RESEARCH ARTICLE

Quantification of fetal organ sparing in maternal low-protein dietary models [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: Maternal malnutrition can lead to fetal growth restriction. This is often associated with organ sparing and long-lasting physiological dysfunctions during adulthood, although the underlying mechanisms are not yet well understood.

Methods: Low protein (LP) dietary models in C57BL/6J mice were used to investigate the proximal effects of maternal malnutrition on fetal organ weights and organ sparing at embryonic day 18.5 (E18.5).

Results: Maternal 8% LP diet induced strikingly different degrees of fetal growth restriction in different animal facilities, but adjustment of dietary protein content allowed similar fetal body masses to be obtained. A maternal LP diet that restricted fetal body mass by 40% did not decrease fetal brain mass to the same extent, reflecting positive growth sparing of this organ. Under these conditions, fetal pancreas and liver mass decreased by 60-70%, indicative of negative organ sparing. A series of dietary swaps between LP and standard diets showed that the liver is capable of efficient catch-up growth from as late as E14.5 whereas, after E10.5, the pancreas is not.

Conclusions: This study highlights that the reproducibility of LP fetal growth restriction studies between laboratories can be improved by careful calibration of maternal dietary protein content. LP diets that induce 30-40% restriction of prenatal growth provide a good model for fetal organ sparing. For the liver, recovery of growth following protein restriction is efficient throughout fetal development but, for the pancreas, transient LP exposures spanning the progenitor expansion phase lead to an irreversible fetal growth deficit.
Keywords
Fetal growth restriction, Developmental origins of health and disease (DOHaD), Small for gestational age (SGA), Intrauterine growth restriction (IUGR), Organ sparing, Brain sparing, Maternal low protein diet, C57BL/6 mice

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Introduction

Moderate nutrient deprivation during animal development results in viable undersized adults. In humans and other mammals, intrauterine growth restriction (IUGR) limits the fetal supply of nutrients and oxygen such that overall growth is decreased but not all organs are equally affected (Barker & Osmond, 1986; Dobbing & Sands, 1971; Gruenwald, 1963). This non-isometric (asymmetric) scaling down of body parts reflects preferential utilization of scarce nutrient resources by certain tissues such as the brain at the expense of others, such as the liver and pancreas. This process is known as organ sparing and, although it is critical for fetal survival, there is a trade off in terms of the suboptimal functions of both the spared and the non-spared organs later in adult life (Hales & Barker, 2001; Hanson & Gluckman, 2014; McMillen & Robinson, 2005; Ravelli et al., 1998; Sharma et al., 2016). The mechanisms of organ sparing are not yet well understood but, in the case of the brain, it is known to involve diverting blood flow away from the periphery (Giussani, 2016; Thornburg, 1991).

Pioneering work in rats by Widdowson and McCance showed that fetal undernutrition has long-lasting effects upon growth trajectories (Widdowson & McCance, 1963; Widdowson & McCance, 1975). More recently, maternal low-protein diets in rodents have proved useful for investigating how fetal nutrition impacts upon organ growth, function and adult physiology (Barbeito-Andres et al., 2019; Berends et al., 2018; Bol et al., 2009; Chen et al., 2010; Gould et al., 2018; King et al., 2019; Langley-Evans et al., 1999; Ozzane & Hales, 2004; Tarry-Adkins et al., 2015; Watkins et al., 2008; Zambrano et al., 2006). One study of C57BL/6 mice examined the impact of a low protein maternal diet during pregnancy and/or postnatal stages upon the organ weights of pups at postnatal day 21 (P21) (Chen et al., 2009). Lowering maternal dietary protein from 20% to 8% during postnatal stages resulted at P21 in significantly smaller kidneys, pancreas, spleen, vastus lateralis, liver and heart but not brain or lungs. In contrast, a low protein (8%) maternal diet during pregnancy, followed by cross-fostering to a standard protein (20%) maternal diet at postnatal stages led to near normal weights at P21 for most organs, although the spleen, heart and thymus were significantly larger (Chen et al., 2009). This and other studies raise the question of how the protein content of maternal diets during pregnancy affects C57BL/6 organ weights at earlier stages, prior to birth.

