

Identification of novel candidate neural genes for diet-induced obesity in outbred heterogeneous stock rats

Thu Hong-Le

University College London

Teresa McGee

University of North Carolina at Chapel Hill

Osborne Seshie

Wake Forest University

Trangdai Bui

Wake Forest University

Gina Giorgio

Wake Forest University

Angela Beeson

Wake Forest University

Benjamin Johnson

University of California, San Diego

Gracie Ang

University of California, San Diego

Oksana Poleskaya

University of California, San Diego

Abraham Palmer

University of California, San Diego

William Valdar

University of North Carolina at Chapel Hill

Richard Mott

University College London

Leah Solberg Woods

leah.solbergwoods@advocatehealth.org

Wake Forest University

Keywords: Gene x diet interactions, genome wide association study, obesity, hypothalamus, heterogeneous stock rats

Posted Date: May 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9695368/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations:

No competing interests reported.

Table 3 To 6 are available in the Supplementary Files section.

Identification of novel candidate neural genes for diet-induced obesity in outbred heterogeneous stock rats

Thu Le¹, Teresa McGee², Osborne Seshie³, Trangdai Bui³, Gina Giorgio³, Angela Beeson³, Benjamin Johnson^{4, 5}, Gracie Ang^{4, 5}, Oksana Polesskaya^{4, 5}, Abraham A. Palmer^{4, 5}, William Valdar², Richard Mott^{1*}, Leah C Solberg Woods^{3*}

¹University College London, Genetics Institute, London, United Kingdom, ²University of North Carolina, Chapel Hill, Department of Genetics, Chapel Hill, North Carolina, USA, ³Wake Forest University School of Medicine, Department of Internal Medicine, Section on Molecular Medicine, Winston-Salem, North Carolina, USA, ⁴University of California San Diego, Department of Psychiatry, San Diego, California, USA, ⁵University of California, San Diego, Institute for Genomic Medicine, San Diego, California, USA

*Corresponding authors

Leah Solberg Woods: leah.solbergwoods@advocatehealth.org

Richard Mott: r.mott@ucl.ac.uk

Abstract

Background

Obesity is caused by genetics, the environment (e.g., diet) and their interactions. The brain, specifically the hypothalamus, plays an important role in obesity, but controlled studies using human brain tissue are not possible. The goal of this study was to conduct a genome wide association study using outbred heterogeneous stock (HS) rats to map genetic loci associated

with diet-induced obesity. This was followed by RNAseq in the ventromedial hypothalamus (VMH) to identify candidate causal obesity genes.

Methods

We measured multiple metabolic traits (including fat and lean mass, fasting glucose, insulin, and lipids, glucose tolerance, food intake and activity levels) after long-term consumption of a low-fat (LFD) or high-fat diet (HFD) in 2000 HS rats, split equally by sex and diet. Rats were genotyped using low-coverage whole genome sequencing. RNAseq data was obtained from the VMH of a sub-set of 400 HS rats split equally by sex and diet. We used linear mixed models to detect physiological and expression quantitative trait loci (pQTLs and eQTLs, respectively) in the full dataset and separately by diet and sex. Genes with cis-eQTLs that overlapped pQTLs were assessed as candidate causal genes through mediation analysis. We also identified VMH genes differentially expressed by diet in both sexes, followed by pathway analysis.

Results

We identified 47 pQTLs where six mapped multiple traits, 11 were diet-specific and 13 were sex-specific. We identified nine candidate causal genes, including *Pcare*, *Rbks*, *Mpv17* and *Gpn1* within a pleiotropic pQTL for multiple adiposity traits on rat chr. 6 and *Tnsfs9* within a pQTL for fat pad weight on rat chr. 9. We also identified *Ccdc77* as a candidate gene within a LFD-specific pQTL for activity levels and *Rtel1* and *Polr3k* within a male-specific pQTL for fatty liver. Genes involved in extracellular matrix and inflammation were dysregulated by diet, particularly in females, while males on HFD showed upregulation of several addiction pathways.

Conclusions

We identified novel candidate genes as neural regulators of diet-induced obesity and related traits and confirmed the importance of accounting for diet and sex in genetic studies of obesity.

Keywords: Gene x diet interactions, genome wide association study, obesity, hypothalamus, heterogeneous stock rats

Introduction

Obesity and overweight are major risk factors for multiple diseases including cardiovascular disease, type 2 diabetes, cancer and stroke (1). There has been a steady increase in prevalence of overweight and obesity (2), where current obesity prevalence in the United States reaches 68.6% (based on body mass index (BMI) and anthropomorphic measures such as waist-hip circumference) (3), emphasizing the dire state of health in the US and a critical need for new obesity treatments. Obesity is caused by genetics (4), the environment (5) and their interactions (GxE) (6, 7). Although human genome-wide association studies (GWAS) for obesity-related traits (e.g., BMI and body fat distribution) have identified many genetic effects (8, 9), GxE interactions (10, 11) are more challenging to identify. Most human studies for obesity-related traits that do account for genetic interactions with diet (GxD) either fail to report significant results (12, 13) or lack reproducibility due to insufficient power (e.g., sample size), high heterogeneity, measurement error and lack of proper controls (14, 15).

Obesity is, in large part, a brain disease where neuronal circuits play a role in regulating food intake and peripheral metabolism (16-18). The hypothalamus, a key brain region involved in satiety signaling and energy homeostasis (19-22), is divided into sub-sections (23). The ventromedial hypothalamus (VMH) is known to play an important role in obesity, lipid metabolism, and glucose production and uptake (24-30). The VMH sends (31) and receives (32, 33) signals from the arcuate nucleus, a region well known for regulating satiety signaling via the leptin-melanocortin pathway (34, 35). Although genes located near BMI-associated loci in human GWAS are enriched within the hypothalamus (36, 37), these studies did not account for diet. Importantly, it is impossible to collect human brain tissue under controlled conditions after a specific diet treatment.

Animal models of human complex disease offer several advantages over human studies, including the ability to regulate environmental exposures such as diet and to collect tissues, including brain, under tightly controlled conditions. Our laboratory uses outbred heterogeneous stock (HS) rats for genetic mapping of obesity and other metabolic traits. HS rats were created from eight inbred founder strains (38), with known differences in metabolic traits (39). They have been bred for over 100 generations, approximating the spectrum of common genetic variation segregating in humans (40, 41). Previous work in our lab has demonstrated the utility of HS rats for understanding genetics underlying obesity when rats were on normal chow (42-44) and shown translational relevance to humans (45-47). Here, we conducted a controlled diet study in a large cohort of HS rats to identify genetic loci for obesity/metabolic health, including those that interact with diet or sex. We then assessed gene expression changes within the VMH in response to diet and employed mediation analysis to identify candidate causal genes within the genetic loci. We also identified genes that were differentially expressed by diet in both sexes, followed by pathway analysis.

Methods

Animals and Diet

The outbred NIH HS rat colony was initiated in 1984 from eight inbred founder strains: ACI/N, BN/SsN, BUF/N, F344/N, M520/N, MR/N, WN/N, WKY/N, and maintained to minimize inbreeding [13]. Rats used in this study were maintained at Wake Forest University school of Medicine (NMcwWfsm:HS, Rat Genome Database number 13673907) (41), weaned at 21 days of age and housed 2 rat of the same sex per cage (except for one week for evaluating food intake) in micro-isolation cages (non-siblings housed together) in a conventional facility. Throughout the course of the study, 1942 HS rats were saved from 824 distinct breeder pairs. To maximize genetic heterogeneity, we randomized only one or two males and females from each breeder pair (two

males and/or females from 234 breeder pairs, with only one male and female from the other breeder pairs), with siblings placed on different diets. We saved 12 experimental animals each week throughout the course of the study (July 2020 through January 2024). All rats were housed at standard temperature and humidity conditions on a 12-hour light/dark cycle with *ad libitum* access to food and water. Breeders and experimental rats up to five weeks of age were fed standard rodent chow (Lab Diet, Prolab RMH 3000, #5P00). At five weeks of age, experimental rats were randomly assigned to either high fat diet (HFD) or low-fat diet (LFD) (Research Diets #12492: 5.21 kcal/g, 60% fat, 20% carbohydrate, 20% protein; or #D12450J: 3.82 kcal/g, 10% fat, 70% carbohydrate, 20% protein; both diets contain 17% sucrose, and the fat source is lard and soybean oil with all other components being matched). All animal experiments were performed using protocols approved by the Institutional Animal Care and Use Committee at Wake Forest University School of Medicine.

Study Design

1942 HS rats were phenotyped as shown in **Table 1**. Each cohort consisted of 12 genetically distinct rats (6 males and 6 females). At five weeks of age, prior to the start of HFD or LFD, baseline adiposity was measured using an EchoMRI. Body weight was then measured weekly. At 12 weeks of age (seven weeks on diet), rats were individually housed for one week to measure food intake. At 15 weeks of age (10 weeks on diet), EchoMRI was used to measure fat and lean mass, followed the next day by an intra-peritoneal glucose tolerance test (IPGTT) to measure fasting glucose, insulin and glucose tolerance. We then implanted a telemeter to measure daily activity. We measured activity when animals were both individually and pair housed. While individually housed, we took a second measurement of individual food intake. Rats were then euthanized after a four-hour fast. Blood was collected for measuring lipids and multiple tissues were collected and snap frozen for subsequent RNA analysis. Spleen was collected for DNA

extraction and genotyping. All experiments were conducted at similar times of day across cohorts. Test details are described below. The full list of phenotypes that were collected is listed in **Supplemental Table 1** with raw phenotype data shown in **Supplemental Table 2**.

Table 1: Phenotype Timeline

Age (weeks)	Time on diet (weeks)	Test
3	0	Wean
5	0	Weigh, baseline EchoMRI, place on LFD or HFD
12	7	Food intake
15	10	EchoMRI, IPGTT
16	11	Food intake, individual and pair-housed daily activity
17	12	Tissue harvest

LFD: low-fat diet, HFD: high-fat diet, IPGTT: intra-peritoneal glucose tolerance test

EchoMRI

Prior to diet assignment and after 10 weeks of LFD or HFD consumption, rats went through EchoMRI analysis (EchoMRI LLC, Houston, TX) to measure fat and lean mass. Non-fasted rats were initially weighed and then each rat was scanned in the machine for two minutes in triplicate. EchoMRI occurred in the morning between 9am-12.

IPGTT

Rats were fasted overnight for 16 hours. Testing began at 10am +/- 30 minutes at which time fasting blood glucose was measured (Contour Next EZ) and serum was saved for subsequent determination of fasting insulin. We then administered an intraperitoneal injection of glucose (1 mg/kg body weight) and measured blood glucose at 15, 30, 60, 90, and 120 minutes after the injection. Glucose sensitivity was calculated by measuring glucose area-under-the-curve (AUC) where:

$$AUC = \sum \frac{(G_t + G_{t+1}) \times \Delta min}{2}$$

Where G is serum glucose levels at time t .

Fasting serum insulin was measured with an ultrasensitive ELISA kit (ALPCO, #80-INSRTU-E10).

Telemeter implantation for activity measurements

Surgeries took place each week on Thursday. Prior to surgery, rats were given oral meloxidyl (1mg/kg) as an analgesic. Rats were then anesthetized with isoflurane gas. Once fully anesthetized, aseptic technique was used to insert a telemeter (Stellar Telemetry Implant E-430001-IMP-05 X 01, TSE systems) subcutaneously under the rat's shoulder. Incision was sutured and the rat was placed in a clean empty cage on a warming pad. Once active and ambulatory, the rat was placed into a separate cage. At this time, rats were housed individually for one week to obtain activity and a second measurement of food intake.

Rats were given through the weekend to recover, and telemeters were activated on Monday using Stellar Commander 1.0.0.7/1 software. Activity was recorded while singly housed from Monday afternoon until Friday morning, after which animals were pair-housed with their previous cage mate and activity recording continued until the following Monday afternoon. We thus obtained 3.5 days of activity when individually housed and 3.5 days of activity when pair-housed.

Euthanasia and tissue harvest

Rats were fasted around 8am, four hours prior to euthanasia. They were then brought to the euthanasia anteroom 40 minutes prior to euthanasia, which generally started at noon. Rats were euthanized by decapitation and blood was collected in serum separator tubes (Fisher Scientific). We weighed and snap froze multiple tissues including: brain, liver, fat pads (retroperitoneal, omental, gonadal), kidneys, heart and pancreas. Brain was removed and snap-

frozen within two minutes of the rat being removed from the cage, while all other tissues were removed and snap-frozen within 10 minutes or less after cage removal.

Prior to freezing, we used visual inspection, employing the following scale to assess degree of fatty liver/damage, as previously described (48):

0 = healthy red coloration, smooth uniform texture

1 = notable yellowing present over 30-75% of the surface area of the liver

2 = liver is uniformly yellowed in coloration

3 = uniform, lighter yellow coloration, visible mild to moderate fibrosis

Low-coverage whole genome sequencing and imputation

To genotype HS rats, we used low-coverage whole genome sequencing. DNA was obtained from spleens, which were collected postmortem. Detailed protocols for spleen collection, DNA isolation, sample processing, preparing sequencing libraries, and performing the sequencing can be found on protocols.io here: <https://www.protocols.io/workspaces/cgord/publications>. DNA was extracted using the DNA Advance kit (Beckman Coulter), then sequencing libraries were prepared using the Twist 96-Plex Library Prep kit (TWIST Bioscience). Reads were aligned to the rat reference genome mRatBN7.2 (GCA_015227675.2 GCF_015227675.2) using BWA-mem v0.7.17. Mapped sequences were used to construct haplotypes and impute biallelic single nucleotide polymorphism (SNP) genotypes using STITCH v1.6.6 39 (49, 50). This process resulted in a high-quality set of ~5 million SNPs with imputation quality scores (INFO) > 0.9. The genotypes are deposited in the University of California Library Digital Collection as Heterogeneous Stock (HS) Rat Genotypes, Version 6, <https://library.ucsd.edu/dc/object/bb65996027> ; the genotypes for the animals from this paper can be found by sub-setting for the animals used in this work.

