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Maternal high-fat diet sex-specifically alters placental morphology and transcriptome in rats: Assessment by next-generation sequencing

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ABSTRACT

Introduction: Maternal nutrition is an extremely important health issue. We evaluated the impact of maternal high fat diet (HFD) on pregnancy outcomes, elucidated how the rat placenta and fetus respond to diet manipulation based on fetal sex, and identified candidate genes and pathways.
Methods: Rats were fed a normal or HFD diet for 10 weeks before conception and during gestation. The placenta was collected on gestational day 21 and sexed. Placental histology was analyzed and placental candidate genes and pathways were identified using whole-genome RNA next-generation sequencing.
Results: Pup weights in both sexes from HFD dams were reduced. The weight of the placenta from the HFD group was also decreased in both sexes, but changes in placental layer distributions were only significant for female fetuses. Maternal HFD altered the placental transcriptome in a sex-specific manner. Activation of the placental renin-angiotensin system (RAS) by maternal HFD was associated with fetal growth restriction in both fetal sexes.
Conclusions: The placenta reacts to maternal HFD by altering the placental layer distribution and gene expression in a sex-specific manner. The male placenta in late gestation is thought to exhibit greater plasticity relative to the female placenta; however, fetuses of both sexes exhibited similar growth restriction. Our data reveal an association between the placental RAS and HFD-induced fetal growth restriction.

1. Introduction

Widespread consumption of high-energy foods has increased dramatically and is linked to a global health burden of metabolic syndrome and adverse maternal and fetal outcomes. Nutrition in pregnancy plays an essential role in placental development, fetal growth, and organogenesis. Nutrition has been shown to impact pregnancy outcomes and elicit long-term consequences on the health of offspring through a process known as developmental programming [1,2]. According to multiple studies, diet-induced maternal obesity may lead to multiple adverse pregnancy outcomes [3], including fetal overgrowth [4] and fetal growth restriction [5]; both exhibit adverse impacts on later life.

Previous studies have demonstrated that a maternal high-fat diet (HFD) affects the metabolic phenotype of offspring [6–9]. The health of offspring is critically dependent on placental function, which mediates the dynamic interaction between embryonic and maternal tissues and ensures successful development of the embryo proper. The ability of the placenta to supply nutrients to the fetus is determined by many factors, including the nutritional state of the mother, utero-placental blood flow, and the expression and function of trophoblast nutrient transporters [1,10]. The intrauterine placental renin-angiotensin system (RAS) is one of the major extrarenal RAS throughout pregnancy,

Abbreviations: Ace, angiotensin converting enzyme-1; Ace2, angiotensin converting enzyme-2; Agt, angiotensinogen; Agtr1a, angiotensin II receptor type 1; ANOVA, analysis of variance; Atp6ap2, (pro)renin receptor; AUC, area under curve; DEGs, differentially-expressed genes; FC, fold change; FPKM, fragments per kilobase million; GD, gestational day; GO, gene ontology; GPT, glutamic-pyruvic transaminase; HFD, high fat diet; Ins2, insulin 2; IPGTT, intraperitoneal glucose tolerance test; ITT, insulin tolerance test; IUGR, intrauterine growth restriction; JZ, junctional zone; KEGG, Kyoto Encyclopedia of Genes and Genomes; LZ, labyrinth zone; NGS, next-generation sequencing; PCR, polymerase chain reaction; RAS, renin-angiotensin system; Ren, renin; RNA, ribonucleic acid; SEM, standard error of the mean; SPSS, statistical package for the social sciences

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regulating uteroplacental blood circulation [11,12], and has been linked with maternal hypoxic stress [13], maternal-fetal blood flow and fetal growth restriction [11,14]. Previous studies have reported several candidate regulatory genes for many of the main phases of placental development, from initiation and maintenance of the trophoblast lineage to the complex functions of the mature placenta [15–18]. Studies of HFD mothers in humans and animal models have reported altered placental growth, gene expression, and nutrient transporters [7,19,20]; however, fewer studies have focused on the effects of maternal HFD on global placental gene expression. Moreover, even fewer studies have focused on the potential programming effects of placenta in a fetal sex-specific manner [7,19]. The evidence to date suggests that sex-specific adaptation of the placenta in response to maternal metabolic status may be central to differences in fetal growth and survival [21,22]. Our previous study found that maternal HFD altered the offspring renal transcriptome in a sex-specific fashion [9]. Therefore, we intended to elucidate whether placental adaptations after maternal HFD exposure are sex-specific, analyze the alterations in placenta growth, and identify candidate genes and pathways using whole-genome ribonucleic acid (RNA) next-generation sequencing (NGS).

2. Materials and methods

2.1. Animals and experimental design

Our animal protocol was approved by the Institutional Animal Care and Use Committee of the Chang Gung Memorial Hospital (approval number 2016062805). Virgin Sprague-Dawley rats (n = 20) aged 7 weeks were obtained from BioLASCO Taiwan Co., Ltd, Taipei, Taiwan and allowed to acclimatize for 1 week before being fed specific diets. The rats were maintained in a light-controlled environment (12-h light/12-h dark cycle, 22 °C) with a relative humidity of 55%, throughout the study. Food and sterile tap water were available *ad libitum*. Female rats were weight-matched and assigned to receive either normal rat chow (0.8 kcal/g, 27.5 kcal% protein; 1.73 kcal/g, 59.7 kcal% carbohydrate; 0.43 kcal/g, 12.6 kcal% fat; Fwusow Taiwan Co., Ltd., Taichung, Taiwan) or HFD (0.92 kcal/g, 16.4 kcal% protein; 1.42 kcal/g, 25.5 kcal % carbohydrate; and 3.22 kcal/g, 58 kcal% fat; #D12331, Research Diets, New Brunswick, NJ, USA) for 10 weeks before mating and during pregnancy. Female rats were mated for 3 days. Gestational day (GD) 1 was considered to be mating day 1. Rats were sacrificed on GD 21 after 8 h fasting. Placentas and fetuses were obtained and weighed. We labeled each placenta and fetus according to position in each uterine horn. Placentas from bilateral proximal uterine horns were used for further study. Some placentas were fixed in 10% formalin in neutral-buffered solution for histological analysis, while others were immediately frozen in liquid nitrogen and stored at −80 °C for quantitative polymerase chain reaction (qPCR) analysis.

2.2. Sex determination

RNA was extracted from two piece of fetal tissue (tail and skin) to established sex. The *Sry* gene was amplified to identify placental sex via qPCR. Male placentas were identified by the presence of *Sry* mRNA (Table S1), and female placentas were identified based on its absence. Primers were designed using GeneTool Software (Biotools, Edmonton, Alberta, Canada).

2.3. Biochemical assays

Plasma samples were used to evaluate total cholesterol and liver enzyme glutamic-pyruvic transaminase (GPT) on an automatic biochemical analyzer. Adiponectin and leptin levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (Adiponectin Rat ELISA Kit Abcam, #ab108784 and Leptin Rat ELISA Kit Abcam, #ab100773) according to manufacturer's instructions.

2.4. Intraperitoneal Glucose Tolerance Test (IPGTT) and Insulin Tolerance Test (ITT)

We performed glucose tolerance testing after dietary manipulation for 8 weeks. After an 8-h fast, hyperglycemia was induced by injection with 50% glucose (2 g/kg body weight). Blood glucose levels were measured in tail vein blood samples using a glucometer (Accu-Chek, Roche, Germany) at five time points: before injection and at 15, 30, 60, and 120 min after the glucose load. The ITT was performed by injecting insulin i.p. (1 U/kg body weight) after a 4-h fast. Blood glucose levels were measured before and 15, 30, 60, and 120 min after insulin injection.

