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Pre-gestational vs gestational exposure to maternal obesity differentially programs the offspring in mice

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Abstract

Aims/hypothesis—Maternal obesity is associated with an increased risk of obesity and impaired glucose homeostasis in offspring. However, it is not known whether a gestational or pre-gestational exposure confers similar risks, and if so, what the underlying mechanisms are.

Methods—We used reciprocal two-cell embryo transfers between mice fed either a control or high-fat diet (HFD) starting at the time of weaning. Gene expression in placenta was assessed by microarray analyses.

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Results—A pre-gestational exposure to a maternal HFD (HFD/control) impaired fetal and placental growth despite a normal gestational milieu. Expression of imprinted genes and genes regulating vasculogenesis and lipid metabolism was markedly altered in placenta of HFD/control. An exposure to an HFD (control/HFD) only during gestation also resulted in fetal growth restriction and decreased placental weight. Interestingly, only a gestational exposure to an HFD (control/HFD) resulted in obesity and impaired glucose tolerance in adulthood.

Conclusions/interpretation—An HFD during pregnancy has profound consequences for the offspring later in life. Our data demonstrate that the mechanism underlying this phenomenon is not related to placental dysfunction, intrauterine growth restriction or postnatal weight gain, but rather an inability of the progeny to adapt to the abnormal gestational milieu of an HFD. Thus, the ability to adapt to an adverse intrauterine environment is conferred prior to pregnancy and it is possible that the effects of a maternal HFD may be transmitted to subsequent generations.

Keywords

Fetal programming; Gestation; Glucose intolerance; Maternal obesity; Pregnancy

Introduction

Obesity in pregnancy is associated with an abnormal intrauterine metabolic milieu that can have long-lasting effects on offspring [1–23]. Maternal obesity in humans is associated with intrauterine growth restriction (IUGR) and large for gestational age (LGA) offspring, and both IUGR and LGA offspring of obese mothers have a high risk of developing obesity in later life [2–9]. We have recently shown that offspring of rats and mice fed a high-fat diet prior to and during pregnancy and lactation develop obesity later in life [12, 13].

Previous studies suggest that obesity has adverse effects on the oocyte and embryo [11, 13–20]. Thus, it is possible that exposure to an adverse metabolic milieu prior to pregnancy accounts for some of the adverse outcomes observed in offspring. In previous studies, we used a novel approach whereby we carried out a series of reciprocal early two-cell embryo transfers between mice fed a control or high-fat diet (HFD). Surprisingly, we found that pre-gestational exposure to obesity programs the brain reward system [24]. The purpose of the current study was to test the hypothesis that pre-gestational exposure to an HFD represents another critical window of exposure resulting in an abnormal metabolic phenotype in offspring.

Methods

Animals and diet

The animal model has been previously described [24]. In brief, female mice at 4 weeks were started on an HFD diet (Harlan TD06414 [Harlan Laboratories, Easton, MA, USA]: 18.4% kJ protein; 21.3% from carbohydrate [sucrose]; 60.3% from fat [lard and soybean oil]; 21.34 kJ/g) or a control diet (Lab Diet5015: 19.8% kJ from protein; 54.9% from carbohydrate [cornstarch]; 25.3% from fat [soybean oil]; 15.48 kJ/g). Food intake was measured weekly. Diets were continued for 10–12 weeks prior to mating. Control dams were mated to C57BL/6-Tg (UBC-GFP) 30Scha/J males (Jackson Labs, Bar Harbor, ME, USA), which

carry a transgene that contains the green fluorescent protein coding region under control of the ubiquitin C promoter. Transfer of both HFD-derived embryos and control-derived embryos into the same recipient minimises effects of variability between recipients of the two donor groups (electronic supplementary material [ESM] Fig. 1).

Two-cell embryos were transferred into pseudopregnant females 24 h after detection of vaginal plugs. Ten HFD-derived and ten control-derived embryos were mixed together, and ten randomly selected embryos were transferred into each oviduct of recipients. Donors and recipients were age matched. Sex determination of embryos was performed by PCR amplification of male-specific *Sry* gene (primer sequences in ESM Table 1). Diets were continued through gestation and lactation and pups were placed on control chow diets at weaning (3 weeks).

These studies were approved by the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia and University of Pennsylvania.

Placenta analyses

Histology—Tissues were fixed overnight at 4°C, dehydrated, and bivalved in sagittal section through the mid placental plane. All tissues were placed in similar orientation prior to embedding in paraffin, and serial sagittal tissue sections, 4 µm thick, were cut through the mid placenta and stained with haematoxylin and eosin. Images were collected using a Leica (Leica Microsystems, Wetzlar, Germany) DMRBE upright microscope with ×2.5, ×5 and ×10 objectives, a 5.0 Mega-Pixel colour CCD camera, and iVision acquisition software (BioVision Technologies, Chester Springs, PA, USA). Micrographs were analysed with ImageJ v1.4.5 (National Institutes of Health, Bethesda, MD, USA). The total cross-sectional area of placenta was outlined from its base at the chorioallantois to the giant cell layer. The total cross-sectional area of labyrinth was outlined based on its characteristic morphological features. Ratios of area of labyrinth to placenta as well as maternal decidua to placenta were calculated [25].