SNP and Founder haplotype dosage imputation and kinship matrix construction

We then derived a subset of 90,363 high-quality tagging SNPs using PLINK v2 (<https://www.cog-genomics.org/plink/2.0/>), such that every other SNP was in Linkage Disequilibrium ($R^2 > 0.95$) with at least one tagging SNP within a window of 100 SNPs. SNPs with mean allele frequency < 0.05 were removed. These sites were used for all subsequent analysis.

Brain dissections and RNA extraction

The VMH was microdissected from frozen brain of 391 HS rats, split equally by sex and diet following coordinates in the Rat Brain Atlas (51). Rats used for RNAseq were the first rat to be euthanized from the cage (to avoid any transcriptomic changes due to the stress of being alone in a cage for several minutes). These animals spanned the phenotypic spectrum for retroperitoneal fat pad weight (RetroFat). Frozen samples were then sent to Azenta Life Sciences (South Plainfield, NJ) for RNA extraction, library preparation and sequencing as described below.

Library Preparation with PolyA selection and Illumina Sequencing

Total RNA was extracted using Qiagen RNeasy Plus Universal micro Kit following manufacturer's instructions (Qiagen, Hilden, Germany). RNA samples were quantified using Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA, USA) and RNA integrity was checked using TapeStation (Agilent Technologies, Palo Alto, CA, USA). RNA sequencing libraries were prepared using the NEBNext Ultra II RNA Library Prep for Illumina using manufacturer's instructions (New England Biolabs, Ipswich, MA, USA). Briefly, mRNAs were initially enriched with Oligod(T) beads. Enriched mRNAs were fragmented for 15 minutes at 94°C. First strand and second strand cDNA were subsequently synthesized. cDNA fragments were end repaired and adenylated at 3'ends, and universal adapters were ligated to cDNA fragments, followed by index addition and library enrichment by PCR with limited cycles. The

sequencing libraries were validated on the Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA) and quantified by using Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA, USA) as well as by quantitative PCR (KAPA Biosystems, Wilmington, MA, USA).

The sequencing libraries were multiplexed and clustered onto a flowcell. After clustering, the flowcell was loaded onto the Illumina HiSeq 4000 or equivalent instrument according to manufacturer's instructions. The samples were sequenced using a 2x150bp Paired End (PE) configuration. Image analysis and base calling were conducted by the Control Software. Raw sequence data (.bcl files) generated from the sequencer was converted into fastq files and de-multiplexed using Illumina's bcl2fastq 2.20 software. One mismatch was allowed for index sequence identification. RNAseq data have been deposited in the Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE330152.

RNAseq quality control:

We used STAR (v2.6.1a) (52) to align RNAseq reads to the reference Rn6.0, PICARD (v2.5.0) to remove PCR duplicates, and featureCount in the Bioconductor package Rsubread to compute gene-level expression counts, which were later normalized by sequencing depth, gene length, and RNA composition using the DESeq2 Bioconductor package (v1.24.0). We excluded very lowly expressed genes with average reads per sample less than one. The normalized expression levels across individuals for each gene were then transformed using the VST variance-stabilising transformation in DESeq2 (53) before being used for eQTL mapping in Matrix eQTL.

Differential expression and pathway analysis

Differential expression was conducted using the DESeq2 (53) packages. Unwanted variation was estimated by deriving three "remove unwanted variation or RUV" factors from control genes

using RUVSeq (54). These control genes are those not associated with Sex, Diet, or Sex_Diet, as determined by the likelihood ratio test comparing the full model $\sim 1 + \text{seqbatch} + \text{Sex} + \text{Diet} + \text{Sex_Diet}$ with the reduced model $\sim 1 + \text{seqbatch}$. Control genes with adjusted $p > 0.1$ were used to calculate RUVg factors W1, W2, and W3, which explained variation not associated with sequence batch (included as a covariate), sex, diet, or sex by diet interactions.

Differential expression was tested in DESeq2 using the model $\sim 1 + W1 + W2 + W3 + \text{seqbatch} + \text{Sex} + \text{Diet} + \text{Sex_Diet}$. The term Sex_Diet is a factorized term to represent interaction effects by looking at differential expression within groups. Significance of each of the main effects, Sex and Diet, and the interaction effects were assessed using the negative binomial Wald test. Log fold changes (LFCs) for Sex and Diet were shrunk with ashR and reported. Significance was defined as adjusted $p < 0.1$ after B-H FDR correction. Interaction analyses used the same framework with a factorized Sex_Diet term and four contrasts: M_1 vs M_0, F_1 vs F_0, M_0 vs F_0, and M_1 vs F_1.

Gene set enrichment analysis was performed with clusterProfiler (55) using ranked, non-shrunk, unadjusted LFCs from the full DESeq2 results. Enrichment scores were normalized to generate normalized enrichment scores (NES); pathways with positive NES were labeled activated and those with negative NES suppressed. GSEA using KEGG analysis was run with clusterProfiler::gseKEGG. All recommended parameters were used, except that the minimum number of genes in a set was set to 3 to accommodate lower-powered subset analyses. Dot plots were ordered by adjusted p-value.

In DESeq2, the model is Gene expression $\sim$ Sex + Diet + Sequencing Batch

Statistical analysis

Statistical analyses largely followed the methodology and code-base in our previous study (43).
We briefly summarize the methods here.

Kinship matrix construction

We estimated a SNP-based genetic relationship matrix (GRM) K_{SNP} from the tagging SNPs in PLINK v2.0, which was used for genetic mapping as well as to estimate heritability and genetic correlations for the physiological traits.

Phenotype correlations and heritability estimates

Each physiological phenotype was quantile-normalized using the rank-inverse Normal transformation and adjusted for covariates (e.g., sex, diet, batch) if they accounted for more than 2% of the total variance (see **Supplemental Table 1**). The resulting residuals were used for correlation and heritability analysis. Phenotypic correlations between traits were computed as Pearson R statistics of the corresponding residuals. Genetic correlations between traits and the additive heritability of each trait was estimated using GCTA (56) using K_{SNP} .

Genetic mapping of physiological traits

GWAS was conducted on all quantile-normalized phenotypes in Supplementary Table 1, including raw tissue weights as well as tissue weights adjusted to body weight (e.g., tissue weight/body weight). Covariates were included explicitly in the genetic mapping (i.e., we did not use residuals). Quantitative trait loci for each physiological trait (named pQTLs in this study) were mapped using the miqtl R package (<https://rdrr.io/github/gkeele/miqtl/>), employing the GRM K_{SNP} across the 90,363 tagging SNPs. A genome-wide significance threshold for each trait was computed from 500 permutations; generally the threshold was in the range $\log P=5-6$. QTL intervals were estimated by finding all SNPs with $R^2 > 0.6$ with the peak SNP of the QTL. The

peak SNP within each pQTL was used for subsequent mediation analysis and to estimate founder haplotype effects.

Sex and diet-specific effects on pQTLs were identified by repeating the miqtl mapping for each trait on subsets of rats categorized by sex or diet, using the same covariates as in the miqtl analysis of all the animals. For pQTL that mapped in only a single diet and not in the other diet or in the complete data, we used ANOVA in lme4qtl (57) to check for the Gene x Diet (GxD) interaction in the full data set by comparing the linear mixed models

$$y = X\alpha + g^{peak}\beta_G + d\beta_D + gd^{peak}\beta_{GxD} + e \quad (a)$$

$$y = X\alpha + g^{peak}\beta_G + d\beta_G + e \quad (b)$$

where g^{peak} is the genotype dosage at the peak SNP with effect β_G under the pQTL, d is an indicator variable indicating the diet with effect β_D and $gd^{peak}\beta_{GxD}$ models the interaction of diet and genotype. The covariance structure of the data is modelled using the GRM K_{SNP} . Those pQTLs for which the p-value of the comparison of the models a vs b is <0.05 are considered as having significant GxD and were designated as diet-specific. A similar process was applied to identify GxS interactions, using sex in place of Diet.

Founder haplotype reconstruction and estimation of QTL haplotype effects

Each HS rat chromosome is a mosaic of the original eight founder haplotypes. The haplotype mosaic was inferred probabilistically for all chromosomes in all rats using the hidden Markov (HMM) model in R/qtI2 (58), given the ~90k tagging SNP genotypes previously calculated for the rats and the SNP alleles at these locations known for the founder strains (49). Since the haplotype reconstruction is probabilistic, it provides, for each rat at each location, a set of haplotype pair (diplotype) probabilities. The diplotype probabilities at the peak SNPs of mapped

pQTLs and eQTLs were used to estimate the effect of haplotype substitutions (haplotype effects) using Diploffect (59). These estimates and their 95% credible intervals were used to determine if peak SNPs for an eQTL had a strain distribution pattern (SDP) consistent with that of the pQTL.

Genetic mapping of expression traits

We mapped eQTLs using MATRIX-eQTL (<https://cran.r-project.org/web/packages/MatrixEQTL/index.html>) including as covariates sex, diet, technician, sequencing batch and the first principal component of the gene expression matrix, the GRM as the individual covariance matrix. A threshold of $\log P=5$ was employed to call cis-eQTLs within each tissue. Cis-eQTL were constrained to lie within 2 Mb of the corresponding gene location.

Mediation analysis and bmediatR

Genes with a cis-eQTL overlapping a pQTL were considered as potential mediators of the corresponding physiological trait if the cis-eQTL contained at least two associated SNPs within the interval of a pQTL (as defined by $R^2 = 0.5$). For these potential mediators, we performed mediation analysis, that is, we tested for evidence that the QTL peak (g) acts on the physiological trait (y) through gene expression (t), either wholly (complete mediation) or in part (partial mediation). This was done in two complementary ways: first, using causal steps, as proposed by Baron and Kenny (60) and used in Hong-Le et al. (43), and modeling g in terms of SNP genotype dosage; and second, as a verification step, using Bayesian model selection via bmediatR (61), and modeling g in terms QTL diplotype probability.

First, for causal steps, consider gene z with a cis-eQTL mapping within the interval of a pQTL. The evidence for mediation is evaluated by comparisons among three models:

$$y = X\alpha + g^{peak}\beta + t_z\gamma + e \quad (1)$$

$$y = X\alpha + g^{peak}\beta + e \quad (2)$$

$$y = X\alpha + t_z\gamma + e \quad (3)$$

where y is the normalized physiological phenotype, t_z is the normalized expression for gene z , and g^{peak} is the SNP genotype dosage for the peak SNP within the pQTL. A Mediator is called when model 1 fits significantly better than model 2 (as assessed by an ANOVA partial F test comparing the models). P values of all tested genes within each QTL are corrected for multiple testing using the Benjamini-Hochberg false discovery rate (BH-FDR) (62), with significant mediators defined as genes with q values < 0.1 . For significant mediators, full mediation is implied when model 1 fails to be significantly more predictive than model 3 (nominal P value < 0.05); otherwise, mediation is considered partial.

355

Second, to further understand mediation mechanisms, all significant mediators discovered through causal steps above were tested using bmediatR. bmediatR considers 8 possible models describing relationships between g , t , and y , including models for complete mediation, partial mediation, and “co-local”, where g affects t and y but independently. Giving each model an equal prior probability of $1/8$, bmediatR then applies Bayesian model selection to obtain posterior probabilities for each model, as well as Bayes factors, reported here on the $\log_{10}$ scale ($\log_{10}BF$), that summarize the extent to which movement from the priors to the posteriors reflects evidence for mediation of any kind (partial or complete). Since this method was used for verification rather than discovery, the Bayes factor thresholds were lenient: $\log_{10}BF > 2$ indicated strong evidence for mediation; $1 < \log_{10}BF \leq 2$ indicated evidence for mediation; $0.5 <$

log₁₀BF ≤ 1 indicated weak evidence for mediation; values between 0.5 and –0.5 reflect a lack of information either way; and any other negative values were considered evidence against mediation. bmediatR was performed with *g*, the QTL state, defined in terms of either genotype (as allele dosage, one parameter) or diplotype probability (i.e., 36 parameters), with interest focused primarily on the latter, since it provides the most complementary perspective. Moreover, mediation analysis was performed on both the full (unstratified) dataset, with covariates for sex and diet, and on the HFD and LFD groups separately (ie, stratified), with a covariate for sex. All pQTL diplotype, transcript, and phenotype sets with evidence of mediation were reported as strong candidates for further analysis if they had evidence for SNP-based mediation through causal steps and diplotype-based evidence through bmediatR.

Results

We first investigated the correlation structure and genetic architecture of the obesity related traits listed in **Supplemental Table 1** in 1,942 HS rats, sampled from both sexes and maintained under either HFD or LFD. We then measured gene expression in hypothalamus tissue of 391 of these animals and related it to the physiological data to determine causal pathways mediating genotype to phenotype and diet.

Adiposity correlates with metabolic health across all groups while correlations with activity levels differ by diet.

