness was directly related with CHA2DS2-VASc scores in patients with sinus rhythm (87). A single-center retrospective study enrolled 202 patients and showed that a thickened EAT was associated with low left atrial appendage flow velocity and had increased risk of thromboembolic phenomena in the presence of AF (88). The mechanism of correlation between EAT and embolic stroke might be explained by EAT-mediated atrial cardiomyopathy, which is characterized by LA enlargement, increased wall stiffness, hypercontractility, endothelial dysfunction, and impaired reservoir function that lead to atrial prothrombotic milieu (84, 86, 89, 90).

4.5 Heart failure

EAT is associated with risk factors for HF, such as obesity, metabolic syndrome, hypertension, and diabetes. Numerous studies focused on the relationship between EAT and HF. A study enrolled 72 type-2 diabetes subjects with normal cardiac function and verified that subjects with higher EAT thickness showed a lower cardiac workload, worse cardiopulmonary function and subclinical cardiac systolic dysfunction after maximal cardiopulmonary exercise test with similar duration of exercise (91). Another study found that HF patients have higher EAT than the control group. Hence, EAT can be considered as a prognostic predictor of HF with preserved ejection fraction (HFpEF) (92). In a prospective multinational PROMIS-HfpEF cohort, increased EAT has been shown to be associated with cardiac structural alterations, adiposity, inflammation, lower insulin sensitivity, and endothelial dysfunction related to HFpEF pathology (93). In addition, a proteomic analysis of EAT from 2,416 HFpEF patients found that EAT proteins such as CD36, POSTN, and TRAP1 were differentially expressed in HFpEF (94). In another study, increased EAT thickness was found closely related with brachial–ankle pulse-wave velocity in HFpEF patients and indicated that

thicker EAT may be independently associated with arterial stiffness (95).

Interestingly, in a multicenter cohort study, EAT thickness was found to be greater in patients with HFpEF than HFrEF/HFmrEF. In addition, the EAT thickness is associated with reduced left atrial and left ventricle function in HFpEF, but with better function in HFrEF/HFmrEF (96). Similar results were also found in post-ablation AF patients (97). However, in patients with non-ischemic cardiomyopathy, EAT volume was found to be greater in the LV reverse remodeling group than in the non-remodeling group, which suggests that EAT volume is an independent predictor of LV reverse remodeling in patients with non-ischemic cardiomyopathy (98). Findings by Hao and colleagues indicate that EAT mediates cardiomyocyte apoptosis after acute myocardial infarction through secretion of complement factor D and activation of poly ADP-ribosepolymerase-1 (99), which may subsequently result in heart failure. A recent study in mice model with preserved ejection fraction found that inflammasome-mediated pyroptosis pathway was activated in the EAT. Moreover, suppression of pyroptosis-related protein gasdermin D in cultured EAT could lower cardiomyocyte inflammation and autophagy (100).

4.6 COVID-19-related cardiac syndrome

The coronavirus disease 2019 (COVID-19) pandemic has spread worldwide with more than 6 million deaths recorded globally (101). Besides pneumonia, myocardial injury is a typical COVID-19-related complication and is present in 20%–30% of patients that contributed to 40% of deaths (102). Patients with larger EAT seem to get higher cardiac risk in COVID-19 patients (103, 104), with worse outcomes (105). Moreover, the type of adipose tissue and its distribution seems to play a crucial role in COVID-19 severity (101). It is well-known that EAT has direct anatomical and functional contiguity with the myocardium, and these two tissues share the same microcirculation, which support the pathophysiology of COVID-19-related cardiac injury. Therefore, clinical studies and practice on COVID-19-related CVDs have focused on cardiac adipose tissue. Recent studies have shown that in patients with COVID-19, higher EAT volume and lower EAT density may be independent predictors of an unfavorable disease prognosis, including cardiovascular complications and death (104, 106, 107). It is reported that EAT is like a highly inflammatory region with dense macrophage infiltrates and highly enriched proinflammatory cytokines, which are overexpressed in COVID-19 patients with CVDs that facilitates viral spread and augments immune response (18, 108, 109). COVID-19-related cardiac injury is characterized by decreased angiotensin-converting enzyme 2 (ACE2) and entry ligand receptor, with pathogenetic role (20, 108). Previous studies indicated that ACE2 deficiency mediates myocardial inflammation, and ACE2 reduction is associated with EAT inflammation (110). In addition, ACE2 downregulation leads to the proinflammatory polarization of M1 macrophages in EAT and results in the dysregulation of the inflammatory response, which is highly observed in COVID-19. Moreover, ICU patients with a higher EAT volume had a higher risk of developing pulmonary embolism compared to those with lower EAT volume (111). Therefore, EAT plays a role in COVID-19-related CVDs and has potential to become a clinically measurable and modifiable therapeutic target.

5 Therapeutic options in EAT

We have discussed that EAT is an independent risk factor and has potential to become a therapeutic target for CVDs. Hence, studies have focused on reducing EAT (Figure 3).

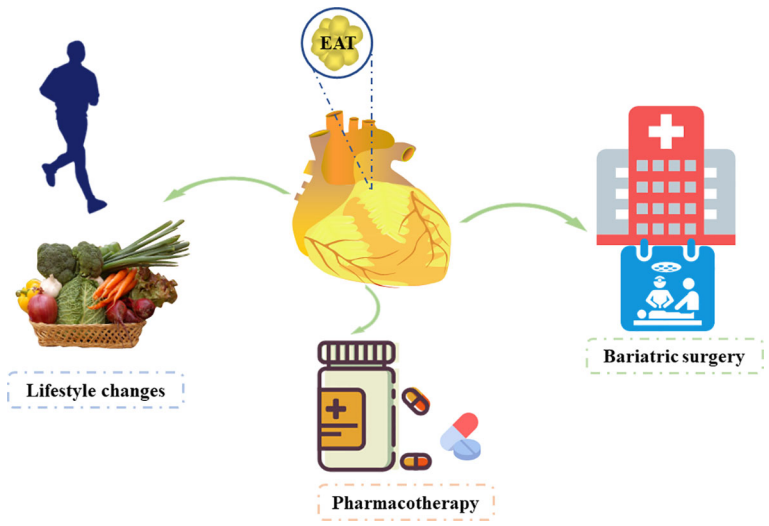

FIGURE 3
Therapeutic options affecting the epicardial adipose tissue.

