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# Olfactomedin 2 deficiency protects against diet-induced obesity

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Declaration of competing interest

The authors declare no conflict of interest. Afia Sultana is currently at the NIH Center for Scientific Review, this work was completed while she was at National Eye Institute.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2021.155122.

## Abstract

**Background and aims:** Olfactomedin 2 (OLFM2; also known as noelin 2) is a pleiotropic protein that plays a major role in olfaction and *Olfm2* null mice exhibit reduced olfactory sensitivity, as well as abnormal motor coordination and anxiety-related behavior. Here, we investigated the possible metabolic role of OLFM2.

**Methods:** *Olfm2* null mice were metabolically phenotyped. Virogenetic modulation of central OLFM2 was also performed.

**Results:** Our data showed that, the global lack of OLFM2 in mice promoted anorexia and increased energy expenditure due to elevated brown adipose tissue (BAT) thermogenesis and browning of white adipose tissue (WAT). This phenotype led to resistance to high fat diet (HFD)-induced obesity. Notably, virogenetic overexpression of *Olfm2* in the lateral hypothalamic area (LHA) induced weight gain associated with decreased BAT thermogenesis.

**Conclusion:** Overall, this evidence first identifies central OLFM2 as a new molecular actor in the regulation of whole-body energy homeostasis.

### Keywords

Olfactomedin 2; Lateral hypothalamic area; Brown adipose tissue; Browning; Thermogenesis; Obesity

## 1. Introduction

Olfactomedin 2 (OLFM2; also known as OlfC or olfactomedin-related endoplasmic reticulum-localized-2, noelin-2) is a secretory glycoprotein that belongs to a family of effectors consisting of at least 13 olfactomedin domain-containing members [1–3], segregated into seven subfamilies. OLFM2, together with olfactomedin 1 and 3, forms the first subfamily, and is primarily expressed in neurons [2,3], even though high levels of expression have been also reported in the skin and adipose tissue [4,5]. Loss of *Olfm2* in null mice (OLFM2 KO) resulted in reduced olfactory sensitivity, abnormal motor coordination and anxiety-related behavior [6]. Here, we have examined the potential role of OLFM2 on energy balance by taking advantage of *Olfm2* null mice.

## 2. Materials and methods

### 2.1. Animals

Male *Olfm2* null and wildtype (WT) littermate mice (C57BL/6; 15 weeks-old) were used for the experiments [6]. They were allowed free access to water and standard laboratory diet (SD) (Scientific-Animal-Food-Engineering, 3% fat, 60% carbohydrates and 16% proteins; Amersfoort, The Netherlands) or high fat diet (HFD) (*D12451*: 45% fat, 35% carbohydrate, 20% protein; Research-Diets, Inc.; New Brunswick, NJ, USA) for 10 weeks (25 weeks-old at the end). The experiments were performed in agreement with the International Law on Animal Experimentation and were approved by the USC Ethical Committee (15010/14/006) (Supplementary materials and methods).

### 2.2. Stereotaxic microinjection of adeno-associated viral vectors

The lateral hypothalamus (LHA) was targeted bilaterally using a 32-gauge needle (Hamilton, Reno, NV) as reported [7–9]. AAV1-Gfp ($5.22 \times 10^{11}$ gc/ml) or AAV1-Olfm2 ($4.48 \times 10^{11}$ gc/ml) (VectorBuilder GmbH; Isenburg, Germany) were delivered at a rate of 100 nl/min for 10 min (Supplementary materials and methods).

### 2.3. Animal measurements

Body temperature, skin temperature, indirect calorimetry, and nuclear magnetic resonance (NRM) were performed as described [7–16] (Supplementary materials and methods).

### 2.4. Analytical methods

Glucose and insulin tolerance tests (GTT and ITT), serum leptin levels, real time PCR, high-performance liquid chromatography (HPLC), BAT UCP1 western blotting and WAT UCP1 immunostaining were performed as described using the same reagents [7–16]. Fluorescent *in-situ* hybridization was performed using RNAscope™ (Advanced Cell Diagnostics; Hayward, CA, USA). Hybridization probes specific to mouse *Olfm2* gene (NM_173777.4) were supplied by the company. Brain sections were labeled with FITC (*Olfm2*) and DAPI (nuclei) (Supplementary materials and methods).

