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Landscape of sex differences in obesity and type 2 diabetes in subcutaneous adipose tissue: a systematic review and meta-analysis of transcriptomics studies

Roxana Andreea Moldovan [a,b], Marta R. Hidalgo [a,c], Helena Castañé [d], Andrea Jiménez-Franco [d], Jorge Joven [d], Deborah J. Burks [e,f], Amparo Galán [e,f,g,*,1], Francisco García-García [a,**,1]

[a] Computational Biomedicine Laboratory, Príncipe Felipe Research Center (CIPF), 46012, Valencia, Spain
[b] Department of Applied Statistics and Operations Research and Quality, Universitat Politècnica de València, Valencia 46022, Spain
[c] Departament de Matemàtiques, Facultat de Matemàtiques, Universitat de València, 46010, Valencia, Spain
[d] Unitat de Recerca Biomèdica, Hospital Universitari de Sant Joan, Institut d'Investigació Sanitària Pere Virgili, Universitat Rovira i Virgili, 43204 Reus, Spain
[e] Molecular Neuroendocrinology Laboratory, Principe Felipe Research Center (CIPF), 46012, Valencia, Spain
[f] CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III, Madrid, Spain
[g] Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de València, 46010, Valencia, Spain

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ABSTRACT

Obesity represents a significant risk factor in the development of type 2 diabetes (T2D), a chronic metabolic disorder characterized by elevated blood glucose levels, and a previous step for its development. Significant sex differences have been identified in the prevalence, development, and pathophysiology of obesity and T2D; however, the underlying molecular mechanisms remain unclear. This study aims to identify sex-specific signatures in obesity and T2D and enhance our understanding of the underlying mechanisms associated with sex differences by integrating expression data.

We performed a systematic review and individual transcriptomic analysis of eight selected studies which included 302 subcutaneous adipose tissue samples. Then, we conducted different gene-level meta-analyses and functional characterizations for obesity and T2D separately, identifying common and sex-specific transcriptional profiles, many of which were previously associated with obesity or T2D.

The obesity meta-analysis yielded nineteen differentially-expressed genes from a sex-specific perspective (e.g., *SPATA18*, *KREMEN1*, *NPY4R*, and *PRM3*), while a comparison of the expression profiles between sexes in T2D prompted the identification and validation of specific transcriptomic signatures in males (*SAMD9*, *NBPF3*, *LDHD*, and *EHD3*) and females (*RETN*, *HEY1*, *PLPP2*, and *PM20D2*). At the functional level, we highlighted the fundamental role of the Wnt pathway in the development of obesity and T2D in females, and the roles of mitochondrial damage and free fatty acids in males.

Overall, our sex-specific meta-analyses supported the detection of differentially expressed genes in males and females associated with the development of obesity and further T2D development, emphasizing the relevance of sex-based information in biomedical data and opening new avenues for research.

Abbreviations: BH, Benjamini & Hochberg; BP, Biological Processes; DGE, Differential Gene Expression Analysis; F, female; FDR, False Discovery Rate; GEO, Gene Expression Omnibus; GO, Gene Ontology; GSEA, Gene Set Enrichment Analysis; ID, Impact of Diabetes; IDM, Impact of T2D in Males; IDF, Impact of T2D in Females; IO, Impact of Obesity; IOF, Impact of Obesity in Females; IOM, Impact of Obesity in Males; logFC, logarithm 2 of the Fold Change; logOR, logarithm 2 of the Odds Ratio; M, Male; qPCR, quantitative PCR; SAT, Subcutaneous Adipose Tissue; SDIO, Sex-Differential Impact of Obesity; SDID, Sex-Differential Impact of T2D; T2D, Type 2 Diabetes; VAT, Visceral Adipose Tissue; WAT, White Adipose Tissue.

* Correspondence to: A. Galán, Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de València, 46010, Valencia, Spain.
** Corresponding author at: Computational Biomedicine Laboratory, Principe Felipe Research Center (CIPF), C/Eduardo Primo Yúfera, 3, 46012, Valencia, Spain.
E-mail addresses: amparo.galan@uv.es (A. Galán), fgarcia@cipf.es (F. García-García).
[1] These authors contributed equally to this work.

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1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to insulin resistance or insulin deficiency. Depending on disease etiology, diabetes has been classified into several categories, with type 2 diabetes (T2D) being the most prevalent, accounting for >90 % of global diabetes cases. T2D results from the dysregulation of carbohydrate, lipid, and protein metabolism due to insulin resistance at the peripheral level and simultaneous pancreatic β-cell dysfunction. It represents a global health concern, with a current incidence of ~9.8 % of the world's population and a steadily increasing trend. Although T2D has a minor genetic component, lifestyle, age, and weight are substantial contributing factors, obesity being recognized as a major risk factor for the onset and progression of the disease [1–4].

Obesity - a chronic, multifactorial, and treatable disorder of neurobehavioral origin - is characterized by increased body fat, leading to adipose tissue dysfunction, which significantly impairs metabolic homeostasis and negatively affects metabolic, biomechanical, and psychosocial health [5]. The literature describes two main types of adipose tissue based on their structural characteristics, coloring, vascularization, and function: white adipose tissue (WAT) and brown adipose tissue [6].

WAT primarily stores energy in the form of triglycerides, the quantity of which increases with age and body weight. Under normal conditions, insulin acts on WAT via the PI3K/AKT signaling pathway, stimulating adipogenesis, inhibiting lipolysis, and increasing fatty acid and glucose uptake [7]. In pathological conditions such as obesity, adipose tissue dysregulation leads to the release of fatty acids and metabolic intermediates into the bloodstream, resulting in increased β-oxidation [8], oxidative stress [9], and the secretion of pro-inflammatory hormones and cytokines [10]. These processes contribute to insulin resistance and T2D development via multiple signaling pathways, including PI3K/AKT, RAS/MAPK, and Wnt [7,11]. Furthermore, inflammation induced by obesity, among others, can trigger diabetes provoking changes in insulin sensitivity [12].

WAT is subdivided into two main deposits: visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) [6]. The distribution and amount of body fat critically influence insulin sensitivity. Marked differences in gene expression patterns related to T2D exist between VAT and SAT, with VAT exhibiting a more pronounced and characteristic immunological pattern even in early disease stages [13]. Conversely, SAT displays a milder inflammatory profile, overexpressing genes in the RAS/MAPK pathway and downregulating peroxisome proliferator-activated receptor gamma (PPAR-γ), thus impairing adipogenesis [14]. These factors substantially elevate T2D risk, positioning SAT not only an early T2D marker, but also a developing risk marker, and a crucial nexus between T2D and obesity despite being less well-characterized than VAT [15].

The historical underrepresentation of sex as a biological variable and the pursuit of unified diagnostic and treatment criteria have led to the neglect of physiological sex differences in many diseases, including diabetes [16]. In relation to our focus, critical aspects of metabolic homeostasis display differential regulation between males and females [17]. Sexual dimorphism, an evolutionary paradigm where females exhibit greater resistance to energy reserve loss in fat form, is crucial in metabolic homeostasis, obesity, and T2D [18]. Genetic sex, prenatal testosterone effects in males, and sex hormone activity during puberty [17,19] contribute to sexual biases in fat distribution, body mass index, obesity prevalence, and T2D susceptibility, progression, diagnosis, and treatment [20–22]. In this context, VAT constitutes the primary type of fat storage in males, whereas females primarily accumulate SAT [20–23]. Adipose tissue constitutes 12–15 % of male body weight but 20–27 % of female's [2]; furthermore, diabetes prevalence in females aged 20–79 remains slightly lower than in males (10.2 % vs. 10.8 %) [3].

While these clinical differences are recognized, the underlying molecular mechanisms remain unknown. Systematic reviews with meta-analysis are essential tools for integrating results to address fundamental biological questions and public data repositories offer new opportunities to explore not only clinical information but also omic data [24–26].

Thus, this study aims to characterize sex-specific differences associated with obesity and its subsequent progression to T2D at the genetic and functional level by integrating gene expression data of SAT and VAT available in public repositories. Our results reveal unique male- and female-specific SAT transcriptional signatures, highlighting critical pathways involved in the development of obesity and the progression from obesity to T2D.