2.5. Histological analysis of the placenta

Placentas were sexed, resulting in four groups: control male, HFD male, control female, and HFD female. Four to six litters per group with one section per placenta were used for analysis. Briefly, 3-μm sections were prepared using a Leica RM2255 microtome. These sections were then stained with hematoxylin and eosin. Sections were scanned using a 3D HISTECH Panoramic SCAN slide scanner and analyzed with Panoramic Viewer software. The cross-sectional area of the entire placenta was obtained, and placental zone analysis was performed using ImageJ at 1.5 × magnification. The volumes of placental zones in each section were calculated (area × thickness of the section). From these measurements, the ratios of the labyrinth zone to whole placenta, junctional zone to whole placenta, and decidua to whole placenta were calculated.

2.6. RNA sequencing

Three placentas, one from each independent litter per group, were pooled for whole-genome RNA NGS analysis performed by Welgene Biotech Co. Ltd. (Taipei, Taiwan) after sex determination. Total RNA was extracted using Trizol® Reagent (Invitrogen, USA). Purified RNA was quantified at OD260 nm using an ND-1000 spectrophotometer (Nanodrop Technology, USA) and quantitated using a Bioanalyzer 2100 with a RNA 6000 LabChip kit (both Agilent Technologies, USA). All procedures were performed according to manufacturers' protocols. Library construction was performed using Agilent's SureSelect Strand Specific mRNA Library Preparation Kit for 75SE (Single-End or Paired-End) sequencing on the Solexa platform. Sequencing was performed with the TruSeq SBS Kit. Raw sequences were obtained from the Illumina Pipeline software bcl2fastq v2.0 and expected to generate 4×10^7 reads per sample. Gene expression levels were calculated as fragments per kilobase million (FPKM) mapped reads. We used the Cuffdiff tool, a program from the Cufflinks package [23] and Benjamini-Hochberg correction for multiple-testing, and then calculated values of the test statistic, i.e. p value and q value (p-value adjusted for false discovery rate), to conduct significance testing. The output files from Cuffdiff were further annotated by adding gene functional descriptions and Gene Ontology (GO) classifications. The reference genome and gene annotations were retrieved from the Ensembl database. GO analysis for significant genes was performed using Kyoto Encyclopedia of Genes and Genomes (KEGG) and NIH DAVID Bioinformatics Resources 6.8 (NIH, Bethesda, MD, USA) to identify regulated biological themes [24].

2.7. Quantitative real-time PCR analysis

NGS/RNA-seq analysis provides an excellent tool for analyzing a large number of genes to focus on a smaller number of genes of interest. We further analyzed the expression of a subset of genes of interest using quantitative real-time PCR (qPCR). RNA was extracted using a previously described procedure [25]. Two-step qPCR was conducted using QuantiTect SYBR Green PCR Reagents (Qiagen, Valencia, CA) on an

iCycler iQ Multi-color Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Several components of the renin-angiotensin system were analyzed, including renin (*Ren*), (pro)renin receptor (*Atp6ap2*), angiotensinogen (*Agt*), angiotensin converting enzyme-1 and -2 (*Ace* and *Ace2*), and angiotensin II receptor type 1 (*Agtr1a*). Insulin 2 (*Ins2*) also was analyzed. Ribosomal 18S RNA was used as a reference in all analyses. Primers were designed using GeneTool Software (Biotools, Edmonton, Alberta, Canada) (Table S1). All samples were run in duplicate. For the relative quantification of gene expression, the comparative threshold cycle (C_T) method was employed. The averaged C_T was subtracted from the corresponding averaged r18S value for each sample, resulting in ΔC_T. $\Delta\Delta C_T$ was achieved by subtracting the average control ΔC_T value from the average experimental ΔC_T. The fold change was established by calculating $2^{-\Delta\Delta CT}$ for experimental versus reference samples. Several genes were selected to further confirm the NGS/RNA-seq results using qPCR with both technical and biological replicates.

2.8. Statistics

Normally distributed data are presented as the mean ± standard error of the mean (SEM). Parameters were compared using one-way analysis of variance (ANOVA) with the LSD *post hoc* test or two-way ANOVA for multiple comparisons, where appropriate. A $P < 0.05$ was considered statistically significant. All analyses were performed using the Statistical Package for the Social Sciences software (SPSS; IBM, Armonk, NY, USA).

3. Results

3.1. Maternal profile and pregnancy outcomes

Body weights of dams were not different between the two dietary groups, but HFD dams exhibited increased total fat mass, especially retroperitoneal fat ($p = 0.001$, Table 1). As shown in Table 1, liver weight, total cholesterol, leptin, and adiponectin levels were similar between groups; however, HFD dams exhibited increased GPT levels ($p = 0.042$). Litter sizes, numbers of stillbirths, and live birth rates were comparable between the two groups. Mean fetal weights and placental weights were reduced in the HFD group. Moreover, the weight of the fetus and placenta were reduced in the HFD group in both sexes (Fig. 1A and B), indicating that maternal HFD caused intrauterine growth restriction (IUGR) and smaller placentas in both sexes.

Table 1
Maternal profile, biochemical analysis and pregnancy outcomes between control and HFD groups.

Variable	Control (n = 8)	HFD (n = 7)	*p value*
Maternal profile			
Body weight (g)	277.5 ± 8	279.8 ± 7	0.838
Total fat mass (g)	12.5 ± 1.1	15.9 ± 0.9	**0.040**[a]
Subcutaneous (g)	6.5 ± 1.1	9.0 ± 0.6	0.092
Mesenteric (g)	2.5 ± 0.1	2.2 ± 0.1	0.141
Retroperitoneal (g)	3.4 ± 0.2	4.6 ± 0.2	**0.001****
Liver weight (g)	10.5 ± 0.9	12.3 ± 1.2	0.350
Maternal biochemical analysis			
GPT (U/L)	33.8 ± 2	48.2 ± 6	**0.042**[a]
Total-Cholesterol (mg/dl)	63.6 ± 2	61.0 ± 2	0.513
Leptin (ng/mL)	1.0 ± 0.2	2.1 ± 0.9	0.255
Adiponectin (μg/mL)	18.9 ± 1.4	17.1 ± 1.2	0.369
Pregnancy outcomes			
Litter size (n)	10.1 ± 0.5	10.4 ± 1.3	0.829
Stillbirth (n)	1	2	–
Live birth rate (%)	98.7 (80/81)	97.3 (73/75)	0.608
Mean fetal weight/litter (g)	2.4 ± 0.1	1.7 ± 0.1	**0.016**[a]
Mean placenta weight/litter (mg)	410 ± 10	363 ± 20	**0.042**[a]

[a] $p < 0.05$, $^{**}p < 0.01$ vs. control.

