Received: 9 September 2024 | Revised: 13 January 2025 | Accepted: 26 January 2025

DOI: 10.1002/oby.24259

ORIGINAL ARTICLE

Obesity Biology and Integrated Physiology

Food insecurity promotes adiposity in mice

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Funding information

Novo Nordisk Foundation, Grant/Award Numbers: NNF17OC0026114, NNF18CC0034900, NNF20SA0035586, NNF20SA0064340, NNF22OC0073778, NNF23SA0084103, NNF21CC0073729; Lundbeck Foundation, Grant/Award Number: R238-2016-2859

Abstract

Objective: The obesity epidemic, driven by a complex interplay of environmental and biological factors, remains a significant global health challenge. Herein, we investigate the impact of food insecurity, characterized by unpredictable food access, on the regulation of body weight and body composition in mice.

Methods: Male and female C57BL/6J mice were subjected to a combination of intermittent fasting and calorie restriction to simulate food insecurity.

Results: Our new model demonstrates that food insecurity increases fat mass and decreases lean mass in both sexes on a standard chow diet. Additionally, high-fat diet-fed male mice exposed to the food insecurity paradigm show decreased lean mass despite being in positive energy balance. Transcriptomic analysis of white adipose tissue from food-insecure male mice revealed upregulation of metabolic pathways associated with fat mass expansion and downregulation of immune response-related transcripts.

Conclusions: These findings underscore the role of food insecurity in driving metabolic adaptations that favor fat storage. Understanding this paradoxical link between food insecurity and adiposity is crucial for developing targeted interventions to address the disproportionate incidence of obesity in socioeconomically disadvantaged populations.

INTRODUCTION

Obesity is estimated to affect more than 1 billion individuals globally and is a major driver of severe comorbidities such as type 2 diabetes, cardiovascular disease, and certain types of cancer [1]. The rising prevalence of obesity is often attributed to environments characterized by easily accessible, hyperpalatable, ultraprocessed, energy-dense foods, coupled with a decline in physical activity [2]. However, recent research has questioned these assumptions [3,4]. Accordingly, obesity prevalence has increased more in rural than in urban populations [5,6], despite less exposure of the rural than the urban populations to this presumed obesogenic environment. Moreover, lack of correlation between densities of fast-food restaurants and obesity prevalence has been reported [7]. Moreover, doubly labeled water studies have demonstrated that energy expenditure from physical activity has remained relatively stable since the late 1980s [8]. Furthermore, an upward trend in birth cohort body mass index throughout the 20th century has been observed, with a marked acceleration after World War II [6,9,10]. This suggests that the obesity epidemic

See Commentary, pg. 1022.

may have begun prior to the rise of the fast-food industry and the increase in sedentary occupations, with other factors beyond the modern environment likely being significant contributors to the obesity epidemic [11].

Evidence has indicated that low socioeconomic status and elevated levels of adversity are closely linked to obesity [12,13]. These conditions often correlate with limited access to safe and nutritious food, referred to in the literature as food insecurity [14]. Today, food insecurity is estimated to affect 2.4 billion people worldwide [15] and is traditionally associated with extreme events such as wars, famines, and epidemics that disrupt food supplies [16]. Historical examples such as the Dutch famine during World War II have demonstrated long-term impacts on metabolic health, linking early-life food shortages to increased rates of obesity in subsequent generations [17]. This is consistent with research highlighting higher obesity rates among low-income, food-insecure populations, particularly women [18,19].

These observations suggest that food insecurity may drive obesity through mechanisms that have evolved to conserve energy during periods of scarcity [14, 20], perhaps especially important in female individuals to guarantee reproductive capacity [21]. In modern contexts, this biological response could increase the risk of obesity among food-insecure individuals. Supporting this, controlled studies have demonstrated that low socioeconomic status and food insecurity can lead to increased food intake and greater adiposity [22]. Also, in animals, unpredictable access to food can stimulate biological responses, resulting in weight gain [23, 24]. Even mild caloric restriction in mice has been associated with increased fat storage [25]. However, the biological mechanisms driving adiposity related to food insecurity remain poorly understood. In order to investigate this phenomenon and elucidate the associated molecular underpinnings, a model that replicates the key aspects of food insecurity, independent of socioeconomic context, is warranted [26].

Herein, we present the development of a novel mouse model to study food insecurity and explore its effects on body weight, body composition, and molecular processes in adipose tissue. Our findings reveal a robust association between an unpredictable food environment and increased adiposity, linking food insecurity to fat accumulation. Accompanying these changes, we observed alterations in the adipose tissue transcriptome, which could underpin the observed shifts in body composition. This study lays the groundwork for understanding the biological mechanisms by which food insecurity promotes adiposity.

Study Importance

What is already known?

- Food insecurity is a condition in which individuals or households lack consistent access to sufficient nutritious, safe, and affordable food.
- Food insecurity is associated with obesity, particularly in women in high-income countries.
- The link between food insecurity and obesity may reflect mechanisms evolved to store fat as a safeguard against future starvation in unpredictable food environments, known as the insurance hypothesis.

What does this study add?

- We have developed a new mouse model to study food insecurity and the physiological, behavioral, and molecular underpinnings.
- Food insecurity in mice increases fat mass and decreases lean mass, indicating adaptive mechanisms for energy storage during perceived scarcity.
- Transcriptomic analysis of white adipose tissue from food-insecure mice revealed upregulation of metabolic pathways associated with fat mass expansion and downregulation of immune response-related transcripts.

How might these results change the direction of research or the focus of clinical practice?

- Our findings highlight the role of food insecurity in driving metabolic adaptations that promote fat storage. As obesity rises among low-income populations with limited access to nutritious food, it is important to better understand the relationship between food insecurity and increased fat mass. This insight could inform targeted interventions to address the disproportionate incidence of obesity in socioeconomically disadvantaged groups.

METHODS

Animals

Ten-week-old C57BL/6J mice (male and female), unless stated otherwise, were obtained from commercial breeders (Janvier Labs) and housed in ventilated cages at constant temperature (22° ± 1°C) with moderate humidity (50% ± 5%). One week before experimental intervention, mice were acclimatized to single housing, unless stated differently. All experiments were done at 22°C with a 12-h light–dark cycle from 6:00 a.m. to 6:00 p.m. Mice had ad libitum access to water and chow (SAFE D30 diet; 3.389 kcal/g: 14.1% fat, 60% carbohydrate, and 26% protein) or, when indicated, a high-fat, high-sucrose diet (HFD) (D12331; 5.56 kcal/g: 58% fat, 25.5% carbohydrate, and 16.4% protein; Research Diets, Inc.), with an exception for the calorie restriction periods. All experiments were approved by the Danish Animal Experimentation Inspectorate (2018-15-0201-01457 and 2023-15-0201-01442) and were performed following Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines [27].

Food insecurity studies

C57BL/6J mice were single- or double-housed, and body weight and food intake were monitored for 1 week before mice were randomized to experimental groups, assuring an identical baseline mean body weight and food intake among groups, unless stated differently. The control group was given ad libitum access to food throughout the protocol. Experimental groups were given different access to food (see online Supporting information for each specific study). When food restriction was required, the food amount was adjusted daily by cutting the food pellets to an appropriate size until reaching the desired weight and provided immediately before the dark phase. During food intake measurements, small food pellets were removed from the grid to avoid food spillage into the cage bedding. Twenty-four-hour fasting periods were achieved by removing all food pellets from the food grid.

Metabolic phenotyping and indirect calorimetry

Single-housed male C57BL/6J mice were acclimatized to metabolic cages (16-channel Promethion, Sable Systems International) for 1 week before the start of the study. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio (RER), energy expenditure (kilocalories per hour), and locomotor activity (beam breaks per hour) were recorded and collected in 15-min bins. Water and chow food were available ad libitum throughout the experiment. Raw data for each mouse were analyzed using the online tool CalR (CalR, version1.3, https://calrapp.org/) and visualized using GraphPad Prism version 10 (GraphPad Software).

Body composition measurements

Body composition (fat and lean mass) was measured by magnetic resonance imaging (MRI) using the minispec LF90 Body Composition Analyzer II (Bruker Corp.). Body weight was measured on a precision scale before the procedure.

Quantitative polymerase chain reaction

Gene expression analysis in the hypothalamus and adrenal glands was performed in control and food insecurity groups at the end of the experiment (4 weeks; $n = 10$). Tissues were quickly dissected in the morning without fasting the animals, flash-frozen on dry ice, and stored at −80°C until analysis. Total RNA was isolated from tissues with phenol/chloroform extraction using QIAzol reagent (QIAGEN) and RNeasy Lipid Tissue Mini Kit (QIAGEN) following the instructions provided by the manufacturer. After extraction, RNA concentration and purity were measured using a NanoDrop 2000 (Thermo Fisher Scientific). A total of 500 ng of RNA was converted into complementary DNA using Superscript III (Thermo Fisher Scientific), following the instructions from the manufacturer. Quantitative polymerase chain reaction (qPCR) was performed using PrecisionPLUS qPCR Master Mix containing SYBR green (Primerdesign; primer expression in online Supporting information). Quantification of mRNA expression was performed according to the delta–delta Ct method and normalized to the housekeeping gene *Hprt* and the control group.