2. Materials and methods

2.1. Systematic review and study selection

A comprehensive systematic review, following the PRISMA guidelines [27], was conducted in March 2023 to identify transcriptomics studies available in the Gene Expression Omnibus (GEO) and ArrayExpress databases (period: 2002–2022) and the PubMed bibliographic search engine. This search was based on the following inclusion criteria: i) keywords: "obesity," "diabetes 2," "type 2 diabetes," "insulin resistance"; ii) organism: "*Homo sapiens*" or "*Mus musculus*"; iii) study type: "expression profiling by array" or "expression profiling by high throughput sequencing"; iv) sample count: ≤ 12 samples; v) tissue: "adipose tissue"; and vi) sex information: "gender" or "sex." Subsequently, studies were filtered based on predefined exclusion criteria: i) experimental designs different from the comparison of cases and controls of obese and non-obese patients and/or insulin-sensitive and insulin-resistant individuals; ii) absence of information or representation of both sexes; iii) insufficient sample size (less than two samples per sex and group); and iv) tissue other than adipose. Two independent reviewers (RAM and AG) examined the articles based on the eligibility criteria described above. The level of agreement was quantified using the kappa coefficient. When there were disagreements, these were resolved by the judgement of the third author (FGG). A more detailed explanation of these criteria can be found in Supplementary File 1.

2.2. Bioinformatic analysis

The *in-silico* workflow involved five steps: i) data retrieval and preprocessing; ii) differential gene expression analysis (DGE); iii) DGE results integration using a meta-analysis-based approach; and iv) functional analysis. Steps i-ii were performed independently for each study, while steps iii-iv integrated data for obesity and T2D separately and obtained disease-specific global results. All bioinformatics and statistical analysis were conducted in R (v4.2.0); Table S1 details packages used.

2.3. Data retrieval and preprocessing

Data were downloaded using the *GEOquery* and *ArrayExpress* packages. For microarray datasets, normalized count matrices obtained through quantile normalization were used and assessed when available. Otherwise, raw CEL files were retrieved, and Robust Multi-array Average preprocessing was applied, including the background correction, quantile normalization, and log2 transformation using the *affy* package. For RNA-seq datasets, raw count matrices were acquired and transformed to log2 counts per million (CPM) using the *limma-voom* method to ensure comparability with microarray data. Probe annotations were mapped to NCBI Gene IDs using the AnnotationDbi package, and in cases of duplicated probe-to-gene mappings, the median expression value was calculated. Additionally, HUGO gene symbols were included to facilitate further interpretation.

Phenotypic variables were harmonized by categorizing patients and samples by sex (M: male; F: female), phenotype (Control: normal weight healthy patients; Obesity: non-diabetic obese patients; T2D: obese with

type 2 diabetes patients), and tissue (SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue).

Exploratory analysis, including box plots, hierarchical clustering based on Pearson correlation and Euclidean distance, and principal component analysis (PCA), were conducted to detect patterns, outliers or batch effects. Detected batch effects were corrected using the *sva* package.

2.4. Differential gene expression analysis

Differential gene expression analysis (DGE) was designed to explore two primary transitions: the progression from healthy lean to obese individuals (obesity comparison/transition/progression), and from obese to T2D obese individuals (T2D comparison/transition/progression). Additionally, the analysis examined the role of sex in these processes through specific comparisons. In summary, performed comparisons include, for obesity: i) the overall impact of obesity (IO): obese vs. control (Ob - C); ii) the impact of obesity on males (IOM): Obese males vs. Control males (Ob.M - C.M); iii) the impact of obesity on females (IOF): Obese females vs. Control females (Ob.F - C.F); iv) the sex-differential impact of obesity (SDIO): the difference between male and female comparisons [(Ob.M - C.M) - (Ob.F - C.F)]. And, for T2D: i) the overall impact of T2D (ID): T2D vs. Obese non-diabetic individuals (T2D - Ob); ii) the impact of T2D on males (IDM): T2D males vs. Obese non-diabetic males (T2D.M - Ob.M); iii) the impact of T2D on females (IDF): T2D females vs. Obese non-diabetic females (T2D·F - Ob.F); iv) the sex-differential impact of T2D (SDID): the difference between male and female comparisons [(T2D.M - Ob.M) - (T2D·F - Ob.F)].

DGE was performed independently for each comparison using the *limma* package. The differential expression statistics were then calculated, and the *p*-values were adjusted for multiple comparisons using the Benjamini & Hochberg (BH) method. A significance cut-off was established at a false discovery rate (FDR) < 0.05.

2.5. Meta-analysis and results integration

Meta-analysis integrated DGE results per disease, contrast and gene using a random effects model. Only genes present in at least two studies were included. LogFC served as the effect size, with the DerSimonian-Laird estimator for heterogeneity. This comprehensive analysis was performed using the *metafor* package. The results yielded a robust measure of expression across the studies in each meta-analysis and provided a BH-adjusted *p*-value for each gene and comparison. Significant genes were identified by applying a threshold of FDR < 0.05 and cross-referenced with obesity and T2D associations in the OpenTargets database (23.02 release).

2.6. Functional characterization

To identify and associate the gene consensus profiles obtained in meta-analysis with specific biological processes (BP), we performed a gene set enrichment analysis (GSEA). For each contrast, a ranked gene list (by logFC) was analyzed using the *clusterProfiler* package with the Gene Ontology (GO) BP database as the reference. The *p*-values were BH-adjusted, and logarithm 2 of the odds ratio (logOR) was used to assess the statistical effect. Significant terms (FDR < 0.05) were grouped by similarity using the *rrvgo* package to simplify result interpretation.

2.7. Experimental validation

2.7.1. Study selection and sample processing

The study included a total of $n = 32$ adult patients with severe obesity (BMI > 35 kg/m2) undergoing bariatric surgery at Hospital Sant Joan (Reus, Spain). Subcutaneous adipose tissue (SAT) biopsies were obtained from male obese ($n = 8$), female obese (n = 8), male obese T2D (n = 8), and female obese T2D (n = 8) patients. Ethical approval was granted by the Ethics Committee of Hospital Universitari Sant Joan (Reus, Spain) (EPIMET083/2018), and informed consent was obtained.

Total RNA was extracted from 100 mg of abdominal fat using a combined protocol including Trizol (Invitrogen) and RNA NucleoSpin® (Macherey-Nagel GmbH & Co) Kit. First-strand synthesis was performed using PrimeScript™ RT Reagent Kit (Perfect Real Time; Takara).

2.7.2. Gene expression analysis

Quantitative PCR was performed on 50 ng cDNA templates using a LightCycler 480 Instrument II (Roche) and TB Green® PreMix ExTaq™ (Tli RNaseH Plus; Takara). Selected genes and primers are listed in Table S2. Relative quantification was performed using the ΔCt method. Statistical analyses were performed inGraphPad Prism 10 (GraphPad Software V 10.0). Results are presented as the arithmetic mean ± the standard error of the mean, and Student's *t*-test was used. Any observed differences were considered significant when: *p*-value <0.05 (*), p-value <0.01 (**) and p-value <0.001 (***).

3. Results

3.1. Systematic review and study selection

After the revision process, we identified 404 studies in GEO and ArrayExpress, of which 82 % were unique records (Supplementary File 2). Based on the predefined exclusion criteria, we selected eight studies for analysis. The parallel search in PubMed involved the review of 230 articles, resulting in three that met the inclusion criteria, which were already represented in the database-derived studies. The level of agreement between the two evaluators who carried out the systematic review was quantified using the kappa coefficient: 0.82. Disagreements were resolved by the judgement of a third reviewer. Fig. 1 outlines the systematic review process including the number of records in each phase and the reasons for exclusion. All *Mus musculus* studies were excluded primarily due to the lack of sex information or the inclusion of only one sex in the study.

Finally, we selected eight *Homo sapiens* studies, which encompassed a total of 327 samples across three experimental groups (Control group (C) - lean individuals (BMI <30 kg/m^2) without any known metabolic disorders, Obese group (Ob) - obese individuals (BMI ≥ 30 kg/m^2) without T2D or other known metabolic comorbidities, and T2D group (T2D) - obese individuals diagnosed with T2D), and two tissues (VAT and SAT) (Table 1). Due to limitations in the sample size for VAT and the pronounced dissimilarities between SAT and VAT observed during the exploratory analysis -consistent with prior findings [10,13,14] VAT samples (25 in total) were excluded to avoid introducing noise in the meta-analysis.

Finally, the analysis included 302 SAT samples, distributed as follows: 46.36 % Control individuals, 48.01 % Obese individuals and 5.63 % T2D patients, giving an overall sex distribution of 44.04 % males and 55.96 % females (Table 2). Further details on the annotation process and gene-level analysis for each study are provided in Table S3. During the exploratory analysis phase, batch effects were identified in samples from GSE2508, GSE20950, and GSE29718 due to differences in sequencing platforms, sample processing times, and sequencing batches. These batch effects were corrected before differential expression analyses.

