**We thank the reviewers for their insightful and carefully considered comments, as considering and addressing these issues makes this a much clearer and more coherent manuscript.. We have substantially revised the manuscript based on these suggestions, by providing extra data and more refined explanations for each of these comments. A point-by point response to each of these comments is presented below.**

## Reviewer 1

The aim of this study was to examine factors predicting weight gain on chow, a synthetic control diet, and a synthetic HFD in C57BL6/J mice. The authors report larger variation in weight gain in mice on HFD that was not present in leptin deficit models, that was unrelated to animal dominance. Weight gain was inversely related to pre-diet weight loss in response to a 16hour fast, please rationale why this was tested as a predictive factor in the introduction. Weight gain was not related to the measured appetite and glucoregulatory hormonal factors. There are a lot of small errors and omissions in this work throughout that require attention. I found some of the figures a bit confusing and don’t understand why some figures are supplementary and others are not.

1. Table 1 - the percentages given as fat, CHO and protein do not add up to 100% (67% for chow, 89 and 91% for CD and HFD so this leads me to believe that it is by weight with fibre missing from the calculation?

**We checked with the vendors and we have updated Table 1 accordingly. The percentages are given as % of calories, but maltodextran was accidentally omitted from the table. Now that it is included, the CD and HFD amounts add up to 100% of the calories. More details about these diets are available at the following links. Furthermore, NCD was erroneously presented as percent weight not percent calories. This has been rectified as well. We apologize for this error. Furthermore, we have indicated a note in the methods section and table legend specifying the seasonal variability in the Teklad diet:**

**Note that there are seasonal variations in the composition of the Teklad diet, and therefore these numbers are reasonable estimates on a batch to batch basis.**

**These changes have also altered the calculations shown in Supplementary Figure 1E, and we have updated both that figure, and the p-values obtained from the mixed linear models in the results section.**

1. But I cannot compute how a 45% fat by weight diet is only 0.7kcals/g less than the 5% by weight fat diet. Please check.

**These values have been now verified with the vendors.**

1. I disagree that a high fat content specifically causes weight gain, even if the calculations from table 1 are correct and there was no difference in energy intakes. Activity levels of mice were not assessed and so this claim should be removed.

**This statement was removed, as the reviewer makes and excellent point regarding our interrogation of energy expenditure without supporting data.**

1. Why was a 16h fast chosen? This should be discussed, this is prolonged and likely induces some stress, could stress susceptibility be a factor related to weight gain? Can cortisol be assessed?

**We chose to examine mice after a 16h fast in order to activate the full breadth of body weight defense mechanisms, outside of food intake. This includes activation of not only glucagon, but also cortisol, and growth hormone dependent catabolic pathways (see** [1, 2]**). At this stage, we did not observe substantial ingestion of the bedding materials, and we and others have previously used this duration of fast in other studies** [3]**. Although it is possible that we may observe altered effects with shorter or longer fasting times, we have not performed those studies. We have addressed this issue in the discussion section (note, references are numbered relative to this document):**

**We tested the effects of fairly prolonged food deprivation, as this duration will activate not only glucagon signaling, but also other catabolic signaling cascades including growth hormone and glucocorticoids** [1, 2]**. In this way, our protocol for food deprivation is likely to engage all of these pathways to defend body weight in the absence of food.**

**We also mentioned in the results section that hormones were altered during fasting in manner consistent with other reports:**

**Futhermore, the fasting/refeeding responses (decreases in total ghrelin, glucagon, GLP-1 and increases with insulin) are consistent with previous data, supportive of a normal fasting response** [4–7]**.**

1. Do the investigators consider that fasting levels of gut related peptides and one refed timpoint is sufficient for proper assessment of appetite related factors? Please discuss. Please clarify the re-fed time taken in the methodology.

**Refeeding was performed for 6h in these animals, as clarified in the methods section:**

**For re-feeding experiments, the indicated food was re-administered to fasted animals for 6 hours *ad libitum* along with water.**

**The reviewer brings up an excellent point regarding the timing of appetite related factors. Although we observed significant elevations in fasting ghrein and glucagon, and a modest elevation of GLP-1 between fasted and refed groups (see Supplementary Figure 2A), a thorough examination of the kinetics of these changes was not performed, and is beyond the scope of this study. These markers do however provide a context to the extent of fasting and re-feeding responses observed in Figure 3. This is mentioned in the results section as described above in comment #4 from this reviewer.**

**Furthermore, we clarify in both the results and discussion section that we determined total ghrelin and not active ghrelin.**

1. I am confused between cohorts 3-6 given in Figure S1 and Cohorts 1-4 in the text please reconcile.

**This was a labeling error and has been fixed in both the manuscript and Figure S1.**

1. Since HFD animals gained more at one location than the other, I do not think it is appropriate to combine these. Do the results hold when HFD groups are separated into cohorts by location? Could this be reason for variability in weight gain on HFD? I am not sure what is meant by “minimal variation across cohorts”? From the figure to me it looks as if there is considerable variation across the HFD cohorts, but within the same cohort is smaller variation?
2. Please also provide data as to all relationships between WL by fasting when given in grams rather than as % in Figure 3, do these results hold?