As expected, fat mass from the EchoMRI positively correlates and lean mass negatively correlates with individual fat pad weights ($r = \pm 0.50 - 0.83$, $p < 0.001$) and with other metabolic traits including fasting insulin (Insulin0; $r = \pm 0.34 - 0.47$, $p < 0.001$), fasting glucose (Gluc0; $r = \pm 0.13 - 0.30$, $p < 0.001$), serum cholesterol (CHOL) ($r = 0.12 - 0.44$, $p < 0.01$) and serum triglycerides (TRIG; $r = 0.22 - 0.47$, $p < 0.001$) across all sex and diet groups (**Figure 1; Supplemental Figure 1; Supplemental Table 3-6**). Fat mass also correlates with body weight

392 (BW), with a stronger correlation in rats on HFD ($r = 0.32 - 0.43$, $p < 0.001$) than those on LFD
393 ($r = 0.14 - 0.26$), $p < 0.01$). Food intake at either 7 weeks, 11 weeks or both correlates with BW
394 ($r = 0.49 - 0.61$, $p < 0.001$) and fat pad weights ($r = 0.09 - 0.38$), independent of sex and diet.
395 Interestingly, food intake positively correlates with lean mass only in rats on LFD ($r = 0.14-0.16$,
396 $p < 0.01$), while a negative correlation between food intake and lean mass is seen in females on
397 HFD ($r = -0.16$), with no correlation between these traits in males on HFD. Correlations with
398 activity levels differ by sex where activity levels negatively correlate with body weight in males
399 on HFD ($r = -0.19$, $p < 0.01$) and with fat mass and fat pad weights ($r = -0.14 - -0.24$, $p < 0.05$) in
400 males on LFD. There is no correlation between activity levels and body weight, fat mass, lean
401 mass or fat pad weights in females. We also estimated phenotypic genetic correlations, to
402 quantify how much phenotypic correlation is attributable to shared additive genetics, and found
403 they generally mirror the phenotypic correlations, although tend to be stronger in animals on
404 HFD relative to those on LFD.

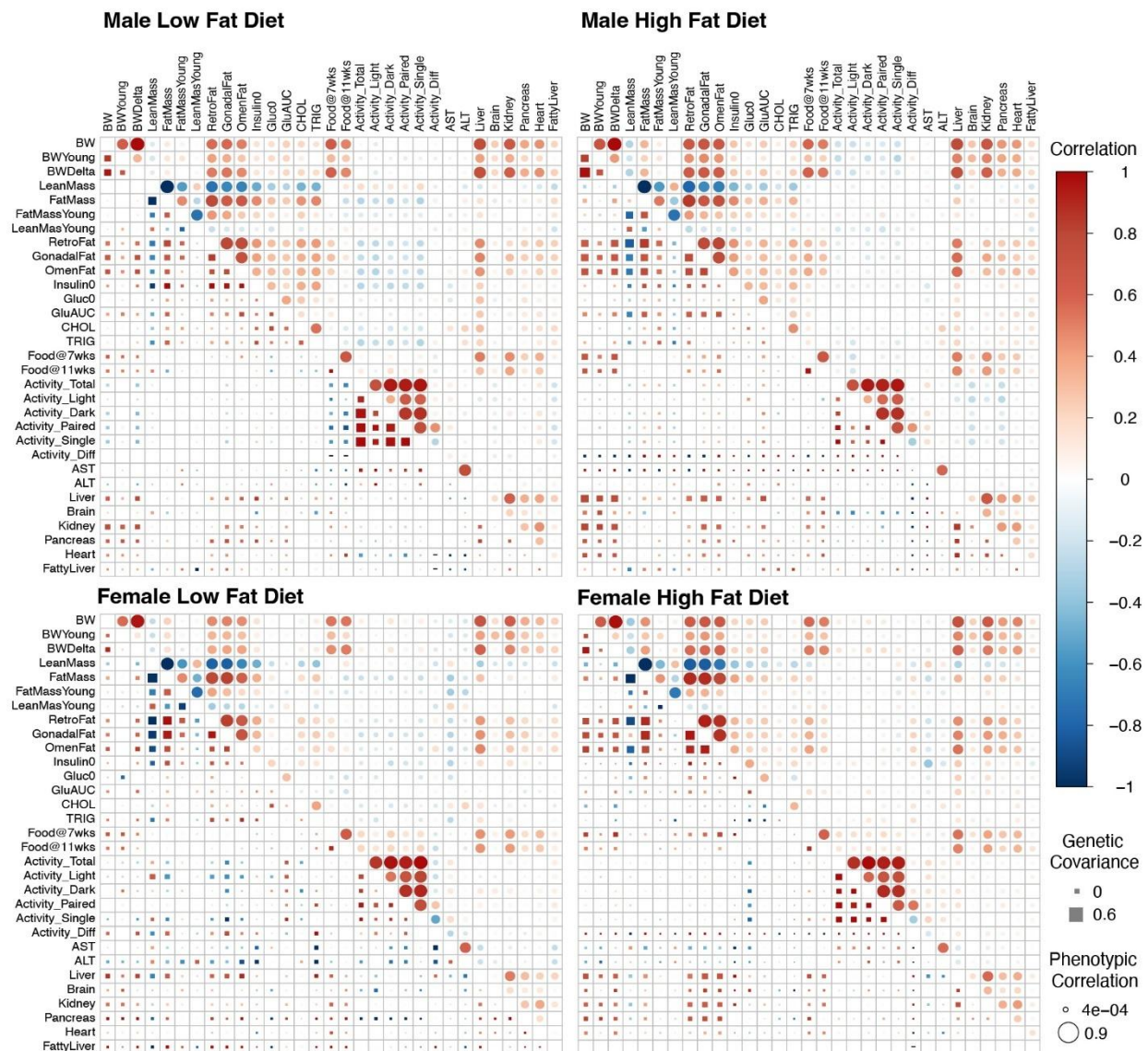


Figure 1. Genetic and phenotypic correlations for physiological traits separated by sex and diet. The upper triangle shows the phenotypic correlations while the lower triangle shows the genetic correlations. Significance levels can be found in Supplemental Figure 1.

Strong heritability of most traits across sex and diet groups

Similar to our previous work, heritability, estimated from the SNP-based additive genetic relationship matrix, is strongest for body weight and fat mass traits, with higher heritability when

on HFD ($h^2 = 0.42 - 0.69$) than LFD ($h^2 = 0.31 - 0.49$) (**Table 2**). Heritability is moderate for metabolic traits (Gluc0, Ins0, CHOL, TRIG, Glucose area under the curve (GlucAUC)), food intake and activity levels, independent of diet ($h^2 = 0.06 - 0.44$). Other than activity during the light period (Activity_Light), activity levels were highly heritable in males on LFD ($h^2 = 0.5 - 0.61$), with moderate heritability in the other groups ($h^2 = 0.12 - 0.43$). Fatty liver was heritable only males ($h^2 = 0.11$ LFD and 0.17 HFD), while serum alanine aminotransferase (AST) was more heritable in females ($h^2 = 0.30$ LFD and 0.27 HFD).

Table 2. Heritability across sex and diet conditions

	Full data	Males Low Fat Diet	Males High Fat Diet	Females Low Fat Diet	Females High Fat Diet
BW	0.46	0.46	0.69	0.31	0.42
BWYoung	0.44	0.42	0.45	0.29	0.25
Heart	0.24	0.2	0.19	0.15	0.3
BWDelta	0.36	0.38	0.6	0.31	0.41
LeanMass	0.47	0.48	0.62	0.57	0.59
FatMass	0.51	0.54	0.62	0.64	0.59
FatMassYoung	0.43	0.36	0.52	0.50	0.2
LeanMasYoung	0.34	0.29	0.37	0.4	0.27
RetroFat	0.46	0.46	0.65	0.45	0.59
GonadalFat	0.47	0.49	0.62	0.46	0.62
OmenFat	0.36	0.34	0.5	0.31	0.51
Insulin0	0.12	0.21	0.16	0.2	0.06
Gluc0	0.25	0.23	0.31	0.23	0.27
GluAUC	0.28	0.33	0.38	0.27	0.19
CHOL	0.31	0.44	0.27	0.36	0.32
TRIG	0.15	0.35	0.2	0.18	0.07
Fatty_Liver	0.09	0.11	0.17	0.04	0
Food@7wks	0.28	0.2	0.42	0.3	0.24
Food@11wks	0.28	0.21	0.25	0.27	0.32
Activity_Total	0.26	0.65	0.31	0.29	0.39
Activity_Light	0.26	0.26	0.43	0.41	0.38
Activity_Dark	0.29	0.61	0.28	0.23	0.23
Activity_Paired	0.2	0.5	0.3	0.28	0.36
Activity_Single	0.25	0.54	0.21	0.12	0.3
AST	0.19	0.06	0.02	0.3	0.27
ALT	0.21	0.06	0.2	0.29	0.13
Liver	0.41	0.3	0.49	0.29	0.43
Brain	0.26	0.26	0.28	0.17	0.1
Kidney	0.39	0.35	0.4	0.36	0.36
Pancreas	0.12	0.2	0.18	0.07	0.22

421

422 **Differentially expressed hypothalamic genes by diet differ by sex**

423 We then asked which genes are differentially expressed (DE) in the VMH between diet, as
424 quantified by DESeq2 (53). We identified 101 genes that are DE by diet using the full dataset
425 with sex as a covariate (false discovery rate (FDR) < 0.1; **Supplemental Table 7**). When
426 stratified by sex, many more genes are DE by diet in females (269) than males (11;
427 **Supplemental Table 7**), likely due to higher intrinsic variation in gene expression in the male
428 data. Pathway analysis in the full dataset demonstrates a downregulation of genes involved in
429 ribosome and oxidative phosphorylation, while there is an upregulation of genes involved in the
430 extracellular matrix (ECM; cytoskeleton in muscle cells, integrin signaling, proteoglycans in
431 cancer, focal adhesion and ECM-receptor interactions), PI3K-Akt signaling, inflammation (e.g.,
432 TGF-beta-signaling, hippo signaling), and addiction (alcoholism, cocaine addiction,
433 amphetamine addiction) among others (**Supplemental Table 8**). When stratified by sex,
434 oxidative phosphorylation, ECM pathways, PI3K-Ak5 signaling and proliferation/inflammation
435 (Hippo signaling, TGF-beta signaling) are dysregulated in females on HFD, while addiction
436 pathways (alcoholism, cocaine addiction, amphetamine addiction), dopamine synapse and
437 cAMP signaling are altered only in males (**Figure 2, Supplemental Tables 9,10**). Ribosomal
438 genes are downregulated in both males and females.

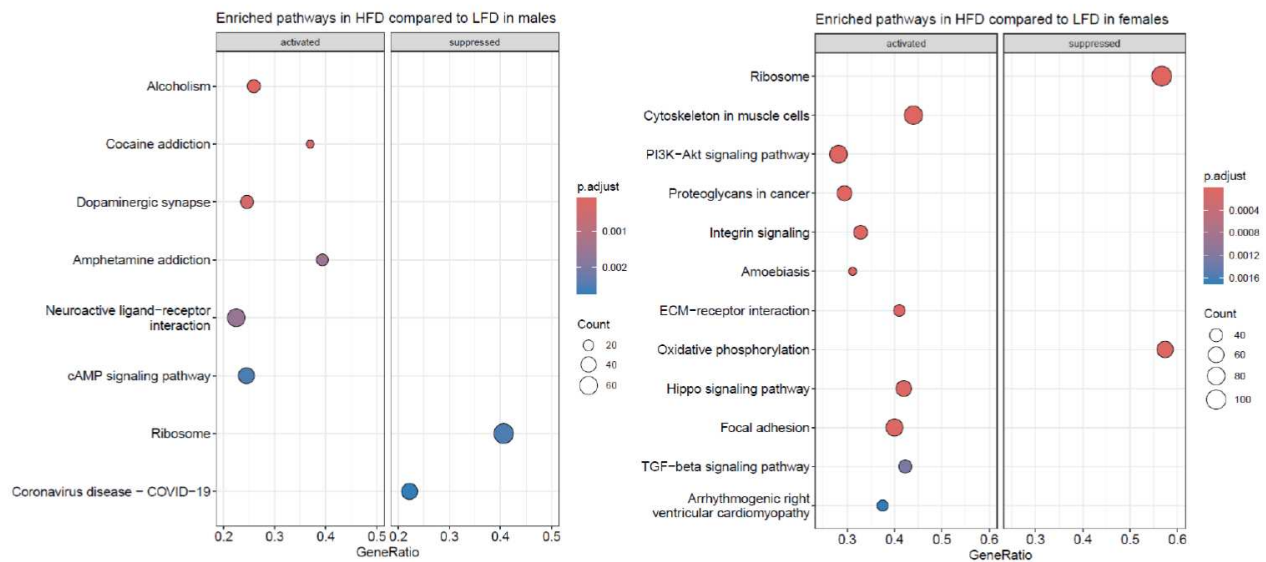


Figure 2. Pathway analysis of RNA sequencing data of the ventromedial hypothalamus showing pathways impacted by high fat diet in male and female HS rats.

Multiple physiological QTL identified in the full dataset

We then mapped physiological QTL (hereafter pQTL) associated with the physiological traits, initially using the full dataset (combining sexes and diets) to identify pQTL. We identified 23 pQTL, of which six are pleiotropic, mapping multiple traits (**Table 3; Figure 3a, b**). These include highly significant loci on rat Chrs. 1:259,628,230bp and 6:27,064,422bp that contain multiple adiposity pQTLs as well as LeanMass and LeanMassDelta. The Chr. 1 locus also contains BW, change in body weight (BWDelta), GlucoseAUC, Brain/BW, Food@11weeks, and CHOL while the Chr. 6 locus maps TRIG and CHOL. Activity levels map to two pleiotropic loci: Chr1:141,239,990 for Activity_Light and activity during the dark period (Activity_Dark) and chr19:37,309,444 for total activity (Activity_Total) and activity while house singly (Activity_Single). A pleiotropic locus on chr18:26,161,137 maps omental fat pad weights (OmenFat), BWDelta and Heart. Finally, a locus on chr1:201,383,401 maps both FatMass and

LeanMass. 17 pQTL map only a single trait. The average mapping interval (defined as $r^2 > 0.6$) width for all loci is 1.97Mb, ranging from 0.11 – 7.98Mb.

