

Author's response to reviews

Title: Lower energy expenditures in infants from obese biological mothers

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Author's response to reviews: see over

Responses to reviewer #1 (Maciej Buchowski)

General comments:

We thank this reviewer again for his/her helpful criticisms. We have addressed the following general comments as follows:

Major Compulsory Revisions:

1. In accordance with the reviewer we reduced the amount of discussions about calorimetric methods and their comparison to the EMTAC. We included more text focusing on the results and additional discussion regarding the ethnic background of our infants and how this might relate to future childhood obesity.
2. As suggested by the reviewer, we included some additional discussion regarding why we only conducted a four-hour morning measurement beginning on page 12, line #14. Additional discussion about the limitations of our study in regards to the ethnic background of our infants begins on page 13, line #21. Finally, we now mentioned the need for further studies on page 14, line #3.
3. As requested by the reviewer we include the main reason why infants only had a 4-hour metabolic measurement in the EMTAC. This appears on page 12, line #16 of the Discussion section.

Minor Essential Revisions:

1. We separated the Aim of the study from the Background in the Abstract as requested by the reviewer.
2. We were not able to include data from other ethnic groups in our analysis. We are the only lab in the country performing these types of studies with an instrument such as the EMTAC. Similar data to ours does not exist. However, we did include text in the discussion suggesting that further studies with a greater number of subjects from all ethnic backgrounds should be conducted utilizing the EMTAC instrument.
3. In regards to the ethnic backgrounds of the infants referred to in reference #14, we now state that infants of “similar ethnic backgrounds” were utilized in that study. This text is now included on page 12, line # 14 of the discussion section.
4. As requested by the reviewer we now clarify in the Methods section on page 7, line #13 on how sleep was determined in the infants and the calculation of sleeping metabolic rate. As presented in the last paragraph of the Results section, infants from obese mothers slept significantly less than those from the overweight group. They also slept less than those from the lean group but this differences was not significant. Since infants in the obese group appeared to consume more energy, we suspect that increased feeding activity might account for the reduction of sleep in these infants. We include some text in this regard in the Discussion section on page 11, line # 10.
5. In regards to the measurement of resting metabolic rate (RMR). This is calculated from a regression of physical activity on energy expenditure throughout the test. Resting metabolic rate is the intercept of this regression equation multiplied by the amount of time the infants are awake

and not feeding. With only four hours of metabolic data, subtracting the time periods that is attributed to sleeping metabolic rate and feeding, the resulting regression equation had a large standard error. Utilization of this equation for the calculation of RMR probably led to errors in the results. Therefore we eliminated any reference to infant RMR in this manuscript. The determination of extrapolated 24-hour energy expenditure and sleeping metabolic rate does not depend on the utilization of the data from physical activity. In our previous study (Rising R, Duro D, Cedillo M, Valois S, Lifshitz F. **Daily metabolic rate in healthy infants.** J Peds 2003, 143:180-185) we were able to utilize close to 15 hours of physical activity data for the calculation of RMR verses an average of about two hours for the present study thus providing more accurate results.

6. We corrected Table #1 according to the reviewer.
7. We added the number of males and females in each group in Table #1
8. We clarified the reference for the equations in Table #2 and describe them in detail in the Methods section of the manuscript beginning on page 8, line #3.
9. In partial agreement with the reviewer, we removed the top photo of Figure #1. However, we feel that it is necessary for readers to see how interaction actually occurs during a metabolic measurement in the EMTAC.

Discretionary Revisions

- Abstract: We changed the abstract Methods section according the recommendations of the reviewer.
- Introduction: We rewrote the last paragraph and omitted the last sentence as recommended by the reviewer.
- Results: We added the text for significance or non-significance as suggested by the reviewer.
- Discussion: We redrafted parts of the Discussion section of the manuscript and decreased the emphasis on technique/methodology as suggested by the reviewer.
- Some details: As suggested by the reviewer, we omitted frequent references to the EMTAC/new instrument.
- Conclusions: We added the text for the Conclusion section of the manuscript that the reviewer recommended.

Responses to reviewer #2 (James Delany)

General comments:

We thank this reviewer again for his helpful criticisms. We have addressed the following general comments as follows:

Major Compulsory Revisions:

1. We agree with the reviewer that there was a problem with sleeping metabolic (SMR) rate being greater than resting metabolic rate (RMR). Resting metabolic rate is calculated from a regression of physical activity on energy expenditure throughout the test. Resting metabolic rate is the intercept of this regression equation multiplied by the amount of time the infants are awake and not feeding (Ravussin et al, 1988). With only four hours of metabolic data, subtracting the time periods that are attributed to sleeping metabolic rate and feeding activities, the resulting regression equation had a large standard error. Utilization of this equation for the calculation of RMR probably led to errors in the results, such as RMR being less than sleeping metabolic rate. Therefore we eliminated any reference to infant RMR in this manuscript. The determination of extrapolated 24-hour energy expenditure and SMR does not depend on the utilization of the data from physical activity. In our previous study (Rising R, Duro D, Cedillo M, Valois S, Lifshitz F. **Daily metabolic rate in healthy infants.** J Peds 2003, 143:180-185) we were able to utilize close to 15 hours of physical activity data for the calculation of RMR verses an average of about two hours for the present study thus providing more accurate results. Therefore, we just present data for 24-hour extrapolated energy expenditure and SMR.