Here, we use C57BL/6J mice to investigate the effects of low protein maternal diets upon fetal growth trajectories, organ weights and organ sparing at embryonic day 18.5 (E18.5). Using diet swap experiments, we define critical time windows during pregnancy, when low dietary protein has a long-lasting impact upon the growth of the pancreas and liver. We also document marked differences in fetal growth restriction on low protein diets between different animal houses.

Methods

Ethics

Animal studies were performed under a UK Home office approved project license (PAA689E24) and in accordance with institutional welfare guidelines and local ethical committees. All efforts were made to ameliorate any suffering and animals fed a low-protein diet were regularly monitored for health status, and also weighed every other day to confirm there was no excessive loss of body mass. All results are reported in line with ARRIVE 2.0 guidelines (Percie du Sert et al., 2020).

Mouse breeding and diets

The C57BL/6J strain of mice was selected as it is inbred and very widely used in genetic studies as well as in models of human diseases. Animals were originally obtained from The Jackson Laboratory and maintained at the MRC National Institute for Medical Research (NIMR), Mill Hill, UK until 2016. From 2016 onwards, C57BL/6J mice were maintained at the Francis Crick Institute (The Crick), London, UK. All mice were housed in the same temperature-controlled room at 21°C with a 12-hour light: dark cycle. Water and food were provided ad libitum and natural matings were set up with up to 8 males and 3 females, of at least 10 weeks of age and 20g body weight, per cage. Timed pregnancies were performed with the morning of the vaginal plug counted as 0.5d post coitus (E0.5) and then dams were placed using alternate allocation to cages with access to either standard chow (control group) or to a low protein diet (experimental group), with up to a maximum of three per cage. Control and experimental groups of animals are visibly different so cannot be blinded from the experimenter during the conduct of the experiment but subsequent statistical analysis, carried out by different individuals, was blinded. The potential confounding effect of circadian rhythms was minimized by switching diets and harvesting all embryos during a fixed time-window of the day. At NIMR the standard diet was #5021 from LabDiet (21.5% protein) and the low protein diet was #4400 from ABdiets, now #100195 from Altromin (8% protein). At the Crick, the standard diet was #2018S from Envigo laboratories (18.6% protein), the 8% low protein diet (Envigo TD.170638) was custom formulated to be similar to that used at NIMR, and additional isocaloric diets were formulated for 6% protein (Envigo TD.180032), 4% protein (Envigo TD.180031) and 3% protein (Envigo TD.180333). The composition of all diets used in this study is provided in the extended data.

Embryo and fetal weights

Dams were killed by cervical dislocation at 18.5 days post coitus and death was confirmed by exsanguination. E18.5 embryos were harvested in ice-cold phosphate-buffered saline (PBS), dried on absorbent tissue, and the body weighed with a readability and repeatability of 0.1mg on an XB 120A analytical balance (Precisa UK). Embryos were photographed in PBS on a Zeiss SV 11 dissecting microscope using a Nikon D700, Lens AF Micro Nikkor 60mm 1:2.8D. Embryos were weighed singly at E10.5, E12.5, E14.5 E16.5 and E18.5 (i.e. the experimental unit is a single animal). However, at E8.5, embryos with 8 somites (Theiler Stage 13) were selected and weighed from a single litter in groups of 4-8 (i.e. the experimental unit is a litter). For each E18.5 embryo, the brain, liver and pancreas were dissected in ice-cold PBS using watchmaker’s forceps (World Precision Instrument...
#14096), dried on absorbent tissue and weighed individually on the XB 120A balance. Embryo and organ weights were paired, and the litter of origin recorded. Sample sizes (n≥8) were decided based on previous experimental results and the published literature, except for E8.5 where n≥2 litters. No animals were excluded from the analysis.