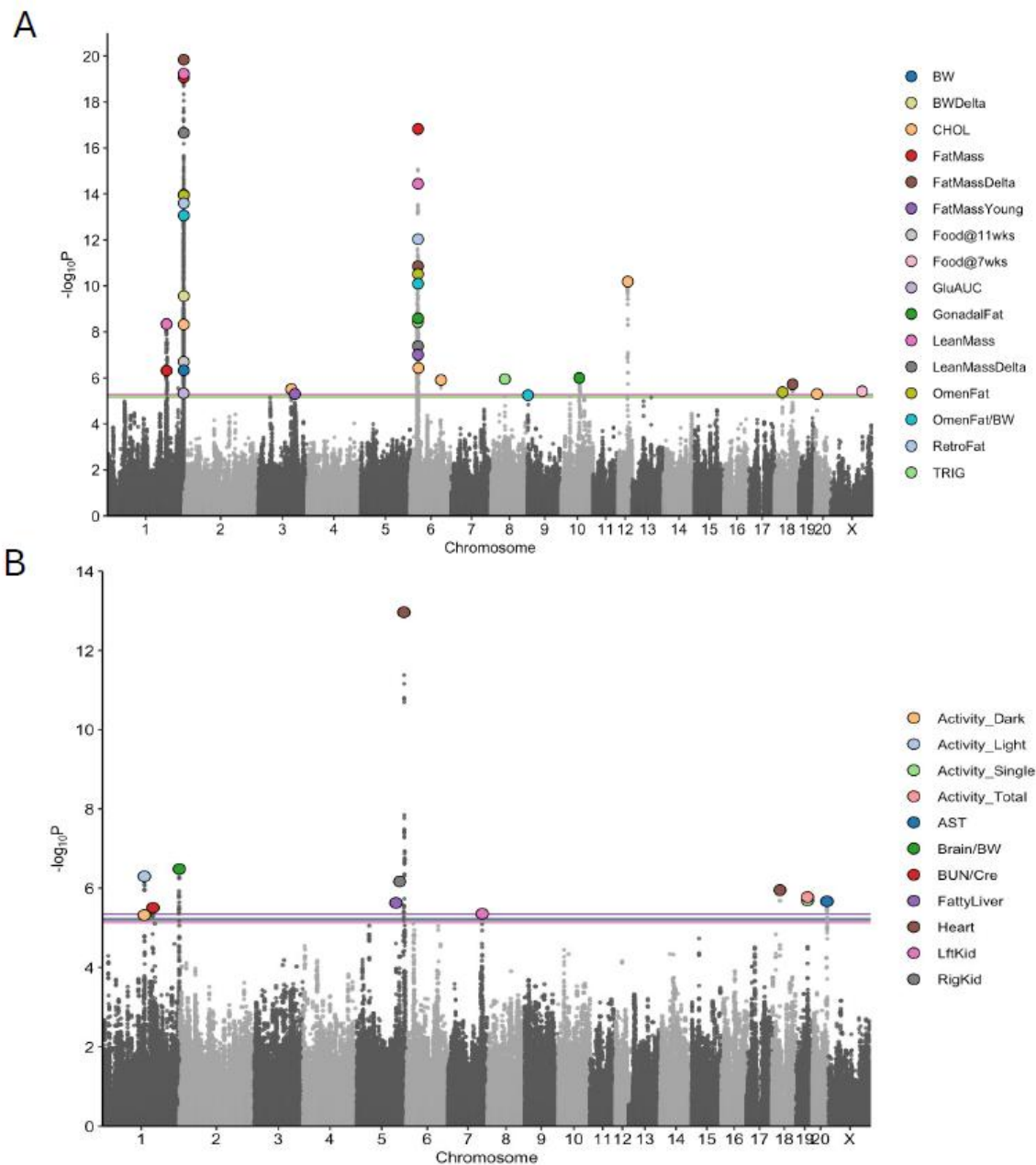


Figure 3. Porcupine plots showing genome-wide scans of multiple metabolic traits using the full dataset. Due to the large number of traits, we show the data in two plots: A) Adiposity, metabolic and food intake traits and B) Activity, tissue weights, and serum biochemistries. The x-axis

shows genomic position and y-axis the significance as $-\log_{10}$ of the P value of the test of association. Traits are color-coded, as indicated in the legend. Thresholds for genome-wide significance are shown as horizontal lines and indicate the genome-wide 10% thresholds, as determined by permutation.

Table 3. Phenotypic QTL in the full dataset: See attached sheet

Identification of diet-specific and sex-specific pQTL

When the rats are stratified by diet, we identify 11 diet-specific pQTL at 10% FDR: six under HFD conditions (right kidney weight (RigKid) chr. 1, CHOL chr. 1, gonadal fat pad weight (GonadalFat) chr. 3, Serum aspartate aminotransferase (ALT) chr. 3, %FatMassDelta chr. 6, Gluc0 chr. 15) and five under LFD conditions (TRIG chr. 1, Activity_Light chr. 4, liver/bw chr. 6, left kidney weight by body weight (lftkid/bw) chr. 7, Insulin0 chr. 12; **Table 4; Figure 4a**). None of these loci are pleiotropic. The average mapping interval width is 1.76Mb with a range from 0.31 – 3.92Mb.

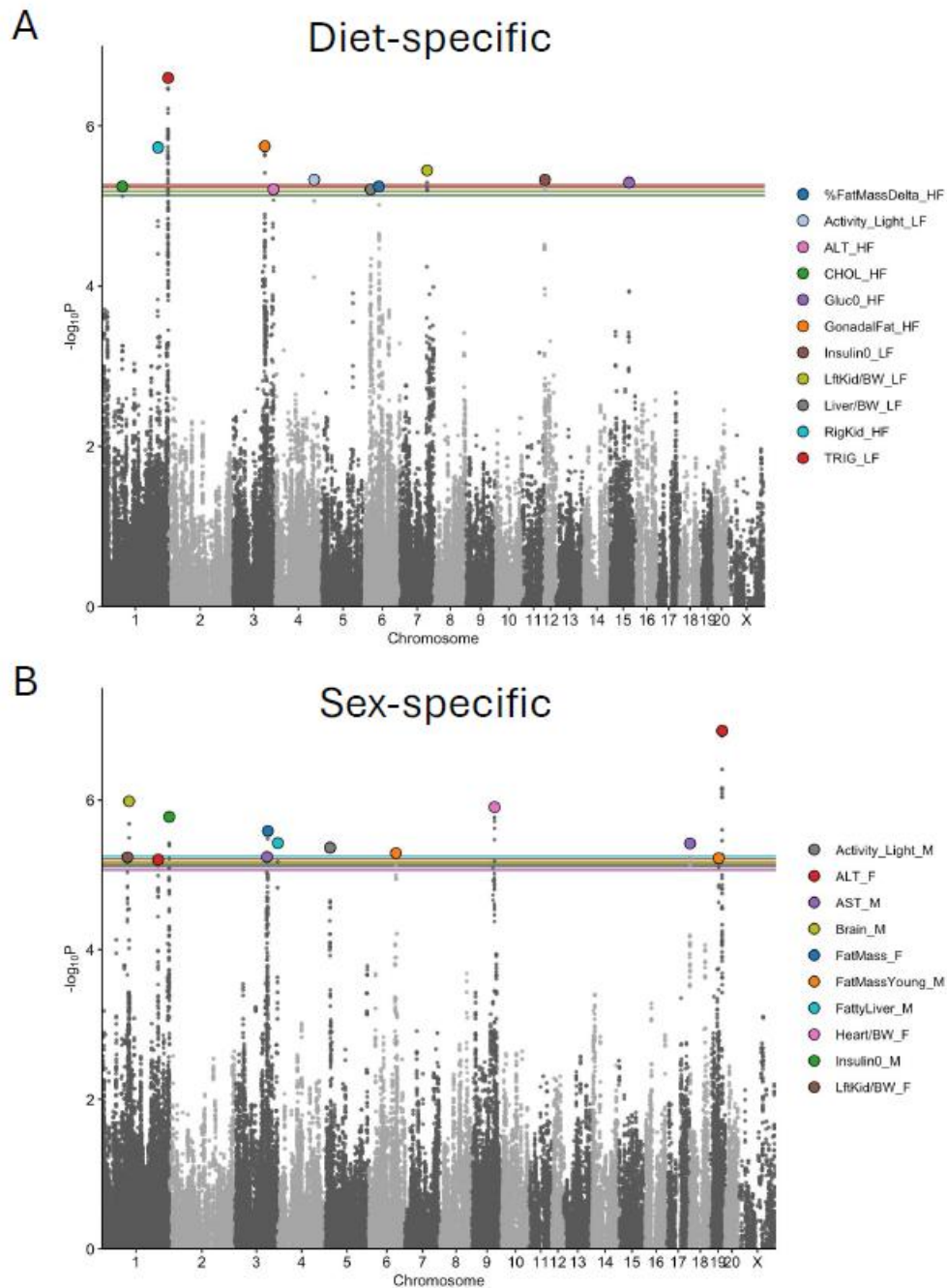


Figure 4. Porcupine plots showing genome-wide scan of multiple metabolic traits that are A) diet-specific and B) sex-specific. The x-axis shows genomic position and y-axis the significance as $-\log_{10}$ of the P value of the test of association. Traits are color-coded, as indicated in the legend. Thresholds for genome-wide significance are shown as horizontal lines and indicate the genome-wide 10% thresholds, as determined by permutation. Only those physiological QTL that

are significant after testing for a diet x SNP or sex x SNP interaction using ANOVA are shown (see also Tables 4 and 5).

Table 4. Diet-specific phenotypic QTL: See attached sheet

If instead the rats are stratified by sex, there are 13 sex-specific pQTL: eight in males (Brain chr. 1, TRIG and Insulin0 chr. 1, AST, chrs. 3 and 18, FattyLiver chr. 3, Activity_Light chr. 5, FatMassYoung chrs 6 and 19) and five in females (ALT chrs. 1 and 19, Lftkid/BW chr. 1, FatMass chr. 3, Heart/BW chr. 9; **Table 5; Figure 4b**). One male-specific locus on Chr1:258,530,115Mb maps both Insulin0 and TRIG. Interestingly, this locus encompasses the same confidence interval as the pleiotropic pQTL for multiple BW and adiposity traits on Chr. 1 in the full data. The average mapping interval is 1.46 with a range from 0.23 – 3.28Mb.

Table 5. Sex-specific phenotypic QTL: See attached sheet

A sub-set of hypothalamic cis-expression QTL are diet-specific

We analyzed 28,213 transcripts with detectable expression levels in the VMH where 4,522 map as cis-eQTL (**Supplemental Table 11**). 66 of these map as diet-specific eQTL (**Supplemental Table 12**).

Identification of candidate causal genes

To identify candidate causal genes within the pQTL, we first used co-localization to identify cis-eQTL that map within the pQTL. We then used mediation analysis (43, 60) and bmediatR (61), followed by correlation analysis and assessment of the SDP to prioritize candidate genes within each locus. The number of hypothalamus-expressed genes within the pQTL ranged from 3-211

(**Supplemental Table 13-15**). Within the pQTL for the full data, we identified nine full mediators (**Table 6**) which correlate with the trait of interest (**Supplemental Tables 16-18**) and are supported by bmediatR (see **Supplemental Figure 2; Supplemental Table 19**), as discussed below.

Table 6. List of Mediators in Full and diet or sex-specific loci: See attached sheet

Within the pleiotropic pQTL on rat chr. 6, there were 96 genes, 14 of which map as cis-eQTL within the VMH. Of these, we identified four full mediators (*Photoreceptor cilium actin regulator (Pcare)*, *GPN-Loop GTPase 1 (Gpn1)*, *Ribokinase (Rbks)* and *Mitochondrial inner membrane protein MPV17 (Mpv17)*) that correlate with the adiposity traits and are supported by bmediatR (**Figure 5; Supplemental Figure 2**), with the strongest evidence for *Pcare* and *Rbks* (**Figure 6**). All four genes share the same SDP among the HS founder strains, where the WKY haplotype results in decreased fat mass and either an increase or decrease in gene expression levels (**Figures 5**). Interestingly, when separated by diet, *Rbks* shows evidence for mediation only under HFD conditions while *Pcare*, *Gpn1* and *Mpv17* show stronger evidence for mediation under LFD conditions (**Figure 6**). We then used the transcriptome wide association analysis feature in RatGtex (<https://ratgtex.org/>), a database that compiles gene expression from HS rats across multiple projects. Expression of all four genes within multiple brain regions strongly associate with adiposity where *Mpv17* and *Rbks* positively associate and *Pcare* and *Gpn1* negatively associate, further supporting our findings here.

No mediators were identified for TRIG or CHOL at this locus indicating that these traits are likely under separate genetic control.

