

RESEARCH ARTICLE

Diet-induced obesity disrupts sexually dimorphic gene expression in mice

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Abstract

Biological sex significantly influences the prevalence, incidence, and severity of numerous human diseases, yet it remains an underappreciated variable in biomedical research. Although sexually dimorphic genes contribute to sex-specific traits and disease manifestations, their regulation under metabolic stress is poorly understood. To explore sex-specific metabolic adaptations, we analyzed responses to high-fat diet (HFD)-induced obesity in male and female mice, focusing on the regulation of sex-biased genes. Despite similar adiposity, HFD-fed males displayed more severe metabolic impairments than females, highlighting divergent metabolic outcomes. To investigate the basis for these sex-specific differences, we performed whole transcriptomic profiling of liver and white adipose tissue (WAT) at early (2 wk) and late (12 wk) stages of HFD exposure. Our analysis revealed marked sex-specific gene expression changes across multiple categories, particularly pronounced in male WAT after prolonged HFD feeding. Strikingly, genes exhibiting sexual dimorphism under normal conditions were preferentially modulated in both sexes, comprising up to 46% of all HFD-regulated genes. This led to a substantial loss of sex-biased gene expression in both liver and WAT after HFD exposure, correlating with metabolic dysfunction. Male-biased genes associated with cilia function and estrogen response were among the most affected, showing significant downregulation in male WAT under HFD. Our findings provide a novel perspective on how obesity disrupts sex-specific gene expression in key metabolic tissues, particularly targeting sex-biased genes. By revealing that a considerable proportion of sex-biased genes exhibit HFD-regulated modulation, our study highlights the critical role of these genes in maintaining metabolic health.

NEW & NOTEWORTHY Biological sex shapes metabolic tissue physiology, largely through sex-biased gene regulation. Our comprehensive transcriptomic analysis reveals that sex-biased genes in liver and white adipose tissue undergo the most significant regulation during obesity-driven metabolic dysfunction, resulting in a loss of their bias. This disruption highlights a previously unrecognized role of sex-biased genes in maintaining metabolic health in both males and females.

insulin resistance; obesity; sex differences; transcriptomics; type 2 diabetes

INTRODUCTION

Sex is a fundamental biological variable that profoundly influences phenotypic traits and gene expression across mammalian tissues, yet it has been historically underrepresented in biomedical research (1–6). Despite recent efforts to address this imbalance (7), many animal and human studies continue to exclude or inadequately report sex-specific data (6), potentially biasing biological interpretations and limiting the generalizability of findings. Large-scale analyses have highlighted particularly pronounced sex effects in adipose tissue, a key organ in metabolic regulation (4, 8), underscoring the need for sex-inclusive research designs. This gap is especially critical in the study of obesity and related

metabolic disorders, where sex-based disparities are well-documented. Obesity frequently co-occurs with metabolic syndrome, type 2 diabetes (T2D), and metabolic dysfunction-associated steatotic liver disease (MASLD), collectively elevating the risk of cardiovascular disease and cancer. Importantly, sexual dimorphism in susceptibility to obesity, insulin resistance, and metabolic traits has been extensively reported in both animal models and human populations (1–5, 9–11).

Animal models, particularly those induced by high-fat diets (HFDs), remain essential tools for investigating the mechanisms underlying obesity and metabolic diseases. However, their relevance is compromised by the persistent underrepresentation of female animals (6), raising concerns



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about the applicability of findings predominantly derived from male subjects. As the global prevalence of obesity rises, currently affecting 13% of the population (12), addressing these sex-based biases becomes increasingly urgent to ensure an accurate and comprehensive understanding of metabolic disease pathophysiology.

The higher resistance to HFD-induced insulin resistance in females compared with males has been described in C57BL/6J mice (13) and in a study examining nearly 100 different mouse strains (3). Sexual dimorphism in metabolic health extends beyond animal models and is also evident in humans. Men exhibit a greater propensity for insulin resistance and metabolic syndrome compared with women (14), a difference that has been attributed to differences in adipose tissue distribution, hormonal regulation, and gene expression (15, 16). Furthermore, research conducted in murine models that distinguish between chromosomal and gonadal sex has revealed intricate processes involved in sex-specific epigenetic, transcriptional, and posttranscriptional control of gene expression in cardiometabolic disorders (17). Therefore, these results underscore the significant disparity in insulin sensitivity between males and females in response to metabolic disturbances in mammals. However, the specific mechanisms underlying this dimorphism are complex and remain incompletely understood.

A major factor intervening in these differences is sex hormones (18). Although a lower susceptibility to T2D has been reported in females of mammals, including humans, this protection is lost in postmenopausal women or animals devoid of ovarian hormones (11), indicating the relevant role of estrogens in female metabolic homeostasis. Conversely, there is a higher prevalence of hypogonadism in obese men (19, 20). However, the elimination of sex hormones by gonadectomy does not completely reverse metabolic sex differences, and their effects are dependent on the target tissue (5, 21, 22). Consequently, there is a complex interplay between sex and metabolism, and the mechanisms of this relationship are different between males and females.

Sex-biased, also known as sexually dimorphic, gene expression plays a crucial role in maintaining organ homeostasis, with numerous genes exhibiting differential regulation between males and females across various tissues. Recent research has identified sex-biased gene expression patterns in multiple organs when comparing males and females in adipose, liver, and other tissues both in rodents (3, 23), in humans (8, 24, 25), and even across different species and developmental stages (26). This sexual asymmetry, primarily driven by genetic mechanisms (27), reflects evolutionary adaptations that have allowed males and females to respond to distinct sex-specific selective pressures (28). These differences influence physiological processes such as metabolism (5, 17, 29, 30), immune response (31), and tissue regeneration (22, 32, 33), highlighting the importance of sex as a biological variable in both health and disease. However, significant gaps remain, particularly in understanding how sex-biased genes are deregulated during physiological challenges such as metabolic stress. Further investigation is needed to uncover the mechanisms driving this deregulation and its implications for sex-specific disease vulnerability and treatment responses.

In this study, we investigate how sex influences the progression of metabolic dysfunction induced by HFD feeding. By performing transcriptomic profiling of liver and WAT at both early (2 wk) and advanced (12 wk) stages of diet-induced obesity, we uncover distinct gene expression patterns between males and females. Our findings reveal that metabolic adaptations to HFD involve sex-specific transcriptional programs, with males and females deploying different gene clusters to cope with metabolic stress. Moreover, we identify a striking loss of sex-biased gene expression in both liver and WAT upon prolonged HFD exposure, suggesting that obesity disrupts fundamental sex-specific regulatory mechanisms. These insights enhance our understanding of the molecular basis of metabolic sex differences and highlight the critical role of sexually dimorphic genes in maintaining metabolic homeostasis.

MATERIALS AND METHODS

Animals and Treatments

C57BL/6J (RRID:MGI:3028467) male and female mice were obtained from Charles River Laboratories. At 8 wk of age, mice were kept on regular diet (RD, control groups) (standard rodent chow, 13% calories from fat, 67% from carbohydrate, and 20% from protein, #2014; Teklad) or fed with high-fat diet (HFD, 45% calories from fat, 35% from carbohydrates, and 20% protein, #D12451; Research diets Inc.) for the periods indicated, which did not provoke adverse effects. Glucose tolerance test (GTT) was determined by intraperitoneal (ip) dose of 2 g/kg glucose after 5-h fasting. Tail blood glucose was measured at the specified time points with reactive strips in a glucometer (#53841; NovaPro). The study design (Supplemental Fig. S1A) involved three randomly assigned cohorts of male and female mice, fed either a RD or HFD for 2, 12, or 16 wk, and euthanized at the respective time points for analysis. The number of animals per group was as follows: male RD 2 wk ($n = 10$), male HFD 2 wk ($n = 10$), female RD 2 wk ($n = 5$), female HFD 2 wk ($n = 5$); male RD 12 wk ($n = 8$), male HFD 12 wk ($n = 11$), female RD 12 wk ($n = 8$), female HFD 12 wk ($n = 10$), male RD 16 wk ($n = 11$), male HFD 16 wk ($n = 15$), female RD 16 wk ($n = 5$), and female HFD 16 wk ($n = 8$), for a total of 106 animals. Animals were monitored for weight, glucose tolerance test (GTT), and insulin levels at 2, 6, 12, and 16 wk of treatment. RNA from liver and visceral WAT from animals at early (2 wk) or advanced HFD feeding (12 wk) was used for RNA-sequencing-based transcriptomic analysis. The minimum sample size for animal groups was determined using the Boston University IACUC Sample Size Calculations spreadsheet (<https://www.bu.edu/research/forms-policies/iacuc-sample-size-calculations/>), based on mean differences and variability observed in previous data regarding the effect of HFD on metabolic parameters. Two animals were excluded from the study due to external causes unrelated to treatment. To minimize bias and confounding variables, animals were randomized before treatment, RD and HFD groups were managed in parallel and all sample processing and data analyses were conducted in a blinded manner.

Animals were housed in ambient and sanitary-controlled conditions at the University of Barcelona School of Medicine

vivarium. All procedures involving animals and their care were approved by the Ethics Committee of the University of Barcelona and local authorities (Generalitat de Catalunya) with the numbers 11207 and 11373, following national and European guidelines for maintenance and husbandry of research animals, which include interventions in the experimental protocols to reduce pain or suffering.