Plasma profiling

Blood was collected from the tail vein or during decapitation and centrifuged at 4°C and 4000 rpm to obtain plasma. Mouse plasma samples were analyzed using enzyme-linked immunosorbent assay (ELISA) to measure leptin (Mouse/Rat Leptin ELISA Kit, #MOB00B, R&D Systems), total ghrelin (Rat/Mouse Ghrelin ELISA Kit, #EZRGRT-91 K, MilliporeSigma), insulin (Ultra-Sensitive Mouse Insulin ELISA Kit, #90080, Crystal Chem Inc.), and corticosterone (DetectX Corticosterone Multi-Format ELISA Kit, #K014-H, Arbor Assays) using manufacturer instructions. Plasma was diluted 1:20 for leptin measurements, 1:2 for total ghrelin measurements, and 1:100 for corticosterone measurements. Plasma glucose was measured using a glucometer. Plasma total cholesterol (LiquiColor Enzymatic Cholesterol Test, #1010-225, Stanbio Laboratory), total glycerol (triglycerides; Triglyceride LiquiColor Test [Enzymatic], #2100-225, Stanbio Laboratory), and nonesterified fatty acids (NEFA-HR [2], #434-91795, #436-91995, #27077000, Fujifilm Wako Chemicals Europe) concentrations were measured using commercially available kits.

RNA sequencing and data analysis

Mice were quickly sacrificed by decapitation, and epididymal white adipose tissue (eWAT) was collected in Eppendorf tubes and kept on dry ice until stored at −80°C. Total RNA was prepared from frozen eWAT of the two experimental groups ($n = 10$ control, $n = 10$ food insecurity) using the RNeasy Micro Kit (QIAGEN) according to the manufacturer's instructions, after tissue disruption in QIAzol reagent. RNA concentration was measured using NanoDrop One (Thermo Fisher Scientific). Bulk RNA sequencing was performed by the Single-Cell Omics platform at the Novo Nordisk Foundation Center for Basic Metabolic Research (online Supporting information). Plots were generated using R Statistical Software (version 4.2.2, R Project for Statistical Computing).

Statistical analysis

Figures were generated and statistical analyses were conducted using GraphPad Prism version 10 (GraphPad Software). Statistical significance was tested by two-way ANOVA corrected for multiple comparisons with Bonferroni test, when appropriate, and a two-tailed $p < 0.05$ was used to indicate statistical significance (*<0.05, **<0.01, ***<0.001, and ****<0.0001 in the figures). Data are expressed as mean ± SEM.

Code availability

The source code used to generate the RNA sequencing plots is available at the following link: https://github.com/claudiagil108/in-food.git

RESULTS

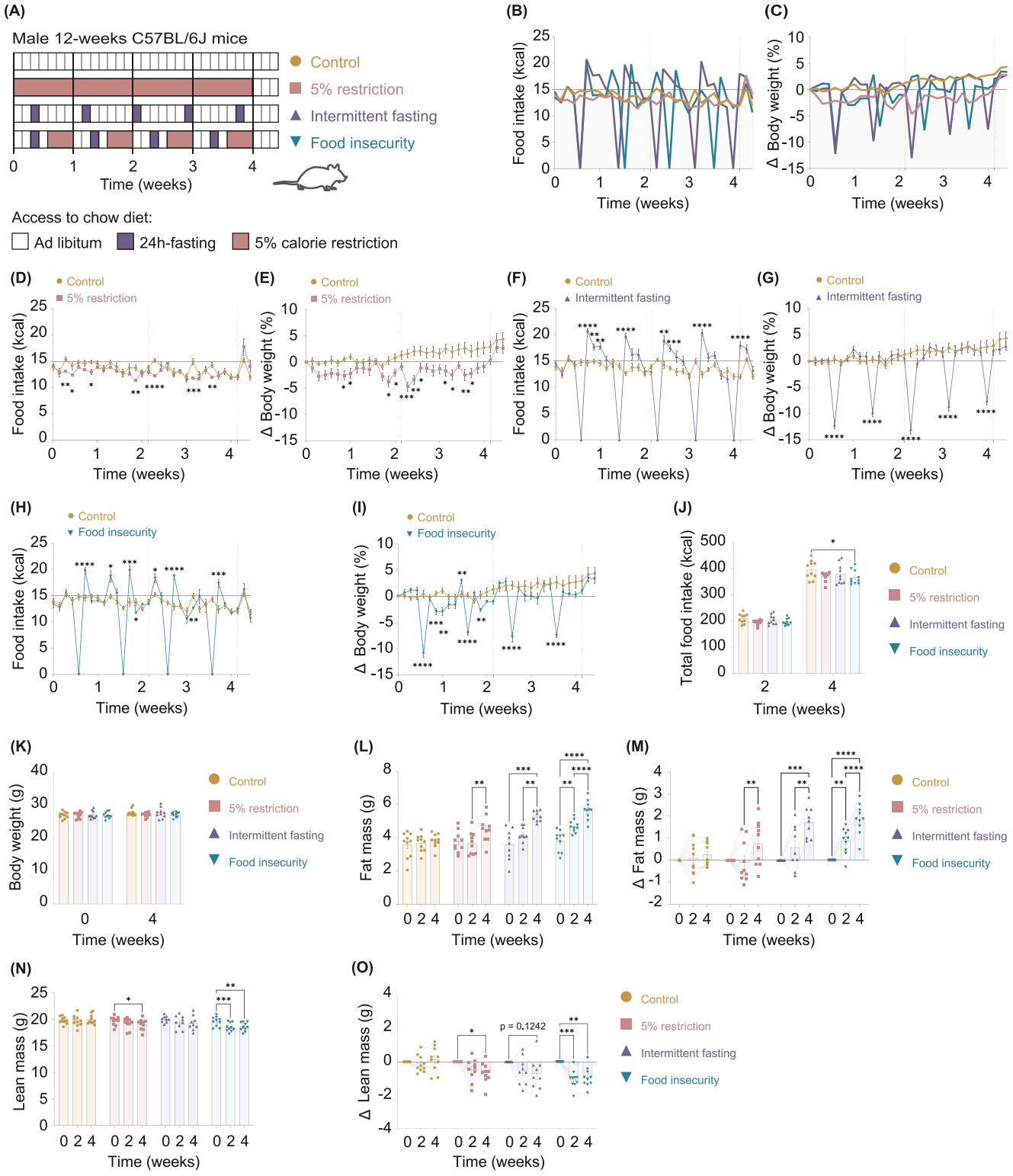
Food insecurity increases fat mass and decreases lean mass in male mice

In order to evaluate feeding interventions mimicking food insecurity, we subjected single-housed, 12-week-old, chow-fed male mice (C57BL/6J) to one of four experimental conditions over a period of 4 weeks: 1) chow diet given ad libitum (control group); 2) chow diet with 5% calorie restriction (5% restriction group); 3) chow diet given ad libitum but with 1 day of fasting per 6 days of protocol (intermittent fasting group); and 4) chow diet given ad libitum but with a weekly combination of 1 day of fasting followed by 1 day of ad libitum refeeding and, subsequently, 3 consecutive days of 5% calorie restriction (food insecurity group; Figure 1A). Food intake was assessed daily in all four groups and reflects the changes in food availability, remaining relatively stable in groups with predictable access to food (control and 5% restriction groups; Figure 1B). Body weight was also monitored daily in all four groups and exhibited dynamic changes in response to food availability, decreasing during periods of food restriction (up to 13% weight loss in response to fasting days) and recovering when food was reintroduced (up to 3% weight gain after fasting days; Figure 1C). A compensatory increase in food intake was observed in the days following the 24-h fasting, with mice eating up to 40% more than usual (Figure 1B). These results underscore the homeostatic mechanisms regulating feeding to preserve body weight in response to fluctuating food availability.

The 5% restriction group exhibited a reduced food intake compared to the control group (Figure 1D). This subtle, but chronic, calorie restriction resulted in a 3% weight loss during the first week of the intervention (Figure 1E). For the 5% restriction group, body weight remained lower than baseline until the end of the intervention (Figure 1E). The intermittent fasting group experienced substantial fluctuations in food intake due to the 24-h fasting periods (Figure 1F), leading to a body weight loss of up to 13% compared to baseline (Figure 1G). Following fasting, body weight recovered rapidly (Figure 1G) due to an increase in food intake that peaked the first day after fasting by being up to 38% higher compared to baseline (Figure 1F). These changes in body weight and the compensatory hyperphagic response to 24-h fasting are similar to what has been previously observed in mice [28]. The food insecurity group displayed similar patterns of food intake fluctuations as the intermittent fasting group, together with slightly lower food intake than the control group during the 5% calorie restriction periods (Figure 1H). These dynamic changes in food intake led to a weight loss of up to 11% during fasting periods (Figure 1I). At the end of the 4-week intervention, total food intake was significantly reduced in the food insecurity group (7.5% less than controls) and also numerically lower than the 5% restriction and the intermittent fasting groups (6.4% and 4.2% lower than controls, respectively; Figure 1J). Despite this, final body weights were similar across all four groups (Figure 1K).

