Neuropharmacology 292 (2026) 110941

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Effects of *Npy1r* limbic conditional knock-out on adipose tissue metabolism

Giacomo Einaudi [a], Alessandra Oberto [b,c], Ilaria Bertocchi [b,c], Eleonora Aimaretti [b], Gustavo Ferreira Alves [b], Elisa Porchietto [b,c], Carlo Cifani [a], Maria Vittoria Micioni Di Bonaventura [a,*], Massimo Collino [b,1], Fausto Chiazza [d,1]

[a] University of Camerino, School of Pharmacy, Pharmacology Unit, Camerino, Italy
[b] University of Turin, Department of Neurosciences "Rita Levi Montalcini", Turin, Italy
[c] University of Turin, Neuroscience Institute Cavalieri-Ottolenghi (NICO), Turin, Italy
[d] University of Eastern Piedmont, Department of Pharmaceutical Sciences, Novara, Italy

ARTICLE INFO

Keywords:
Neuropeptide Y
Obesity
Metabolic syndrome
High fat diet
White adipose tissue

ABSTRACT

Neuropeptide Y (NPY) is a key regulator of energy homeostasis, acting through various receptor subtypes in both central and peripheral systems. Increasing interest has been directed toward exploiting NPY as a pharmacological target in obesity. While the orexigenic role of NPY in the hypothalamus is well established, its downstream effects on peripheral metabolism remain less defined, particularly when perturbations to the system are introduced.

Previously, we observed that female mice with limbic NPY-Y1 receptor gene (*Npy1r)* knockout (KO) under different dietary conditions (standard, SD, or high-fat diet, HFD) accumulated more subcutaneous white adipose tissue (WAT) compared to wild-type in the absence of gonadal hormones, despite no changes in food intake. To deepen the mechanisms underlying these effects, we conducted molecular analyses on WAT of these mice.

We found that *Npy* gene expression was upregulated in WAT of HFD-fed mice, regardless of genotype. However, NPY peptide levels were reduced in both KO and HFD groups, suggesting post-transcriptional regulation of NPY under metabolic stress. NPY-Y2 receptor gene *(Npy2r)* expression in WAT was significantly increased in both KO and HFD while *Npy1r* expression in WAT remained unchanged across groups.

Genes involved in WAT metabolism were similarly upregulated in both KO and HFD mice, indicating that limbic *Npy1r* KO mimics some of the metabolic effects induced by HFD. Correlation analysis suggests that dysregulated NPY signalling may promote increased lipid storage and reduce energy expenditure.

Overall, these findings highlight the complex interplay between central and peripheral NPY signalling emphasizing the importance of caution when investigating therapeutic strategies targeting single NPY receptors.

Overall, these findings highlight the complex interplay between central *Npy1r* signalling and peripheral adipose tissue regulation. They also emphasize the importance of caution when investigating new therapeutic strategies targeting single NPY receptors, as central interventions may provoke maladaptive metabolic responses in peripheral tissues.

1. Introduction

Obesity and metabolic disorders are nowadays dominating pathologies in the western world, due to changes in lifestyle and numerous other contributing factors (Lin and Li, 2021). Until recently, the clinical management of obesity was primarily done via behavioural strategies, but some highly successful pharmacological approaches have changed the landscape of available options (Ahima, 2016; Müller et al., 2022). Therapeutic peptides, particularly those that mimic the Glucagon-Like Peptide-1, have found great success as weight loss therapies (Popoviciu et al., 2023; Wang et al., 2023). Advanced therapies have tried to add onto the moiety for Glucagon-Like Peptide-1 agonism an activity of other peptides involved in metabolism. Dual agonists acting on Glucose-dependent Insulinotropic Polypeptide, Glucagon, and other hormone receptors are currently in use in the clinic; all these research efforts have shed light on the importance of peptides in metabolic

* Corresponding author. School of Pharmacy, University of Camerino, via Madonna delle Carceri 9, Camerino, 62032, Italy.
E-mail address: mariavittoria.micioni@unicam.it (M.V. Micioni Di Bonaventura).
[1] These authors share senior authorship.

https://doi.org/10.1016/j.neuropharm.2026.110941
Received 3 February 2026; Received in revised form 12 March 2026; Accepted 19 March 2026
Available online 20 March 2026

disorders (Alfaris et al., 2024; Boughton and Murphy, 2013; Melson et al., 2024). Numerous other peptides have been identified to play a role in metabolism, food consumption, and energy intake (Ignot-Gutiérrez et al., 2024; Prinz and Stengel, 2017; Wang et al., 2022). Some of them are neuropeptides that act locally, while others have hormonal activities, with intricate interplays between the central and peripheral systems.

Among them, Neuropeptide Y (NPY), part of the pancreatic polypeptide family, is widely expressed in the central and peripheral nervous systems, as well as in non-neuronal tissues (Kumari et al., 2024; Heilig and Widerlöv, 1990). NPY is involved in a variety of physiological processes, most notably the feeding behaviours and energy expenditure (Holzer et al., 2012; Lin et al., 2015; Loh et al., 2017). NPY is known as one of the most important orexigenic mediators; in the arcuate nucleus of the hypothalamus, under the influence of mediators like ghrelin and insulin, its release promotes food intake (Kohno and Yada, 2012; Loh et al., 2017). NPY effects are mediated by a family of G-protein-coupled receptors (Y1, Y2, Y4, Y5, and Y6), each with distinct function and tissue distribution (Blomqvist and Herzog, 1997; Brothers and Wahlestedt, 2010; Fetissov et al., 2004). In addition to its well-established role as a central neurotransmitter, NPY is also involved in both catecholaminergic and non-catecholaminergic neurons signalling to peripheral tissues where its receptors are expressed. However, the exact mechanisms by which NPY modulates autonomic activity remain incompletely understood (Zhang et al., 2014; Kumari et al., 2024; Statello et al., 2017).

In recent years, inconsistent findings have emerged, particularly from animal studies, largely due to diverse experimental settings in which NPY seems to play contrasting roles depending on feeding conditions and sex (Bertocchi et al., 2020; Yang et al., 2008; Zhang et al., 2014; Zhu et al., 2024). One of the most intricate aspects is the interplay between central and peripheral NPY's effects, particularly when perturbations of the system are induced (Oberto et al., 2022). In this context, a better understanding of the physiological consequences of targeting NPY signalling is crucial for the development of effective interventions.

The present study, building on our previous work (Oberto et al., 2022), aims to investigate whether the central deletion of the NPY-Y1 receptor gene (*Npy1r*) induces compensatory or downstream effects in peripheral tissues, with a particular focus on white adipose depots. In our prior study, we demonstrated that selective genetic ablation of *Npy1r* in the limbic system of female mice disrupted the central regulation of energy homeostasis via a hypothalamic-related pathway in the absence of gonadal hormones. Indeed, specifically, ovariectomized (ovx) *Npy1r* knockout (KO) mice fed either with standard diet (SD) or high fat diet (HFD) showed a significant increase in subcutaneous white adipose tissue (WAT) compared to wild-type (WT) mice. These findings suggested an interaction between hypothalamic *Npy1r* signalling and gonadal hormones in regulating peripheral metabolic function. However, the molecular mechanisms underlying these observations remained unclear. Therefore, in this study, we extend these findings to elucidate the potential pathways through which central NPY signalling influences adipose tissue metabolism.

2. Materials and methods

2.1. Specific aim and experimental design

In this study, we aimed to investigate the impact of *Npy1r* limbic KO, alone or in combination with HFD feeding, on the peripheral signalling of NPY and on adipose tissue function. Specifically, we used an already established model from which we collected WAT samples to perform the following analysis:

1) Quantitative Real Time-Polymerase Chain Reaction (qPCR) was performed to evaluate the gene expression of Npy, Npy1r, NPY-Y2 receptor (*Npy2r*), NPY-Y5 receptor (*Npy5r*), and key genes involved in adipose tissue function, Sterol regulatory element-binding transcription factor 1 *(Srebf1)*, Carnitine palmitoyltransferase I (*Cpt1a),* Peroxisome proliferator-activated receptor alpha (*Ppara),* Uncoupling Protein 1 *(Ucp1),* and Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*Ppargc1a).*
2) Enzyme-Linked Immunosorbent Assay (ELISA) to measure subcutaneous WAT concentration of NPY.
3) Correlation analysis between NPY pathway genes linking them to adipose tissue metabolism

Detailed procedures are described in the following paragraphs.

2.2. Animals

All animal procedures were conducted in accordance with Italian legislation (D.L.26/2014) and European Directive 2010/63/EU, with approval by the Animal Welfare Body (Organismo Preposto al Benessere Animale, OPBA) of the University of Turin, Turin, Italy and by the Italian Ministry of Health (approval n. 574/2016-PR). The animal cohort and procedure used to induce *Npy1r* conditional inactivation are reported in detail in the previously published study (Oberto et al., 2022). Briefly, inactivation of the *Npy1r* gene in the juvenile forebrain was achieved in mice carrying gene-targeted floxed *Npy1r* alleles and an inducible Cre recombinase transgene controlled by a doxycycline sensitive, synthetic transcriptional activator (tTA). Early *Npy1r* inactivation was prevented via chronic doxycycline treatment of pregnant females from conception. After birth, pups were fostered by doxycycline-free dams, which activated tTA, induced Cre expression and, subsequently, led to *Npy1r* gene inactivation in forebrain cells expressing the alpha subunit of calcium-calmodulin kinase type 2. Seven-week-old (P48) female mice were sham operated (cycling) or ovx and maintained on a SD. Shortly after, starting from postnatal day 60, mice were switched to a HFD or maintained on SD until sacrifice. Mice's body weight, food and energy intake were monitored twice a week; circulating leptin levels were measured before sacrifice, using a commercially available ELISA kit. Results from these analyses were reported in a prior publication (Oberto et al., 2022). After euthanasia, subcutaneous WAT was collected, weighed and immediately frozen for further biomolecular analysis.

