International Journal of Women's Health

Dovepress
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ORIGINAL RESEARCH

Beyond BMI: A Mendelian Randomization Study of the Causal Effects and Mediating Pathways of Regional Adipose Tissue Depots on Polycystic Ovary Syndrome

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Background: Previous studies suggest that increased body fat is associated with polycystic ovary syndrome (PCOS) risk. Recent evidence highlights that the distribution of adipose tissue may play a more critical role in predicting PCOS risk compared to total fat mass; however, causal relationships remain unclear. This Mendelian randomization (MR) study aimed to investigate the causal associations between body mass index (BMI)-independent regional adipose tissue distribution and PCOS risk.

Methods: Female-specific data on regional adipose tissue depots (n = *19,273*), independent of BMI and height, including gluteofemoral adipose tissue (GFAT), abdominal subcutaneous adipose tissue (ASAT), visceral adipose tissue (VAT), and related adipose tissue ratios, were derived from large-scale genome-wide association studies. Independent genetic instruments were selected based on genome-wide significance ($P < 5 \times 10^{-8}$), and the Steiger test confirmed causal direction. Causal associations were validated through meta-analysis combining discovery and replication PCOS datasets. Additionally, two-step mediation analysis was performed to investigate five potential mediators: sex hormones, lipid metabolism, glucose metabolism, adipose-specific factors, and inflammatory markers.

Results: Genetically predicted higher GFAT volume demonstrated a significant causal protective effect on PCOS risk (OR = 0.845, 95% CI: 0.735–0.971). This protective effect was predominantly mediated through reductions in fasting insulin (58.37%, 95% CI: 27.66–89.08%) and leptin (51.75%, 95% CI: 33.54–75.41%). Other mediators included the homeostasis model assessment of insulin resistance (HOMA-IR; 37.20%), sex hormone-binding globulin (SHBG; 24.74%), bioavailable testosterone (BT; 11.99%), and triglycerides (TG; 9.52%). Additionally, suggestive evidence driven by a single genetic instrument linked higher VAT/ASAT, VAT/GFAT, and ASAT/GFAT ratios to increased PCOS risk (OR > 1). Sensitivity analyses and supplementary methods confirmed the robustness of these findings.

Conclusion: This study provides causal evidence supporting the protective role of GFAT against PCOS and identifies critical metabolic and hormonal pathways as mediators. These results highlight the significance of adipose tissue distribution patterns in the pathogenesis of female endocrine disorders.

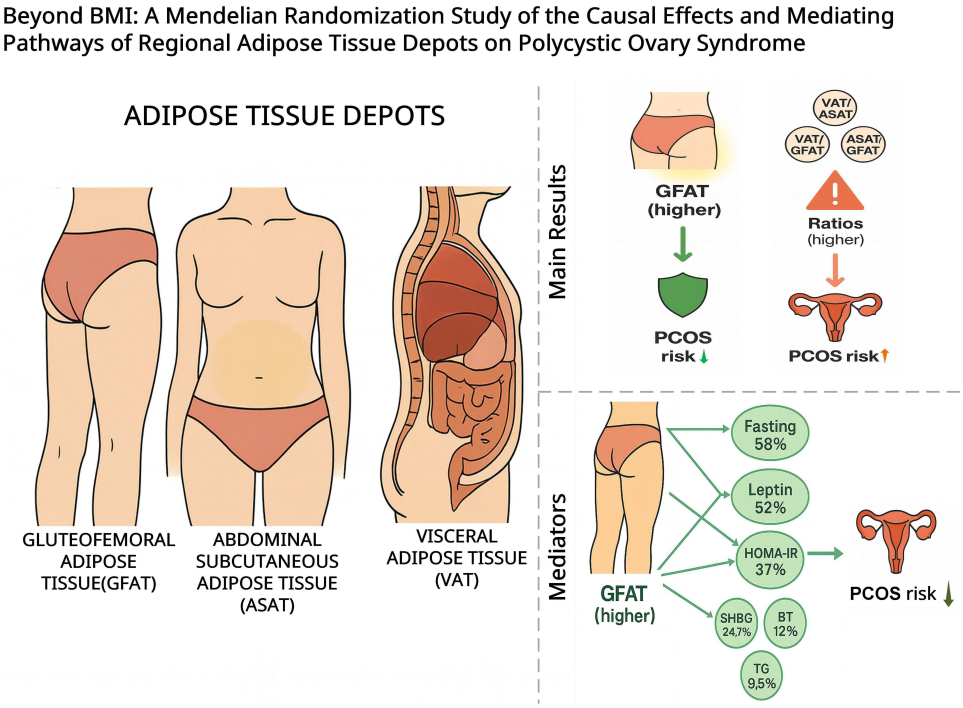
Keywords: Mendelian randomization, polycystic ovary syndrome, gluteofemoral adipose tissue, abdominal subcutaneous adipose tissue, visceral adipose tissue

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age, characterized by irregular menstrual cycles, hyperandrogenism, and polycystic ovarian morphology.[1] According to the World Health Organization (WHO), PCOS affects approximately 6–13% of reproductive-aged women globally; however, up to 70% of cases remain undiagnosed. It is a major cause of anovulatory infertility.[2] Patients with PCOS frequently experience

Received: 9 July 2025
Accepted: 15 September 2025
Published: 25 September 2025

Graphical Abstract



various adverse health outcomes, including reproductive dysfunction, metabolic disturbances, social stigmatization, and psychological disorders, which may persist throughout their lifetime.[3,4] The economic and health burden of PCOS is substantial, with health-care expenditures in the United States alone estimated at approximately 8 billion USD in 2020.[5] The precise etiology and pathogenesis of PCOS remain unclear due to its complexity involving interactions between genetic and environmental factors, thus complicating prevention strategies.[6] Therefore, identifying risk factors and elucidating the underlying mechanisms of PCOS are essential for developing effective early detection and intervention strategies.