Biochemical Measurements

Insulin levels were determined by ELISA (Ultra-Sensitive Mouse Insulin ELISA Kit, #90080; CrystalChem). Hepatic triglyceride content was measured after organic solvent extraction (34). Briefly, a preweighed frozen piece of liver (100 to 200 mg) was homogenized in 1 mL of isopropanol, vortexed for 1 min, and spun after 2,000 g at 4°C, and the amount of triglyceride of the supernatant was measured with the Triglycerides-LQ GPO-POD Kit (#41030; Spinreact).

Histological Analysis

Fresh tissue samples were fixed in neutral-buffered 10% formalin for 24 h, and afterward were sequentially dehydrated in 70 to 100% ethanol and embedded in paraffin. Tissue blocks were sliced at 5 µm and stained for general morphological evaluation with standard hematoxylin and eosin (Sigma-Aldrich). In addition, tissue samples were immediately frozen after euthanasia in cryopreservation medium OCT (Sakura Inc.) and stored at -80°C. Frozen sections of 10–15 µm thickness were stained with Oil Red O (Sigma-Aldrich) to determine the presence of neutral lipid infiltration. Images were taken with a Nikon Eclipse E600 microscope and collected with Olympus Cell[^]D software v. 3.4.

Adipocyte size in hematoxylin & eosin-stained sections was measured by the ImageJ (RRID:SCR_003070) plugin Adiposoft (35), on at least 3 random fields for each individual, counting from 400 to 1,100 adipocytes per field, with an average of 600 adipocytes/field. Histological preparations were performed at the HCB-IDIBAPS Biobank Core.

RNA Isolation and qPCR

Tissue samples were homogenized using TRIzol reagent (Invitrogen), phases were separated with chloroform, and RNA was isolated from the aqueous phase using RNeasy columns (Qiagen). RNA was quantitated, and its purity was assessed using Nanodrop microvolume spectrophotometer (Thermo Fisher Scientific). Real-time PCR was performed using the SensiFAST SYBR Hi-ROX One-Step Kit (Meridian) following manufacturer's instructions. Quantification of a given gene, expressed as relative mRNA level compared with a reference sample, was calculated after normalization to a housekeeping gene that showed no variation with treatment. Oligonucleotides for target genes analyzed were designed using Primer3Plus bioinformatics resource (36). Oligos for qPCR sequences are listed in Supplemental Table S1.

RNA-Seq Analysis

RNA sequencing (RNA-seq) was performed at the Genomics core facility of IDIBAPS using liver and WAT samples from 3 mice per group. Messenger RNA strand-specific RNA libraries were created from 200 ng of total RNA using the Illumina

Stranded mRNA Prep Ligation and sequenced in paired-end mode with a read length of 2 × 50 base pairs on an Illumina NextSeq2000 (Illumina, Inc.) for RNA-seq analysis. The resulting median of 40 million paired-end reads for each sample passed the quality control checks performed in FastQC software (RRID:SCR_014583). Raw reads were mapped to the reference genome (Mus_musculus_GRCm38) using the pseudo-aligner Kallisto. Transcript counts were imported to R statistical software using Tximport package (RRID:SCR_016752). Data were then filtered and normalized for exploratory analysis with EdgeR using the trimmed mean of M values (TMM) normalization method (37). The iDEP web-based tool was used for preliminary analyses (38). Differentially expressed genes (DEGs) were identified using the DESeq2 package (RRID:SCR_015687) (39), following low count filtering and normalization via the DESeq2 built-in median-of-ratios method (40). DEGs were defined as those with an adjusted *P* value lower than 0.05 by false discovery rate (FDR), and a fold change between the RD and HFD was set to 1.5 for liver comparisons and 2.0 for WAT. For the identification of sex-dimorphic genes, an FDR of 0.05 and a fold change of 2.0 was set for the comparison between males and females in both tissues. Venn diagrams and intersections between groups were obtained with DeepVenn (41). The enrichment of DEGs in Gene Ontology categories, KEGG pathways, and the GSEA Hallmark lists of curated genes (42) was determined by g: Profiler (RRID:SCR_006809) (43). Heatmaps were performed using Splot (www.bioinformatics.com.cn/srplot).

RNA-Seq datasets generated and analyzed during the current study are available in the NCBI GEO repository under accession number GSE262523. The datasets used during the current study are available from the corresponding author on reasonable request.

Statistical Analysis

All results are expressed as means ± standard deviation (SD). Statistical significance was determined by two-tailed Student's *t* test when only two groups were compared or with one-way ANOVA with Dunnett multiple comparison test for comparison of three or more groups. For comparison involving two variables, the two-way ANOVA with Dunnett post-hoc analysis was used to compare the groups. If the data failed the normality test, nonparametric tests were used. For the comparison of proportions of a contingency table, we performed the Chi-square test. Statistical significance was set for a *P* value < 0.05. Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, RRID:SCR_002798).

RESULTS

Females Exhibit Better Glucose Metabolism than Males upon High-Fat Diet Feeding, despite Similar Adiposity Induction

We first aimed to analyze the distinct temporal metabolic adaptations in males and females during the progression of an obesogenic diet (Supplemental Fig. S1A). Both sexes exhibited increased body weight after 6–7 wk of HFD (Supplemental Fig. S1B), and the relative body weight was significantly elevated