2.3. qPCR protocol

Total RNA was extracted from WAT using the Fatty Tissue RNA Purification Kit (#36200, Norgen Biotek Corp, Ontario, Canada) following the manufacturer's protocol. The concentration and purity of the RNA were assessed using the NanoDrop system (Thermo Fischer Scientific, Madison, Wisconsin, USA). Subsequently, 800 ng of total RNA were reverse-transcribed into complementary DNA using the SensiFAST™ cDNA Synthesis Kit 50 reactions (#BIO-65053 Meridian Bioscience, Memphis, Tennessee, USA).

qPCR was performed using 100 ng of complementary DNA to evaluate the transcript levels of the target genes. Real-time was performed through the iTaq Universal SYBR Green Supermix (Bio-rad Laboratories Inc., Hercules, California, USA) according to the manufacturer's instructions. qPCR reaction was carried out with CFX Connect Real-Time PCR Detection System (Bio-Rad). Relative gene expression was obtained after normalization to 18s using the standard formula $2^{-\Delta\Delta CT}$ as previously described (Delconti et al., 2025; Livak and Schmittgen, 2001). Primer sequences are detailed in Table S1 (Supplementary material).

2.4. ELISA analysis

NPY WAT quantification was performed using the Mouse Neuropeptide Y (NPY) ELISA Kit (#MBS455063, MyBioSource, San Diego, California, USA) following the manufacturer's protocol.

2.5. Statistical analysis

Shapiro-Wilk and Bartlett tests were performed to check the normal distribution of data. Statistical differences in gene expression and NPY concentration were determined by two-way Analysis of Variance (ANOVA) (genotype and diet), followed by multiple comparisons and Bonferroni's post hoc tests. Cycling or ovx animals were independently analysed.

Correlation analysis for the gene expression was performed within each cohort of animals. Data were analysed with Spearman's rank correlation analysis for non-parametric variables; R coefficients are presented. Additionally, to estimate the relationship between NPY concentration or WAT weight with the various gene expression, a simple linear regression analysis was performed within the cycling groups. The equation of the interpolated line is reported in Tables 1 and 2.

All the analyses were performed using GraphPad Prism 10, after exclusion of outliers from the datasets. Outliers were identified using the Robust Regression and Outlier Removal method implemented in GraphPad Prism 10 (Motulsky and Brown, 2006). A Q value of 1% was used in these analyses. When flagged as outliers individual datapoints, rather than subjects, were excluded from the analysis. The threshold for statistical significance in the ANOVA and correlation analysis was set at $p < 0.05$. Data are presented as mean ± Standard Error of the Mean (SEM).

3. Results

3.1. Npy1r limbic conditional KO is associated with increased levels of Npy mRNA and reduced protein expression in WAT

To investigate changes in the subcutaneous WAT, we first analysed *Npy* gene expression in this tissue. Factorial ANOVA revealed a significant effect of the diet on *Npy* expression in both cycling ($F(1, 28) = 26.38$; $P < 0.0001$) and ovx ($F(1, 28) = 9.394$; $P = 0.0048$) mice (Fig. 1A–B). In cycling animals, a significant genotype–diet interaction was observed ($F(1, 28) = 10,47$; $P = 0.0031$) (Fig. 1A). Multiple comparison analysis revealed a significant increase in *Npy* gene expression in all groups of cycling animals compared to WT-SD controls ($P = 0.0111$ vs SD-KO, $P < 0.0001$ vs HFD-WT, $P = 0.0003$ vs HFD-KO), suggesting that both factors contributed to elevated *Npy* transcription (Fig. 1A). On the contrary, in ovx mice, although HFD tended to increase *Npy* expression, no statistically significant differences were found in *Npy* gene expression among the groups ($P = 0.0904$ SD-WT vs HFD-WT, $P = 0.2703$ SD-KO vs HFD-KO) (Fig. 1B).

When NPY peptide concentrations were measured in subcutaneous WAT, the highest levels were observed in SD-WT mice, in both cycling and ovx groups (Fig. 1C and D). In contrast, in all the other groups subjected to either central *Npy1r* deletion or HFD feeding, NPY levels were markedly reduced ($P = 0.034$ vs SD-KO, $P = 0.007$ vs HFD-WT, $P = 0.007$ vs HFD-KO) with a significant interaction between genotype and diet ($F(1, 27) = 6.759$; $P = 0.0149$). Additionally, NPY concentrations were comparable between cycling and ovx mice under the same dietary and genetic conditions, suggesting that gonadal hormone status did not significantly influence NPY protein levels in WAT under these experimental conditions (Fig. 1C and D).

3.2. Npy2r expression was increased in WAT of Npy1r KO and HFD cycling mice

In regularly cycling mice, NPY receptor expression in WAT was mostly influenced by the HFD feeding. Specifically, the two-way ANOVA analysis revealed a significant decrease in the expression of *Npy1r* in HFD mice independently of genotype ($F(1, 28) = 7,671$; $P = 0.0098$ for diet; $P = 0.3528$ for genotype; $P = 0.1000$ for interaction) (Fig. 2A). In contrast, *Npy2r* expression was upregulation in both SD-KO and HFD-WT mice compared to SD-WT ones ($P = 0.0349$ vs SD-KO, $P = 0.0479$ vs HFD-WT) without further increase in HFD-KO due to an observed interaction between diet and genotype factors ($F(1, 27) = 5.353$; $P = 0.0285$) (Fig. 2C). Similar effects were observed in the expression of *Npy5r,* with a reported interaction between diet and genotype ($F(1, 28) = 8.268$; $P = 0.0076$) (Fig. 2E). However, HFD consumption also independently increased the expression of *Npy5r* ($F(1, 28) = 33.12$ $P < 0.0001$); multiple comparison analysis revealed significant increase in the expression in HFD-WT ($P < 0.0001$) and HFD-KO ($P = 0.0386$) compared to SD-WT. Neither diet nor genotype had a significant effect on the expression of any of the NPY receptors analysed in ovx mice (Fig. 2B–D, F).

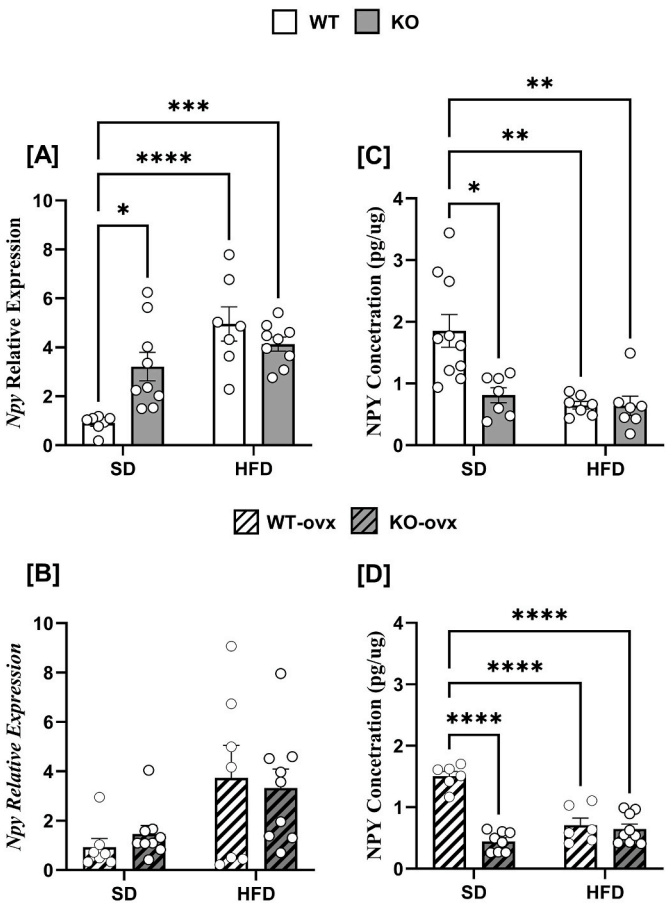

Fig. 1. NPY relative gene expression and protein concentration in the subcutaneous WAT of cycling [**A-C**] or ovx [**B-D**] mice, respectively. Gene expression data are reported as $2^{-\Delta\Delta cT}$ values. NPY Concentration in subcutaneous WAT analysed by ELISA. Data are reported as pg per µg of sample analysed. Data are reported as Mean ± SEM (n = 10). Data were analysed by two-way ANOVA in a 2 (diet: ND, HFD) x 2 (genotype: WT, KO) factorial design. Bonferroni's post hoc tests were used to follow up on significant interactions or main effects from the factorial ANOVAs. $^{*}P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$ $^{****}P < 0.0001$.

