

## **Author's response to reviews**

**Title:** Body Mass Index, Percent Body Fat, and Regional Body Fat Distribution in Relation to Leptin Concentrations in Healthy, Non-Smoking Postmenopausal Women in a Feeding Study

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**Author's response to reviews:** see over

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Re: MS: 6062648521172541

BMI, Percent Body Fat, and Regional Body Fat Distribution in Relation to Leptin Concentrations in Healthy, Non-Smoking Postmenopausal Women in Feeding Study.

We are happy that the reviewers read our manuscript very carefully and would like to thank them. We have addressed, point by point, the issues raised by the reviewers. Changes to the manuscript are made in ***bold and italics***.

*Reviewer 1: "In this study, the authors aimed at examine the association between serum leptin concentrations and adiposity estimated by BMI and dual energy x-ray absorptiometry measures in postmenopausal women. They found that, under controlled dietary conditions, higher levels of adiposity were associated with higher concentrations of serum leptin. The study, although limited by its design, is well designed and performed. We feel that its usefulness is very limited, since it adds very limited informations to the body of knowledge on postmenopausal body composition and leptin levels. The authors state that their study is the first to report the associations between DEXA body fat dfistribution and lean body mass in relation to serum leptin concentrations in which energy was balanced to mantain weight of the women. They do not take into consideration the work of other authors (Di Carlo et al., 2004, Tommaselli et al., 2006) that, although did not use a particular diet, studied women that did not increase their weight. The results of this study simply confirm the enormous amount of data present in the literature."*

**It is true that the results of our study confirm the finding from several studies of a positive association between adiposity and leptin concentrations. However, because both diet and body weight variations are known to alter serum leptin levels, it is important to control for these factors when studying the associations between body composition and leptin levels. As stated in the discussion section of our paper, to our knowledge our study is the first study to evaluate the associations between BMI as well as DEXA adiposity in relation to serum leptin levels in healthy postmenopausal women under controlled dietary conditions in which energy was balanced to maintain weight of the women. In addition, smoking, hormone replacement therapy (HRT), and compounds with estrogenic properties are known to affect leptin concentrations secondary to their effects on body composition. Thus, smoking, HRT, and other estrogenic compounds are confounders of the relationship between adiposity and leptin concentration in postmenopausal women. The study by Di Carlo et al. (2004) and Tomamaselli et al. (2006), are excellent studies, but both of these studies assessed the treatment effects of HRT and estrogenic compounds (tibolone and raloxifine) on body composition changes on leptin levels. In addition, neither of these studies accounted for the effects of diet.**

Reviewer 2: “*This manuscript describes the relationship between body mass index, fat distribution and leptin levels in healthy non-smoking postmenopausal women during weight maintenance. It is a nice contribution to the literature and requires only minor changes. It could however be significantly improved upon if the authors included measurements of other important hormones such as ghrelin and adiponectin. The authors should include these measurements if they are available. In addition, did the authors assess physical activity levels and if so did this impact the associations? The authors need to explain clearly that the results presented in this manuscript refer to the control arm of the feeding study only and remove any reference to 'each dietary period'. The methods used by the authors to assess the associations are appropriate and are sufficient to replicate the work.*”

Unfortunately, we do not have measures of ghrelin and adiponectin. While we did not assess physical activity levels, the calorie intakes of the women were adjusted to maintain their body weight, and thus we indirectly compensated for the effects of energy expenditure. Our study was conducted in the control arm of the feeding study. We used the term diet period to indicate the study period when the women crossed over from one arm of the study to another after the washout period. **We have now changed the term diet period to study period throughout the text.**

#### Minor essential revisions

1. *TITLE: Please define BMI in the title.*

**Done**

2. *ABSTRACT:*

*In the final sentence of the 'methods' section the term 'each diet period' does not need to be used as only the control group of the other study was used.*

**Diet period is now changed to study period.**

3. *Why were the results for the association between lean body mass and leptin not included here?*

**This is shown in Table 3.**

4. *INTRODUCTION*

*Sentence 5: Were other hormones measures (ghrelin, adiponectin), if so please include here.*

**Ghrelin and adiponectin were not measured.**

5. *Last sentence on the page 3 'In addition...and circulating leptin concentration' has already been mentioned.*

*We have now removed this sentence.*

6. Sentence beginning 'Indeed, there may be cross talk.... Please explain and expand upon what is meant here.

**Adipose tissue plays a central role in energy homeostasis and metabolism, behaving as an endocrine organ. Skeletal muscles (lean mass) have similar functions with regards to metabolism. It is suggested that cytokines and leptin derived from both adipose tissue and lean mass play a critical role in maintaining the ratio of lean mass to fat mass. Thus, leptin could play a role in the cross-talks or conversations between adipose tissue and lean mass (Argiles et al. 2005).**

#### *MATERIALS AND METHODS*

7. Please make it clear that the group used in the study was the control arm of the feeding study.

#### *Done*

8. Why were the fat measurements taken at week 4 and not at week 8? '...measures of BMI were taken on the same day.' How were these measured? Who assessed BMI?

