# Response to Reviewer 2

1. In methods section, lipolysis assay: Please indicate whether the assay was performed immediately on fresh tissue or on fresh tissue that had been snap frozen (lines 85-87).

**Fresh adipose tissue was used for lipolysis assay. This information was added to the methods section.**

1. Table 2 describes clinical characteristics of CD and control patients and some of the same data is graphically presented in Figure 1A. The redundancy should be corrected.

**Removed weight, BMI and abdominal circumference from Table 2 as requested.**

1. For the gene expression studies, results should be clarified in a few instances to better describe which results were and were not statistically significant. While it is possible that a larger sample size would have yielded statistically significant results, only speculations can be made in these cases. As it stands now with the text claiming significant changes and the graphs lacking asterisks for some of the “increased” genes, it is confusing to the reader.

**The reviewer is correct in that throughout the manuscript we mention genes that did not quite reach statistical significance. We chose to do this, in general, for two reasons. In one set of cases, we want to mention similar genes (for example *DGAT1* and *DGAT2*) and how they may be similarly affected by glucocorticoid exposure. In other cases, we wanted to mention in the results/discussion sections some key genes that may be of interest. For example, we mention *AGPAT2,* a gene which was elevated, but did not quite reach significance. As we were limited by our sample size, we realize that some genes that may be relevant drivers of the phenotype would not be statistically significant. Based on our design, we would have been expected to only identify a gene that is changed by 50% to be statistically significant only 23% of the time. We therefore believe it is important to mention biologically significant genes and notate them appropriately rather than only discuss genes that were statistically significant.**

**Furthermore, we set what we believe to be a very high standard for statistical significance. As now described on the methods and materials section our definition of significance for qPCR was:**

**Statistical tests were performed as described below based on tests of normality and homoscedasticity, then p-values were adjusted for multiple comparisons based on the number of genes tested for each tissue across this manuscript.**

**This means that we are not using the nominal p-values anywhere throughout the manuscript, and we are correcting for multiple comparisons in all figures.**

**Throughout the paper, asterisks indicate significance (after adjusting for multiple hypothesis testing). Where genes are increased but are not denoted with an asterisk, they are not statistically significant. This could be due 1) to our conservative adjustments for multiple hypothesis testing (see above) or 2) the DEseq2 algorithms exclusion of genes with wide variance from final calculations (see previous response for more details). In these instances, the genes are “increased” but we do not claim that they are increased beyond our level of statistical significance. Full gene expression statistics are listed in Supplementary Table 1.**

a.      Lines 283-284 and figure 4A, are ACSL3, ACSL4, ELOV1, ELOV6 genes significantly increased as indicated in the text? They don’t look to be increased.  If so, please indicate on graph with an asterisk and perhaps change the scale for these data points so the reader can see the values  If not, please reword the text accordingly. Also, ACSL5 is mentioned in the text but not shown on the graph. Correct by adding to graph or using “data not shown”.

***ACSL3/4* genes are increased but not in a manner that is statistically significant (padj=0.12 and 0.10 respectively). *ACSL5* is not mentioned in the revised manuscript and was only modestly changed (5% reduced, padj=0.76). *ELOVL1* and 6 are increased but *ELOVL1* was not found to be statistically significant after adjusting for multiple hypothesis testing while *ELOVL6* was not tested by DESeq algorithm (see previous response for details).**

b.      Lines 284-87 and figure 4B, please adjust as described above in comment 3a. If FADS1, b. FADS2, HSD17B are significantly increased please show and asterisk. If not please correct the text.   If close to significant give the p value and explain.

***FADS1* and *FADS2* were not evaluated by the DESeq algorithm and therefore no statistical tests were provided, and no p-values are reported. This is described in the methods section:**

**To ensure that we did not miss any genes that had a high fold change, but that DESeq2 did not perform statistical tests for, we manually inspected genes that had a expression at >50 reads, fold change >2.5 but no p-value calculated. These genes included *FADS1, FADS2, ELOVL6*, *SPP1*, *BMP3* and *AACS* (see Supplementary Table 1).**

***HSD17B12*** **was elevated by 43% (padj=0.07) and therefore was not statistically significant and is not denoted as such.**

c.      Lines 286-88, same as above. DGAT 1 and 2 are listed but only DGAT 2 is shown. GPD1 and AGPAT2 don’t look to be significantly increased. Where is LPIN1?

***DGAT1* (81% increase, with nominal p-value=0.46) and *DGAT2* (2.1 fold increase padj=0.01) were both increased. *GPD1* (34% increase padj=0.09) and *AGPAT2* (37% increase padj=0.16) were not significantly increased and were not denoted as such. We removed mention of *LPIN1* (25% increase, padj=0.53)**

d.      Line 302, please indicate that perilipin expression data are not shown

**Added note that this data is not shown.**

e.      Lines 305-309 and Figure 4E, SRD5A1 and SRD5A3 do not look elevated. It may be a scale issue, but doesn’t look to be the case. Same for NSDHL. If scale is the issue, please modify the chart with an inset or scale change to clearly show the data. Or change to a table. This is confusing to the reader. STS is not shown on the graph, please explain as “not shown” or add.

***SRD5A1* (30% increase, padj=0.24), *SRD5A3* (30% increase, padj=0.14) were increased but not significantly and are denoted as such. NSDHL was removed from the graph, as it was expressed at very low levels. STS (23% increase, padj=0.47) was removed from the discussion and is not presented on the graph. We have also re-graphed Figure 4E on separate scales for clarity.**

f.      Lines 312-14, and Figure 4F. Same as above: Fasn, Acss2, Acs1, Dgat, Agpat2, Acaca1 don’t appear to be increased (by definition of statistical significance or other in some cases). Please clarify this textually and visually.