Overall, these results indicate that in cycling animals, both HFD and central *Npy1r* deletion influence the expression of *Npy* and its receptors in subcutaneous WAT, with diet emerging as the dominant modulator. Notably, no hormone-related effects were observed in ovx animals, suggesting a limited contribution of gonadal hormones in regulating peripheral NPY signalling under these experimental conditions.

3.3. Gene expression of lipid metabolism markers is modulated by dietary intervention and genetic manipulation of Npy1r

We next assessed the expression of key markers of lipid metabolism in WAT. In cycling mice, both *Npy1r* KO and HFD independently increased the expression of a gene of a major molecular modulators of

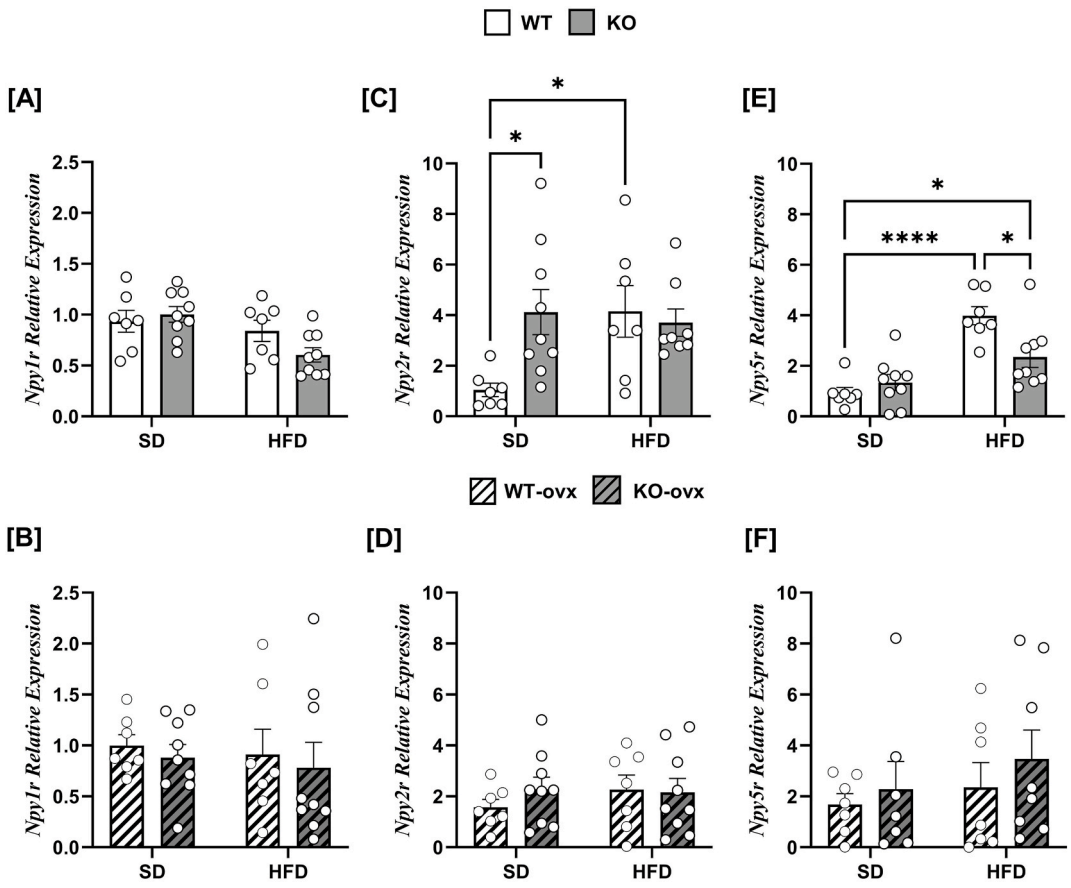

Fig. 2. NPY receptors relative gene expression in the subcutaneous WAT of cycling or ovx mice, respectively. [**A-B**] *Npy1r* [**C-D**] *Npy2r* [**E-F**] *Npy5r*. Data are reported as $2^{-\Delta\Delta cT}$ values. Data are reported as Mean ± SEM (n = 10). Data were analysed by two-way ANOVA in a 2 (diet: ND, HFD) x 2 (genotype: WT, KO) factorial design. Bonferroni's post hoc tests were used to follow up on significant interactions or main effects from the factorial ANOVAs. *$P < 0.05$ ***$P < 0.001$ ****$P < 0.0001$.

lipid metabolism *Srebf1* (F (1, 27) = 7.866; P = 0.0092 for diet; P = 0.0072 for genotype) (Fig. 3A). *Cpt1a* expression was significantly influenced by *Npy1r* KO (F (1, 27) = 5.458; P = 0.0271) while the expression of *Ppara* was non-significantly increased by both diet and genotype (F (1, 27) = 3.210; P = 0.0844), (Figure C-E), suggesting parallel and potentially overlapping influences of central *Npy1r* deletion and dietary fat on WAT metabolic gene expression. Interestingly, the central KO of *Npy1r* induced transcriptional changes in WAT metabolic regulators that mirrored those typically associated with HFD exposure.

Conversely, ovariectomy did not significantly impact the expression of these metabolic genes, indicating that the absence of gonadal hormones did not modulate these specific pathways under our experimental setting (Fig. 3B–D, F). This suggests that ovariectomy may affect adipose tissue metabolism through mechanisms distinct from peripheral NPY pathways.

As expected, HFD feeding resulted in significant downregulation of the thermogenic mediators *Ucp1* (F (1, 28) = 22.66; P < 0,0001) and *Ppargc1a* (F (1, 27) = 153; P = 0.0005), an effect that was independent of genotype and gonadal hormones (Fig. 3G, H, I, J).

3.4. NPY protein concentration is inversely correlated with its gene expression and WAT weight

Correlation analyses with WAT Weight (reported in Oberto et al., 2022) were performed to highlight the potential involvement of the NPY pathway in the WAT metabolism. Since ovariectomy defines a distinct endocrine condition and did not substantially modify the concentration of NPY or any of the NPY pathway genes in our dataset, correlation analyses presented here were focused on the cycling cohorts. A strong inverse correlation emerged between NPY protein levels and subcutaneous WAT weight, suggesting a potential protective role of NPY against adipose tissue expansion (R = −0.7255; P < 0.0001) (Fig. 4A and Table 1). Interestingly, NPY concentrations also showed an inverse correlation with both its own gene expression (R = −0.3921; P = 0.0354) and that of its receptor *Npy2r* (R = −0.3974; P = 0.0363) (Fig. 4B–D and Table 1), possibly reflecting the presence of a feedback regulatory mechanism. On the contrary, no significant correlations were detected between NPY level and the expression of *Npy1r* (R = −0.00985; P = 0.9595) (Fig. 4C) or *Npy5r* (R = −0.0208; P = 0.9215) (Table 1), hinting at a limited involvement of these receptors compared to *Npy2r* in our setting.

Additionally, we explored the relationship between NPY concentrations, and the expression of key genes involved in lipid metabolism. NPY concentration had a notable but non-significant correlation with the expression of the β-oxidation genes *Cpt1a* (R = −0.3491; P = 0.0805) and a significant one with *Ppara* (R = −0.4323; P = 0.03) (Fig. 4E–F and Table 1), further supporting a link between reduced NPY availability in WAT and altered lipid catabolism. Other correlations with metabolic genes were weak and not statistically significant (Fig. 5G–H and Table 1).

Consistent with the opposite trend between *Npy* gene expression and concentration, we recorded a direct correlation between *Npy* peptide expression and WAT weight (R = 0.4420; P = 0.007) (Fig. 5A and Table 2) as well as a positive correlation between WAT weight and *Npy2r* expression (R = 0.4114; P = 0.0174) (Fig. 5C and Table 2). Notably, an inverse correlation was detected between WAT weight and *Npy1r*