3.2. Sex differences in obesity and T2D transcriptomic signatures

We studied separately the transition from lean to obese (obesity transition) and from non-diabetic obese to T2D obese (T2D transition). For the obesity transition, we analyzed the overall impact of obesity (IO) by comparing all obese vs. all lean individuals, the impact of obesity in males (IOM) by comparing obese males vs. lean males, the impact of obesity in females (IOF) by comparing obese females vs. lean females, and the sex-differential impact of obesity (SDIO) by comparing IOM and IOF. Analogously, for the T2D transition, we analyzed the overall impact

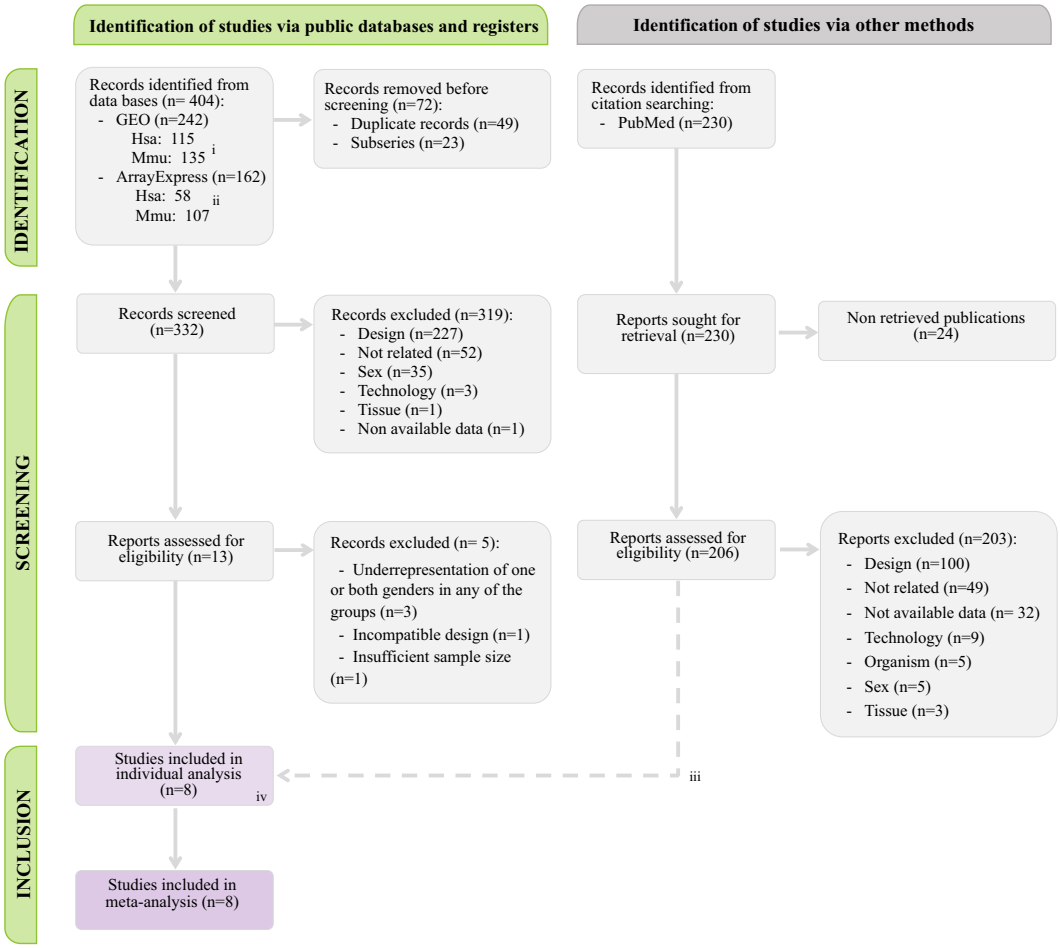


Fig. 1. Flow diagram for the systematic review following PRISMA Statement guidelines. i) 8 out of 242 studies identified in GEO included samples of both, *Homo sapiens* and *Mus musculus*.; ii) 3 out of 162 studies identified in ArrayExpress included samples of both, *Homo sapiens* and *Mus musculus*; iii) Three studies were identified through bibliographic searching, all of them were already represented in the database-derived studies; iv) All samples included in the meta-analysis belong to *Homo sapiens*.

Table 1
Description of selected studies. Identifier accession in the database, database source, kind of platform, described phenotypes (control/lean individual - C, non-T2D obese - Ob, and T2D obese - T2D), tissue (SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue) and technology used are detailed for each study. All features can be found in the GEO and ArrayExpress databases.

Study accession	Database	Platform	Phenotypes	Tissue	Type
E_MEXP_1425 [28]	ArrayExpress	Affymetrix GeneChip Human Genome U133 Plus 2.0 [HG-U133_Plus_2]	C, Ob	SAT	Microarray
GSE2508 [29]	GEO	[HG_U95Av2] Affymetrix Human Genome U95 Version 2 Array [HG_U95A] Affymetrix Human Genome U95A Array [HG_U95B] Affymetrix Human Genome U95B Array [HG_U95C] Affymetrix Human Genome U95C Array [HG_U95D] Affymetrix Human Genome U95D Array [HG_U95E] Affymetrix Human Genome U95E Array	C, Ob	SAT	Microarray
GSE20950 [30]	GEO	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	Ob, T2D	SAT & VAT	Microarray
GSE29718 [31]	GEO	[HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]	C, Ob	SAT & VAT	Microarray
GSE64567 [32]	GEO	Illumina HumanHT-12 V4.0 expression beadchip	C, Ob	SAT	Microarray
GSE92405 [33]	GEO	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array [CDF: Brainarray HGU133Plus2_Hs_ENTREZG_v18]	C, Ob	SAT	Microarray
GSE141432 [34]	GEO	Illumina NextSeq 500 (*Homo sapiens*)	C, Ob, T2D	SAT	RNA-seq
GSE205668 [35]	GEO	Illumina HiSeq 2500 (*Homo sapiens*)	C, Ob	SAT	RNA-seq

of T2D (ID) by comparing all T2D obese vs. all non-T2D obese individuals, the impact of T2D in males (IDM) by comparing T2D obese males vs. non-T2D obese males, the impact of T2D in females (IDF) by comparing T2D obese females vs. non-T2D obese females, and the sex-differential impact of T2D (SDID) by comparing IDM and IDF.

Each comparison computes a logFC indicating the magnitude and direction of the change. In IO, IOM, IOF, ID, IDM and IDF comparisons, positive logFC values indicate an absolute increase of the values in the

Table 2
Distribution of samples in studies by tissue, sex, and experimental group. C: control; Ob: non-diabetic obese; T2D: type 2 diabetic obese; SAT: subcutaneous adipose tissue.

Study	SAT						Total
	C		Ob		T2D		
	M	F	M	F	M	F	
E_MEXP_1425	8	5	9	5	0	0	27
GSE2508	10	10	9	10	0	0	39
GSE20950	0	0	2	8	4	5	19
GSE29718	4	4	5	2	0	0	15
GSE64567	10	19	9	26	0	0	64
GSE92405	9	17	9	17	0	0	52
GSE141432	3	6	4	4	4	4	25
GSE205668	22	13	12	14	0	0	61
Total	66	74	59	86	8	9	302

first compared group with respect to the second one (obese males > lean males in IOM, as instance) and negative values indicate an absolute decrease of the values in the first compared group with respect to the second one (obese males < lean males in IOM). In SDIO and SDID comparisons, positive logFC values indicate a relative increase of the values in males with respect to females in the disease, which may be due to 5 different scenarios (Fig. S1): the gene is upregulated in males and downregulated in females; the gene is upregulated in males and not significant in females; the gene is upregulated in both males and females, but the increase is higher in males; the gene is downregulated in females and not significant in males; or the gene is downregulated in both males and females, but the decrease is higher in females. Reversely, negative logFC values indicate a relative decrease of the values in males with respect to females in the disease, which may be due to the reverse 5 previous scenarios.