**Furthermore, we checked the data in Figure 2C, and determined that there is no significant correlation between any of these hormones on either percent or absolute weight gain. This is noted in the results section as well:**

****

**Effects of fasted pre-diet hormone levels on absolute weight gain.** The correlations between these hormones and percent weight gain is still shown in Figure 2C. All correlations are p< 0.20 and rho2 < 0.14

**We observed no significant correlation between pre-diet hormone levels and either percent or absolute weight gain in both HFD and CD fed mice (Figure 2C and Table 2).**

**Since absolute weight gain data are not included in the revised manuscript they are presented to the right**

1. Why were there 3 fasting weight loss periods reported in Figure 3A? This was only done twice 12 weeks apart? There were also 2 outliers, did results include these individuals? Figure 3B can be deleted.
2. The last 3 paragraphs of the results section could be shortened to one. Please clarify the relative contribution of fasting response and diet to weight gain.
3. Please be consistent with terminology utilising NCD or “chow”

**NCD is now used throughout the manuscript when referring to the normal chow diet rather than “chow”.**

1. Why use “trend” to described the reduction in food intake, this P was <0.05?
2. Figure 3B doesn’t match the listed figure should be 1C and 1D?

**This has been fixed, we thank the reviewer for noticing this error.**

1. Figures 2 is not significant and could be removed.
2. Why were NCD not included on Figure 4?

**As noted in Supplementary Figure 1 and Figure 3 there are dramatic differences in the weight gain and feeding responses in NCD fed animals compared to CD fed animals. This is likely due to the different nature of the ingredients (grain for chow, chemically defined ingredients for CD/HFD). We therefore decided that it would be difficult to interpret fasting responses in this system, and that the appropriate control for HFD was the CD. We therefore did not perform these studies using animals kept on chow throughout. This is explicated in the results section as such:**

**Due to the dramatically different fasting responses observed in NCD mice compared with CD mice (See Figure 3C and E) we only performed these studies comparing the more chemically comparable CD and HFD diets**.

1. Data is not split in SFig2 for hormone/diet differences but is stated in the results section. Please include or remove entirely.

**Supplementary figure 2 now clearly indicates which diet/feeding status changes were statistically significant. We have clarified the meaning of the asterisks in the figure legend. We have also added more details regarding these hormonal changes being consistent with previous reports, and for which changes we observed statistically significant differences:**

**Significant differences of were detected between several hormones (resistin, total ghrelin, GLP-1 and leptin) as well as fasting glucose levels between these diets. These are consistent with previous reports of HFD induced changes relative to NCD diets for resistin and leptin** [8, 9]**.**

## Reviewer 2

In this manuscript the authors have described the physiological effects of dietary manipulation in a common inbred strain of laboratory mice. The aim of this study was to control the genetic background, environment and diet of these laboratory animals as closely as possible in order to assess the amount of variability that is not due to genetic differences. Although this work is compare different genetic mouse, the data presented are not novel and some results are not consistent with the conclusive statements of the Authors.

**Although, it was not specified which results are not novel or which results are not supported by the authors, we believe that these very likely overlap with some of the comments and clarifications suggested by reviewer #1. This is especially true for reviewer 1’s comments 1, 3 and 7 which we have dealt with above.**

I saw this paper published on biorxiv already. <http://biorxiv.org/content/early/2014/04/23/004283>

## Other Changes

* Based on a suggestion of someone who read the article on biorxiv, we tested the assumption that the pre-diet hormones and body weight gain (both absolute and percent) fit a normal distribution. We did this via a Shapiro-Wilk test, and determined that the data were not normally distributed. Therefore rather than use Pearson’s R to calculate correlations we used Spearman’s Rank Order test, which does not presume normality between covariates. This alters the values in Table 2, and is described as such in the methods section:

**Potential correlations were tested by determining Spearman’s rho after finding that both absolute and percent weight gain did not fit a normal distribution (Shapiro-Wilk test p<0.05).**

## References

1. Ho, K. Y., Veldhuis, J. D., Johnson, M. L., Furlanetto, R., Evans, W. S., Alberti, K. G., and Thorner, M. O. (1988). Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. J. Clin. Invest. *81*, 968–975.

2. Luque, R. M., Park, S., and Kineman, R. D. (2007). Severity of the catabolic condition differentially modulates hypothalamic expression of growth hormone-releasing hormone in the fasted mouse: potential role of neuropeptide Y and corticotropin-releasing hormone. Endocrinology *148*, 300–9.

3. Lu, B., Bridges, D., Yang, Y., Fisher, K., Cheng, A., Chang, L., Meng, Z., Lin, J., Downes, M., Yu, R. T., et al. (2014). Metabolic Crosstalk: molecular links between glycogen and lipid metabolism in obesity. Diabetes.

4. Holst, J. J., Orskov, C., Nielsen, O. V, and Schwartz, T. W. (1987). Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. FEBS Lett. *211*, 169–74.

5. Kreymann, B., Ghatei, M. A., Williams, G., and Bloom, S. R. (1987). Glucagon-Like Peptide-1 7-36: A Physiological Incretin in Man. Lancet *330*, 1300–1304.

6. Tschöp, M., Weyer, C., Tataranni, P. a, Devanarayan, V., Ravussin, E., and Heiman, M. L. (2001). Circulating ghrelin levels are decreased in human obesity. Diabetes *50*, 707–9.

7. Tschöp, M., Smiley, D. L., and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. Nature *407*, 908–13.

8. Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., Patel, H. R., Ahima, R. S., and Lazar, M. a (2001). The hormone resistin links obesity to diabetes. Nature *409*, 307–12.

9. Frederich, R. C., Hamann, A., Anderson, S., Löllmann, B., Lowell, B. B., and Flier, J. S. (1995). Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat. Med. *1*, 1311–4.