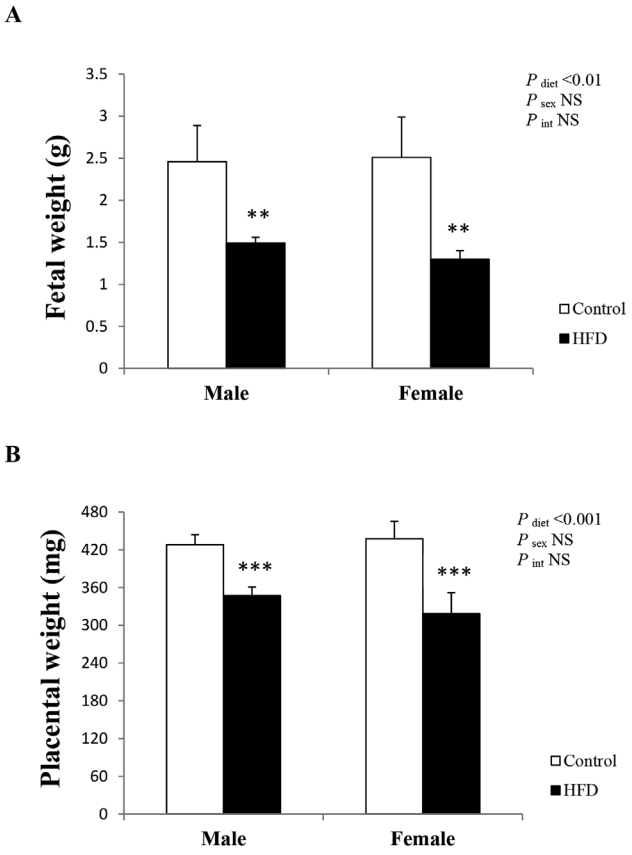

Fig. 1. Fetal weight (A) and placental weights (B) between control and HFD groups on GD 21 days. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control, n = 8–11/group.

3.2. Glucose tolerance

The HFD group had significantly impaired glucose tolerance relative to the control group at 15, 60, and 120 min, as well as increased area under curve (AUC) values (Fig. 2A). Similarly, ITT revealed that the HFD group had significantly increased blood glucose levels upon fasting and at 15 min and 30 min. In addition, increased glucose AUC values were observed over the course of the study (Fig. 2B), indicating that the HFD group exhibited reduced insulin sensitivity.

3.3. Placental layer distribution

Abnormal distribution of placental layers can be indicative of pathology. The mature placenta in rodents is composed of three broad zones, including the maternal decidua on the outside, the junctional zone (JZ) and the inner labyrinth zone (LZ), which mediates direct nutrient exchange between fetal and maternal blood. Our results revealed a significant increase in junctional zone thickness ($p = 0.034$, Fig. 3B) and the volume of the junctional zone (HFD female: 0.00857 mm^3 vs. control female: 0.00430 mm^3; $p < 0.001$) in female placenta from HFD dams, whereas no differences in labyrinth and decidua layers were noted among the four groups.

3.4. Maternal HFD alters the placental transcriptome

We used whole transcriptome analysis and analyzed differential gene expression induced by maternal HFD and the sexual dimorphism in placenta. The mean depth of coverage (X) was 103X for control male, 86X for HFD male, 109X for control female, and 86X for HFD female groups, respectively. More than 10300 transcripts were identified in each group. The mappability of genes relative to the rat reference

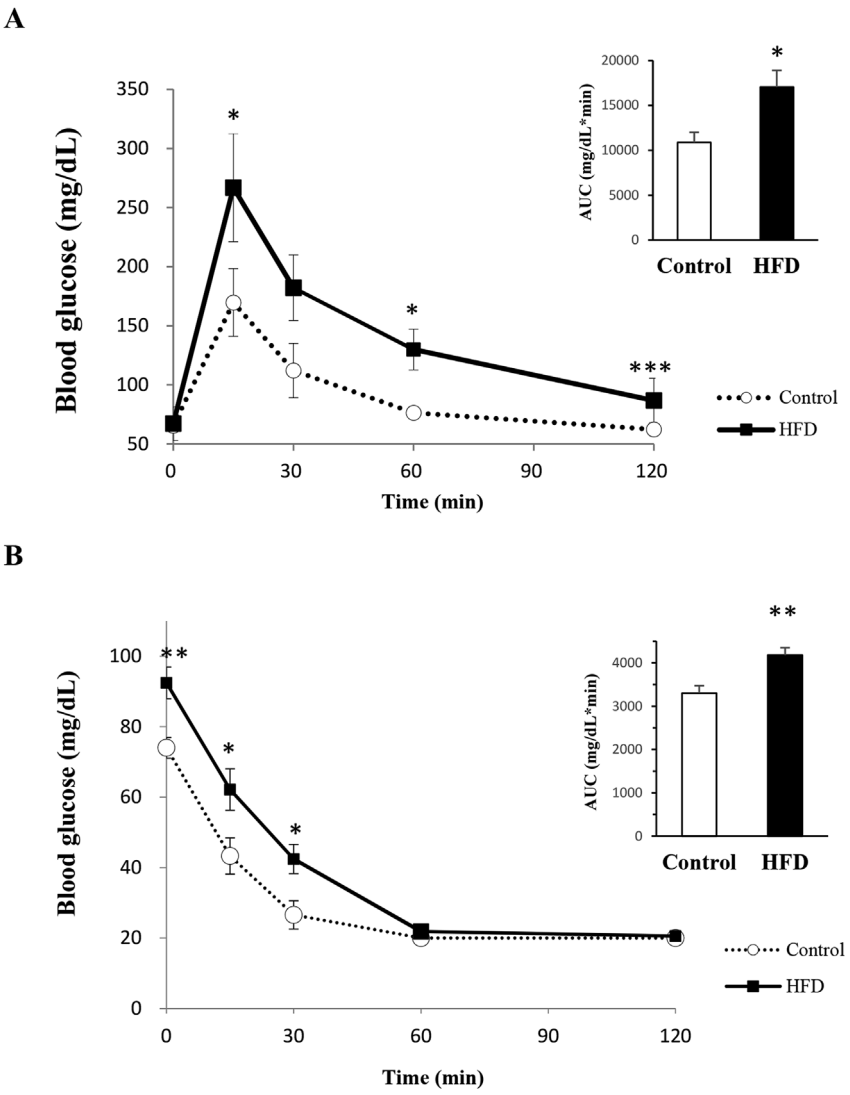

Fig. 2. Comparing glucose tolerance in the HFD and control groups after diet manipulation for 8 weeks. (A) Intraperitoneal Glucose Tolerance Test and (B) Insulin Tolerance Test. Data were analyzed by repeated measures ANOVA with *post hoc* least significant difference testing. The integrated AUC values were calculated using the trapezoidal method. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs. control, n = 6–8/group at each time point.

genome was 85.6% for control male, 82.5% for HFD male, 85.7% for control female, and 83.4% for HFD female groups, respectively. The criteria for the differential expressed genes (DEGs) were (1) genes that are changed by FPKM > 0.3 in either the control or HFD groups, and (2) minimum 2-fold difference in normalized read counts between groups. A summary of the sequencing data is shown in supplementary material. Among the DEGs, maternal HFD induced alterations in 1598 and 893 genes in the placental transcriptome of males and females, respectively (Fig. 4A). A total of 556 altered genes were shared between these groups. We next used DAVID v6.8 to identify functionally related gene groups and gain more biological insight using our gene lists. We identified 51 significantly KEGG pathways altered by HFD exposure in male placentas and 31 significantly KEGG pathways in female placentas. Among these pathways, we observed 21 overlapping pathways, including focal adhesion, ECM-receptor interaction, complement and coagulation cascades, and PI3K-Akt signaling pathway (Fig. 4B and C).

