Author's response to reviews

Title: The relationship between body composition and cardiovascular risk factors in young Australian men

Authors:

Selma C Liberato (selma.liberato@gmail.com)
Louise Maple-Brown (louise.maple-brown@menzies.edu.au)
Josefina Bressan (ibrm@ufv.br)
Andrew Hills (ahills@mmri.mater.org.au)

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Author's response to reviews: see over
Reply to reviewers

Reviewer 1

Major Compulsory Revisions:
1. Lines 32-33 (Abstract) and line 90: The statement “significant knowledge gaps exist in young people” is ambiguous. Clarification is needed. Do the authors feel that young adults, as a group, are ignorant of the relationships between risk factors and cardiovascular disease (CVD)? Or do the authors believe that the relationships between risk factors and CVD in young adults have been insufficiently studied? Or both?

Reply: Thanks for your comment. We don’t feel that young adults, as a group, are ignorant of the relationships between risk factors and CVD. The sentence was reworded to reflect that there is a knowledge gap in the relationships between risk factors and CVD in young adults. The revised sentence reads “Despite clear evidence of the coexistence of several risk factors in young people as children and an understanding of the importance of the health behaviors in controlling CV disease, there are limited data on the relationships between risk factors and CV disease in young people. Therefore further study is required.”

2. Lines 66-67: The authors should clarify the statement, “the rate of CV disease is accelerating . . . .” Does this statement apply to both fatal and nonfatal CVD?

Reply: The sentence has been amended to reflect that the rate of CV disease is accelerating everywhere. Yes, the statement applies to both fatal and non-fatal CVD. The revised sentence reads “The rate of CV disease is accelerating worldwide and one of the causes is the dramatic increase in the prevalence of obesity with its related complications of hypertension, hyperlipidemia, diabetes and atherosclerotic vascular disease.”

3. Methods Section: Indicate what criteria, other than age, were used in selecting subjects for this study. Were they students at Queensland University of Technology? Were they compensated for participating?

Reply: Description of the participant inclusion criteria and recruitment has been included as follows. “Participants were recruited by flyers posted in shopping centers and education centers as well advertisement in local newspapers. Inclusion criteria to participate in the study were age between 18 and 25 year and absence of any chronic disease. The participants were volunteers and were not compensated.

4. Lines 103-107: The implication is that body fat content was the only measurement of body composition reported. On the other hand, Table 1 and the Results section indicate that “fat-free mass” also was determined. A brief description of the “fat-free mass” procedure is needed.
Reply: A sentence describing how “fat-free mass” was determined has been added as follows. “Body fat content was determined from DEXA whereas body fat-free mass was calculated by subtracting body weight from body fat content.”

5. Line 141: Table 2, column 1 indicates that PAEE was calculated by subtracting RMR from EE. Line 141 states, “PAEE was calculated by subtracting EE from RMR.” Correction is needed
Reply: This has been corrected. The revised sentence reads “Physical activity energy expenditure (PAEE) was calculated by subtracting RMR from EE.”

6. Line 227: The term “better blood lipid profiles” is vague. The authors should specify how the blood lipid profiles of their subjects were “better” compared to those reported in other studies.
Reply: how the blood lipid profiles of the participants of the current study were “better” compared to those reported in other studies has been specified as follows “Participants in the current study had better blood lipid profiles including lower TC, LDL-C and triglycerides and higher HDL-C values compared to other studies examining young men”

7. Lines 271-272: This sentence implies that the lean men had reduced cardio-respiratory fitness and increased body fat compared to the overweight men. This is not true according to data in Tables 1 and 2. Correction is needed.
Reply: The sentence has been reworded. The revised sentence reads “The association between reduced cardio respiratory fitness and increased body fat in overweight men observed in the current study has been reported in other studies”

8. Table 2, EI, RMR, EE and PAEE values: All show 5 or 6 significant figures, which suggests an unrealistically high degree of accuracy in these measurements. Can kcal levels really be measured to the nearest 1.0 kcal? Three or 4 significant figures would be more appropriate.
Reply: EI, RMR, EE and PAEE values have been revised to 3 significant figures.

9. Table 2: The units for RMR, EE, PAEE and VO2max are incorrect because the time component is missing. E.g., RMR values should be should be expressed as “kJ/hr” or as “kcal/hr,” not as “kJ” or “kcal.”
Reply: RMR, EE, PAEE and VO2max values have been amended.

10. What follow-up work is suggested by the results of this study?
Reply: The following sentence suggesting follow-up work has been added “Further studies investigating the effect of MUFA consumption including food sources in CV risk factors in a large sample of broader age range and ethnicity and both genders, would are warranted.”