16S microbiome sequencing

Faecal pellets were collected directly from C57BL/6J mice at six weeks of age maintained at the NIMR or the Crick animal facility on either the #2018S or #5021 diet. Pellets were snap frozen in liquid nitrogen and stored at -80 °C. DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen) and normalised to a concentration of 5μg/μl. V3/V4 specific primers were used to amplify the ~550bp amplicon and DNA was purified using AMPure beads (Beckman Coulter Life Sciences). A second polymerase chain reaction step was used to attach Illumina index primers (Illumina) and sequencing adaptors, before purifying the DNA with AMPure beads again. Libraries were measured for purity and quantity on the Nanodrop 1000 (Thermo Fisher Scientific) before denaturation. Sequencing was carried out on the Illumina MiSeq (Illumina) as per the manufacturer’s instructions for 16s metagenomics sequencing library preparation. The MiSeq provides an on-instrument analysis of the fastq files using the MiSeq Reporter Software, which classifies observed organisms via alignment to the Greengenes database.

Data analysis and statistical methods

All graphs and statistical analyses were generated using RStudio Version 1.2.5042 (2020-04-01). Boxplots were made using the ggplot2 package and show the median with the first and third quartiles of the interquartile range, and whiskers extending from the hinge by 1.5x interquartile range. The plot in Figure 1 was also made using the ggplot2 package, with the black line passing through the mean (large black dot), and the error bars showing the standard deviation (SD). In all graphs, individual data points are coloured according to the independent litter of origin. For statistical analyses, the data were modelled by a linear mixed-effects model (LMM) using restricted maximum likelihood (REML) from the lme4 package, or by a general linear mixed-effects model (GLMM) using maximum likelihood (Laplace Approximation) from the glmm package. Experimental variables such as diet, stages, and sex were allocated as fixed effects, whereas independent litters were allocated as a random effect to take account of within litter and between litter variances. The goodness-of-fit of the model to the data were evaluated using the quantile-quantile (QQ)-plot and QQ-line functions in R. Statistical inference for fixed effects was determined by one-way or two-way analysis of variance (ANOVA), followed by a Wald Chi-Square test using the car package, and adjusted for multiple comparisons using estimated marginal means (EMMs) and corrected with Bonferroni post-hoc tests from the emmeans package.

Figure 1. Fetal growth curves for standard (STD) and low protein (LP) maternal diets. (A) The 8% LP maternal diet (#4400) decreased the mass of E18.5 embryos by ~40% relative to the STD diet (#5021). (B) Growth trajectories of embryos from E8.5 to E18.5 on STD and LP diets. In this and all subsequent figures embryos from different litters are indicated with different coloured data points and asterisks indicate statistical significance between STD and LP weights at the indicated stages (* p < 0.05, ** p < 0.01, ***p < 0.001 and **** p < 0.0001). C57BL/6J mice were housed in the animal facility at National Institute for Medical Research (NIMR). Details of all diets are provided in Table S1. The source data and statistical analysis for this and all subsequent figures are provided in Table S2 and Table S3 (Serpente et al., 2021).
Asterisks on all graphs show statistical significance (* p < 0.05, ** p < 0.01, ***p < 0.001 and **** p < 0.0001). The source data used in all graphs are provided in Table S2 (Serpente et al., 2021). For each graph, the descriptive statistics (mean and SD, or the median and range), statistical approach used, allocation of fixed and random effects, choice of post-hoc ANOVA test and statistical significance are provided in Table S3 (Serpente et al., 2021).

Results

Prenatal growth parameters for the LP maternal diet

C57BL/6J dams were maintained for the duration of pregnancy on either a standard (STD) or low protein (LP) diet, containing 21.5% or 8% protein respectively (Table S1, Serpente et al., 2021). Consistent with previous studies in rats (Claycombe et al., 2015; Desai et al., 1996), we observed in mice that fetal body size at E18.5 is substantially decreased by LP maternal diet (Figure 1A). A prenatal time course of body weights revealed that LP maternal diet significantly decreased growth compared to STD diet at all stages from E12.5 to E18.5 (Figure 1B). At E18.5, this resulted in ~40% lower body weights for LP compared to STD fetuses. We also quantified how LP maternal diet alters organ sizes at E18.5, approximately one day before birth. Accurate masses were determined for the brain, liver and pancreas indicating that all organs from LP fetuses have significantly (p<0.0001) lower masses than their counterparts from STD fetuses (Figure 2). Non-isometric decreases in organ weights at E18.5 are indicative that prenatal LP induces positive sparing of the brain (77% of STD value) but negative sparing of the liver (40% of STD value) and pancreas (32% of STD value), relative to the body (60% of STD value), which is defined as neutral sparing (Figure 2). These findings together show that restricting the protein content of the maternal diet throughout pregnancy in C57BL/6 mice results in fetal growth restriction and robust organ sparing.

