# Reviewer 2

The manuscript by Gunder et al. describes and interesting mouse model, which combines obesity and excess of glucocorticoids. They investigate the effect of obesity and dexamethasone on several parameters including water and food consumption, body weight and fat mass, Then, they analyze several muscle features and insulin effect on blood glucose.

The manuscript is well written and well organized. The part dealing with muscle features is sound and rather exaustive.

My only concern is about the poor description of glucose metabolism in the animal model.

In particular, they only performed an insulin tolerance test to evaluate insulin resistance. I do understand that the use of hyperinsulinemic clamp is not easy and obvious to perform, but they should at least further investigate the mechanisms of insulin resistance.

**This is an excellent point and we are providing two new pieces of data to address this important point in the revised manuscript. First we have done glucose clamp experiments in this model and published the results from the obese animals in Harvey et al 2018. To summarize, we found that there was strongly impaired glucose infusion rates, driven by elevated endogenous glucose production and impaired suppression of EGP by insulin. This was concordant with a lack of suppression of NEFA levels by insulin. We observed decreased peripheral 2-deoxyglucose uptake in muscle and adipose tissues. For the lean animals, we did not publish the data because we found a suppression of insulin clearance in lean animals. This was not observed in obese animals. As such the lean animals were chronically exposed to higher insulin levels. These data are presented here, and as you can see there is only mild glucose intolerance in lean dexamethasone treated mice. Future experiments are planned to investigate the phenomena of differential insulin clearance. We have clarified the findings in Harvey et al in the revised discussion section of this manuscript:**

../../../../../../../../Desktop/CushingAcromegalyStudy/manuscript/Obesity-Glucocorticoids/Endo%20clamp%20Respo**Glucose Clamp Data in NCD-fed Mice:** Insulin clearance (A), plasma insulin concentrations (B), area under the glucose infusion rate curve (C), hepatic glucose production (D) and glucose turnover (E) for lean mice during at basal and during euglycemic clamp following 3 weeks of dexamethasone (n=10) or vehicle (n=13) treatment. For clamp experiments, insulin was infused at 4 mU/kg/min following a prime continuous infusion of 40mU/kg bolus. All mice were fasted for 5-6 hours prior to experiments.

**In our previous study we demonstrated vie euglycemic hyperinsulinemic clamps that obese dexamethasone treated mice were insulin resistant (as determined by suppressed glucose infusion rates), had lowered muscle glucose uptake, and had elevated endogenous glucose production. Based on elevated lipolysis in these mice, we posited that this is due to indirect promotion of glucose production by adipocyte lipolysis [21].**

For instance, Does the lack of In vivo insulin effect relate to defects in muscle insulin action. What about insulin effect on canonical targets (muscle vs liver vs adipose tissue)?

**To the reviewer’s second question, we have added new data about pAkt signaling in these muscles. Consistent with other reports, we did not detect any differences in proximal insulin signaling. We have added these data to the revised manuscript as the new Figure C-D:**

**To test whether proximal insulin signaling was affected in either group, we evaluated muscle lysates from gastrocnemius tissues at the end of a hyperinsulinemic euglycemic clamp. We found that the relative phosphorylation of Akt at Ser 473 was unchanged between water and dexamethasone treatments, in either group (Figure 4C-D). This is consistent with prior work demonstrating that proximal insulin signaling is largely unaffected by glucocorticoids [28,29].**

How the authors address the mechanism of dexa-induced defects in insulin action of obese vs lean animals

**We agree that we have not defined the mechanism by which obesity modifies glucocorticoid actions in muscle. We have made this explicit in the revised description. We believe that the data presented in Figure 3 supports the hypothesis that obesity causes more transactivation of critical GR-dependent genes but as yet do not have a clear biochemical mechanism to why. We are pursuing this question aggressively and look forward to identifying and sharing these answers in forthcoming work. We speculate about a few options in the revised discussion:**

**One hypothesis is that obesity remodels the chromatin landscape, allowing for easier GR access to genes involved in modulating muscle size and function. Indeed, obesity alters the packing and accessibility of DNA in adipocytes [14,21,41] and therefore may have a similar effect in muscle in which Glucocorticoid Response Elements are more easily bound by GR causing increased glucocorticoid action. Another potential mechanism is that the effects of GR-dependent signaling are enhanced by insulin resistance by FOXO dephosphorylation**