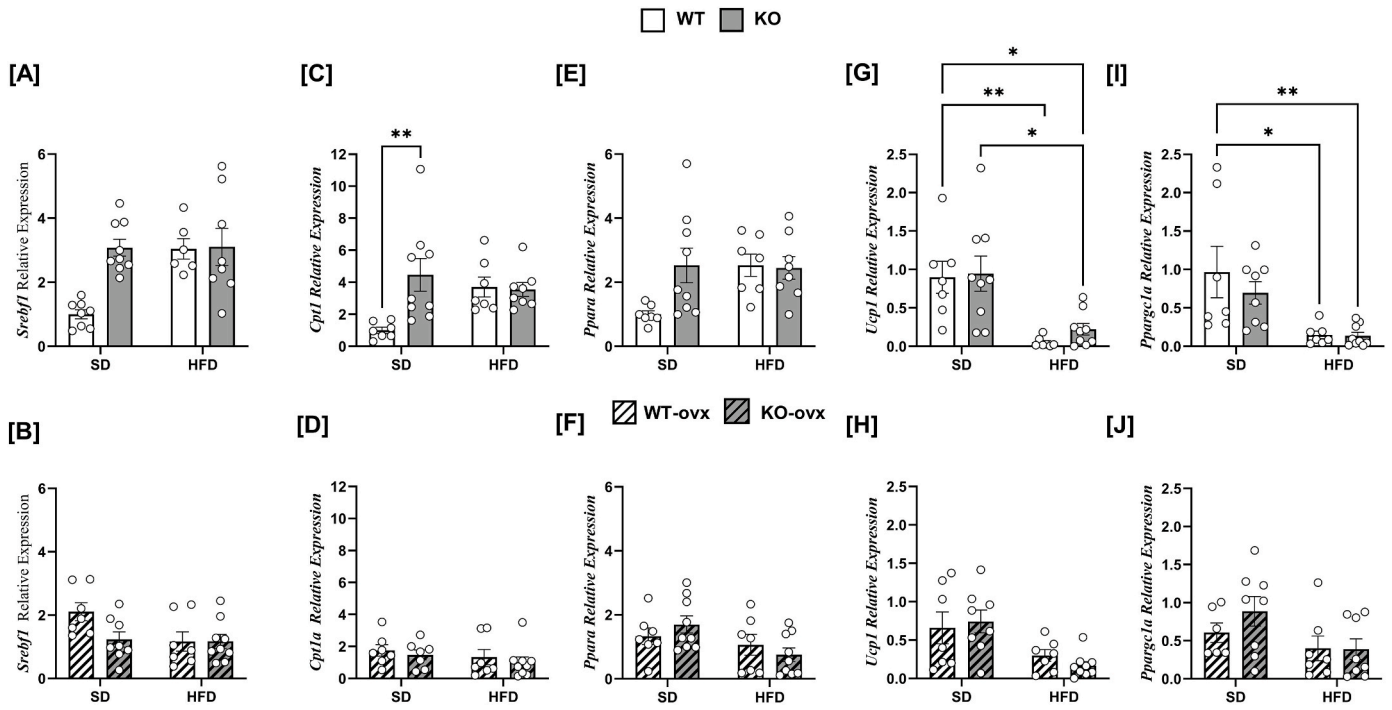

Fig. 3. Relative gene expression in the subcutaneous WAT of cycling or ovx mice respectively. [**A-B**] *Srebf1* [**C-D**] *Cpt1a* [**E-F**] *Ppara* [**G-H**] *Ucp1* [**I-J**] *Ppargc1a*. Data are reported as $2^{-\Delta\Delta cT}$ values. Data are reported as Mean ± SEM (n = 10). Data were analysed by two-way ANOVA in a 2 (diet: ND, HFD) x 2 (genotype: WT, KO) factorial design. Bonferroni's post hoc tests were used to follow up on significant interactions or main effects from the factorial ANOVAs. *$P < 0.05$ **$P < 0.01$.

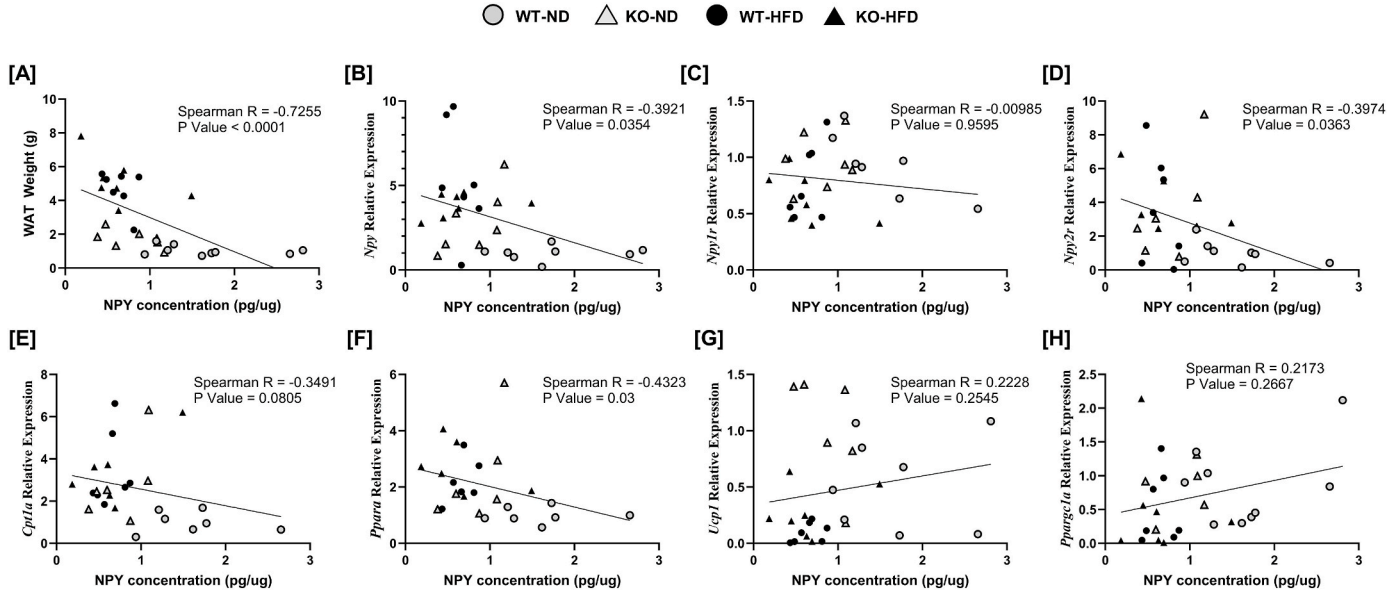

Fig. 4. Graphical representation of the correlation analysis between NPY concentration, WAT weight [**A**], *Npy* [**B**], *Npy1r* [**C**], *Npy2r* [**D**], *Cpt1a* [**E**], *Ppara* [**F**], *Ucp1* [**G**], *Ppargc1a* [**H**]. Data were analysed with Spearman's rank correlation analysis for non-parametrical variables; r coefficients are reported. The graphs represent the sample distribution among the 4 *cycling* groups with the predicted relationship depicted by the interpolated line.

expression (R = −0.4261; P = 0.0107) (Fig. 5B and Table 2), revealing potentially opposing roles of *Npy1r* and *Npy2r* in the regulation of adipose tissue remodelling.

3.5. NPY pathway genes are correlated with those of lipid metabolism in a diet-dependent manner

We next performed a correlation analysis between the expression of components of the NPY pathway and genes involved in adipose tissue function. Correlations were first explored in the full dataset and subsequently within stratified subgroups defined by ovarian status, diet, and genotype. This stratification approach was used to determine whether associations observed at the whole-cohort level were robust across conditions or instead context-dependent and potentially masked by biological heterogeneity.

When considering all the cohort of mice we observed significant direct correlations between *Npy2r* expression with *Srebf1* (R = 0.54; P < 0.0001), *Cpt1a* (R = 0.65; P < 0.0001), and *Ppara* (R = 0.53; P < 0.0001)

Table 1
Correlation analysis between NPY concentration, WAT weight and relative gene expression among all the cycling mice. Data were compared by Spearman's rank correlation; r and P values are reported. A linear regression analysis was performed to predict the relationship between the variables. The equation of the interpolated line is reported, as well as the *P*-value.

	NPY Concentration			
	Spearman's r	*p* Value	Equation	*p* Value
WAT Weight	−0.7255	0.0001	Y = −1.530X + 4.677	0.0282
Relative Gene Expression				
Npy	−0.3921	0.0354	Y = −2.020X + 5.008	0.0002
Npy1r	−0.0099	0.9595	Y = −0.07561X + 0.8716	0.5196
Npy2r	−0.3974	0.0363	Y = −1.757X + 4.535	0.0466
Npy5r	−0.0208	0.9215	Y = −0.05495X + 1.259	0.8641
Srebf1	−0.0878	0.6832	Y = −0.2924X + 1.526	0.4399
Cpt1a	−0.3491	0.0805	Y = −0.7864X + 3.354	0.2183
Ppara	−0.4323	0.0309	Y = −0.7275X + 2.745	0.0994
Ucp1	0.2228	0.2545	Y = 0.1277X + 0.3432	0.3782
Ppargc1a	0.2173	0.2667	Y = 0.2587X + 0.4132	0.1476

(Fig. 6A). An inverse correlation was found between *Npy* and *Ucp1* (R = −0.40; P = 0.0022), together with positive ones between *Npy1r* and *Ppargc1a* (R = 0.35; P = 0.005), and between *Npy5r* and *Srebf1* (R = 0.37; P = 0.008) (Fig. 6A). A first stratification was performed between cycling and ovx cohorts (Fig. 6B and C). This analysis revealed that, except for a positive correlation between *Npy2r* and *Srebf1,* which was also detected in ovx mice (R = 0.47; P = 0.018), the majority of NPY pathway–related correlations were observed in cycling animals. In this subgroup, *Npy* expression was positively correlated with *Npy2r* (R = 0.38, P = 0.03), as well as with the expression of lipogenic and β-oxidation genes, including *Srebf1* (R = 0.43; P = 0.015), *Cpt1a* (R = 0.51; P = 0.004), and *Ppara* (R = 0.51; P = 0.003) (Fig. 6B). Not surprisingly, *Npy2r* expression was also positively correlated with all these metabolic genes, reinforcing its potential involvement in lipid metabolism (Fig. 6B). Strong inverse correlations were instead observed between *Npy* expression and genes involved with thermogenesis, namely *Ucp1* (R = −0.54; P = 0.001), and *Ppargc1a* (R = −0.367; P = 0.033). However, the expression of these two genes positively correlated with *Npy1r* (R = 0.37; P = 0.029 for *Ucp1,* R = 0.40; P = 0.020 for *Ppargc1a*) expression, suggesting a possible role for this receptor in modulating the thermogenic phenotype of adipose tissue.