Critical windows of maternal protein intake for the growth of the fetal liver and pancreas

To map the developmental windows during pregnancy when maternal dietary protein intake is the most critical for fetal body and organ masses at E18.5, we utilized a series of twelve dietary interventions (Figure 3). The maternal diet was switched from STD to LP (interventions 1-3) or from LP to STD (interventions 7-9) at three different fetal stages: E7.5, E10.5 and E14.5. These same fetal stages were also used to delineate transient exposure windows to LP (interventions 4-6) or STD diet (interventions 8-12). For body mass, we observed the expected general tendency to decrease as a function of the duration of fetal exposure to maternal LP diet. A switch from STD to LP diet as late as E14.5 (intervention 3) was sufficient to produce a strong and significant (p<0.0001) decrease in body mass, which is in line with the exponential fetal growth curve (Figure 1B). Consistent with this, the complementary dietary switch from LP to STD diet at E14.5 (intervention 9) was sufficient to increase body mass significantly (p=0.0012) compared to the continuous LP regime.

Brain mass at E18.5 was largely preserved across all dietary regimes except for continuous LP, where there was a significant (p<0.0001) reduction of ~30%, albeit less than the ~40% decrease of the overall body (Figure 3). In contrast to brain sparing, liver and pancreas masses at E18.5 were decreased significantly (p<0.0001) following all three switches from STD to LP diet (interventions 1-3, Figure 3). Conversely, after a diet swap from LP to STD at E14.5 (intervention 9), the liver but not the pancreas was able to catch up to approximately

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**Figure 2.** E18.5 body and organ masses for STD and LP maternal diets. Graphs show mass (g) at E18.5 for pancreas, liver, body and brain on STD (#5021) and 8% LP (#4400) maternal diets. For each organ, percentages correspond to LP/STD mass (x100) and positive (green) or negative (red) sparing, relative to the body (neutral, grey), is indicated. Asterisks indicate significant (p<0.0001) differences in weight between STD and 8% LP maternal diets. C57BL/6J mice were housed in the animal facility at National Institute for Medical Research (NIMR). Details of all diets are provided in Table S1 (Serpente et al., 2021).
Strikingly, we also observed that transient windows of LP exposure from either E7.5 or E10.5 until E14.5 (interventions 5 and 6) led to significant (p<0.0001) and substantial decreases in pancreas but not liver mass (Figure 3). Together, these findings show that fetal growth of the pancreas is more sensitive than that of the liver to short periods of maternal protein deprivation.

Differential effects of LP diets on fetal growth in different animal facilities

During the course of this study, we moved animal facilities from the former MRC National Institute for Medical Research to the Francis Crick Institute. At the Crick, we used the same strain of mice (C57BL/6J) and an identical LP diet with 8% protein but we were unable to replicate the ~40% fetal growth restriction observed at NIMR. Surprisingly, at the Crick, the 8% LP diet had no significant effect upon E18.5 body mass (Figure 4A). It is not clear which factor(s) are responsible for the observed difference in the fetal growth response to protein restriction between NIMR and the Crick. However, 16S sequencing of the fecal microbiome of the C57BL/6J colony did reveal a substantial difference in the composition of the major bacterial phyla at the Crick compared to NIMR. In particular, although swapping STD diets (#5021 and #2018S, Table S1, Serpente et al., 2021) at NIMR did not substantially change the adult

Figure 3. Critical fetal windows for dietary protein-dependent organ growth. Graphs show body and organ (brain, liver, pancreas) masses at E18.5 for maternal STD (#5021) and 8% LP (#4400) diets, or following the indicated series (1-12) of maternal STD-LP diet swaps at E7.5, E10.5 and/or E14.5. Brain mass is decreased substantially by continuous LP but not by shorter LP exposures. In contrast, liver and pancreatic masses are very sensitive to short developmental exposures to LP. Note that the liver but not the pancreas is able to undergo catch up growth to approximately STD size after a late diet swap from LP at E14.5. Asterisks indicate those maternal dietary manipulations with significant differences in body/organ weight from continuous STD diet. C57BL/6J mice were housed in the animal facility at National Institute for Medical Research (NIMR). Details of all diets are provided in Table S1 (Serpente et al., 2021).
Figure 4. Fetal body masses on standard and low-protein maternal diets. (A) Fetal body weights at E18.5 from dams fed on STD (#2018S) diet or the isocaloric diets STDiso (#TD.180332), 8%LP (#TD.170638), 6%LP (#TD.180032), 4%LP (#TD.180031), or 3%LP (#TD.180033). At the Crick, the 4%LP diet (indicated in bold) approximately recapitulates the percentage decrease in fetal mass observed with the 8%LP diet at NIMR (Figure 2). (B) Male and female body and brain weights and brain:body ratios on STD and 4% LP maternal diets are comparable at E18.5. Asterisks indicate significant differences in body or organ weight from continuous STD diet. C57BL/6J mice were housed in the BRF at the Crick. Details of all diets are provided in Table S1.
at postnatal stages, however, undernutrition is known to be more spared from maternal protein restriction than cell lar amounts of cerebral DNA (Leuba & Rabinowicz, 1979).

In contrast to the brain, we found that pancreas and liver masses are sensitive to even short fetal exposures to LP. For the liver, LP to STD diet switches even as late as E14.5 highlighted an impressive capacity for catch-up growth. For the pancreas, however, these experiments revealed that the ability to catch up growth after protein restriction is lost at some point between E7.5 and E10.5. In this regard, it is interesting that pancreas but not liver size is known to be fixed early in fetal development and constrained by the size of the progenitor cell pool (Stanger et al., 2007). More specifically, all of the Pdx1-expressing progenitors required to make the pancreas are generated from E8.5 to E12.5 (Stanger et al., 2007). We therefore surmise that maternal LP experienced by the fetus during the window of progenitor expansion may limit the size of the progenitor pool, in turn leading to a long-lasting deficit in pancreas size. This may be one contributing factor explaining the observed association between poor fetal growth and adult pancreatic malfunction (Hales & Barker, 2001).

Calibration of dietary protein is important for standardization of fetal growth restriction
A striking finding of our study was that the 8% LP diet decreased the fetal body mass of C57BL/6J mice by 40% at NIMR, yet it had little or no effect at the Crick. The protein content of the diet had to be titrated down to 4% in order to get a comparable amount of fetal growth restriction at the Crick. It remains unclear which differences between animal facilities are relevant but substantial changes in microbiota were observed and could be a contributing factor. Regardless of the underlying cause, our findings highlight that variability between animal facilities poses a serious problem to the reproducibility of maternal nutrition studies in rodent models. One step towards improving reproducibility is to titrate the protein content of isocaloric diets to a level that replicates a standard decrease in fetal body mass, such as the 40% that was used in this study. In conclusion, our LP study highlights one aspect of the much wider challenge of environmental standardization in animal experiments (Richter et al., 2009).

Data availability
Underlying data
Figshare: Extended data for “Quantification of fetal organ sparing in maternal low-protein dietary models”. https://doi.org/10779/crick.c.5532651.v2

This collection contains the following underlying data:
- Table S1 csv files
- Table S2 csv files
- Table S3 csv files

Extended data
Figshare: Extended data for “Quantification of fetal organ sparing in maternal low-protein dietary models”. https://doi.org/10779/crick.c.5532651.v2
This collection contains the following extended data:
- FigS1.ai – Figure S1
- Serpente Table S1
- Serpente Table S2
- Serpente Table S3

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Reporting guidelines
ARRIVE Compliance Questionnaire and ARRIVE Guidelines 2.0: author checklist are both deposited at Figshare.

Extended data for “Quantification of fetal organ sparing in maternal low-protein dietary models”. https://doi.org/10779/crick.c.5532651.v2

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References
Stanger BZ, Tanaka AJ, Melton DA: Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. Nature. 2007; 448(7130): 886–891. Published Abstract | Publisher Full Text
Tarry-Adkins JL, Fernandez-Twinn DS, Madsen R, et al.: Coenzymine Q10 Prevents...


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Reviewer Report 07 February 2022

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The study by Serpente and co-authors showed a differential effect of maternal low protein diet during gestation on body and organ weight in a mouse model, with the brain positive spared and the liver and the pancreas more affected. The study also explored the critical windows for each organ during prenatal growth, confirming that the timing and extension of nutrient restriction induce different responses across organs.

In particular, the results suggest that the pancreas is more sensitive than the liver to short periods of maternal protein deprivation. Although the mechanisms of organ sparing were not examined, the authors suggest that the timing of cellular processes such as the early proliferation of progenitors in the pancreas could partly explain the narrower window for a catch-up of this organ compared to the liver. The effect of the redistribution pattern of fetal circulation in IUGR also need to be discussed (e.g., Tchirikov et al., 2002; Ebbing et al., 2009).

Interestingly, the effect of an 8% protein diet differs between two animal facilities, which is associated with differences in the microbiome. A review of the literature on experimental models of nutrient restriction reveals variable and even some contradicting results, and this study provides one possible explanation for such differences that deserves further research. This finding is relevant not only for replicating controlled conditions in experimental designs but also for the implications for studying the effect of malnutrition in human populations.

A previous study with a similar design shows that the effect of low protein diets is not homogeneous across cell types or brain regions (Barbeito et al., 2019). Given the detailed analysis of critical windows performed in this study, it would be interesting to explore whether they induce different responses at the cell level beyond the effect on overall brain size.

References


**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Have experience studying the effects of maternal malnutrition on fetal growth, both in human populations and mice.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 02 November 2021

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The study showed that maternal 8% LP diet induced substantial growth retardation in fetal mice starting at E12.5 with the pancreas and liver being most affected and the brain relatively spared. However, in a second animal facility, it required a 4% LP diet to get equivalent organ and body...
weight changes. The microbiome of the mothers differed between the two facilities. A series of
dietary transitions between LP and standard diets throughout gestation showed that the liver was
capable of catch-up growth from as late as E14.5 but after E10.5, the pancreas is was destined to
be growth retarded.

This paper confirms the impact of maternal LP diet on fetal and organ size that has been reported
many times in both mouse and rat. It adds some new data on the ontogeny of the retardation of
fetal weight in utero and the critical windows for organ impact between pancreas and liver. The
paper also shows that the impact of LP diet on fetal development is not just dependent on animal
strain but can be affected by animal facility, and that the protein content of the LP diet may need
to be titrated between different institutions to get equivalent effects on organ phenotype. This
may or may not be related to maternal microbiome. The paper, therefore, adds new data but is
somewhat limited on mechanism.

The various LP diets used were isocalorific but was the methionine content normalized?

While the pancreatic weight was especially sensitive to LP exposure, was there an equivalent
decrease in pancreatic endocrine cell mass as measured by islet mass? Within the islets was the
make-up of all endocrine cells equally affected at E18.5, or was the beta cell contribution
particularly vulnerable? This might indicate key ontogeny points at which the lineage development
of the beta cells are substantially compromised.

While brain weight is spared in the embryos after LP exposure, what about neuronal
development? There may be long-term neurological deficits which could be supported or refuted
in the Discussion from previous literature.

As the microbiome was measured the Discussion could include any evidence that this is directly
connected via maternal physiology to fetal organ development.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes
**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Have experience with low protein diet administered to pregnant mice and rats and the development of the endocrine pancreas in the offspring before and after birth.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.