We would like the thank the reviewers for their comments and suggestions. The revised manuscript clarifies the issues brought up by the reviewers with several new pieces of data. For clarity we have noted in this response how the manuscript has been modified.

Associate Editor's Comments:  
Both reviewers requested dexamethasone measurements and reference to human pharmacology. Ideally these studies might be performed on leftover serum from animals used in these studies. If none is available, measurements on comparably treated animals is acceptable or historical data but only if measured in your laboratory. I note that steroid hormone measurements, including synthetic dexamethasone, must conform to journal policies.  
  
**Reviewer Comments:  
Reviewer 1: This is a solid study reporting on the combination of obesity and chronically elevated glucocorticoids leading to exacerbations in metabolic function. I feel this work is potentially worthy of publication with the inclusion of the following data:**  
In order to confidently draw comparisons between the DEX-treated chow-fed versus high-fat fed groups, it is important to demonstrate that the method of DEX administration (via the drinking water) results in comparable elevations in serum DEX. There is a possibility that the exacerbated metabolic function observed in the high-fat fed mice was simply due to increased consumption of the DEX-treated drinking water in these animals. As such, a measurement of serum DEX, in both the DEX-treated chow-fed and DEX-treated high-fat fed groups should be included.

We the amounts of dexamethasone these mice were consuming (via measurement of drinking water throughout the study; 1A-C of this response) as well as the serum concentrations (via LC-MS; D). The obese dexamethasone-treated mice did consume modestly more dexamethasone when compared to lean mice when normalized by body weight. To our surprise, as the study went on the HFD mice specifically drank more water (and dexamethasone), even though they started with lower water consumption (Figure 1C of this response). This was reflected in serum concentration which was determined from blood at the end of the study. The increase in dexamethasone consumption may reflect that the mayincreasedThese new data are described in the revised methods:

**Methods for dex intake and quantification**

…and results sections:

**Results**

We are grateful to the reviewer for probing us to look into this trend, as this presents a significant limitation to our study. We have addressed in the revised manuscript, although do not believe that this fully accounts for the more phenotype observed in these mice for several reasons now indicated in the revised discussion:

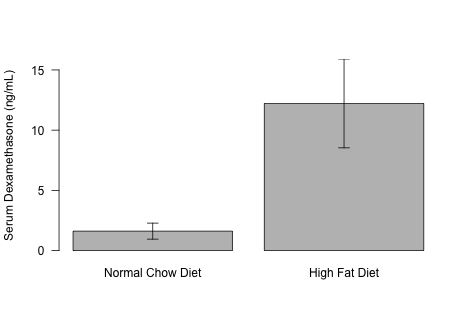
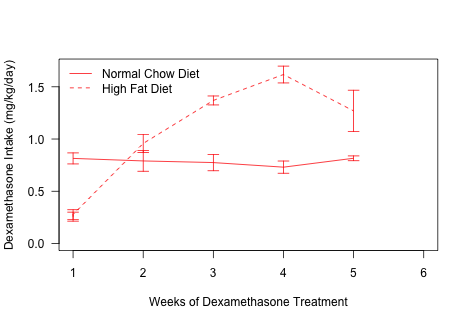
**The obese, dexamethasone treated animals consumed increasing fluids, including dexamethasone as the study progressed (Figure XX) resulting in increased serum dexamethasone at sacrifice (Figure XX). This was unexpected and may be due to the increased urination, and water requirement in severely diabetic animals, as has been documented previously** (1). **This is an important limitation to our study, although we note that several phenotypes including fasting glucose, liver triglycerides, hepatic lipogenic gene expression, and adipose tissue mass changed in different directions in lean and obese animals, and therefore is unlikely due to an increased dose of dexamethasone.**

We also note that we have observed increased blood glucose and glycerol levels with less than one week of dexamethasone exposure in a smaller scale time course experiment (Figure 2 of this response). At this stage, dexamethasone consumption is lower in the HFD group than the NCD group. While we are willing to include these data in the revised manuscript if necessary, the small n (4 animals per group at each time point) is less rigorous than we would prefer, and it will take approximately 3 months to repeat this time course.



B

A



C

D

Minor points

1. The figures 1A, 1B, 1C and 1D appear to be mislabeled in the legend.

Fixed in both the legends document and main documentThere are typographical errors on both lines 278 & 406.  
Fixed 278, removed sentence with typo in 406 as it regarded patients (acknowledgements).   
  
**Reviewer 2: Authors assessed some metabolic effects of increased glucocorticoid in combination with obesity induced by hyper-caloric feeding (in mice). Authors speculate that this combination of events is present in "many individuals". Therefore they propose that pre-clinical studies on this topic are needed. The results are very descriptive, in line with expectation, and no mechanism of action has been identified. Thus, this study is very descriptive and its results expected.**  
  
Main criticisms:  
1) If authors wanted to mimic the clinical glucocorticoid treatment in mice, then was the increase in circulating glucocorticoid content experimentally-induced in mice comparable to the level seen in humans undergoing glucocorticoid therapy?

Response: We measured intake of dexamethasone weekly throughout the study and found that mice were receiving less than 1mg/kg/d. Though this is at the high end, it is within the clinical range administered to humans, which is generally from 0.75-9mg/d and up to 3mg/kg/d (~210mg for an average American male), depending on the patient’s condition (2,3). As mentioned above, the obese mice had higher intake of dexamethasone and that was matched with elevated serum concentrations; however, these values were within range of serum cortisol concentrations observed in Cushing’s syndrome patients (4,5), even when accounting for the increased potency of dexamethasone in comparison to cortisol.

