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Peri-conceptional obesogenic exposure induces sex-specific programming of disease susceptibilities in adult mouse offspring

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ABSTRACT

Vulnerability of the fetus upon maternal obesity can potentially occur during all developmental phases. We aimed at elaborating longer-term health outcomes of fetal overnutrition during the earliest stages of development. We utilized Naval Medical Research Institute (NMRI) mice to induce pre-conceptional and gestational obesity and followed offspring outcomes in the absence of any postnatal obesogenic influences. Male adult offspring developed overweight, insulin resistance, hyperleptinemia, hyperuricemia and hepatic steatosis; all these features were not observed in females. Instead, they showed impaired fasting glucose and a reduced fat mass and adipocyte size. Influences of the interaction of maternal diet * sex concerned offspring genes involved in fatty liver disease, lipid droplet size regulation and fat mass expansion. These data suggest that a peri-conceptional obesogenic exposure is sufficient to shape offspring gene expression patterns and health outcomes in a sex- and organ-specific manner, indicating varying developmental vulnerabilities between sexes towards metabolic disease in response to maternal overnutrition.

Abbreviations: Acaca, Acetyl-Coenzyme A carboxylase 1; *Acly*, ATP citrate lyase; *Actb*, Beta-actin; ANOVA, Analysis of variance; AUC, Area under the curve; *Bax*, Bcl2-associated X protein; *Bcl2*, B cell leukemia/lymphoma 2; *Bscl2*, Berardinelli–Seip congenital lipodystrophy 2 (also known as *seipin*); BW, Body weight; CD, Control diet; *Cd36*, Cd36 antigen; CET, Central European Time; *Cidea*, Cell death-inducing DNA fragmentation factor, alpha subunit-like effector A; *Cpt1*, Carnitine palmitoyltransferase 1; CT, Computed tomography; dpc, Day post coitum; EEC, European Economic Commission; FA, Fatty acid; *Fabp4*, Fatty acid binding protein 4; *Fasn*, Fatty acid synthase; GR, Glucocorticoid receptor; GTT, Glucose tolerance test; H&E, Hematoxylin–eosin; *Hes1*, Hairy and enhancer of split 1; HFD, High-fat, high-calorie diet; HMW, High-molecular-weight; HOMA-IR, Homeostatic model assessment of insulin resistance; HP, Heat production; *Lep*, Leptin; mat-CD, Exposure to maternal control diet; mat-HFD, Exposure to maternal high-fat, high-calorie diet; MD, Maintenance diet; MDA, Malondialdehyde; *Me1*, Malic enzyme 1; *Mest*, Mesoderm-specific transcript/imprinted paternally expressed gene 1 (also known as *Peg1*); MRI, Magnetic resonance imaging; N, Nitrogen; NAFLD, Non-alcoholic fatty liver disease; NMRI, Naval Medical Research Institute; NEFA, Non-esterified fatty acid; *Nr3c1*, Nuclear receptor subfamily 3, group C, member 1 (also known as *Gr*, glucocorticoid receptor); *Nr1h3*, Nuclear receptor subfamily 1, group H, member 3 (also known as *Lxra*, liver X receptor alpha); NRL, Nose–rump-length; PFA, Paraformaldehyde; *Plin2*, Perilipin 2; *Pnpla2*, Patatin-like phospholipase domain-containing protein 2 (also known as *Atgl*, adipose triglyceride lipase); *Ppara*, Peroxisome proliferator activated receptor alpha; *Pparg*, Peroxisome proliferator activated receptor gamma; *Ppia*, Peptidylprolyl isomerase A; RER, Respiratory exchange ratio; ROI, Region of interest; *Scd2*, Stearoyl-Coenzyme A desaturase 2; S.e.m., Standard error of the mean; *Sfrp5*, Secreted frizzled-related sequence protein 5; *Srebf1*, Sterol regulatory element binding transcription factor 1; TBARS, Thiobarbituric acid-reactive substances; *Ube2d2*, Ubiquitin-conjugating enzyme E2D 2; VCO_2, Carbon dioxide production; VO_2, Oxygen consumption

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1. Introduction

Human epidemiological studies show that the offspring's risk of developing obesity in later life is strongly associated with maternal obesity [1]. Among the various perinatal risk factors for the development of childhood obesity, pre-conceptional maternal obesity confers the strongest risk [2,3]. This finding is supported by evidence of a decreased offspring obesity risk subsequent to maternal weight-loss surgery [4].

It seems likely that more than one critical time window during development plays a role in programming offspring disease risks [5]. To address this hypothesis, animal studies are needed in order to separate the wide range of confounding exposures and factors during the developmental phases that can influence offspring health outcome. Various animal models have already been used to address the transgenerational impact of diet-induced obesity by fetal exposures throughout pregnancy and also the lactation period [5], which represents a critical time window for programming in rodents [6,7]. In humans, this period of developmental plasticity most likely corresponds to the third trimester of pregnancy and may expand into postnatal life [8]. However, only very few rodent studies have specifically investigated the transgenerational effects of maternal pre-gravid obesity and during the gestational period [9,10], although an impact on oocyte and early embryo development has been suggested [11,12].

Whereas rodent offspring outcomes have so far mostly been determined in males [13], few studies have analyzed the impact of an adipogenic exposure during the pregnancy and lactation period by comparing both sexes separately, as reported from rat [14–16] and mouse models [17–19]. In humans, associations between parental body mass index and children's weight and body fat are reported to be sex-dependent, an observation not being explained as yet [20,21]. Developmental components to sex-specific differences in human disease risk have also been reported for several common disorders [22], such as in diabetes [23].

Most rodent studies utilized inbred mouse strains such as C57BL/6J that upon obesogenic feeding develop pronounced obesity already being complicated by hyperinsulinemia, hyperleptinemia, and dyslipidemia [17,24,25]. However, in the human situation, a substantial proportion of fertile obese women still have largely compensated metabolic homeostasis [26,27]. We hypothesized that exposure during the earliest developmental stages of life even to milder forms of maternal obesity is already sufficient to induce programming of long-term health risks in offspring of each sex. Thus, we aimed at utilizing a mouse model showing less pronounced maternal adiposity during early developmental stages. For this purpose, we took advantage of the outbred Naval Medical Research Institute (NMRI) mouse stock that is less vulnerable to the consequences of high-fat feeding than the C57BL/6J strain [28].

In the offspring of NMRI dams, we assessed long-term phenotypic outcomes, metabolic features, and underlying gene expression profiles in each sex separately and at different life times. Offspring were transferred to foster dams to specifically preclude programming influences during lactation and were subsequently grown up in the absence of any additional postnatal high-fat diet exposure. Nevertheless, in later life of offspring we demonstrated signs of metabolic disease associated with differential gene expression patterns that strikingly differ between sexes and that are evoked by the obesogenic environment of dams in the pre-conceptional period and early phase of development.

2. Materials and methods

2.1. Experimental design

Male and female NMRI mice (RjHan:NMRI; originally Swiss mice transferred to the US Naval Medical Research Institute, then to the Central Institute for Laboratory Animal Breeding, Hannover, Germany in 1958) were purchased from Janvier (Le Genest St Isle, France). This outbred stock is characterized by an albino appearance, a rapid growth rate, a high success of reproduction, and large litter sizes [29]. Mice were maintained under specific pathogen-free conditions in the closed barrier facility of the Gene Center Munich at 23 °C, 40% humidity and with a 12 h light/dark cycle (lights on at 7 AM). All animals had free access to their specific rodent diet and water *ad libitum*. All experiments were approved by the Committee on Animal Health and Care of the local governmental body of the state of Bavaria and performed in strict compliance with the European Economic Commission (EEC) recommendations for the care and use of laboratory animals (European Communities Council Directive of 24th November 1986 [86/609/EEC]).

28 female NMRI mice at 3 weeks of age were randomly distributed into a total of 3 groups. Two groups (7 mice each) received the D12492 high-fat, high-calorie diet (HFD) (E15741-34; Ssniff, Soest, Germany) or the control diet to D12492 (CD) (Ssniff; Table 1, Supplemental Fig. A.1). We used the term "HFD" being aware that apart from a high-saturated fat content, the carbohydrate composition was characterized by a ratio of 10:1 of sugar to starch. The third group (foster mothers, 14 mice) received a standard maintenance rodent diet (MD) (V1536, Ssniff; Table 1) after arrival to the animal facility (Supplemental Fig. A.1). Body weight was monitored every three days and body composition was measured weekly. At the age of 12 weeks, mice were mated and screened for vaginal plugs every morning and evening. Females of the experimental groups remained on their specific diets and those of the foster group were on CD during pregnancy and lactation to ensure that any postnatal dietary exposure of offspring was only to CD.

The term "peri-conceptional" was used to refer to maternal obesity prior to conception and during the earliest developmental phases including early gestational periods equivalent to the first and second trimesters in humans. Pregnant females were weighed and their body composition was analyzed every three days. All animals were allowed to give birth naturally. Within 12 h after birth, each litter of the two experimental groups (HFD, CD) was adjusted to a size of 8 animals by culling surplus pups, and directly transferred to one of the dams of the foster group, which gave birth the same day and whose pups were removed (Supplemental Fig. A.1). All litters in each maternal group were greater than 8 pups and none of the dams were excluded due to large variations in litter size. To avoid any selection influence of the experimenter, offspring were randomly chosen regardless of sex, size or other features.

Offspring were weighed every three days. At day 21, all offspring were weaned onto CD. Thereafter, body composition of offspring was monitored weekly and from week eight onwards every two weeks up to 5 months of life, when mice became too large for analysis of body composition. At age 9 months, offspring were sacrificed. In a separate

Table 1
Diet compositions.

	High-fat, high-calorie diet (HFD)	Control diet (CD)	Maintenance diet (MD)
Crude nutrients (g/100g)[a]			
Crude protein	24.1	24.1	19.3
Crude fat	34.0	5.1	3.4
Crude fiber	6.0	6.4	5.0
Crude ash	6.1	6.2	6.5
N free extracts	27.0	54.7	55.3
Myristic acid (C14:0)	1.03	0.06	0.01
Palmitic acid (C16:0)	8.06	0.70	0.47
Stearic acid (C18:0)	5.61	0.37	0.08
Oleic acid (C18:1)	12.13	1.44	0.62
Starch	2.2	26.0	37.5
Sugar / dextrines	22.4	26.1	4.7
Energy (%) derived from			
Protein	19.0	27.0	33.0
Carbohydrates	21.0	60.0	58.0
Fat	60.0	13.0	9.0
Total energy (MJ/kg)	21.4	15.1	13.0

N, nitrogen.
[a] Single diet components are denoted in g per 100 g diet.