3.5. Sex differences in the placental transcriptome

By further analysis of the DEGs between males and females, we identified a total of 287 and 218 genes in males and females, respectively, that reached a minimum two-fold difference between sexes in the control diet and HFD groups (Figure S1). Among them, a total of 23 genes were shared between groups. We observed one significant KEGG pathway, cell adhesion molecules (CAMs), that was related to sex differences in the control group, and one gene in this pathway, *Selp* (fold change FC = 0.36), that is important in regulation of blood pressure and metabolic syndrome [26,27]. In the HFD group, the KEGG pathway for vitamin digestion and absorption was significantly associated with sex differences (Figure S1), including *Cubn* (FC = 2.01), which is involved in the catabolism of lipoproteins and vitamin B_{12} absorption in intestine and kidney [28,29], and has an essential role in transport of nutrients during embryogenesis [30].

We observed that dietary manipulation had a significant impact on the placental transcriptomes regardless of sex, but that male placental transcriptomes were more likely to be altered relative to female ones. We further analyzed the DEGs with a focus on genes that were previously reported to be altered by maternal HFD, obesity and/or fetal growth restriction, including the RAS [14,31], AMPK signaling [4,32], mTOR signaling [4,7,33], and insulin signaling [34,35] pathways. Among these genes, 16 DEGs (1 up- and 15 down-regulated) were shared between both fetal sexes, and all exhibited the same trend in fold changes (Table S2). In addition, *Ins2* expression was significantly different between fetal sexes in HFD groups (FC = 2.6); *Ins2* has been

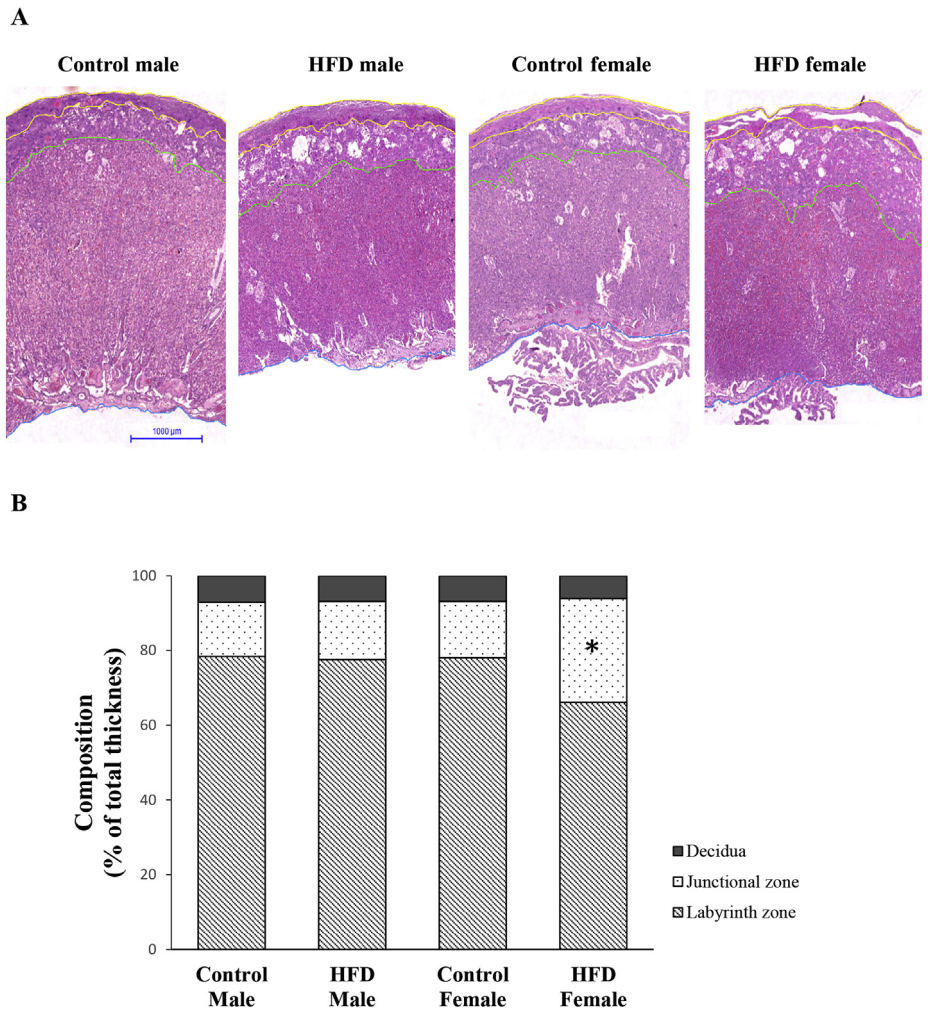

Fig. 3. Thickness and composition of placental layers. (A) Histological appearance of the placental layers in four groups at GD 21. (B) Mean percentage of total thickness of the decidua, junctional zone and labyrinth zone in four groups. Control male (n = 6 litters), HFD male (n = 6 litters), control female (n = 5 litters), HFD female (n = 4 litters) *$p < 0.05$ vs. control male, control female and HFD male group.

implicated in negative regulation of feeding behavior (GO:2000252) and hormone activity (GO:0005179).

3.6. Maternal HFD changes expression RAS and Ins2 in placenta

In both sexes, placental mRNA expression of *Ren*, *Atp6ap2*, *Ace*, *Ace2,* and *Agtr1a* was significantly increased in the HFD group compared with the control group (Fig. 5). In addition, maternal HFD significantly reduced placental *Ins2* mRNA expression relative to both male and female control groups. We also validated several transcripts using qPCR in male and female placenta that were observed to change significantly in the NGS dataset. Comparison of the expression results obtained using two platforms revealed good correspondence for most pairs (Figure S2).

4. Discussion

The aim of our study was to determine the effects of maternal HFD on rat placental histology and gene expression in a sex-specific manner. Our major findings are: (1) Maternal HFD increased the retroperitoneal fat component and GPT level, coinciding with maternal insulin resistance. (2) Both male and female pup weights from HFD dams were reduced compared with the control group, consistently exhibiting IUGR. (3) The weight of the placenta from the maternal HFD group was also reduced in both sexes, but changes in placental layer distributions were only significant in females. (4) The phenomenon of HFD-induced fetal growth restriction may be associated with a placental RAS activation programming effect. (5) Maternal HFD altered the placental transcriptome in a sex-specific manner as demonstrated by 1598 and 893 DEGs in males and females, respectively. These results also suggested that dietary manipulation had a greater impact on the placental transcriptomes than did fetal sex, and the placenta from male fetuses exhibited greater changes than the female placenta.