### 2.5. Statistical analysis

Data are expressed as mean ± SEM. Statistical significance was determined by Student's *t*-test using, $P < 0.05$ was significant (Supplementary materials and methods).

## 3. Results

### 3.1. Standard diet fed Olfm2 null mice showed negative energy balance

*Olfm2* null mice displayed lower body weight (Suppl. Fig. 1A) and feeding (Suppl. Fig. 1B), lower adiposity (Suppl. Fig. 1C), increased energy expenditure (EE; Suppl. Fig. 1D) and reduced respiratory quotient (RQ; Suppl. Fig. 1E), indicating a shift to fat metabolism. Total locomotor activity (LA) was decreased in the OLFM2 KO mice (Suppl. Fig. 1F) and while central LA (central part of the cage) was similar in WT and OLFM2 KO mice (Suppl. Fig. 1G), peripheral LA (peripheral parts of the cage) was markedly reduced in OLFM2 KO mice (Suppl. Fig. 1H), both in the dark phase and total, indicating anxiety.

### 3.2. Standard diet fed Olfm2 null mice showed increased thermogenesis

*Olfm2* null mice had higher body (Suppl. Fig. 2A) and BAT temperature (Suppl. Fig. 2B–C), confirming increased BAT thermogenesis. Absence of OLFM2 did not globally impact either glucose tolerance, with only a slight worse handling at time 30 min (Suppl. Fig. 2D–E) nor insulin sensitivity (Suppl. Fig. 2F–G).

### 3.3. High fat diet fed Olfm2 null mice showed negative energy balance

*Olfm2* null mice fed a HFD displayed a lower body weight (Fig. 1A), decreased feeding (Fig. 1B), a trend to lower adiposity, as well as decreased BAT and sWAT weight (Fig. 1C–D). Leptin levels trended also lower in OLFM2 KO mice (Fig. 1E). *Olfm2* null displayed

increased EE (Fig. 1F) and reduced RQ (Fig. 1G). No changes were found in total LA (Suppl. Fig. 3A), central LA or peripheral LA (Fig. 3B–C).

### 3.4. High fat diet fed Olfm2 null mice showed increased thermogenesis and browning

*Olfm2* null mice fed a HFD displayed higher body (Fig. 1H) and BAT temperature (Fig. 1I–J) than WT controls. In keeping with this, UCP1 protein levels (Fig. 1K–L) and mRNA expression of thermogenic/oxidative markers (Fig. 1M) were also elevated in the BAT of *Olfm2* null animals. OLFM2 KO mice had higher BAT levels of norepinephrine (NE), but not dopamine (DA) or serotonin (5-HT) (Fig. 1N). A nonsignificant increase in UCP1 staining was detected in gWAT (Suppl. Fig. 4A–B) without changes in the adipocyte area (Suppl. Fig. 4C). Analysis of sWAT demonstrated a significant elevation in the UCP1 immunoreactivity (Suppl. Fig. 4D–E) and decreased adipocyte area (Suppl. Fig. 4F). Thus, global lack of OLFM2 promoted the browning of sWAT.

### 3.5. High fat diet fed Olfm2 null mice showed improved insulin resistance

While glucose tolerance was similar in both genotypes (Suppl. Fig. 5A–B), the lack of OLFM2 improved insulin resistance in HFD fed mice (Suppl. Fig. 6C–D). Therefore, global genetic ablation of *Olfm2* was protective against DIO and insulin resistance.

### 3.6. Olfm2 overexpression in the LHA induced positive energy balance and decreased BAT thermogenesis

RNAscope™ analysis of *Olfm2* expression in WT mice (Fig. 2A) confirmed [6] high expression in several brain regions, such as the neocortex, piriform cortex, amygdala, thalamus, and hypothalamus. Here, we found a high mRNA expression of *Olfm2* in the arcuate (ARC), dorsomedial (DMH) and the lateral hypothalamic area (LHA), with lower expression levels in the ventrolateral part of the ventromedial nucleus of the hypothalamus (VMH). Considering the role of the LHA in the regulation of thermogenesis [7–9,16], we first unsuccessfully tried to knockdown/silencing within this area, despite we used several lentiviral and AAV-based strategies (data not shown). Thus, we overexpressed OLFM2 in the LHA of WT mice fed a SD using also virogenetics. *Olfm2 in vivo* overexpression (Fig. 2B) promoted feeding-independent weight gain (Fig. 2C–E), associated with decreased BAT thermogenesis (Fig. 2F–G), exactly the opposite phenotype induced by OLFM2 deficiency in OLFM2 global KO null mice.