In contrast to the stable total body mass, remarkable changes in body composition were observed. All three intervention groups exhibited significant increases in fat mass compared to the control group, which maintained the same level of adiposity throughout the protocol (Figure 1L,M and Figure S1A,B). The food insecurity group displayed the most striking effect with a 49% increase in absolute fat mass (Figure 1L,M and Figure S1A) and a shift in body fat percentage from 14% at baseline to 21% at week 4 (Figure S1B). Food insecurity also resulted in a marked reduction in absolute lean mass (5% less than baseline; Figure 1N,O and Figure S1C), and both changes in absolute fat and lean mass differed significantly from the control group after 2 and 4 weeks (Figure S1A,C). Whereas both the 5% restriction and intermittent fasting groups increased their absolute fat mass after 4 weeks by 20% and 45%, respectively (Figure 1L,M), only the latter showed a significant difference in absolute fat mass from the control group at week 4 (Figure S1A). The 5% restriction group exhibited a mild reduction in absolute lean mass (3.4% less at week 4 than at baseline; Figure 1N,O) that was not significantly different from the control group at week 4 (Figure S1C). The intermittent fasting group displayed a numerical but nonstatistical reduction in absolute lean mass (Figure 1N,O) and a significant reduction in lean mass percentage (Figure S1D). In summary, the food insecurity intervention induced a dramatic change in body composition, characterized by increased fat mass and decreased lean mass. Interestingly, the increased adiposity in food-insecure mice occurred despite a lower total energy intake. In order to investigate whether the food insecurity paradigm impacts whole-body energy expenditure and substrate metabolism, we subjected mice to either food insecurity or control conditions while monitoring them in metabolic cages (Figure S2). As expected, a decrease in energy expenditure was observed during the fasting phase of the protocol. However, during the subsequent 5% calorie restriction period, we observed a counterintuitive increase in energy expenditure (Figure S2G,H). Total energy expenditure over the 4 weeks was not changed by food insecurity (Figure S2I,J). The RER decreased during fasting compared to the control group. During refeeding, RER was elevated and remained elevated throughout the 5% calorie restriction period until the next fasting phase (Figure S2K,L). Although the animals appeared to exhibit increased locomotion during the very first fasting bout, there was no change in total locomotor activity or its pattern over the 4-week experiment (Figure S2M–P). In order to evaluate whether food insecurity impacts markers of metabolic health or hormones involved in glucose and energy homeostasis, we measured circulating levels of leptin, ghrelin, glucose, insulin, cholesterol, triglycerides, nonesterified fatty acids, and corticosterone after 4 weeks (Figure S3A–H). Except for slight decreases in plasma glucose and triglycerides, food insecurity had no significant effect on any of these markers. Food insecurity led to increased expression of *Crh*,

Crhr1, and *Igf1* in the hypothalamus, suggesting alterations in stress response and potentially growth signaling pathways. In order to further explore the hypothalamus-pituitary–adrenal (HPA) axis, we measured genes associated with stress and energy homeostasis and found increased expression of *Cyp11a1*, *Cyp11b2*, and *Cyp21a1* in the adrenal glands, indicating enhanced steroidogenesis, particularly in glucocorticoid and mineralocorticoid production. Together, these findings suggest that food insecurity activates both hypothalamic and adrenal pathways involved in stress and metabolic regulation (Figure S3I,J).



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Importantly, the food insecurity protocol was replicated four times, consistently resulting in increased fat mass and, in three out of the six experiments, a statistically significant decrease in lean mass (Figure S3K). Owing to the unpredictable nature of food availability in the food insecurity group, and the impact on body composition that aligns with prior work in other species [29,30], we decided to continue with this protocol for our subsequent mouse studies.

Food insecurity induces fat accumulation in female mice

In order to evaluate the impact of the food insecurity paradigm on female body weight and body composition, we subjected female mice to a similar feeding protocol as male mice, comparing a food insecurity group against a control group (Figure 2A). Similar to male mice, female mice experienced large fluctuations in body weight and food intake in response to fasting and refeeding cycles (Figure 2B,C), with a body weight loss up to 9% on fasting days (Figure 2D). Food insecurity in female mice also gave rise to pronounced hyperphagia on the refeeding days, with mice eating up to 64% more than baseline (Figure 2C). However, unlike male mice, the body weight of food-insecure female mice "overshot" during the refeeding period after the fasting days, surpassing the stable body weight level of control mice by up to 3.3% (Figure 2D). Despite these fluctuations in food intake and body weight, total food intake and final body weight were similar between the food insecurity and the control groups (Figure 2E,F).

The food insecurity group displayed a 26% increase in absolute fat mass (Figure 2G,H and Figure S4A) and a change in body fat percentage from 16% at baseline to 20% at week 4 (Figure S4B). Moreover, a 6% decrease in absolute lean mass was observed in the food-insecure female mice (Figure 2I,J and Figure S4C). The control group also showed subtle changes in body composition, including a transient increase in fat mass after 2 weeks (Figure 2G,H and Figure S4B) and a decrease in lean mass by the end of the protocol (Figure 2I,J and Figure S4C,D), but these effects were less pronounced compared to the food insecurity group. Of note, the changes in body composition of the double-housed female mice from this experiment were replicated in single-housed female mice (Figure S3K).

Food insecurity does not exacerbate fat accumulation but reduces lean mass in male mice fed an HFD

In order to explore the interaction between food insecurity and HFD-induced obesity, we subjected HFD-fed male mice to our 4-week food insecurity protocol, comparing an HFD-food insecurity group to an HFD-control group (Figure 3A). Similar to chow-fed mice, HFD-fed mice exhibited pronounced changes in food intake and body weight in response to fasting and refeeding cycles (Figure 3B). HFD-fed animals also exhibited compensatory hyperphagia following fasting, with a food intake up to 55% higher than baseline (Figure 3C) that enabled body weight regain (Figure 3D). Despite a slight reduction in total food consumption in the HFD-food insecurity group (5% lower than the HFD-control group; Figure 3E), there was no significant difference in body weight between the groups after the 4-week intervention (Figure 3F). Both the control group and the group exposed to food insecurity gained fat mass during the intervention, but with no difference between the groups (Figure 3G,H and Figure S5A,B). Interestingly, despite the pronounced positive energy balance, the food insecurity group exhibited a significant reduction in absolute lean mass in response to the intervention, which was not observed in the control group (Figure 3I,J and Figure S5C,D). No differences in plasma corticosterone levels were detected (Figure S5E).

In order to determine whether exposure to food insecurity potentiates a subsequent susceptibility to diet-induced obesity, male chow-fed mice were subjected to the 4-week food insecurity or control protocol, followed by a 3-week recovery period with ad libitum chow diet and, subsequently, 3 weeks of exposure to ad libitum HFD (Figure S6A). No significant differences in food intake or body weight were observed between groups during the HFD feeding period (Figure S6B–F). After the HFD feeding period, both groups exhibited a similar increase in fat mass (Figure S6G–I), whereas the food insecurity group displayed a more pronounced increase in absolute lean mass (Figure S6J–L). In order to further investigate the potential interaction between food insecurity and diet-induced obesity, female mice were subjected to a similar experimental paradigm of food insecurity followed by HFD feeding, albeit with only a 3-day recovery period between the food insecurity paradigm and the exposure to HFD (Figure S7A). No differences in HFD intake, body weight gain, or body composition were observed between the food insecurity group and

FIGURE 1 A model of food insecurity in mice. (A) Schematic representation of the 4-week food insecurity protocol in single-housed chow-fed male mice ($n = 10$ per group). (B) Daily food intake and (C) body weight change of all the groups. The horizontal gray line in panel B represents the baseline food intake from the pre-experiment week. (D) Daily food intake and (E) body weight change of control and 5% restriction groups. The horizontal gray line in panel D represents the baseline food intake from the pre-experiment week. (F) Daily food intake (kilocalories) and (G) body weight change of control and intermittent fasting groups. In panel F, y values $= 0$ are $p < 0.0001$, and the horizontal gray line represents the baseline food intake from the pre-experiment week. (H) Daily food intake and (I) body weight change of control and food insecurity groups. In panel H, y values $= 0$ are $p < 0.0001$, and the horizontal gray line represents the baseline food intake from the pre-experiment week. (J) Total food intake and (K) final body weight of all the groups. (L) Absolute fat mass, (M) fat mass change, (N) absolute lean mass, and (O) lean mass change of all the groups at baseline and after 2 and 4 weeks of the protocol. In panels M and O, the gray lines represent paired values for individual animals. One mouse was removed from the intermittent fasting group body composition data (panels L, M, N and O) due to a malfunctioning MRI reading in week 2. In panels B through I, the dashed vertical lines represent body composition measurements. Differences among groups (D–K) and within groups (L–O) are indicated with asterisks after two-way ANOVA (group × time): p values, *<0.05, **<0.01, ***<0.001, and ****< 0.0001. Data are shown as mean ± SEM. [Color figure can be viewed at wileyonlinelibrary.com]