We found that fetuses of both sexes from HFD dams exhibited growth restriction, consistent with our previous report [9]. Moreover, placental histology revealed that junctional zone thickness was exclusively increased in female placenta with a relatively reduced percentage of the labyrinth zone. The result was similar to results reported by others [22,36]. Kim et al. reported that diet-induced maternal obesity significantly decreased labyrinth thickness and cell proliferation in mid-to late-gestation age [22]. This is likely to be significant because in the rodent placenta, syncytiotrophoblast cells form the nutrient transport surface within the labyrinth layer, which regulates maternal-fetal nutrient and oxygen exchange [15]. However, the junctional zone, composed of trophoblast giant and spongiotrophoblast cells, produces several hormones and angiogenic factors [37] that regulate maternal blood flow and hormone production [15,38]. The placenta is a vital source of steroid hormones, and progesterone and estrogens are the main hormones of pregnancy regulating insulin and glucose homeostasis, lipid profile, and appetite [39]. Maliqueo et al. reported that

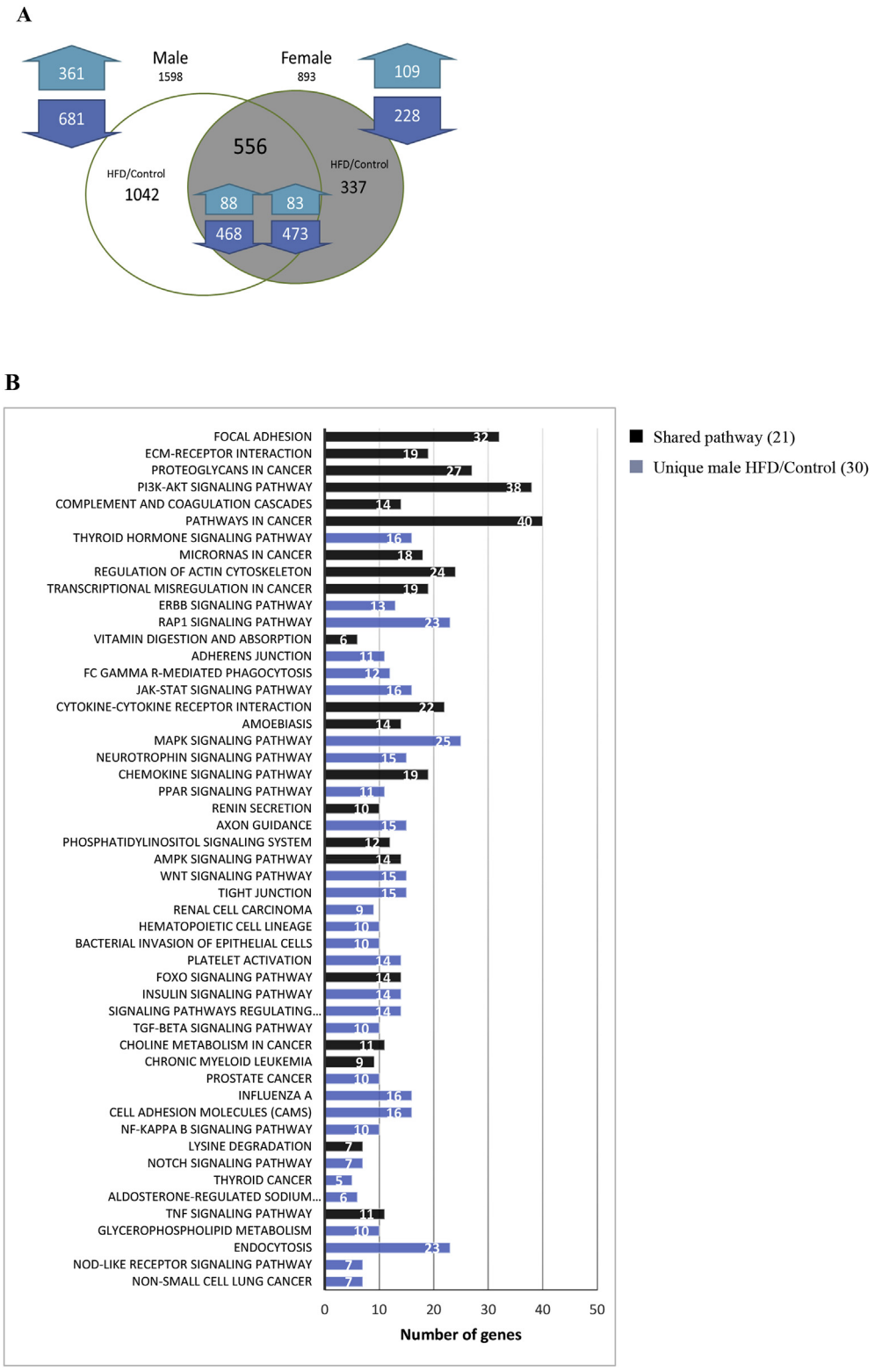

Fig. 4. Transcriptome profiling of rat placenta at GD 21 using next-generation sequencing (NGS) analysis. (A) Venn diagram depicting unique and shared sets of DEGs of HFD vs. control diet between male (white circle) and female placenta (grey circle). (B) Histogram presentation of the 51 KEGG functional annotations for the DEGs in male HFD/Control. (C) Histogram presentation of the 31 KEGG functional annotations for the DEGs in female HFD/Control.

obesity during pregnancy altered the maternal serum progesterone and testosterone concentrations depending on fetal sex [40]. Our data also found that HFD altered female placental transcriptomes involved in ovarian steroidogenesis (Fig. 4C), negative regulation of feeding behavior (GO:2000252), and hormone activity (GO:0005179). *Ins2* expression was significantly altered in the HFD group with fetal sex differences. *Ins2* encodes insulin that plays a critical role in the regulation of carbohydrate and lipid metabolism. Therefore, we postulate that our observations of fetal sex-related increasing placental junctional zone thickness and alteration of *Ins2* gene expression can be attributed