Since ovx cohorts showed correlations mostly among non-NPY related gene, we further stratified the cycling cohorts to determine whether these correlations were specifically driven by dietary intervention or by *Npy1r* deletion, performing subgroup analysis within distinct experimental conditions. As shown in Fig. 7A–D, we identified some divergent patterns across subgroups. Most correlations were driven by SD-fed mice: *Npy* and *Npy2r* were positively correlated with each other (R = 0.73; P = 0.003) and with genes involved in lipid metabolism (Fig. 7A). Interestingly, in this subgroup *Npy1r* was also positively correlated with *Srebf1* (R = 0.47; P = 0.05), *Cpt1a* (R = 0.62; P = 0.01), *Ppara* (R = 0.57; P = 0.04), *Ucp1* (R = 0.52; P = 0.041), and with *Npy2r* (R = 0.81; P < 0.0001) (Fig. 7A). These relationships were not observed in HFD-fed mice (Fig. 7B). Regarding the analysis of the subgroup with different genotypes, no marked differences were observed between genotypes, mostly reflecting those of the cycling subgroup (Fig. 7C–D). These findings highlight, the complexity of the NPY signalling, responding differently depending on metabolic context, such as dietary fat exposure.

4. Discussion

NPY is implicated in metabolic regulation mostly due to its activity in the hypothalamus, where activation of the receptor NPY1R, and to a lesser extent NPY5R, stimulates food intake Blomqvist and Herzog, 1997; Stanley et al., 1992; Gerald et al., 1996). Based on these central orexigenic effects, *Npy1r* KO has been proposed as a potential experimental strategy to suppress appetite and reduce food intake. However, results from previous studies have been inconsistent. Rather than producing a reduction in weight gain, *Npy1r* deficiency has frequently been associated with increased body weight and adiposity, ultimately promoting systemic metabolic dysregulation (Kushi et al., 1998; Paterlini

Table 2
Correlation analysis between WAT weight and relative gene expression among all the *cycling* mice. Data were compared by Spearman's rank correlation; r and P values are reported. A linear regression analysis was performed to predict the relationship between the variables, the equation of the interpolated line is reported as well as the P value.

	WAT Weight			
	Spearman's r	*p* Value	Equation	*p* Value
Relative Gene Expression				
Npy	0.4420	0.0070	Y = 0.4511X + 2.137	0.0235
Npy1r	−0.4261	0.0107	Y = −0.0725X + 1.099	0.0097
Npy2r	0.4114	0.0174	Y = 0.4590X + 1.734	0.0309
Npy5r	0.0842	0.6522	Y = 0.03431X + 1.145	0.7252
Srebf1	0.1496	0.4855	Y = −0.0876X + 1.428	0.5640
Cpt1a	0.4074	0.0186	Y = 0.2424X + 2.063	0.1202
Ppara	0.2530	0.1554	Y = 0.0757X + 1.904	0.4722
Ucp1	−0.4423	0.0078	Y = −0.1029X + 0.7425	0.0046
Ppargc1a	−0.4129	0.0137	Y = −0.0704X + 0.8085	0.1413

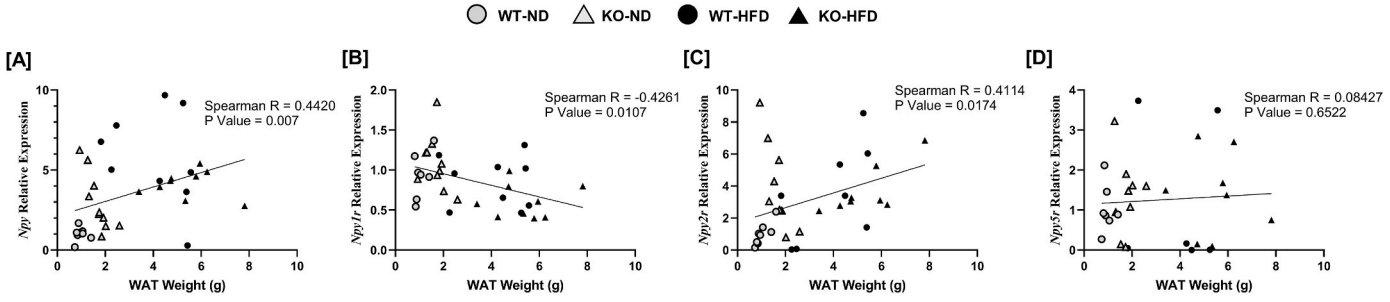

Fig. 5. Graphical Representation of the correlation analysis between WAT weight, relative gene expression of *Npy* [**A**] and its receptors [**B-D**]. Data were analysed with Spearman's rank correlation analysis for non-parametrical variables; r coefficients are reported. The graphs represent the sample distribution among the 4 *cycling* groups with the predicted relationship depicted by the interpolated line.

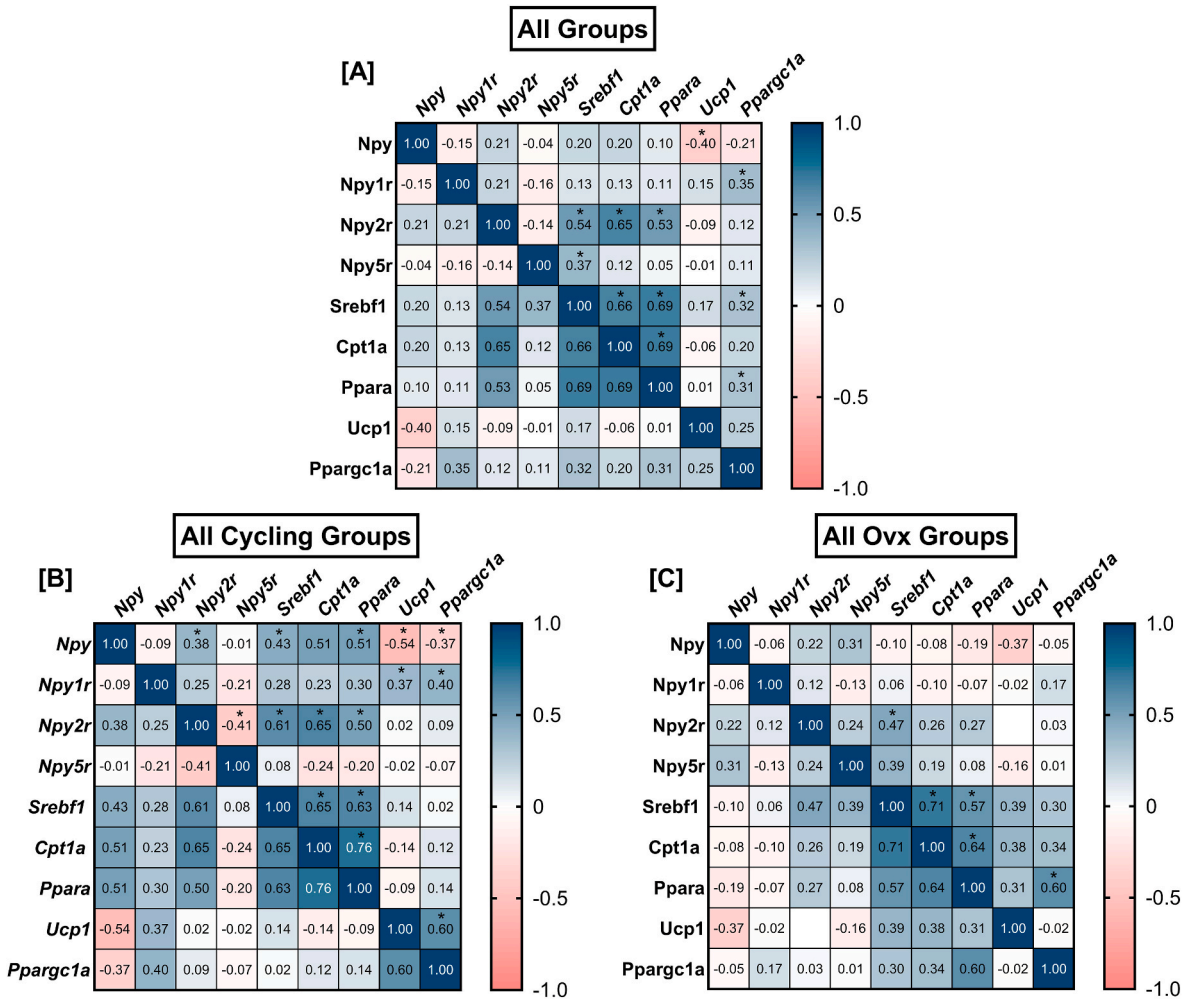

Fig. 6. Correlation analysis between the relative gene expression in subcutaneous WAT. Data were compared by Spearman's rank correlation; r coefficient values ranging from −1 to +1 are reported. Correlations were drawn among all groups [**A**] or between the two subgroups of cycling [**B**] or ovx mice [**C**]. *P < 0.05 demarks a statistically significant correlation.

et al., 2021; Pedrazzini, 2004). Until recently, these paradoxical effects have been attributed to compensatory activation of other NPY receptor subtypes in the absence of *Npy1r* signalling, which may sustain or even exacerbate the metabolic phenotype. To avoid such compensatory mechanisms, our earlier studies employed a conditional *Npy1r* KO restricted to the limbic system and induced only during adulthood (Bertocchi et al., 2011; Oberto et al., 2022).