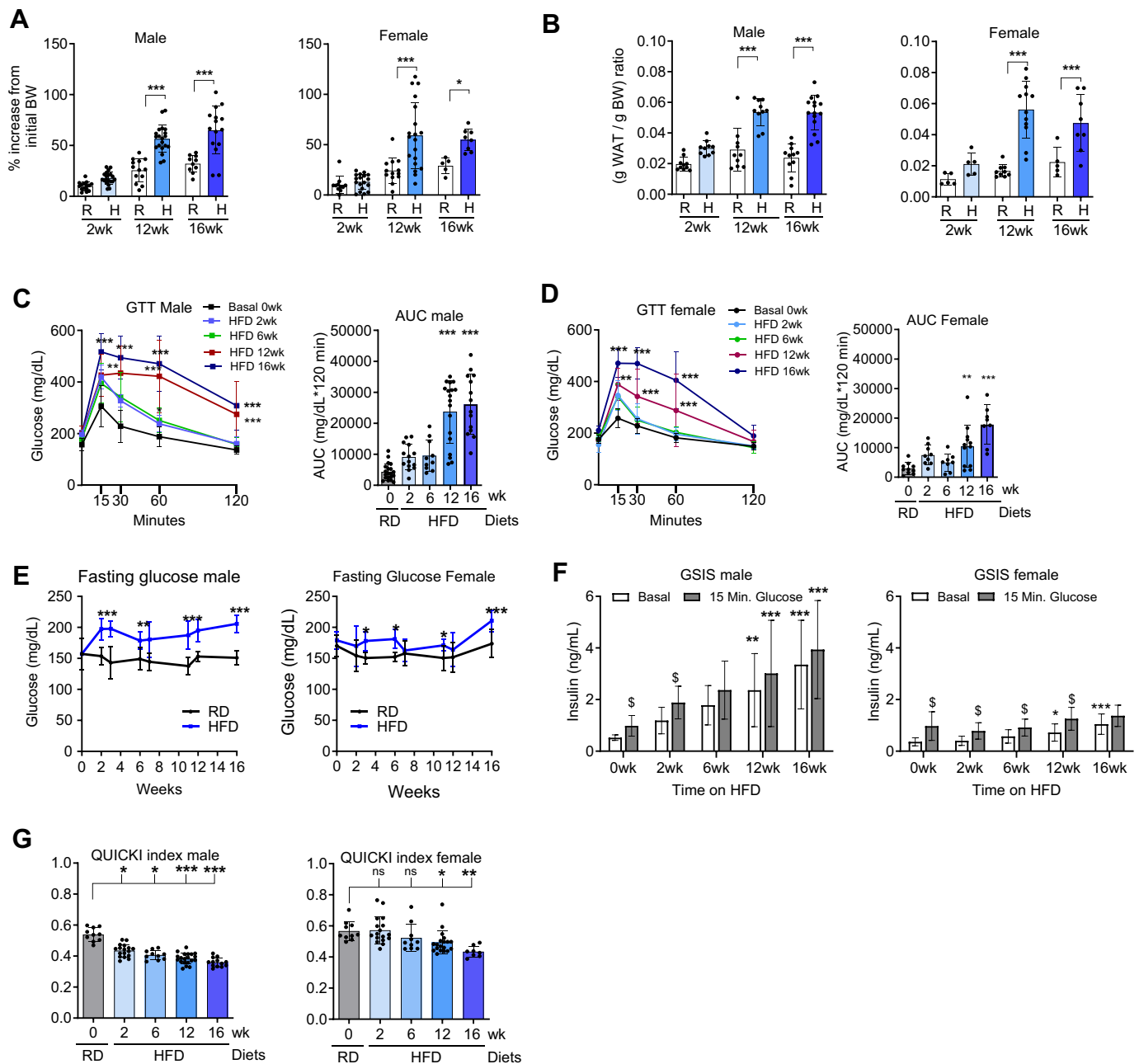


Figure 1. High-fat diet (HFD) increases adiposity in a comparable degree in both sexes, but glucose metabolism and insulin sensitivity are more severely affected on males than on females. **A:** percentage of increase in body weight (BW), respect to baseline body weight in males and females at different time points (2 wk, 12 wk, or 16 wk) of regular (R) or HFD (H) feeding in mice ($n = 5\text{--}20$ mice/group and time point). **B:** relative visceral WAT weight corrected by BW ratio, in both sexes at the different time points of regular or HFD feeding. **C:** glucose tolerance test (GTT) performed in male animals at baseline or after 2, 6, 12, and 16 wk of HFD feeding and the corresponding area under the curve (AUC) calculations. The GTT was performed after 5 h fast with 2 g of glucose per kg of BW ($n = 10\text{--}20$ mice/group and time point). **D:** GTT performed in female animals ($n = 8\text{--}12$ mice/group and time point). **E:** basal (5-h fasting) glucose levels in males ($n = 5\text{--}10$ mice/group and time point) and females ($n = 5\text{--}20$ mice/group and time point) fed with regular (RD) or HFD over the 16 wk of diet. **F:** plasma insulin levels before and 15 min after glucose administration in the GTT ($n = 8\text{--}15$ mice/group and time point), in males or females at baseline and at different time points (2 wk, 6 wk, 12 wk, or 16 wk) of HFD. *, **, and ***Statistically significant differences compared with the initial stage (0 wk) at basal or stimulated insulin levels, respectively, on a one-way ANOVA with Dunnett multiple comparison test. \$Statistically significant difference in a Student's *t* test for comparison from basal to glucose-stimulated insulin levels at that specific time point. **G:** progression of the QUICKI insulin sensitivity index over the HFD feeding period in males and females. Data represent the means $\pm$ SD. Differences between groups were tested by the one-way ANOVA multiple comparison test with Dunnett post-hoc test. GTT curves were tested for differences using a two-way ANOVA multiple comparison with Dunnett post-hoc test. Statistical significance is represented as: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. WAT, white adipose tissue.

after 12 wk of HFD (Fig. 1A) primarily due to heightened relative adiposity (Fig. 1B). Although females had smaller adipocytes on a regular diet, HFD led to an enlargement of adipocyte size in both sexes, with comparable mean sizes after 16 wk (Supplemental Fig. S1, B and C). Relative food and energy intake remained equivalent between sexes under both diets (Supplemental Fig. S1, E and F).

Despite a comparable increase in relative adiposity, male mice displayed a pronounced progression of glucose intolerance on HFD, as evidenced by glucose tolerance tests (Fig. 1C) and by basal hyperglycemia from week 2 onward (Fig. 1E), whereas females exhibited a more moderate progression (Fig. 1, D and E). Moreover, males also showed hyperinsulinemia starting at week 2, which became significant by week 12, with glucose-stimulated insulin secretion diminished from week 6 (Fig. 1F). The insulin sensitivity index QUICKI (44) significantly dropped in males upon HFD feeding (Fig. 1G). Conversely, females maintained their insulin levels until week 6 and preserved their glucose-stimulated response up to week 12, exhibiting moderate basal hyperinsulinemia at advanced stages of HFD. Female insulin levels at week 16 were four times lower than those of males, highlighting a pronounced sex-specific response in insulin sensitivity upon the HFD challenge.

Liver steatosis, a key marker of metabolic syndrome and MASLD, corresponded with increased glucose intolerance and hyperinsulinemia in males after 12 wk of HFD, with

hepatic triglycerides significantly elevated (Fig. 2A), and macrosteatosis evident by week 16 (Fig. 2C). In contrast, female mice exhibited a moderate rise in hepatic triglycerides as early as the second week of HFD, but this rise persisted at levels less pronounced than those in males throughout the HFD exposure (Fig. 2, B and C).

Adaptive Responses to a HFD Are Associated with Distinct Patterns of Gene Expression in the Liver of Males and Females

To better understand HFD-induced phenotypic changes, we performed RNA-seq on liver and adipose tissue transcriptomes in male and female mice at early (2 wk) and advanced (12 wk) stages of obesity. Pairwise comparisons identified differentially expressed genes (DEGs) regulated by HFD at each time point in males and females. DEGs were then categorized as common to both sexes, exclusive to males, or exclusive to females, and further classified as up- or downregulated.

A 2-wk HFD exposure significantly regulated numerous DEGs in the liver of both sexes. Among these, 371 were exclusive to males, 185 to females, and only 42 were shared between sexes (Fig. 3, A–C). Bubble plots highlight the enrichment of up- or downregulated DEGs in specific pathways for male-exclusive (Fig. 3D), common DEGs (Fig. 3E), or DEGs exclusive to females (Fig. 3F).

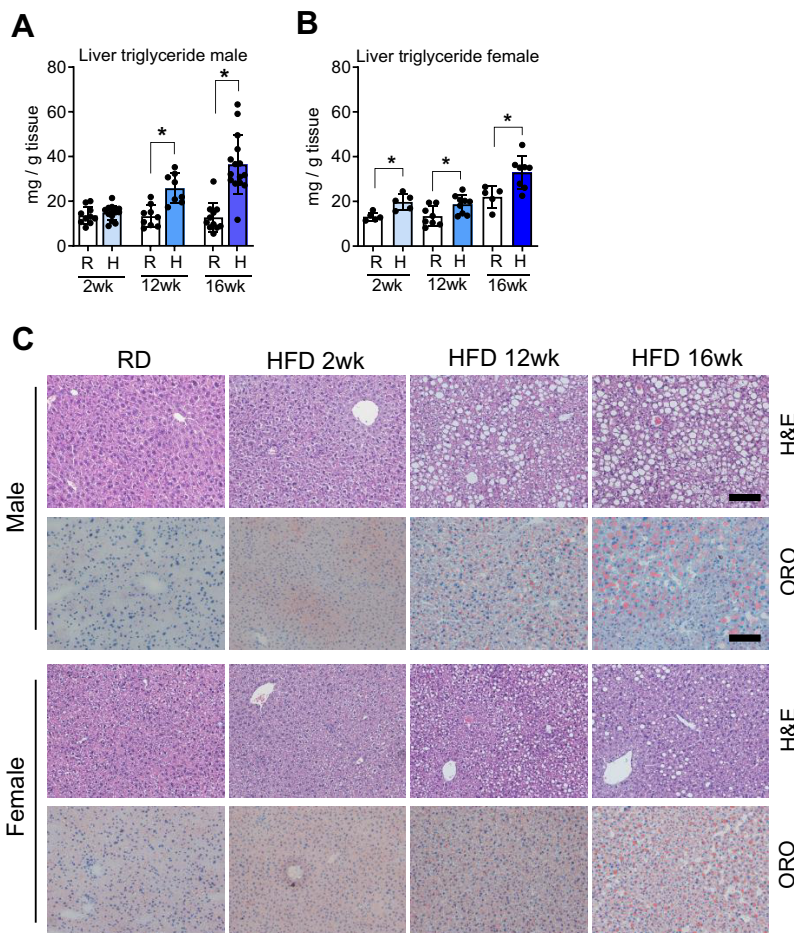
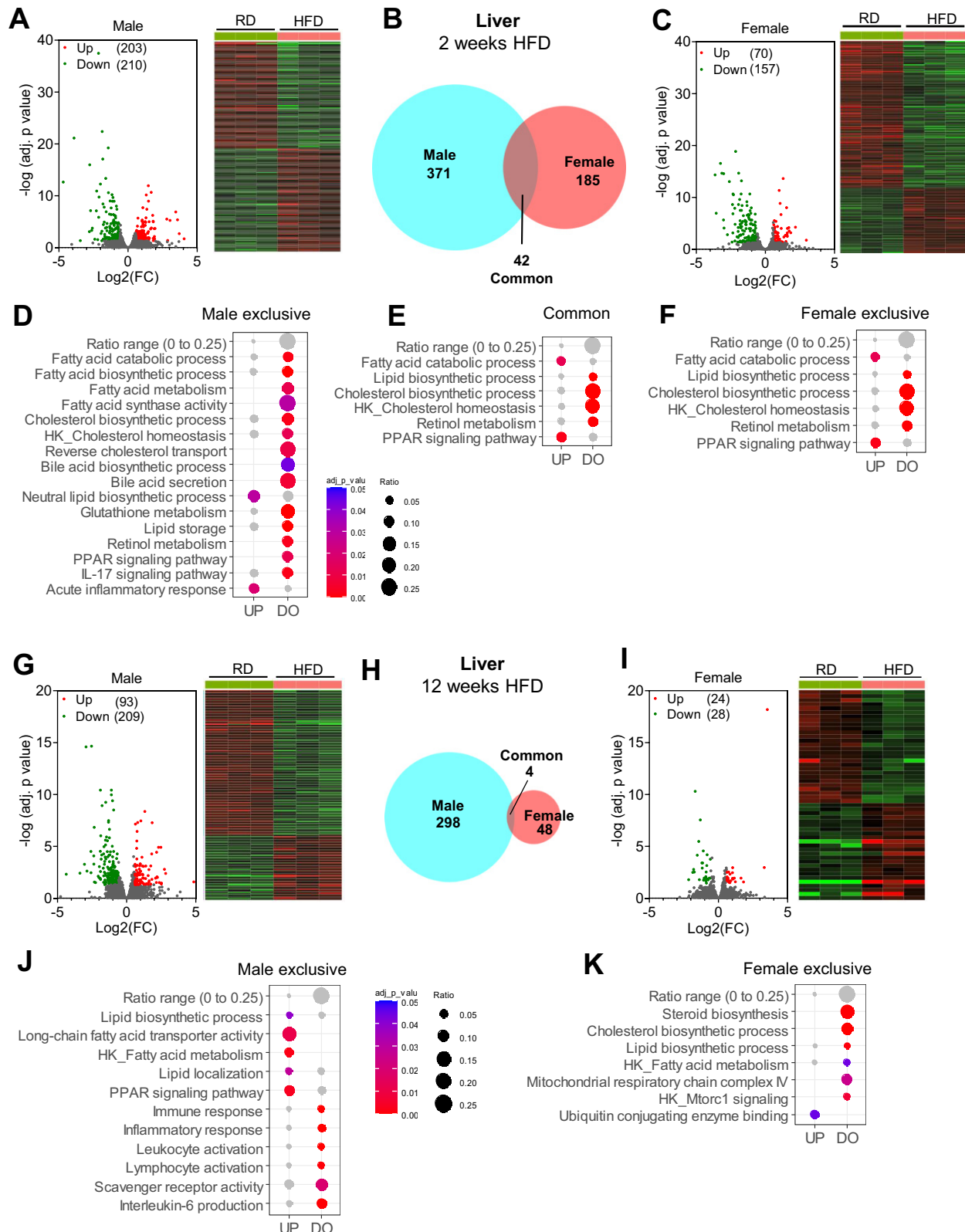