Minor Essential Revisions:

1. Line 49: Define the abbreviation “EI” so the Abstract can stand alone.
Reply: It has been corrected.
2. Line 55: Change “in blood lipids” to “on blood lipids.”
   Reply: It has been corrected.

3. Line 58: Insert “dietary” before “fatty acids.”
   Reply: It has been corrected.

4. Line 75: Insert “low levels of” before “high density lipoprotein cholesterol.”
   Reply: It has been corrected.

5. Line 106: Change “corresponds with” to “correspond to.” Grammatically, because the subject of the sentence, BMI (body mass indices), is plural, the verb “correspond” should be used.
   Reply: It has been corrected.

6. Line 113: RMR must include a time measurement as part of the units, e.g., “100 kcal/hr,” not “100 kcal.”
   Reply: It has been corrected.

7. Line 131: Suggest revising “recording household estimates in food record, and entered into . . . ." to “recording household estimates into a food record and entering into . . . .” Delete comma after “record.”
   Reply: It has been corrected.

8. Line 149: A reference is needed for the “Borg scale.” Some readers may be unfamiliar with this.
   Reply: A reference has been included.

9. Line 161: Define “WC.” Include this term in the List of Abbreviations.
   Reply: WC is defined in methods section.

10. Line 163: A period should be inserted after “MUFA,” and “therefore” should be capitalized. (As written, line 163 is a grammatically improper run-on sentence.)
    Reply: It has been corrected.

11. Line 169: The P value should be shown as “0.05,” not as “0.05.”
    Reply: It has been corrected.

12. Lines 175 and 181: Insert “subjects” after “overweight” and after “lean.”
    Reply: It has been corrected.

13. Line 183: Insert “(Table 2)” after “alcohol intake.”
    Reply: It has been corrected.

14. Line 189: The units for VO2max should be “mL of O2/min,” not “mL of O2.”
    Reply: It has been corrected.

15. Line 209: Insert “ratios” after “PAEE/BW.”
    Reply: It has been corrected.
16. Line 217: The word “factor” should be plural.  
Reply: It has been corrected.

17. Line 218: Insert “high” before “percentage.”  
Reply: It has been corrected.

18. Line 231: Change “behavior” to “behavioral.”  
Reply: It has been corrected.

19. Line 234: “Risk factor” at the end of the line should be plural.  
Reply: It has been corrected.

20. Line 240: Revise “population young as children” to “populations as young as children.”  
Reply: It has been corrected.

21. Line 259: Insert “and” between “groups” and “similar.”  
Reply: It has been corrected.

22. Lines 272-273: Need to state that the “reduced cardio respiratory fitness and increased body fat” were seen in the overweight men, who showed lower VO2max levels compared to the lean men. Increased VO2max values seen in the lean men is associated with increased cardio respiratory fitness.  
Reply: It has been stated. The final sentence reads “Lean men in the current study had a higher mean VO2max than the overweight men. Increased VO2max has been associated with increased cardio respiratory fitness. The association between reduced cardio respiratory fitness and increased body fat observed in overweight men in the current study has been reported in other studies.”

23. Lines 278: The term “MUFA” is usually considered to be plural (monounsaturated fatty acids). Therefore, “has” should be changed to “have.” Also, insert “lipid” after “blood.”  
Reply: They have been corrected.

24. Line 281: Add “of EI” after “23%.”  
Reply: It has been corrected.

25. Lines 283-284: Change “was” to “were.” What level(s) of dietary MUFA were associated with 20% reduced risk in coronary heart disease events, according to reference 75?  
Reply: It has been corrected. Levels of MUFA are not provided in the reference.

26. Line 286: Change “intake of MUFA favorable affect CV risk factors” to “intake of MUFA favorably affects CV risk factors.” “Intake” is a singular noun.  
Reply: It has been corrected.

27. Line 289: Insert “sources” after “animal.”  
Reply: It has been corrected.
28. Lines 290-291: The wording “a Mediterranean predominantly from animal plant” is confusing. Do the authors mean “a Mediterranean diet in which MUFAs were primarily from animal sources” or “a Mediterranean diet in which MUFAs were primarily from plant sources,” i.e., referring to two types of Mediterranean diets?
   Reply: It has been corrected.

29. Line 297: Should the sentence read, “Normal and overweight participants may be misclassified using BMI.” rather than, “Normal and overweight participants are misclassified using BMI”?
   Reply: It has been corrected.

30. Figures 1 and 2, vertical axes: The designation “CV risk factors” is confusing. How about “Average number of CV risk factors per subject”?
   Reply: It has been changed.

