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Metabolic Effects of Maternal High-Fat Diet on Glucose Regulation and Leptin Resistance in Mice: A Research Protocol

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Abstract

Introduction: Maternal physiological systems undergo critical adaptations during pregnancy to maintain homeostasis. Leptin, an adipocyte-derived hormone, is a critical regulator of energy balance and metabolism, particularly in the early stages of pregnancy. Previous studies demonstrate that hyperleptinemia induces leptin resistance, suppresses feelings of satiety, and increases the risk of gestational diabetes. This study aims to investigate the role of leptin resistance in maternal obesity with mouse models. By understanding leptin resistance, which remains underexplored, pathways can be identified to reduce the risks of excessive weight gain during pregnancy and transmission of health complications to offspring.

Methods: The study will utilize female wild-type (C57BL/6) mice of similar ages, maintained in a controlled environment. The experimental group will receive a high-fat diet (HFD) to elevate leptin levels, while the control group will be fed a standard diet. Initial measurements of body mass, food intake, and plasma leptin levels will be recorded to establish a baseline. These measurements will be taken weekly to examine the relationship between leptin levels and the anthropometric data of the mice. To specifically assess leptin resistance, food intake and body mass will be closely monitored in mice exhibiting hyperleptinemia.

Anticipated Results: Results are anticipated to demonstrate that leptin resistance impairs maternal metabolic adaptations during pregnancy, leading to altered glucose homeostasis and increased fat mass. Elevated leptin levels are associated with increased adiposity, increasing the risk of maternal obesity. Mice with hyperleptinemia will exhibit increased food intake and body mass, indicating a state of leptin resistance. Persistent weight gain and increased food consumption in these mice will suggest leptin resistance, contributing to metabolic dysregulation and an increased risk of gestational diabetes and obesity. Offspring may exhibit higher birth weights and metabolic dysfunction, supporting the Developmental Origins of Health and Disease (DOHaD) hypothesis.

Conclusion: Understanding the role of leptin in pregnancy can identify pathways involved in gestational disorders. The investigation aims to inform therapeutic strategies targeting leptin signaling to prevent the transmission of health complications to offspring. By elucidating mechanisms of leptin resistance, the study aims to contribute to interventions that mitigate risks associated with maternal obesity during pregnancy.

Keywords: leptin; diabetes; glucose; obesity; leptin resistance; pregnancy; high-fat diet; gestational metabolism

Introduction

Maternal physiology undergoes profound adaptations during pregnancy to sustain fetal development and ensure metabolic homeostasis. A central aspect of these changes involves hormonally mediated metabolic regulation [1]. Among these key regulators is the adipocyte-derived hormone leptin, which governs appetite, energy balance, and metabolic function [2]. In non-pregnant individuals, elevated leptin levels suppress hunger and enhance energy expenditure [2]. However, during pregnancy, leptin concentrations rise significantly, a critical adaptation that ensures sufficient energy availability for fetal growth and underscores leptin's essential role in gestation [3].

Leptin's effects on satiety are primarily mediated by its binding to the long-form leptin receptor (Ob-Rb) in the hypothalamus and initiating a signalling cascade [4]. This activates the Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway, where JAK2 phosphorylates Ob-Rb and enables recruitment of STAT3 [4]. Phosphorylated STAT3 dimerizes and translocates to the nucleus to regulate target genes that suppress appetite and promote energy expenditure, such as pro-opiomelanocortin (POMC), while downregulating

orexigenic signals such as neuropeptide Y (NPY) and Agouti-related peptide (AgRP) [4].

Beyond its metabolic effects, leptin acts as a pleiotropic hormone, influencing diverse physiological processes independent of body weight. These include vascular function, bone and cartilage growth, and immune modulation [3]. Notably, leptin plays a pivotal role in neonatal development. During gestation, it is secreted by both the placenta and fetal adipose tissue, where it contributes to the regulation of growth, neurodevelopment, and long-term metabolic programming of offspring [5]. Postnatally, milk-borne maternal leptin may offer protective benefits; adequate exposure during lactation has been linked to reduced susceptibility to obesity and metabolic dysfunction in adulthood [6].

Pregnancy-induced metabolic adaptations increase adipose tissue mass, elevating leptin secretion and contributing to hyperleptinemia [7,8]. However, prolonged exposure to high leptin levels can induce leptin resistance, a condition characterized by impaired leptin signaling and blunted cellular responses [9]. This is often due to downregulation or dysfunction of the Ob-Rb receptor or impaired activation of downstream effectors such as STAT3, disrupting hypothalamic appetite regulation [4]. Consequently, despite elevated circulating leptin, resistance disrupts the hormone's regulatory effects on appetite, fat storage, and glucose metabolism [10]. This creates a positive feedback loop; impaired leptin signaling promotes increased caloric intake, further expanding adipose tissue and driving additional leptin secretion. While this adaptation ensures energy availability for fetal growth and lactation, excessive weight gain and metabolic dysregulation heighten the risk of complications in both the mother and offspring [3].

Leptin resistance during pregnancy can confer lasting implications on maternal health. While it may contribute to gestational diabetes during pregnancy, postpartum, persistent leptin resistance may hinder metabolic recovery, contributing to chronic weight retention and difficulties with weight loss [11]. This metabolic dysfunction increases susceptibility to insulin resistance, type 2 diabetes, and cardiovascular disease [12]. Furthermore, disrupted leptin signaling can impair appetite regulation, complicating efforts to maintain healthy dietary habits [13]. These effects create a self-perpetuating cycle that exacerbates obesity and metabolic syndrome after pregnancy.

The Developmental Origins of Health and Disease (DOHaD) hypothesis posits that fetal and early postnatal environments shape long-term health outcomes via developmental plasticity [14]. Maternal hyperleptinemia and leptin resistance may program offspring energy homeostasis, influencing fetal metabolism, appetite regulation, and adiposity, thereby increasing risks of obesity and metabolic disorders [15,16]. Early postnatal life represents a critical window of vulnerability, where nutritional and hormonal fluctuations can predispose offspring to leptin resistance, altered dietary preferences, and metabolic disease, particularly if postnatal conditions mismatch intrauterine programming [17,18].