Figure 2. HFD induces different degrees of liver steatosis in male and female mice. **A:** liver tissue triglyceride levels in male animals after 2, 12, and 16 wk of regular (RD) or HFD feeding ($n = 8$ – 15 mice/group and time point), measured as described in methods. **B:** liver tissue triglyceride levels in females ($n = 5$ – 9 mice/group and time point). **C:** representative liver sections stained with hematoxylin-eosin (H&E) or Oil red o (ORO) of male and female animals on RD or 2 wk, 12 wk, or 16 wk of HFD. HFD, high-fat diet.

Pathway analysis showed limited overlap between sexes, with shared pathways such as retinol metabolism featuring both sex-specific and common DEG regulation (Fig. 3, D–F). Similar patterns were observed in cholesterol metabolism and

fatty acid catabolic processes, which often displayed divergent regulation between males and females (Fig. 3, D–F).

After 12 wk of HFD, sex profile divergence in the liver became even more pronounced (Fig. 3, G–I). However,



pathways related to lipid transport and handling were found to be upregulated exclusively in male DEGs (Fig. 3J), consistent with the emergence of lipid steatosis after 12 wk of HFD (Fig. 2). Conversely, females exhibited predominantly downregulated cholesterol and lipid synthesis pathways, similar to findings at the 2-wk HFD stage (Fig. 3K). However, the most significantly enriched pathways in the liver were different between the early and advanced stages (Fig. 2). Key DEGs from diverse gene categories were validated in a larger cohort by qPCR (Supplemental Fig. S3A).

HFD Induces Sex-Divergent Gene Profile in Visceral White Adipose Tissue

After 2 wk of HFD, an early expansion of WAT mass and an increase in adipocyte size occurred in both sexes (Fig. 1, Supplemental Fig. S1C), which were associated with a significant regulation of DEGs in WAT in males (Fig. 4A) and females (Fig. 4C). However, consistent with liver findings, only a small portion of DEGs overlapped between sexes (Fig. 4B). Common DEGs exhibited downregulated metabolic and lipid pathways, alongside upregulated extracellular matrix pathways in both sexes, indicating active tissue remodeling (Fig. 4, D–F) (4, 32, 33). Likewise, pathways related to lipid, carbohydrate, and nucleotide metabolism were downregulated in both sexes, but the specific DEGs varied. In addition, mitochondrial-related DEGs were enriched at both common and sex-specific downregulated DEGs at early and advanced HFD, pointing to a downregulation of genes related to this central organelle in WAT by different mechanisms in males and females.

At 12 wk, the WAT transcriptome showed dramatic changes (Fig. 4, G–I), yet only 9% of DEGs overlapped between the sexes. The common upregulated DEGs were enriched in pathways related to the extracellular matrix and angiogenesis, indicating tissue remodeling and showing additional enrichment in the innate immune response and the TLR-4 pathway (Fig. 4K).

Male-specific upregulated DEGs were highly enriched in inflammatory and immune-related pathways, reflecting activation of innate and adaptive immune systems (Fig. 4J). Remarkably, none of these categories were found to be enriched in female WAT (Supplemental Fig. S4). Downregulated male-specific DEGs were enriched in oxidative phosphorylation and estrogen response genes (42), indicating HFD-associated suppression of these pathways, which were not significantly affected in females. In

addition, male-specific downregulated genes highlighted pathways related to cilia function, previously linked to obesity and metabolic health (45). Conversely, female-specific DEGs were enriched in upregulated pathways associated with amino acid metabolism, contrasting with male-specific downregulation in the same category (Supplemental Fig. S4). A subset of DEGs across gene categories was validated via qPCR in a larger cohort (Supplemental Fig. S3B).

Sex-Dimorphic Genes Are Preferentially Regulated in Response to HFD

In our analysis of the transcriptome, sex emerged as the primary factor distinguishing animal groups in both tissues, as shown by principal component analysis (PCA). In the liver, PC1 revealed a pronounced separation between sexes (Fig. 5A), with WAT displaying a similar but less marked separation (Fig. 5B).

This transcriptomic divergence reflects a strong underlying sex bias, primarily driven by differences in sex-dimorphic gene expression (23, 26). To quantify this bias, we identified sex-biased genes in basal conditions, defined as those with significantly different expression between males and females. A substantial number of sex-biased genes were identified in both liver and WAT (Fig. 5, C and D), defined as genes differentially expressed in males (male-biased) or in females (female-biased).

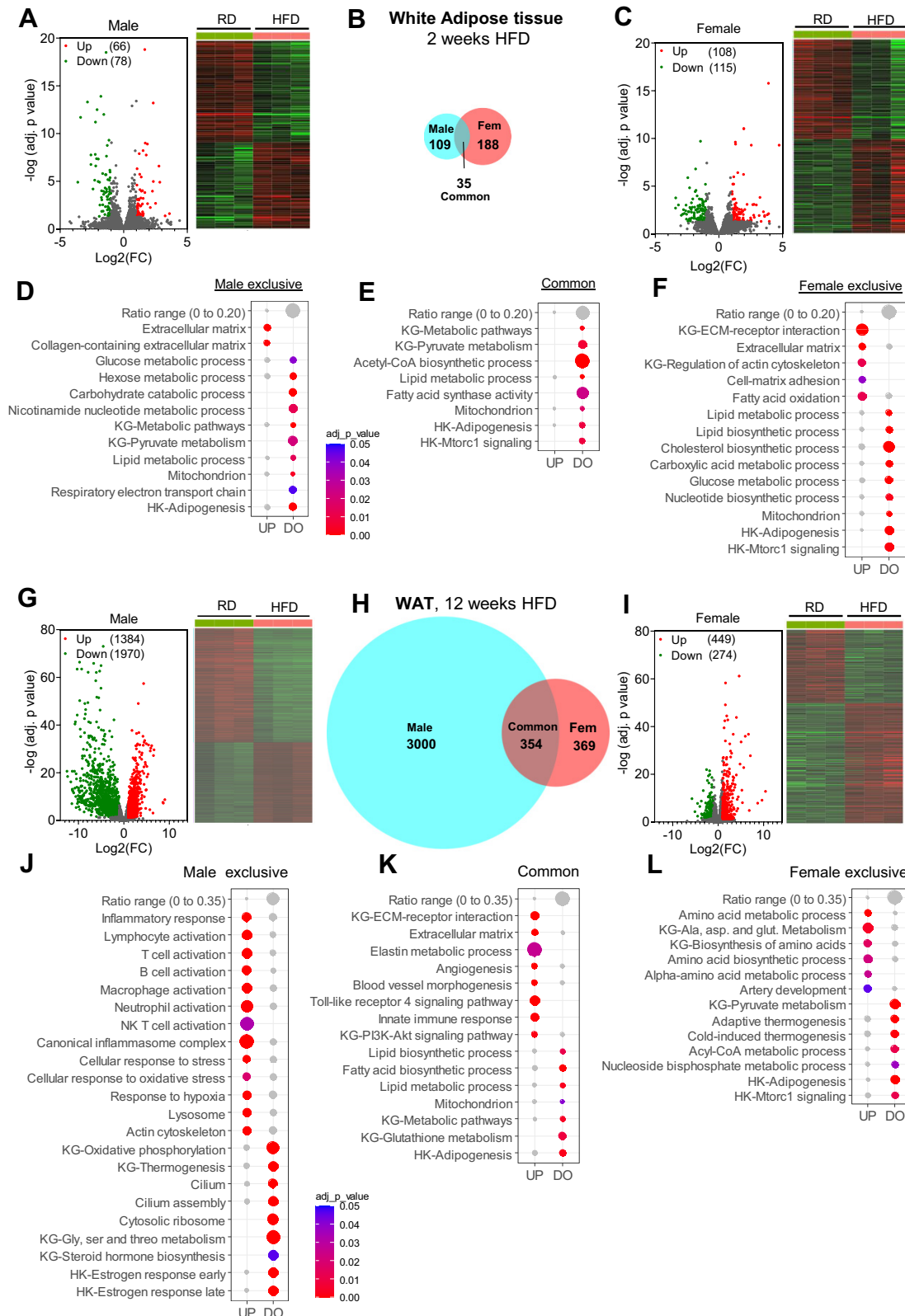
Enrichment analyses revealed that these genes clustered into distinct biological pathways, many of which were significantly enriched for either male- or female-biased genes. Notably, some pathways included both, suggesting sex-divergent transcriptional strategies within shared functional gene categories (Fig. 5, E and F). For instance, cilium-related gene categories were exclusively enriched for male-biased genes in WAT, whereas lipid metabolism pathways in both tissues contained a mixture of sex-biased genes, reflecting sex-specific regulatory programs involved in lipid handling.