We feel that reporting uncorrected data will not add anything to the interpretation of the results. All energy expenditure data is usually reported as “Daily” or for 24-hours. This allows comparison to other metabolic studies. A four-hour metabolic measurement, uncorrected for differences in body size and composition, does not allow determination of true metabolic rate differences among the three groups of infants that were of similar body weight.

In partial agreement with the reviewer we do present the amount of time infants spend asleep during the test. We present it as the percentage of time during the 4-hour (240 minutes) that the infant was observed to be asleep. The rest of the time the infants were active in some way whether feeding, playing or just lying still. For this study we did not record the actual amount of time infants spent just awake but inactive.

As we mentioned above we felt that calculating RMR during a four-hour test was not accurate. Therefore, we just eliminated presentation of this data from the manuscript.

2. In accordance with the reviewers comments we clarified the discussion of the advantages of the EMTAC instrument. We present part of this on Page 3, line # 14. We present some additional information on Page 12, line #10 of the Discussion section. The main advantage is the installation of the hand access ports around the infant enclosure. This allows unrestricted access to the infant by parents, thus eliminating problems with separation anxiety during the four-hour metabolic test. Previous studies of energy expenditure utilized instruments that had several methodological problems. For example, three instruments utilized in previous studies (Moon et al, Appl. Physiol.

1993;74:476-484; Butte et al, Am. J. Clin. Nutr. 1995;62:1047S-1052S; and Roberts et al, NEJM 1988; N Engl J Med 1988, 318:461-466) had no means for parents to interact with their infants during metabolic testing. Without a means for parental interaction infants may have been stressed during testing thus causing artificially increased energy expenditure. This could lead to inaccurate interpretation of the infants true energy needs. In an attempt to compensate for the possible separation stress of infants, metabolic testing usually only lasted one to two hours. Moreover, some tests were done while the infant was asleep. The doubly labeled water technique utilized as a companion technique might contribute to errors due to the greater body water pool turnover infants in comparison to adults. The technique can over/underestimate total daily energy expenditure by as much as 10% in adults (Rising et al, Am. J. Nutr. Nutr 1994; 59:800-804). Having one single indirect calorimetric method to determine all of the components of energy expenditure, as well as physical activity, offers an advantage of a simple, accurate technique that might be useful in a clinical setting.

3. As found by the reviewer, the errors in the interpretation of References #11 and #12 have been corrected. Additionally Reference #12 was replaced with the correct reference as suggested by the reviewer.
4. The reviewer suggests we correct the metabolic data for infants in each group by utilizing body weight as the dominator. However, this is not appropriate because the relationship between body weight and energy expenditure does not pass through zero. There have been several studies reported by Dr. Eric Ravussin that suggest that it is better to utilize fat-free and fat mass in derived regressions to correct metabolic data. The problem with just using body weight is that infants with similar body weights, as in the case among the three groups of infants in our study, may have different proportions of metabolically active fat-free to the assumed non-metabolically active fat mass. Therefore, it is necessary to utilize the residuals of the regression equations based on fat-free and fat mass to correct the metabolic data. If age is a statistical predictor, than that should also be included in regression equations when correcting metabolic data. We have validated this methodology for correcting metabolic data in several of our previous studies (reference #s 14, 15, 21, 23 and 30). Moreover, it has been validated in adults (reference #s 9 and 10). Finally, the high correlations between fat-free mass, as calculated in our study, and 24-hour extrapolated energy expenditure ($r=0.94$) and SMR ($r=0.93$) is evident of the appropriateness of our methodology for correcting metabolic data. These same relationships have been found in several previous studies by Dr. Eric Ravussin. We do present both these correlations in the Results section of the manuscript.
5. We agree with the reviewer in that we have over step our bounds in the discussion of the accuracy of the doubly-labeled water technique. Therefore, we eliminated most of the references to the accuracy of the technique in the manuscript. We just mentioned in the Introduction that the technique was part of a study reported by Roberts et al, 1988.
6. In agreement with the reviewer we addressed the concerns regarding the ethnic background of our infants. We cite an additional study (reference #38) that explains similar results in teenagers. Moreover, we do suggest in the last paragraph of the Discussion section that due to the minimum number of Caucasian or Afro-American infants in our study, additional infant metabolic measurements should be made in those from these ethnic backgrounds.

Minor Essential Revisions

1. We corrected the second paragraph on page 3 according to the reviewer's suggestion.
2. In partial agreement with the reviewer, we removed the top photo of Figure #1. However, we feel it is necessary for readers to see how interaction actually occurs during a metabolic measurement in the EMTAC.