DNA and RNA extraction—Genomic DNA and total RNA were extracted using Allprep DNA/RNA Mini Kit (Qiagen, Valencia, CA, USA). Concentrations were determined with NanoDrop (Thermo Fischer Scientific, Waltham, MA, USA), and RNA was bioanalysed for integrity (Agilent, Santa Clara, CA, USA) and samples with RNA integrity number scores less than 7.5 were excluded.

Real-time PCR—Real-time PCR was performed using TaqMan (Life Technologies, Grand Island, NY, USA) primers (ESM Table 1) [12, 13]. *Gapdh* expression (there were no differences in expression between the four study groups) was assayed for each sample in parallel with the gene of interest.

Microarray analysis—RNA was isolated from embryonic day (E)12.5 placentas and complementary DNA was generated using Ambion WT Expression Kits (Ambion, Austin, TX, USA) and labelled with GeneChip WT Terminal Labeling Kits (Affymetrix 900671, Santa Clara, CA, USA). Samples were hybridised to GeneChip Mouse Exon 1.0ST arrays (Affymetrix 901168) and scanned (GeneChip Scanner 3000 7G System, Affymetrix). Probe

intensity data were normalised and summarised to transcript cluster ID level using RMA-Partek Genomics Suite (Partek, St Louis, MO, USA). Sample variation was visualised using principal components analysis. Pairwise comparisons were made between groups and false discovery rates (FDR) were calculated along with adjusted p values by the Benjamini and Hochberg step-up method. The results were filtered with FDR <0.15 and fold change >1.5. Ingenuity Pathway Analysis software (Qiagen, Redwood City, CA) was used to evaluate functional pathways.

Metabolic analyses

In dams at 13 weeks of age, glucose tolerance tests (GTTs) and body composition measurements (NMR-LF50, Bruker) were performed in a separate group of animals from those used for embryo transfer. In offspring at 13 weeks of age, GTTs and body composition measurements (NMR-LF50, Bruker, Billerica, MA, USA) were performed. Animals were fasted for 6–8 h, 2 g/kg of glucose was given i.p. and blood glucose was sequentially measured in tail vein blood via clipping of the distal tail (Freestyle Glucometer-Abbott, Princeton, NJ, USA). Fasting serum leptin (ELISA, Millipore, Billerica, MA, USA), insulin (RIA, Millipore) and NEFA (NEFA-HR kit, Wako Diagnostics, Richmond, VA, USA) were measured in dams and offspring. Liver was harvested at 13 weeks of age from offspring and snap frozen for triacylglycerol analyses using Cayman kits (Cayman Chemical, Ann Arbor, MI, USA).

Statistical analysis

Results are expressed as means \pm SEM. All data sets were analysed for normality. A two-tailed Student's t test was performed for normally distributed data, and a Mann–Whitney U test performed on non-parametric data. A two-way ANOVA was used to determine if there was an interaction between embryo donor exposures and embryo recipient exposures. Statistical analysis was performed using Graphpad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). A power calculation was performed prior to the study and a post-hoc power calculation was performed for fetal, placental and adult weights.

Results

HFD causes obesity and glucose intolerance in dams

Body weights of dams at initiation of diets (at 4 weeks of age) were not different between study groups (donors 18.2 \pm 1.1 g; recipients 20.1 \pm 0.9 g); however, at breeding, HFD dams were significantly heavier, had higher leptin and NEFA levels, and were glucose intolerant. Fasting blood glucose levels were not significantly different between groups (Table 1). Food intake (3–4 g per day) was also similar between groups, resulting in increased energy intake by HFD-fed dams.

Similar to what has previously been shown [26], there was a significant decrease in the number of female progeny at E12.5 in HFD/control and HFD/HFD groups (Table 2). Interestingly, by postnatal day 14, there were no differences in female:male sex ratios between groups as there was increased mortality in male newborn pups compared with females. It is not clear why males have increased postnatal mortality, but postnatal mortality

is generally increased in human males [27]. In addition, there were no differences in average litter size between groups.

Exposure to a maternal HFD causes fetal growth restriction and abnormal placentation

A pre-gestational and gestational exposure to a maternal HFD (HFD/control, control/HFD and HFD/HFD) similarly decreased male and female fetal weight at E12.5 and E17.5 (Fig. 1a, b). Furthermore, there were no significant differences between fetal weight in control/HFD and HFD/HFD groups. Thus, the effect of a maternal HFD on fetal weight was similar in both donor groups, and not exacerbated or mitigated by a pre-gestational HFD exposure (two-way ANOVA $p=NS$; Fig. 1a, b). However, HFD/control offspring displayed a greater increase (a nearly 11-fold increase in weight for both male and female offspring) in weight gain over the course of gestation as compared with control/control, control/HFD and HFD/HFD progeny (approximately 8–9-fold increase in all three groups; ESM Fig. 2a, b).

Consistent with what was observed for fetal weight, a pre-gestational and gestational exposure to a maternal HFD decreased placental weight at E12.5 in males ($p<0.05$), with a trend toward significance in females (Fig. 1c, d). However, in contrast to fetal weights, by the end of gestation, placenta weights were increased in all HFD groups compared with control/control. Furthermore, this effect does not appear to be a result of an interaction between pre-gestational and gestational exposures, indicating that the gain in placental mass during gestation is solely a result of in utero exposure (two-way ANOVA $p=NS$). Finally, the change in placental weight over gestation was similarly increased in HFD/control, control/HFD and HFD/HFD compared with control/control (ESM Fig. 2c, d).

Neither a pre-gestational nor gestational exposure to maternal HFD impacted the relative proportion occupied of each layer of placenta at E12.5 (Fig. 1e). However, the depth of placenta as a proportion of the placenta/decidua unit was similarly decreased in all exposed groups as compared with control ($p<0.05$; Fig. 1f; ESM Fig. 3).

Mismatch between the pre-gestational and gestational environments impairs placental function

Fetal:placental weight ratios can be a marker of placental function and an indicator of the ability of placenta to support the demands of fetal growth [28]. At E17.5, when fetal growth is primarily dependent on placental function, the fetal:placental weight ratio was decreased for female ($p<0.02$) and male ($p<0.005$) gestations in all exposed groups (Fig. 2a, b). This decrease was observed as early as E12.5 in control/HFD and HFD/HFD females ($p<0.03$) and in HFD/control males ($p<0.04$; Fig. 2a, b). This effect does not appear to be a result of an interaction between pre-gestation and gestational exposures, as a combination of the two exposures does not exacerbate or mitigate the observed effect (two-way ANOVA $p=NS$).

In utero exposure to maternal HFD increases weight and body fat content in adult offspring

While a pre-gestational exposure to an HFD resulted in growth-restricted fetuses and newborn pups (Fig. 3), there was no effect on adult weight (Fig. 4), and body fat content and serum leptin levels were similar between HFD/control and control/control offspring (Fig. 4).

By contrast, a gestational exposure to a maternal HFD resulted in increased body fat and elevated leptin levels in male and female adult animals compared with control/control (Fig. 4). There was no interaction between pre-gestational and gestational exposures that would account for observed differences in body composition or serum leptin levels (two-way ANOVA $p=NS$).

A mismatch in pre-pregnancy and gestational nutritional environments impairs glucose tolerance

Surprisingly, exposure to a maternal HFD resulted in impaired glucose tolerance only in adult control/HFD offspring and these animals had a significant increase in AUC_{glucose} compared with control/control offspring (females, $p<0.01$; males $p<0.03$; Fig. 5a–d). This effect was not observed in HFD/HFD offspring. Two-way ANOVA analysis showed that the AUC_{glucose} was impacted by an interaction between a pre-gestational and gestational exposure ($p<0.05$). Similarly, fasting plasma insulin levels and hepatic triacylglycerol levels were only significantly increased in control/HFD males and females (Fig. 5e–h). Fasting plasma NEFA levels and food consumption did not differ between groups ($p>0.05$).

Pre-gestational and gestational HFD alters placental gene expression

We performed gene expression profiling of E12.5 placentas to determine whether the transcriptome profile was associated with differences in adult phenotypes. Overall, exposure to a maternal HFD in utero had a more profound effect on gene expression in placenta than a pre-gestational exposure. A total of 1,026 genes (ESM Tables 2, 3, 4 and 5) were significantly enriched in the HFD recipients compared with controls (FDR <0.05 , $p<0.05$). By contrast, far fewer genes were significantly enriched as a result of a pre-gestational exposure to a maternal HFD (177 genes, FDR <0.05 , $p<0.01$).

Expression profiles of HFD/control were compared with control/control revealing enrichment of functional annotations including genes involved in solute transport, steroid biosynthesis, lipid metabolism, tissue development and vascularisation (Table 3, ESM Table 2). Interestingly, expression of a number of imprinted genes was significantly decreased in HFD/control including *Slc22a3* and *Slc22a18*, which encode organic ion and solute carriers; *Dio3* (iodothyronine deiodinase), which encodes a protein that plays an essential role in regulation of thyroid hormone inactivation during embryological development; and *Cts6* (cathepsin 6), which is within the *H19/Igf2* imprinted region. Also of note, was the finding that expression of *Cyp17a1* was markedly increased. *Cyp 17a1* encodes a key biosynthesis enzyme of oestrogen, which is critical in regulating adipogenesis and adipocyte development and is a candidate gene for obesity susceptibility in humans [29, 30].

Expression of *Maoa* (monoamine oxidase A) was reduced by 1.54-fold ($p<0.01$). Monoamine oxidase A regulates maternal monoamine neurotransmitter transfer across the placenta to the fetus, and decreased expression may contribute to the changes in the reward circuitry that we observed in brains of adult offspring of HFD/control animals [24]. Despite relatively high FDR values in the array data, when expression of *Cyp17a1*, *Maoa*, *Dio3*, and *Cts6* were validated using quantitative PCR, we found significant changes in expression (2–3-fold vs control/control [$p<0.01$]; ESM Table 6).

Many more genes were differentially expressed in HFD/HFD than in control/HFD vs control/control, suggesting that a pre-gestational and gestational exposure to HFD is more consequential. However pathways that were affected were very similar between the two groups (Table 3, ESM Tables 3, 4 and 5). Genes whose expression was decreased have functions in biological processes that regulate angiogenesis (*Fbln7*, members of the carcinoembryonic antigen gene family and *Flt3*), trophoblast invasion (genes that encode cathepsins and matrix metalloproteinases) and fetal well-being (*Psg18*, *Psg19*, *Psg27* and *Psg29*). Low levels of pregnancy-specific glycoproteins are associated with spontaneous abortion [31], intrauterine growth retardation and preeclampsia [32].

By far the largest group of genes with increased expression only in the HFD/HFD placenta have roles in inflammation and include *Aim2*, *C2*, *Ccl8*, *Cd3g*, *Clec2d*, *Dusp14*, *Gpmb*, *Il2ra*, *Lbp*, *Masp1*, *Pik3cg*, *Serping1* and *Tnfsf9* (ESM Table 5). Interestingly, several genes regulating production of ceramide and sphingosine metabolism were also significantly upregulated only in HFD/HFD placenta (including *A4galt*, *Degs2*, *Ppp2r5a* and *Slpr1*). Ceramide and sphingosine are lipid signalling molecules with diverse functions such as cell death and lipid homeostasis [33]. These data suggest that a pre-gestational and gestational exposure magnifies inflammation and lipid abnormalities in placenta.

While gene expression profiles were very similar between control/HFD and HFD/HFD, a number of interesting genes were only dysregulated in control/HFD placenta, some of which may play a role in the difference in phenotypes between these two groups. These include *Meg3*, *Lpar4* and *Rsad2*. Expression of *Meg3*, a long non-coding RNA and imprinted gene is decreased in islets from patients with type 2 diabetes [34]. *Lpar4* encodes the receptor for lysophosphatidic acid, a glycerophospholipid that has been linked to insulin resistance [35]. *Rsad2* regulates adipocyte differentiation and decreased levels are found in adipose tissue of diet-induced obese mice [36].

To gain a better understanding of the common factors that were associated with fetal growth restriction in the three exposure groups, we identified genes that were differentially expressed in all three treatment groups compared with control/control. Expression of transporters (*Slc22a18* and *Slc5a3*), genes involved in placental invasion, extracellular matrix degradation and vascular remodelling (*Mmp12*, *Amot*, *Cts6* and *Mgp*) were all similarly downregulated in placenta from the three IUGR groups. Interestingly, several of these have been identified as being dysregulated in pre-eclampsia, which is often associated with maternal obesity in humans that is characterised by poor placental invasion and abnormal vascular remodelling [37].

Of significance was our finding that expression of 11β -*Hsd2* (also known as *Hsd11b2*), which encodes 11β -hydroxysteroid dehydrogenase, was substantially reduced in placenta of all three groups. 11β -hydroxysteroid dehydrogenase inactivates cortisol to cortisone and protects the developing fetus from the deleterious effects of circulating maternal glucocorticoids. Levels are significantly reduced in placentas of IUGR pregnancies in humans [38] and in animal models of uteroplacental insufficiency [38].

A targeted approach was taken to determine whether maternal HFD alters expression of genes regulating fetal and placental growth, nutrient detection and transport. Insulin-like growth factor (IGF)-1 and IGF-2 are important regulators of fetal and placental development [39]. There were no significant differences in *Igf1* expression between the four groups at either age (Fig. 6). By contrast, gestational exposure to an HFD (control/HFD and HFD/HFD) resulted in a two-fold increase in *Igf1r* expression at E12.5, whereas a pre-gestational or gestational exposure to an HFD was associated with decreased expression of *Igf2* and *Igf2r* (two-way ANOVA analysis showed a significant interaction between pre-gestational and gestational exposures [$p=0.04$]; Fig. 6). This is surprising as *Igf1r* and *Igf2* are usually coordinately regulated. Thus, the biological significance of these findings is unclear. At E17.5, expression of *Igf2* and *Igf2r* remained decreased in all three HFD exposed groups, but the interaction was lost and the two-way ANOVA was not significant. Expression of *Igf1r* normalised and was similar between all four groups (Fig. 6).

Pre-gestational or gestational exposure to maternal HFD resulted in decreased expression of different classes of placental nutrient transporters. At E12.5, expression of *Mtor*, *Slc38a1* (an Na⁺-dependent system A neutral amino acid transporter), *Glut1* (also known as *Slca1*) and *Cd36* (a long-chain fatty acid translocase), were modestly reduced in placenta compared with control/control; however, there was no interaction between exposure groups (two-way ANOVA NS; Fig. 6). *Mtor* and *Slc38a1* expression had normalised by day 17.5 in placenta in all groups. By contrast, *Cd36* expression was persistently decreased in pre-gestational HFD exposure groups. Thus, these data suggest that changes in nutrient transport are not responsible for differences in metabolic phenotype between groups. It remains to be determined if these changes in expression reflect changes in nutrient transport.

Discussion

It has been well described that obesity in pregnancy results in multiple phenotypic consequences for offspring, including obesity, diabetes and cardiovascular disease [2–13, 40, 41]. However, the window of exposure to a maternal HFD that is responsible for conferring these effects has not been well defined. Using reciprocal embryo transfers, we have been able to separate a pre-gestational exposure to a maternal HFD from the effects of an in utero exposure.

The first novel observation is that a pre-gestational exposure to maternal HFD restricts fetal and placental growth during gestation. The fetus remains growth restricted and surprisingly, so does the newborn pup. Thus, even in the face of a normal gestational milieu, a pre-pregnancy exposure to a maternal HFD impairs fetal and placental growth. These findings continue to broaden the window of susceptibility to the adverse effects of maternal HFD to include the oocyte and its pre-conception environment. These data suggest that it is possible that effects of maternal obesity may be transmitted through the germline to subsequent generations and this has profound implications for human health. The effects of maternal obesity can also be transmitted to future generations through gestational exposure. Female offspring of obese dams may develop insulin resistance and glucose intolerance during pregnancy, thus affecting the F2 generation and inducing a vicious cycle.

Despite being growth restricted and displaying catch-up growth after birth, HFD/control offspring do not display glucose intolerance. It is possible that with age or diet or other stressors, glucose tolerance will become impaired in this group as well. Interestingly, in our previous studies a pre-gestational exposure to obesity also programs the reward system in adult offspring [24]. While food intake was not increased during adulthood, food preferences may be affected later in life, as other investigators have found [42, 43]. It is also possible that there are other metabolic and phenotypic abnormalities such as impaired beta-cell function, energy expenditure, physical activity or behaviour changes that characterise these offspring, and additional phenotypic characterisation will be carried out in the future.

One of the most interesting findings in the HFD/control group was differential expression of several imprinted genes in placenta. This suggests that an HFD prior to pregnancy may re-program imprinted marks during oocyte and early embryonic development. The acquired imprint instability may be carried into the next generation and increase the risk of chronic diseases in adulthood. Further, altered expression of several imprinted genes has been associated with human obesity and in animal models of obesity [44].

The second novel observation is that exposure to a maternal HFD solely during gestation has consequences during gestation and into adulthood. The findings of previous studies using different paradigms have suggested that an abnormal metabolic phenotype in the adult results from fetal programming as a result of IUGR, postnatal programming as a result of accelerated catch-up growth in the newborn period, or is secondary to obesity in adulthood [10, 45]. Our data would suggest that these are not primary factors contributing to the impaired glucose tolerance of adult (13 weeks old) control/HFD animals. In our model, both control/HFD and HFD/HFD animals (sharing an identical uterine environment) display IUGR, have similar postnatal growth curves and an obese body composition as adults, but have discordant glucose tolerance. The difference between groups is that the control/HFD animals, derived from control-fed dams, lack the pre-gestational exposure. Consequently, these embryos have not co-adapted with their gestational exposure, and therefore are ill prepared for the antenatal stress of fetal life in a high-fat environment, which is consistent with the concept of predictive adaptive programming [46]. Predictive adaptive programming has usually been discussed in the context of limited maternal nutrient availability. However, our data suggest that this response also applies to maternal nutrient excess.

Surprisingly, HFD/HFD female offspring were not glucose intolerant in adulthood; however, they had significantly higher body fat content than control/control, which places them at high risk for later development of glucose intolerance. However, HFD/HFD males did have glucose intolerance, similar to what we have previously observed in male offspring of obese dams [13]. As ageing is known to affect adiposity and glucose homeostasis [47], it is possible that glucose intolerance will develop with age in females and worsen over time in males.

We observed few sex differences in metabolic and growth variables in adulthood between the groups except for weight (female gestational exposure to an HFD produced increased weight compared with a control gestational exposure). However, despite weight differences, body fat content was similarly increased in both sexes exposed to an HFD during gestation.

It is possible that other metabolic or behavioural phenotypes may differ with age between sexes and this remains to be determined.

In conclusion, obesity during pregnancy has profound consequences for the offspring later in life. Our data demonstrate that the mechanism underlying this phenomenon is not related to placental dysfunction, IUGR or postnatal weight gain, but rather an inability of progeny to adapt to the abnormal gestational milieu of an HFD. Thus, the ability to adapt to an adverse intrauterine environment is conferred prior to pregnancy and it is possible that the effects of maternal obesity may be transmitted to subsequent generations.

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Abbreviations

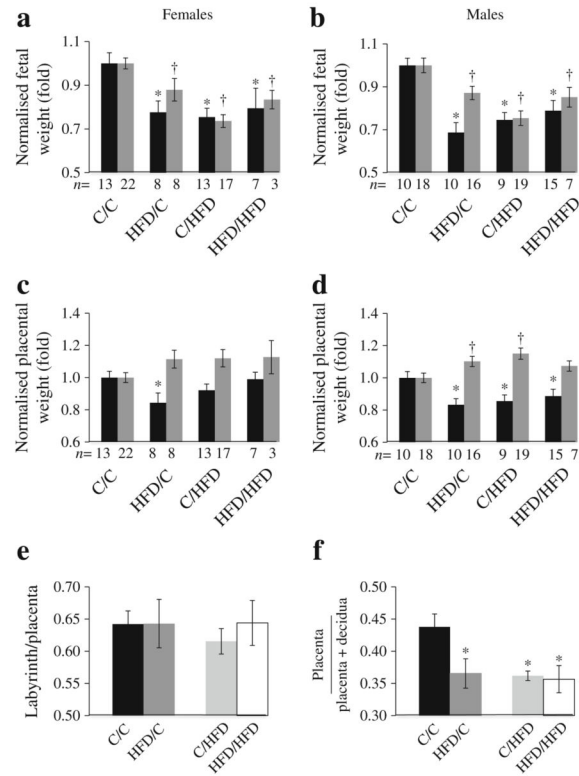
FDR	False discovery rate
GTT	Glucose tolerance test
HFD	High-fat diet
IUGR	Intrauterine growth restriction
LGA	Large for gestational age