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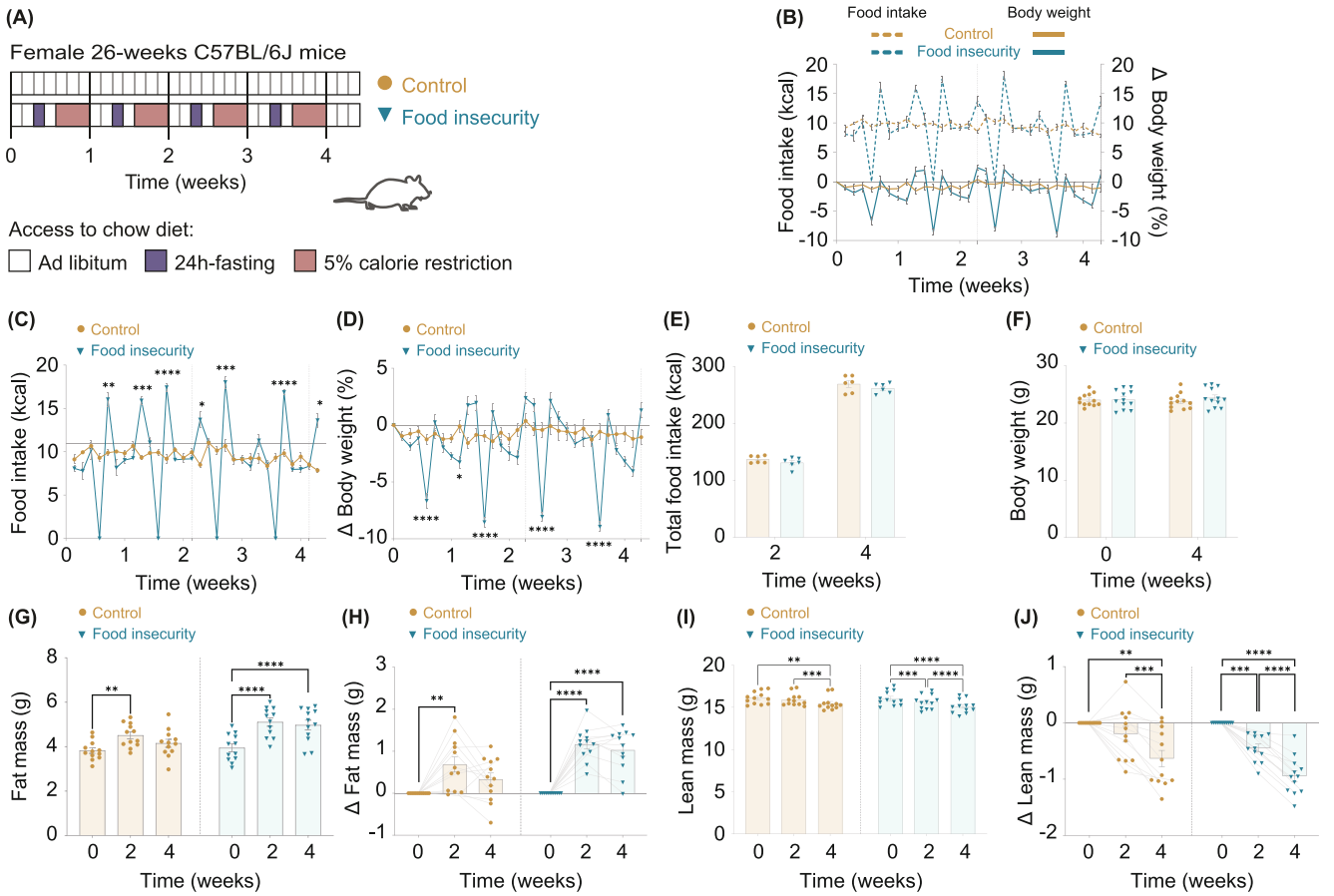

FIGURE 2 Female mice exposed to food insecurity. (A) Schematic representation of the 4-week food insecurity protocol in double-housed chow-fed female mice ($n = 12$ per group). (B,C) Daily food intake and (B,D) body weight change of control and food insecurity groups. In panel C, y values $= 0$ are $p < 0.0001$, and the horizontal gray line represents the baseline food intake from the pre-experiment week. (E) Total food intake and (F) final body weight of control and food insecurity groups. (G) Absolute fat mass, (H) fat mass change, (I) absolute lean mass, and (J) lean mass change of control and food insecurity groups at baseline and after 2 and 4 weeks of the protocol. In panels H and J, the gray lines represent paired values for individual animals. In panels B through D, the dashed vertical lines represent body composition measurements. Differences among groups (C–F) and within groups (G–J) are indicated with asterisks after two-way ANOVA (group × time): p values, *<0.05, **<0.01, ***<0.001, and ****<0.0001. Data are shown as mean ± SEM. [Color figure can be viewed at wileyonlinelibrary.com]

the control group (Figure S7B–L). These findings suggest that 4 weeks of food insecurity do not affect subsequent susceptibility to short-term HFD-induced weight gain in male or female mice.

Effects of food insecurity on WAT transcriptome

In order to gain a deeper understanding of the molecular mechanisms driving the increased fat mass associated with food insecurity, we performed RNA sequencing on eWAT from control and food-insecure male mice (Figure 1A). Comparative analysis revealed a substantial transcriptional remodeling with 2101 differentially expressed genes (false discovery rate < 0.05 and |logFC| > 0.5), of which 1062 were downregulated and 1039 were upregulated in the food insecurity group (Figure 4A and Figure S8). Pathway enrichment analyses identified multiple pathways linked to immune and inflammatory responses (Figure S8B,C) such as defense response, adaptive immune response, and inflammatory response, among the most downregulated biological pathways (Figure 4B and Figure S8A). Conversely, metabolic pathways such as oxidative phosphorylation, fatty acid metabolic processes, and adipogenesis were significantly upregulated (Figure 4C and Figure S8A). Notably, genes involved in lipogenesis (*Elovl6* and *Cidea*) and the circadian clock (*Ciart*) were among the top upregulated genes (Figure 4D), whereas immune and inflammatory response genes (*Camp*, *Cd7*, *Ccl17*, *C7*, and *Rgs1*) were enriched in the most downregulated genes (Figure 4E). These transcriptional adaptations suggest that WAT undergoes metabolic and immune reprogramming in response to food insecurity, potentially contributing to increased fat accumulation.

DISCUSSION

The paradoxical link between food insecurity and obesity may reflect mechanisms evolved to store fat as a safeguard against future starvation

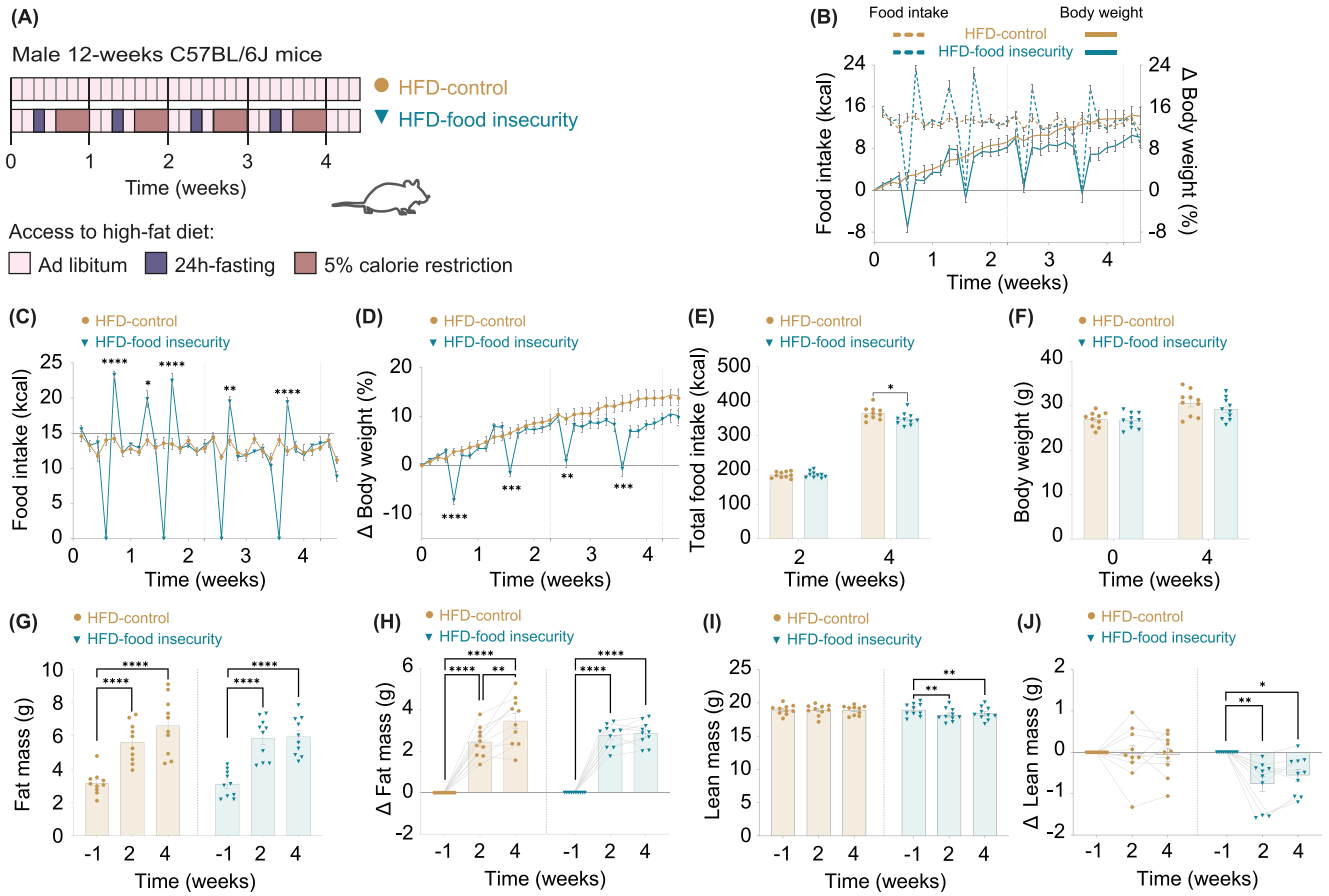

FIGURE 3 HFD-fed mice exposed to food insecurity. (A) Schematic representation of the 4-week food insecurity protocol in single-housed HFD-fed male mice ($n = 10$ per group). (B,C) Daily food intake and (B,D) body weight change of HFD-control and HFD-food insecurity groups. In panel C, y values $= 0$ are $p < 0.0001$, and the horizontal gray line represents the baseline food intake from the pre-experiment week. (E) Total food intake and (F) final body weight of HFD-control and HFD-food insecurity groups. (G) Absolute fat mass, (H) fat mass change, (I) absolute lean mass, and (J) lean mass change of HFD-control and HFD-food insecurity groups at baseline (1 week before week 0) and after 2 and 4 weeks of the protocol. The gray lines in panels H and J represent paired values for individual animals. In panels B through D, the dashed vertical lines represent body composition measurements. Differences among groups (C–F) and within groups (G–J) are indicated with asterisks after two-way ANOVA: p values, *<0.05, **<0.01, ***<0.001, and ****<0.0001. Data are shown as mean ± SEM. HFD, high-fat diet. [Color figure can be viewed at wileyonlinelibrary.com]

in unpredictable food environments, a concept known as the insurance hypothesis [14]. In environments where food access is sporadic, adaptive strategies prioritizing fat accumulation to enhance survival chances might be advantageous even if it results in increased adiposity when food becomes more abundant. However, in modern obesogenic environments, this adaptive phenotype may contribute to the progression of obesity and its many comorbidities. Our understanding of this phenomenon and its underlying biological processes remains limited [31].