Significant differential expression was observed in three of seven studies for SDIO. However, no significant differences were detected for

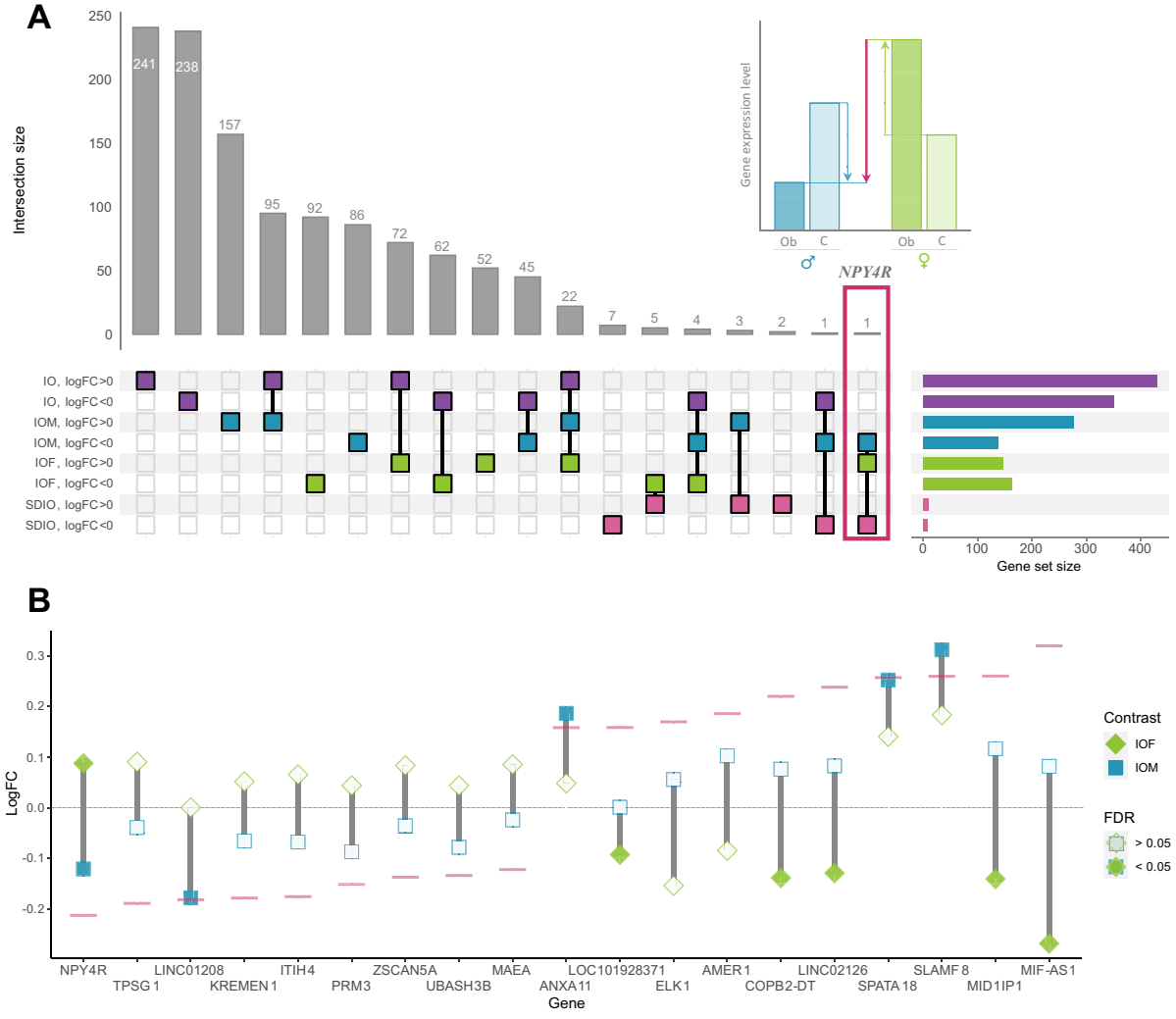


Fig. 2. Transcriptomic profiles in the obesity meta-analysis. (A) Upset plot of significantly altered genes. Horizontally, the number of significant genes per comparison is displayed and separated by logFC. The purple, blue, green, and pink bars denote the IO, IOM, IOF, and SDIO comparisons, respectively, while the white and gray background represent positive and negative logFC values. The intersections between these groups are displayed vertically. The upper-right corner bar plot illustrates the variation of *NPY4R* expression levels. Blue and green arrows denote the variation in expression levels in the IOM and IOF comparisons, respectively, while the pink arrow indicates the overall effect of these changes in the SDIO comparison. (B) Exploration of the expression patterns of the nineteen differentially expressed genes in the SDIO comparison. Squares and rhombi represent the logFC obtained in the meta-analysis of IOM and IOF comparisons, respectively, while the pink line depicts the effect of these changes in the SDIO comparison. Significantly affected genes in the IOM and IOF comparisons are represented by filled squares and rhombi, respectively. Note that the mentioned colors refer to the online version of the article; please refer to the figure legend for further information. IOM: impact of obesity in males; IOF: impact of obesity in females. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

SDID in any individual study. Table S4 summarizes the results of the DGE analyses per study.

We performed a meta-analysis for each comparison which comprised 37,226 genes in obesity and 20,161 genes in T2D, including genes present in at least two independent studies (Table 3). Surprisingly, genes significant in males and females differ greatly for both obesity and T2D transitions. For obesity, only 3.49 % of differentially expressed genes were common between IOM and IOF comparisons, comprising 22/428 upregulated and 4/300 downregulated genes (Fig. 2). For T2D, 7.64 % of differentially expressed genes overlapped between IDM and IDF comparisons, including 49/639 upregulated and 66/982 downregulated genes (Fig. 3 and Table 3). The results of the meta-analysis for each of the genes and contrasts can be found in Supplementary file 3 for obesity and Supplementary file 4 for T2D.

Table 3
Meta-analysis results. Number of significant genes by comparison: IO (impact of obesity), IOM (impact of obesity in males), IOF (impact of obesity in females), SDIO (sex-differential impact of obesity), ID (impact of diabetes), IDM (impact of T2D in males), IDF (impact of T2D in females), SDID (sex-differential impact of T2D). Significant genes are separated by the sign of their logarithm 2 of fold change (logFC). The last row reports the number of genes included in each meta-analysis. The significant genes were identified when the adjusted *p*-value (False Discovery Rate) < 0.05.

	Obesity				T2D			
	IO	IOM	IOF	SDIO	ID	IDM	IDF	SDID
logFC > 0	430	277	147	10	713	105	534	0
logFC < 0	350	137	163	9	1268	217	765	0
Total	780	414	310	19	1981	322	1.299	0
Analyzed genes	37,226				20,161			

3.3. Transcriptomic profiles of sex differences in obesity

Exploring sex-specific differences in the impact of obesity (SDIO) led to the identification of nineteen differentially expressed genes. Comparative analysis of the SDIO results with individual sex comparisons (IOM and IOF) provided valuable insight into the behavior of these genes during the development of obesity in males and females (Fig. 2).

The SDIO analysis revealed differences in gene expression linked to various physiological scenarios arising from sex-specific responses to obesity (Fig. S1 and Fig. 2A). Fig. 2B depicts the differences in the logFC of the differentially expressed genes in the SDIO comparison. Among the identified genes, 14/19 exhibited opposite expression patterns between males and females. *NPY4R*, *TPSG1*, *KREMEN1*, *ITIH4*, *PRM3*, *ZSCAN5A*, *UBASH3B*, and *MAEA* displayed upregulated expression in obese females and downregulated expression in obese males, whereas *ELK1*, *AMER1*, *COPB2-DT*, *LINC02126*, *MID1IP1*, and *MIF-AS1* displayed upregulated expression in obese males and downregulated expression in obese females. *LINC01208* and *LOC101928371* exhibited significant downregulation exclusively in one sex, whereas *ANXA11*, *SPATA18*, and *SLAMF8* were consistently upregulated in both sexes, with significantly higher levels in males (Table 4). Notably, *NPY4R* displayed a significantly opposite pattern in the sex comparisons - upregulated expression in the IOF comparison and downregulated expression in the IOM comparison (Fig. 2A, B).

3.4. Transcriptomic profiles of sex differences in type 2 diabetes

The analysis of sex-specific differences in the impact of T2D (SDID) did not reveal significant differences in gene expression. However, comparisons between IDM and IDF highlighted meaningful trends (Fig. 3). Out of 20,161 analyzed genes, 66 were significantly downregulated, and 49 were significantly upregulated in both sexes. While no genes displayed significant opposite patterns (e.g., upregulated in one sex and downregulated in the other), non-significant opposite tendencies were noted in specific genes (Fig. 3).