Here, we used the female mice investigated in Oberto et al., to further explore the role of the NPY system in metabolic regulation beyond its well-established role in hypothalamic control of feeding behaviour (Oberto et al., 2022). This study extends our previous findings providing some new evidence in the complex interplay between central and peripheral NPY pathways in the regulation of adipose tissue accumulation and lipid metabolism.

So far, NPY has been implicated in promoting adipose tissue hypertrophy, lipogenesis and lipolysis under various conditions (Yang et al., 2008; Zhang et al., 2014). Based on these findings, we initially hypothesized that the observed metabolic alterations could result from a direct involvement of the NPY pathway within adipose tissue itself. Consistent with this idea, analysis of *Npy* gene expression in the WAT revealed a significant upregulation in HFD-fed mice, independent of both genotype and gonadal hormones. A genotype-dependent interaction was observed in sham-operated animals only. Further analysis of NPY receptor expression revealed a significant increase in *Npy2r* levels in both HFD and *Npy1r* KO groups. Interestingly, *Npy2r* expression positively correlated with that of *Npy* in cycling mice, suggesting a coordinated regulatory mechanism in response to diet and genotype. These findings may align with previous reports supporting a functional role for *Npy2r* receptor in adipose tissue metabolism (Baker et al., 2009; Kuo et al., 2007; Shi et al., 2011). In contrast to *Npy2r*, *Npy1r* expression in WAT was not significantly affected by diet, genotype, or hormonal status. Although *Npy1r* has been implicated in mediating NPY's actions when co-released with norepinephrine, enhancing typical sympathetic responses, its specific function within adipose tissue remains controversial (Zhang et al., 2014).

Recent studies have suggested that NPY may promote the proliferation of mural cells, precursors of thermogenic adipocytes in both brown and white fat, thus potentially playing a protective role against diet-induced metabolic stress (Chao et al., 2011; Zhu et al., 2024). Conversely, other findings indicate that NPY may suppress brown adipose tissue thermogenesis, thereby contributing to the development of metabolic derangements (Chao et al., 2011). Our data add further insights to this complex picture, showing an upregulation of *Npy* mRNA expression in the WAT of HFD-fed mice, paralleled by a significant reduction in NPY peptide levels in both *Npy1r* KO and HFD groups, regardless of hormonal status. This apparent mismatch between mRNA and protein levels may reflect a compensatory upregulation of transcription in response to diminished peptide availability. However, the lack of corresponding peptide accumulation suggests impaired translation or reduced peptide stability, pointing to the involvement of

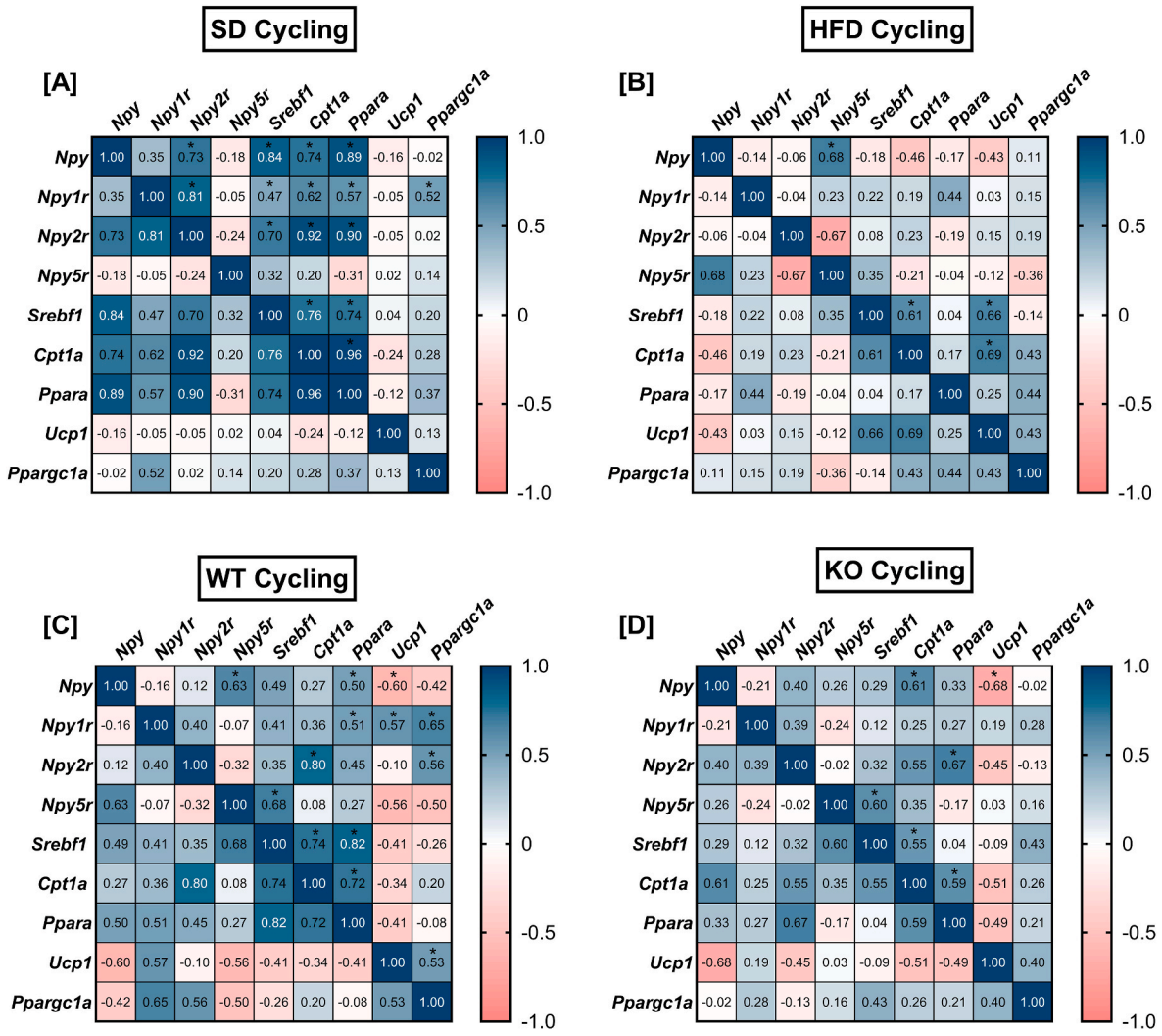

Fig. 7. Correlation analysis between the relative gene expression in subcutaneous WAT. Data were compared by Spearman's rank correlation; r coefficient values ranging from −1 to +1 are reported. Correlations were drawn among cycling groups between subgroups of mice that shared the same genotype or diet: SD-WT and SD-KO [**A**]; HFD-WT and HFD-KO [**B**]; SD-WT and HFD-WT [**C**]; SD-KO and HFD-KO [**D**]. *P < 0.05 demarks a statistically significant correlation.

post-transcriptional regulatory mechanisms under conditions of metabolic stress. Interestingly, our data also revealed an inverse correlation between NPY concentration in WAT and tissue weight, suggesting that NPY levels may be higher under physiological conditions and decline in the context of excessive fat accumulation.

When we analysed the expression of genes involved with β-oxidation and lipogenesis, we found increased expression of *Cpt1a*, *Ppara* and *Srebf1* in both KO and HFD-fed animals, indicating a dysregulation in lipid metabolism in KO mice, in line with that observed in HFD-fed group. These findings are in keeping with previous evidence showing that global *Npy1r* deletion induces metabolic derangements like those caused by HFD (Pedrazzini, 2004). Differently, the expression of genes predominantly involved in energy expenditure, namely *Ucp1* and *Ppargc1a*, was decreased exclusively in HFD-fed mice, while no changes were found in KO animals.