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**Fig. 1.**

Female (a, c) and male (b, d) fetal (a, b) and placental (c, d) weight (black bars, E12.5; grey bars, E17.5) normalised to C/C and expressed as means±SEM. * $p < 0.05$ vs C/C at E12.5; † $p < 0.05$ vs C/C at E17.5. Six C and five HFD litters at each age. (e) Labyrinth/placenta ratios and (f) placenta/(placenta+decidua) ratios. $n=6-8$ placentas from each group. Data are expressed as means±SEM. * $p < 0.05$ vs C/C. C, control

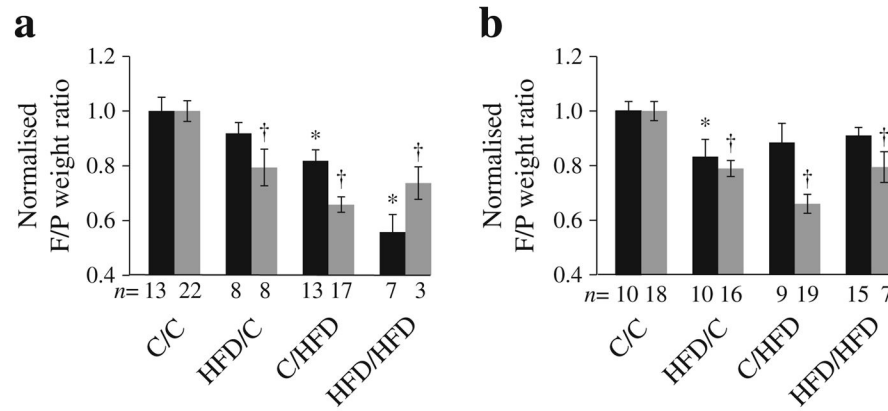


Fig. 2. F:P weight ratios were calculated for female (a) and male (b) mice for each group (black bars, E12.5; grey bars, E17.5). * $p < 0.05$ vs C/C at E12.5; † $p < 0.05$ vs C/C at E17.5. Six C and five HFD litters at each age. C, control; F/P, fetal/placental

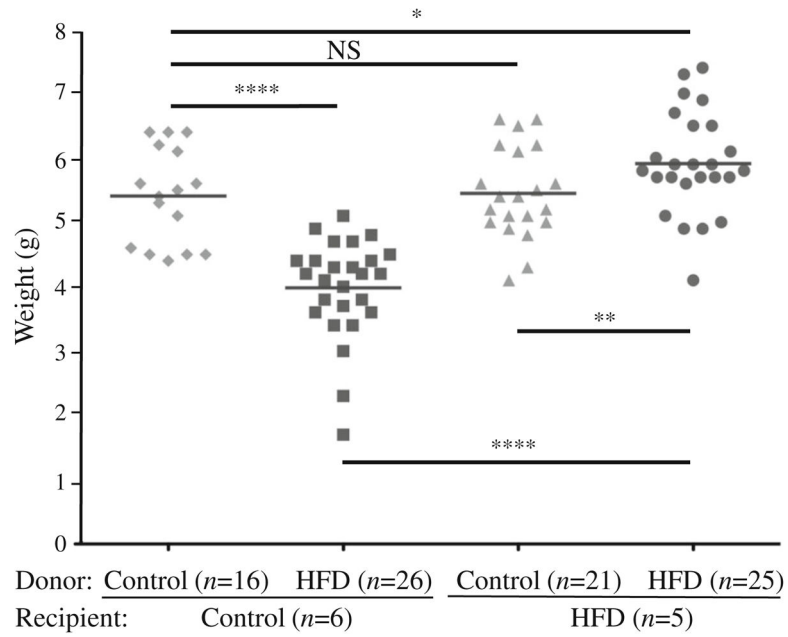


Fig. 3. Newborn weights. Six control and five HFD litters. Repeated measures were performed on individual animals from 2–13 weeks of age. (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$). The horizontal lines through the data points represent the mean

