Author's response to reviews

Title: Endothelial Dysfunction in Obese Non-Hypertensive Children Without Evidence of Sleep Disordered Breathing

Authors:

Rakesh Bhattacharjee (rakesh.bhattacharjee@sickkids.ca)
Wadha H Alotaibi (waltobaib@yahoo.com)
Leila Kheirandish-Gozal (lgozal@peds.bsd.uchicago.edu)
Oscar Sans Capdevila (osans@hsjdbcn.org)
David Gozal (dgozal@peds.bsd.uchicago.edu)

Version: 2 Date: 5 November 2009

Author's response to reviews: see over
- We thank all 3 Reviewers for their constructive criticisms. We have modified the manuscript according to their suggestions. A point by point response to each of the comments follows below.

**Reviewer's report**

**Title:** Evidence of Endothelial Dysfunction in Obese Non-Hypertensive Children  
**Reviewer:** Aaron S Kelly  
**Reviewer’s report:**

Summary:
The authors measured endothelial function in normal weight and obese pre-pubertal children without obstructive sleep apnea syndrome and hypertension. There was no difference between groups in the magnitude of reperfusion. Obese children had delayed reperfusion compared to normal weight peers. The authors conclude that pediatric obesity, independent of OSAS and hypertension, is associated with endothelial dysfunction.

**Major Comments:**
1. The authors might consider adding OSAS to the title since this is major focus of the paper.  
   - The title has been modified as requested.

2. It is unlikely that only 60 seconds of occlusion will result in maximal reactive hyperemic blood flow. Therefore, the magnitude of reperfusion was likely underestimated.  
   - It is possible, yet unproven, that extending the occlusion for longer periods of time would lead to increased reperfusion kinetics. However, the selection of a 60-second occlusion was based on child comfort during testing and the reduced likelihood of the child moving during such assessment. Selection of longer occlusion times in children posed to be quite difficult and discomforting for children, and the test secondarily becomes laden with motion artifact, thereby compromising the data acquisition and validity. Further, while studies investigating endothelial dysfunction in adults select longer occlusion times, since the degree of vascular sclerosis is minimal in children in comparison to adults, the investigators anticipated robust differences even at shorter occlusion times. Finally, we have already demonstrated the marked reproducibility of the test over time (6 months).

3. It is unclear why two tests of endothelial function were performed. Ischemic pre-conditioning likely influenced the results of the second test.  
   - Selection of two tests was based on ascertainment of reproducibility of the test for any given child. The reviewer is correct in assuming the possibility of ischemic preconditioning exerting an influence. However, we found no evidence of such phenomenon since there were no significant differences between test 1 and test 2.

4. Reproducibility of the endothelial function technique, in this lab, should be
presented.
- Please see above.

5. Has this technique been validated against coronary blood flow or other standard techniques for measuring endothelial function? Is it nitric oxide-dependent?
- To the best of our knowledge this test has not been validated against coronary blood flow in children. The likelihood of conducting coronary perfusion studies in children is very low and imposes formidable challenges. Determination of the mechanisms of endothelial dysfunction in children, including ascertaining perfusion responses to nitric oxide and non-nitridergic pathways are currently underway.

6. Statistics: adjustments should be made for age, gender, and race.
- There were no significant differences found between both obese and non-obese children for these specific demographic factors (see Table 1), hence no adjustments are really needed.

7. Importantly, the main conclusion seems flawed and is not supported by the data. There were no differences between groups for magnitude of reactive hyperemia (endothelial function). Although differences in time to peak hyperemia were observed, it is unknown what this means and is unclear if it has any relevance to cardiovascular risk. It may simply be that the obese, because they have more tissue to reperfuse, have a delayed blood flow response.
- Measurement of capillary perfusion is dependent on capillary density, skin pigmentation, skin thickness and as such is quite dependent on probe location. While efforts were made to replicate the location in all children, these factors have significant effects on magnitude of reactive hyperemia. As such, this technique delineates endothelial function by specific kinetic responses as opposed to actual magnitude-amplitude of the response as suggested by this reviewer.

8. The following comment is too strong based upon the evidence in this study: “...our identification of abnormal endothelial function would suggest that these homeostatic processes have already failed...”
Manuscript text modified as requested.