31. Lines 200-201, 574-575: Slashes needed between “plant” and “animal” (plant/animal) to indicate ratios.
   Reply: It has been corrected.

32. Table 1: The P value for “Body fat (%)” is listed as “0.000.” Shouldn’t this P value be “<0.001”?
   Reply: It has been corrected.

33. Line 596, Table 2: Why were VO_{2max} levels not measured for 4 subjects (1 lean, 3 overweight)?
   Reply: The following sentence explaining the reason was added to the results section.
   Four participants had measurements VO_{2max} missing due to technical problems with the equipment or due to inability of the participant to complete the last session of measurements.

Discretionary Revisions
1. Line 30, Abstract (also Line 63, Introduction, and elsewhere): I suggest abbreviating “cardiovascular disease” as “CVD” rather than as “CV disease.” Nutrition Journal may have a preferred abbreviation.
   Reply: CV was preferred because it could be used for CV risk factors. We are happy to revise to CVD if the editor prefers.

2. Line 102: I suggest using “circumference” singular, not plural (two times in this line).
   Reply: It has been corrected.

3. Line 184: For the sentence, “Lean men consumed more energy relative to BW”, add parenthetically that the lean subjects had a larger EI/BW ratio than did the overweight subjects.
   Reply: It has been corrected.
4. Lines 207 and 223: Insert “waist” before “circumference” or use abbreviation “WC.”
Reply: Circumferences refer to both waist and hip.

5. Line 267: Consider changing “figures” to “ratios.”
Reply: It has been changed.

6. Line 269: Consider changing “than” to “compared to.”
Reply: It has been changed.

Quality of written English: Needs some language corrections before being published.
Reply: Language has been corrected.

Reviewer 2

1. Is the question posed by the authors new and well defined?
The question is not new, although the authors adequately define the conceptual question in the very last sentence of the Introduction.
Reply: The abstract and the introduction have been reworded to improve definition of the conceptual question as follows: “Despite clear evidence of the coexistence of several risk factors in young people as children and an understanding of the importance of the health behaviors in controlling CV disease, there are limited data on the relationships between risk factors and CV disease in young people. Therefore further study is required.”

2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work?
Most methods are well described and sufficient to replicate the work. However, this is not the case for DEXA measurements.
Reply: Description of DEXA measurement has been expanded as follows:
“Body composition was measured by dual-energy X-ray absorptiometry (DEXA) (DPX-Plus; Lunar Corp, Madison, WI). The participant removed shoes any materials that could attenuate the x-ray beam, such jewellery, watches and clothes with zippers and laid on his back in the centre of the table. Participants remained motionless in the supine position while the scanning arm of the DEXA passed over their body from head to toe in parallel 1-cm strips. DEXA measurements were made using a constant potential x-ray source of 76 kVp and a cerium filter that produces dual-energy peaks of 38 and 62 keV. The soft tissue mass (fat and lean tissue) is measured pixel-by-pixel as a beam of photons penetrates the participant’s body. Body fat content was determined from DEXA whereas body fat-free mass was calculated by subtracting body weight from body fat content. The DEXA values were used to classify participants into two groups: a) lean (body fat <20%) and b) overweight (body fat ≥20%). BMI >25 and >30 kg.m⁻² correspond to body fat percentage values of approximately 20% and 25% in men, respectively [19-21].”

In addition, there is evidence that indexes which take into account stature do a better job of controlling for misclassification of obesity than BMI or percent fat-mass (Kelly TL, Wilson KE, Heymsfield SB. 2009. Dual energy X-ray absorptiometry body composition reference values from NHANES. PLoS One
This concern casts concern on how the two groups—lean and overweight—are formed.

Reply: BMI does not account for variation in body fat distribution and abdominal fat mass and its limitations have been mentioned in several studies. Three participants were misclassified in this study when BMI was used and this has also been reported in other studies. We used DEXA whole body measurement (head to toe in parallel 1-cm strips) and according to the suggested reference above (Kelly et al., 2009), DEXA is the only widely available technology capable of providing regional measures of fat and lean mass, and it has been shown that fat and lean distribution may predict health outcomes. DEXA is capable of separating body mass into fat and lean components, thereby permitting the evaluation of fat mass without the confounding influence of other tissue constituents (Kelly et al., 2009). This reference also mention that studies have shown that lean mass and weight scale with height to approximately the power of two, establishing an analytic framework for height-scaled indices and proposes the use of FMI (fat mass/height^2).