Obesity is recognized as a critical factor associated with PCOS, exacerbating insulin resistance and potentially leading to more severe hyperandrogenism.[7] Body Mass Index (BMI) is commonly used clinically to assess body composition and preliminarily identify high-risk individuals. Approximately half of PCOS patients are overweight or obese; however, 40–50% of PCOS patients maintain a normal BMI but exhibit excessive truncal-abdominal fat deposition compared to BMI-matched healthy controls, suggesting that fat distribution may be more critical than total body fat.[8] Traditional BMI measures cannot adequately reflect specific fat distribution patterns; thus, alternative metrics such as waist circumference (WC), hip circumference (HC), and waist-to-hip ratio (WHR) have been proposed. Mendelian Randomization (MR) analyses have supported causal associations of BMI, WC, and HC—but not WHR—with PCOS.[9–11] Furthermore, according to the WHO, body fat distribution is a superior predictor of metabolic abnormalities compared to overall obesity.[12] Recent large-scale imaging and genetic studies have further refined fat distribution into gluteofemoral adipose tissue (GFAT), abdominal subcutaneous adipose tissue (ASAT), and visceral adipose tissue (VAT), revealing distinct genetic backgrounds and biological functions for each fat depot.[13] Accumulating evidence indicates that GFAT is metabolically protective relative to abdominal depots.[14–17] GFAT functions as a "metabolic sink" for excess free fatty acids, limiting ectopic lipid deposition and insulin resistance,[16] and is associated with a more favorable endocrine milieu—higher adiponectin and leptin, lower pro-inflammatory cytokines, higher aromatase activity, and higher circulating SHBG with lower free androgens—whereas visceral adiposity is linked to

hyperinsulinemia, inflammation, and reduced hepatic SHBG synthesis.[14,15,17] These contrasts provide a biologic rationale to hypothesize that depot-specific adiposity differentially influences PCOS risk. Clarifying the causal relationships between different fat distributions and PCOS risk could provide novel targets and directions for preventive and intervention strategies.

Since conventional observational studies struggle to distinguish causal relationships from mere associations, the present study employed MR (an epidemiological approach utilizing genetic variants significantly associated with exposure factors as instrumental variables) to investigate BMI-independent fat distribution's causal relationship with PCOS. Genetic variants used as instrumental variables (IVs) follow a random distribution similar to randomized controlled trials when transmitted from parents and are independent of environmental and other genetic factors, thus minimizing traditional confounding.[18] Therefore, MR effectively reduces confounding bias and prevents reverse causality. Additionally, this study incorporates genetic mediation analyses to explore potential biological pathways linking fat distribution to PCOS. The study aims to elucidate the causal effects of regional fat depots on PCOS and identify modifiable mediators, providing scientific evidence for understanding PCOS pathogenesis and developing targeted prevention and intervention strategies.

Methods

Study Design

The reporting of this study adheres to the STROBE-MR guidelines[19](Supplementary material-STROBE-MR checklist). Selection of single nucleotide polymorphisms (SNPs) from large-scale genome-wide association study (GWAS) data adheres to the three core assumptions of MR: 1) relevance, with significant associations to exposure phenotypes; 2) independence from confounding factors; and 3) exclusivity of effects on outcomes through exposure pathways only.[20] The exposures considered in this study are MRI-derived volumes of GFAT, ASAT, and VAT, adjusted for BMI and height. Initially, two-sample MR analyses were conducted to assess their causal relationships with PCOS. Additionally, the study evaluated three fat distribution ratios—VAT/ASAT, VAT/GFAT, and ASAT/GFAT—to comprehensively represent the relative distribution patterns among various anatomical regions and potentially offer additional explanatory power beyond individual fat volumes. For exposures demonstrating significant causal associations, genetic mediation analyses were further conducted to explore the roles of five potential mediator categories: sex hormone levels, lipid metabolic characteristics, glucose metabolism traits, adipose-specific factors, and inflammatory markers. Moreover, reverse causality analyses were performed to assess whether PCOS could causally affect fat distribution.

Selection of Genetic Instruments and Data Sources

All data utilized in this study were publicly available GWAS summary statistics. Ethical approval, clinical registration, and patient consent were previously obtained in original studies. This study makes exclusive use of secondary data that are openly accessible to the public; consequently, neither ethics-committee approval nor clinical trial registration was necessary. Under the "Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects" (China, in force since 18 February 2023), projects are exempt from ethical review when they satisfy either of the following criteria (Article 32, Items 1 and 2): ① they analyse lawfully obtained public-domain data or information gathered through non-invasive observation of public behaviour; ② they employ datasets that have been fully anonymised. Because our investigation meets both conditions, formal ethical review was not required. Detailed data sources are summarized in Table 1.

The regional fat distribution phenotypes were derived from MRI data of up to 38,965 participants in the UK Biobank. Agrawal et al employed an automated deep convolutional neural network-based model for adipose tissue segmentation and volume calculation.[13] To minimize the influence of general obesity, all adiposity phenotypes were adjusted for BMI and height using sex-specific linear regression models. The resulting residuals, after standardization, were employed as phenotypes in subsequent genetic analyses. Publicly available GWAS data included overall and sex-specific analyses (male and female). These adjusted volumes, independent of BMI and height, effectively represented localized fat accumulation, demonstrating near-zero correlation with BMI and providing precise characterization of region-specific