While leptin's role in regulating energy balance is well-documented, its complex and evolving functions in pregnancy, particularly in maternal obesity, leptin resistance, and intergenerational metabolic effects, remain less understood. This study aims to investigate the role of leptin resistance during pregnancy by using a mouse model to elucidate underlying mechanisms and their implications for both mothers and offspring. It is hypothesized that leptin resistance is mediated by downregulated Ob-Rb expression and impaired JAK2/STAT3 signaling, resulting in blunted central leptin responses [4]. Additionally, it is hypothesized that a maternal high-fat diet (HFD) induces hyperleptinemia and central leptin resistance, leading to systemic metabolic dysregulation during pregnancy—including impaired glucose tolerance, increased adiposity, and beta-cell hypertrophy—which persist postpartum and are transmitted to offspring, predisposing them to early-onset obesity, insulin resistance, and hepatic steatosis through disrupted hypothalamic leptin signaling and altered energy homeostasis.

Methods

Animal Model

The study will utilize female wild-type C57BL/6 mice, a well-characterized model for their robust metabolic response to HFD and frequent breeding, making them an ideal model for studying leptin resistance [19]. In addition, C57BL/6 mice offer a translational framework for investigating these mechanisms, as their genetic and physiological traits closely mirror human metabolic adaptations, including susceptibility to diet-induced obesity and leptin resistance [20].

Mice will be 8 to 10 weeks of age at the beginning of the experiment to control age-related variations in metabolism. They will be maintained in a controlled environment with a 12-hour light-dark cycle, temperature set at 22ºC ± 1ºC, and humidity between 40-60% with water provided ad libitum. Pregnant females will be singly housed after copulation plus detection (E0.5) to prevent inter-dam aggression and allow individual monitoring of food intake. While single housing can affect stress and metabolism, adult female C57BL/6 mice have not shown to exhibit significant corticosterone elevation under these conditions [21]. Housing conditions will be monitored regularly.

Graphical Abstract

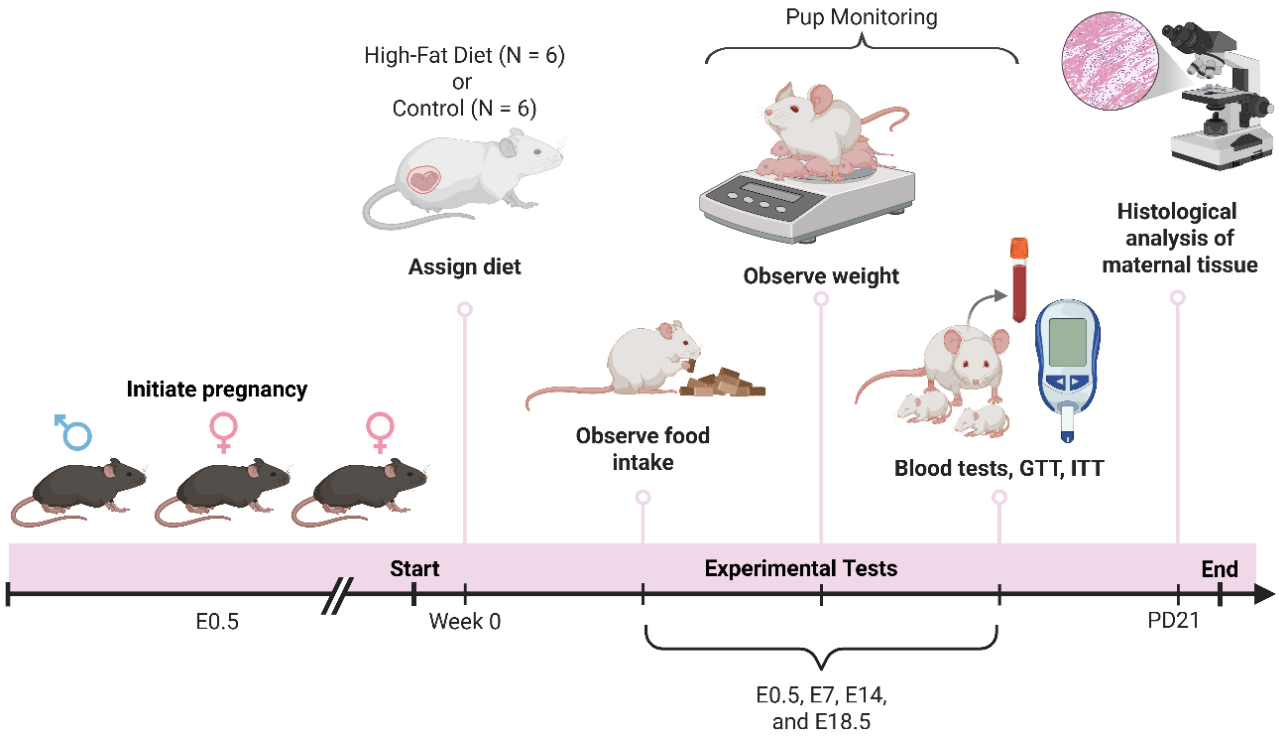

Figure 1. Graphical Abstract for the Experimental Procedure. Pregnancy will be initiated in the mice. At Week 0, pregnant dams will be assigned HFD or control diet (N = 6 per group). Experimental tests will be performed to monitor food intake, weight, and blood glucose and insulin. Histological analysis of maternal tissue will be performed at postnatal day 21 (PD21). Figure created with BioRender (https://www.biorender.com/).

Pregnancy Induction

To initiate pregnancy, female mice will be paired with age-matched male C57BL/6 mice in a 2:1 ratio, allowing for natural breeding. Pregnancy will be confirmed by the presence of a copulation plug, marking embryonic day 0.5 (E0.5). Mice will then be randomly assigned to either the HFD or control diet group (Figure 1). Gestational age will be carefully tracked, with the study being conducted within the range, E18.5 to E20. Embryos beyond E20 may be too large, while those before E18.5 may not be fully developed [22].

Experimental Design

The study will consist of two groups: an experimental group and a control group. The control group will contain C57BL/6 mice fed a standard diet (13% kcal from fat, LabDiet 5053) to serve as the control for comparison. The experimental group will contain C57BL/6 mice fed a high-fat diet (60% kcal from fat, Research Diets D12492) to induce hyperleptinemia and potentially leptin resistance during their perinatal period. A GraphPad power analysis will determine the required sample size for statistical significance. Since pilot data is unavailable, a sample size of six mice per group (N = 6) will be used. Dietary intervention will begin on E0.5 and continue until the end point of the study (Table 1). All mice will be monitored daily for general health and behavior (Figure 2). Researchers performing metabolic measurements, leptin assays, and histological analyses will be blinded to group allocation to minimize bias during data collection and interpretation.