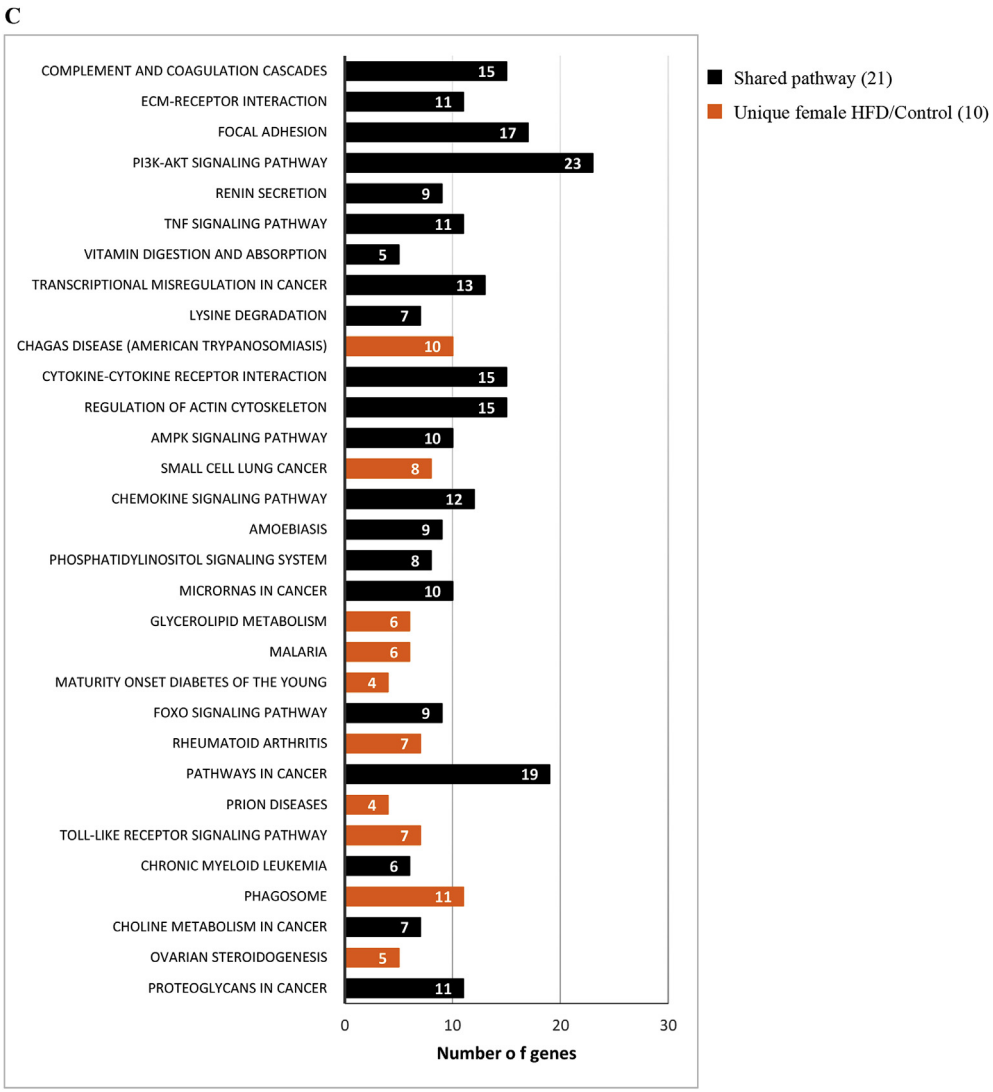

Fig. 4. (*continued*)

to sex-specific placental steroid hormones-gene-diet interactions.

Higher number of male placental DEGs were altered than female placental DEGs. Since the KEGG pathway of vitamin digestion and absorption is involved in lipid and vitamin B_{12} transport and absorption during embryogenesis, our data suggest that diet manipulation alters placental morphology and transcriptome via multiple mechanisms. The placenta is a transient organ that serves as a buffer system to maintain optimal nutrient status and oxygenation to the fetus, so our data potentially suggest that the female placenta exhibited a reduced ability to adapt to the poor intrauterine environment caused by maternal HFD. This is consistent with our observation of fewer changes in female placental gene expression relative to those of male fetuses. In other words, the male placenta is more sensitive to *in utero* perturbations; however, whether the consequences of these changes in placenta are protective or adverse remains unknown. Although it is possible that the male placenta has a relatively greater ability to restore the normal placental layer distribution, this remains to be determined. Further placental zonal specific gene expression study is likely to elucidate the impacts of sex differences in response to maternal HFD.

The uteroplacental RAS is one of the major extrarenal RAS in pregnancy, and a functional local RAS is critical for the regulation of uteroplacental blood flow, prostaglandin synthesis, and estradiol secretion [11,41]. Several reports have associated disturbances of the uteroplacental RAS with IUGR and preeclampsia [42,43]. Shibata et al. reported that activation of the angiotensin II type 1 receptor (AT1-R) by angiotensin II reduced the expression of the placental system A amino acid transporter, which may contribute to IUGR [14]. In addition, lifelong HFD or maternal obesity has been associated with abnormal placental vasculature [44,45], altered trophoblast invasion [46], and local placental inflammation [22,47]. From our NGS/whole genome sequencing data, we found two downregulated DEGs in the HFD group in both fetal sexes, *Agt* and *Rapgef1,* that have been related to blood vessel development (GO:0001568). We found that maternal HFD altered RAS in placenta by increasing mRNA expression of *Ren*, *Atp6ap2*, *Ace,* and *Agtr1a* via the ACE/ANGII/AT1R axis. This finding is consistent with previous reports and suggests that disturbances of fetoplacental RAS by maternal HFD exposure may cause poor vascularization of the placenta and placental dysfunction, in turn compromising fetal growth.

We observed that maternal HFD resulted in excess maternal fat deposition, increased GPT levels, and an increased glucose AUC value in IPGTT and ITT, which reduced insulin sensitivity. Abnormal liver function has been associated with insulin resistance and nonalcoholic fatty liver disease [48], and this could be a risk factor for metabolic syndrome [49]. Our data are consistent with maternal HFD alteration of several placental transcriptomes involved in glucose metabolism, including the PI3K-Akt and insulin signaling pathways, which regulate glucose metabolism.

HFD has often been used to promote obesity in rodents; however,

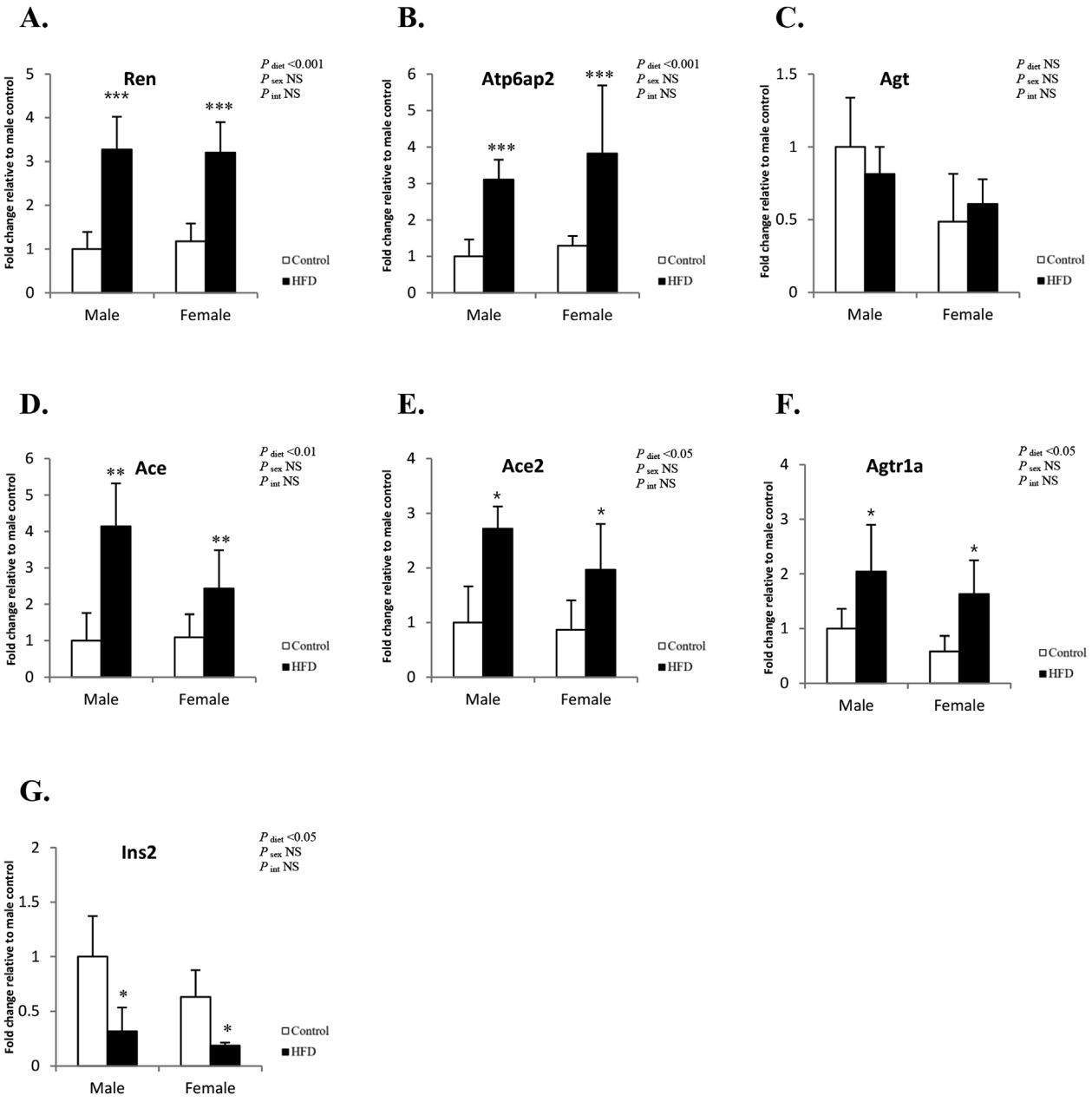

Fig. 5. Effects of maternal high-fat diet on mRNA levels of (A–F) RAS and (G) *Ins2* genes in placentas from male and female fetuses. The bar graphs present the two-way ANOVA results using maternal diet and fetal sex as two factors. N (pups/L) = 10–17/4-6 per group; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs. control male.