Using the proposed index (FMI = fat mass/height^2) in our data would have resulted in the same classification for all participants except for 2 participants classified as healthy weight would have been classified as overweight (>6 kg/m^2). All tables and figures were done using the proposed index to classify the participants into lean and OW groups and are shown in the Appendix 1 (at the end of this reply). There were minor changes:

- Lean group is younger than OW group (Table 1)
- HDL-C is no longer significantly lower in OW compared to lean participants. On the other hand TC/HDL-C ratio is now significantly lower in lean participants than in OW participants (Table 1).
- Lean group has a higher PAL compared to OW group (Table 2)

There were no changes in the main findings that “The likelihood of multiple CV risk factors is greater among those with high body fatness and low MUFA intake” and that “intake of MUFA favorably affects CV risk factors regardless of the source” (Figures 1 and 2).

It is not known whether or not the use of the proposed FMI classification scheme will confer benefits over BMI in terms of predicting obesity-related morbidity or mortality. The FMI classifications presented in the paper are based on prevalence data, not disease risk, and therefore the clinical utility of the FMI classification scheme will not be known until data relating disease risk to FMI becomes available. For these reasons, we have not incorporated any of the data presented in Appendix 1 into the revised manuscript, but would be very happy to do so if the Editor or reviewer requested.

The following sentences have been added “Normal and overweight participants may be misclassified using BMI and therefore use of body fat estimated by DEXA rather than use of BMI to classify the participants to each group was an advantage of the current study. DEXA is the only widely available technology capable of providing regional measures of fat and lean mass, separating body mass into fat and lean components, thereby permitting the evaluation of fat mass without
the confounding influence of other tissue constituents [78]. In addition, and it has been shown that fat and lean distribution may predict health outcomes [78]. Studies have shown that lean mass and weight scale with height to approximately the power of two, establishing an analytic framework for height-scaled indices and use of FMI (fat mass/height^2) has been proposed. However it is not known whether or not the use of the proposed FMI classification scheme will confer benefits over BMI in terms of predicting obesity-related morbidity or mortality. The FMI classifications were based on prevalence data, not disease risk, and therefore the clinical utility of the FMI classification scheme will not be known until data relating disease risk to FMI becomes available [78].”

The BP measurement seems to be a single assessment without standardization common to laboratory and epidemiological studies; e.g., three measurements preceded by a resting period and at least five minutes between measurements.
Reply: Description of BP measurement has been expanded as follows “Sitting BP was assessed taking one measurement by the same investigator after 10-min rest with a sphygmanometer”.

The use of two difference technologies for the RMR measurements is potentially a major problem. There is no description of what was done, or could be done, to compensate for the stated and known difference between methods. For example, were more subjects in the “lean” group measured with one technology than the other, thus creating a bias caused solely by the measurement system?
Reply: A similar proportion of lean and overweight participants were assessed using each of the methods and therefore likelihood of measurement bias was reduced. Description of RMR measurement has been added to clarify the issue as follows “RMR measures are less than 100 kcal/d lower using the Deltatrac compared to Moxus according to a study conducted in the same laboratory using the same equipments [22]. A similar proportion of lean and overweight participants were assessed using each of the methods and therefore likelihood of measurement bias was reduced.”

With a difference in means in RMR between the two groups of ~140 kCal, how much of this is due to measurement bias?
Reply: The likelihood of measurement bias is small because a similar proportion of lean and overweight participants were assessed using each of the methods. Description of RMR measurement has been added to clarify the issue. A sentence explaining this issue has been added as follows “A similar proportion of lean and overweight participants were assessed using each of the methods and therefore likelihood of measurement bias was reduced.”

Were a subset of subjects measured on both devices; and if so, how do measurements compare?
Reply: None of the participants were measured on both devices as the intention was to use only one device (Deltatrac II metabolic cart) but halfway through the Deltatrac II metabolic cart broke and the RMR was assessed in the second device for the remaining of the participants.
However, a study conducted in the same laboratory around the same time assessed participants in both devices and showed that RMR measures are less than 100 kcal lower using the Deltatrac compared to Moxus. This explanation has been added as follow “RMR measures are less than 100 kcal/d lower using the Deltatrac compared to Moxus according to a study conducted in the same laboratory using the same equipments [22]. A similar proportion of lean and overweight participants were assessed using each of the methods and therefore likelihood of measurement bias was reduced.”

The use of the word “cohort” and “cross-sectional” in the title of this paper is misleading. These are terms commonly used to identify large groupings from a defined population—usually geographical—with a defined strategy to recruit from this population into a sampling frame. There is no description of how these 35 young men came to be part of the study, and the concern is whether some type of selection bias could be driving the recruitment with the a priori hypothesis influencing selection. Were athletes recruited to identify the lean group, while the overweight group is selected from known “couch potatoes”? In addition, a common problem in modern day observational studies is advertising the hypothesis, a reliance on volunteerism, and resultant statistical associations being driven by selection biases. To what extent could some of the statistical associations being reported result from such a selection process, and to what extent could such associations be consistent with biology or with bias?