Table 1. Cohort Structure, Dietary Regimens, and Dosage Information

N =	Cohort	Group	Diet	% kcal from fat
6	Control	C57BL/6 mice	Standard Diet	13% kcal from fat
6	Experimental	C57BL/6 mice	High-Fat Diet (HFD)	60% kcal from fat

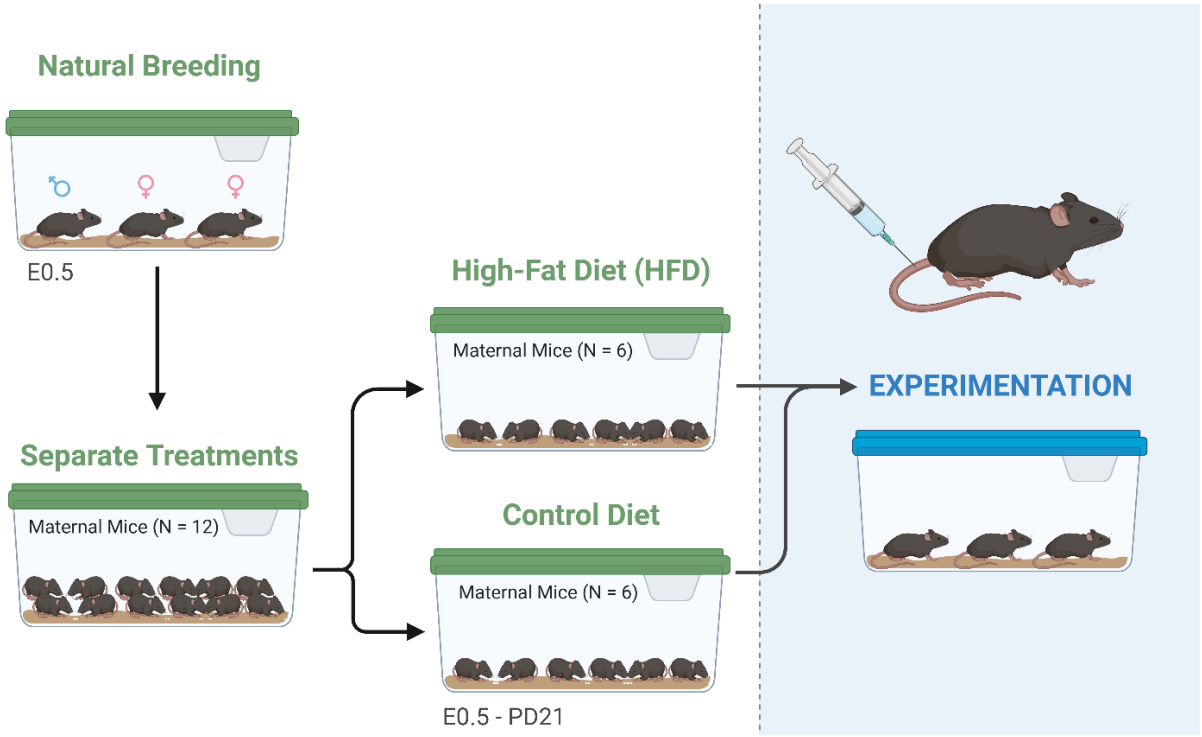

Figure 2. Maternal Mouse Model for Dietary Manipulation and Experimental Assessment. Two female and one male C57BL/6J mice were housed together to facilitate natural breeding. Upon detection of a vaginal plug (E0.5), pregnant females were separated and randomly assigned to one of two dietary groups: a high-fat diet (HFD; 60% kcal from fat) or a control diet (13% kcal from fat), with N = 6 per group. Maternal mice remained on their respective diets from Embryonic day 0.5 (E0.5) to postnatal day 21 (PD21). Experimental endpoints included metabolic testing and tissue collection to assess the impact of maternal diet on glucose regulation and organ morphology. Figure created with BioRender (https://www.biorender.com/).

Pre-Treatment Baseline Measurements

Baseline measurements will be collected for all mice in the experimental and control groups seven days prior to dietary intervention (Week 0). Mice will be weighed using an electronic balance to establish an initial body weight. Body mass will be recorded weekly throughout the 30-day study period using the same electronic balance. Food intake will be measured weekly by weighing the food provided and food remaining at the end of each week. Weekly average food intake will be calculated for each mouse. Blood samples will be collected via tail vein bleed at baseline to assess initial plasma leptin levels using an ELISA kit (R&D Systems) [23]. Plasma will be stored at -80˚C until analysis.

Leptin Resistance Assessment

To specifically assess leptin resistance, blood samples will be collected at baseline and E0.5, E7, E14, and E18.5 via tail vein bleed. Persistently elevated leptin levels in the experimental group, despite continued HFD consumption, will indicate the development of leptin resistance [24]. On E18.5, mice from the experimental group (N = 6) and control group (N = 6) will be injected intraperitoneally with leptin, and food intake will be measured over the subsequent 24 hours. A significant reduction in food intake in control mice but not in the experimental group will indicate leptin resistance [24].

Metabolic and Physiological Assessments

At baseline and E0.5, E7, E14, and E18.5, body composition (fat mass vs. lean mass) will be assessed using quantitative magnetic resonance (QMR) imaging in mothers. To assess dysfunction, glucose tolerance tests (GTT) and insulin tolerance tests (ITT) will be performed on the mothers at E0.5, E7, E14, and E18.5 (Figure 3). For GTT, mice will be fasted for 6 hours before an intraperitoneal injection of glucose (dose: 1.5 g/kg body mass), and blood glucose levels will be measured at 0, 15, 30, 60, and 120 minutes using a glucometer (Figure 3). For ITT, mice will receive an intraperitoneal injection of insulin (dose: 0.75 IU/kg body mass), and glucose levels will be measured at the same time points (Figure 3). Impaired glucose clearance in the experimental group will suggest metabolic dysfunction associated with leptin resistance [25].