To widen the picture on the role of NPY under the different experimental conditions, we performed correlation analysis in progressively smaller subgroups of mice. When analysing all the cohorts of mice we observed positive correlations between *Npy2r* expression with *Srebpf1*, *Cpt1a*, and *Ppara*. Moreover, we observed positive correlations between *Npy1r* and *Pppargc1a*, pointing to different role of the receptors in adipose tissue metabolism. When we stratified between cycling and ovx mice, we observed stronger correlations between NPY-related pathway and genes involved in adipose tissue metabolism mainly in the cycling cohorts, while this correlation was absent in the ovx groups. This evidence allowed us to speculate that ovx induced changes in adipose tissue metabolism beyond those related to the NPY pathway genes. When we analysed cycling mice, indeed, we found strong direct correlations between *Npy* and *Npy2r* expression with *Srebf1*, *Cpt1a* and *Ppargc1a*, while in this subgroup *Npy* was inversely correlated with *Ucp1* and *Ppargc1a*. Conversely, *Npy1r* expression was found to have a strong direct correlation with *Ucp1* and *Ppargc1a*, supporting previous evidence attributing to *Npy1r* a relevant role in promoting energy expenditure and browning of adipose tissue (Yang et al., 2008). Furthermore, when analysing dietary subgroups separately, NPY seemed to play a more relevant role in the SD subgroups, since *Npy, Npy1r* and *Npy2r* expression were all strongly correlated to *Srebf1*, *Cpt1a* and *Ppargc1a*. These correlations were prominent in SD-fed mice but absent in HFD-fed ones. While *Npy1r* KO induced significant changes in gene expression levels, stratified correlation analyses within WT and KO subgroups did not reveal marked differences in the overall correlation structure. This suggests that *Npy1r* deletion affects the magnitude of gene expression without substantially altering the relationships among NPY-related and metabolic genes.

Our findings are consistent with a context-dependent homeostatic role of NPY under physiological conditions, which become attenuated or reorganized under HFD or *Npy1r* deletion (Kuo et al., 2007; Zhang et al.,

2014).

Regarding the different receptors, we can hypothesize a relevant role of NPY2R that showed changes in gene expression in both KO and HFD mice, while numerous positive correlations with the genes involved in adipose tissue metabolism were observed in the SD cohorts. Although *Npy1r* expression was mostly unchanged in our experiments, its positive correlations with genes related to thermogenesis still point to a role in adipose tissue homeostasis, different from other subtypes. Lastly, *Npy5r* expression showed minimal modulation under the conditions examined, suggesting a limited contribution in this experimental context.

Altogether, here we identified context-specific correlation patterns linking NPY pathway components to genes involved in lipid metabolism and thermogenesis. These associations were predominantly confined to cycling and SD-fed animals, supporting the concept that NPY exerts distinct regulatory roles in adipose tissue depending on genotype, nutritional, and hormonal status.

A limitation of the present study is that transcriptional analyses were restricted to female WAT. Sex-specific differences in adipose regulation are well established, and whether comparable NPY-related molecular mechanisms operate in male WAT remains to be determined. Addressing sex-dependent regulation, in our and other models of NPY modulation, will therefore be an important objective for future investigations. Additionally, although we reported evidence implicating NPY in thermogenic regulation, the present work did not investigate brown adipose tissue depots, which are known to have a predominant role in these mechanisms. Future studies incorporating depot-specific molecular and functional assessments will be necessary to determine whether the context-dependent regulatory patterns described here extend to thermogenic adipose depots.

5. Conclusions

Our data support a role for NPY signalling in regulating lipid metabolism. Under physiological conditions, NPY might exert a protective influence on adipose tissue function, which appears to be altered, or even lost, under pathological metabolic conditions. Our work demonstrates that NPY function is highly context-dependent, varying according to both dietary and genetic contexts, however the specific mechanisms underlying these interactions remain to be elucidated.

Although NPY is widely recognized for its role in regulating energy intake, energy expenditure, and adipose tissue homeostasis, and has been proposed as a promising pharmacological target, our findings underscore the complexity of its signalling network. Interventions targeting individual components of the NPY system could produce unintended consequences, as suggested by our and other findings. Strategies that appear beneficial may disrupt the delicate balance between central and peripheral pathways. Taken together, our data suggest that therapeutic modulation of the NPY system should be approached with caution, considering its intricate and context-dependent metabolic effects.

CRediT authorship contribution statement

Giacomo Einaudi: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alessandra Oberto:** Methodology, Investigation, Conceptualization. **Ilaria Bertocchi:** Methodology, Investigation, Conceptualization. **Eleonora Aimaretti:** Investigation. **Gustavo Ferreira Alves:** Investigation. **Elisa Porchietto:** Investigation. **Carlo Cifani:** Writing – review & editing, Visualization, Validation, Supervision, Resources. **Maria Vittoria Micioni Di Bonaventura:** Writing – review & editing, Visualization, Validation, Supervision, Resources. **Massimo Collino:** Writing – review & editing, Visualization, Validation, Supervision, Resources. **Fausto Chiazza:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation, Conceptualization.

Ethical statement

Animals were housed according to general guidelines on protecting animals used for scientific purposes (EU Directives 201/63/EU) with approval by the Animal Welfare Body (Organismo Preposto al Benessere Animale, OPBA) of the University of Turin, Turin, Italy and by the Italian Ministry of Health (approval n. 574/2016-PR).

Funding sources

This work has received funding from the Italian Ministry for University and Research in the framework of the 2022 Program for Research Projects of National Interest (PRIN grant n. 20228HRTJ2 CUP D53D23013290006 to I.B., grant n. 20224PR8HE CUP D53D23013220006 to M.C., grant n. PRIN2022LHMF43 CUP J53D23012220001 to M.V.M.D.B., and grant n. 2022SZ2FMK CUP J53D23012440006 to C.C.) and by the national project "Dipartimenti di Eccellenza 2023-2027", awarded to the Department of Neuroscience "Rita Levi Montalcini" (University of Turin).

Declaration of competing interest

All authors have no conflicts of interest to declare concerning this article research, authorship, and/or publication.

Acknowledgements

Not applicable.

Glossary

ANOVA = Analysis of Variance
Cpt1a = Carnitine palmitoyltransferase I gene
ELISA = Enzyme-Linked Immunosorbent Assay
HFD = High Fat Diet
KO = Knockout
NPY = Neuropeptide Y
Npy = Neuropeptide Y gene
Npy1r = NPY-Y1 receptor gene
Npy2r = NPY-Y2 receptor gene
Npy5r = NPY-Y5 receptor gene
Ovx = ovariectomized
Ppargc1a = Peroxisome proliferator-activated receptor gamma coactivator 1 alpha gene
Ppara = Peroxisome proliferator-activated receptor alpha gene
qPCR = Quantitative Real Time-Polymerase Chain Reaction
SD = Standard Chow Diet
SEM = Standard Error of the Mean
Srebf1 = Sterol regulatory element-binding transcription factor 1 gene
tTA = synthetic transcriptional activator
Ucp1 = Uncoupling Protein 1 gene
WAT = White Adipose Tissue
WT = Wild-Type

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2026.110941.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

Ahima, R.S., 2016. Pharmacotherapy of obesity and metabolic syndrome. In: Metabolic Syndrome. Springer International Publishing, pp. 797–809. https://doi.org/10.1007/978-3-319-11251-0_44.

Alfaris, N., Waldrop, S., Johnson, V., Boaventura, B., Kendrick, K., Stanford, F.C., 2024. GLP-1 single, dual, and triple receptor agonists for treating type 2 diabetes and obesity: a narrative review. eClinicalMedicine 75. https://doi.org/10.1016/j.eclinm.2024.102782.

Baker, S.B., Cohen, M., Kuo, L., Johnson, M., Al-Attar, A., Zukowska, Z., 2009. The role of the neuropeptide Y2 receptor in liporemodeling: Neuropeptide Y-mediated adipogenesis and adipose graft maintenance. Plast. Reconstr. Surg. 123 (2), 486–492. https://doi.org/10.1097/PRS.0b013e3181954c80.

Bertocchi, I., Oberto, A., Longo, A., Mele, P., Sabetta, M., Bartolomucci, A., Palanza, P., Sprengel, R., Eva, C., 2011. Regulatory functions of limbic Y1 receptors in body weight and anxiety uncovered by conditional knockout and maternal care. Proc. Natl. Acad. Sci. USA 108, 19395–19400. https://doi.org/10.1073/pnas.1109468108.

Bertocchi, I., Oberto, A., Longo, A., Palanza, P., Eva, C., 2020. Conditional inactivation of Npy1r gene in mice induces sex-related differences of metabolic and behavioral functions. Hormones Behav. 125, 104824. https://doi.org/10.1016/j.yhbeh.2020.104824.

Blomqvist, A.G., Herzog, H., 1997. Y-receptor subtypes—how many more? Trends Neurosci. 20 (7), 294–298. https://doi.org/10.1016/S0166-2236(96)10081-4.

Boughton, C.K., Murphy, K.G., 2013. Can neuropeptides treat obesity? A review of neuropeptides and their potential role in the treatment of obesity. Br. J. Pharmacol. 170, 7. https://doi.org/10.1111/bph.2013.170.issue-7.

Brothers, S.P., Wahlestedt, C., 2010. Therapeutic potential of neuropeptide Y (NPY) receptor ligands. EMBO Mol. Med. 2, 429–439. https://doi.org/10.1002/emmm.201000100.

Chao, P.T., Yang, L., Aja, S., Moran, T.H., Bi, S., 2011. Knockdown of NPY expression in the dorsomedial hypothalamus promotes development of brown adipocytes and prevents diet-induced obesity. Cell Metab. 13, 573–583. https://doi.org/10.1016/j.cmet.2011.02.019.