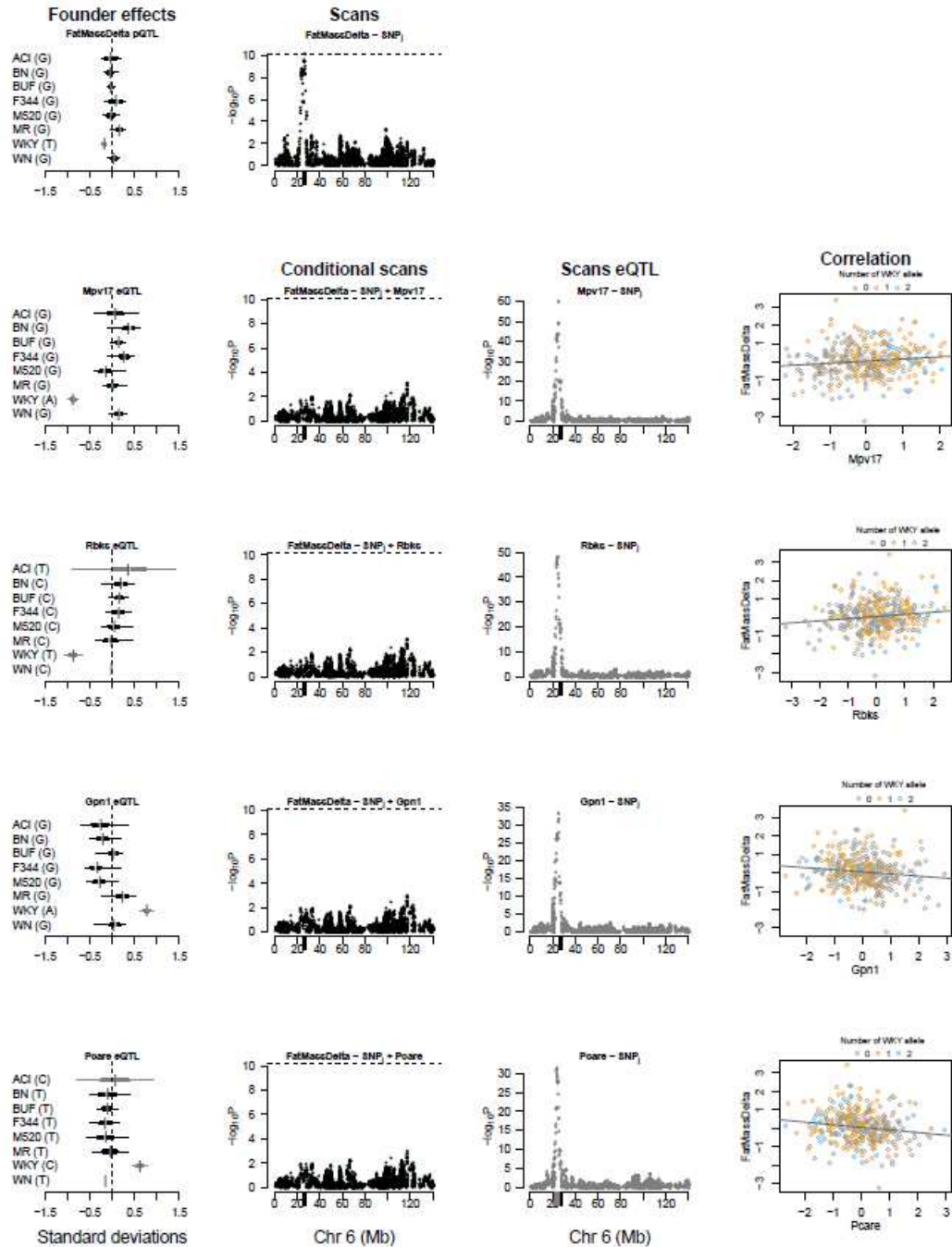


Figure 5. Four genes expressed in the ventromedial hypothalamus (VMH) are identified as mediators within the pleiotropic adiposity locus on rat chromosome 6. The first column shows estimated founder haplotype effects at the peak SNP, shown as box-whisker plots. Note that the WKY haplotype leads to decreased FatMassDelta (change in fat mass from time of diet start), decreased VMH expression of *Mpv17* and *Rbks* and increased expression of *Pcare* and *Gpn1*.

The second column shows the significant peak on rat chromosome 6 (where the dashed horizontal lines represent maximum log(P value) of the physiological QTL), followed by conditional scans where expression levels of each of the four mediators are included in the model. Note that significance level of the FatMassDelta peak drops completely for each of the four genes. The third column shows expression QTL mapping within the VMH for each of the four genes. The fourth column shows the correlation between FatMassDelta vs. gene expression in VMH across 391 rats, using a scatter-plot color-coded by genotype of the WKY allele at the peak SNP of the FatMassDelta pQTL.

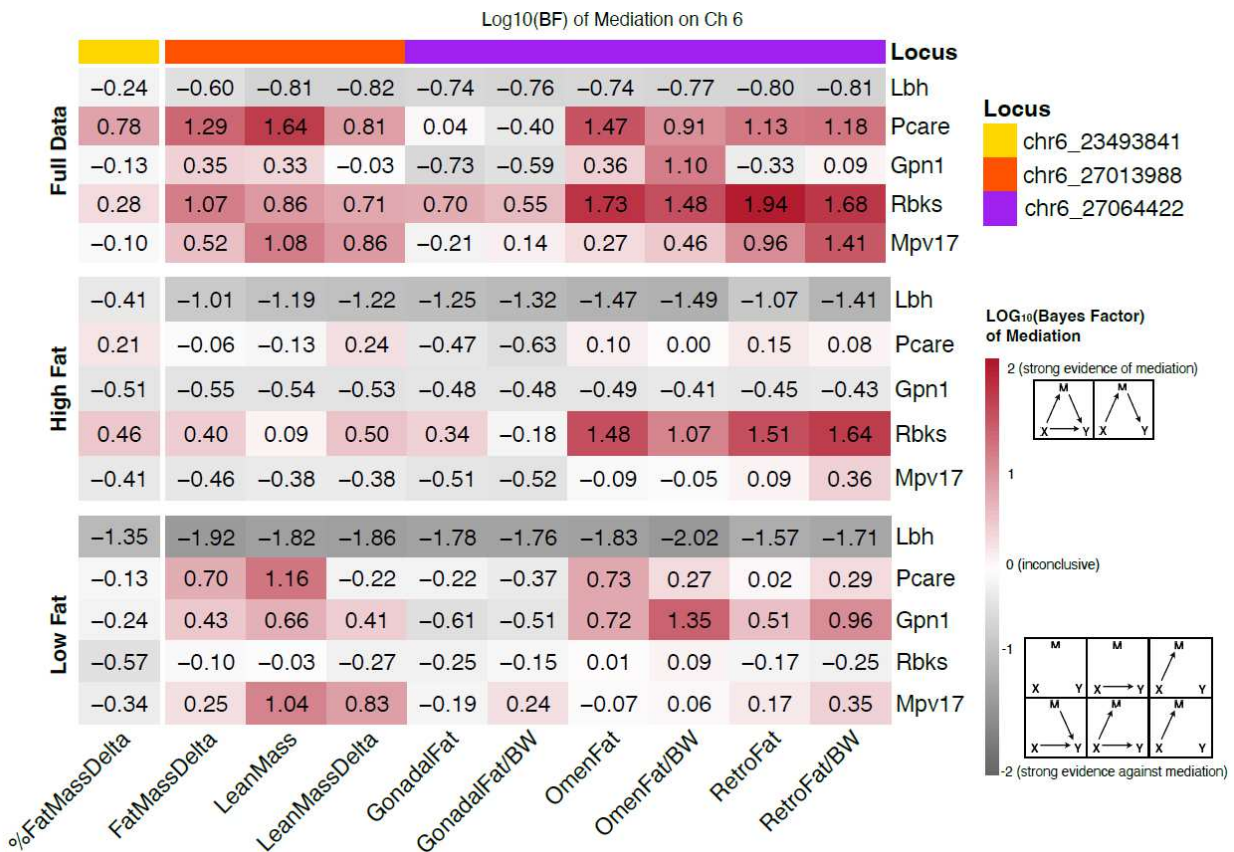


Figure 6. bmediatR results for the pleiotropic adiposity locus on rat chromosome 6. Each table column corresponds to a QTL locus (X) and phenotype (Y) combination, and each row to a gene whose expression level (M) is potentially mediating the effect of X on Y. Each table cell

quantifies the evidence for mediation on the log₁₀BF scale, with positive numbers favoring mediation, negative numbers favoring non-mediation, and zero being inconclusive (see scale on right, and Methods). Evidence for mediation is shown for diplotype-based analyses on both stratified (lower two subtables) and unstratified (top subtable) data, illustrating where inferred relationships are the same or different between the HFD and LFD groups. Color coding indicates strength of the mediation where darker red indicates strong evidence of mediation and grey indicates evidence against mediation.

Within the Chr. 9 locus for OmenFat/BW, there were 44 genes, 11 of which map as cis-eQTL within the VMH. Of these, only one gene, *Tumor necrosis factor superfamily member 9 (Tnfsf9)*, was identified as a full mediator that correlates with OmenFat/BW ($r = 0.13$, $p = 0.01$). The M520 and WKY haplotypes share a C at this locus, leading to decreased OmenFat/BW relative to the other founder haplotypes, with the M520 haplotype leading to decreased *Tnfsf9* expression levels and the WKY haplotype not impacting expression levels, at least at the peak marker (**Figure 7**). *Tnfsf9* is strongly supported by bmediatR (see **Supplemental Figure 2**).

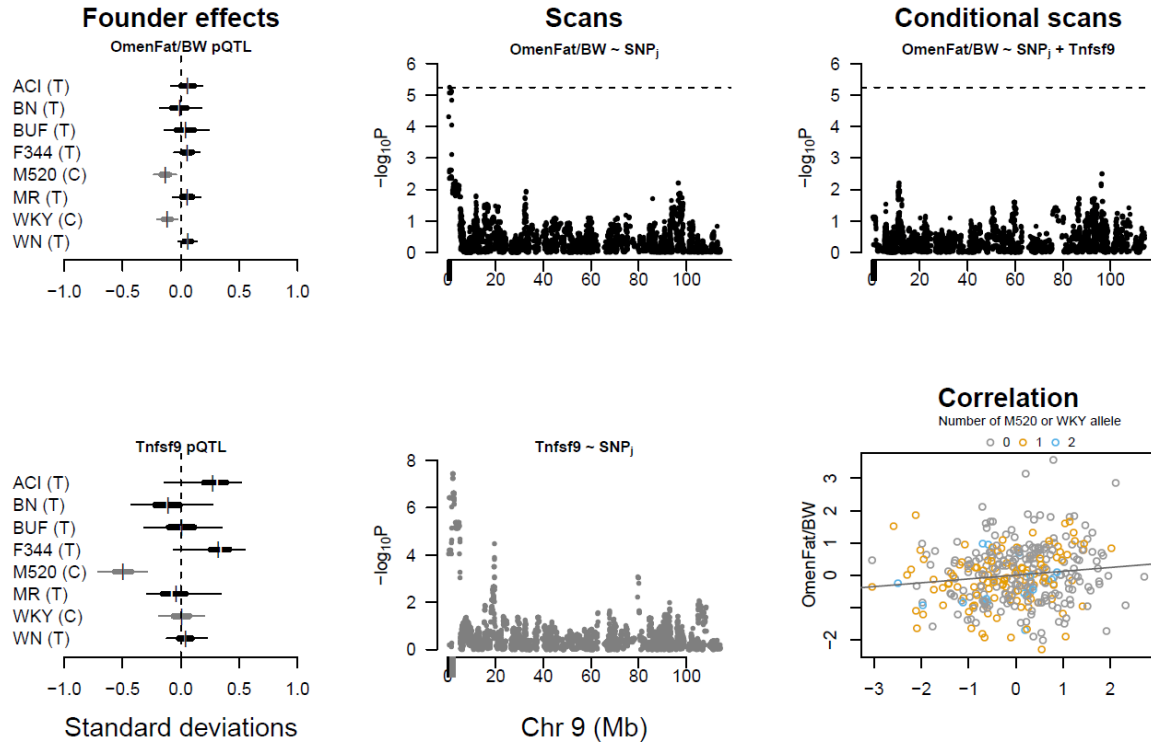


Figure 7. VMH expression of *Tnfsf9*, identified as a full mediator for the Omental Fat Pad Weight over body weight (Omenfat/BW) locus on rat chromosome 9. The first column shows estimated founder haplotype effects at the peak SNP, shown as box-whisker plots. Note that the M520 and WKY haplotypes lead to decreased OmenFat/BW, with the M520 haplotype also leading to decreased VMH expression of *Tnfsf9*. The second and third columns show the significant peak on rat chromosome 9 (where the dashed horizontal lines represent maximum $-\log(P$ value) of the physiological QTL), followed by conditional scans where *Tnfsf9* expression levels are included in the model, resulting in disappearance of the peak. Also pictured is expression QTL mapping within the VMH for *Tnfsf9* and the correlation between OmenFat/BW vs. *Tnfsf9* gene expression in the VMH across 391 HS rats, using a scatter-plot color-coded by genotype of the M520/WKY allele at the peak SNP of the OmenFat/BW pQTL.

Within the pleiotropic locus on rat Chr. 1 there are 27 genes, five of which map to the VMH. ENSRNOG00000065260 was identified as a partial mediator for BWDelta and Brain/BW, but not the other traits that map to this locus. BmediatR shows strong support for this gene, indicating it is likely a full mediator (**Supplemental Figure 2**).

There were ten genes within the pleiotropic chr. 18 locus for BWDelta, OmenFat and Heart, where only *Epb41/4a* is expressed in the VMH (**Supplemental Table 13**). Although *Epb41/4a* expression levels in the VMH correlate with BWDelta, this gene is not supported as a mediator.

Within the LFD-specific locus for Activity_Light on chr. 4, there were 12 genes, six of which map as cis-eQTL (**Supplemental Table 14**). Of these, only *Coiled-Coil Domain-Containing Protein 77 (Ccdc77)* was identified as a full mediator that also correlates with the trait (0.31, $p < 0.01$) and is supported by bmediatR (**Supplemental Figure 2**). The M520 and WKY haplotypes at this locus increase activity levels and *Ccdc77* expression levels. (**Figure 8; Table 6**). Within the male-specific locus for FattyLiver on Chr. 3, there were 47 genes; only five map as cis-eQTL within the VMH (**Supplemental Table 15**). Of these, *RNA Polymerase III Subunit K (Polr3k)* and *Regulator of telomere elongation helicase 1 (Rtel1)* were identified as mediators that correlate with the trait ($r = 0.29$, $p < 0.01$ and $r = -0.28$, $p < 0.01$) and are supported by bmediatR (**Supplemental Figure 2**). The ACI and MRI haplotypes at this locus lead to increased fatty liver score, with decreased expression of *Rtel1* and increased expression of *Polr3k* (**Figure 9; Table 6**).