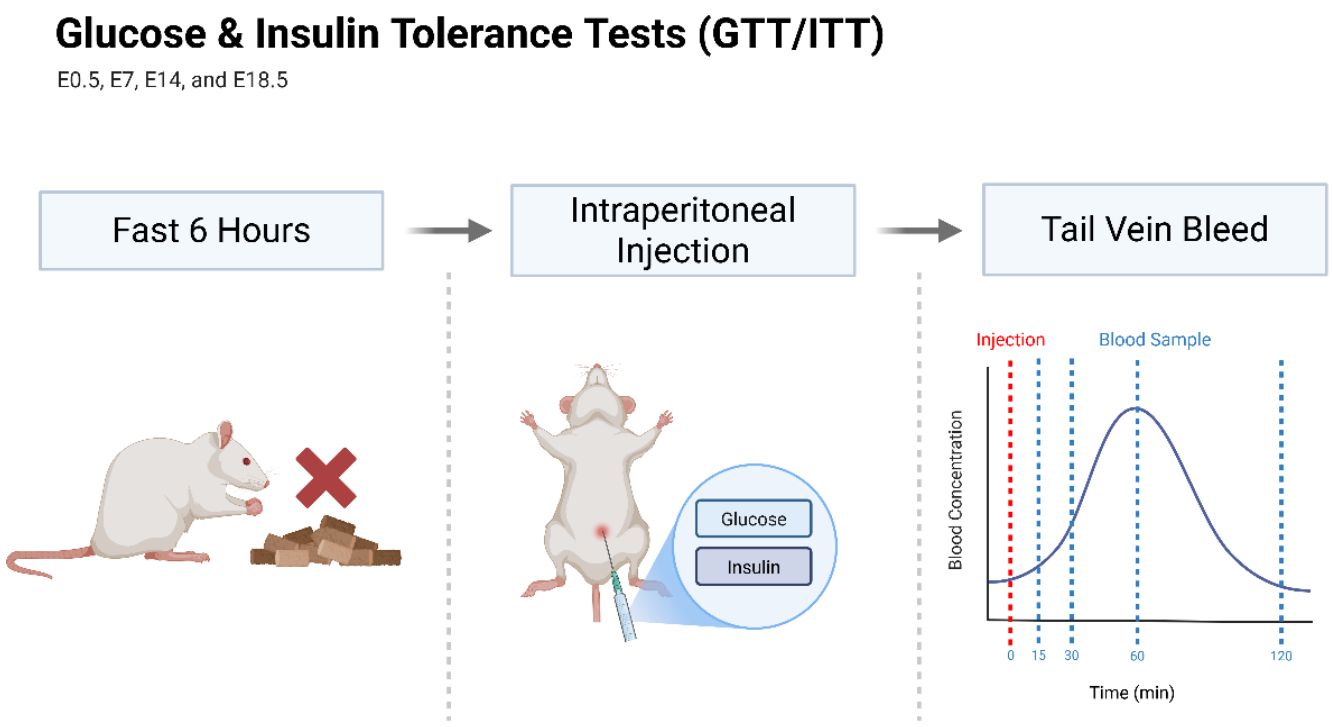

Figure 3. Glucose and Insulin Tolerance Testing in Pregnant Mice. Mice were fasted for 6 hours before intraperitoneal injection of glucose or insulin to assess metabolic function at key gestational timepoints (E0.5, E7, E14, and E18.5). Blood glucose levels were monitored via tail vein sampling at baseline and multiple timepoints post-injection (0, 15, 30, 60, and 120 minutes) to evaluate glucose clearance and insulin sensitivity. Data from these tests were used to identify diet-induced impairments in glucose homeostasis during pregnancy. Figure created with BioRender (https://www.biorender.com/).

Pregnancy Monitoring

Mice will be monitored for pregnancy and general health throughout gestation. Body mass, food intake, and plasma leptin levels will be measured at E0.5, E7, E14, and E18.5. Timed mating will be used to ensure accuracy in gestational tracking, with mating confirmed by the presence of a copulation plug. Data will be excluded if maternal mice give birth before E18.5 or carry past-term beyond E20, ensuring that only data from pregnancies with a normal gestational period are included [26]. This controls for abnormal or preterm births.

Pup Monitoring

Pups will be monitored during the early postnatal period to assess the developmental impact of maternal hyperleptinemia and leptin resistance. At weaning (PD21), body weight and food intake will be recorded to evaluate early growth trends [27]. GTTs and ITTs will be performed using age-appropriate protocols to assess glucose metabolism and insulin sensitivity in offspring. Blood samples will be collected for leptin analysis using an ELISA kit, allowing comparison of circulating leptin levels between pups from the HFD and control group dams.

Histology Analysis

At the end point of the study, mice will be euthanized using sodium pentobarbital via a single-dose injection [28]. Maternal mice will be sacrificed after the completion of pregnancy on PD21 for the measurement of any postnatal physiological changes. Histological analyses will focus on structural and morphological differences in tissues (Table 2). Tissues from organs, including the pancreas, brain, and liver, will be harvested (Figure 4).