In the liver, the sex-dimorphic genes accounted for 6% of the total expressed genes, whereas in the WAT they accounted for up to 13.7% in animals on RD (see Fig. 6, A and B). Strikingly, upon HFD exposure, the proportion of sex-dimorphic genes among HFD-regulated genes increased significantly in both tissues (Fig. 6, C and D), indicating that sex-dimorphic DEGs are preferentially responsive to HFD. This pattern is particularly evident in WAT, where 46.7% and 38.1% of HFD-regulated genes in males and females, respectively, correspond to sex-biased genes.

Figure 3. HFD induces differential changes in the liver transcriptome in male and female mice. *A*: volcano plot and heatmap of differentially expressed genes (DEGs) in the liver of male mice after 2 wk of HFD feeding. *B*: proportional Venn diagram displaying the number of DEGs changed by HFD exclusively in males, exclusively in females, or changed in both sexes (common). *C*: volcano plot and heatmap of DEGs in the liver of female animals after 2 wk of HFD feeding. *D*: bubble plot depicting the most relevant pathway and gene categories significantly enriched in the set of DEGs (upregulated or downregulated) by 2 wk of HFD feeding, only in males. The size of the circle represents the ratio of DEGs that fall into a particular category respect to the total number of genes in that category, whereas the color of the adjusted *P* value from 0 (red) to 0.05 blue, or nonsignificant (gray). Gene categories are from Gene Ontology unless they are from GSEA HALLMARK (HK) or from Kegg Pathways (KG). *E*: bubble plot of the pathways or gene categories significantly enriched in upregulated or downregulated genes in both males and females (common). *F*: bubble plot of the pathways or gene categories significantly enriched in upregulated or downregulated genes in exclusively females. *G*: volcano plot and heatmap of differentially expressed genes in the liver of male animals after 12 wk of HFD feeding. *H*: Venn diagram displaying the number of differentially expressed genes changed by HFD exclusively in males, exclusively in females, or changed in both sexes. *I*: volcano plot and heatmap of differentially expressed genes in the liver of female animals after 12 wk of HFD feeding. *J*: bubble plot of the pathways or gene categories significantly enriched in upregulated or downregulated genes exclusively in males. *K*: bubble plot of the pathways or gene categories significantly enriched in upregulated or downregulated genes exclusively in females. RNA-seq analysis was performed using liver samples from 3 mice/group. HFD, high-fat diet.

A detailed analysis of the proportions of male-biased and female-biased genes among HFD-responsive DEGs revealed dynamic changes over the course of the diet in both tissues (Fig. 6, E and F). After 2 wk of HFD, the proportions of male-

and female-biased DEGs were relatively similar in the liver of male mice (Fig. 6E). However, the liver of female mice exhibited a striking pattern in which nearly all HFD-upregulated DEGs were male-biased genes, whereas most HFD-



downregulated DEGs were female-biased genes, uncovering a loss of female bias in the set of genes responding to HFD (Fig. 6E). This trend persisted at 12 wk, although the male liver began to display a higher proportion of regulated female-biased genes.

In the context of WAT (Fig. 6F), male animals exposed to HFD for two weeks showed a greater proportion of female-biased genes within the HFD-regulated genes. The WAT of female mice exhibited the same striking pattern observed in the liver, in which nearly all HFD-upregulated DEGs were male-biased genes, whereas most HFD-downregulated DEGs were female-biased. Strikingly, at the advanced stage of HFD exposure, both sexes downregulated genes biased toward their own sex while upregulating those biased toward the opposite sex, indicating a reduction in transcriptomic sex bias. Remarkably, in male WAT, over half (55%) of the genes downregulated after 12 wk of HFD were sex-biased, with nearly all being male-biased, indicating a pronounced reduction in male-specific gene expression (Fig. 6F). Pathway enrichment analysis at this stage revealed that only a small number of inflammatory pathways showed significant enrichment in sex-biased genes (Fig. 6G), suggesting that male WAT inflammation arises from secondary triggers. However, other gene categories (such as estrogen response and cilium) were downregulated specifically in male WAT, with these pathways being enriched in sex-dimorphic genes. Conversely, female WAT showed enrichment of sex-dimorphic DEGs in upregulated amino acid metabolism and downregulated lipid-related processes (Fig. 6H).

HFD Significantly Reduces Sexually Dimorphic Profiles in Liver and Visceral Adipose Tissue

Analysis of sex-dimorphic DEGs in male WAT after 12 wk of HFD revealed a notable reduction in sex bias within specific gene categories. One of the most prominent categories exhibiting this property includes cilium-related genes. These genes, which were expressed at higher levels in males under RD and early HFD exposure, were downregulated by HFD to levels similar to females, which consistently showed low expression in this category and no response to HFD (Fig. 7, A and B). This effect was also evident in the estrogen response category (Fig. 7B). Conversely, females displayed lower expression levels in amino acid metabolic genes compared with males in basal conditions and at the early HFD stage. However, after 12 wk of HFD, these

genes were upregulated to similar levels as those observed in males (Fig. 7C). Nevertheless, this loss of sex bias was not observed in other pathways that were not significantly enriched in sex-dimorphic genes, such as oxidative phosphorylation (Fig. 7D) and inflammatory response (Fig. 7E). In these pathways, although males and females responded in the same direction, the magnitude of change was significantly greater in males.

In line with this, we selected the DEGs displaying sex bias in RD and checked whether their sex bias was maintained under HFD. In the liver, a significant proportion of sex-biased DEGs lost their bias with HFD exposure (Fig. 8A). Strikingly, this effect was even more pronounced in the WAT, where most of the sex-dimorphic genes observed in RD lost their bias in HFD (Fig. 8B), graphically confirming the dramatic loss of the sex-dimorphic gene expression in both tissues and both sexes upon HFD exposure.

DISCUSSION

Despite established differences in biological traits and treatment outcomes between sexes (5, 46–48), female subjects remain underrepresented in metabolic research, limiting the applicability of male-derived findings. To address this gap, we investigated how sex shapes metabolic and transcriptional responses to obesogenic and diabetogenic challenges, aiming to clarify mechanisms underlying differential disease susceptibility and progression of metabolic disease.

At a phenotypic level, our findings align with previous studies (13, 49), demonstrating significant sexual dimorphism in the response to an HFD in C57BL/6J mice, affecting insulin sensitivity and its downstream effects. Notably, female mice exhibited greater protection against glucose intolerance and insulin resistance despite experiencing a similar relative increase in WAT mass. This resilience may stem from the ability of female mice to sustain energy expenditure under HFD conditions, unlike males, who show a reduction in energy expenditure compared with low-fat diet controls (13). This divergence may explain the relatively lower adiposity and hepatic triglyceride accumulation in females, despite comparable relative energy intake. These observations support previous findings that females exhibit greater resistance to HFD-induced metabolic dysfunctions (3, 5, 13), and it is consistent with the lower incidence of T2D in pre-menopausal women (14, 48).

Figure 4. HFD induces differential transcriptome changes in male and female mice in visceral WAT. **A:** volcano plot and heatmap of DEGs in visceral white adipose tissue of male mice after 2 wk of HFD feeding. **B:** Venn diagram displaying the number of DEGs changed by HFD exclusively in males, exclusively in females, or changed in both sexes (common). The size of the circles is proportional to the number of DEGs. **C:** volcano plot and heatmap of DEGs in white adipose tissue of female animals after 2 wk of HFD feeding. **D:** bubble plot depicting the most relevant pathway and gene categories significantly enriched in the set of genes differentially expressed (upregulated or downregulated) by HFD only in males, after 2 wk of HFD. Gene categories are from Gene Ontology, unless they are from GSEA HALLMARK (HK) or from Kegg Pathways (KG). **E:** bubble plot of the pathways or gene categories significantly enriched in upregulated or downregulated genes in both males and females. **F:** bubble plot of the pathways or gene categories significantly enriched in upregulated or downregulated genes exclusively in females. **G:** volcano plot and heatmap of differentially expressed genes in visceral white adipose tissue of male animals after 12 wk of HFD feeding. **H:** Venn diagram displaying the number of differentially expressed genes changed by HFD exclusively in males, exclusively in females, or changed in both sexes. The area of the Venn circles is proportional to the number of genes depicting the same scale as in (B). **I:** volcano plot and heatmap of differentially expressed genes in visceral white adipose tissue of female animals after 12 wk of HFD feeding. **J:** bubble plot of the pathways or gene categories significantly enriched in upregulated or downregulated genes exclusively in males. **K:** bubble plot of the pathways or gene categories significantly enriched in upregulated or downregulated genes in both males and females (common). **L:** bubble plot of the pathways or gene categories significantly enriched in upregulated or downregulated genes exclusively in females. RNA-seq analysis was performed using WAT samples from 3 mice/group. DEG, differentially expressed gene; HFD, high-fat diet.