The great majority of genes significantly dysregulated in one sex present an analogous behavior in the other sex, either significant or not. However, we found a group of interesting exceptions in columns 5, 6, 9 and 10 of the upset plot in Fig. 3. We found a set of 63 IDF downregulated genes which were non-significantly upregulated in IDM. Moreover, five of them had previous associations with obesity, seven with T2D, and five with both disorders, including GFAP and RETN

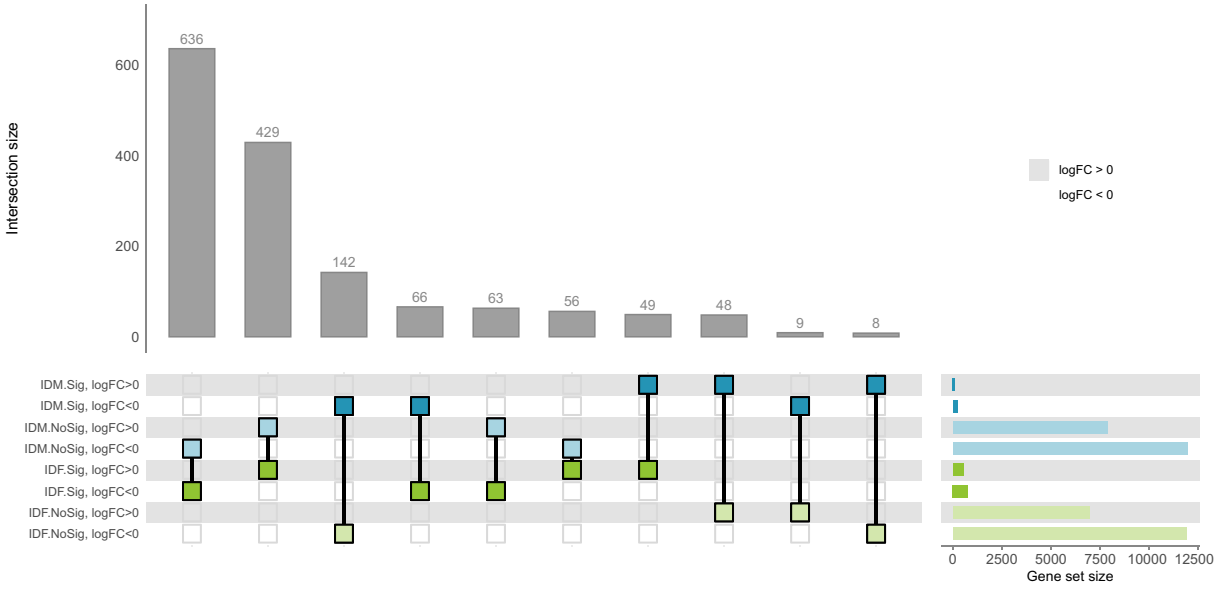


Fig. 3. Transcriptomic profiles in the T2D meta-analysis. Upset plot comparing expression profiles by sex for IDM and IDF comparisons. Horizontally, the number of significant genes per comparison is displayed, separated by logFC and adjusted *p*-value. Intersections between these groups are displayed vertically. The IDM and IDF comparisons are denoted by blue and green color bars, respectively. Positive and negative logFC values are represented by white and gray backgrounds, respectively. Significant values (FDR < 0.05) are opaque. Note that the mentioned colors refer to the online version of the article; please refer to the figure legend for further information. IDM: impact of T2D in males; IDF: impact of T2D in females. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Significantly affected genes identified in the obesity meta-analysis. Significant genes are grouped by comparison and categorized by the sign of the logFC. Genes are ordered based on the FDR and their absolute value of the effect size. In cases with a large set of significant genes, only the first 20 for each category are shown, along with other relevant and significant genes that offer valuable insights for this study. Genes are highlighted based on their known associations with obesity (in bold), type 2 diabetes (underlined), or both, as extracted from the Open Targets Platform [36].

Comparison	logFC > 0	logFC < 0
IO	*WDR41*, ***FCGR2B***, *LAMB2*, *SERPINH1*, *RNASE6*, *AGTRAP*, *AP2S1*, *HCLS1*, *IRF8*, *TYK2*, *ALOX5AP*, *TMEM109*, *CAPZB*, *IL10RA*, ***VSIG4***, ***PLA2G7***, *HYCC1*, *GPR137B*, ***ACP5***, *NOP2*, (+410)	*TEX51*, *MTHFS*, ***INSR***, ***TMPRSS6***, *KYAT3*, ***MAOA***, *SLC6A5*, *ECPAS*, *URAHP*, *LOC105373554*, *MPDZ*, *RPLP2*, *ARX*, *PPP4R1L*, *LINC02833*, *ACADSB*, *CACHD1*, *DCST1-AS1*, *ST6GALNAC6*, *APTR*, (+330, including: *LINC01208*)
IOM	*ANXA11*, ***CCND1***, *ACTN1*, *DDX41*, *YKT6*, *LOC101929398*, *STAT1*, *CPVL*, *IGLC2*, ***PLA2G7***, ***ANXA5***, *S100A11*, *TAPBPL*, *ITGB5*, *PLXNB2*, *SEC31A*, *CAPZB*, *GPR137B*, ***ACP5***, *LAMB2*, (+257, including: *SLAMF8*, ***SPATA18***)	*CRH*, *LINC01208*, *MCCC1*, *TMOD1*, *HADHB*, ***ECHS1***, ***HK2***, *USP32P2*, ***MAMLD1***, *TEX51*, *CASD1*, *COPG2IT1*, ***MAOA***, *PKP2*, *SALL2*, *LINC01788*, *PCDHA5*, *RPS3AP20*, *TAC4*, *TRMT9B*, (+117, including: ***INSR***, ***NPY4R***)
IOF	*HCLS1*, ***CD163***, ***CHTOP***, ***LYN***, *ARHGAP17*, *PRSS23*, ***PPRC1***, *RNASE6*, ***PLA2G7***, *IGHG2*, *PLA2G4C*, ***ABCC5***, *IGHV3–23*, *GAB3*, *PPP4R1*, *PUS1*, *BCYRN1*, *SEPTIN9*, *FKBP15*, ***FCGR2B***, (+127, including: ***NPY4R***)	*IGHD6–6*, *MTHFS*, *TRGV5*, *STAU2-AS1*, ***RAD21***, *ISCU*, *RSPH10B2*, ***NDUFA2***, *OPHN1*, *ST6GALNAC6*, *POLR3GL*, *GKAP1*, *TEX51*, *LINC02266*, *DCAF12L1*, ***RBL2***, ***EIF4EBP2***, *PRDM12*, *RTP1*, *NKAIN3*, (+143, including: ***INSR***, *LOC101928371*, *MIF-AS1*, *COPB2-DT*, *LINC02126*, *MID1IP1*)
SDIO	*LOC101928371*, *MIF-AS1*, *SLAMF8*, *AMER1*, *COPB2-DT*, ***SPATA18***, *LINC02126*, *ANXA11*, *MID1IP1*, *ELK1*	***NPY4R***, ***KREMEN1***, *LINC01208*, *ZSCAN5A*, *UBASH3B*, ***ITIH4***, *TPSG1*, *PRM3*, ***MAEA***

Table 5
Significantly affected genes identified in the T2D meta-analysis. Significant genes are grouped by comparison and categorized by the sign of the logFC sign. Genes are ordered based on the FDR and their absolute value of the effect size. In cases with a large set of significant genes, only the first 20 for each category are shown, along with other relevant and significant genes that offer valuable insights for this study. Genes are highlighted based on their known associations with obesity (in bold), type 2 diabetes (underlined), or both, as extracted from the Open Targets Platform [36].