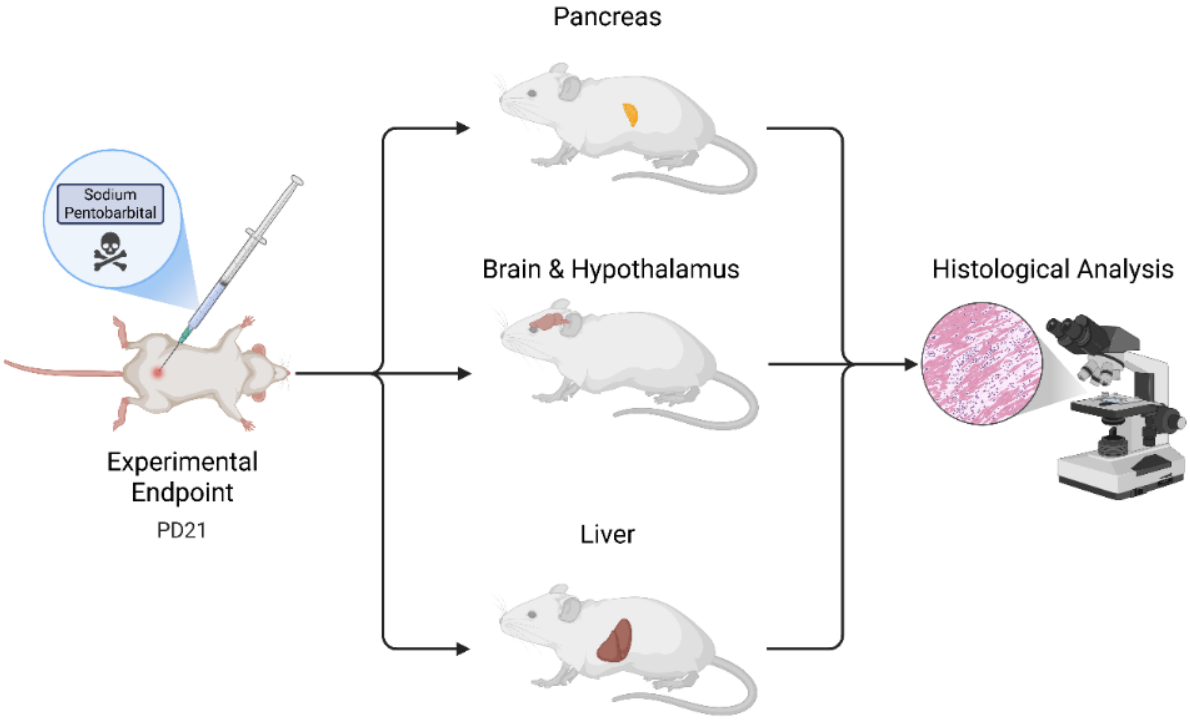

Figure 4. Tissue Collection and Histological Analysis on Postnatal Day 21 (PD21). At the experimental endpoint (PD21), offspring will be euthanized using sodium pentobarbital. Following euthanasia, the pancreas, liver, and brain (including the hypothalamus) will be dissected and collected. These tissues will be fixed, processed, and embedded for histological analysis to assess structural and cellular changes associated with maternal dietary intervention. Figure created with BioRender (https://www.biorender.com/).

Table 2. Experimental Treatment Groups and Expected Outcomes

Group	Diet	Assessed Tissue	Assays Performed	Expected Outcomes
Control	Standard	Pancreas	IHC, insulin staining immunoassays	Normal insulin expression and glucose metabolism
Experimental	HFD		IHC, insulin staining immunoassays	Altered insulin expression and impaired glucose tolerance, indicating insulin resistance
Control	Standard	Hypothalamus	P-STAT3 IHC	Normal leptin signaling in arcuate nucleus
Experimental	HFD		P-STAT3 IHC	Decreased P-STAT3 staining, indicating disrupted leptin signaling
Control	Standard	Liver	Oil Red O staining	Low lipid accumulation
Experimental	HFD		Oil Red O staining	Increased lipid accumulation and early signs of hepatic dysfunction

Pancreas

Pancreatic tissue will be examined for beta-cell morphology, insulin staining, and inflammatory markers in maternal mice. Pancreatic beta cells are crucial for insulin production and may demonstrate changes in morphology under conditions of leptin resistance or metabolic stress [24]. Immunohistochemistry (IHC) will be performed on pancreatic tissue to assess beta-cell morphology. Insulin staining will be performed to assess the functional impact of leptin resistance on insulin

secretion and beta-cell function. Finally, markers such as TNF-α and interleukin-6 (IL-6) will be assessed via ELISA, as obesity and leptin resistance frequently promote inflammation [30].

Brain (Hypothalamus)

Leptin typically acts on several brain nuclei within the hypothalamus due to its role in regulating appetite and energy balance [31]. Thus, female mice will likely show alterations in leptin signaling pathways under hyperleptinemic conditions. Using phospho-signal transducer and activator of transcription (P-STAT3) IHC, leptin-responsive cells in the brain will be mapped from a cross-section of the hypothalamus [10]. To further assess leptin sensitivity, hypothalamic tissue will also be collected at the experimental endpoint (PD21) to quantify leptin receptor (Ob-Rb) expression. Western blot analysis will be performed to evaluate Ob-Rb protein levels. This will help determine whether HFD-induced hyperleptinemia leads to central leptin receptor downregulation.

Liver

The liver plays a central role in lipid metabolism and is highly sensitive to metabolic alterations induced by HFD. To assess this, liver cryosections will be stained with Oil Red O, a lipid-specific dye that highlights neutral lipid deposits [32]. Quantification of Oil Red O staining will provide a visual marker for lipid accumulation.

Statistical Analysis

Group comparisons will be conducted using two-way repeated measures ANOVA for time-course data and unpaired t-tests for endpoint measurements. Data will be presented as means ± standard deviations (SD). Statistical significance will be set at $p < 0.05$. GraphPad software will be used for all statistical analyses.

Results

Plasma Leptin and Leptin Resistance Development

It is expected that mice on the HFD will exhibit increased plasma leptin levels, increased food intake, and significant weight gain, consistent with the development of leptin resistance. The control group is expected to maintain normal food intake, lower blood leptin levels, and stable body weight [33]. Mice with leptin resistance will likely demonstrate impaired glucose tolerance and increased adiposity, highlighting the metabolic dysregulation that contributes to maternal obesity and gestational complications [33].

Metabolic and Physiological Adaptations

Mice in the HFD group are expected to demonstrate a progressive increase in body weight [33]. A higher fat mass percentage and lower lean mass in the HFD group are also expected to be revealed during QMR imaging [33]. Food intake is predicted to be significantly greater in HFD mice. Additionally, glucose tolerance tests may show impaired glucose clearance in the HFD group, with significantly higher blood glucose levels at all timepoints post-glucose injection (15, 30, 60, and 120 minutes), indicating gestational glucose intolerance [34]. Insulin tolerance tests may reveal a diminished hypoglycemic response in HFD mice, suggesting insulin resistance.

Pregnancy Outcomes

HFD-fed mice may experience altered pregnancy outcomes, including increased gestational weight gain and prolonged gestation length compared to controls [35]. Pups born to mothers under HFD are expected to demonstrate elevated body weight compared to pups born to mothers under the normal diet, indicating early metabolic alterations [36]. GTT and ITT results may reveal impaired glucose clearance and reduced insulin sensitivity, mirroring the metabolic phenotype of their mothers. Maternal plasma leptin levels are predicted to remain elevated post-partum, suggesting persistent metabolic dysregulation beyond pregnancy [16].These findings suggest that maternal hyperleptinemia and leptin resistance during gestation can disrupt neonatal energy homeostasis, predisposing offspring to obesity and insulin resistance.