In order to investigate this, we developed a new mouse model of food insecurity and demonstrated that mice exposed to a food-insecure environment for 4 weeks exhibit increased fat mass and decreased lean mass compared to control animals with ad libitum access to food. This effect of food insecurity on body composition aligns with previous animal and human studies, which have demonstrated increased adiposity without changes in food intake or body weight [20].

A key finding of this study is the observed increase in adiposity in response to the food insecurity paradigm. Notably, we also found that intermittent fasting alone promoted an increase in adiposity. Although intermittent fasting is typically regarded as beneficial for metabolic health [32], an increase in adiposity has previously been observed in female mice subjected to a 2-week intermittent fasting intervention [28]. Accordingly, in our model, the recurring 24-h fasting bouts might partly drive the food insecurity-related adiposity. Interestingly, we also observed a similar, although less pronounced, increase in fat and decrease in lean mass when mice were subjected to a subtle, but chronic, 5% energy restriction. This effect aligns with previous research by Li et al., who reported a 69% increase in fat mass in female mice after 3 weeks of a continued 5% calorie-restricted diet and a 44% increase when exposed for 4 weeks, whereas lean mass decreased by 12% and 16%, respectively [25]. Even a 20% calorie restriction has been reported to increase adiposity in female mice chronically exposed to food restriction for more than 6 months [33]. In another study, male mice subjected to a 20% calorie restriction increased their fat mass by 59% over a 7-week intervention, which

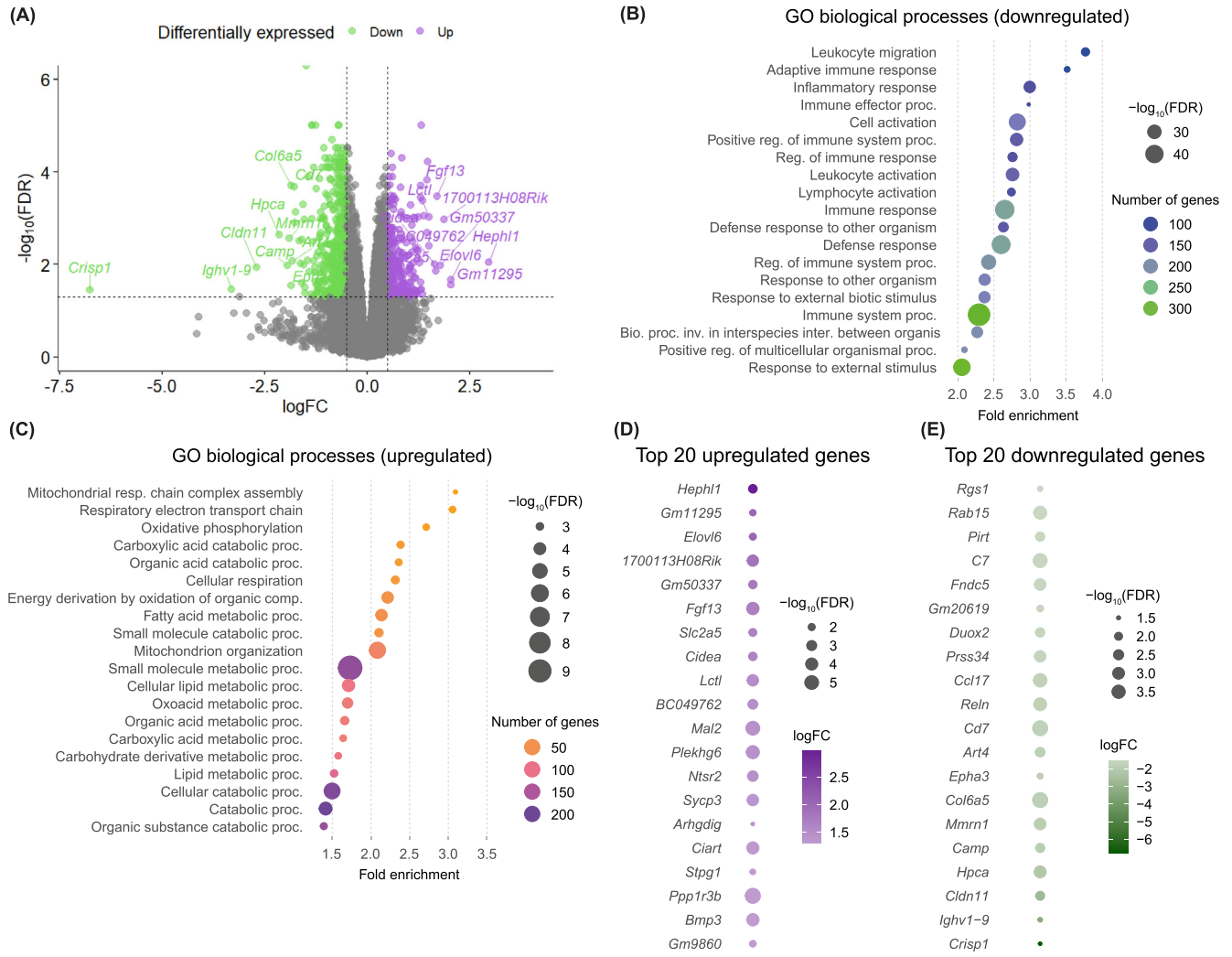

FIGURE 4 Impact of food insecurity on the adipose tissue transcriptome. (A) Volcano plot representing DEGs in eWAT (food insecurity vs. control) at day 31 of the protocol. DEGs after |logFC| > 0.5 and FDR > 0.05 cutoffs are highlighted in green (downregulated genes, "Down") and purple (upregulated genes, "Up"). Genes that did not pass those cutoffs are colored in gray. Labeled are the top 10 down- and upregulated genes based on logFC. (B) Top 20 downregulated and (C) upregulated GO terms (biological processes) from eWAT RNA sequencing data (food insecurity vs. control). (D) Top 20 upregulated and (E) downregulated genes with respective logFC (represented by color intensity) and $-\log_{10}$(FDR) value (represented by dot size). Statistical analysis is described in the *Methods* section. DEG, differentially expressed gene; eWAT, epididymal white adipose tissue; FC, fold change; FDR, false discovery rate; GO, Gene Ontology. [Color figure can be viewed at wileyonlinelibrary.com]

was similar to a 62% increase observed in ad libitum-fed controls [34]. However, unlike the control group, the calorie-restricted mice did not experience an increase in lean mass [34]. Smith Jr. et al. observed a decrease in lean mass and, consequently, an increased fat mass percentage when HFD-fed mice were exposed to chronic or intermittent mild calorie restriction for 12 months [35]. Collectively, these results suggest that mild energy restriction in mice triggers physiological adaptations that prioritize fat storage over lean mass preservation, thereby elevating the overall energy reserves of the organism to increase the chance of survival in periods of insufficient food supply.

The mechanisms behind the observed changes in body composition are unclear, but a shift in fuel partitioning might play a role [36]. The role of fuel partitioning in fat mass accumulation is evident from earlier studies, which have shown that calorie restriction in some rodent models of obesity is linked to increased metabolic efficiency. In these studies, despite a negative energy balance, a larger fraction of ingested calories is stored as fat. Studies in classical genetic models of obesity such as ob/ob mice [37], db/db mice [38], *Mc4r*-knockout mice [39], and fa/fa rats [40], but also in ventromedial hypothalamus-lesioned rats [41], have reported an increase in fat mass in the context of calorie restriction. This might relate to a decrease in whole-body energy expenditure [42] but could also involve an increased macronutrient absorption from the gut [43] and/or decreased loss of glucose, amino acids, and other energy-providing metabolites via kidneys or other excretory routes [44]. Another explanation for the increase in fat mass might be a "collateral fattening" [45], driven as a response to the loss of lean mass.