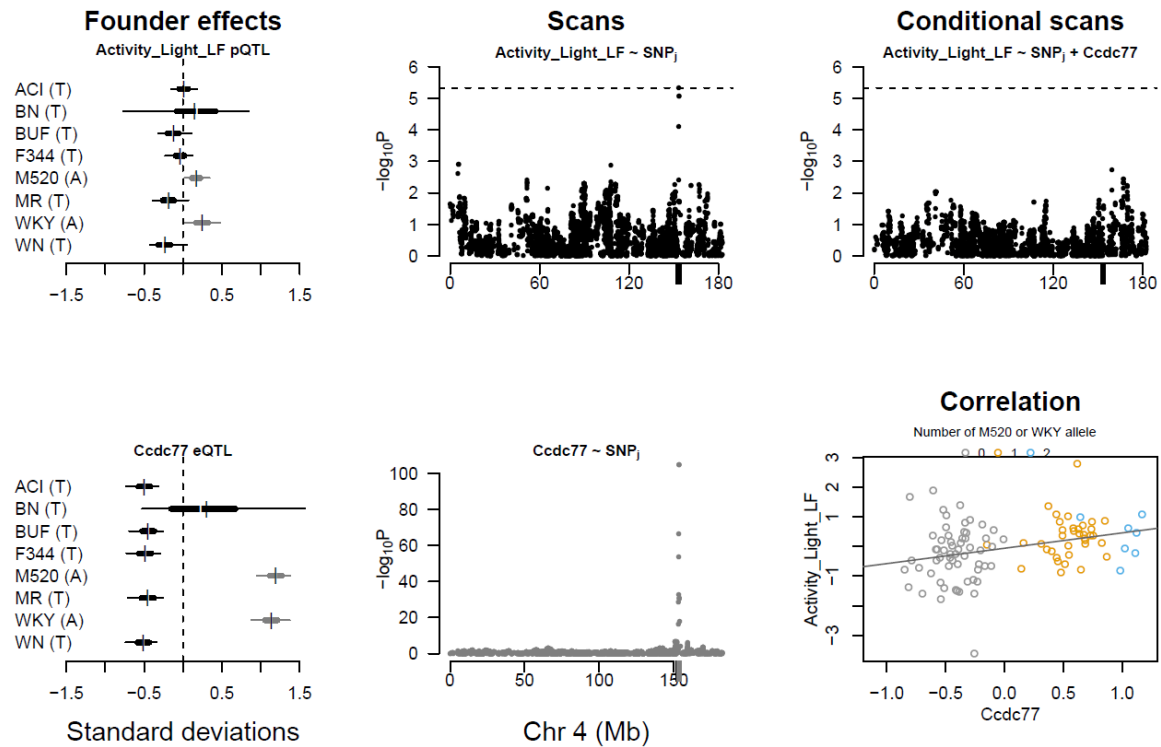


Figure 8. VMH expression of *Ccdc77* is identified as a full mediator for the low-fat diet-specific physiological QTL for activity levels during the light phase (Activity_Light) on rat chromosome 4. The first column shows estimated founder haplotype effects at the peak SNP, shown as box-whisker plots. Note that the M520 and WKY haplotypes lead to increased Activity_Light and increased VMH expression of *Ccdc77*. The second and third columns show the significant peak on rat chromosome 4 (where the dashed horizontal lines represent maximum log(P value) of the physiological QTL), followed by conditional scans where *Ccdc77* expression levels are included in the model, resulting in disappearance of the peak. Also pictured is expression QTL mapping within the VMH for *Ccdc77* and the correlation between Activity_Light vs. *Ccdc77* gene expression in the VMH using a scatter-plot color-coded by genotype of the M520/WKY allele at the peak SNP of the Activity_Light pQTL.

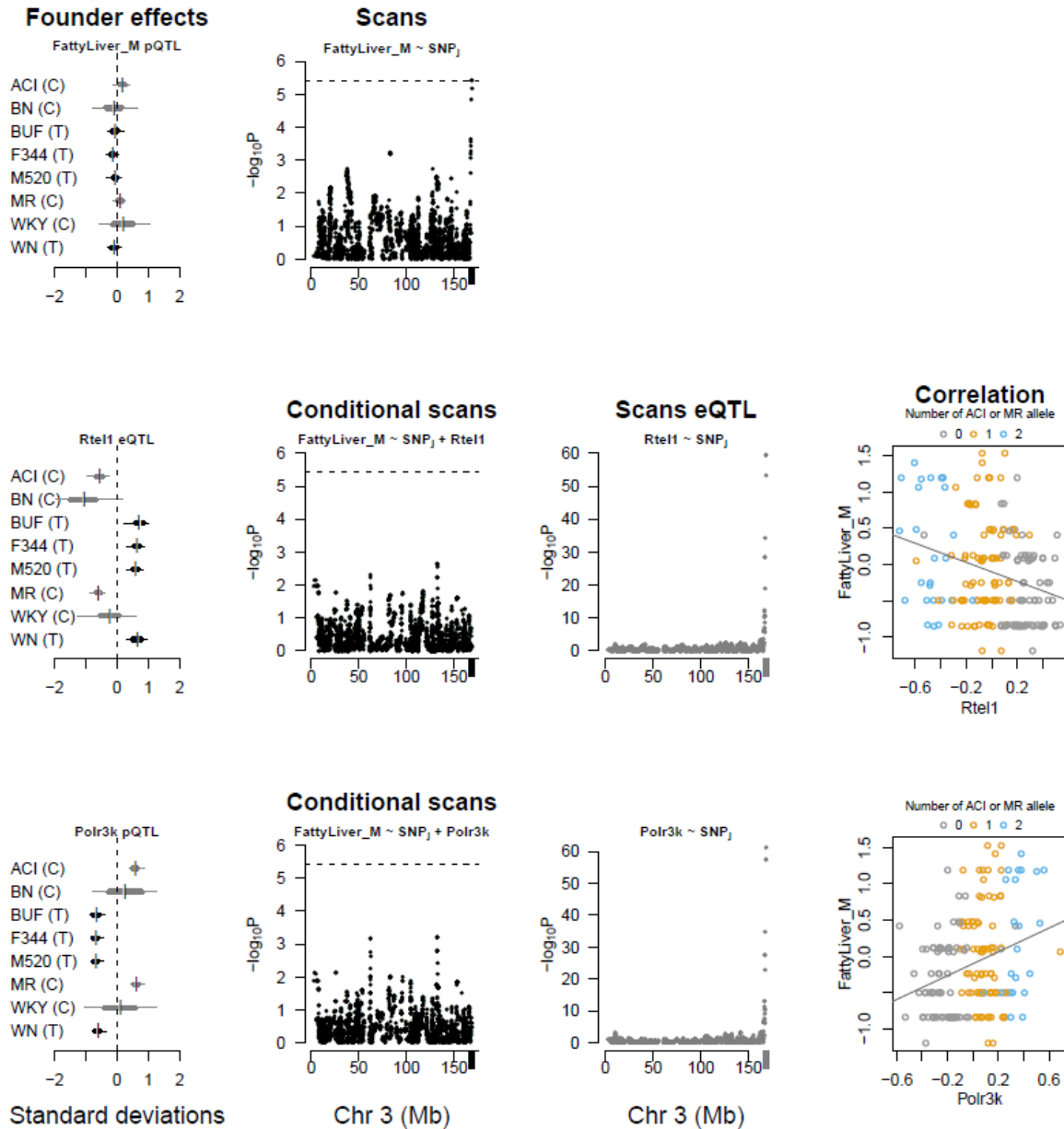


Figure 9. VMH expression of *Rtel1* and *Polr3k* are identified as full mediators for the fatty liver score (FattyLiver) locus on rat chromosome 3. The first column shows estimated founder haplotype effects at the peak SNP, shown as box-whisker plots. Note that the ACI and MR haplotypes lead to increased FattyLiver where BN and WKY share the C allele at this location, but do not seem to impact the phenotype, although large error bars indicate insufficient information. The C allele also leads to decreased VMH expression of *Rtel1* and increased expression of *Polr3k*. The second column show the significant peak on rat chromosome 3

(where the dashed horizontal lines represent maximum log(P value) of the physiological QTL), followed by conditional scans where *Rtel1* and *Polr3k* expression levels are included in the model, making the peak disappear. The third column shows expression QTL mapping within the VMH of *Rtel1* and *Polr3k*. The fourth column shows the correlation between FattyLiver vs. *Rtel1* and *Polr3k* gene expression in the VMH using a scatter-plot color-coded by genotype of the ACI/MR allele at the peak SNP of the FattyLiver pQTL.

Discussion

By employing a controlled diet study in an outbred rat model, we identified novel candidate genes within the VMH that may play a causal role in driving obesity. We also demonstrated the role of gene-diet and gene-sex interactions on several metabolic traits and identify candidate genes within some loci. Furthermore, we found that HFD alters the VMH transcriptome, leading to disruptions in oxidative phosphorylation, ECM remodeling, and inflammation. Males, but not females, also show disruptions in addiction pathways.

Our work identifies both novel and known pQTL for multiple metabolic traits. Using mediation analysis and other tools (correlation analysis, bmediatR, SDP, RatGtex), we identify several novel candidate VMH genes that may underlie these pQTL. Specifically, we found four candidate genes (*Pcare*, *Gpn1*, *Rbks* and *Mpv17*) as mediators within a pleiotropic locus on rat chromosome 6 for adiposity (including fat mass, lean mass and all fat pad weights) in the full data-set. *Pcare* and *Rbks* show the strongest evidence of association, where *Rbks* plays a larger role under HFD conditions and the other genes may play a larger role under LFD conditions.

Although none of these genes have previously been associated with adiposity (potentially because this is the first genetic study of adiposity to collect VMH under controlled conditions), there are several reasons why these genes are plausible candidates. *Rbks* plays a vital role in the creation of ATP, acting in the first step of ribose metabolism where increased *Rbks* activity leads to decreased cellular ribose. Previous work has shown that increased ribose in the small intestine leads to increased gut motility and resistance to weight gain in mice fed a HFD (63), in support of our work showing a positive association between *Rbks* and adiposity. Other work has shown that, in the liver, *Rbks* associates with Metabolic dysfunction-associated steatotic liver disease (MASLD), a disease strongly linked to obesity (64). *PCARE* is a ciliary protein that is highly expressed in the photoreceptors but also with strong expression in other brain regions including the hypothalamus. Ciliopathies and ciliary genes, particularly those on neuronal primary cilia, are strongly linked to obesity (65), making this a highly plausible candidate gene. *Mpv17* is a mitochondrial inner membrane protein that is involved in mitochondrial DNA replication and function (66). *Mpv17l2*, a related mitochondrial membrane protein, has recently been identified as a potential candidate in human obesity (67). Given this and the key importance of mitochondrial function in obesity (68), this is also a highly plausible candidate. *Gpn1* is involved in gene transcription and DNA repair, playing a vital role in cell function (69). Future work is needed to verify the role of these VMH genes on adiposity. Given that it is impossible to conduct a controlled study using brain tissue in humans, despite a known crucial role for the brain in obesity genetics (36, 37), our findings may prove highly relevant.

Of interest, the chromosome 6 locus has previously been reported (42-44), and our prior work identified and verified two causal genes (*Adcy3* and *Krtcap3*) within this locus. Specifically, we have previously shown that a coding variant within the transmembrane domain of *Adcy3* leads to increased adiposity in a rat model (70), as does decreased whole-body expression of *Krtcap3* (71, 72). Together, this work highlights the complexity of this region, demonstrating that six (or

more) genes from various tissues may be playing a causal role in driving increased fat mass within a single QTL.

In addition to replicating the chromosome 6 locus, we also replicate a previously identified locus for RetroFat and TRIG on rat chromosome 1 (42, 44). In the current study, we map multiple additional traits to this locus, including fat mass, lean mass, all fat pad weights, CHOL, brain weight and food intake, showing that this locus drives multiple related phenotypes. Of interest, both TRIG and Insulin0 map to this locus, but only in males, supporting our previous work in males on chow diet (43, 73). This region is 1.99Mb and contains 27 genes. VMH ENSRNOG00000065260 was identified as a mediator for BWGain and Brain/BW, but not the other traits at this locus. Previous work by our group had identified (43) and verified (74) adipose tissue *Grk5* as a causal gene for fat mass and TRIG within this region, indicating that adipose tissue may play a more important role than the VMH at this QTL.

We also identified *Tnfsf9* as a candidate VMH mediator within a locus for omental fat pad weight on rat chromosome 9. *Tnfsf9* is a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. TNFSF9 has been shown to trigger microglia activation, promoting inflammation after acute ischaemic stroke (75). Given that obesity is known to lead to activation of the immune system resulting in a state of chronic inflammation (45), particularly in the hypothalamus where inflammation is known to occur in response to HFD feeding (76), *Tnfsf9* is a highly plausible candidate gene at this locus.