5.1 Lifestyle intervention

EAT is exacerbated by several unhealthy life styles, such as sedentary life, weight gain, and an unbalanced diet (112, 113). Lifestyle intervention based on dietary control and regular physical activity is an essential "first-line" strategy for the clinical management of obesity. Physical exercise and strict diet control reduce visceral fat, including EAT, and improve cardiac function (114, 115). A recent study showed that bad childhood experience is associated with increased EAT in children with depression and reduced physical exercise (116). Studies have indicated that regular physical activity is an effective non-invasive strategy for reducing EAT that may provide beneficial effects on the cardiovascular system (113, 117, 118). Several studies showed that aerobic exercise decreases EAT thickness significantly in obese men (118, 119). Another study from India showed that the 12-week regular Taekwondo training reduces the EAT thickness significantly in elderly women with hypertension (120). Another study from Turkey enrolled 74 obese women and found that long-term, sustained weight loss reduces epicardial fat thickness significantly as assessed by echocardiography, which can be used as an indicator of metabolic profile for weight reduction in obese women (121). Moreover, the decrease in epicardial fat thickness was significantly higher in patients who reversed their metabolic syndrome diagnosis with weight loss than in those whose metabolic syndrome status was unchanged. Iacobellis et al. reported significant reduction in epicardial fat in severely obese patients after 6 months of low-calorie diet (122). However, a systematic review and meta-analysis conducted by Rabkin and Campbell (123) showed that diet and bariatric surgery markedly reduced EAT, but this was not achieved with exercise. Moreover, a reduction in body mass index was significantly associated with reduced EAT by diet-based interventions.

5.2 Medical treatment

The impact of medical treatment on EAT is worth investigating. The use of statin is associated with decreased adipokine release from visceral EAT.

Parisi et al. (124) reported that statin therapy was significantly associated with lower EAT thickness and with lower levels of EAT-secreted inflammatory mediators. Of note, there was a significant correlation between EAT thickness and its proinflammatory status. Among lipid-lowering agents, atorvastatin has more significant effect than simvastatin and ezetimibe (125). Other studies also demonstrated that statins reduce EAT volume (124, 126). Additionally, antidiabetic drugs such as GLP-1A (127, 128), metformin (129, 130), and SGLT2 inhibitors (131, 132) were also proved to reduce EAT. For individuals with severe obesity, bariatric surgery is the most reliable treatment. It is well-known that different depots of adipose tissue and visceral fat change after bariatric surgery. Weight loss following bariatric surgery is associated with EAT reduction (133). Hunt et al. reported that severely obese subjects have lower EAT during a 14-year follow-up after bariatric surgery (134).

6 Conclusion

The cardiovascular system is widely affected by EAT. The expansion and remodeling of EAT contributes to vascular dysfunction and CVDs significantly. The evolving field of non-invasive imaging technique-based EAT composition analysis showed great potential for the stratification of CVD risk. Therefore, it is critical to identify strategies that are capable of reducing cardiovascular risk by modulating EAT mass, distribution, and function. At present, there is growing interest regarding EAT. In the future, the assessment of EAT may become part of the clinical practice to help clinicians identify patients at great risk of developing CVDs and to provide information on their clinical and therapeutic prognosis.

Author contributions

CL reviewed the literature and drafted this review. XL reviewed the literature and corrected the figures. BA, LC, and WL reviewed the literature, gave critical comments, and revised the manuscript. YW gave critical comments and revised the manuscript. YW and HZ reviewed the literature, gave critical comments, revised the manuscript, and took charge of project supervision and administration. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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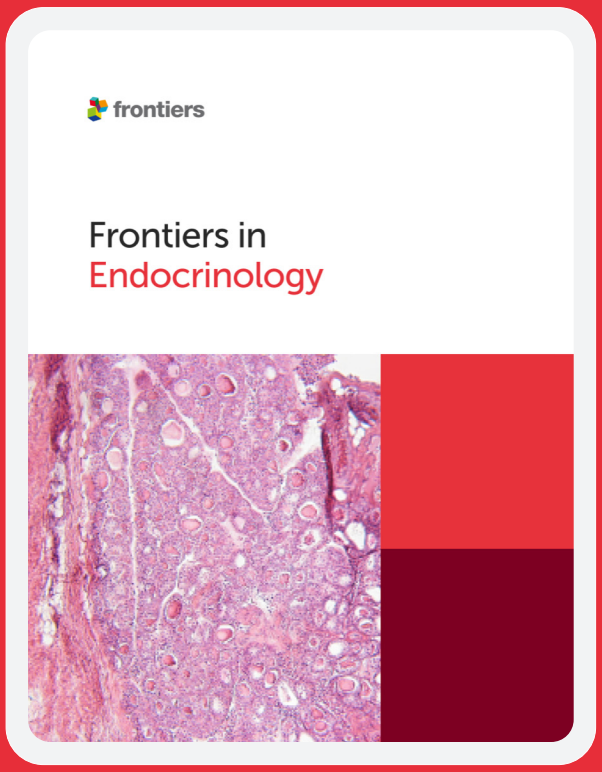

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