## 4. Discussion

Here, we performed a whole metabolic phenotyping of *Olfm2* null mice. Our data demonstrate that OLMF2 is involved in the regulation of energy metabolism. We also investigated the possible role of central OLFM2 on these effects. Our expression studies demonstrated that *Olfm2* mRNA is highly expressed in several hypothalamic nuclei. Given the catabolic and thermogenic phenotype that we found in the *Olfm2* null mice and the key role of LHA regulating BAT thermogenesis [7,8,16], we hypothesized that the observed effects could be mediated by OLFM2 deficiency in that hypothalamic area and therefore, its genetic overexpression may trigger the opposite phenotype. Our results, showing increased body weight and decreased BAT thermogenesis in mice with selective LHA overexpression,

indicated that OLFM2 modulation in the LHA is sufficient to trigger metabolic changes at whole body level. Notably, these effects are opposite to those observed after global deletion of *Olfm2* gene. Whether this is a consequence of a direct action of OLFM2 within this hypothalamic region or in different brain areas where LHA neurons send projections will require further studies. However, it is likely that metabolic disturbances observed in the *Olfm2* null mice have a hypothalamic origin. Second evidence supporting the brain-centered function of OLFM2 is that KO mice showed a higher NE levels in the BAT, suggesting elevated adrenergic tone, commonly associated with sympathetic nervous activity, which is driven and modulated by several brain areas, among them the LHA [7,8]. A limitation of our study is the lack of data showing whether selective knockdown/silencing of *Olfm2* could recapitulate the phenotype of global *Olfm2* null mice. Generation of conditional *Olfm2* mice in specific hypothalamic nuclei, among them the LHA, will help to clarify the exact role of central OLFM2.

## 5. Conclusions

Our data reveal for the first time a role for OLMF2 in the regulation of energy homeostasis, with OLFM2 KO mice showing a catabolic phenotype protective from DIO. Notably, OLFM2 effects on energy homeostasis are likely driven by its central function.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## Data availability

Data that support the findings of this study are available from the corresponding authors upon request.