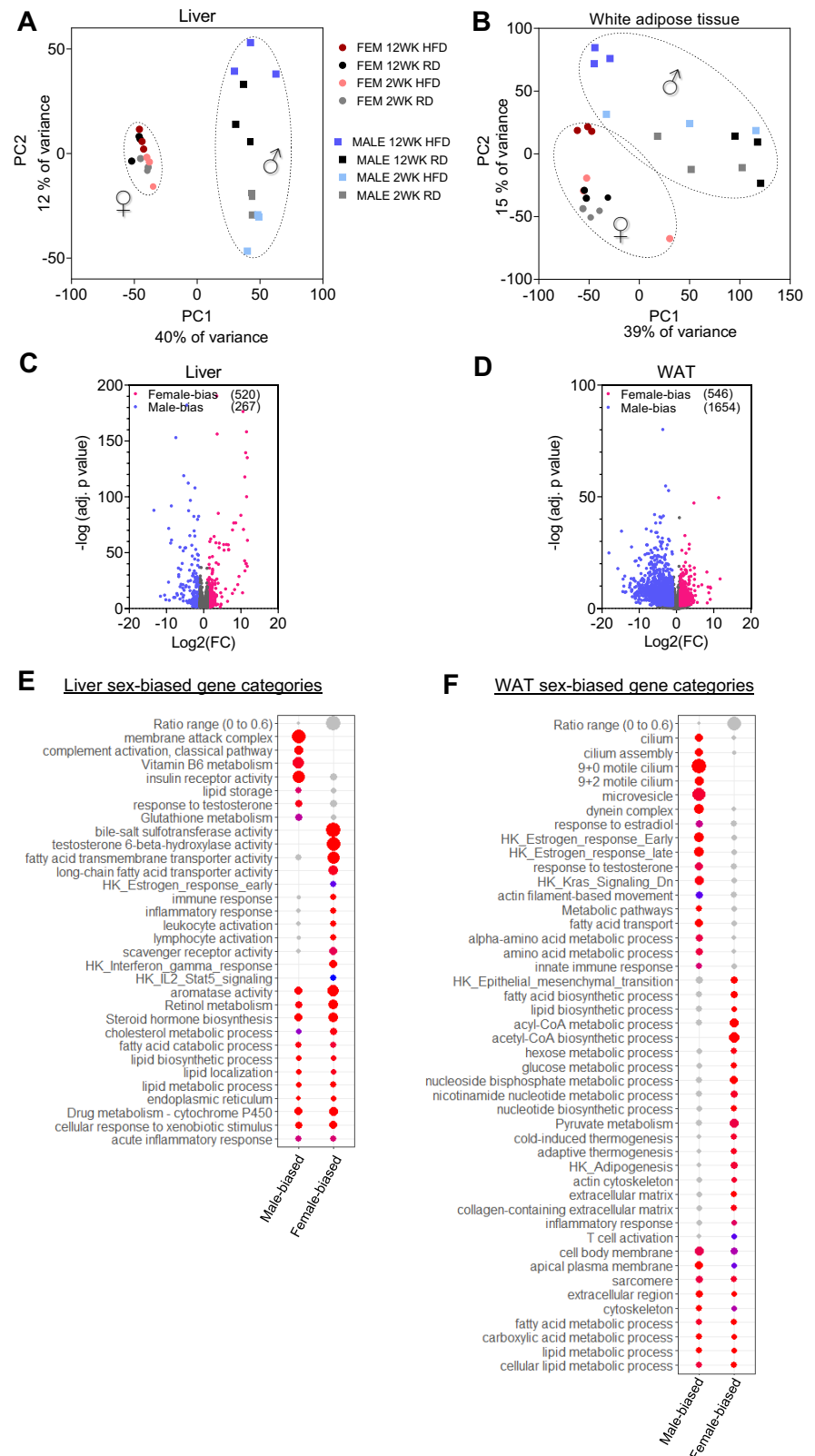


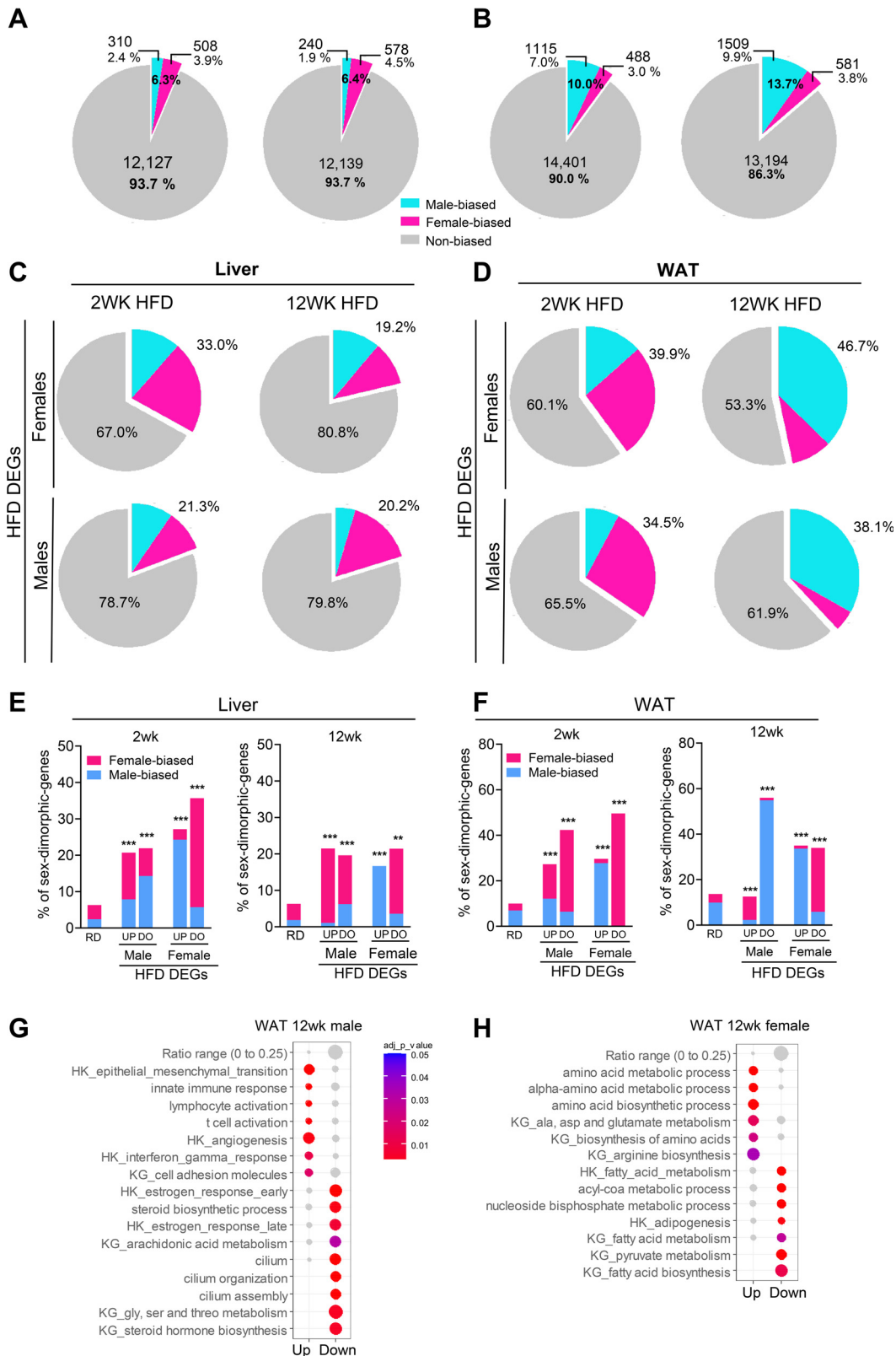
Figure 5. Sex-dimorphic genes are differentially expressed in female vs. male in liver and WAT. **A:** principal component analysis (PCA) of the transcriptome of all liver samples from male and female mice fed with regular diet (RD) or HFD for 2 or 12 wk. Square dots represent males and rounded dots represent females; values depicted for the first two principal components. **B:** PCA of the WAT transcriptome as in (A). **C:** volcano plot showing differentially expressed genes between male and female livers. Male-biased genes (blue) and female-biased genes (purple) are indicated, along with nonbiased genes. Expression was compared globally between sexes in RD groups at 2 and 12 wk. **D:** volcano plot illustrating male- and female-biased genes identified in white adipose tissue (WAT). **E:** Bubble plot highlighting significantly enriched pathways and gene categories among sex-biased genes in liver. Categories are primarily from Gene Ontology (GO), with additional sets from GSEA Hallmark (HK) and KEGG pathways (KG). **F:** bubble plot showing pathway enrichment analysis of male- and female-biased gene sets in WAT. RNA-seq analysis was performed using liver and WAT samples from 3 mice/group. HFD, high-fat diet; WAT, white adipose tissue.