Table 1 Detailed Information of Data Sources

Phenotype	GWAS Catalog ID	Ref	Ancestry	Sex	Participants
VAT	GCST90205221	35773277	EUR(96.9%)	Female	19,273 individuals
ASAT	GCST90205222	35773277	EUR(96.9%)	Female	19,273 individuals
GFAT	GCST90205223	35773277	EUR(96.9%)	Female	19,273 individuals
VAT/ASAT ratio	GCST90205224	35773277	EUR(96.9%)	Female	19,273 individuals
VAT/GFAT ratio	GCST90205225	35773277	EUR(96.9%)	Female	19,273 individuals
ASAT/GFAT ratio	GCST90205226	35773277	EUR(96.9%)	Female	19,273 individuals
PCOS	GCST90454205	40069456	EUR	Female	5,171 cases and 283,185 controls
PCOS	GCST90483500	40229599	EUR	Female	14,467 cases and 430,267 controls
FSH	GCST90483475	40229599	EUR	Female	51,396 individuals
LH	GCST90483478	40229599	EUR	Female	41,650 individuals
E2	GCST90483481	40229599	EUR	Female	92,969 individuals
Bioavailable testosterone	GCST90012102	32042192	EUR	Female	188,507 individuals
SHBG	GCST90012107	32042192	EUR	Female	189,473 individuals
Leptin	GCST90007312	32917775	EUR	Female	27,987 individuals
Adiponectin	GCST001465	22479202	EUR	Both(44%male,56%female)[a]	39,883 individuals
LDL-C	GCST90239658	34887591	EUR	Both(48%male,52%female)[a]	1,320,016 individuals
HDL-C	GCST90239652	34887591	EUR	Both(48%male,52%female)[a]	1,320,016 individuals
TG	GCST90239664	34887591	EUR	Both(48%male,52%female)[a]	1,320,016 individuals
TC	GCST90239676	34887591	EUR	Both(48%male,52%female)[a]	1,320,016 individuals
HOMA-IR	GCST005179	20081858	EUR	Both(45%male,55%female)[a]	37,037 individuals
FI	GCST90002238	34059833	EUR	Both(48%male,52%female)[a]	151,013 individuals
CRP	GCST90029070	35459240	EUR	Both(51%male,49%female)[a]	575,531 individuals

Notes: [a]Derived from sex-combined GWAS that were adjusted for sex and principal components, these mediators represent effects after controlling for sex differences. Therefore, they are considered appropriate for analyses. Ref: PMID.

adiposity. GWAS identified 250 independent common genetic variants, including 39 novel loci predominantly significant in females. Only female-specific GWAS data (n = *19,273*) were used in this study. SNP selection involved genome-wide significance thresholds (*p-value < 5e-08*) and stringent LD clumping ($r^2 < 0.001$ within 10MB), followed by harmonization with outcome datasets. SNPs with intermediate allele frequencies or allele inconsistencies were excluded. When SNPs from the exposure data were absent in the outcome datasets, the TwoSampleMR package automatically identified proxy SNPs. MR-Steiger filtering was conducted, outliers and weak genetic instruments (F-statistic < 10) were excluded. Because the regional adiposity phenotypes were inverse-normally transformed, causal estimates reflect a one-standard-deviation (1 SD) change on the Z-score scale—ie, a shift from the population median to the ~84th percentile. In the female UK Biobank imaging cohort, this corresponds, on average, to ≈ 1.5 L of VAT, 3.3 L of ASAT, and 3.2 L of GFAT after BMI- and height-adjustment.

The PCOS datasets included discovery and replication cohorts. The discovery dataset originated from Gualdo et al (5171 cases and 283,185 controls), derived from the Estonian Biobank and FinnGen consortium without sample overlap with exposure data.[21] The largest PCOS GWAS to date—from Venkatesh et al (14,467 cases, 430,267 controls)—contains a UK Biobank subset that overlaps with the exposure GWAS: 3746 cases and 260,413 female controls, totalling 19,273 shared participants (≈ 7% of the 264,159 individuals in the replication sample). As this proportion is well below the 15% threshold generally considered to induce appreciable winner's-curse bias, this GWAS was retained exclusively as a replication dataset.[22] Although the GWAS by Day et al (GCST007089) included a large sample comprising 10,074 cases and 103,164 controls (non-overlapping with UK Biobank), approximately 77% of the participants were derived from the 23andMe cohort, for which full summary statistics are not publicly available. Therefore, the present study utilized the dataset from Gualdo et al as the primary discovery dataset for subsequent analyses.

Mediator data were obtained from large consortium and GWAS datasets,[22–29] classified into five categories: (1) Sex hormones (proxied by follicle-stimulating hormone [FSH], luteinizing hormone [LH], estradiol [E2], bioavailable testosterone [BT], and sex hormone-binding globulin [SHBG]); (2) Lipid metabolism traits (proxied by low-density

lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], triglycerides [TG], and total cholesterol [TC]); (3) Glucose metabolism traits (proxied by homeostasis model assessment of insulin resistance [HOMA-IR] and fasting insulin [FI]); (4) Adipose-specific factors (proxied by leptin and adiponectin); and (5) Inflammation levels (proxied by C-reactive protein [CRP]). Sex hormone and leptin data were female-specific, while other mediators were derived from sex-combined GWAS adjusted for sex and principal components, representing effects controlled for sex differences. Thus, these mediators were considered appropriate for analyses involving female-specific outcomes (PCOS).

Statistical Analysis

Inverse variance weighting (IVW) was the primary analytical method.[30] False discovery rate (FDR) correction via the Benjamini–Hochberg procedure identified significant causal evidence ($P_{FDR\text{-}IVW}$ and *p-value* both <0.05) and suggestive evidence (*p-value* < 0.05, $P_{FDR\text{-}IVW}$ > 0.05). Heterogeneity was assessed using Cochran's Q test and quantified by I^2 statistics to guide method selection.[31] Supplementary analyses included MR-Egger, Weighted Median, dIVW,[32] cML, and BWMR[33]. Given limitations in single-cohort representativeness and statistical power, causal effects from two cohorts were combined using the "meta" package, applying stringent Bonferroni correction (significant if *p-value* < 0.00416 [0.05/12]). Horizontal pleiotropy was examined using MR-Egger regression[34] and MR-PRESSO,[35] with leave-one-out analyses verifying the robustness of positive findings. Considering limited statistical power compared to observational studies, explained variance (R^2) of exposure variables was calculated, and two-sample outcome statistical power was assessed via the online tool mRNA.

In mediation analyses, total, direct, and mediation effects were estimated to identify mediators.[36] Total effects represented overall impacts of exposures on outcomes, direct effects excluded mediation pathways, and mediation effects, calculated as the product of exposure-to-mediator and mediator-to-outcome effects, indicated indirect pathway contributions. Mediation proportion was the mediation-to-total effect ratio (mediation effect/total effect), with confidence intervals derived using the Delta method. Mediators were considered significant at *p-value* < 0.05.