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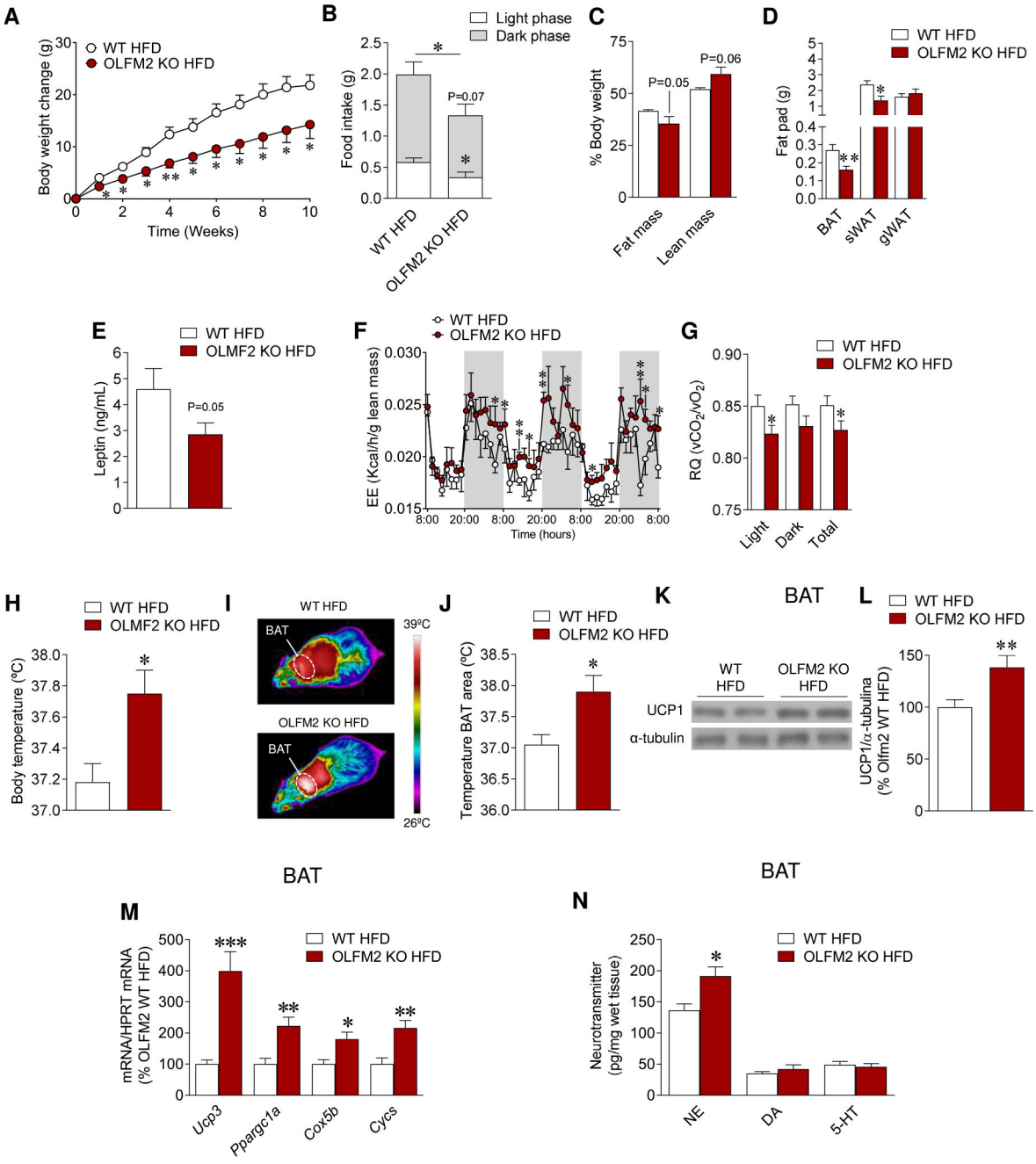


**Fig. 1.**

Effect of global OLFM2 deficiency on energy balance in high fat diet fed mice.

(A) Body weight change after HFD exposure, (B) food intake, (C) body composition, (D) fat pads weight, (E) leptin serum levels, (F) energy expenditure (EE), (G) respiratory quotient (RQ), (H) body temperature, (I) representative thermal images, (J) temperature of the BAT area, (K) western blot representative images, (L) BAT UCP1 protein levels, (M) BAT mRNA levels of thermogenic/oxidative markers and (N) BAT neurotransmitter levels of WT and OLFM2 KO mice fed a HFD. Data are expressed as MEAN ± SEM. N = 6 mice/group in all the panels, except for body composition data (C) where N = 5 mice/group. Statistical

significance was determined by Student's *t*-test. *P < 0.05, **P < 0.01, ***P < 0.001 *vs.* WT HFD. In panel B: i) the asterisk (*P < 0.05) above the horizontal middle line indicates the statistical difference WT HFD *vs.* OLFM2 KO HFD mice in total food intake (light phase + dark phase); ii) the asterisk (*P < 0.05) above the OLFM2 KO HFD white bar indicate the statistical difference WT HFD *vs.* OLFM2 KO HFD mice in the light phase food intake; iii) the P = 0.07 above the OLFM2 KO HFD grey bar indicates the P value between WT HFD *vs.* OLFM2 KO HFD mice in the dark phase food intake.