some researchers did not identify significant differences in body weight with HFD [50]. Our data showed IUGR from HFD dams and a similar fetal restriction outcome was reported by Sasson et al. [51], which was related to vasculogenesis and lipid metabolism. Our previous study demonstrated that maternal HFD altered female offspring steroidogenesis and coincided with upregulation of the imprinted gene *Igf2* [52]. We subjected dams to HFD for 10 weeks prior to breeding and observed that maternal insulin resistance and increased fat mass were induced. Therefore, our study is a model to test the impacts of chronic maternal HFD, rather than acute effects of maternal HFD. This discrepancy may be explained by the different study period lengths, maternal phenotypes, and programming impacts on the reproductive profile.

In conclusion, the placenta reacts and adapts to maternal HFD in a sex-specific manner by altering placental layer distribution and gene expression. Male placentas in the late gestational period are thought to exhibit plasticity under the adverse maternal conditions induced by HFD; however, we observed that the both sexes had similar growth restrictions in late gestation. The effect of IUGR may be associated with placental RAS activation in both sexes. In summary, this study provides a greater understanding of the role of the placenta and contributes to the identification of relevant pathways in a maternal HFD model. However, the long-term deleterious consequences of maternal HFD in male and female offspring in adult life require further study to clarify the effects of postnatal insults.

Disclosure

The authors declare no conflict of interest.

Author contributions

Conception and design: YJL, LTH, and YLT. Animal treatment, collection, and measurements: YJL, LTH, JMS, MMT, HRY, ICL, and

YLT. Analysis and interpretation of data: YJL, JMS, MMT, HRY, CCT, and YLT. Drafting and/or revising the article critically for important intellectual content: YJL, LTH, and YLT. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Supplementary data