The observed phenotypes resulting from the food insecurity intervention might be reflective of reduced energy expenditure, which may stem from loss of muscle mass, lowered body temperature, and/or decreased physical activity, factors previously associated with intermittent fasting in mice [28]. The contribution of decreased energy expenditure to increased adiposity was previously suggested by Myers et al., when food-insecure rats sustained more fat mass than food-secure rats during a 24-h fasting period that followed the intervention [24]. In a study by Wiersma and Verhulst, zebra finches exhibited a decrease in daily energy expenditure consequential to a reduction in food availability [46]. The decrease in energy expenditure could be linked to reduced energy allocation to secondary processes such as reproduction, somatic growth, and immunity [47]. Somatic growth impairments were previously seen together with an increase in fat mass in food-scarcity studies in birds [29] and in calorie-restricted db/db mice and ventromedial hypothalamus-lesioned rats [38,41], suggesting an increased partitioning of energy toward storage in adipose tissue. Immune system perturbations in food-insecure animals are supported by our transcriptomic assessments, showing suppressed expression of numerous immune system-related genes in adipose tissue in response to food insecurity. Immunity impairments were also observed in birds after 1 month of unpredictable food access, coinciding with an increased body and fat mass [30]. Although reduced immune system activity might help lower energy expenditure,

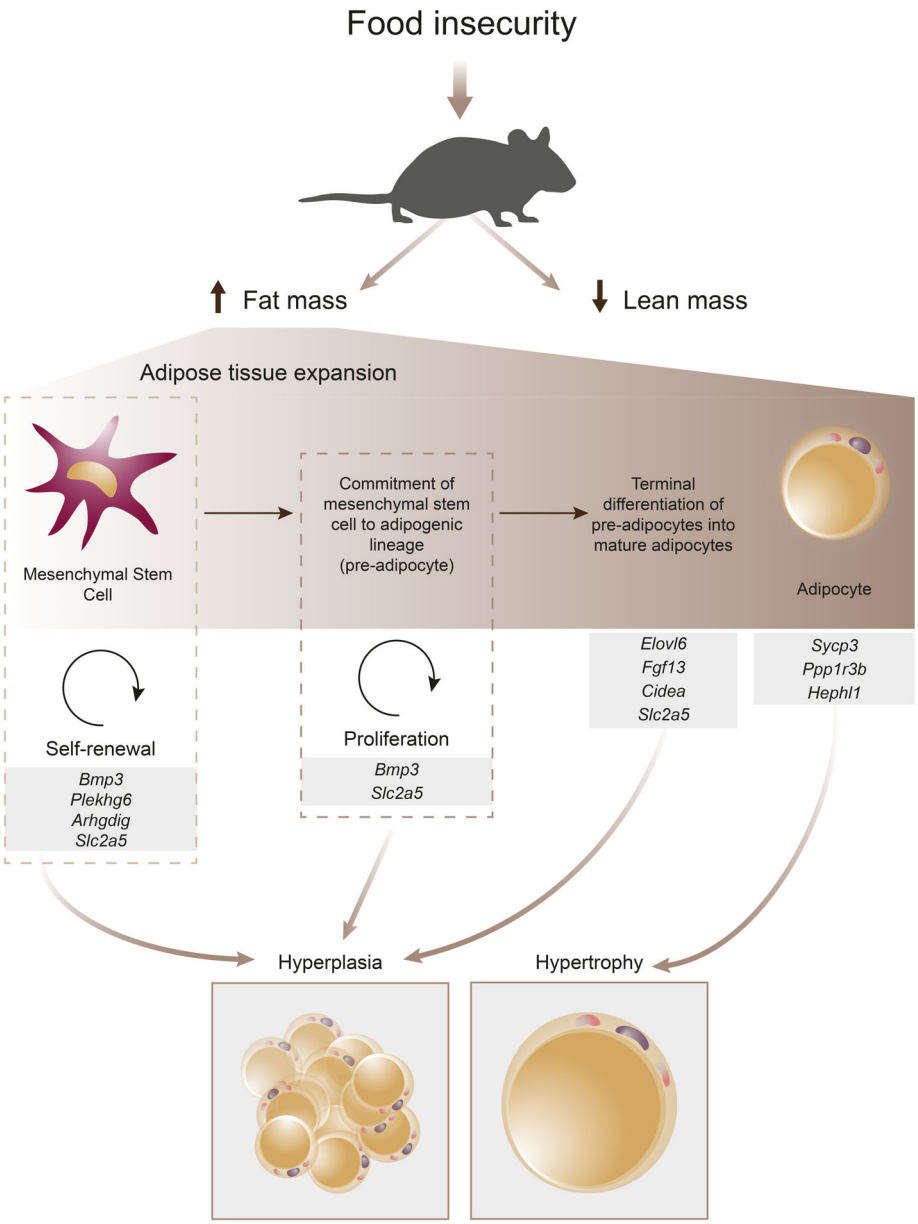

FIGURE 5 Food insecurity-driven adipose tissue expansion hypothesis. Hypothesis for the adipose tissue expansion in the context of food insecurity based on selected top upregulated genes in epididymal white adipose tissue of food-insecure male mice (Figure 4D; represented in gray boxes). [Color figure can be viewed at wileyonlinelibrary.com]

one might also speculate whether some of the downregulated immunological processes are required to accommodate the increase in adiposity seen in food-insecure mice.

The most upregulated transcripts in eWAT of food-insecure mice also highlight potential molecular mechanisms linked to food insecurity-driven adiposity. Adipose tissue growth occurs by expansion of individual adipocytes (hypertrophy) and/or by increasing the number of fat cells (hyperplasia) [48]. The latter involves differentiation from precursors, which comprises the commitment of mesenchymal stem cells to the adipogenic lineage and terminal differentiation of committed cells (preadipocytes) into mature fat cells (Figure 5) [49]. Several of the top upregulated hits (*Bmp3*, *Plekhg6*, *Arhgdig*, and *Slc2a5*) might trigger hyperplastic adiposity by promoting mesenchymal stem cells and preadipocytes proliferation directly or by enhancing cytokinesis (Figure 5) [50–54]. In addition to increasing the pool of precursor cells, food insecurity might also more directly stimulate adipocyte differentiation, as suggested by the increased expression of classical genes driving adipogenesis (*Elovl6*, *Cidea*, and *Fgf13*) [55–58] or the *Slc2a5* gene, which encodes the main fructose transporter and has been linked to obesity development (Figure 5) [59,60]. Food insecurity-related adipose growth might also be supported by alterations in angiogenesis, glycogen synthesis, and/or iron metabolism, as suggested by increased expression of *Sycp3*, *Ppp1r3b*, and *Hephl1* in our food-insecure mice (Figure 5) [58,61,62].

Unpredictable access to food has previously been associated with stress [63] and an amplified propensity for fat storage in birds [29,30] and rodents [24,64]. Stress, however, is a complex and multifaceted physiological phenomenon that can have divergent effects on feeding behavior and metabolism. This complexity is further underscored by the difficulty of modulating stress in rodents to accurately replicate human stress conditions [65,66]. Nevertheless, some rodent studies have pointed to direct links between stress and adiposity [67–69]. An increase in adiposity was observed in HFD-fed rodents exposed to several stressors, including single housing [67], another (i.e., unfamiliar or aggressive) mouse [67], and cold exposure [68]. In chow-fed mice, social crowding (eight vs. four mice per cage) also led to increased adiposity, characterized by larger fat depots and increased adipocyte size, despite no changes in overall body weight [70]. Moreover, stress has been linked to food insecurity in humans [71–73], potentially reflecting the unpredictability of food access and a lack of control over daily circumstances. Further translational research is needed to clarify the causal relationships among stress, food insecurity, and obesity. In context, it is worth noting that distinguishing preclinical models of food insecurity from models of binge eating can be challenging due to overlapping characteristics. Similarly, intermittent fasting protocols share many similarities with these interventions, such as intermittent food access, stress-related phenotypes, and rapid overconsumption following restriction. However, binge eating models typically involve limited access to highly palatable foods, which leads to a loss of control when food is freely available, and do not generally include caloric restriction. Instead, they often feature excessive consumption of palatable foods that surpasses homeostatic energy needs [74]. Future studies should also explore the potential disruption of circadian rhythms in our food insecurity paradigm, as well as the subtle cold stress from conducting experiments below the thermoneutral zone, both of which may influence the findings.

In conclusion, we have developed a mouse model simulating food insecurity, characterized by intermittent food restriction and unpredictable access, that reveals key parallels with human food insecurity, notably increased adiposity. Our findings indicate that food insecurity is associated with alterations in adipose tissue gene expression related to adipogenesis and immune function. Although these adaptive mechanisms may have conferred evolutionary benefits by promoting energy storage in adipose tissue during periods of food scarcity, they may inadvertently contribute to the current rise in obesity rates. As obesity continues to escalate among low-income populations with limited access to nutritious foods, it is crucial to unravel the intricate relationship between food insecurity and increased fat mass. This understanding might provide new clues for developing targeted interventions to address the disproportionate incidence of obesity in socioeconomically disadvantaged populations.

AUTHOR CONTRIBUTIONS

Cláudia R. E. Gil and Christoffer Clemmensen designed the project with input from Jens Lund. Cláudia R. E. Gil conducted the animal studies. Cláudia R. E. Gil analyzed the data. Jens Lund, Jan J. Żylicz, Pablo Ranea-Robles, Thorkild I. A. Sørensen, and Christoffer Clemmensen provided input to the studies. Cláudia R. E. Gil, Jens Lund, Pablo Ranea-Robles, and Christoffer Clemmensen wrote the manuscript. All authors interpreted data and reviewed and approved the final manuscript.

ACKNOWLEDGMENTS

We thank previous and current members of the Clemmensen Group for scientific discussions, as well as the staff at the Single-Cell Omics platform (SCOP) and the Rodent Metabolic Phenotyping platform (RMPP) at the Novo Nordisk Foundation Center for Basic Metabolic Research (CBMR) for technical expertise and support.