Comparison	logFC > 0	logFC < 0
ID	*RIMBP3*, *PELP1-DT*, *ERLNC1*, *KIF5A*, *BCAN*, *RDH12*, *LINC00896*, *LINC00870*, *PIP*, *GPR45*, *WNT7A*, *SPINT1*, *SLCO4A1-AS1*, *KIR2DL4*, *TRAV20*, *VENTXP1*, *RSPH9*, *B3GALT5*, *LINC01271*, *LINC02777*, (+693)	*CYMP*, *SNX15*, *NACAD*, *SULT4A1*, *LINC00382*, *MSX2*, *AQP2*, *CAMK2N2*, *MTERF2*, *LOC284379*, *PATE2*, *NECAB3*, *PNCK*, *FBXO38-DT*, *OR13C4*, ***SMOC1***, *RYR3-DT*, *SMIM43*, *OCA2*, *KRTAP5-AS1*, (+1248, including: *LDHD*, ***PLPP2***, ***LRP4***)
IDM	*ERLNC1*, *PELP1-DT*, *KIF5A*, *GDPD1*, *GPR45*, *NFASC*, *PLAC4*, *CD3E*, *VENTXP1*, *TRAV20*, *RDH12*, ***SLC12A3***, *AQP6*, *LINC02530*, *LINC00896*, ***CD27***, ***ERBB2***, *MARCHF8*, *LINC02532*, ***BLK***, (+85, including: *SAMD9*, *EHD3*, ***ZDHHC19***)	*USP43*, *ZMIZ1-AS1*, *LUZP2*, *TBL1Y*, *PABPC3*, *SMIM43*, *FAM209B*, *LINC00382*, *LOC284379*, *GAMT*, *SPON1*, *SLC66A1L*, *PECR*, *MTERF2*, *EFCAB12*, *SETD9*, *ITGA11*, *CAMK2N2*, *AQP2*, ***SARDH***, (+197, including: *LDHD*, *NBPF3*, *PRKN*)
IDF	*RIMBP3*, *FBXO2*, *SLC9A2*, *KIF5A*, *LINC00896*, *XKR7*, *BCAN*, *RDH12*, ***PAX5***, ***CRLF2***, *PLPP4*, *SPINT1*, ***EN2***, *LINC01271*, *LINC02777*, *TTLL1-AS1*, *CD1A*, *HOXC11*, ***BLK***, *CALHM1*, (+514, including: ***GFAP***, ***RETN***)	*LINC02450*, *ITGA2*, *NOVA1*, *GOLGA8IP*, *PPIL6*, *ITGA11*, *RDH8*, *LIMS2*, *NACAD*, *SNX15*, *JARID2-AS1*, *LINC00382*, *CREBZF*, *A1BG*, *OR5AK4P*, *FBXO38-DT*, *BICRA*, ***ACVR2A***, *MSX2*, ***PLPP2***, (+745, including: ***HEY1***, ***PLPP2***, *PM20D2*, *VAX2*, *RECK*, ***LRP4***, ***TCF7L2***)
SDID	-	-

(Table 5). We also found a set of 56 IDF upregulated genes which appeared as non-significantly downregulated in IDM. Of them, eight are related to obesity, two with T2D, and two with both disorders, including the Wnt signaling component VAX2.

As for the IDM signature, nine genes showed significant downregulation and eight significant upregulation with non-significant opposite tendency in IDF, including *ZDHHC19,* an obesity-related marker [32]. No significant signature was found for SDID (Table 5).

3.5. Functional patterns in obesity and type 2 diabetes

We conducted a GSEA on the gene patterns in each comparison using BP GO terms to provide a functional and integrative perspective on obesity and T2D. Using an FDR < 0.05 cut-off, we identified 2962 (IO), 2719 (IOM), 1808 (IOF), and 31 (SDIO) significantly altered processes in obesity, and 756 (ID), 792 (IDM), 536 (IDF), and 48 (SDID) significant altered processes in T2D.

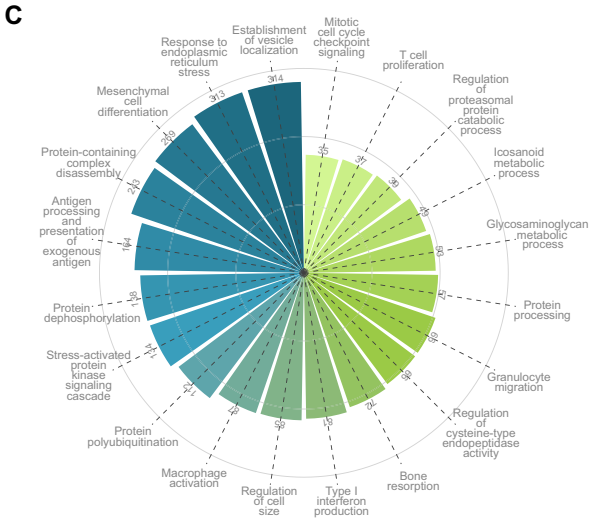
In the context of obesity, the 31 significantly enriched functions in the SDIO comparison were all related to the activation and regulation of the immune system and inflammation, displaying a negative logOR denoting the enrichment of these functions in females in the development of obesity compared to males (Fig. 4A).

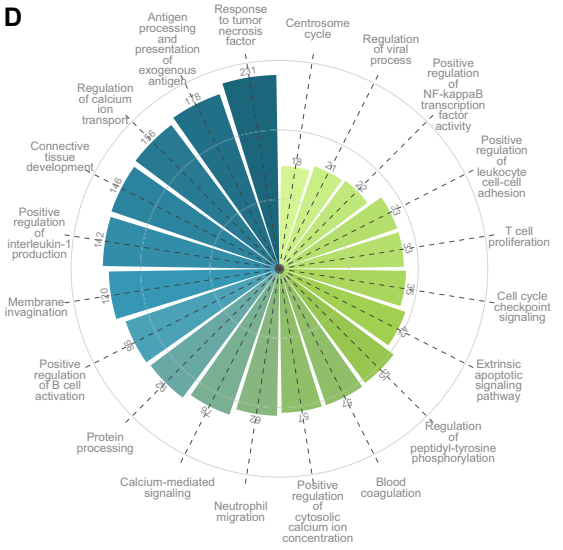
Notably, the IOM comparison also revealed enriched biological processes with a negative logOR associated with lipid transport and metabolism, including "*Lipid import into the cell*," "*Alditol phosphate metabolic process*," and "*Long-chain fatty acid import into the cell*."

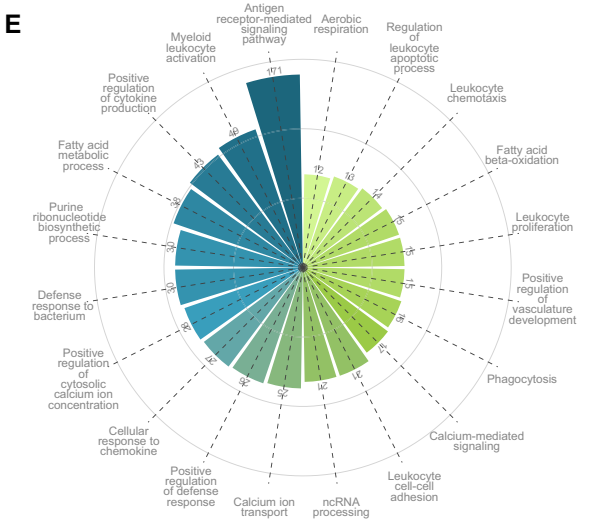
To address the large number and hierarchical structure of enriched terms, we grouped them based on distance. Alongside the role of immunity, we observed an increase in stress-related processes and the role of calcium in the development of obesity in both sexes. These functions are grouped in IOM under the terms "*Establishment of vesicle localization*," "*Response to endoplasmic reticulum stress*," "*Stress-activated protein kinase signaling cascade*," and "*Regulation of cell size*," totaling 627 terms (Fig. 4C). Similarly, in the IOF comparison, the terms "*Calcium-mediated signaling*," "*Positive regulation of cytosolic calcium ion concentration*," and "*Regulation of calcium ion transport*" displayed enrichment, with a total of 291 functions (Fig. 4D). In addition, in males, the term "*Protein-containing complex disassembly*" highlights processes related to mitochondrial damage, including those associated with mitochondria and the electron transport chain; in contrast, the number of functions associated with mitochondrial processes in females is negligible.

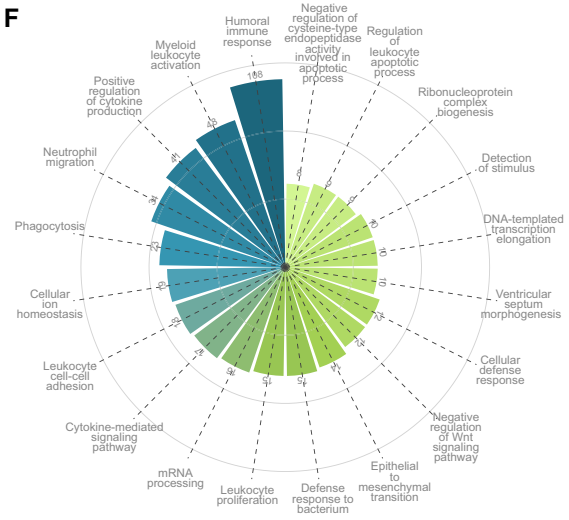
In the context of T2D, both sexes highlighted the relevant role of immunity (Fig. 4B), consistent with the obesity results. However, the deregulation of biological processes such as "*Regulation of cellular ketone metabolic process*," "*Neutral lipid biosynthetic process*," "*Acylglycerol biosynthetic process*," "*Triglyceride biosynthetic process*," "*Triglyceride metabolic process*" and "*Mitochondrial respiratory chain complex assembly*" led to substantial changes in the SDID comparison.