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References

[1] I. Sandovici, K. Hoelle, E. Angiolini, M. Constancia, Placental adaptations to the maternal-fetal environment: implications for fetal growth and developmental programming, Reprod. Biomed. Online 25 (2012) 68–89.
[2] A.N. Sferruzzi-Perri, E.J. Camm, The programming power of the placenta, Front. Physiol. 7 (2016) 33.
[3] L. Higgins, S.L. Greenwood, M. Wareing, C.P. Sibley, T.A. Mills, Obesity and the placenta: a consideration of nutrient exchange mechanisms in relation to aberrant fetal growth, Placenta 32 (2011) 1–7.
[4] F.J. Rosario, T.L. Powell, T. Jansson, Activation of placental insulin and mTOR signaling in a mouse model of maternal obesity associated with fetal overgrowth, Am. J. Physiol. Regul. Integr. Comp. Physiol. 310 (2016) R87–R93.
[5] L. Radulescu, O. Munteanu, F. Popa, M. Cirstoiu, The implications and consequences of maternal obesity on fetal intrauterine growth restriction, J Med Life 6 (2013) 292–298.
[6] F. Gaccioli, V. White, E. Capobianco, T.L. Powell, A. Jawerbaum, T. Jansson, Maternal overweight induced by a diet with high content of saturated fat activates placental mTOR and eIF2alpha signaling and increases fetal growth in rats, Biol. Reprod. 89 (2013) 96.
[7] L. Song, B. Sun, G.J. Boersma, Z.A. Cordner, J. Yan, T.H. Moran, et al., Prenatal high-fat diet alters placental morphology, nutrient transporter expression, and mtorc1 signaling in rat, Obesity 25 (2017) 909–919.
[8] Y.L. Tain, Y.J. Lin, J.M. Sheen, I.C. Lin, H.R. Yu, L.T. Huang, et al., Resveratrol prevents the combined maternal plus postweaning high-fat-diets-induced hypertension in male offspring, J. Nutr. Biochem. 48 (2017) 120–127.
[9] Y.L. Tain, Y.J. Lin, J.M. Sheen, H.R. Yu, M.M. Tiao, C.C. Chen, et al., High fat diets sex-specifically affect the renal transcriptome and program obesity, kidney injury, and hypertension in the offspring, Nutrients 9 (2017).
[10] I.L. Aye, T.L. Powell, T. Jansson, Review: adiponectin–the missing link between maternal adiposity, placental transport and fetal growth? Placenta 34 (Suppl) (2013) S40–S45.
[11] A.H. Nielsen, K.H. Schauser, K. Poulsen, Current topic: the uteroplacental renin-angiotensin system, Placenta 21 (2000) 468–477.
[12] A.C. Cooper, G. Robinson, G.P. Vinson, W.T. Cheung, F. Broughton Pipkin, The localization and expression of the renin-angiotensin system in the human placenta throughout pregnancy, Placenta 20 (1999) 467–474.
[13] R. Goyal, R. Lister, A. Leitzke, D. Goyal, C.P. Gheorghe, L.D. Longo, Antenatal maternal hypoxic stress: adaptations of the placental renin-angiotensin system in the mouse, Placenta 32 (2011) 134–139.
[14] E. Shibata, R.W. Powers, A. Rajakumar, F. von Versen-Hoynck, M.J. Gallaher, D.L. Lykins, et al., Angiotensin II decreases system A amino acid transporter activity in human placental villous fragments through AT1 receptor activation, Am. J. Physiol. Endocrinol. Metab. 291 (2006) E1009–E1016.
[15] J. Rossant, J.C. Cross, Placental development: lessons from mouse mutants, Nat. Rev. Genet. 2 (2001) 538–548.
[16] A.H. El-Hashash, D. Warburton, S.J. Kimber, Genes and signals regulating murine trophoblast cell development, Mech. Dev. 127 (2010) 1–20.
[17] D. Hu, J.C. Cross, Development and function of trophoblast giant cells in the rodent placenta, Int. J. Dev. Biol. 54 (2010) 341–354.
[18] J.G. Knott, S. Paul, Transcriptional regulators of the trophoblast lineage in mammals with hemochorial placentation, Reproduction 148 (2014) R121–R136.
[19] C.M. Reynolds, M.H. Vickers, C.J. Harrison, S.A. Segovia, C. Gray, Maternal high fat and/or salt consumption induces sex-specific inflammatory and nutrient transport in the rat placenta, Phys. Rep. 3 (2015).
[20] V. King, N. Hibbert, J.R. Seckl, J.E. Norman, A.J. Drake, The effects of an obesogenic diet during pregnancy on fetal growth and placental gene expression are gestation dependent, Placenta 34 (2013) 1087–1090.
[21] A. Tarrade, P. Panchenko, C. Junien, A. Gabory, Placental contribution to nutritional programming of health and diseases: epigenetics and sexual dimorphism, J. Exp. Biol. 218 (2015) 50–58.
[22] D.W. Kim, S.L. Young, D.R. Grattan, C.L. Jasoni, Obesity during pregnancy disrupts placental morphology, cell proliferation, and inflammation in a sex-specific manner across gestation in the mouse, Biol. Reprod. 90 (2014) 130.
[23] C. Trapnell, A. Roberts, L. Goff, G. Pertea, D. Kim, D.R. Kelley, et al., Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks, Nat. Protoc. 7 (2012) 562–578.
[24] da W. Huang, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, Nat. Protoc. 4 (2009) 44–57.
[25] Y.L. Tain, M.S. Wu, Y.J. Lin, Sex differences in renal transcriptome and programmed hypertension in offspring exposed to prenatal dexamethasone, Steroids 115 (2016) 40–46.
[26] H. Wang, J. Nawata, N. Kakudo, K. Sugimura, J. Suzuki, M. Sakuma, et al., The upregulation of ICAM-1 and P-selectin requires high blood pressure but not circulating renin-angiotensin system in vivo, J. Hypertens. 22 (2004) 1323–1332.
[27] E. Straface, L. Gambardella, E. Canali, A. Metere, N. Gabrielli, R. Arcieri, et al., P-Selectin as a new gender associated biomarker in patients with metabolic syndrome, Int. J. Cardiol. 145 (2010) 570–571.
[28] E.I. Christensen, H. Birn, Megalin and cubilin: multifunctional endocytic receptors, Nat. Rev. Mol. Cell Biol. 3 (2002) 256–266.
[29] R. Kozyraki, Cubilin, a multifunctional epithelial receptor: an overview, J. Mol. Med. (Berl.) 79 (2001) 161–167.
[30] K.A. Burke, E. Jauniaux, G.J. Burton, T. Cindrova-Davies, Expression and immunolocalisation of the endocytic receptors megalin and cubilin in the human yolk sac and placenta across gestation, Placenta 34 (2013) 1105–1109.
[31] R.A. Irani, Y. Xia, The functional role of the renin-angiotensin system in pregnancy and preeclampsia, Placenta 29 (2008) 763–771.
[32] M.J. Zhu, B. Han, J. Tong, C. Ma, J.M. Kimzey, K.R. Underwood, et al., AMP-activated protein kinase signalling pathways are down regulated and skeletal muscle development impaired in fetuses of obese, over-nourished sheep, J. Physiol. 586 (2008) 2651–2664.
[33] T. Jansson, I.L. Aye, D.C. Goberdhan, The emerging role of mTORC1 signaling in placental nutrient-sensing, Placenta 33 (Suppl 2) (2012) e23–e29.
[34] M. Ruiz-Palacios, A.J. Ruiz-Alcaraz, M. Sanchez-Campillo, E. Larque, Role of insulin in placental transport of nutrients in gestational diabetes mellitus, Ann. Nutr. Metab. 70 (2017) 16–25.
[35] K.R. Howell, T.L. Powell, Effects of maternal obesity on placental function and fetal development, Reproduction 153 (2017) R97–R108.
[36] A.N. Sferruzzi-Perri, O.R. Vaughan, M. Haro, W.N. Cooper, B. Musial, M. Charalambous, et al., An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory, FASEB J. 27 (2013) 3928–3937.
[37] M.G. Achen, J.M. Gad, S.A. Stacker, A.F. Wilks, Placenta growth factor and vascular endothelial growth factor are co-expressed during early embryonic development, Growth Factors 15 (1997) 69–80.
[38] J.C. Cross, How to make a placenta: mechanisms of trophoblast cell differentiation in mice–a review, Placenta 26 (Suppl A) (2005) S3–S9.
[39] T. Napso, H.E.J. Yong, J. Lopez-Tello, A.N. Sferruzzi-Perri, The role of placental hormones in mediating maternal adaptations to support pregnancy and lactation, Front. Physiol. 9 (2018) 1091.
[40] M. Maliqueo, G. Cruz, C. Espina, I. Contreras, M. Garcia, B. Echiburu, et al., Obesity during pregnancy affects sex steroid concentrations depending on fetal gender, Int. J. Obes. 41 (2017) 1636–1645.
[41] A.M. Poisner, The human placental renin-angiotensin system, Front. Neuroendocrinol. 19 (1998) 232–252.
[42] L. Anton, D.C. Merrill, L.A. Neves, D.I. Diz, J. Corthorn, G. Valdes, et al., The uterine placental bed Renin-Angiotensin system in normal and preeclamptic pregnancy, Endocrinology 150 (2009) 4316–4325.
[43] H.D. Mistry, L.O. Kurlak, F. Broughton Pipkin, The placental renin-angiotensin system and oxidative stress in pre-eclampsia, Placenta 34 (2013) 182–186.
[44] R.D. Pereira, N.E. De Long, R.C. Wang, F.T. Yazdi, A.C. Holloway, S. Raha, Angiogenesis in the placenta: the role of reactive oxygen species signaling, BioMed Res. Int. 2015 (2015) 814543.
[45] E.K. Hayes, A. Lechowicz, J.J. Petrik, Y. Storozhuk, S. Paez-Parent, Q. Dai, et al., Adverse fetal and neonatal outcomes associated with a life-long high fat diet: role of altered development of the placental vasculature, PLoS One 7 (2012) e33370.
[46] E.K. Hayes, D.R. Tessier, M.E. Percival, A.C. Holloway, J.J. Petrik, A. Gruslin, et al., Trophoblast invasion and blood vessel remodeling are altered in a rat model of lifelong maternal obesity, Reprod. Sci. 21 (2014) 648–657.
[47] K.A. Roberts, S.C. Riley, R.M. Reynolds, S. Barr, M. Evans, A. Statham, et al., Placental structure and inflammation in pregnancies associated with obesity, Placenta 32 (2011) 247–254.
[48] R.K. Schindhelm, M. Diamant, S.J. Bakker, R.A. van Dijk, P.G. Scheffer, T. Teerlink, et al., Liver alanine aminotransferase, insulin resistance and endothelial dysfunction in normotriglyceridaemic subjects with type 2 diabetes mellitus, Eur. J. Clin. Investig. 35 (2005) 369–374.
[49] R.K. Schindhelm, M. Diamant, J.M. Dekker, M.E. Tushuizen, T. Teerlink, R.J. Heine,

Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease, Diabetes Metab Res Rev 22 (2006) 437–443.
[50] P.A. Kakimoto, A.J. Kowaltowski, Effects of high fat diets on rodent liver bioenergetics and oxidative imbalance, Redox Biol 8 (2016) 216–225.
[51] I.E. Sasson, A.P. Vitins, M.A. Mainigi, K.H. Moley, R.A. Simmons, Pre-gestational vs gestational exposure to maternal obesity differentially programs the offspring in mice, Diabetologia 58 (2015) 615–624.
[52] Y.-J. Lin, C.-C. Tsai, L.-T. Huang, J.-M. Sheen, M.-M. Tiao, H.-R. Yu, et al., Detrimental effect of maternal and post-weaning high-fat diet on the reproductive function in the adult female offspring rat: roles of insulin-like growth factor 2 and the ovarian circadian clock, J. Assist. Reprod. Genet. 34 (2017) 817–826.