Analyses were conducted in R (version 4.2.3), utilizing packages meta, TwoSampleMR, MVMR, MendelianRandomization, and MRPRESSO.

Results

Selection and Strength Assessment of Genetic Instruments

This study identified 52 genetic variants associated with BMI and height-independent regional adipose tissue distribution and corresponding ratios. Phenotypic variance explained (R^2) ranged from 0.87% to 4.80%: 9 SNPs for VAT (R^2≈2.05%), 3 for ASAT (R^2≈0.87%), 17 for GFAT (R^2≈4.80%), 10 for VAT/ASAT ratio (R^2≈2.41%), 6 for VAT/GFAT ratio (R^2≈1.17%), and 7 for ASAT/GFAT ratio (R^2≈1.74%). All selected SNPs passed the Steiger directionality test, ensuring the correct direction of association, and demonstrated F-statistics greater than 10, indicating robust genetic instruments. One SNP (rs9660318) was identified as an outlier and excluded. The remaining SNPs satisfied the three core MR assumptions. Detailed SNP information is provided in Table S1.

Two-Sample Mendelian Randomization Analysis

In the discovery dataset with no sample overlap (Figure 1), genetically predicted BMI- and height-independent GFAT volumes were significantly associated with a 15.5% reduction in PCOS risk per SD increase (OR = 0.845, 95% CI: 0.735–0.971, P_{FDR} = 0.036). Additionally, higher VAT/ASAT and ASAT/GFAT ratios were significantly associated with increased PCOS risk (OR = 1.298 per SD increase, 95% CI: 1.069–1.576, P_{FDR} = 0.027; OR = 1.435 per SD increase, 95% CI: 1.127–1.826, P_{FDR} = 0.018, respectively). However, no significant causal associations were observed for VAT volumes, ASAT volumes, or VAT/GFAT ratio (al*l p-value* > 0.05 and P_{FDR} > 0.05). Complementary analyses (dIVW, cML, BWMR) largely supported the primary results with consistent directions and significant associations, except for VAT/ASAT ratio. The weighted median method was consistent in direction, achieving significance only for ASAT/GFAT ratio. MR-Egger produced larger effect sizes and wider confidence intervals, yet no nominally significant results were observed due to limited statistical power under the assumption of invalid instruments.

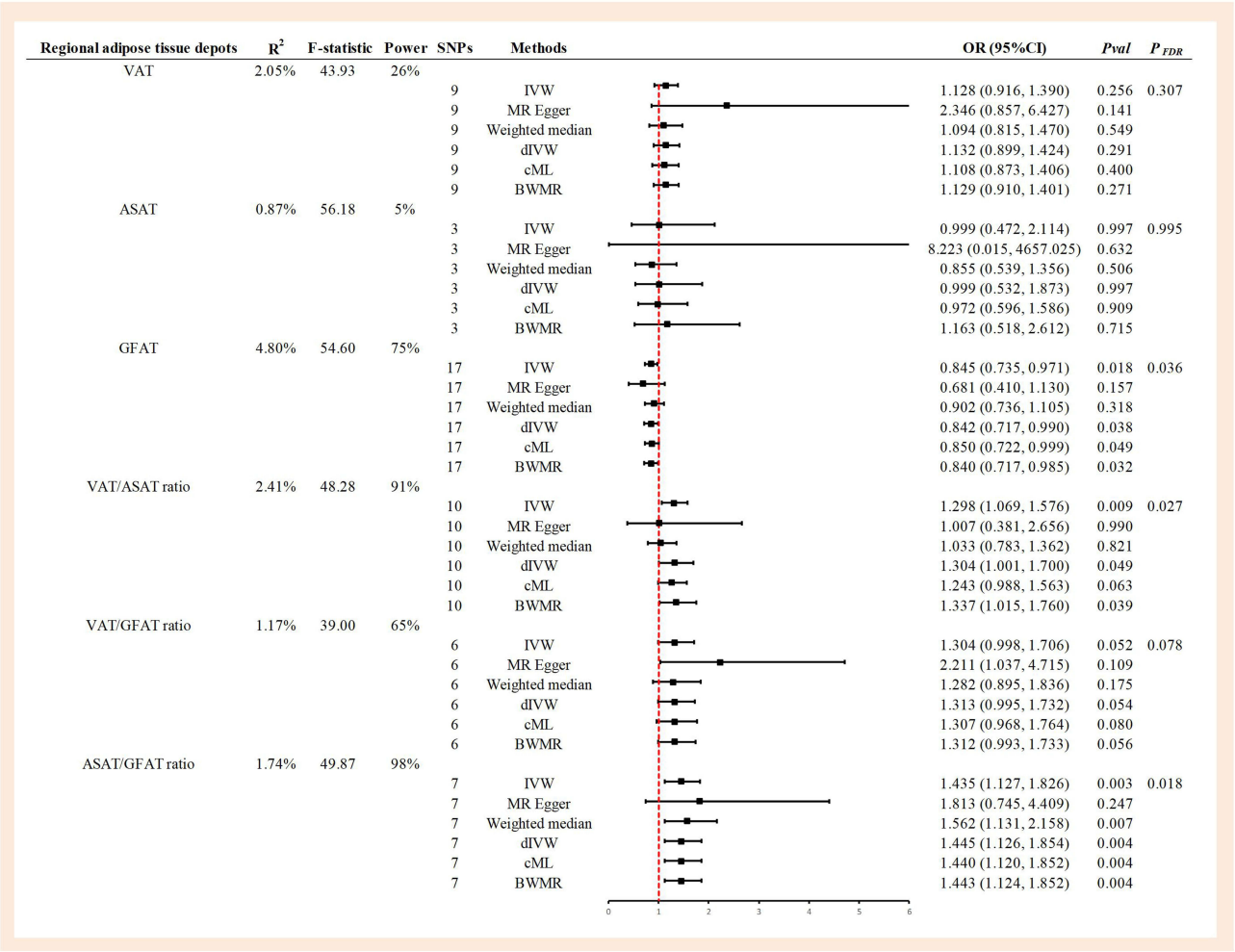


Figure 1 Mendelian randomization analysis of different regional adipose tissue depots on PCOS. VAT, ASAT, and GFAT values were adjusted for BMI and height. Causal associations were considered statistically significant only when both the FDR-adjusted *p-value* (P_{FDR}) and the original p-value (Pval) were less than 0.05.