In addition to identifying candidate neural genes for adiposity and other metabolic traits, our work identifies QTL that interact with diet. Interestingly, all adiposity traits (fat mass, lean mass, fat pad weights) have increased heritability when on HFD relative to LFD, with no differences in heritability by diet for the other traits. We identified 11 diet-specific loci, six under HFD and five

under LFD conditions. We identified *Ccdc77* as a candidate mediator within a QTL on rat chromosome 4 for Activity_Light (e.g., activity only during the light phase) only under LFD conditions. Although very little is known about *Ccdc77*, a gene located in the centrosome, previous work has found that this gene falls within a locus that has previously been associated with autism spectrum disorder (77), a disorder which can impact general activity. *Ccdc77* gene expression levels are also associated with comorbidities of type 1 diabetes, including hypothyroidism (78), a condition often associated with lethargy. Future studies are necessary to verify the role of *Ccdc77* in activity. The current work is important because although gene-diet interactions are known to influence obesity, human studies for GxD lack reproducibility (14, 15, 79). Furthermore, although previous studies in mice have shown strong gene-diet interactions for obesity traits (80-82), to our knowledge previous mapping studies have considered only one diet condition. Thus, we expect that this study will lay the groundwork for future gene-diet interaction studies in rodents and other models.

In addition to the diet-specific loci, we identified 13 sex-specific QTL (eight in males and five in females), supporting previous work demonstrating robust gene-by-sex interactions in mice (83) and humans (84, 85). Of particular interest, we identified two potential VMH mediators at a male-specific locus on rat chromosome 3 for fatty liver score, a trait that was heritable only in males. Expression of *Rtel1* negatively correlates with fatty liver and expression of *Polr3k* positively correlates with fatty liver. *Rtel1* is involved in the stability, protection and elongation of telomeres and telomere length has previously been associated with non-alcoholic fatty liver disease (86, 87), providing support for this gene. *Polr3k* is involved in gene transcription and has previously been linked to fatty liver disease, but in the opposite direction of our work where a knock-out of MAF1, a global repressor of *Polr3k*, leads to protection against diet-induced obesity and non-alcoholic fatty liver disease (88, 89).

In addition to QTL mapping and gene identification, this work demonstrates that genes involved in oxidative phosphorylation, ECM remodeling, inflammation/proliferation and addiction are disrupted within the VMH under HFD conditions. These pathways are unsurprising and supported by previous work in brain tissue of C57BL/6J mice (90-92). We have also seen disruptions to similar pathways in adipose and liver tissue in HS rats on normal chow with varying degrees of adiposity (45) while others have seen similar changes in adipose, liver and muscle after HFD feeding in baboons (93). Interestingly, when separated by sex, we see that ECM remodeling and inflammation/proliferation pathways are found only in females while addiction pathways, dopaminergic synapse and cAMP signaling were found only in males, implicating alterations to the reward pathway, as previously shown by several studies (94, 95). It is surprising that ECM remodeling and inflammation pathways are unaltered in males and we suspect that this may be due to the larger variation in gene expression levels found in the male population, potentially masking these differences. To our knowledge, this is one of the first studies to look at differences in gene expression in response to HFD separately in males and females, particularly within an outbred population.

Correlation data show that fat mass correlates strongly with metabolic phenotypes across all sex and diet groups, as expected. Food intake positively correlates with lean mass in animals on LFD, but not HFD, indicating that energy consumed is more likely to turn into muscle/bone only when on LFD. We also saw that activity levels positively correlate with lean mass and negatively correlate with fat mass in males on LFD, demonstrating that male rats that move more are leaner with lower fat mass. This relationship was not seen in females.

There are some limitations to this work. Although this study is extremely well-powered to identify general effects, power is lower for diet-specific and sex-specific loci. Thus, future studies will be needed to increase power and verify these findings. Although there is plausible evidence from

the literature for most of the candidate genes identified, future studies are required for validation (e.g., knock-down or over-expression strategies). There is a chance that the candidate mediators are not tissue specific and that the findings here represent global gene expression (e.g., it is possible that *Rbks* plays a larger role in the small intestine and *Rtel1* plays a larger role in liver than they do in the brain). That said, previous work looking at RNAseq in liver and adipose tissue of HS rats identified candidate causal genes that differed from those identified here (43, 44), implicating an important role for the current candidate genes specifically in the VMH.

Conclusions

In conclusion, our work identifies diet-specific and sex-specific genetic loci associated with multiple metabolic traits in an outbred rat model. We used RNAseq from the VMH to identify novel candidate causal genes for multiple traits. These include *Pcare*, *Rbks*, *Mpv17* and *Gpn1* within a pleiotropic locus for fat mass on rat chromosome 6 and *Tnfsf9* within a locus for Omenfat on rat chromosome 9. We also identified *Ccdc77* as a candidate gene within a LFD-specific locus for activity and *Rtel1* as a candidate gene within a male-specific locus for fatty liver score. We further show that addiction related pathways are found in male, but not females on HFD relative to LFD. Together these results demonstrate the importance of accounting for both diet and sex in genetic studies for obesity. Identification of novel candidate genes within the VMH has the potential to lead to novel therapeutic targets for obesity and emphasizes the importance of controlled studies using brain tissue in the study of obesity.

List of Abbreviations

Activity_Dark: activity during the dark period

Activity_Light: activity during the light period

Activity_Single: activity while house singly

776 Activity_Total: total activity
777 ALT: Serum aspartate aminotransferase
778 AST: Serum alanine aminotransferase
779 BW: body weight
780 BWDelta: change in body weight from time of high fat diet start to end of study
781 BWYoung: body weight at 5 weeks of age
782 BMI: body mass index
783 BUN/Cre: Serum blood urea nitrogen/creatinine ratio
784 CHOL: serum cholesterol
785 DE: differential expression
786 Gluc0: fasting blood glucose
787 GlucAUC: Glucose area under the curve during intra-peritoneal glucose tolerance test
788 GxD: gene by diet interaction
789 GxE: gene by environment interaction
790 GonadalFat: fat mass of the gonadal fat pad
791 GWAS: genome-wide association study
792 HMM: hidden Markov model
793 HFD: high fat diet
794 HS: heterogeneous stock
795 Insulin0: fasting serum insulin
796 IPGTT: intra-peritoneal glucose tolerance test
797 LFD: low-fat diet
798 LftKid: weight of the left kidney
799 OmenFat: weight of the omental and mesenteric fat pads
800 NES: normalized enrichment score
801 RetroFat: retroperitoneal fat mass

802 RigKid: weight of the right kidney
803 RUV: remove unwanted variation
804 SNP: single nucleotide polymorphism
805 SDP: strain distribution pattern
806 TRIG: serum triglycerides
807 TNF: tumor necrosis factor
808

809 **Declarations**

810 **Ethics approval and consent to participate:** All animal experiments were ethically performed
811 using protocols approved by the Institutional Animal Care and Use Committee at Wake Forest
812 University School of Medicine.

813 **Consent for publication:** Not Applicable

814 **Availability of data and materials:** The datasets generated and/or analyzed during the current
815 study are available at the following:

816 Publicly available in a repository:

817 Genotype data are uploaded onto the University of California Library Digital Collection as
818 Heterogeneous Stock (HS) Rat Genotypes, Version 6,
819 <https://library.ucsd.edu/dc/object/bb65996027>

820 RNAseq data have been deposited in the Gene Expression Omnibus
821 (<https://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE330152 and in RatGtex
822 (<https://ratgtex.org/>).

823 Included in the paper or Supplementary Information:

824 Raw phenotyping data are provided in Supplementary Table 2.

825

826

Competing interests: The authors declare that they have no competing interests

Funding: This work is supported by grants to Leah C. Solberg Woods (R01 DK120667), William Valdar (R35 GM127000), Abraham A. Palmer (P50 DA037844, P30 DA060810).

Authors' contributions: LSW designed the study, oversaw experiments and wrote the manuscript. TL and TM ran the statistical analysis under guidance of RM and WV. OS, TB and GG conducted all animal experiments. AB provided rats from the HS rat colony. BJ and GA ran the low-coverage whole genome sequencing under guidance of OP and AP. All authors read and approved the final manuscript.

Acknowledgements: Not Applicable

References

1. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med*. 2017;377(1):13-27.
2. Collaborators GUOF. National-level and state-level prevalence of overweight and obesity among children, adolescents, and adults in the USA, 1990-2021, and forecasts up to 2050. *Lancet*. 2024;404(10469):2278-98.
3. Fourman LT, Awwad A, Gutierrez-Sacristan A, Dash CA, Johnson JE, Thistle AK, et al. Implications of a New Obesity Definition Among the All of Us Cohort. *JAMA Netw Open*. 2025;8(10):e2537619.
4. Bouchard C. Genetics of Obesity: What We Have Learned Over Decades of Research. *Obesity (Silver Spring)*. 2021;29(5):802-20.
5. Verde L, Frias-Toral E, Cardenas D. Editorial: Environmental factors implicated in obesity. *Front Nutr*. 2023;10:1171507.
6. Boye C, Nirmalan S, Ranjbaran A, Luca F. Genotype x environment interactions in gene regulation and complex traits. *Nat Genet*. 2024;56(6):1057-68.
7. Kim MS, Shim I, Fahed AC, Do R, Park WY, Natarajan P, et al. Association of genetic risk, lifestyle, and their interaction with obesity and obesity-related morbidities. *Cell Metab*. 2024;36(7):1494-503 e3.
8. Pulit SL, Stoneman C, Morris AP, Wood AR, Glastonbury CA, Tyrrell J, et al. Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry. *Hum Mol Genet*. 2019;28(1):166-74.
9. Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, et al. Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. *Hum Mol Genet*. 2018;27(20):3641-9.

10. Namba S, Sonehara K, Koyanagi YN, Kikuchi T, Ojima T, Edahiro R, et al. A cross-population compendium of gene-environment interactions. *Nature*. 2026.
11. Westerman KE, Majarian TD, Giulianini F, Jang DK, Miao J, Florez JC, et al. Variance-quantitative trait loci enable systematic discovery of gene-environment interactions for cardiometabolic serum biomarkers. *Nat Commun*. 2022;13(1):3993.
12. Robinson MR, English G, Moser G, Lloyd-Jones LR, Triplett MA, Zhu Z, et al. Genotype-covariate interaction effects and the heritability of adult body mass index. *Nat Genet*. 2017;49(8):1174-81.
13. Tyrrell J, Wood AR, Ames RM, Yaghootkar H, Beaumont RN, Jones SE, et al. Gene-obesogenic environment interactions in the UK Biobank study. *Int J Epidemiol*. 2017;46(2):559-75.
14. Franks PW, Merino J. Gene-lifestyle interplay in type 2 diabetes. *Curr Opin Genet Dev*. 2018;50:35-40.
15. Westerman KE, Walker ME, Gaynor SM, Wessel J, DiCorpo D, Ma J, et al. Investigating Gene-Diet Interactions Impacting the Association Between Macronutrient Intake and Glycemic Traits. *Diabetes*. 2023;72(5):653-65.
16. Butler MJ, Eckel LA. Eating as a motivated behavior: modulatory effect of high fat diets on energy homeostasis, reward processing and neuroinflammation. *Integr Zool*. 2018;13(6):673-86.
17. Coll AP, Yeo GS. The hypothalamus and metabolism: integrating signals to control energy and glucose homeostasis. *Curr Opin Pharmacol*. 2013;13(6):970-6.
18. Liu CM, Kanoski SE. Homeostatic and non-homeostatic controls of feeding behavior: Distinct vs. common neural systems. *Physiol Behav*. 2018;193(Pt B):223-31.
19. Ferrario CR, Munzberg-Gruening H, Rinaman L, Betley JN, Borgland SL, Dus M, et al. Obesity- and diet-induced plasticity in systems that control eating and energy balance. *Obesity (Silver Spring)*. 2024;32(8):1425-40.
20. Ferreira-Hermosillo A, de Miguel Ibanez R, Perez-Dionisio EK, Villalobos-Mata KA. Obesity as a Neuroendocrine Disorder. *Arch Med Res*. 2023;54(8):102896.
21. Purnell JQ, le Roux CW. Hypothalamic control of body fat mass by food intake: The key to understanding why obesity should be treated as a disease. *Diabetes Obes Metab*. 2024;26 Suppl 2:3-12.
22. Shefer G, Marcus Y, Stern N. Is obesity a brain disease? *Neurosci Biobehav Rev*. 2013;37(10 Pt 2):2489-503.
23. Na ES. Epigenetic Mechanisms of Obesity: Insights from Transgenic Animal Models. *Life (Basel)*. 2025;15(4).
24. Dhillon H, Zigman JM, Ye C, Lee CE, McGovern RA, Tang V, et al. Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron*. 2006;49(2):191-203.
25. Frohman LA, Bernardis LL. Effect of hypothalamic stimulation on plasma glucose, insulin, and glucagon levels. *Am J Physiol*. 1971;221(6):1596-603.
26. Gaur A, Pal GK, Ananthanarayanan PH, Pal P. Role of Ventromedial hypothalamus in high fat diet induced obesity in male rats: association with lipid profile, thyroid profile and insulin resistance. *Ann Neurosci*. 2014;21(3):104-7.