Examination of the functional profiles obtained in IDM (Fig. 4E) and IDF (Fig. 4F) again highlights the role of fatty acid metabolism in obese males, which encompasses over fifty biological processes under the terms "*Fatty acid metabolic process*" or "*Fatty acid beta-oxidation*." Conversely, T2D females exhibit evidence of fourteen enriched biological processes related to the negative regulation of the Wnt signaling pathway. Based on these results, we compiled 164 genes associated with this pathway - of which fifteen displayed a significant decrease in IDF, while only *PRKN* displayed significant changes in IDM (Table 5).









(caption on next page)

Fig. 4. Gene Set Enrichment Analysis Results for Biological Processes Gene Ontology. (A and B) The enriched processes identified in GSEA for (A) obesity and (B) T2D have been ordered based on logOR; dot plots represent the top five over- and under-expressed terms per comparison. (C-F) The color indicates the effect's magnitude and direction, while transparency corresponds to the FDR (lower significance results in less opacity). The circle bar size represents the number of genes associated with each enriched term. Graphical summary of the critical functions obtained in the (C) IOM, (D) IOF, (E) IDM, and (F) IDF comparisons. BP GO terms were grouped based on distance using the REVIGO methodology. The top twenty groups with the highest number of associated significant BP GO terms are represented, along with the number of processes associated and the name assigned to each after analysis. IOM: impact of obesity in males; IOF: impact of obesity in females; IDM: impact of T2D in males; IDF: impact of T2D in females.

3.6. SDIO and T2D in silico signatures experimental validation in obese subjects

To validate in silico results, we analyzed RNA from SAT of obese and T2D obese patients by quantitative PCR (qPCR) (Fig. 5). We first selected potential gene expression signatures from the SDIO *in-silico* results (Table 4) to validate differential gene expression between obese males and females. Overall, the expression of the *SPATA18*, *KREMEN1*, *NPY4R,* and *PRM3* genes confirmed the in silico results meta-analysis (Fig. 5A).

We then employed qPCR analysis to confirm a T2D signature as revealed by differential expression profile between T2D and obese males (*SAMD9*, *NBPF3*, *LDHD*, and *EHD3*) and females (*RETN*, *HEY1*, *PLPP2*, and *PM20D2*) (Fig. 5B). We selected genes according to significant differences (Table 5), playing key metabolic roles, and found to be referenced for its expression in SAT in the Genotype-Tissue Expression (GTEx) Portal.

In all cases, we selected *RPL19* as the reference gene due to its previously demonstrated stability and consistency in WAT when considering sex as a biological variable [37].

4. Discussion

Although it is well known that there are significant differences between sexes in the prevalence, development, and pathophysiology of obesity and T2D, their molecular basis remains unclear. Understanding the impact of sex-specific differences in disease remains critical to improving outcomes. To the best of our knowledge, we have conducted the first systematic review and meta-analysis on WAT with sex as a central perspective, revealing gene signature and functions involved in obesity and T2D progression using publicly available data.

We used an *in-silico* approach, analyzing transcriptomic studies that included WAT samples from patients of both sexes including control, obese and T2D subjects. We analyzed data via eight meta-analyses and subsequent functional analyses, identifying common patterns and genes specific to each disorder and the more significant or exclusive involvement of specific markers and biological processes in the development of obesity and T2D in SAT in relation to sex.

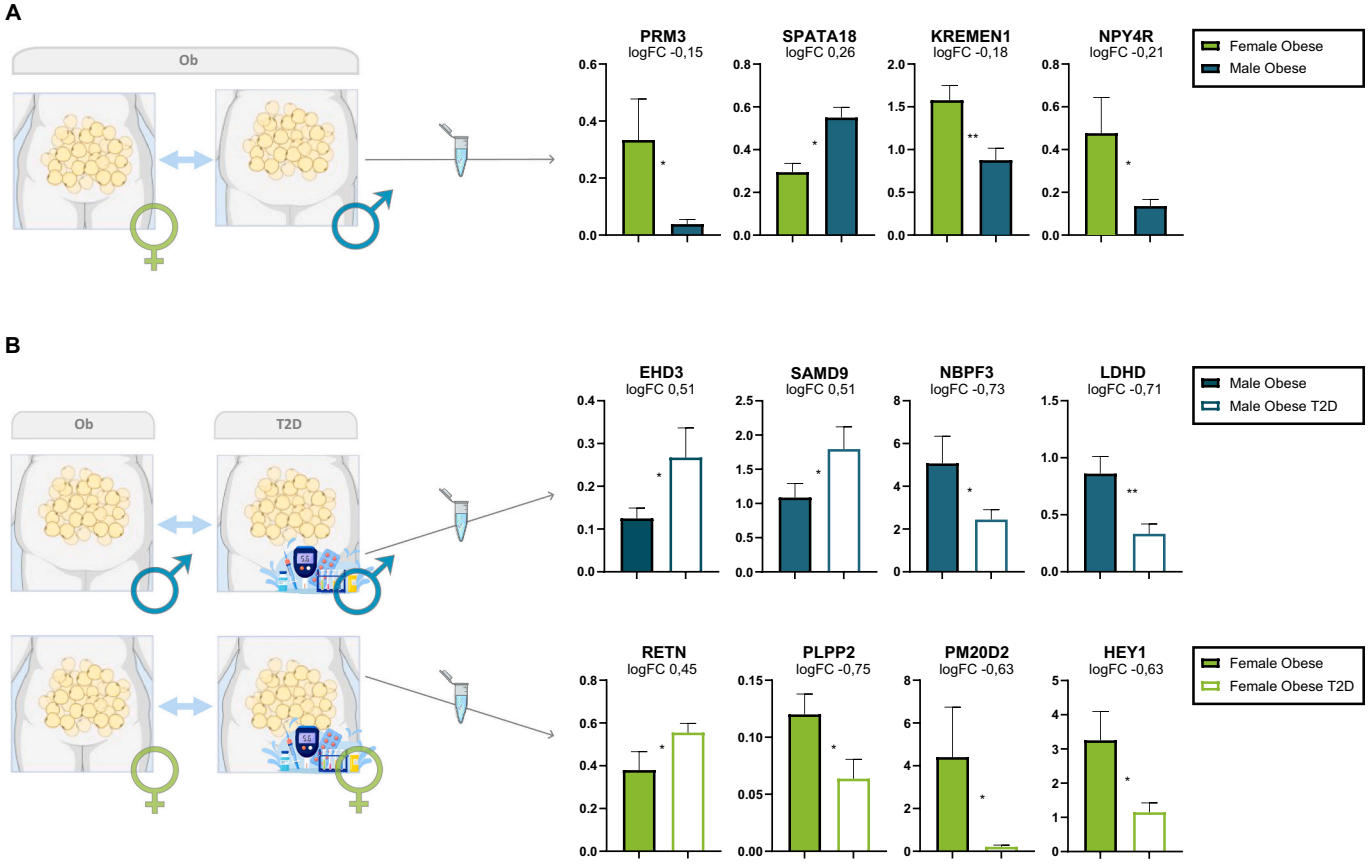


Fig. 5. Gene expression-mediated validation of the *in-silico* results. Relative gene expression analysis in human SAT samples from obese and T2D obese males and females by qPCR. After obtaining informed consent, SAT samples were obtained during surgery, and RNA was extracted and used for real-time PCR. (A) Relative gene expression analysis for obesity in male and female obese samples. (B) Relative gene expression analysis for T2D in males (upper panel) and females (lower panel). In silico-*analyses*, *fold-change* results are shown for each gene. Each experimental group is represented by an $n = 8$. Student's *t*-test applied for significance: (*) *p*-value<0.05, (**) p-value<0.01.

4.1. Transcriptomic landscape of sex differences in obesity

Our meta-analysis identified 1185 potential markers involved in weight gain and obesity. Of these, 18.8 % had been previously associated with obesity, 18.5 % with T2D, and 8.4 % with both, according to data extracted from OpenTargets (Table 4), adding robustness to our results, and highlighting the novelty of the remaining 54.3 % of our findings. Our analysis revealed 26 dysregulated genes with analogous change in both sexes (Fig. 2A), including *INSR*, which plays a significant role in insulin resistance and T2D development/progression [7].