Significant heterogeneity was detected solely in ASAT analyses (I^2 = 80%, Cochran's Q-*p-value* = 0.005), prompting the use of random-effects models, whereas fixed-effects models were applied in other analyses without heterogeneity evidence. Neither MR-Egger nor MR-PRESSO identified evidence of horizontal pleiotropy (*p-value* > 0.05; Table S2). Adequate statistical power was confirmed for all positive results. Leave-one-out analyses confirmed the robustness of GFAT results, but indicated rs73221948 (CDCA2, EBF2) and rs3936510 (C5orf67) drove VAT/ASAT and ASAT/GFAT ratios' associations with PCOS, respectively (Figure S1). Reverse causation analyses yielded no significant findings (*p-value* > 0.05; Table S3).

Replication dataset analyses validated the causal associations observed between GFAT volumes, ASAT/GFAT ratio, and PCOS, showing enhanced genetic effects and statistical significance (Table S4). A meta-analysis of both datasets with stringent Bonferroni correction (Figure 2) robustly supported the protective role of BMI- and height-independent GFAT volumes against PCOS (OR = 0.845 per SD increase, 95% CI: 0.735–0.971, *p-value* = 3.65e-04). Elevated VAT/ASAT, VAT/GFAT, and ASAT/GFAT ratios were also associated with increased PCOS risk. Given findings from leave-one-out analyses and novel evidence for VAT/GFAT ratio, these ratio-based results should be cautiously interpreted as suggestive.

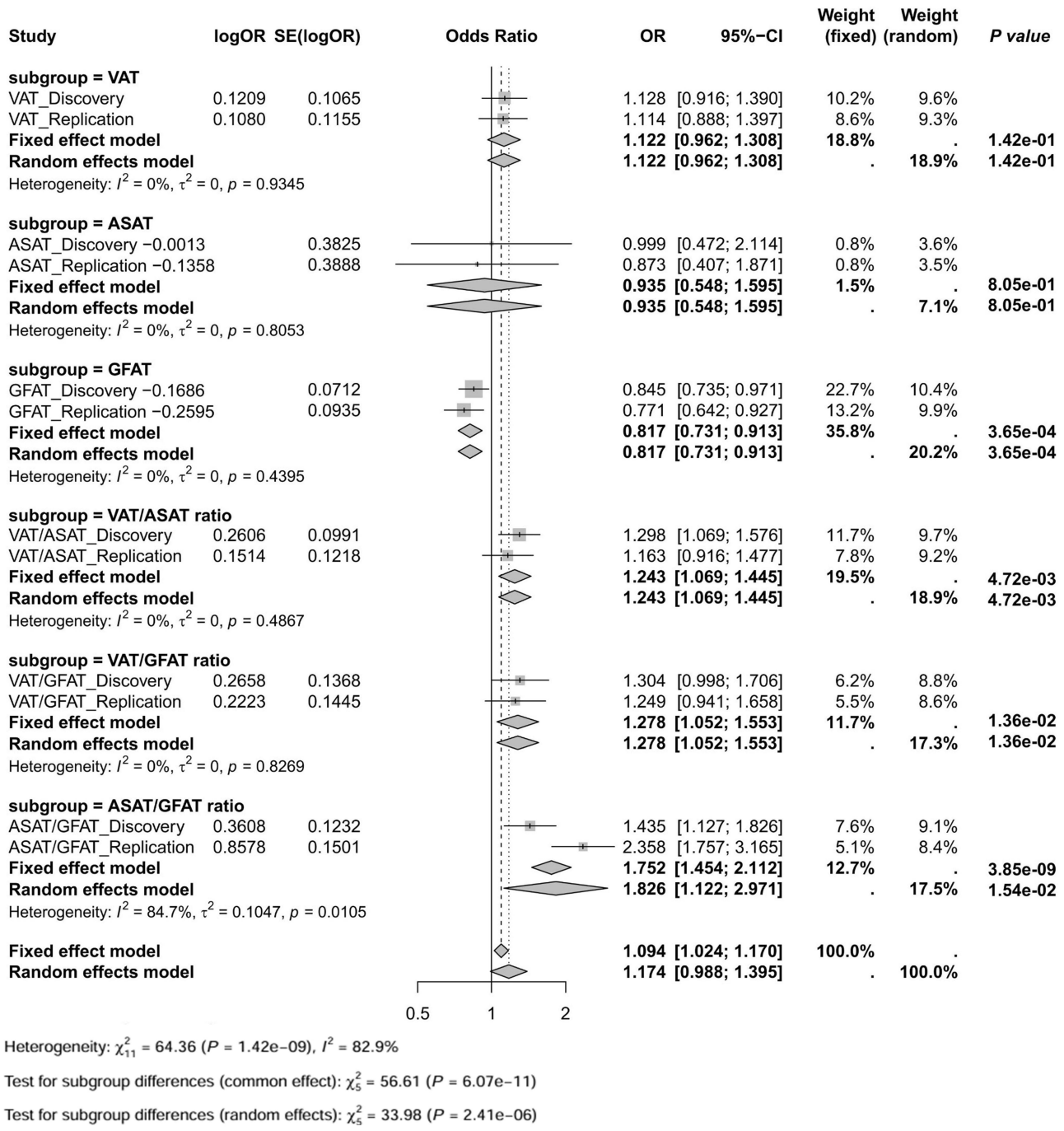


Figure 2 Meta-analysis combining discovery and replication datasets. The I² statistic was calculated to assess heterogeneity and determine the appropriate effect model: a fixed-effect model was applied when I² < 50%, otherwise a random-effect model was used. Statistical significance in the meta-analysis was determined using a more stringent Bonferroni correction; a *p-value* < 0.00416 (0.05/12) was considered indicative of a significant causal association.