In the liver, HFD induced steatosis, an early hallmark of MASLD (50). Hepatic steatosis presented differences between sexes in both triglyceride accumulation and lipid droplet storage under HFD conditions. These sex-specific disparities in steatosis and MASLD susceptibility found in animals

and humans arise from complex mechanisms, primarily linked to differences in sex hormones between males and females (51–55). However, other mechanisms, including sex-specific insulin levels (56) and temporal patterns of pituitary growth hormone secretion (57), also regulate liver gene expression.

Strikingly, although histological and compositional differences were notable, sex-specific transcriptional changes in both liver and WAT were even more pronounced and evolved dynamically over time. In early HFD exposure, only

a minor fraction of genes regulated by HFD in the liver was shared between males and females. Although the early response in both sexes involved pathways related to lipid metabolism, distinct sets of genes were regulated in each



sex, revealing sex-specific transcriptional programs to cope with the metabolic challenge induced by HFD. At later stages of HFD feeding, transcriptomic divergence became even more apparent. For instance, males displayed upregulated lipid metabolic pathways in the liver, correlating with increased steatosis resembling lipogenesis patterns in MASLD (58). In contrast, the downregulation observed in females suggests a compensatory mechanism aimed at managing excessive lipid accumulation.

Beyond sex dimorphism, our data revealed that the transcriptomic response to HFD evolves over time, with early and late phases involving largely distinct gene sets within each sex. In the liver, early downregulation of lipid, retinol, and bile acid metabolism pathways was either reversed or replaced by upregulation at advanced HFD feeding. In contrast, WAT exhibited early alterations in lipid and carbohydrate metabolism pathways, followed by a shift toward new transcriptional programs during prolonged HFD exposure. The latter changes included pathways related to inflammation, amino acid metabolism, cilia, and mitochondria, which were mostly sex-biased. These dynamic, tissue-specific shifts underscore the sex specificity of the differential functional and cellular adaptation of liver and WAT to metabolic stress over time.

WAT exhibited particularly dynamic responses to HFD, especially at later stages. Previous studies have reported a greater adiposity in males compared with females (59), although this feature is strain-dependent (46). In our cohorts, no sex differences in relative adiposity were observed, suggesting that sex-specific metabolic adaptations to HFD are not attributable to differences in adipose tissue expansion.

A notable observation in male WAT was the marked activation of inflammatory pathways after 12 wk of HFD, consistent with its role in driving male-specific metabolic disturbances (49, 60, 61). However, inflammatory DEGs upregulated by HFD showed no exclusive male bias, indicating secondary activation process, whereas these genes were moderately upregulated in females as well. Since the expression of inflammatory genes in WAT primarily reflects their expression in infiltrating immune cells, these results highlight an additional mechanism by which the whole tissue transcriptome is shaped by changes in tissue cellular composition. This cell composition-driven regulation is particularly relevant in WAT, as demonstrated in both animal models and humans (62, 63). In support of this, recent studies have shown that endothelial cells in adipose tissue exhibit significant sex-biased gene expression and phenotypic differences, reinforcing the contribution of sex-specific cellular functions to WAT physiology (64).

Consistent with previous studies (5, 22, 23, 65, 66), sex strongly shaped transcriptome profile in both tissues. A key novelty of this study lies in the observation that clusters of sex-dimorphic genes were highly responsive to HFD in males and females, at both the early and advanced stages. About 6% and 14% of all expressed genes exhibited sex-biased expression in the liver and WAT under a normal diet, respectively, aligning with prior findings in the human transcriptome across multiple tissues (1%–13%) (8). Importantly, the proportion of sex-dimorphic genes among those impacted by HFD increased dramatically in both the liver and WAT after 12 wk of HFD. This reached up to 60% of downregulated genes in the WAT of HFD-fed male mice and 40% of downregulated genes in the liver of female mice. This underscores the importance of sex-dimorphic gene regulation in driving phenotypic adaptations to HFD, consistent with the enrichment for hormone-related and other transcription factor binding sites in the promoters of sex-biased genes (8).

HFD modulated sex-biased gene expression in a time- and sex-dependent manner. At the early stages of HFD, males predominantly regulated genes that were biased in both sexes, whereas females upregulated male-biased genes and downregulated female-biased genes. This led to a net reduction in female-biased gene expression in both tissues. At the advanced stage of HFD, both sexes exhibited repression of their own sex-biased genes and induction of genes biased toward the opposite sex, resulting in a convergence of transcriptomic profiles. In contrast, certain sex-dimorphic gene expression patterns, such as the heightened inflammatory response in male WAT, arose specifically under HFD as secondary responses to the dietary intervention.

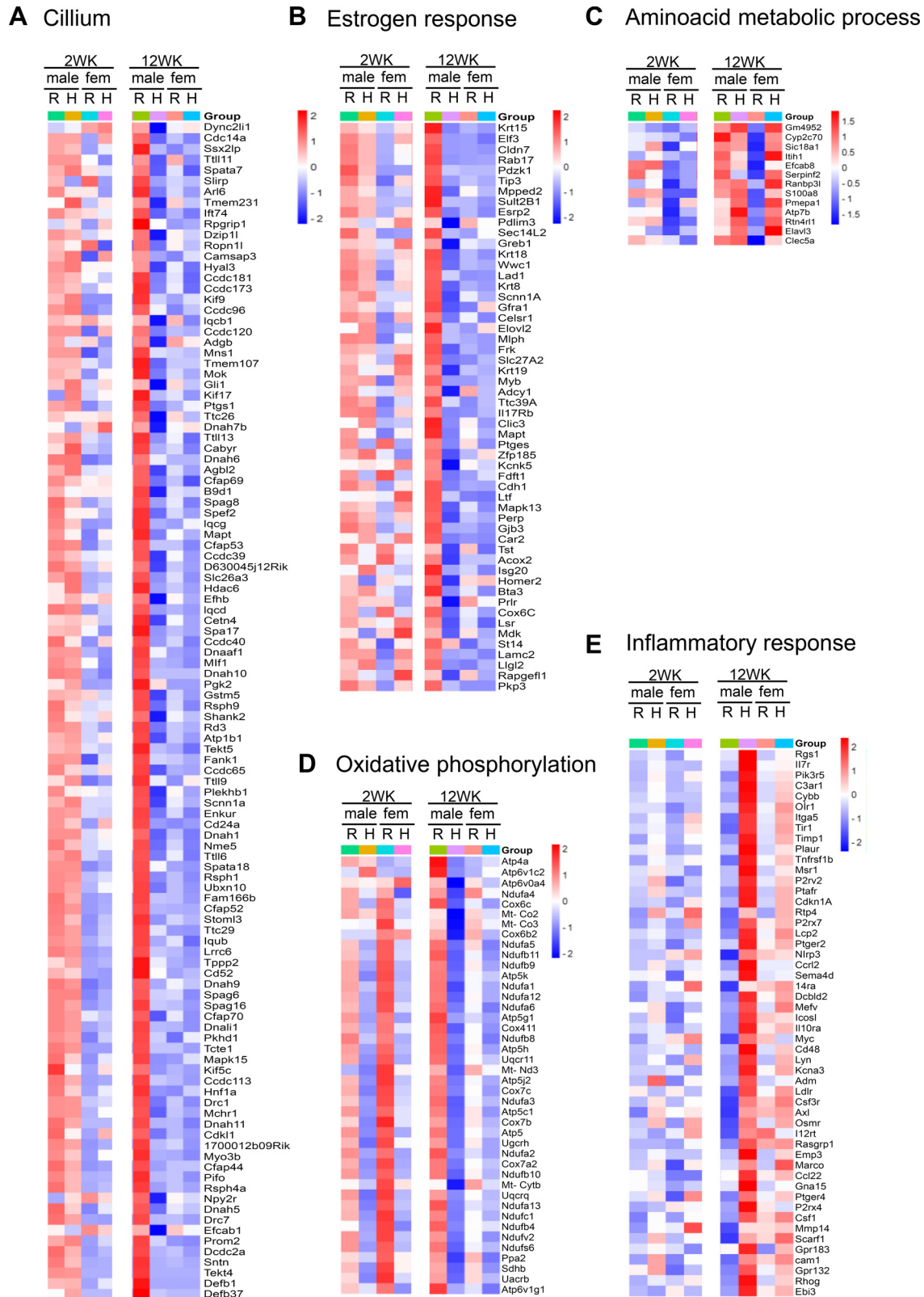
This HFD-induced reduction in sex-biased gene expression was associated with the progression of metabolic disturbances. Mechanistically, this likely reflects the sex-dimorphic gene regulation as a component of metabolic homeostasis. Supporting this, sex hormones and their receptors have been identified as the primary drivers of sex-biased gene expression in these tissues, although organizational effects of gonadal sex, sex chromosome complement, and their interactions also contribute significantly to shaping sex-specific transcriptomic profiles (67). Notably, the influence of sex hormones is tissue-specific, with estradiol exerting a dominant effect on gene regulation in adipose tissue, and testosterone having a greater impact on liver transcriptomes (67).