27. Goncalves GHM, Tristao SM, Volpi RE, Almeida-Pereira G, de Carvalho Borges B, Donato J, et al. STAT3 but Not ERK2 Is a Crucial Mediator Against Diet-Induced Obesity via VMH Neurons. *Diabetes*. 2021;70(7):1498-507.
28. Shimazu T, Fukuda A, Ban T. Reciprocal influences of the ventromedial and lateral hypothalamic nuclei on blood glucose level and liver glycogen content. *Nature*. 1966;210(5041):1178-9.
29. Sun JS, Yang DJ, Kinyua AW, Yoon SG, Seong JK, Kim J, et al. Ventromedial hypothalamic primary cilia control energy and skeletal homeostasis. *J Clin Invest*. 2021;131(1).
30. Wang Q, Zhang B, Stutz B, Liu ZW, Horvath TL, Yang X. Ventromedial hypothalamic OGT drives adipose tissue lipolysis and curbs obesity. *Sci Adv*. 2022;8(35):eabn8092.
31. Timper K, Bruning JC. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. *Dis Model Mech*. 2017;10(6):679-89.
32. King BM. The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. *Physiol Behav*. 2006;87(2):221-44.
33. Sternson SM, Shepherd GM, Friedman JM. Topographic mapping of VMH --> arcuate nucleus microcircuits and their reorganization by fasting. *Nature neuroscience*. 2005;8(10):1356-63.
34. Baldini G, Phelan KD. The melanocortin pathway and control of appetite-progress and therapeutic implications. *J Endocrinol*. 2019;241(1):R1-R33.
35. Fitzpatrick M, Solberg Woods LC. Adenylate cyclase 3: a potential genetic link between obesity and major depressive disorder. *Physiol Genomics*. 2024;56(1):1-8.
36. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518(7538):197-206.
37. Turcot V, Lu Y, Highland HM, Schurmann C, Justice AE, Fine RS, et al. Protein-altering variants associated with body mass index implicate pathways that control energy intake and expenditure in obesity. *Nat Genet*. 2018;50(1):26-41.
38. Hansen C, Spuhler K. Development of the National Institutes of Health genetically heterogeneous rat stock. *Alcohol Clin Exp Res*. 1984;8(5):477-9.
39. Wagner VA, Holl KL, Clark KC, Reho JJ, Lehmler HJ, Wang K, et al. The Power of the Heterogeneous Stock Rat Founder Strains in Modeling Metabolic Disease. *Endocrinology*. 2023;164(12).
40. Solberg Woods LC. QTL mapping in outbred populations: successes and challenges. *Physiol Genomics*. 2014;46(3):81-90.
41. Solberg Woods LC, Palmer AA. Using Heterogeneous Stocks for Fine-Mapping Genetically Complex Traits. *Methods Mol Biol*. 2019;2018:233-47.
42. Chitre AS, Polesskaya O, Holl K, Gao J, Cheng R, Bimschleger H, et al. Genome-Wide Association Study in 3,173 Outbred Rats Identifies Multiple Loci for Body Weight, Adiposity, and Fasting Glucose. *Obesity (Silver Spring)*. 2020;28(10):1964-73.
43. Hong-Le T, Crouse WL, Keele GR, Holl K, Seshie O, Tschannen M, et al. Genetic Mapping of Multiple Traits Identifies Novel Genes for Adiposity, Lipids, and Insulin Secretory Capacity in Outbred Rats. *Diabetes*. 2023;72(1):135-48.

44. Keele GR, Prokop JW, He H, Holl K, Littrell J, Deal A, et al. Genetic Fine-Mapping and Identification of Candidate Genes and Variants for Adiposity Traits in Outbred Rats. *Obesity* (Silver Spring). 2018;26(1):213-22.
45. Crouse WL, Das SK, Le T, Keele G, Holl K, Seshie O, et al. Transcriptome-wide analyses of adipose tissue in outbred rats reveal genetic regulatory mechanisms relevant for human obesity. *Physiol Genomics*. 2022;54(6):206-19.
46. Tsaih SW, Holl K, Jia S, Kaldunski M, Tschannen M, He H, et al. Identification of a novel gene for diabetic traits in rats, mice, and humans. *Genetics*. 2014;198(1):17-29.
47. Wright SN, Leger BS, Rosenthal SB, Liu SN, Jia T, Chitre AS, et al. Genome-wide association studies of human and rat BMI converge on synapse, epigenome, and hormone signaling networks. *Cell Rep*. 2023;42(8):112873.
48. Ling W, Quan J, Lin J, Qiu T, Li J, Lu Q, et al. Grading fatty liver by ultrasound time-domain radiofrequency signal analysis: an in vivo study of rats. *Exp Anim*. 2018;67(2):249-57.
49. Chen D, Chitre AS, Nguyen KH, Cohen K, Peng B, Ziegler KS, et al. A Cost-effective, High-throughput, Highly Accurate Genotyping Method for Outbred Populations. *bioRxiv*. 2024.
50. Davies RW, Flint J, Myers S, Mott R. Rapid genotype imputation from sequence without reference panels. *Nat Genet*. 2016;48(8):965-9.
51. Paxinos G, C W. *The Rat Brain in Stereotaxic Coordinates*: Academic Press; 2013.
52. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;29(1):15-21.
53. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.
54. Risso D, Ngai J, Speed TP, Dudoit S. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nat Biotechnol*. 2014;32(9):896-902.
55. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16(5):284-7.
56. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet*. 2011;88(1):76-82.
57. Ziyatdinov A, Vazquez-Santiago M, Brunel H, Martinez-Perez A, Aschard H, Soria JM. lme4qtl: linear mixed models with flexible covariance structure for genetic studies of related individuals. *BMC Bioinformatics*. 2018;19(1):68.
58. Broman KW, Gatti DM, Simecek P, Furlotte NA, Prins P, Sen S, et al. R/qtl2: Software for Mapping Quantitative Trait Loci with High-Dimensional Data and Multiparent Populations. *Genetics*. 2019;211(2):495-502.
59. Zhang Z, Wang W, Valdar W. Bayesian modeling of haplotype effects in multiparent populations. *Genetics*. 2014;198(1):139-56.
60. Baron RA, Kenny DA. The Moderator-Mediator Variable Distinction in Social Psychological Research: Conceptual, Strategic, and Statistical Considerations. *Journal of Personality and Social Psychology*. 1986;51(6):1173-82.
61. Crouse WL, Keele GR, Gastonguay MS, Churchill GA, Valdar W. A Bayesian model selection approach to mediation analysis. *PLoS Genet*. 2022;18(5):e1010184.

62. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society*. 1995;57(1):289-300.
63. Liu Y, Li TR, Xu C, Xu T. Ribose Accelerates Gut Motility and Suppresses Mouse Body Weight Gaining. *Int J Biol Sci*. 2016;12(6):701-9.
64. Liu J, Hu S, Chen L, Daly C, Prada Medina CA, Richardson TG, et al. Profiling the genome and proteome of metabolic dysfunction-associated steatotic liver disease identifies potential therapeutic targets. *medRxiv*. 2023.
65. Vaisse C, Reiter JF, Berbari NF. Cilia and Obesity. *Cold Spring Harb Perspect Biol*. 2017;9(7).
66. Dalla Rosa I, Camara Y, Durigon R, Moss CF, Vidoni S, Akman G, et al. MPV17 Loss Causes Deoxynucleotide Insufficiency and Slow DNA Replication in Mitochondria. *PLoS Genet*. 2016;12(1):e1005779.
67. Hemerich D, Svenstrup V, Obrero VD, Preuss M, Moscati A, Hirschhorn JN, et al. An integrative framework to prioritize genes in more than 500 loci associated with body mass index. *Am J Hum Genet*. 2024;111(6):1035-46.
68. Marino F, Petrella L, Cimmino F, Pizzella A, Monda A, Allocca S, et al. From Obesity to Mitochondrial Dysfunction in Peripheral Tissues and in the Central Nervous System. *Biomolecules*. 2025;15(5).
69. Gonzalez-Gonzalez R, Guerra-Moreno JA, Cristobal-Mondragon GR, Romero V, Pena-Gomez SG, Montero-Moran GM, et al. Human Gpn1 purified from bacteria binds guanine nucleotides and hydrolyzes GTP as a protein dimer stabilized by its C-terminal tail. *Protein Expr Purif*. 2017;132:85-96.
70. Fitzpatrick MK, Szalanczy A, Beeson A, Vora A, Scott C, Grzybowski M, et al. Protein-coding mutation in *Adcy3* increases adiposity and alters emotional behaviors sex-dependently in rats. *Obesity (Silver Spring)*. 2025;33(1):91-103.
71. Szalanczy AM, Fitzpatrick M, Beeson A, Bui T, Dyson C, Eller S, et al. Chronic stress from adolescence to adulthood increases adiposity and anxiety in rats with decreased expression of *Krtcap3*. *Frontiers in genetics*. 2023;14:1247232.
72. Szalanczy AM, Goff E, Seshie O, Deal A, Grzybowski M, Klotz J, et al. Keratinocyte-associated protein 3 plays a role in body weight and adiposity with differential effects in males and females. *Frontiers in genetics*. 2022;13:942574.
73. Solberg Woods LC, Holl KL, Oreper D, Xie Y, Tsaih SW, Valdar W. Fine-mapping diabetes-related traits, including insulin resistance, in heterogeneous stock rats. *Physiol Genomics*. 2012;44(21):1013-26.
74. Seramur ME, McDonald B, Davis M, Reeves TE, Solberg Woods LC, Chuang Key CC. GRK5 is required for adipocyte differentiation through ERK activation. *Int J Obes (Lond)*. 2025;49(5):855-63.
75. Li Z, Gao T, Wang J, Zhang X, Zhang Y, Zhang L, et al. Ferroptosis mediated by TNFSF9 interferes in acute ischaemic stroke reperfusion injury with the progression of acute ischaemic stroke. *J Neurochem*. 2024;168(6):1030-44.
76. Szalanczy AM, Key CC, Solberg Woods LC. Genetic variation in satiety signaling and hypothalamic inflammation: merging fields for the study of obesity. *J Nutr Biochem*. 2022;101:108928.

77. Silva IM, Rosenfeld J, Antoniuk SA, Raskin S, Sotomaior VS. A 1.5Mb terminal deletion of 12p associated with autism spectrum disorder. *Gene*. 2014;542(1):83-6.
78. Mussa BM, Srivastava A, Rajan R, Venkatachalam T, Al-Habshi A, Abdelgadir E, et al. Saliva as a potential and non-invasive approach to identify upregulated genes associated with comorbidities of T1DM: a brief report. *Eur J Med Res*. 2025;30(1):955.
79. Shin J, Zhou X, Tan JTM, Hypponen E, Benyamin B, Lee SH. Lifestyle Modifies the Diabetes-Related Metabolic Risk, Conditional on Individual Genetic Differences. *Frontiers in genetics*. 2022;13:759309.
80. Kreznar JH, Keller MP, Traeger LL, Rabaglia ME, Schueler KL, Stapleton DS, et al. Host Genotype and Gut Microbiome Modulate Insulin Secretion and Diet-Induced Metabolic Phenotypes. *Cell Rep*. 2017;18(7):1739-50.
81. Parks BW, Nam E, Org E, Kostem E, Norheim F, Hui ST, et al. Genetic control of obesity and gut microbiota composition in response to high-fat, high-sucrose diet in mice. *Cell Metab*. 2013;17(1):141-52.
82. Parks BW, Sallam T, Mehrabian M, Psychogios N, Hui ST, Norheim F, et al. Genetic architecture of insulin resistance in the mouse. *Cell Metab*. 2015;21(2):334-47.
83. Norheim F, Hasin-Brumshtein Y, Vergnes L, Chella Krishnan K, Pan C, Seldin MM, et al. Gene-by-Sex Interactions in Mitochondrial Functions and Cardio-Metabolic Traits. *Cell Metab*. 2019;29(4):932-49 e4.
84. Lagou V, Magi R, Hottenga JJ, Grallert H, Perry JRB, Bouatia-Naji N, et al. Sex-dimorphic genetic effects and novel loci for fasting glucose and insulin variability. *Nat Commun*. 2021;12(1):24.
85. Winkler TW, Justice AE, Graff M, Barata L, Feitosa MF, Chu S, et al. The Influence of Age and Sex on Genetic Associations with Adult Body Size and Shape: A Large-Scale Genome-Wide Interaction Study. *PLoS Genet*. 2015;11(10):e1005378.
86. Donati B, Valenti L. Telomeres, NAFLD and Chronic Liver Disease. *Int J Mol Sci*. 2016;17(3):383.
87. Tang L, Li D, Ma Y, Cui F, Wang J, Tian Y. The association between telomere length and non-alcoholic fatty liver disease: a prospective study. *BMC Med*. 2023;21(1):427.
88. Bonhoure N, Byrnes A, Moir RD, Hodroj W, Preitner F, Praz V, et al. Loss of the RNA polymerase III repressor MAF1 confers obesity resistance. *Genes Dev*. 2015;29(9):934-47.
89. Willis IM, Moir RD, Hernandez N. Metabolic programming a lean phenotype by deregulation of RNA polymerase III. *Proc Natl Acad Sci U S A*. 2018;115(48):12182-7.
90. Deng G, Morselli LL, Wagner VA, Balapattabi K, Sapouckey SA, Knudtson KL, et al. Single-Nucleus RNA Sequencing of the Hypothalamic Arcuate Nucleus of C57BL/6J Mice After Prolonged Diet-Induced Obesity. *Hypertension*. 2020;76(2):589-97.
91. Kim JK, Hong S, Park J, Kim S. Metabolic and Transcriptomic Changes in the Mouse Brain in Response to Short-Term High-Fat Metabolic Stress. *Metabolites*. 2023;13(3).
92. Yoon G, Cho KA, Song J, Kim YK. Transcriptomic Analysis of High Fat Diet Fed Mouse Brain Cortex. *Frontiers in genetics*. 2019;10:83.
93. Lin W, Wall JD, Li G, Newman D, Yang Y, Abney M, et al. Genetic regulatory effects in response to a high-cholesterol, high-fat diet in baboons. *Cell Genom*. 2024;4(3):100509.

- 1076 94. Wallace CW, Fordahl SC. Obesity and dietary fat influence dopamine
1077 neurotransmission: exploring the convergence of metabolic state, physiological stress, and
1078 inflammation on dopaminergic control of food intake. *Nutr Res Rev.* 2022;35(2):236-51.
1079 95. Wang X, Li H. Chronic high-fat diet induces overeating and impairs synaptic
1080 transmission in feeding-related brain regions. *Front Mol Neurosci.* 2022;15:1019446.
1081

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables36582026.docx](#)
- [SupplementalTables5122026.xlsx](#)
- [SupplementalFiguresCombined.pdf](#)