Reply: The title has been reworded to delete the words “cohort” and “cross-sectional” from the title. Description of how the participants were selected has been included. The likelihood of selection bias was minimum by recruiting participants from diverse sources. None of the participants were athletes and because the participants were recruited from different contexts the likelihood of any of the reported statistical associations being due to selection process is small. The following has been added to the methods regarding participant recruitment “Participants were recruited by flyers posted in shopping centers and education centers as well advertisement in local newspapers. Inclusion criteria to participate in the study were age between 18 and 25 year and absence of any chronic disease.”

The aforementioned comments should be construed as "Major Compulsory Revisions"

3. Are the data sound and well controlled?
See preceding concerns detailed about methods.
Reply: Methods section has been reworded and all preceding concerns/suggestions addressed.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
How results are reported appear to be straightforward and without dissimulation.

5. Are the discussion and conclusions well balanced and adequately supported by the data?
There is no discussion of the potential limitations of this study, which are potentially noteworthy as detailed in preceding sections. Too much of a stretch is being made in trying to cover almost every aspect of CV risk. If the aforementioned methodological concerns can be adequately addressed, this paper would be much better as a focused effort on the MUFA results. This concern falls under "Major Compulsory Revision."

Reply: The discussion on the limitations of this study has been expanded as follow “A small sample size was a limitation of the current study. Another limitation is the fact that RMR of half of the participants was assessed in different equipment due to technical problems. However the likelihood of measurement bias is small because similar proportion of lean and overweight participants was assessed using each of the equipments.”

The aforementioned methodological concerns and others raised by the other reviewer have been addressed.

6. Do the title and abstract accurately convey what has been found? No. See preceding concerns.  
Reply: Title and abstract have been reworded to address preceding suggestions/concerns.

7. Is the writing acceptable? The writing is excellent, with no suggestion of English as a second language or major difficulties in communication of either overarching concepts or specific detail.
### Appendix 1

**Table 1** – Body composition, blood pressure and blood lipid measures of young men classified by Fat Mass Index (fat mass/height^2) were lean are those < 6 kg/m2 and OW are those > 6 kg/m2

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<tr>
<th>Subjects</th>
<th>Lean</th>
<th>Lean SD</th>
<th>OW</th>
<th>OW SD</th>
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</thead>
<tbody>
<tr>
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<td>10.3</td>
<td>85.0</td>
<td>12.1</td>
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<td>Age</td>
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<td>22.7</td>
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<td>27.2</td>
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<td>5.8</td>
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<td>60207.8</td>
<td>8954.1</td>
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**Table 2** – Reported food intake, resting metabolic rate (RMR), daily energy expenditure (EE) (kcal/day), physical activity energy expenditure (PAEE) (kcal/day), physical activity level (PAL) and oxygen consumption maximum (VO_{2max}) in young men classified by Fat Mass Index (fat mass/height^2) were lean are those < 6 kg/m2 and OW are those > 6 kg/m2

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Lean</th>
<th>Lean SD</th>
<th>OW</th>
<th>OW SD</th>
</tr>
</thead>
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<td>525.1</td>
<td>2599.5</td>
<td>571.2</td>
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<td>EI/BW</td>
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<td>7.8</td>
<td>31.3</td>
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<td>2.2</td>
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<tr>
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<td>282.5</td>
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<td>661.0</td>
<td>3586.5</td>
<td>568.3</td>
</tr>
<tr>
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<td>21.1</td>
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<td>20.8</td>
</tr>
<tr>
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<td>409.2</td>
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<td>396.4</td>
</tr>
<tr>
<td>PAEE/BW</td>
<td>25.7</td>
<td>5.0</td>
<td>19.3</td>
<td>5.0</td>
</tr>
<tr>
<td>PAL ave</td>
<td>2.0</td>
<td>0.1</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>VO2 max</td>
<td>55.8</td>
<td>6.2</td>
<td>47.4</td>
<td>6.2</td>
</tr>
</tbody>
</table>
Figure 1 using % of fat from DEXA to classify the participants in OW or lean groups

Figure 2 using % of fat from DEXA to classify the participants in OW or lean groups

Figure 1 using % of fat from DEXA/ (height)^2 to classify the participants in OW or lean groups

Figure 2 using % of fat from DEXA/ (height)^2 to classify the participants in OW or lean groups