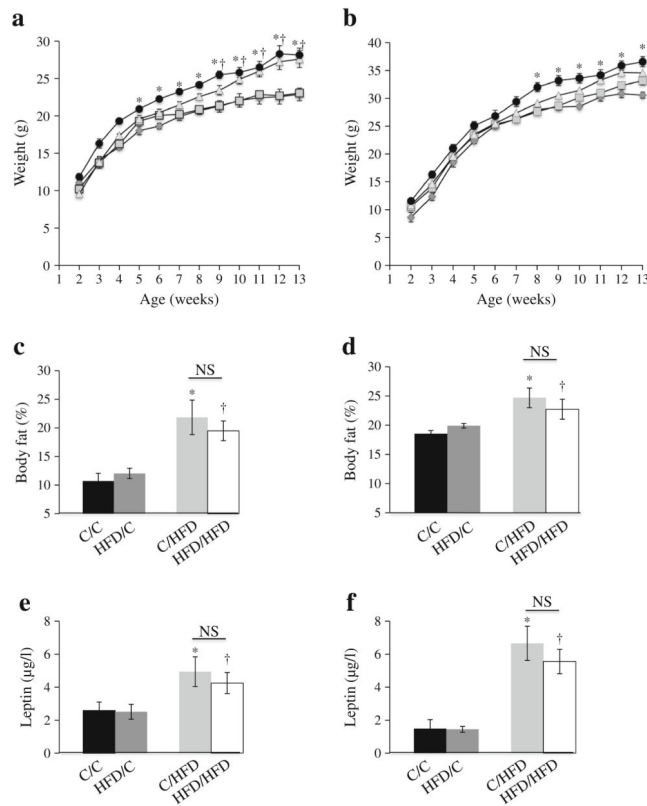


Fig. 4. Adult weight and percentage body fat. Female (**a**) and male (**b**) offspring were weighed weekly. Repeated measures were performed on individual animals from 2–13 weeks of age. Black circles, HFD/HFD; triangles, C/HFD; squares, HFD/C; diamonds, C/C. Percentage body fat (**c**, **d**) and leptin levels (**e**, **f**) of female (**c**, **e**) and male (**d**, **f**) progeny at 13 weeks of age. Data are expressed as means±SEM. * $p < 0.05$ C/HFD vs C/C; † $p < 0.05$ HFD/HFD vs C/C. C, control

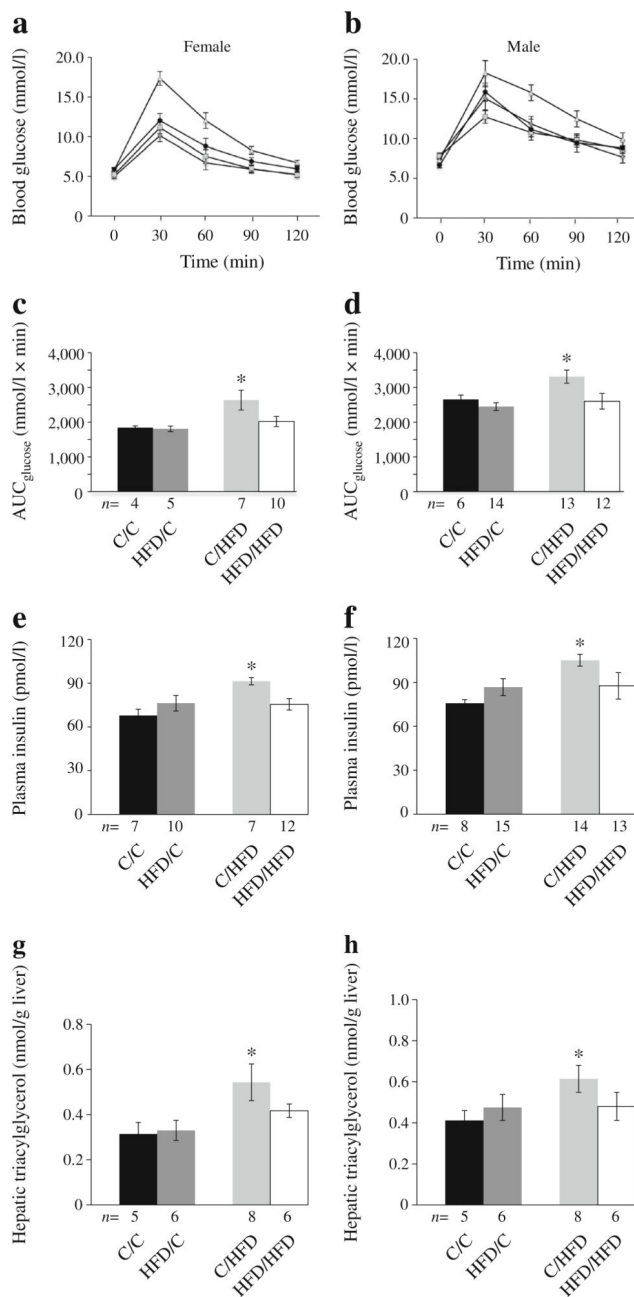


Fig. 5. Metabolic abnormalities in 13-week-old offspring. 2 h glucose tolerance tests in females (**a**) and males (**b**). Black circles, HFD/HFD; triangles, C/HFD; squares, HFD/C; diamonds, C/C. AUC_{glucose} in females (**c**) and males (**d**). Fasting plasma insulin levels in females (**e**) and males (**f**). Fasting hepatic triacylglycerol levels in females (**g**) and males (**h**). Data are expressed as means ± SEM. **p* < 0.05 vs C/C. C, control