Delconti, M., Ravasenga, T., Dionisi, M., Romanazzi, T., Chiazza, F., Giatti, S., Lim, D., Genazzani, A.A., Riva, B., Meregalli, C., Cavaletti, G., Distasi, C., 2025. Paclitaxel activates SOCE/ICRAC in dorsal root ganglion neurons: implications for paclitaxel-induced peripheral neuropathy. Cell Calcium 130, 103040. https://doi.org/10.1016/j.ceca.2025.103040.

Fetissov, S.O., Kopp, J., Hökfelt, T., 2004. Distribution of NPY receptors in the hypothalamus. Neuropeptides 38, 175–188. https://doi.org/10.1016/j.npep.2004.05.009.

Gerald, C., Walker, M.W., Criscione, L., et al., 1996. A receptor subtype involved in neuropeptide Y–induced food intake. Nature 382, 168–171. https://doi.org/10.1038/382168a0.

Heilig, M., Widerlöv, E., 1990. Neuropeptide Y: an overview of central distribution, functional aspects, and possible involvement in neuropsychiatric illnesses. Acta Psychiatr. Scand. 82, 95–114. https://doi.org/10.1111/j.1600-0447.1990.tb01366.x.

Holzer, P., Reichmann, F., Farzi, A., 2012. Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut–brain axis. Neuropeptides 46, 261–274. https://doi.org/10.1016/j.npep.2012.08.005.

Ignot-Gutiérrez, A., Serena-Romero, G., Guajardo-Flores, D., Alvarado-Olivarez, M., Martínez, A.J., Cruz-Huerta, E., 2024. Proteins and peptides from food sources with effect on satiety and their role as anti-obesity agents: a narrative review. Nutrients 16, 3560. https://doi.org/10.3390/nu16203560.

Kohno, D., Yada, T., 2012. Arcuate NPY neurons sense and integrate peripheral metabolic signals to control feeding. Neuropeptides 46, 315–319. https://doi.org/10.1016/j.npep.2012.09.004.

Kumari, R., Pascalau, R., Wang, H., Bajpayi, S., Yurgel, M., Quansah, K., Hattar, S., Tampakakis, E., Kuruvilla, R., 2024. Sympathetic NPY controls glucose homeostasis, cold tolerance, and cardiovascular functions in mice. Cell Rep. 43, 113674. https://doi.org/10.1016/j.celrep.2024.113674.

Kuo, L.E., Kitlinska, J.B., Tilan, J.U., Li, L., Baker, S.B., Johnson, M.D., Lee, E.W., Burnett, M.S., Fricke, S.T., Kvetnansky, R., Herzog, H., Zukowska, Z., 2007. Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. Nat. Med. 13, 803–811. https://doi.org/10.1038/nm1611.

Kushi, A., Sasai, H., Koizumi, H., Takeda, N., Yokoyama, M., Nakamura, M., 1998. Obesity and mild hyperinsulinemia found in neuropeptide Y Y1 receptor-deficient mice. Proc. Natl. Acad. Sci. USA 95, 15659–15664. https://doi.org/10.1073/pnas.95.26.15659.

Lin, X., Li, H., 2021. Obesity: epidemiology, pathophysiology, and therapeutics. Front. Endocrinol. 12, 706978. https://doi.org/10.3389/fendo.2021.706978.

Lin, X., Qi, Q., Zheng, Y., Huang, T., Lathrop, M., Zelenika, D., Bray, G.A., Sacks, F.M., Liang, L., Qi, L., 2015. Neuropeptide Y genotype, central obesity, and abdominal fat distribution: the POUNDS LOST trial. Am. J. Clin. Nutr. 102, 514–519. https://doi.org/10.3945/ajcn.115.107276.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Loh, K., Zhang, L., Brandon, A., Wang, Q., Begg, D., Qi, Y., Fu, M., Kulkarni, R., Teo, J., Baldock, P., Brüning, J.C., Cooney, G., Neely, G., Herzog, H., 2017. Insulin controls food intake and energy balance via NPY neurons. Mol. Metabol. 6, 574–584. https://doi.org/10.1016/j.molmet.2017.03.013.

Melson, E., Ashraf, U., Papamargaritis, D., Davies, M.J., 2024. What is the pipeline for future medications for obesity? Int. J. Obes. 48. https://doi.org/10.1038/s41366-024-01473-y.

Motulsky, H.J., Brown, R.E., 2006. Detecting outliers when fitting data with nonlinear regression: a new method based on robust nonlinear regression and the false discovery rate. BMC Bioinf. 7, 123. https://doi.org/10.1186/1471-2105-7-123.

Müller, T.D., Blüher, M., Tschöp, M.H., DiMarchi, R.D., 2022. Anti-obesity drug discovery: advances and challenges. Nat. Rev. Drug Discov. 21, 201–223. https://doi.org/10.1038/s41573-021-00337-8.

Oberto, A., Bertocchi, I., Longo, A., Bonzano, S., Paterlini, S., Meda, C., Della Torre, S., Palanza, P., Maggi, A., Eva, C., 2022. Hypothalamic NPY–Y1R interacts with gonadal hormones in protecting female mice against obesity and neuroinflammation. Int. J. Mol. Sci. 23, 6351. https://doi.org/10.3390/ijms23116351.

Paterlini, S., Panelli, R., Gioiosa, L., Parmigiani, S., Franceschini, P., Bertocchi, I., Oberto, A., Bartolomucci, A., Eva, C., Palanza, P., 2021. Conditional inactivation of limbic neuropeptide Y1 receptors increases vulnerability to diet-induced obesity in male mice. Int. J. Mol. Sci. 22, 8745. https://doi.org/10.3390/ijms22168745.

Pedrazzini, T., 2004. Importance of NPY Y1 receptor-mediated pathways: assessment using NPY Y1 receptor knockouts. Neuropeptides 38, 267–275. https://doi.org/10.1016/j.npep.2004.05.007.

Popoviciu, M.S., Păduraru, L., Yahya, G., Metwally, K., Cavalu, S., 2023. Emerging role of GLP-1 agonists in obesity: a comprehensive review of randomised controlled trials. Int. J. Mol. Sci. 24, 10449. https://doi.org/10.3390/ijms241310449.

Prinz, P., Stengel, A., 2017. Control of food intake by gastrointestinal peptides: mechanisms of action and possible modulation in the treatment of obesity. J. Neurogastroenterol. Motil. 23, 180–196. https://doi.org/10.5056/jnm16194.

Shi, Y.C., Lin, S., Castillo, L., Aljanova, A., Enriquez, R.F., Nguyen, A.D., Baldock, P.A., Zhang, L., Bijker, M.S., Macia, L., Yulyaningsih, E., Zhang, H., Lau, J., Sainsbury, A., Rzog, H., 2011. Peripheral-specific Y2 receptor knockdown protects mice from high-fat diet-induced obesity. Obesity 19, 2137–2148. https://doi.org/10.1038/oby.2011.99.

Stanley, B.G., Magdalin, W., Seirafi, A., Nguyen, M.M., Leibowitz, S.F., 1992. Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the Y1 receptor mediating this peptide's effect. Peptides 13, 581–587. https://doi.org/10.1016/0196-9781(92)90091-8.

Statello, R., Carnevali, L., Paterlini, S., Gioiosa, L., Bertocchi, I., Oberto, A., Eva, C., Palanza, P., Sgoifo, A., 2017. Reduced NPY Y1 receptor hippocampal expression and signs of decreased vagal modulation of heart rate in mice. Physiol. Behav. 172, 31–39. https://doi.org/10.1016/j.physbeh.2016.07.017.

Wang, L., Wang, N., Zhang, W., Cheng, X., Yan, Z., Shao, G., Wang, X., Wang, R., Fu, C., 2022. Therapeutic peptides: current applications and future directions. Signal Transduct. Targeted Ther. 7, 904. https://doi.org/10.1038/s41392-022-00904-4.

Wang, J.Y., Wang, Q.W., Yang, X.Y., Yang, W., Li, D.R., Jin, J.Y., Zhang, H.C., Zhang, X.F., 2023. GLP-1 receptor agonists for the treatment of obesity: role as a promising approach. Front. Endocrinol. 14, 1085799. https://doi.org/10.3389/fendo.2023.1085799.

Yang, K., Guan, H., Arany, E., Hill, D.J., Cao, X., 2008. Neuropeptide Y is produced in visceral adipose tissue and promotes proliferation of adipocyte precursor cells via the Y1 receptor. FASEB J. 22 (7), 2452–2464. https://doi.org/10.1096/fj.07-100735.

Zhang, W., Cline, M.A., Gilbert, E.R., 2014. Hypothalamus-adipose tissue crosstalk: neuropeptide Y and the regulation of energy metabolism. Nutr. Metab. 11, 27. https://doi.org/10.1186/1743-7075-11-27.

Zhu, Y., Yao, L., Gallo-Ferraz, A.L., Bombassaro, B., Simões, M.R., Abe, I., Chen, J., Sarker, G., Ciccarelli, A., Zhou, L., Lee, C., Sidarta-Oliveira, D., Martínez-Sánchez, N., Dustin, M.L., Zhan, C., Horvath, T.L., Velloso, L.A., Kajimura, S., Domingos, A.I., 2024. Sympathetic neuropeptide Y protects from obesity by sustaining thermogenic fat. Nature 634, 243–250. https://doi.org/10.1038/s41586-024-07504-2.