**The DEXA measurements were done at week 4 at the mid point of the study. This is how the study was approved to be conducted. Since the study employed an isocaloric weight maintenance diet, it would not matter whether the DEXAs were done baseline or at the end of week 8. Weight and height were converted to BMI.**

#### *Subjects*

9. Please include the socio economic status of the participants?

**Unfortunately, we did not collect data on the socioeconomic status of the women. This was viewed as a sensitive question and it was not asked.**

#### *Diets and Feeding*

10. Why was weight measured on each weekday, and which of these measures was used to calculate BMI? Please provide information on anthropometry, how was weight and height measured? Was waist and hip measurements taken and if so what is the association between waist: hip ratio and leptin concentrations?

**The weight that was measured on each weekday was done purely to monitor the weights of the women to adjust calories. The women had to maintain their weights. These measurements were done that the Beltsville Human Nutrition Unit at USDA in Maryland. Waist-to-hip ratio measurements were not done, and in retrospect this was a limitation.**

#### *Biological sample collection and analysis*

11. Again '...in each dietary period' is mentioned although there is only one dietary period.

**We have now changed to *study period*.**

12. Please include additional information regarding the handling of blood samples, e.g. time between sampling and analysis and provide the coefficients of variation for the assay.

**We clarify in the method section of the manuscript that the fasting blood samples, collected in the morning before breakfast were taken almost immediately the laboratory housed within the same complex for serum extraction. The serum was extracted, aliquoted, and stored in a  $-70^{\circ}\text{C}$  freezer. Within batch coefficient of variation for the leptin assay was less than 15%.**

#### STATISTICAL ANALYSIS

13. 'Additional models estimated percent changes in serum leptin concentrations...' The authors need to re-phrase this sentence as repeated measures would be necessary to have information to show percent change in leptin.

**We are not sure we follow clearly the comment/suggestion here. We are not measuring percent change in leptin concentrations over time (but cross sectionally), which would require repeated measures at each time point. We have continuous measures (continuous variable) for BMI, and DEXA adiposity, therefore, we used simple statistical modeling to assess the effects of changes in BMI (one-unit change), changes in total fat (one percent change), changes in body fat distribution (1000g changes at a time) to compute percent change in serum leptin concentrations. In a linear regression model, for example:**

$$E[\ln(\text{leptin})] = B_0 + B_1(\text{BMI})$$

**For log transformed data, a one unit change in BMI is associated with:  $\exp(B_1) - 1$ , which is the percent change in leptin.**

14. The authors say that race was included as a categorical variable (African American, yes/no)... 'Were Asian participants excluded from this analysis?

**African American, yes/no is only an example of how we handled race. We have made the clarifications in the text.**

15. Again 'dietary period' is mentioned when there is only one dietary period.

**Done.**

#### RESULTS

16. Was lean body mass correlated with BMI or total fat mass? Did the authors assess the association between lean body mass and leptin concentrations while adjusting for total fat?

**Yes indeed, there was a weaker correlation between lean mass and BMI than BMI and total fat mass. The statistical model for lean mass is shown in the footnotes to Table 3. In model 1, we adjusted for known breast cancer risk factors – age, race, family history of breast cancer, parity and menarche < 12 years. In model 2, we added BMI to the variables in model 1. Thus, we did not include total mass, but included BMI, which is significantly and highly correlated with total fat mass in our study. As a result, statistically, the point estimates would not change much whether we adjusted for BMI or total fat mass.**

#### *DISCUSSION*

*17. The third sentence states that 'Higher lean mass was also associated with higher serum leptin concentrations...' but this is only when BMI is not included in the model, please include in the discussion that the association is not present after adjusting for BMI. Also could the authors provide some potential reasons for this?*

**Done.**

*18. The authors mention in sentence 5 that a 'study similar to ours...' although they have mentioned earlier that theirs is the 'first study'. Could they please include and highlight the differences between their study and that of Havel et al.*

**We are sorry for this confusion and now clarified the distinctions. Unlike our study, about 50% the women in the Havel et. al. study were on HRT. Because we used DEXA scans, our study also had a large array of adiposity measures.**

*19. Page 11: First sentence: please provide references for these studies.*

**Done.**

*20. Page 11: the sentence beginning with 'Further, our results...' has been repeated earlier in the discussion section, please delete this sentence.*

**We have now removed this sentence.**

*21. Page 11: the sentence beginning with 'Our adiposity measures...' should be in the methods section of this paper.*

**We have already described in the method section how we computed percent change in serum leptin concentrations. We think the discussions regarding adiposity measures on different scales is appropriate because it is important for readers to understand why we used multiple  $R^2$  to summarize the results as well.**

*22. It needs to be stated that the results are not generalisable to all group as the participants were non-smoking, postmenopausal, women who were not taking hormone replacement therapy.*

**Done.**

*23. Was habitual alcohol intake assessed and if so can the authors include this in their analysis?*

**None of the women drank alcohol other than what was given during the actual study.**

*24. Page 13: second sentence: 'Because of the well-known...' what are the authors referring to here, please elaborate.*

**Well-know arguments for the limitations in the use of BMI include the inability of BMI to distinguish between body fat and muscle mass. That is why we gave the example, in the following sentence, that for a given BMI Asians have higher body fat content.**

*TABLES*

*25. Table 3: The footnote says '... for each hormone...' were other hormones measured and of so can the authors include these in their manuscript.*

**We meant leptin. We have now made the necessary corrections.**