Minor Comments: 1. Figure 2, panel 3: it would be helpful to report the correlation adjusted for either BMI or body fat.
No adjustments were made since the 2 groups were matched.
Reviewer’s report
Title: Evidence of Endothelial Dysfunction in Obese Non-Hypertensive Children
Version: 1 Date: 6 October 2009
Reviewer: Stijn Verhulst

Reviewer’s report:
This is a well written, interesting and important study which compared markers of endothelial dysfunction between obese and non-obese children. The authors excluded or controlled for a number of confounding conditions including hypertension, sleep apnea and a family history of cardiovascular disease. I have some comments and suggestions for the authors:

1. The authors have chosen to exclude children with sleep apnea. Although I can understand this approach, I think that it would be very interesting to look at endothelial function in obese children with and without sleep apnea.
   - We absolutely agree; however, such study would be beyond the scope of the present manuscript. The impact of the concurrent presence of sleep apnea in obese and non-obese children is currently being prospectively investigated using a pre- and post-OSA intervention approach.

2. Is the InBody 320 scale which was used in the present study to determine the percentage body fat validated in children?

3. Uric acid could be an important molecule in the relation between obesity, sleep apnea, hypertension and endothelial dysfunction. Do the authors have any data on uric acid or on other markers of oxidative stress in this population?
   - We are keenly aware of the studies by Dr Styjn group on uric acid, obesity and sleep apnea in children. Unfortunately, data pertaining to uric acid are not available.

4. Subjects with fasting glucose > 120 mg/dl were excluded? Why did the authors did not chose the cut-off of the ADA which is 126 mg/dl? An oral glucose tolerance test was not performed, so it remains possible that some subjects did have type 2 diabetes.
   - It is indeed possible that some children may have type 2 diabetes due to the fact that an OGTT was not done. However, we find that in most obese children, overt diabetes is relatively infrequent and usually manifests as fasting glycemic levels that exceed 130 mg/dl. Independently, both groups of children underwent the same selection criteria.

5. In my experience, many of the variables collected in the present study are not normally distributed. Therefore, I wonder if non-parametric testing is not indicated?
   - The Reviewer raises an important issue in this type of research; non
parametric testing of the data did not alter any of the significance levels and in fact due to such tests being more “liberal” further accentuated the differences. Since the conclusions are the same, we opt to retain the current analytical procedures.

6. It is surprising that none of the metabolic variables were different between both groups. Can the authors explain this finding?
   - Indeed this was a rather surprising finding, although this may be explained by the fact that none of the obese children had obstructive sleep apnea. Also, while children statistically met criteria for obesity, there was a paucity of children with extreme morbid obesity in this cohort.
Reviewer's report

Title: Evidence of Endothelial Dysfunction in Obese Non-Hypertensive Children

Version: 1 Date: 22 October 2009
Reviewer: Keiran Mather

Reviewer's report:

This is a well done paper using laser doppler on dorsal hand skin to evaluate post-occlusion hyperemia as a measure of endothelial function, comparing obese versus non-obese children. This is done in the context of a larger trial of sleep apnea, and the authors have taken this opportunity to carefully exclude subjects with underlying OSA, a potential confounder in similar prior work. The authors found that time to maximal post-occlusion flow response was prolonged in obese subjects, and related to BMI, to percent fat by bioimpedance, and to triglycerides. The design and presentation are clean overall, and the paper reads well. The results are concordant with finding in other obese populations using other methods, but unique in the carefully defined pediatric population studied. I found both the introduction and the discussion wordy (not off topic, just needs shortening overall).

Major compulsory revision:
My main issues had to do with a lack of discussion/presentation of validation details regarding the doppler methodology. Although this is arguably moot given the positive findings, it would be of value to objectively present whether the method itself has been compared against other approaches, and whether the study-specific changes to methodology (short occlusions) have been systematically evaluated. In general, do measures of skin microvascular responses relate to other measures of vascular function and/or to meaningful outcomes?

- The reviewer brings up a very important question that lends itself to further research queries. While the findings presented in this manuscript attempted to correlate endothelial function to certain specific atherogenic markers determined by blood sampling, and although we explored such correlations in the context of sleep apnea in children (e.g., Kim J, Bhattacharjee R, Snow AB, Capdevila OS, Kheirandish-Gozal L, Gozal D. Myeloid related protein 8/14 levels in children with obstructive sleep apnoea. Eur Respir J. 2009 Jul 16. [Epub ahead of print]), correlation of this novel assay of endothelial function to other assays of vascular function would be necessary. However, none of the other tests has actually been validated in children. Furthermore, the main problem of the other existing unvalidated tests is inherent to the fact that all such measures of endothelial function are invasive and present therefore a formidable obstacle from the ethical standpoint and from the pragmatic standpoint when assessing pediatric cohorts.

Discretionary revision:
A similar methodologic issue comes in a lack of detail of what parameters are extracted from the impedance measures. Which measures are direct and which are calculated? What is 'dry lean mass' and is it importantly different from overall percent body fat?
- All information from Anthropometry including dry lean mass were extrapolated from impedance measurements using InBody 320.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable