We thank the reviewers for their time and expertise considering this study. We appreciate your insights and have made the recommended adjustments to aid in clarity of findings and in rationale for the work. The revisions include A<B<C<D<E<F. The specific requests are listed by reviewer below

* our point-by-point rebuttal responding to each question must be posted as a separate document.
* Two versions of your revised manuscript. (1) A marked-up copy to be used for editor and reviewer purposes that indicates all changes made to the text with either highlighting, colored text, or tracked changes and (2) A “clean”, un-marked copy that has all color and mark-up removed that will be used by production, should your manuscript be accepted.
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**Reviewer 1**:

This is an interesting study that examined the effect of GDF-15 deficiency on multiple pregnancy -related outcomes in GDF-15 KO mice. Surprisingly, GDF15-KO dams had no difference in pregnancy-related weight gain, food intake, insulin sensitivity and neonatal outcomes compared to WT dams, suggesting that GDF15 is not a major player in pregnancy-associated metabolic changes and pregnancy outcomes in mice. The study is well designed with multiple parameters investigated.  
A few comments to the authors:

1. **Figure 2A & B: If insulin tolerance is reduced in pregnancy why does fasting glucose tend to be lower?**

Insulin resistance and lower blood glucose, both fasting and fed, during pregnancy compared to non-pregnant mice are well-documented phenomemons1,2. A combination of placentally derived hormones, such as placental lactogens, reduced hypoglycemic counter-regulatory measures in the islet, and large feto-placental demand for glucose result in lower glucose levels for dams. We believe that our data provide strong evidence that our model is consistent with other rigorous pregnancy related glycemia studies. To address this in the manuscript, we made the following change:

Lines 207-208: “Consistent with other murine models of pregnancy, fasting blood glucose in pregnant dams tended to be lower than non-pregnant females (Figure 2B, p=0.20) (35,36).”

1. **Figure 2 D & E: If dexamethasone in drinking water reduces insulin tolerance why does fasting glucose tend to be lower?**

The poorer glycemic health and peripheral glucose disposal in response to exogenous insulin delivery is consistent with previously published work on dexamethasone treated animals from our group3. We believe the reduced glucose is a reflection of reduced insulin-resistance associated postprandial glycogen accretion in the fed state, resulting in mild hypoglycemia in the fasted state.

**Reviewer 2:**

The paper GDF15 knockout does not substantially impact perinatal body weight or neonatal outcomes in mice by Mulcahy et al sets out to establish how loss of GDF15 from the maternal-fetal dyad affects pregnancy and pup development. The subject of this paper is timely. The physiology and/or pathophysiology of rise in GDF15 that occurs in pregnancy is unknown, and understanding this is critical to our understanding of GDF15-GFRAL biology. As such, even though this paper predominantly presents negative findings, the core observation that loss of GDF15 does not grossly alter pregnancy-related outcomes are still of significant interest to the field. However, there are some aspects of the paper where the rationale is unclear and the findings are of much less interest. I commend the authors on their careful design of the GDF15 KO pregnancy studies.

1. **The rationale for use of dexamethasone (dex) in pregnancy to induce insulin resistance is lacking. There is no introduction of the model or its utility in the introduction. Dex is rarely given in pregnancy due to concerns related to fetal organ development, except acutely in cases where fetal lung maturation is of critical importance due to impending pre-term delivery. Insulin resistance, gestational diabetes and underlying type 2 diabetes are of broad translational relevance during pregnancy, however this is generally secondary to obesity in the human population, and as such a diet induced obese model would seem more appropriate for this study.**

We thank the reviewer for suggesting we provide more robust rationale. We were interested in the contribution of GDF15 levels in relation to insulin resistance of pregnancy before we investigated the effect of genetic knockouts. To establish the contribution of physiological state to GDF15 levels and insulin sensitivity, we evaluated pregnant vs non-pregnant females. We then wanted to examine the contribution of the stress of pregnancy alone in contrast with known elevated stressors in the context of pregnancy. We anticipated pregnancy would raise GDF15 levels, but thought pregnancies associated with chronic stress could have an additive effect on GDF15. Although we agree a DIO model would be insightful, we felt evaluating GDF15 in the frame of stress was a more important first step for our argument and was cogent with the current literature suggesting GDF15 acts as a sentinel of somatic stress. We agree with the reviewer that dexamethasone is rarely given except in the case of pre-term delivery for lung maturation. However, our team has previously used dexamethasone as it is a more specific GR agonist, and not subject to HPA downregulation (such as models of chronic intermittent stress). Therefore we consider this a more consistent model for maternal stress and its effects on pregnancy outcomes4. Dexamethasone treatment (both injected and orally administered) has been used for decades to understand the effect of stress on gestational5, placental6,7, and offspring health8. We made the following changes to the body of the manuscript to better articulate this rationale.

Line 67-75: “GDF15 elevations in circulation are thought to be sentinels of stressors present in the body. Comparisons between non-pregnant and pregnant individuals and between healthy versus chronic stress during pregnancy (like dexamethasone administration) are understudied in murine models Given the sometimes-conflicting human data and lack of evaluation of physiological state and chronic stress compounding physiological state, we sought to characterize GDF15 in circulation comparing pregnant, non-pregnant, and stressed pregnant females while assessing glycemic health. We also sought to define the effects of *Gdf15* loss of function during the course of healthy murine pregnancy, including effects on weight gain, food intake, insulin sensitivity, and neonatal outcomes.”

1. **Specific comments relating to Fig 2: Why are the ITTs expressed as % of baseline and not mmol/l or mg/dl as is the convention? Expressing as mg/dl would remove the need for fig 2B and 2E, and allow for interpretation of the actual glycemic state of the mice.**

We included the raw glucose curves for both experiments below. As you can see, we see more pronounced insulin resistance in the dex-treated dams. We see a greater rate of glucose drop in the pregnant animals compared to non-pregnant.