Mediation Analysis

Based on the significant causal relationships identified, mediation analyses further examined associations between GFAT volumes (adjusted for BMI and height) and five mediator categories in PCOS (Table 2). Specifically, GFAT influenced PCOS risk by reducing BT, FI, leptin, HOMA-IR, and TG levels and increasing SHBG levels (Figure 3A). FI and leptin emerged as significant mediators, explaining the largest proportions of mediated effects (58.37%, 95% CI: 27.66–89.08%; and 51.75%, 95% CI: 33.54–75.41%, respectively). Additional mediators, ranked by the proportion of

Table 2 Mediation Mendelian Randomization Analysis

Mediator	Confidence Interval of Total Effect (95% CI)	Confidence Interval of Direct Effect (95% CI)	Confidence Interval of Mediation Effect (95% CI)	Proportion of Mediation Effect (%)	Confidence Interval of the Proportion of Mediation Effect	*Pval*
FSH	−0.169 (−0.308, −0.029)	−0.149 (−0.292, −0.007)	−0.019 (−0.046, 0.008)	11.40%	−4.60% ~ 27.40%	0.150
LH	−0.169 (−0.308, −0.029)	−0.160 (−0.301, −0.018)	−0.009 (−0.033, 0.016)	5.25%	−9.37% ~ 19.87%	0.439
E2	−0.169 (−0.308, −0.029)	−0.168 (−0.308, −0.028)	−0.001 (−0.015, 0.013)	0.46%	−7.84% ~ 8.76%	0.824
BT	−0.169 (−0.308, −0.029)	−0.148 (−0.289, −0.008)	−0.020 (−0.033, −0.008)	11.99%	4.62% ~ 19.35%	0.001
SHBG	−0.169 (−0.308, −0.029)	−0.127 (−0.268, 0.014)	−0.042 (−0.060, −0.024)	24.74%	14.18% ~ 35.30%	4.03E-06
Leptin	−0.169 (−0.308, −0.029)	−0.026 (−0.221, 0.169)	−0.143 (−0.279, −0.007)	51.75%	33.54% ~ 75.41%	0.039
Adiponectin	−0.169 (−0.308, −0.029)	−0.155 (−0.297, −0.012)	−0.014 (−0.042, 0.015)	8.30%	−8.61% ~ 25.20%	0.332
LDL-C	−0.169 (−0.308, −0.029)	−0.169 (−0.308, −0.029)	0.000 (−0.004, 0.004)	−0.03%	−2.39% ~ 2.33%	0.979
HDL-C	−0.169 (−0.308, −0.029)	−0.168 (−0.308, −0.029)	0.000 (−0.004, 0.003)	0.20%	−1.97% ~ 2.36%	0.853
TG	−0.169 (−0.308, −0.029)	−0.153 (−0.293, −0.012)	−0.016 (−0.027, −0.005)	9.52%	2.77% ~ 16.28%	0.005
TC	−0.169 (−0.308, −0.029)	−0.168 (−0.307, −0.028)	−0.001 (−0.004, 0.002)	0.59%	−1.46% ~ 2.64%	0.569
HOMA-IR	−0.169 (−0.308, −0.029)	−0.231 (0.382, −0.080)	0.063 (0.005, 0.120)	37.20%	3.10% ~ 71.40%	0.029
FI	−0.169 (−0.308, −0.029)	−0.070 (−0.219, 0.079)	−0.098 (−0.150, −0.047)	58.37%	27.66% ~ 89.08%	1.81E-04
CRP	−0.169 (−0.308, −0.029)	−0.168 (−0.308, −0.029)	0.000 (−0.001, 0.001)	0.001%	−0.55% ~ 0.68%	0.745

