Reviewer(s)' Comments to Author:  
Reviewer: 1  
  
Comments to the Author  
Harvey et al focus in the current manuscript on the interaction between obesity and glucocorticoid treatment and their combined effects on glucose metabolism and lipolysis. They additionally describe the effects of glucocorticoids on lipolysis and ATGL in mice.  
  
I have several issues with the paper.  
  
1) the human study is not adequately described in the paper. It should not be necessary to look up the reference for readers of this manuscript. I feel that the human data could be left out, but described in the introduction as unpublished. Stratification of groups with total n=19 in total, is fairly risky. Also, in the abstract significance is described while in fact P > 0.05. This is misleading. All in all, I feel that the human data are weak at best (<1% of total data here presented) and poorly described (what were the characteristics of the patients?).

Best to put human data in supplements or ‘data not shown’ and edit manuscript accordingly.

2) the mice data are more comprehensive and the abstract should be written accordingly.  
While I feel that the experiments are very nicely done, the added novelty to the current literature (both human and rodent) is very limited. Increased lipolysis has been described many times in humans (using tracers, not only NEFA levels measurements) and also in mice (why did the authors use tracers for glucose but not for glycerol?). Also, the role of ATGL has been highlighted previously. That overweight and glucocorticoids enhance this process seems logical. All in all, the performed analyses do not bring a big move forward in this field. The same goes for the liver measurements.

Need to work on the text in intro and discussion to make a stronger case for the novelty of our findings.  
  
Reviewer: 2  
  
Comments to the Author  
This study presents metabolic disturbances induced by coexistence of obesity and glucocorticoid administration. The authors first show human data indicating Cushing’s diseases coexisted with obesity is metabolically deleterious. Next the authors show in vivo and in vitro data indicating combination of high fat diet and glucocorticoid administration induces insulin resistance, exacerbated fatty liver, fat pad loss and lipolytic effects in adipocytes.  
These are interesting observations because the manifestation of glucocorticoid effects might be different in obesity compared to lean state. However, this manuscript has some flaws as indicated below.

Major comments are as follows:  
1) Human data are insufficient. The authors should also present other metabolic profiles, such as body composition or waist circumference since the authors subsequently discuss the amount of adipose tissue and lipolysis.

Move human data to supplementary or mention as data not published. Our other option is to include the AST and ALT, as well as the waist circumference data to show that we do have more data, though since both reviewers had an issue with this I think putting in the supplement and focusing more on the mouse data would be best.

2) To clarify whether the observed effects of dexamethasone are specific in obesity, mice fed with normal chow diet and treated by dexamethasone or vehicle need to be analyzed and compared with mice fed with high fat diet. I think this may be the same thing Quynh was suggesting that we aren’t even trying to show here?

Data should be added in Figure 1 E-H, Supplementary Figure 1G and H.

Not sure what this means?

3) In Figure 3A and B, the authors should explain the time courses for body weight and fat mass of mice fed with high fat diet. Dexamethasone-treated mice are comparable with vehicle-treated mice in body weight and fat mass in initial 2 weeks. Meanwhile, in later period, dexamethasone-treated mice decrease body weight and fat mass. What causes these time-dependent metabolic changes of dexamethasone? This point needs to be discussed together with validity of the timing for analyzing the effects of dexamethasone on organ weights or gene expressions.

Systemic insulin resistance happens quite early and then after about a week of treatment you start to see lean mass loss.

Additionally, it is unacceptable that the body weight and fat mass slightly decrease in vehicle treated normal chow mice in initial 1 to 2 weeks.

I guess we should explain that these mice were initially put in the CLAMS and single-housing may have led to the decrease in weight in all including the vehicle-treated

4) Glucocorticoid receptor is expressed in muscle and liver as well as adipocyte. It is inadequate to focus on only Pnpla2/ Atgl expression in adipocyte, Srebp1 and Fasn gene expression in liver. Please show more data about the signaling or expression changes in adipocyte, liver and muscle related to lipid or glucose metabolism and discuss metabolic changes from the view of inter-organ communication.

I think this is unnecessary aside from maybe including the hepatic gluconeogenic gene expression data

Minor comments are as follows:  
5) Legends should be added to Figure 3A-C.

Dave could you please put figure legends in here?

6) In Figure 2C, mice treated with dexamethasone for 6 weeks fed with normal chow diet show no apparent fatty liver. Additionally, in Figure 3A, combination of normal chow diet and dexamethasone decreases body weight. However, it is reported that treating with corticosterone for 5 weeks induces body weight gain, fat pad increase and histological fatty liver (J Endocrinol. 2013 Oct 28;219(3):231-41.). Please explain these discrepancies.

In our hands, lean mice provided dex do not start to gain fat mass until 5-6 weeks in and then continue from there (Hochberg paper). In the paper cited, both doses lead to increases in fat mass; however, we are unsure of the dose the mice were getting as they do not measure amount of water consumed. We used a concentration of 0.057ug/mL (Dex plus extra—need to find out exact dose of dex) and was estimated to give ~1mg/kg/day according to amount consumed, the actual ug/ml dose we used is much lower than the amount used in the Fansson et al paper. The lean mice measured here were older and were sacrificed at the end of the 5th week of treatment (i.e. about 6 weeks treated), it is likely had we left them on dexamethasone longer we would have begun to see an increase in fat mass in the dexamethasone treated group.

7) The authors should present some examples of mice not analyzed because of symptoms in high fat diet and dexamethasone group. What symptoms were seen in 16 mice in 32?

Lethargy and extreme weight loss, matched with decreased food intake.