FUNDING INFORMATION

This work was supported by the Lundbeck Foundation (fellowship R238-2016-2859) and the Novo Nordisk Foundation (grant numbers NNF17OC0026114 and NNF22OC0073778). Cláudia R. E. Gil is supported by the Copenhagen Bioscience PhD Program (https://cphbiosciencephd.org/) funded by the Novo Nordisk Foundation (grant number NNF20SA0035586). Jens Lund is supported by the BRIDGE Translational Excellence Programme (www.bridge.ku.dk) funded by the Novo Nordisk Foundation (grant number NNF20SA0064340). The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW) is supported by the Novo Nordisk Foundation (grant number NNF21CC0073729). The Novo Nordisk Foundation Center for Basic Metabolic Research is an independent Research Center, based at the University of Copenhagen, Denmark, and is partially funded by an unconditional donation from the Novo Nordisk Foundation (www.cbmr.ku.dk; grant numbers NNF18CC0034900 and NNF23SA0084103).

CONFLICT OF INTEREST STATEMENT

Christoffer Clemmensen is a cofounder of Ousia Pharma, a biotech company developing therapeutics for the treatment of obesity. The other authors declared no conflicts of interest.

DATA AVAILABILITY STATEMENT

Epididymal white adipose tissue bulk RNA sequencing data generated in this study have been submitted for Gene Expression Omnibus (GEO) and are publicly available as of the date of publication under accession number GSE274149. All other data generated in this study are provided in online Supporting information. Please direct all data and material requests to Christoffer Clemmensen.

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REFERENCES

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. *Lancet*. 2024;403:1027-1050. doi:10.1016/S0140-6736(23)02750-2
2. Schwartz MW, Seeley RJ, Zeltser LM, et al. Obesity pathogenesis: an Endocrine Society scientific statement. *Endocr Rev*. 2017;38:267-296. doi:10.1210/er.2017-00111
3. Sørensen TIA. An adiposity force induces obesity in humans independently of a normal energy balance system-a thought experiment. *Philos Trans R Soc Lond B Biol Sci*. 2023;378:20220203. doi:10.1098/rstb.2022.0203
4. Speakman JR, Sørensen TIA, Hall KD, Allison DB. Unanswered questions about the causes of obesity. *Science*. 2023;381:944-946. doi:10.1126/science.adg2718
5. NCD Risk Factor Collaboration (NCD-RisC). Rising rural body-mass index is the main driver of the global obesity epidemic in adults. *Nature*. 2019;569:260-264. doi:10.1038/s41586-019-1171-x
6. Sonne-Holm S, Sorensen TI. Post-war course of the prevalence of extreme overweight among Danish young men. *J Chronic Dis*. 1977;30:351-358. doi:10.1016/0021-9681(77)90029-7
7. Mazidi M, Speakman JR. Higher densities of fast-food and full-service restaurants are not associated with obesity prevalence. *Am J Clin Nutr*. 2017;106:603-613. doi:10.3945/ajcn.116.151407
8. Speakman JR, de Jong JMA, Sinha S, et al. Total daily energy expenditure has declined over the past three decades due to declining basal expenditure, not reduced activity expenditure. *Nat Metab*. 2023;5:579-588. doi:10.1038/s42255-023-00782-2
9. Komlos J, Brabec M. The trend of BMI values of US adults by deciles, birth cohorts 1882-1986 stratified by gender and ethnicity. *Econ Hum Biol*. 2011;9:234-250. doi:10.1016/j.ehb.2011.03.005
10. Pedersen MM, Ekstrøm CT, Sørensen TIA. Emergence of the obesity epidemic preceding the presumed obesogenic transformation of the society. *Sci Adv*. 2023;9:eadg6237. doi:10.1126/sciadv.adg6237
11. Davis RAH, Plaisance EP, Allison DB. Complementary hypotheses on contributors to the obesity epidemic. *Obesity (Silver Spring)*. 2018;26:17-21. doi:10.1002/oby.22071
12. Dhurandhar EJ. The food-insecurity obesity paradox: a resource scarcity hypothesis. *Physiol Behav*. 2016;162:88-92. doi:10.1016/j.physbeh.2016.04.025
13. Hemmingsson E, Nowicka P, Ulijaszek S, Sørensen TIA. The social origins of obesity within and across generations. *Obesity Rev*. 2022;24(1):e13514. doi:10.1111/OBR.13514
14. Nettle D, Andrews C, Bateson M. Food insecurity as a driver of obesity in humans: the insurance hypothesis. *Behav Brain Sci*. 2017;40:e105. doi:10.1017/S0140525X16000947
15. FAO, IFAD, UNICEF, WFP and WHO. *The State of Food Security and Nutrition in the World 2023: Urbanization, Agrifood Systems Transformation and Healthy Diets Across the Rural–Urban Continuum*. FAO; 2023. doi:10.4060/cc3017en
16. Crandall AK, Madhudi N, Osborne B, Carter A, Williams AK, Temple JL. The effect of food insecurity and stress on delay discounting across families: a COVID-19 natural experiment. *BMC Public Health*. 2022;22:1576. doi:10.1186/s12889-022-13969-1
17. Ravelli G-P, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. *N Engl J Med*. 1976;295:349-353. doi:10.1056/NEJM197608122950701
18. Hernandez DC, Reesor LM, Murillo R. Food insecurity and adult overweight/obesity: gender and race/ethnic disparities. *Appetite*. 2017;117:373-378. doi:10.1016/j.appet.2017.07.010
19. Townsend MS, Peerson J, Love B, Achterberg C, Murphy SP. Food insecurity is positively related to overweight in women. *J Nutr*. 2001;131:1738-1745. doi:10.1093/jn/131.6.1738
20. Bateson M, Pepper GV. Food insecurity as a cause of adiposity: evolutionary and mechanistic hypotheses. *Philos Trans R Soc Lond B Biol Sci*. 2023;378:20220228. doi:10.1098/rstb.2022.0228
21. Power ML, Schulkin J. Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins. *Br J Nutr*. 2008;99:931-940. doi:10.1017/S0007114507853347
22. Cardel MI, Pavela G, Janicke D, et al. Experimentally manipulated low social status and food insecurity alter eating behavior among adolescents: a randomized controlled trial. *Obesity (Silver Spring)*. 2020;28:2010-2019. doi:10.1002/oby.23002
23. Lin WC, Liu C, Kosillo P, et al. Transient food insecurity during the juvenile-adolescent period affects adult weight, cognitive flexibility, and dopamine neurobiology. *Curr Biol*. 2022;32:3690-3703.e5. doi:10.1016/j.cub.2022.06.089
24. Myers KP, Majewski M, Schaefer D, Tierney A. Chronic experience with unpredictable food availability promotes food reward, overeating, and weight gain in a novel animal model of food insecurity. *Appetite*. 2022;176:106120. doi:10.1016/j.appet.2022.106120
25. Li X, Cope MB, Johnson MS, Smith DL, Nagy TR. Mild calorie restriction induces fat accumulation in female C57BL/6J mice. *Obesity (Silver Spring)*. 2010;18:456-462. doi:10.1038/oby.2009.312
26. Wilbrecht L, Lin WC, Callahan K, Bateson M, Myers K, Ross R. Experimental biology can inform our understanding of food insecurity. *J Exp Biol*. 2024;227(suppl 1):jeb246215. doi:10.1242/jeb.246215
27. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol*. 2020;pbio.3000410. doi:10.1371/journal.pbio.3000410
28. Zhang LN, Mitchell SE, Hambly C, Morgan DG, Clapham JC, Speakman JR. Physiological and behavioral responses to intermittent starvation in C57BL/6J mice. *Physiol Behav*. 2012;105:376-387. doi:10.1016/j.physbeh.2011.08.035
29. Andrews C, Zuidersma E, Verhulst S, Nettle D, Bateson M. Exposure to food insecurity increases energy storage and reduces somatic maintenance in European starlings (Sturnus vulgaris). *R Soc Open Sci*. 2021;8(9):211099. doi:10.1098/rsos.211099
30. Cornelius EA, Vézina F, Regimbald L, et al. Chickadees faced with unpredictable food increase fat reserves but certain components of

their immune function decline. *Physiol Biochem Zool*. 2017;90:190-200. doi:10.1086/689913