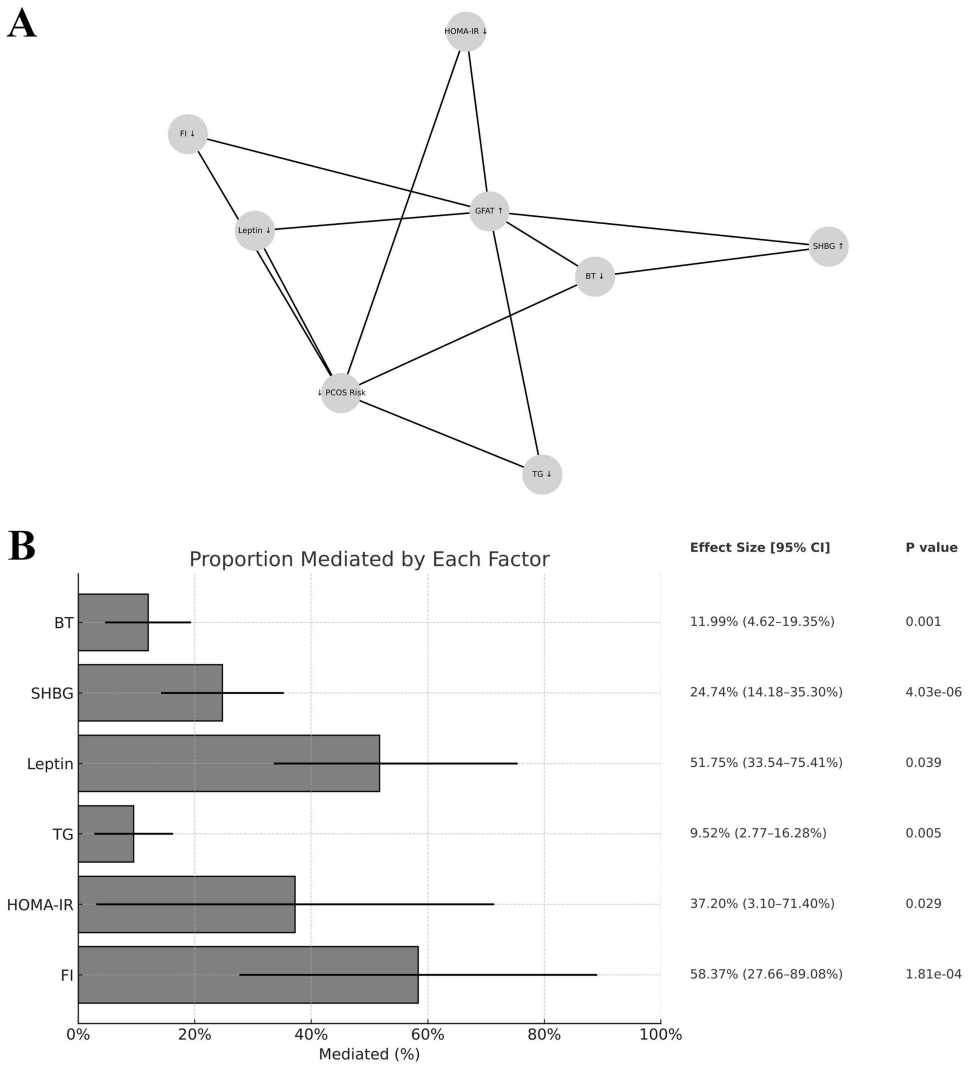


Figure 3 Mediation Mendelian randomization analysis of the GFAT–PCOS association. (**A**) Directed network summarizing significant pathways. Node symbols indicate the direction of change associated with higher GFAT: "↑" = increase, "↓" = decrease (eg, SHBG ↑, BT ↓). "↓ PCOS Risk" denotes a reduced risk of PCOS. Arrows depict the putative causal directions inferred from two-step MR (GFAT → mediator and mediator → PCOS). The protective effect of higher GFAT is consistent with decreases in BT, FI, leptin, HOMA-IR, and TG and an increase in SHBG. (**B**) Proportion of the total GFAT→PCOS effect mediated by each statistically significant mediator (|indirect effect| ÷ total effect), shown as point estimates with 95% confidence intervals (error bars) and two-sided P values.

effect mediated, were HOMA-IR (37.20%, 95% CI: 3.10–71.40%), SHBG (24.74%, 95% CI: 14.18–35.30%), BT (11.99%, 95% CI: 4.62–19.35%), and TG (9.52%, 95% CI: 2.77–16.28%; Figure 3B).

Discussion

This MR study extends beyond traditional obesity metrics, such as BMI and WHR, to investigate causal associations and modifiable mediation pathways involving regional adipose tissue depots and ratios in PCOS. Our findings demonstrated robust evidence supporting a protective effect of BMI- and height-independent GFAT volumes against PCOS, mediated through BT, FI, leptin, HOMA-IR, TG, and SHBG. Additionally, suggestive evidence linked elevated VAT/ASAT, VAT/GFAT, and ASAT/GFAT ratios to increased PCOS risk. Reverse causation analyses revealed no evidence of PCOS genetic susceptibility influencing adipose tissue distribution.

This study provides robust causal evidence supporting a protective effect of GFAT volume on PCOS risk, independent of BMI and height. These results indicate that women with greater GFAT volume, at a given overall obesity level, exhibit a lower risk of developing PCOS. In women, gluteofemoral fat distribution ("pear-shaped" obesity) is increasingly recognized for its metabolically protective role. Compared to abdominal VAT, higher GFAT quality is independently

associated with improved insulin sensitivity and favorable metabolic profiles.[17] Women with higher GFAT demonstrate lower FI levels, reduced HOMA-IR, and lower circulating TG. Conversely, abdominal fat accumulation, particularly visceral fat, promotes insulin resistance and hypertriglyceridemia.[14,16] Mechanistically, GFAT acts as a metabolic "sink", safely sequestering excess free fatty acids and preventing ectopic lipid deposition in the liver and muscle, thus mitigating lipotoxicity and insulin resistance.[16,37]

GFAT also confers protective effects through distinctive endocrine functions. This lower-body subcutaneous adipose depot is associated with a healthier adipokine profile, characterized by elevated adiponectin and leptin levels and reduced pro-inflammatory cytokines, opposite to visceral fat.[14,17] Indeed, GFAT secretes higher leptin levels than VAT, and leptin concentrations rise with increasing GFAT, negatively correlating with the waist-to-hip ratio.[38] Additionally, the "pear-shaped" fat distribution in women typically coincides with higher circulating SHBG and lower free BT levels, reflecting a more favorable endocrine-metabolic state.[15] In contrast, abdominal obesity, especially visceral obesity, suppresses hepatic SHBG synthesis due to hyperinsulinemia, thereby elevating free testosterone—a common feature in insulin-resistant conditions like metabolic syndrome or PCOS.[15] Moreover, GFAT exhibits higher aromatase activity, particularly in postmenopausal women, fostering a more estrogenic environment that may counteract some harmful effects of visceral fat.[39] These findings are further supported by our mediation analysis. Collectively, these distinctions highlight GFAT as an active endocrine organ rather than merely a passive energy reservoir, actively modulating insulin sensitivity, lipid metabolism, and sex hormone bioavailability, thus safeguarding female metabolic health.

Recent research has indicated that lean women with PCOS, who have normal-range BMI, typically accumulate more fat in trunk, whole-body, abdominal, ASAT, and VAT regions.[8] This is supported by previous MR studies identifying a causal relationship between VAT and PCOS risk.[40,41] Interestingly, our study did not observe nominally significant associations between VAT and PCOS risk. This discrepancy might primarily stem from key methodological differences. Firstly, the VAT GWAS data utilized in our analysis were adjusted for BMI and height, thus specifically evaluating fat distribution effects independent of BMI. In contrast, previous studies frequently employed unadjusted VAT data, potentially capturing indirect pathways mediated by BMI. Secondly, our study used female-specific GWAS data, more suitable for investigating PCOS—a female-specific disorder. Prior research often relied on sex-combined GWAS data, which, despite adjustments for sex and age principal components, may lack sensitivity and biological relevance when examining traits with pronounced sexual dimorphism, such as fat distribution and reproductive disorders.