**As denoted by the lack of asterisks, these genes are not statistically significantly increased. P-values for all adipose tissue qPCR are shown in the table below. As you can see, several of these are nomimally statistically significant, but after adjusting for the number of genes tested by qPCR in these samples, and these multiple hypotheses, statistical significance was not achieved (see Table 1 of this response).**

**Table 1: Pairwise tests for qPCR analysis of WAT.** The q-value used to denote significance in this manuscript is the p-value from the appropriate statistical test (shown in “Test”), adjusted by the method of Benjamini and Hochberg for all the genes tested throughout the manuscript (n=24). The columns marked shapiro are the p-values from the Shapiro-Wilk tests, which evaluate normality and the column marked levene indicates the p-value from Levene’s test, which evaluates equality of variances.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **shapiro-water** | **shapiro-dex** | **levene** | **Test** | **p-value** | **q-value** |
| *Acaca* | 0.004 | 0.970 | 0.471 | Wilcoxon | 0.018 | 0.070 |
| *Aco1* | 0.253 | 0.152 | 0.073 | Student | 0.195 | 0.260 |
| *Acsl1* | 0.068 | 0.306 | 0.006 | Welch’s | 0.129 | 0.193 |
| *Acss2* | 0.018 | 0.131 | 0.304 | Wilcoxon | 0.412 | 0.449 |
| *Agpat2* | 0.664 | 0.054 | 0.652 | Student | 0.059 | 0.133 |
| *Dgat2* | 0.818 | 0.550 | 0.331 | Student | 0.031 | 0.094 |
| *Dhcr24* | 0.000 | 0.087 | 0.138 | Wilcoxon | 0.003 | 0.047 |
| *Dhcr7* | 0.003 | 0.513 | 0.423 | Wilcoxon | 0.018 | 0.070 |
| *Fasn* | 0.002 | 0.190 | 0.707 | Wilcoxon | 0.226 | 0.285 |
| *Gpam* | 0.053 | 0.353 | 0.587 | Student | 0.005 | 0.047 |
| *Gpd1* | 0.001 | 0.123 | 0.437 | Wilcoxon | 0.006 | 0.047 |
| *Idh1* | 0.207 | 0.144 | 0.024 | Welch’s | 0.083 | 0.133 |
| *Ldhb* | 0.064 | 0.087 | 0.868 | Student | 0.009 | 0.057 |
| *Mdh1* | 0.080 | 0.889 | 0.164 | Student | 0.076 | 0.133 |
| *Me1* | 0.024 | 0.162 | 0.696 | Wilcoxon | 0.078 | 0.133 |
| *Nr3c1* | 0.311 | 0.359 | 0.363 | Student | 0.403 | 0.449 |
| *Psmd1* | 0.011 | 0.117 | 0.786 | Wilcoxon | 0.078 | 0.133 |
| *Psmd8* | 0.085 | 0.030 | 0.461 | Student | 0.073 | 0.133 |
| *Rplp0* | 0.053 | 0.016 | 0.191 | Student | 0.036 | 0.096 |
| *Rplp13a* | 0.381 | 0.863 | 0.957 | Student | 0.542 | 0.565 |
| *Scd1* | 0.005 | 0.021 | 0.208 | Wilcoxon | 0.191 | 0.260 |
| *Scd2* | 0.000 | 0.534 | 0.749 | Wilcoxon | 0.026 | 0.090 |
| *Scd3* | 0.019 | 0.004 | 0.338 | Wilcoxon | 0.851 | 0.851 |
| *Scd4* | 0.000 | 0.087 | 0.547 | Wilcoxon | 0.412 | 0.449 |

4.      Line 323, should this read “Figure 5C” instead of “5B” ? For Figure 5C, the increases look significant, but no asterisks. Please clarify this to the reader by stating the p values or adding asterisks.

**The reference should have been to 5C, we thank the reviewer for noting this. See the previous point regarding gene expression changes in WAT by qPCR.**

5.      Lines 325-26, where are the data that are described? If not shown please indicate.

**These data are shown in Figures 5A and 5B. Note the coloring of the pathways indicates the fold change.**

6.      Lines 335-37 and Figure 6B, there are clear increases.  If not significant simply mention the variability among mice in response to dexamethasone.

# These were indeed not significant (see Table 2 of this response). We have noted in the results section that this is due to variability in dexamethasone responsiveness.

# Table 2: Pairwise tests for qPCR analysis of skeletal muscle.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **shapiro-water** | **shapiro-dex** | **levene** | **Test** | **p-value** | **q-value** |
| *Fbxo32* | 0.541 | 0.021 | 0.302 | Wilcoxon | 0.200 | 0.247 |
| *Trim63* | 0.766 | 0.079 | 0.227 | Student | 0.247 | 0.247 |
| *Psmd8* | 0.683 | 0.969 | 0.214 | Student | 0.065 | 0.196 |

7.      Lines 341-43 and Figure 6E. If data are shown please discuss briefly which proteasomal pathway and aa catabolism genes were induced.

**We have now noted in the revised results section:**

**Among the amino acid catabolism genes, *AOX1* (96% increase, padj=0.03), *OXCT1* (40% increase, padj=0.04) and *BCAT1* (80% increase, padj=0.048) were all significantly upregulated.**

8.      In Figure 7, non-significant data (negative results) are shown. Perhaps omit the graph use “data not shown”. It is unclear why this is shown. Insulin data do not look significant. Are they? If not use p values or clarify in some manner.

**These data are indeed non-significant. We think that this is important exclusionary data, indicating that there is no transcriptional downregulation of insulin signaling genes, or increase in ceramides, two potential mechanisms of glucocorticoid-induced insulin resistance. We therefore believe that this is worth including in the main manuscript, but are willing to move them to the supplement at the editor’s discretion.**