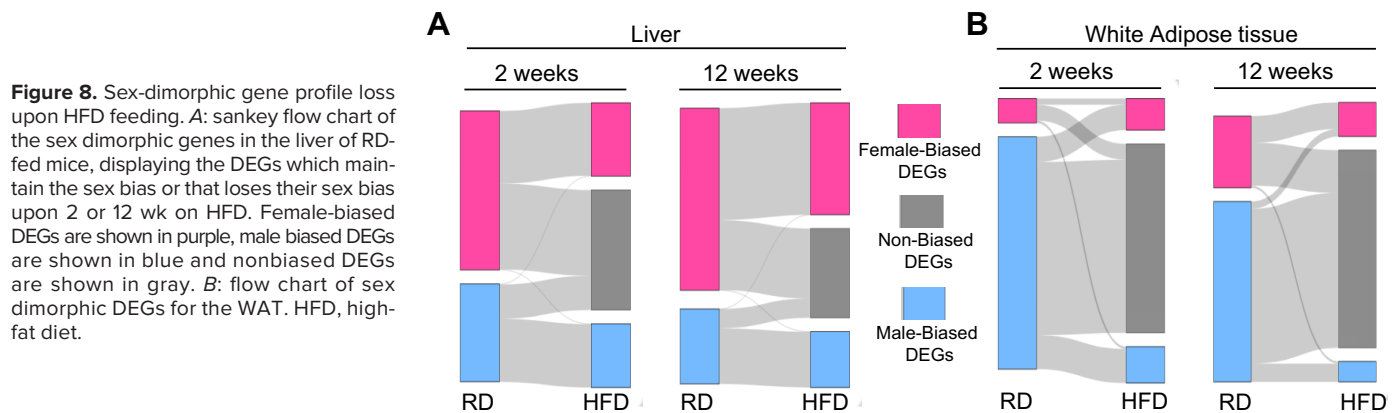
The loss of sex dimorphism induced by HFD mirrors metabolic alterations observed in humans with either testosterone excess in women or deficient levels in men (68). Indeed, HFD-induced obesity lowers male fertility by

Figure 6. Sex-dimorphic genes are proportionally enriched in the HFD-regulated genes. *A*: pie chart displays the number and percentage of male-biased (blue), female-biased (purple), or nonbiased (gray) genes respect to the globally expressed genes, in the liver at 2 and 12 wk. *B*: pie charts of the proportion and numbers of male-biased and female-biased genes in the visceral WAT. *C*: pie chart displaying the proportion of sex-dimorphic and non-biased DEGs within the HFD-regulated DEGs in the liver at 2 and 12 wk, either in female or male animals. *D*: pie chart of sex-dimorphic and nonbiased DEGs within the HFD-regulated DEGs in visceral WAT. *E*: percentage of sex-dimorphic genes within all expressed genes in the liver in regular diet (RD) and within the HFD-regulated genes (HFD DEGs), either up- or downregulated, in males or in females. Each bar displays the percentage of male-biased (light blue) or female-biased (purple). Significance of a Chi-square test comparing the percentage of sex-biased genes in each set of DEGs with the percentage in RD is shown as (***) for $P < 0.001$ or (**) for $P < 0.01$. *F*: percentage of sex-dimorphic genes in visceral WAT, as depicted in (*E*). *G*: pathways or gene categories significantly enriched in the subset of sex-biased DEGs, found upregulated (UP) or downregulated (Down) by 12 wk of HFD in the visceral WAT of male animals. *H*: pathways or gene categories of sex biased DEGs, in visceral WAT of females after 12 wk of HFD. DEG, differentially expressed gene; HFD, high-fat diet; WAT, white adipose tissue.

reducing the testosterone-to-estradiol ratio in mice (69, 70), whereas in men, low androgen levels are strongly associated with obesity (19, 20, 71), suggesting a complex interaction between sex hormones and metabolic homeostasis (72,

73). Furthermore, aging-related declines in sex hormone activity contribute to higher metabolic disease incidence (10, 48), representing a bidirectional relationship between sex hormone action and metabolic health.





Estrogens and particularly via estrogen receptor alpha ($ER\alpha$), play a pivotal role in female metabolic health (60, 74, 75). $ER\alpha$ enhances mitochondrial function across various glucoregulatory tissues, including muscle (76, 77), white and brown adipose tissue (78), liver (52), macrophages (79), and pancreatic islets (80), and in the brain (81–83), impacting behavior. This explains, at least in part, the premenopausal women protection against T2D compared with age-matched men (10, 11). However, the progressive loss of the female-biased gene profile in liver and WAT under HFD suggests diminished $ER\alpha$ protection in advanced HFD stages.

Although sex hormones are key drivers of sex-specific responses, additional mechanisms also play significant roles in shaping these differences. For example, the removal of sex hormones reverses most sex-related transcriptomic differences in the male liver but has limited effects on WAT and in the female liver (5). Moreover, the WAT transcriptome maintains a strong sex bias even in the absence of circulating sex hormones, indicating a tissue-specific effect of hormonal regulation and the involvement of other factors in shaping sex-dimorphic transcriptomes. These additional mechanisms include the influence of sex chromosomes, mitochondrial inheritance, epigenetic regulation, genotype-by-sex interactions, tissue-specific cellular composition (e.g., by immune infiltration as discussed above), behavior, and environmental factors (10, 17, 48).

These mechanisms appear particularly relevant in WAT. The concept of an “obesogenic memory” in WAT, driven by epigenetic changes (84) and reflected in its transcriptome, has been demonstrated in male mice following HFD and exercise intervention (85). This memory is associated with impaired mitochondrial function in WAT in male animals, whereas female resistance to HFD has been linked to sustained mitochondrial activity in WAT (5, 86). Although our

data showed HFD downregulation of mitochondrial genes in both sexes, this effect was significantly greater in male WAT, consistent with superior mitochondrial preservation in females under HFD conditions. In this regard, it has been shown that epigenetic modifications driven by the X-linked gene KDM5 histone demethylase modulate preadipocyte programming, affecting cell cycle and mitochondrial function programs, which contribute to sex differences in adipose tissue function (87).

Our data revealed sex-specific activation of cellular pathways in response to HFD, offering novel mechanistic insights. In male WAT, genes related to cilium structure and function were significantly regulated. This pathway, previously linked to obesity (45) and WAT expansion (88, 89), may represent a male-specific mechanism of adipose tissue remodeling under HFD, which remains poorly understood and merits further investigation.

Overall, our findings demonstrate that male and female mice exhibit fundamentally different transcriptional responses to HFD, driven by both sex-specific gene programs and dynamic tissue adaptations. These differences reflect sex-specific strategies to cope with metabolic perturbations. Moreover, in the absence of metabolic perturbations, the transcriptomes of both sexes show significant sex bias, with many of the responsible genes for this bias undergoing substantial regulation in response to HFD. Remarkably, HFD-induced metabolic changes result in a reduction of the sex bias in the profile in both sexes, correlating with the extent of metabolic disruption. These results suggest that preserving the sex bias in each sex is pivotal for maintaining metabolic homeostasis in both sexes. Moreover, these findings emphasize the importance of incorporating sex-specific considerations into research and clinical practice, paving the way for precision therapies targeting sex-dimorphic pathways to address obesity and related metabolic disorders.

Figure 7. Heatmaps of expression of gene categories with sex bias in visceral white adipose tissue. **A:** relative expression for the genes significantly changed in WAT of male animals after 12 wk of HFD, compared with their expression at 2 wk and with the expression in females, which are within the category “GO:CC cilium.” Each row of the heatmap displays the mean of each group for the expression levels of a specific gene, showing upregulation in red or downregulation in blue, in visceral WAT. **B:** heatmap for the HFD-DEGs found in males after 12 wk of HFD in WAT, corresponding to the category “Hallmark-estrogen response.” **C:** heatmap for the HFD-DEGs found in females after 12 wk of HFD in WAT, corresponding to the category “GO:BP amino acid metabolic process.” **D:** heatmap for the HFD-DEGs found in males after 12 wk of HFD in WAT, corresponding to the category “Hallmark-oxidative phosphorylation.” **E:** heatmap for the HFD-DEGs found in males after 12 wk of HFD in WAT, corresponding to the category “GO:BP - Inflammatory response.” GO, Gene Ontology; HFD, high-fat diet; WAT, white adipose tissue.

DATA AVAILABILITY

RNA-Seq datasets generated and analyzed during the current study are available in the NCBI GEO repository under accession number GSE262523.

SUPPLEMENTAL MATERIAL

Supplemental Fig. S1–S4: <https://doi.org/10.6084/m9.figshare.29469533.v1>.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

V.R., A.N., and J.-M.S. conceived and designed research; V.R., S.M.-R., H.M., A.G.-B., and A.H.-G. performed experiments; V.R., S.M.-R., H.M., A.G.-B., L.B., A.H.-G., E.E., and J.-M.S. analyzed data; V.R., S.M.-R., H.M., L.B., A.H.-G., E.E., M.C., I.M.-R., and A.N. interpreted results of experiments; V.R. prepared figures; V.R. and J.-M.S. drafted manuscript; V.R., S.M.-R., H.M., L.B., M.C., I.M.-R., A.N., and J.-M.S. edited and revised manuscript; V.R., S.M.-R., H.M., A.G.-B., L.B., A.H.-G., E.E., M.C., I.M.-R., A.N., and J.-M.S. approved final version of manuscript.

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