Histological Analysis

Pancreas

Histological examination of pancreatic sections is expected to reveal significant beta-cell hypertrophy in HFD mice compared to controls, indicating a compensatory response to increased insulin demand under leptin-resistant conditions [37]. Insulin immunostaining is expected to show elevated expression in the experimental group, reflecting heightened beta-cell activity [38]. However, chronic hyperleptinemia may also lead to beta-cell dysfunction, and some pancreatic islets may display irregular morphology, including signs of degranulation [38].

Brain (Hypothalamus)

PSTAT-3 immunohistochemical (IHC) staining of the hypothalamic sections are expected to demonstrate reduced activation of leptin-responsive neurons in the arcuate nucleus of HFD mice compared to controls [38]. The suppression of leptin signaling in the HFD group would support the development of hypothalamic leptin resistance. Additionally, general disruptions in hypothalamic structures may be observed, such as altered neuronal organization or reduced density of leptin-sensitive regions [38]. These structural and signaling changes could reflect impaired central regulation of energy balance and appetite in response to prolonged hyperleptinemia.

Liver

Liver sections stained with Oil Red O are expected to exhibit greater lipid accumulation in the HFD group, confirming hepatic steatosis [39]. Increased expression of lipogenic and inflammatory markers is also expected to be

observed, indicating early stages of liver dysfunction [39]. These findings suggest that chronic exposure to HFD and elevated leptin levels disrupts hepatic metabolism and contributes to systemic insulin resistance and inflammation, consistent with the onset of metabolic syndrome.

Discussion

The expected findings from this study will provide critical insights into leptin resistance during pregnancy and its metabolic implications for maternal and offspring health. We expect HFD to induce hyperleptinemia and leptin resistance, contributing to metabolic dysfunction, excessive gestational weight gain, and impaired glucose regulation. These findings align with previous research, demonstrating that leptin resistance is a key factor in metabolic disorder development, particularly gestational diabetes mellitus (GDM) [40].

Leptin Resistance and Metabolic Adaptations in Pregnancy

Expected results for maternal mice on HFD include elevated plasma leptin levels and increased food intake, indicating leptin resistance. Thus, leptin's satiating effects are suppressed, leading to increased caloric intake and adipose accumulation. The increase in adiposity exacerbates leptin release, further contributing to hyperleptinemia and ultimately, leptin resistance. These results align with existing literature, suggesting chronic leptin elevation impairs hypothalamic signaling [41].

The expected impaired glucose tolerance observed in HFD-fed mice supports the implications of leptin resistance in metabolic dysregulation. Glucose tolerance and insulin sensitivity tests that reveal higher blood glucose levels and diminished insulin responses in HFD-fed mice indicate a predisposition to insulin resistance [33]. These findings are particularly relevant in the context of human pregnancy, where leptin resistance has been strongly linked to GDM [40]. With rising rates of maternal obesity and GDM, understanding the underlying mechanisms of leptin resistance is crucial for developing targeted interventions [42].

Long-Term Health Implications for Maternal and Offspring Health

Beyond pregnancy, persistent leptin resistance can have significant long-term health consequences for mothers. Consistently elevated leptin levels post-pregnancy suggests prolonged metabolic dysregulation, increasing the risk of chronic weight gain, obesity, and metabolic syndrome [11]. Epidemiological studies have reported a higher incidence of type 2 diabetes and cardiovascular disease in women with a history of GDM, reinforcing the importance of early intervention strategies to mitigate disease [43].

This study also highlights transgenerational effects of maternal hyperleptinemia and leptin resistance. Elevated leptin levels during gestation may alter fetal metabolic programming, predisposing offspring to obesity and metabolic disorders later in life. The study expects that the offspring of HFD-fed mothers will exhibit higher birth weights and altered metabolic profiles, consistent with previous research linking maternal obesity to the DOHaD hypothesis [14]. Early postnatal exposure to hyperleptinemia may further exacerbate leptin resistance, creating a positive feedback loop that promotes excessive weight gain and metabolic dysfunction in offspring [26, 27]. Although this study primarily focuses on maternal outcomes and basic offspring metrics, histological examination of offspring tissues can be reserved for future studies to further elucidate the long-term effects of leptin resistance.

Limitations & Future Directions

While this study offers valuable insights into the role of leptin resistance in pregnancy-related metabolic outcomes, several limitations must be acknowledged. Firstly, the reliance on a relatively small sample size (N = 6 per group) may limit statistical power and generalizability, particularly in detecting subtle inter-individual variations in metabolic response.

Additionally, repeated tail vein bleeding for leptin and glucose measurements introduces potential stress-related confounders. Stress alters endocrine function, elevate glucocorticoid levels, and adversely affect pregnancy outcomes, including fetal development and gestational length [44]. Handling stress may also affect food intake and maternal behavior, influencing leptin secretion and metabolic assessments. Another potential stressor is post-copulation housing. Pregnant dams were singly housed to monitor food intake and prevent aggression. Although isolation can influence stress, prior studies report no significant increase in corticosterone in singly housed adult female C57BL/6 mice [21]. While all groups undergo the same protocol, we expect their effects to be normalized. Nonetheless, future studies could incorporate less invasive blood sampling methods.

Furthermore, leptin resistance is mediated centrally through impaired Ob-Rb receptor signaling and disrupted JAK2/STAT3 pathway activation in the hypothalamus, which blunts satiety signaling despite elevated leptin levels [4]. Future work should measure hypothalamic Ob-Rb expression and STAT3 phosphorylation to clarify the molecular mechanisms underlying leptin resistance observed in this model.

Finally, while the C57BL/6 mouse model is well-characterized, interspecies differences remain. Extrapolating findings to human pregnancy must be done cautiously. Controlled environmental conditions and genetic homogeneity in animal models do not fully encapsulate the complexity of human maternal metabolism, which is influenced by environmental and genetic factors.

Conclusions

This study proposes a comprehensive protocol to investigate the role of leptin resistance in maternal obesity

using a well-characterized murine model. By inducing hyperleptinemia through HFD and assessing metabolic, hormonal, and developmental parameters across gestation and the early postnatal period, this research aims to elucidate the physiological consequences of disrupted leptin signaling during pregnancy. Through metabolic assessments, histological analyses, and offspring monitoring, the study will provide valuable insights into how maternal leptin resistance contributes to gestational disorders and intergenerational transmission of metabolic dysfunction. Ultimately, these findings may inform future strategies aimed at mitigating the effects of maternal obesity and improving both maternal and offspring health outcomes through targeted interventions in leptin signaling pathways.