31. Cardel MI, Pavela G, Dhurandhar E, Allison DB. Future research directions for the insurance hypothesis regarding food insecurity and obesity. *Behav Brain Sci*. 2017;40:e110. doi:10.1017/S0140525X16001357
32. Ezpeleta M, Cienfuegos S, Lin S, et al. Time-restricted eating: watching the clock to treat obesity. *Cell Metab*. 2024;36:301-314. doi:10.1016/j.cmet.2023.12.004
33. Zhang G, Deighan A, Raj A, et al. Intermittent fasting and caloric restriction interact with genetics to shape physiological health in mice. *Genetics*. 2021;220:iyab157. doi:10.1101/2021.04.02.438251
34. Hambly C, Speakman JR. Contribution of different mechanisms to compensation for energy restriction in the mouse. *Obes Res*. 2005;13:1548-1557. doi:10.1038/oby.2005.190
35. Smith DL Jr, Yang Y, Mestre LM, et al. Impact of sustained calorie restriction and weight cycling on body composition in high-fat diet-fed male and female C57BL/6J mice. *Obesity (Silver Spring)*. 2024;32:959-968. doi:10.1002/oby.24015
36. Friedman MI, Sørensen TIA, Taubes G, Lund J, Ludwig DS. Trapped fat: obesity pathogenesis as an intrinsic disorder in metabolic fuel partitioning. *Obes Rev*. 2024;25:e13795. doi:10.1111/obr.13795
37. Coleman DL. Increased metabolic efficiency in obese mutant mice. *Int J Obes (Lond)*. 1985;9:69-73.
38. Cox JE, Powley TL. Development of obesity in diabetic mice pair-fed with lean siblings. *J Comp Physiol Psychol*. 1977;91:347-358. doi:10.1037/h0077322
39. Ste Marie L, Miura GI, Marsh DJ, Yagaloff K, Palmiter RD. A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. *Proc Natl Acad Sci U S A*. 2000;97:12339-12344. doi:10.1073/pnas.220409497
40. Zucker LM. Efficiency of energy utilization by the Zucker hereditarily obese rat 'fatty' (38569). *Proc Soc Exp Biol Med*. 1975;148:498-500. doi:10.3181/00379727-148-38569
41. Cox JE, Powley TL. Intragastric pair feeding fails to prevent VMH obesity or hyperinsulinemia. *Am J Physiol*. 1981;240:E566-E572. doi:10.1152/ajpendo.1981.240.5.E566
42. Trayhurn P, Arch JRS. Is energy expenditure reduced in obese mice with mutations in the leptin/leptin receptor genes? *J Nutr Sci*. 2020;9:e23. doi:10.1017/jns.2020.19
43. Nilaweera KN, Speakman JR. Regulation of intestinal growth in response to variations in energy supply and demand. *Obes Rev*. 2018;19(1):61-72. doi:10.1111/obr.12780
44. Lund J, Gerhart-Hines Z, Clemmensen C. Role of energy excretion in human body weight regulation. *Trends Endocrinol Metab*. 2020;31:705-708. doi:10.1016/j.tem.2020.06.002
45. Dulloo AG, Miles-Chan JL, Schutz Y. Collateral fattening in body composition autoregulation: its determinants and significance for obesity predisposition. *Eur J Clin Nutr*. 2018;72:657-664. doi:10.1038/s41430-018-0138-6
46. Wiersma P, Verhulst S. Effects of intake rate on energy expenditure, somatic repair and reproduction of zebra finches. *J Exp Biol*. 2005;208:4091-4098. doi:10.1242/jeb.01854
47. Halsey LG, Areta JL, Koehler K. Does eating less or exercising more to reduce energy availability produce distinct metabolic responses? *Philos Trans R Soc Lond B Biol Sci*. 2023;378:20220217. doi:10.1098/rstb.2022.0217
48. Hirsch J, Batchelor B. Adipose tissue cellularity in human obesity. *Clin Endocrinol Metab*. 1976;5:299-311. doi:10.1016/s0300-595x(76)80023-0
49. Cristancho AG, Lazar MA. Forming functional fat: a growing understanding of adipocyte differentiation. *Nat Rev Mol Cell Biol*. 2011;12:722-734. doi:10.1038/nrm3198
50. Adra CN, Manor D, Ko JL, et al. RhoGDIgamma: a GDP-dissociation inhibitor for rho proteins with preferential expression in brain and pancreas. *Proc Natl Acad Sci U S A*. 1997;94:4279-4284. doi:10.1073/pnas.94.9.4279
51. Cernea M, Tang W, Guan H, Yang K. Wisp1 mediates Bmp3-stimulated mesenchymal stem cell proliferation. *J Mol Endocrinol*. 2016;56:39-46. doi:10.1530/JME-15-0217
52. Guan H, Arany E, van Beek JP, et al. Adipose tissue gene expression profiling reveals distinct molecular pathways that define visceral adiposity in offspring of maternal protein-restricted rats. *Am J Physiol Endocrinol Metab*. 2005;288:E663-E673. doi:10.1152/ajpendo.00461.2004
53. Stewart A, Guan H, Yang K. BMP-3 promotes mesenchymal stem cell proliferation through the TGF-beta/activin signaling pathway. *J Cell Physiol*. 2010;223:658-666. doi:10.1002/jcp.22064
54. Wu D, Asiedu M, Adelstein RS, Wei Q. A novel guanine nucleotide exchange factor MyoGEF is required for cytokinesis. *Cell Cycle*. 2006;5:1234-1239. doi:10.4161/cc.5.11.2815
55. Barneda D, Planas-Iglesias J, Gaspar ML, et al. The brown adipocyte protein CIDEA promotes lipid droplet fusion via a phosphatidic acid-binding amphipathic helix. *Elife*. 2015;4:e07485. doi:10.7554/eLife.07485
56. Du Y, Ma J, Wang Y, et al. MiR-421 regulates goat intramuscular preadipocytes differentiation via targeting FGF13. *Anim Biotechnol*. 2022;33:1333-1343. doi:10.1080/10495398.2021.1898414
57. Matsuzaka T, Shimano H. Elovl6: a new player in fatty acid metabolism and insulin sensitivity. *J Mol Med*. 2009;87:379-384. doi:10.1007/s00109-009-0449-0
58. Morton NM, Nelson YB, Michailidou Z, et al. A stratified transcriptomics analysis of polygenic fat and lean mouse adipose tissues identifies novel candidate obesity genes. *PLoS One*. 2011;6:e23944. doi:10.1371/journal.pone.0023944
59. Du L, Heaney AP. Regulation of adipose differentiation by fructose and GluT5. *Mol Endocrinol*. 2012;26:1773-1782. doi:10.1210/me.2012-1122
60. Fiorentino TV, de Vito F, Suraci E, et al. Obesity and overweight are linked to increased sodium-glucose cotransporter 1 and glucose transporter 5 levels in duodenum. *Obesity (Silver Spring)*. 2023;31:724-731. doi:10.1002/oby.23653
61. Kitano H, Chung JY, Noh KH, et al. Synaptonemal complex protein 3 is associated with lymphangiogenesis in non-small cell lung cancer patients with lymph node metastasis. *J Transl Med*. 2017;15:138. doi:10.1186/s12967-017-1241-5
62. Zhang Z, Funcke JB, Zi Z, et al. Adipocyte iron levels impinge on a fat-gut crosstalk to regulate intestinal lipid absorption and mediate protection from obesity. *Cell Metab*. 2021;33:1624-1639. e9. doi:10.1016/j.cmet.2021.06.001
63. Reneerkens J, Piersma T, Ramenofsky MR. An experimental test of the relationship between temporal variability of feeding opportunities and baseline levels of corticosterone in a shorebird. *J Exp Zool*. 2002;293:81-88. doi:10.1002/jez.10113
64. Spaulding MO, Hoffman JR, Madu GC, et al. Adolescent food insecurity in female rodents and susceptibility to diet-induced obesity. *Physiol Behav*. 2024;273:114416. doi:10.1016/j.physbeh.2023.114416
65. Rabasa C, Dickson SL. Impact of stress on metabolism and energy balance. *Curr Opin Behav Sci*. 2016;9:71-77. doi:10.1016/j.cobeha.2016.01.011
66. Razzoli M, Pearson C, Crow S, Bartolomucci A. Stress, overeating, and obesity: insights from human studies and preclinical models. *Neurosci Biobehav Rev*. 2017;76:154-162. doi:10.1016/j.neubiorev.2017.01.026
67. Bartolomucci A, Cabassi A, Govoni P, et al. Metabolic consequences and vulnerability to diet-induced obesity in male mice under chronic social stress. *PLoS One*. 2009;4:e4331. doi:10.1371/journal.pone.0004331

68. Aldiss P, Lewis JE, Lupini I, et al. Cold exposure drives weight gain and adiposity following chronic suppression of brown adipose tissue. *Int J Mol Sci*. 2022;23(3):1869. doi:10.3390/ijms23031869
69. Moles A, Bartolomucci A, Garbugino L, et al. Psychosocial stress affects energy balance in mice: modulation by social status. *Psychoneuroendocrinology*. 2006;31:623-633. doi:10.1016/j.psyneuen.2006.01.004
70. Lin E-JD, Sun M, Choi EY, Magee D, Stets CW, During MJ. Social overcrowding as a chronic stress model that increases adiposity in mice. *Psychoneuroendocrinology*. 2015;51:318-330. doi:10.1016/j.psyneuen.2014.10.007
71. Allen NL, Becerra BJ, Becerra MB. Associations between food insecurity and the severity of psychological distress among African-Americans. *Ethn Health*. 2018;23:511-520. doi:10.1080/13557858.2017.1280139
72. Carter KN, Kruse K, Blakely T, Collings S. The association of food security with psychological distress in New Zealand and any gender differences. *Soc Sci Med*. 2011;72:1463-1471. doi:10.1016/j.socscimed.2011.03.009
73. Bermúdez-Millán A, Pérez-Escamilla R, Segura-Pérez S, et al. Psychological distress mediates the association between food insecurity and suboptimal sleep quality in Latinos with type 2 diabetes mellitus. *J Nutr*. 2016;146:2051-2057. doi:10.3945/jn.116.231365
74. Hildebrandt BA, Ahmari SE. Breaking it down: investigation of binge eating components in animal models to enhance translation. *Front Psychiatry*. 2021;12:728535. doi:10.3389/fpsyt.2021.728535

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Gil CRE, Lund J, Żylicz JJ, Ranea-Robles P, Sørensen TIA, Clemmensen C. Food insecurity promotes adiposity in mice. *Obesity (Silver Spring)*. 2025;33(6):1087-1100. doi:10.1002/oby.24259