**Write something for the discussion section**

2) What is the novelty of this study?

To our knowledge this is the first paper to investigate chronically elevated glucocorticoids in the context of pre-existing obesity and compare to the lean phenotype. We show that obesity results in a more dramatic phenotype, including increased insulin resistance and lipolysis, as well as metabolic disturbances not noticed in lean mice given dexamethasone, such as excess hepatic lipid accumulation and pronounced fasting hyperglycemia. Additionally, we provide glucose clamp data that illustrate the main attributing factors to the hyperglycemia and insulin resistance in obese, dexamethasone-treated mice is hepatic glucose production. Lastly, we show that lipolysis is highly correlated with the increased metabolic perturbations both at the physiological (i.e. enhanced glycerol release) and molecular level (elevated ATGL transcripts and protein expression); moreover, obese dexamethasone-treated mice have reduced suppression of lipolysis in the presence of insulin when compared to obese controls. While these data agree with some published studies, we believe that these are valuable data to the research community. We have also added new data in this revision addressing the role of HSL and Perilipin phosphorylation in obese, dexamethasone treated animals. As can be seen in the new Supplementary Figure 2, both HSL and Perilipin phosphorylation on PKA sites is attenuated. This is described in the revised results section:

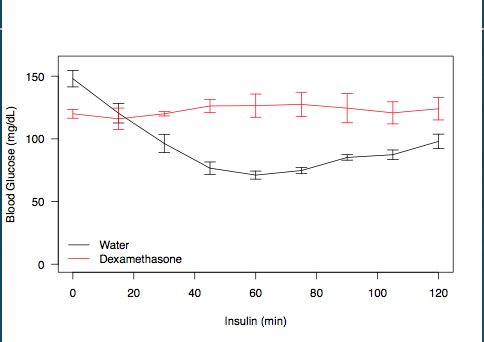
**Say something in the results**

And mentioned in the discussion in terms of the molecular links between glucocorticoids and lipolysis:

**Say something in the discussion**

3) Fig. 1A: In relative terms, insulin-induced changes in glycemia are similar between the 4 groups. Please, show data as percentage change over basal.

Response: Dexamethasone leads lower glucose clearance in both lean and obese mice; however, in the lean mice this difference is not significant between the treatment groups (A). It is important to note that the insulin dose was high (2.5 U/kg; generally, for chow mice we give 0.75-1.0 U/kg) since obesity is known to cause insulin resistance and these animals may not respond to lower doses which may prevent the observance of changes between the obese treatment groups. Since we wanted to compare all 4 groups we administered the same dose. However, at a lower dose of insulin we do see significantly lower glucose clearance in the dexamethasone-treated, chow-fed mice when compared to controls (B).



A B

4) Fig. 1C-F: What is the effect of glucocorticoid treatment on these parameters in NCD mice? Are these effects exacerbated in HFD?

Response: It is difficult to interpret data from the chow-fed (lean) animals in the clamp as the controls and dexamethasone treated animals had differences insulin clearance rates. Therefore, even though the groups were given the same dose, the dexamethasone-treated group cleared insulin more slowly than controls resulting in increased circulating levels throughout the clamp. With that said, dexamethasone-treated mice had higher circulating insulin yet similar glucose responses as controls and these data are consistent with what we observe with the ITT. Unfortunately, we cannot compare the lean mice to the obese mice for these particular data due to the discrepancy in circulating insulin.

1. **Lee SM, Bressler R.** Prevention of diabetic nephropathy by diet control in the db/db mouse. *Diabetes* 1981;30(2):106–111.

2. **Tyrrell JB, Findling JW, Aron DC, Fitzgerald PA, Forsham PH.** An overnight high-dose dexamethasone suppression test for rapid differential diagnosis of Cushing’s syndrome. *Ann.Intern.Med.* 1986;104:180–186.

3. **Fleseriu M, Biller BMK, Findling JW, Molitch ME, Schteingart DE, Gross C, Auchus R, Bailey T, Biller BMK, Carroll T, Colleran K, Fein H, Findling JW, Fleseriu M, Hamrahian A, Katznelson L, Kerr J, Kipnes M, Kirschner L, Koch C, Lerman S, Lyons T, McPhaul M, Molitch ME, Schteingart DE, Vaughan TB, Weiss R.** Mifepristone, a Glucocorticoid Receptor Antagonist, Produces Clinical and Metabolic Benefits in Patients with Cushing’s Syndrome. *J. Clin. Endocrinol. Metab.* 2012;97(6):2039–2049.

4. **Martin NM, Dhillo WS, Banerjee A, Abdulali A, Jayasena CN, Donaldson M, Todd JF, Meeran K.** Comparison of the dexamethasone-suppressed corticotropin-releasing hormone test and low-dose dexamethasone suppression test in the diagnosis of cushing’s syndrome. *J. Clin. Endocrinol. Metab.* 2006;91(7):2582–2586.

5. **Papanicolaou DA, Yanovski JA, Cutler GB, Chrousos GP, Nieman LK.** Distinguishes Cushing ’ s Syndrome from Pseudo-Cushing. *Endocrinol. Metab.* 2009;83(4):1163–1167.

Please also see: https://reference.medscape.com/drug/decadron-dexamethasone-intensol-dexamethasone-342741