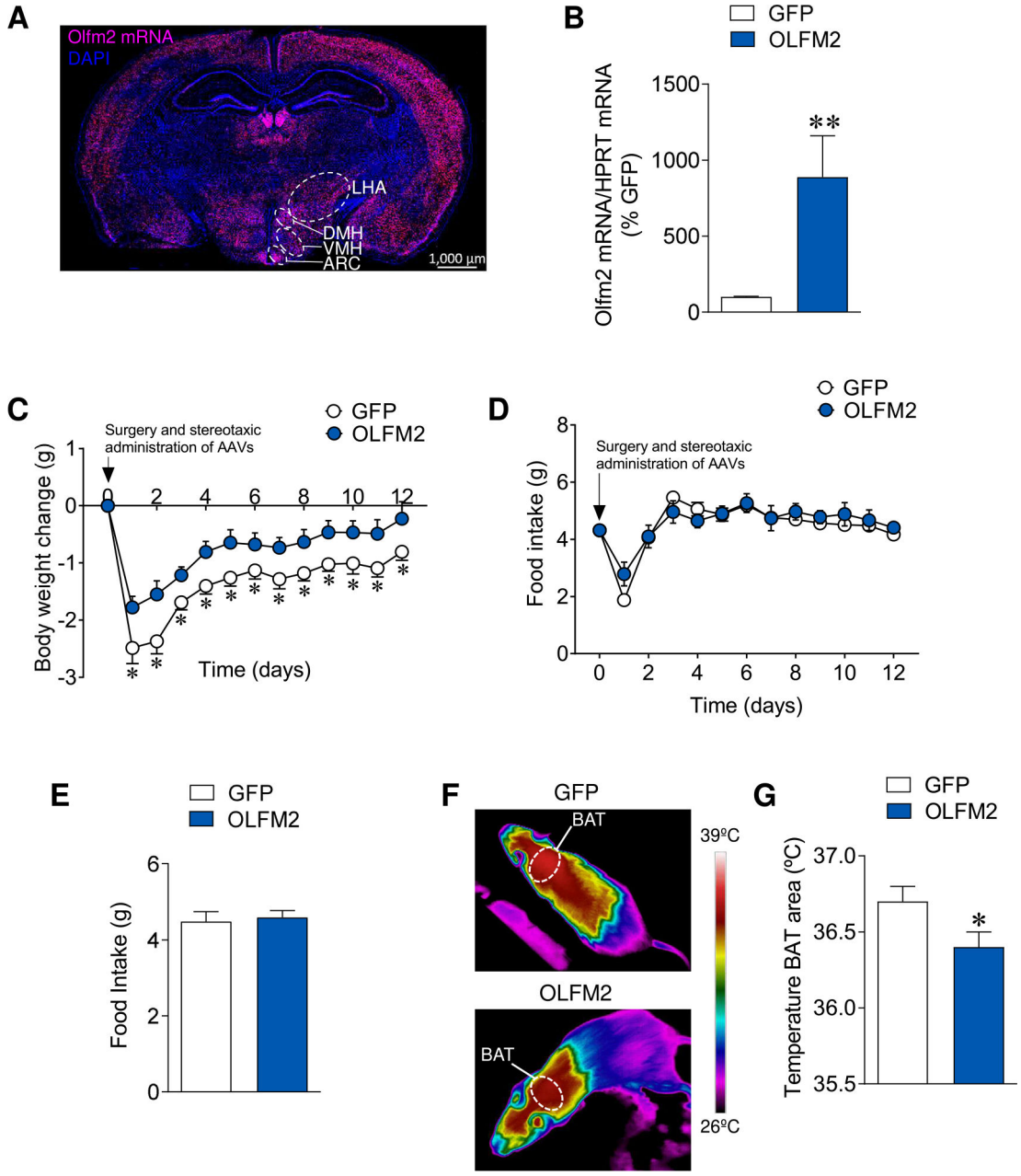


**Fig. 2.**

Effect of *Olfm2* overexpression within the lateral hypothalamic area on energy balance in standard diet fed mice.

(A) Representative confocal micrographs depicting Olfm2 mRNA (magenta) and DAPI (blue) fluorescence in the brain coronal section of a WT mice. Scale bar = 1000 µm, (B) *Olfm2* mRNA levels in the LHA (N = 8–11 mice/group), (C) body weight change, (D) daily food intake (N = 11 mice/group), (E) average food intake (N = 11 mice/group), (F) representative thermal images and (G) temperature of the BAT area (N = 8–11 mice/group) after stereotaxic administration of AAVs encoding GFP or OLFM2 in the LHA of mice

fed a SD. Data are expressed as MEAN ± SEM. Statistical significance was determined by Student's t-test. *P < 0.05, **P < 0.01 *vs.* GFP.