In terms of sex differences in the impact of obesity, we identified 19 sex-differentially dysregulated markers (Fig. 2); of note, five represent long non-coding RNAs (*LOC101928371*, *LINC02126*, *LINC01208*, *COPB2-DT*, and *MIF-AS1*) [38]. Among these, *MIF-AS1* has a pro-inflammatory role in obesity and is involved in the development of insulin resistance [39]. These results also included genes related to immune responses, such as *SLAMF8*, *ZSCAN5A*, *UBASH3B*, *TPSG1*, *MAEA*, and *ANXA11*, which are involved in macrophage and lymphocyte activation, mast cell differentiation, and pro-inflammatory cytokine production [40,41]. Its implications in T2D susceptibility [42], progression [43], and complications [44] have been widely reported. Finally, we also identified among this set diabetes-associated genes as *ITIH4* and *PRM3* [36], and our experimental validation confirmed that they were overexpressed in females compared to males in SAT samples (Fig. 5A).

Sex-specific patterns in mitochondrial processes were also evident. For instance, *SPATA18*, a critical gene involved in the induction of mitophagy and the regulation of mitochondrial quality in response to DNA damage, was upregulated in obese males but not in obese females (Fig. 2; Fig. 5A), suggesting greater susceptibility to mitochondrial damage and the development of T2D in males [45]. Similarly, *ELK1*, which encodes a transcription factor essential for T2D development that becomes activated by phosphorylation via the MAPK/ERK1/2 pathway, promoting adipogenesis and insulin resistance in WAT [46,47], and *MID1IP1*, whose increased expression has been associated with lipogenesis and fatty acid accumulation [48], exhibited male-specific upregulation, supporting the idea of higher risk of T2D development in obese males.

We also found such sex differences in *KREMEN1* and *AMER1*, two genes involved in the Wnt β-catenin pathway, which regulates metabolism and homeostasis, inhibiting adipogenesis and increasing insulin sensitivity via negative regulation of *PPAR-γ* and *GSK3β* [7]. Altered transcriptional regulation of these two genes may inhibit the Wnt β-catenin pathway, a key regulator in T2D development, through sex-specific mechanisms [49,50].

Finally, we identified *NPY4R* as a sex-specific molecular marker associated with diet-induced obesity. Our study revealed the significant upregulation of *NPY4R* expression in obese females and downregulation in obese males (Fig. 2; Fig. 5A). This gene's role in females has been linked to an orexigenic effect and to a positive correlation with body mass index and abdominal circumference [51,52], representing a novel finding in the context of sex-specific studies.

Functionally, we observed the enrichment of inflammatory processes in obese patients of both sexes, but more significantly in females (Fig. 4); furthermore, we observed a sex-neutral enrichment in processes related to stress and the role of calcium in the development of obesity. Meanwhile, obese males exhibited processes related to mitochondrial damage, which supports our gene-level findings.

4.2. Transcriptomic landscape of sex differences in type 2 diabetes

Our meta-analysis identified 2542 potential markers for T2D, of which 15.2 % were previously linked to obesity, 16.2 % to T2D, and 5.6 % to both conditions (Table 5). Individual comparisons by sex, IDM and IDF identified sex-specific differentially expressed genes not revealed in the global comparison. The number of results in IDF comparison was higher because of greater sample representation of females in the included studies. Although the limited number of studies and samples prevented the identification of differentially expressed genes in the study of sex differences in diabetes (SDID), comparing profiles by sex allowed us to identify novel SAT markers, and draw some interesting conclusions.

In males, *FAM83E* and *RASAL1,* both associated with Ras/MAPK signaling pathway, emerged as potential indicators linked to body weight, T2D, and complications such as diabetic nephropathies [53,54]. Other male-specific dysregulated genes included *EHD3* (involved in protein biosynthesis and transport) [55], *SAMD9* (involved in inflammatory response through TNF-alpha) [36], both upregulated in T2D SAT samples; and *NBPF3*, which is generally overexpressed in spermatids in testis, downregulated in male T2D SAT samples, thus suggesting a potential connection between T2D and fertility (Fig. 3; Fig. 5B).

In females, significant specific dysregulated genes included *GFAP, RETN* and *VAX2*, relevant genes in the context of T2D previously associated with sex-specific patterns [56–59], with *RETN* being validated in SAT samples (Fig. 5B). Other female-specific upregulated genes like *PLPP2*, *PM20D2,* and *HEY1* were linked to adipose tissue metabolism at the lipidic, proteic and transcriptional levels, respectively [38,60].

Functional analysis highlighted sex-neutral immunity-related processes in obesity and T2D. However, T2D males exhibited greater dysregulation of biological processes related to fatty acid biosynthesis and β-oxidation, and mitochondrial processes as evidenced by *LDHD* upregulation (Fig. 3; Fig. 4B; Fig. 5B). Of note, these findings agree with the results obtained for *SPATA18* in obese patients.

Examining functional profiles by sex (Fig. 4E-F) revealed the presence of fourteen significant functions associated with the Wnt signaling pathway in T2D females, which do not display significance in males. These biological processes are linked to fifteen genes, including key regulators of both canonical and non-canonical Wnt pathways as *RECK* and *LRP4* previously associated with metabolism, insulin response, and diabetes [11] or *VAX2,* a dominant-negative Wnt antagonist, underscoring its significant role in T2D development in females. Other involved genes include *TRABD2B*, *TMEM64*, *APCDD1L*, *TCF7L1*, *ARHGEF19*, *WLS*, *BCL9*, *TBX18*, *DRD2*, *GSK3A* and *NFATC1*.

Additionally, *KREMEN1*, previously identified as sex-differentially dysregulated in obesity, exhibits non-significantly increased expression in females and decreased expression in males. These findings emphasize the significant role of the Wnt signaling pathway in the development of obesity-related T2D, particularly in females, and its strong association with sex, as observed in obesity.

4.3. Strengths and limitations

Sex-related differences have been documented in the clinical and pathophysiological aspects of metabolic diseases and disorders, including obesity and T2D [17,22]. However, sex is often perceived more as a bias rather than a biological variable in biomedical research. Encouragingly, recent efforts by the National Institutes of Health [61] and the European Union [62] emphasize the importance of sex as a variable in research.

Our study advances the understanding of sex differences in obesity and T2D development in human SAT, but it is not without limitations. The exclusion of VAT and mice WAT data due to insufficient sex-specific information limited our scope to human SAT. Additionally, despite recent efforts to publish open data under the FAIR (Findable, Accessible, Interoperable, Reusable) principles [63], data availability remains limited. During our search, 24 % of identified publications were not retrievable due to restricted access or missing datasets. This, along with the lack of homogeneity in clinical and phenotypic data, prevented the integration of variables like age, body mass index, or insulin resistance index, which could have impacted disease variability. These limitations restricted the number of studies and samples analyzed, and forced a higher representation of females in the study. This imbalance led to a loss of statistical power, especially when studying the impact of T2D.

Despite these limitations, our methodology ensured the robustness and reliability of the identified molecular differences by accounting for inter-sample variability, thereby facilitating their extrapolation to the general population. This led to the discovery of both novel and previously reported sex-specific target genes with implications for disease diagnosis, prognosis, and clinical trial design. Furthermore, our findings emphasize the necessity of incorporating sex as a biological variable to achieve more accurate and clinically relevant insights into metabolic diseases.

5. Conclusions

Sex differences and their implications remain largely underexplored in biomedical research. By integrating transcriptomic public data from SAT through a meta-analysis approach, our study identified both shared patterns and potential sex-specific signatures involved in the development of obesity and its progression to T2D.

Our findings emphasize the critical role of sex in shaping the molecular mechanisms underlying these metabolic disorders, providing new insights into their pathophysiology. This study, in addition to underlining the importance of considering sex in biomedical research, has generated novel biological knowledge that will allow for a more appropriate translatability of the applications of personalized medicine in the management of this group of patients. This relevant information will be useful to characterize the progression of T2D, its diagnosis and prognosis, by incorporating sex as a crucial clinical variable. Furthermore, it will enable the design of specific therapeutic strategies and personalized lifestyle interventions in the treatment of obesity and T2D.

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CRediT authorship contribution statement

Roxana Andreea Moldovan: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Marta R. Hidalgo:** Writing – review & editing, Writing – original draft, Software, Methodology. **Helena Castañé:** Investigation. **Andrea Jiménez-Franco:** Investigation. **Jorge Joven:** Investigation. **Deborah J. Burks:** Writing – review & editing, Funding acquisition. **Amparo Galán:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Conceptualization. **Francisco García-García:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Consent for publication

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data and results generated in the various steps of the meta-analyses are freely available on the Metafun-SDT2D platform (https://bioinfo.cipf.es/metafun-SDT2D), and all scripts are available in the GitHub repository https://github.com/Roxyam/T2D-Meta-Analysis.

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