List of Abbreviations
DOHaD: developmental origins of health and disease
GDM: gestational diabetes mellitus
GTT: glucose tolerance test
IHC: immunohistochemistry
IL-6: interleukin-6
ITT: insulin tolerance test
HFD: high-fat diet
PD21: postnatal day 21
P-STAT3: phospho-STAT3
QMR: quantitative magnetic resonance
TNF-α: tumor necrosis factor-alpha

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Ethics Approval and/or Participant Consent
This research protocol did not require ethics approval and/or participant consent.

Authors' Contributions
CH: Made substantial contributions to the design and concept of the proposed study and gave final approval of this version for publication.
LD: Made substantial contributions to the design and concept of the proposed study and provided extensive revisions.

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References
[1] Walewska E, Hamada Z, Pérez-García V, Galvão A. Altered uterine leptin signalling in obese mothers: from impaired decidualisation to pregnancy complications. Reproduction 2025;169. https://doi.org/10.1530/REP-24-0319
[2] Dornbush S, Aeddula NR. Physiology, Leptin [Internet]. StatPearls; 2023. [cited 2025 April 2] Available from: https://www.ncbi.nlm.nih.gov/books/NBK537038/
[3] Pérez-Pérez A, Toro A, Vilariño-García T, Maymó J, Guadix P, Dueñas JL, et al. Leptin action in normal and pathological pregnancies. J Cell Mol Med 2017;22:716. https://doi.org/10.1111/jcmm.13369
[4] Liu Z, Xiao T, Liu H. Leptin signaling and its central role in energy homeostasis. Front Neurosci 2023;17:1238528. http://doi.org/10.3389/fnins.2023.1238528
[5] Pico C, Jilkova ZM, Kus V, Palou A, Kopecky J. Perinatal programming of body weight control by leptin: putative roles of AMP kinase and muscle thermogenesis. Am J Clin Nutr 2011;94:S1830–7. http://doi.org/10.3945/ajcn.110.000752
[6] Picó C, Palou M, Pomar CA, Rodríguez AM, Palou A. Leptin as a key regulator of the adipose organ. Rev Endocr Metab Disord 2021;23:13. http://doi.org/10.1007/s11154-021-09687-5
[7] Armistead B, Johnson E, Vanderkamp R, Kula-Eversole E, Kadam L, Drewlo S, et al. Placental Regulation of Energy Homeostasis During Human Pregnancy. Endocrinology 2020;161:bqaa076. http://doi.org/10.1210/endocr/bqaa076
[8] Sáinz N, Barrenetxe J, Moreno-Aliaga MJ, Martínez JA. Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. Metabolism 2015;64:35–46. http://doi.org/10.1016/j.metabol.2014.10.015
[9] Amitani M, Asakawa A, Amitani H, Inui A. The role of leptin in the control of insulin-glucose axis. Front Neurosci 2013;7:51. http://doi.org/10.3389/fnins.2013.00051
[10] Valleau JC, Sullivan EL. The Impact of Leptin on Perinatal Development and Psychopathology. J Chem Neuroanat 2014;0:221. http://doi.org/10.1016/j.jchemneu.2014.05.001
[11] Vilariño-García T, Polonio-González ML, Pérez-Pérez A, Ribalta J, Arrieta F, Aguilar M, et al. Role of Leptin in Obesity, Cardiovascular Disease, and Type 2 Diabetes. Int J Mol Sci 2024;25:2338. http://doi.org/10.3390/ijms25042338
[12] Yeung AY, Tadi P. Physiology, Obesity Neurohormonal Appetite And Satiety Control [Internet]. StatPearls; 2023 [cited 2025 April 30]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK555906/