From a translational perspective, incorporating depot-specific adiposity metrics—particularly GFAT volume and VAT-to-GFAT ratios—into imaging- or bioimpedance-based risk models could improve early identification of women at heightened PCOS risk, even at normal BMI. These metrics may guide personalised management: patients with a low GFAT-to-VAT balance might benefit from metabolic-targeted strategies (eg, visceral-fat–reducing diet–exercise programmes, GLP-1 receptor agonists, insulin-sensitising agents), whereas those with adequate GFAT reserves but persistent hyperandrogenism may remain candidates for conventional hormonal modulation. Future interventional trials stratified by regional fat distribution are warranted to determine whether shifting adiposity towards a more gluteofemoral pattern, or selectively lowering VAT, improves reproductive and metabolic outcomes in PCOS. Looking ahead, adolescent screening for at-risk fat-distribution patterns (eg, a low GFAT-to-VAT balance) could enable earlier lifestyle or metabolic interventions to reduce subsequent PCOS risk.

Several strengths characterize this study. Notably, it extends beyond conventional obesity phenotypes to systematically evaluate the multidimensional impact of biologically defined, BMI-independent fat distribution on PCOS. Comprehensive quality control measures, including supplementary validation methods and sensitivity analyses, were implemented. Additionally, our approach integrated discovery and replication cohorts followed by meta-analysis, enhancing the generalizability and robustness of our findings. The mediation analyses further elucidated multiple metabolic and endocrine pathways, offering early clinical signals for PCOS risk assessment and encouraging therapeutic exploration shifting from hormone replacement to targeted metabolic modulation. Our mediation-MR interpretation relies on standard assumptions—valid instruments for each step, linearity without strong effect modification, and no unmeasured mediator–outcome confounding beyond the genetic instruments—which warrant confirmation as larger female-specific datasets become available. Future laboratory and clinical experiments—such as leptin- or insulin-modulation

studies in animal models or pilot trials—will be essential to confirm the mechanistic pathways suggested by our genetic mediation findings.

However, this study has several limitations. First, consistent with most MR studies, all exposure, mediator, and outcome GWAS were derived from participants of European ancestry, which restricts the transferability of our findings. Allele-frequency differences, linkage-disequilibrium architecture and environmental exposures vary across populations, potentially altering instrument strength and pleiotropic profiles. Searches of the GWAS Catalog up to 20 June 2025 revealed no sufficiently powered, publicly accessible summary data for non-European groups (eg, East Asians). Future work should therefore generate ancestry-specific GWAS of regional adiposity using large resources such as the China Kadoorie Biobank and apply the present multistage MR-mediation framework in multi-ethnic settings, followed by trans-ethnic MR meta-analyses to test for heterogeneity and assess replicability. Second, several mediator GWAS (eg, LDL-C, HOMA-IR, CRP) were sex-combined datasets adjusted for sex and principal components because fully female-specific data were unavailable; residual sexual heterogeneity may have attenuated or inflated certain indirect-effect estimates, so these mediation results should be considered provisional until female-only GWAS become available. Third, although horizontal pleiotropy was evaluated with MR-PRESSO and MR-Egger, it can never be fully ruled out. To strengthen inference, we additionally applied three complementary, pleiotropy-robust estimators—dIVW, cML and BWMR—all of which produced effect estimates concordant with the primary IVW results. Fourth, although the replication GWAS included a modest UK Biobank overlap (≈ 7% of its sample) with the exposure GWAS, this is below the commonly accepted 15% threshold for substantial bias; nonetheless, a discovery-only sensitivity analysis yielded directionally consistent estimates, suggesting minimal impact. Finally, while MR offers robust statistical evidence for causation, it cannot capture dynamic lifestyle or environmental modifiers of adipose distribution; therefore, triangulation with observational, prospective and family-based MR studies —ideally incorporating longitudinal data on diet, physical activity, and other exposures—is required to corroborate and fully contextualise these relationships.

Conclusion

To encapsulate, this MR study explored causal associations between regional adipose tissue depots, independent of BMI and height, and PCOS risk. GFAT exhibited significant protective effects, with subsequent mediation analyses highlighting various metabolic and hormonal pathways involved, particularly through reductions in FI, leptin, HOMA-IR, BT, and TG levels, along with increased SHBG levels. Additionally, suggestive evidence indicated increased PCOS risk associated with elevated VAT/ASAT, VAT/GFAT, and ASAT/GFAT ratios. Reverse analyses found no causal influence of PCOS genetic susceptibility on regional adiposity distribution. Clinically, integrating GFAT volume and VAT-to-GFAT ratios into imaging-based screening algorithms could improve early risk stratification and guide metabolic-targeted interventions for PCOS. Nevertheless, because our results are derived from European-ancestry cohorts and rely on genetic inference, replication in diverse populations and functional studies (eg, adipose-tissue or endocrine interventions) are needed before routine clinical application. These findings support using adipose tissue distribution as an early risk marker and therapeutic target, promoting a shift in PCOS treatment strategies from singular hormonal control toward comprehensive metabolic regulation.

Data Sharing Statement

The data used in this study are from publicly available downloadable GWAS data, available from Table 1.

Acknowledgments

We thank all GWAS participants and investigators for publicly making the summary statistics data available.

Ethics Statement

This study makes exclusive use of secondary data that are openly accessible to the public; consequently, neither ethics-committee approval nor clinical trial registration was necessary. Under the "Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects" (China, in force since 18 February 2023), projects are exempt from ethical review when they satisfy either of the following criteria (Article 32, Items 1 and 2): ① they analyse lawfully

obtained public-domain data or information gathered through non-invasive observation of public behaviour; ② they employ datasets that have been fully anonymised. Because our investigation meets both conditions, formal ethical review was not required.

Funding

This work was supported by Medical Science Research Project of Hebei (20211066).

Disclosure

The authors report no conflicts of interest in this work.

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