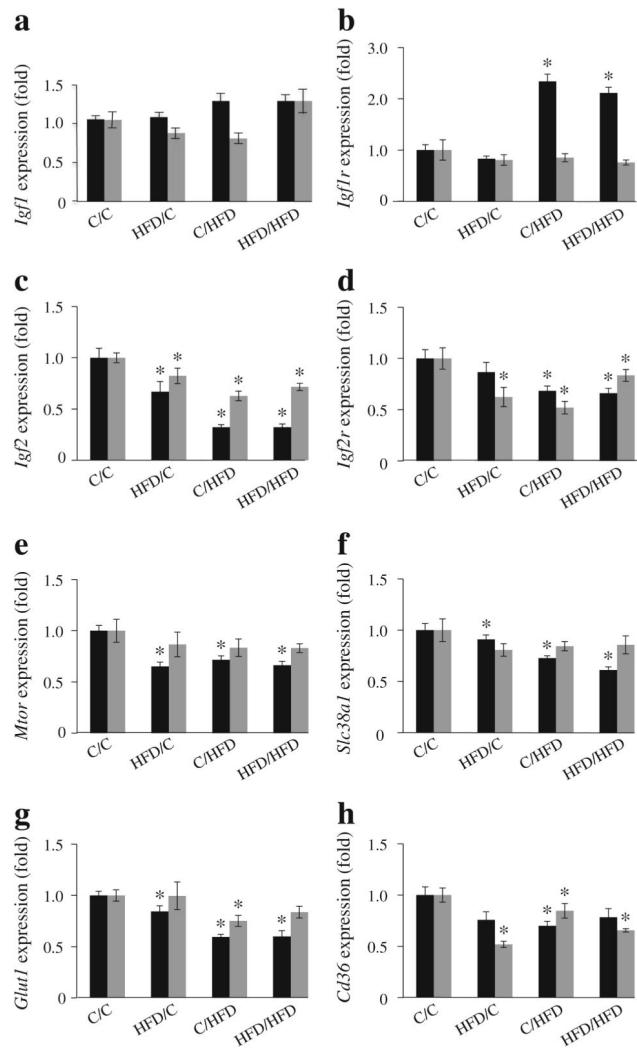


Fig. 6. Placental gene expression patterns. Gene expression was measured by quantitative PCR in placenta at E12.5 (black bars) and E17.5 (grey bars). $n=7-11$. Fold change values for each time point are relative to C/C and normalised to *Gapdh*. * $p < 0.05$ vs C/C at each age. C, control

Table 1

Characteristics of the control and HFD dams

Variable	Control (n=14)	HFD (n=37)	p value
Donors			
Weight (g)	20.56±0.24	36.42±0.86	<0.0001
2 h GTT, AUC (mmol/l×min) (n=10 each group)	785±31	1,262±70	<0.0001
Fasting blood glucose (mmol/l) (n=10 each group)	5.47±0.12	5.33±0.18	0.5614
Leptin (µg/l) (n=10 each group)	2.68±0.25	57.14±5.35	<0.0001
Recipients			
n	21	25	
Pre-pregnancy			
Weight (g)	27.04±0.75	43.37±0.77	<0.0001
2 h GTT, AUC (mmol/l×min) (n=10 each group)	747±13	1,198±104	0.0082
Fasting blood glucose (mmol/l) (n=10 each group)	4.88±0.26	4.37±0.33	0.3234
Leptin (µg/l) (n=10 each group)	4.47±1.18	19.65±3.19	0.0017
E12.5			
Fasting blood glucose (mmol/l) (n=10 each group)	5.74±0.28	7.09±0.01	0.0522
Leptin (µg/l) (n=10 each group)	8.99±1.77	33.34±9.63	0.0167
E17.5			
Fasting blood glucose (mmol/l) (n=10 each group)	6.41±0.65	6.07±0.41	0.6607
Leptin (µg/l) (n=10 each group)	9.01±0.75	41.9±7.91	0.0251

Data are presented as means±SEM

Table 2

Distribution of progeny and fraction of female fetuses across groups at E12.5

Group	Males (n)	Females(n)	Female fraction of total
Control/control	18	23	0.56
HFD/control	30	18	0.38**
Control/HFD	30	32	0.52
HFD/HFD	30	14	0.32**

**
 $p < 0.01$ vs control diet

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Table 3Results of ingenuity functional annotation clustering of genes enriched in each treatment group^a

Treatment group	Functional annotation	<i>p</i> value	Genes
HFD/C vs C/C ^b	Lipid metabolism	2.70×10 ⁻⁴	2
	Steroid metabolism	9.00×10 ⁻⁴	2
	Tissue development	4.19×10 ⁻⁴	3
	Growth failure	8.89×10 ⁻³	4
C/HFD vs C/C ^c	Cellular development	1.06×10 ⁻⁶	34
	Protein degradation	5.29×10 ⁻⁶	10
	DNA degradation	7.94×10 ⁻⁶	9
	Vasculogenesis	4.39×10 ⁻⁵	15
HFD/HFD vs HFD/C ^d	Tissue development	1.12×10 ⁻⁶	57
	Immune response	3.57×10 ⁻⁶	38
	Vasculogenesis	7.82×10 ⁻⁵	18
HFD/HFD vs C/C ^e	Inflammatory response	9.24×10 ⁻¹⁰	67
	Cell death	8.79×10 ⁻⁴	110
	Vasculogenesis	1.31×10 ⁻⁶	30

^aBased on the most meaningful terms^bFiltering based on FDR <0.40, fold change >1.5^cFiltering based on FDR <0.15, fold change >1.5^dFiltering based on FDR <0.40, fold change >1.5^eFiltering based on FDR <0.15, fold change >1.5

C, Control