Author's response to reviews

Title: Chronic and acute effects of walnuts on antioxidant capacity and nutritional status in humans: a randomized, cross-over pilot study

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Author's response to reviews: see over
March 25, 2010

Enclosed please find our revised manuscript, “Chronic and acute effects of walnuts on antioxidant capacity and nutritional status in humans: a randomized, cross-over pilot study,” by Diane L. McKay, C-Y. Oliver Chen, Kyung-Jin Yeum, Nirupa R. Matthan, Alice H. Lichtenstein, and Jeffrey B. Blumberg for consideration of publication in Nutrition Journal.

We respectfully thank all of the reviewers for their helpful comments, and provide a point-by-point discussion of each below.

Sincerely,

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Author response to reviewers’ report

Title: Effects of walnuts on antioxidant capacity and nutritional status in humans: a randomized, cross-over pilot study

Version: 1 Date: 3 January 2010
Reviewer: Andrew Collins

Minor revisions:
Not surprisingly, there is a tendency to dwell on effects that are not statistically significant, but suggestive. This should be resisted. In the Conclusions, as well as the Abstract, the (albeit very weak) statement that ‘these data indicate a trend in a direction that suggests a dose-response relationship’ is really not justified.

Author response: This statement has been deleted from the Abstract and Conclusion sections.

Page 5, para. 3, lines 5-7: I am not convinced of the logic of or need for the 2 days’ abstinence from polyphenol-rich foods.

Author response: Polyphenol-rich foods have been shown to increase plasma markers of antioxidant capacity and reduce oxidative stress. We wanted to ensure the effects we observed were due to the walnuts alone, and not to any other polyphenolic-rich foods consumed by our subjects. Reports in the literature
indicate that most polyphenolic metabolites are cleared from plasma and urine within 48 hours.

Figures 1 and 2: error bars should be included. In figure 2, the changes in linolenic acid are larger but apparently less significant than those in linoleic acid. Does this reflect a much higher inter-individual variability?

**Author response:** Error bars have been added to Figures 1 and 2.

Discretionary revisions:
Page 6, para. 2, line 2: The range of BMIs is very large, and includes some obese and some underweight subjects. Restricting the study to subjects with normal BMI would have been preferable.

**Author response:** The qualifying BMI range for our subjects was 18.5-35 kg/m², which includes normal weight, overweight, and stage I obese subjects. It does not include underweight subjects or those with a more extreme degree of obesity. We included subjects who were overweight and obese in order to enhance the generalizability of our study findings, as they represent 2/3 of the U.S. adult population. As indicated in Table 2, the actual mean BMI of our subjects was ~27 and the SD (~3.7) was not particularly large.

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Minor Essential Revisions
1. It would be helpful if dietary data were presented. Were there any changes in the macronutrient profile of the diets on the two treatments?

**Author response:** Under Results, at the end of paragraph 1, we do indicate the following: “Data from the food frequency questionnaires revealed no significant differences in subjects’ dietary intake within or between each intervention phase.” Please see our response to Dr. Thomson.

2. It is interesting that the lower dose of walnuts appeared to improve the blood lipid profile while the effect was lost at the higher dose. Please discuss.

**Author response:** Changes in blood lipid profiles were not the primary outcome of this pilot study and, as such, we did not elaborate on this finding. The discrepancy could have been due to variability in subjects’ background diets, or the small sample size.

3. It may be helpful to use absolute values in some of the tables. Were there any
baseline value differences for any of the analytes? Have the authors considered using a mean baseline (i.e. mean baseline from both treatment periods).

**Author response:** There were no between-group differences for any of the analytes at baseline. We also ran the analyses for both interventions using a common baseline, and found no discernable differences.

4. Lipid ratios should be presented in Table 5.

**Author response:** We have now added changes in the total cholesterol-to-HDL ratios for both interventions to Table 6 (formerly Table 5).

5. Body weight data should be presented.

**Author response:** Baseline BMI values are included in Table 2. The following sentence has been added to the paragraph 1 of our Results section: “No significant changes in body weight were observed with either intervention.”

Discretionary Revisions
1. For the Figures, grams should be used instead of ounces.

**Author response:** We have changed oz to grams on Figures 1 and 2.

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Version: 1 Date: 30 December 2009
Reviewer: Jessica Grieger

2. Minor Essential Revisions
Methods:
a: what is the rationale for using a 6 week washout period in between walnut consumption visits?

**Author response:** According to ATP III guidelines, 4-6 wk is sufficient to see an effect on the lipid profile due to changes in diet. We chose this amount of time in order to prevent carryover effects from the first intervention.

b: the BMI criteria is broad (18.5-35 kg/m2), what was the basis for this?

**Author response:** Please see our response to Dr. Collins.

Discussion:
The discussion is well written and discusses the results in terms of previous studies and acknowledges reasons for the null effects obtained. I think it would be more appropriate to state up front that the population was reazonably healthy and this may be a good reason as to why some antioxidant markers had no
effect. Leaving it to the end may underestimate why significant results may be more often found in unhealthier populations.

**Author response:** In the Abstract (under Methods), and once again in the body of the paper under Methods (Study Design and Intervention, pg 5, line 1) we do state that our subjects are “generally healthy men and postmenopausal women.”

Is it worth mentioning why there was no within or between group differences found for the folate and magnesium levels after walnut doses?

**Author response:** We do stress in the Abstract (under Discussion), Discussion (pg 16, ln 3) and Conclusions (pg 16, ln 11) that subjects were well-nourished, and it is unlikely that the amount of folate and magnesium provided by the walnuts was sufficient to see any significant change.

Conclusions:
Add a line or two about some significant results found.

**Author response:** The following sentence was added to the Conclusion section: “However, improvements in linoleic acid and pyridoxal phosphate were observed with chronic consumption, while total plasma thiols were enhanced acutely.”

3. Discretionary Revisions
Results:
a: Would it be possible to split baseline values of ORAC/FRAP to assess responders and non-responders following walnut consumption?

**Author response:** This is a good suggestion. However, the results would be questionable for such a small subset of subjects.
Author response: We agree that measuring isoprostanes is a more accepted biomarker for lipid peroxidation. Given the high cost of the isoprostane assay, and limited funding for this pilot study, we chose to assess lipid peroxidation with a more cost-effective assay.

> 3. Are the data sound and well controlled? YES -but sample size precludes making conclusions.
Please describe the baseline diet - given the selection of healthy controls there is the likelihood that the diets of the subjects were also healthy at baseline and addition of walnuts would provide little additional "exposure" to healthy food constituents to influence oxidative stress levels.

Author response: We have added a new table (Table 3) listing subjects’ dietary intake data at baseline.

> 4. Does the manuscript adhere to the relevant standards for reporting and data deposition? yes

> 5. Are the discussion and conclusions well balanced and adequately supported by the data? generally

> 6. Do the title and abstract accurately convey what has been found? generally and qualifies the pilot nature of the research. The title would be improved by adding the short and long term assessment of antioxidant capacity - the assessment of both short and long term effects is somewhat unique for these studies.

Author response: We have changed the title to: “Chronic and acute effects of walnuts on antioxidant capacity and nutritional status in humans: a randomized, cross-over pilot study.

However the conclusion that a controlled feeding intervention is needed seems not to be supported by the manuscript which documented good adherence to walnut intervention based on FA measures.

Author response: Although adherence to the walnut regimen was good, the variability in subjects’ self-selected background diets may have precluded our ability to see any changes with walnuts alone. Therefore, we feel it would be appropriate to control for background diet in future studies.

> 7. Is the writing acceptable? yes well-written