**A graph of a pregnancy and insulin tolerance

Description automatically generatedA graph of a patient's level

Description automatically generated**

1. **Fig 2C - the statistics are not clear. I believe there is likely a main effect of pregnancy and it is indeed well established that GDF15 is elevated in pregnancy, however the legend says paired t tests were used which does not make any sense in looking at the comparison marked on 2C. Dex treated dams are lighter in the second half of pregnancy, so it seems likely the results in 2E reflect body weight differences rather than anything else.**

The method used in 2C is two-way ANOVA with test for interaction, assessing the effect of time of collection (ZT1 vs ZT 13) and pregnancy status. The test revealed a significant effect of pregnancy (p=0.007), but not of time (p=0.98). There was no evidence of interaction (p=0.48).We changed the language of the passage from results and from the figure legend below,

line 209-214: “We found that GDF15 is 49% (54 ±18.8 pg/dL) elevated in pregnant animals compared to non-pregnant mice (**Figure 2C,** p=0.007), but does not differ based on collection time (p=0.98).”   
line 213-215: “Figure 2C) GDF15 levels at ZT1 and ZT13 in pregnant and non-pregnant females, assessed by two way anova for effect of time and of pregnancy status.”

1. **The results text states that "Both genotypes had a rapid increase in food intake in the final week of pregnancy, with smaller increases in the Gdf15-/- dams", the lines in Fig 3E overlap completely, so this is not substantiated.**

We edited the manuscript to remove the language stating a significant difference.

**line 239-240: “**Both genotypes had a rapid increase in food intake in the final week of pregnancy.”

1. **For fig 3, With regards to fig 3D, I suggest showing this as % change from delivery day - Given the higher body weight and variation in 3F around 20-25 day mark I wonder if this would account for the variability in weight loss.**

We recalculated the postnatal weight loss as a percentage of delivery day and saw that the variability is still large. We have changed it in the manuscript to,

Lines 241-243: “*Gdf15-/-* dams had 51% lower percent postnatal weight loss than *Gdf15+/+* dams with high levels of variability, but this failed to reach statistical significance (**Figure 3D**, p=0.14; **Figure 3F**)”

1. **For fig 5C, what is the p value? Text states p=0.05, figure legend states \*p<0.05. Given alpha is set at 0.05, p=0.05 is not actually significant.**

Thank you for pointing out that inconsistency. The p-value is 0.05, so we have removed the asterisk.

1. **Overall comments on graphs: x axis labels should be aligned with sampling timepoints within the graphs.**

We updated x-axes for insulin tolerance tests, body weight, and food intake measurements to better reflect days of collection for data.

1. **More complete labelling would be helpful.  
   I strongly applaud the authors for their use of gender neural language "expectant parents" in the discussion, but suggest "expectant gestational parents" may be more accurate and avoid confusion.**

Thank you for the positive feedback and the suggestion:

Line 306: “Elevated circulating levels of GDF15 have been documented in **expectant gestational parents** with normal weight status compared to those with obesity”

1. **There are a number of sentences in both the discussion and introduction that could be edited for clarity of thought.**

**References**

1. Rossi G, Lapaczewski P, Diamond MP, Jacob RJ, Shulman GI, Sherwin RS. Inhibitory effect of pregnancy on counterregulatory hormone responses to hypoglycemia in awake rat. *Diabetes*. 1993;42(10):1440-1445. doi:10.2337/diab.42.10.1440

2. Zhang Z, Piro AL, Dai FF, Wheeler MB. Adaptive Changes in Glucose Homeostasis and Islet Function During Pregnancy: A Targeted Metabolomics Study in Mice. *Front Endocrinol*. 2022;13. doi:10.3389/fendo.2022.852149

3. Harvey I, Stephenson EJ, Redd JR, Tran QT, Hochberg I, Qi N, Bridges D. Glucocorticoid-Induced Metabolic Disturbances Are Exacerbated in Obese Male Mice. *Endocrinology*. 2018;159(6):2275-2287. doi:10.1210/en.2018-00147

4. El Habbal N, Mulcahy MC, Redd JR, Bridges D. Effects of Dexamethasone on Offspring Survival and Intrauterine Growth Restriction. *Journal of the Endocrine Society*. 2021;5(Supplement\_1):A748-A749. doi:10.1210/jendso/bvab048.1522

5. Namdar Ahmadabad H, Kayvan Jafari S, Nezafat Firizi M, Abbaspour AR, Ghafoori Gharib F, Ghobadi Y, Gholizadeh S. Pregnancy outcomes following the administration of high doses of dexamethasone in early pregnancy. *Clin Exp Reprod Med*. 2016;43(1):15-25. doi:10.5653/cerm.2016.43.1.15

6. Lee JY, Park SJ, Kim SH, Kim MH. Prenatal administration of dexamethasone during early pregnancy negatively affects placental development and function in mice1. *Journal of Animal Science*. 2012;90(13):4846-4856. doi:10.2527/jas.2012-5090

7. Vaughan OR, Sferruzzi-Perri AN, Coan PM, Fowden AL. Adaptations in Placental Phenotype Depend on Route and Timing of Maternal Dexamethasone Administration in Mice1. *Biology of Reproduction*. 2013;89(4):80, 1-12. doi:10.1095/biolreprod.113.109678

8. Vaughan OR, Phillips HM, Everden AJ, Sferruzzi-Perri AN, Fowden AL. Dexamethasone treatment of pregnant F0 mice leads to parent of origin-specific changes in placental function of the F2 generation. *Reprod Fertil Dev*. 2015;27(4):704-711. doi:10.1071/RD14285