[13] Lacagnina S. The Developmental Origins of Health and Disease (DOHaD). Am J Lifestyle Med 2019;14:47. https://doi.org/10.1177/1559827619879694
[14] Briffa JF, McAinch AJ, Romano T, Wlodek ME, Hryciw DH. Leptin in pregnancy and development: a contributor to adulthood disease? Am J Physiol Endocrinol Metab 2015;308:E335–50. http://doi.org/10.1152/ajpendo.00312.2014
[15] Biesiada LA, Glowacka E, Krekora M, Sobantka S, Krokocka A, Krasomski G. The impact of excessive maternal weight on the nutritional status of the fetus – the role of leptin. Arch Med Sci 2016;12:394. http://doi.org/10.5114/aoms.2016.59267
[16] Gomes RM, Bueno FG, Schamber CR, de Mello JCP, de Oliveira JC, Francisco FA, et al. Maternal diet-induced obesity during suckling period programs offspring obese phenotype and hypothalamic leptin/insulin resistance. J Nutr Biochem 2018;61:24–32. http://doi.org/10.1016/j.jnutbio.2018.07.006
[17] Tamashiro KLK, Terrillion CE, Hyun J, Koenig JI, Moran TH. Prenatal Stress or High-Fat Diet Increases Susceptibility to Diet-Induced Obesity in Rat Offspring. Diabetes 2009;58:1116. https://doi.org/10.2337/db08-1129
[18] Charles River. C57BL/6 DIO Mice for Metabolic Research [Internet]. Charles River; n.d. [cited 2025 Mar 8]. Available from: https://www.criver.com/products-services/research-models-services/preconditioning-services/custom-diets/c57bl6-dio-mice-metabolic-research?region=24
[19] Rydell-Törmänen K, Johnson JR. The Applicability of Mouse Models to the Study of Human Disease. Mouse Cell Culture 2018;1940:3. https://doi.org/10.1007/978-1-4939-9086-3_1
[20] Smolensky I, Zajac-Bakri K, Mallien AS, Gass P, Guzman R, Inta D. Effects of single housing on behavior, corticosterone level and body weight in male and female mice. Lab Anim Res 2024;40:1–12. http://doi.org/10.1186/s42826-024-00221-7
[21] Chen VS, Morrison JP, Southwell MF, Foley JF, Bolon B, Elmore SA. Histology Atlas of the Developing Prenatal and Postnatal Mouse Central Nervous System, with Emphasis on Prenatal Days E7.5 to E18.5. Toxicol Pathol 2017;45:705. https://doi.org/10.1177/0192623317728134.
[22] NC3Rs. Blood sampling: Mouse [Internet]. NC3Rs; 2013 [cited 2025 Mar 8]. Available from: https://nc3rs.org.uk/3rs-resources/blood-sampling/blood-sampling-mouse
[23] Vasselli JR, Scarpace PJ, Harris RBS, Banks WA. Dietary Components in the Development of Leptin Resistance. Advances in Nutrition 2013;4:164. https://doi.org/10.3945/an.112.003152
[24] Denroche HC, Huynh FK, Kieffer TJ. The role of leptin in glucose homeostasis. J Diabetes Investig 2012;3:115. https://doi.org/10.1111/J.2040-1124.2012.00203.x
[25] Murray SA, Morgan JL, Kane C, Sharma Y, Heffner CS, Lake J, et al. Mouse Gestation Length Is Genetically Determined. PLoS One 2010;5:e12418. http://doi.org/10.1371/journal.pone.0012418
[26] Curley JP, Jordan ER, Swaney WT, Izraelit A, Kammel S, Champagne FA. The Meaning of Weaning: Influence of the Weaning Period on Behavioral Development in Mice. Dev Neurosci 2009;31:318. https://doi.org/10.1159/000216543
[27] Laferriere CA, Pang DS. Review of Intraperitoneal Injection of Sodium Pentobarbital as a Method of Euthanasia in Laboratory Rodents. J Am Assoc Lab Anim Sci 2020;59:254. https://doi.org/10.30802/AALAS-JAALAS-19-000081
[28] Kim J, Oh C-M, Kim H. The Interplay of Adipokines and Pancreatic Beta Cells in Metabolic Regulation and Diabetes. Biomedicines 2023, Vol 11, Page 2589 2023;11:2589. http://doi.org/10.3390/biomedicines11092589
[29] Pérez-Pérez A, Sánchez-Jiménez F, Vilariño-García T, Sánchez-Margalet V. Role of Leptin in Inflammation and Vice Versa. Int J Mol Sci 2020;21:5887. https://doi.org/10.3390/IJMS21165887
[30] Münzberg H, Flier JS, Bjørbæk C. Region-Specific Leptin Resistance within the Hypothalamus of Diet-Induced Obese Mice. Endocrinology 2004;145:4880–9. https://doi.org/10.1210/en.2004-0726
[31] Du J, Zhao L, Kang Q, He Y, Bi Y. An optimized method for Oil Red O staining with the salicylic acid ethanol solution. Adipocyte 2023;12:2179334. https://doi.org/10.1080/21623945.2023.2179334
[32] Reilly AM, Yan S, Huang M, Abhyankar SD, Conley JM, Bone RN, et al. A high-fat diet catalyzes progression to hyperglycemia in mice with selective impairment of insulin action in Glut4-expressing tissues. J Biol Chem 2021;298:101431. https://doi.org/10.1016/j.jbc.2021.101431
[33] Eyth E, Basit H, Swift C. Glucose Tolerance Test. StatPearls Publishing; 2023. https://doi.org/10.5005/jp/books/14203_21
[34] Akhaphong B, Gregg B, Kumusoglu D, Jo S, Singer K, Scheys J, et al. Maternal High-Fat Diet During Pre-Conception and Gestation Predisposes Adult Female Offspring to Metabolic Dysfunction in Mice. Front Endocrinol (Lausanne) 2022;12:780300. https://doi.org/10.3389/fendo.2021.780300
[35] Smoothy J, Larcombe AN, Chivers EK, Matthews VB, Gorman S. Maternal high fat diet compromises survival and modulates lung development of offspring, and impairs lung function of dams (female mice). Respir Res 2019;20:1–18. http://doi.org/10.1186/s12931-019-0976-3

[36] Bartolomé A. The Pancreatic Beta Cell: Editorial. Biomolecules 2023;13:495. http://doi.org/10.3390/biom13030495
[37] Martin SS, Qasim A, Reilly MP. Leptin Resistance: A Possible Interface of Inflammation and Metabolism in Obesity-Related Cardiovascular Disease. J Am Coll Cardiol 2008;52:1201–10. http://doi.org/10.1016/j.jacc.2008.05.060
[38] Martínez-Uña M, López-Mancheño Y, Diéguez C, Fernández-Rojo MA, Novelle MG. Unraveling the Role of Leptin in Liver Function and Its Relationship with Liver Diseases. Int J Mol Sci 2020;21:9368. https://doi.org/10.3390/ijms21249368
[39] Pérez-Pérez A, Vilariño-García T, Guadix P, Dueñas JL, Sánchez-Margalet V. Leptin and Nutrition in Gestational Diabetes. Nutrients 2020, Vol 12, Page 1970 2020;12:1970. https://doi.org/10.3390/nu12071970
[40] Martin RL, Perez E, He YJ, Dawson R, Millard WJ. Leptin resistance is associated with hypothalamic leptin receptor mRNA and protein downregulation. Metabolism 2000;49:1479–84. https://doi.org/10.1053/meta.2000.17695
[41] Pantham P, Aye ILMH, Powell TL. Inflammation in Maternal Obesity and Gestational Diabetes Mellitus. Placenta 2015;36:709. https://doi.org/10.1016/j.placenta.2015.04.006
[42] Yu Y, Soohoo M, Sørensen HT, Li J, Arah OA. Gestational Diabetes Mellitus and the Risks of Overall and Type-Specific Cardiovascular Diseases: A Population- and Sibling-Matched Cohort Study. Diabetes Care 2021;45:151. https://doi.org/10.2337/dc21-1018
[43] Yam KY, Naninck EFG, Abbink MR, la Fleur SE, Schipper L, van den Beukel JC, et al. Exposure to chronic early-life stress lastingly alters the adipose tissue, the leptin system and changes the vulnerability to western-style diet later in life in mice. Psychoneuroendocrinology 2017;77:186–95. https://doi.org/10.1016/j.psyneuen.2016.12.012
[44] Meek LR, Dittel PL, Sheehan MC, Chan JY, Kjolhaug SR. Effects of stress during pregnancy on maternal behavior in mice. Physiol Behav 2001;72:473–9. https://doi.org/10.1016/S0031-9384(00)00431-5

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