Reviewer’s report

Title: Exercise training with dietary counselling increases mitochondrial chaperone expression in middle-aged subjects with impaired glucose tolerance

Version: 1 Date: 11 October 2007

Reviewer: Kathryn H Myburgh

Reviewer’s report:

General

The background is logically written and, in general, supports the study. Some specific comments are provided.

This study was part of a larger study and 22 subjects volunteered. This is likely a good number for this complexity of work, however, it must be discussed how this could have influenced your results.

The methods are sound and the data presented are for the most part relevant and good. A few specific comments are provided.

However, the discussion does not reflect the standard of the data.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Introduction: Although it is commendable that it is not too long, it should be expanded (briefly) in some aspects. See specific comments.

2. Where relevant do power analyses to determine if this may have affected your conclusions).

3. There is little or no discussion of the comparison between the two groups, which was an objective of the study.

4. P4 Sentence 3: This sentence is too general: in which tissue/system was oxidative stress measured in the rats? If the HSP responses were impaired, what was the challenge? Or do you mean in a baseline condition in response to the diabetes itself?

5. PKB/Akt – what is the relevance of this part of the sentence? Explain.

6. P5 Sentences 2 and 3 are too generalized. Expand each sentence with one more sentence explaining more specifically e.g. can you give relevant examples of some cellular functions and adaptations that are regulated by ROS in their capacity as 2nd messengers? Similarly, for the next sentence, please explain e.g. why you state that ROS is involved in i) pathophysiology of insulin resistance
ii) pathophysiology of diabetes and iii) the complications of diabetes. Or, is the only evidence that it is involved related to the indirect evidence in the following sentence? This is not clear.

7. P5 Par 2 Sentence 2: Very generalized statement with no references. If very little is known, it should be easy to actually mention exactly what has been done in humans and which animal models have provided relevant information or investigated parameters relevant to your study.

8. You have used various markers for oxidative stress in your study, yet none are mentioned in the Background in terms of prior use in a similar population or any papers indicating their acceptance as relevant and repeatable markers (in serum and muscle). What was your purpose in measuring serum urate? I was under the impression that urate is in fact an anti-oxidant. Please discuss the various possible interpretations of a plasma urate concentration.

Methods

9. P6 There is too little information on the subject selection “based on 2 oral glucose tolerance tests”. What exactly was your definition of IGT? What cut-offs were used?

10. P7 There is insufficient information regarding the training intervention, particularly since it was individualized, it is also possible that some individuals did a lot less exercise. What was adherence of the subjects in this sub-study? Please provide data on the range of training volume actually performed.

11. P7: Was the biopsy taken under resting conditions? When was the last exercise session prior to the biopsy? It would be helpful to the reader if you could specify the typical size of the muscle sample required to perform these analyses. Was one homogenate used for all further analyses? Or were separate small pieces used for the various analyses? Were any homogenates or extract aliquots refrozen between assays?

12. P8: MHC analysis: Too little information on the gel electrophoresis method. This is an incorrect reference. Reference the method you have used.

Results

13. It is unclear why the comprehensive dietary analyses are provided. The effect of dietary intervention was not a priority in the introduction or the discussion. Limit to essentials. I suggest you remove everything except the total energy intake and the vitamin C and E intakes.

14. P11: Is there any relevance to the differences in dietary fat and fibre intake between the slow and fast twitch muscle subject groups? If this is important to explain fat soluble vitamin absorption, then you need to make this one of the issues of investigation. Otherwise, remove data that isn’t relevant.

15. P12. Correlations should be presented graphically for proper evaluation of
the spread of data. I suggest that you provide a graph with 2 panels: indicating the positive relationship in the one group and the lack of relationship in the other group. A

16. A second correlation graph that would be relevant to see is the relationship between VO2max (do you mean the final Vo2max, or the change in VO2max?) and the increased GRP75 (again, do you mean the change in GRP75? Or the new, elevated value post-intervention?). Again, it would be relevant to have two panels: one for the significant result and one for the lack of correlation in the other group.

Discussion

17. Par 1 is lightly confusing because in the introduction you have emphasized the roles of HSP60 and GRP75 as chaperones involved in trafficking and processing, whereas here you are emphasizing a cytoprotective role, which you assigned to the 70kDa HSP family in the introduction, whereas here you are assigning the HSP72 and 90 the roles of chaperones.

18. P13 Sentence 2: Remove the whole sentence. “Although the IGTslow group represents a type of ordinary people”. Rephrase appropriately. E.g. refer to this fibre type distribution as more common (or if you meant less prone to type II diabetes?) and provide references of a larger population study.

Of course the fibre type of the 2 groups differed since you selected them to differ. Explain properly why you are discussing results presented in reference number 19 – does this reference report on the exact same subject cohort? Then say so, since in this paragraph you start out specifically stating “In this study”... If a change in MHC proportion post-intervention for these subjects was not statistically significant, then you cannot say that one type of MH increased and another decreased. How many subjects would have been required to make this conclusion? If these data are presented elsewhere, do they warrant a paragraph of discussion on their own?

19. P14: It is confusing that you are discussing results presented elsewhere. You can’t try to correlate some data presented in one paper and other data presented in the current paper. It is more important that you attempt to explain the last sentence, since you objective here was to compare the two groups.

20. P14 last Par and P15 first Par: This is a confusing piece of writing: It is a very long paragraph – what is the actual point you wish to make? That the mitochondria are dysfunctional? Or that mitochondria are not generated? Or that dysfunctional mitochondria are generated? Is there sufficient evidence to distinguish? Which DNA damage is reduced? Mitochondrial DNA? If the oxidative enzymes did not increase and only mRNA was elevated (which genes?) it is very possible that mitochondrial biogenesis did not increase. It is unclear why you have a sentence here about the anti-oxidative processes. Which genes are you talking about? Anti-oxidant enzymes, or? Are they cytosolic or mitochondrial in
location? Do you mean matrix or membrane? Mitochondrial function and biogenesis are too loosely linked, when you are actually discussing biogenesis. Suddenly adding some discussion on HSP72 doesn’t fit in this paragraph. The relation of HSP72 and anti-oxidant defence is not explained.

21. If your study used a similar type of exercise to ref 46, and reference 46 found a decrease in HSP72 expression, then how does this explain why you found no change? Discuss other possibilities: your study duration was 18 months – are changes possibly transient?

22. P16: How do you explain this? HOMA-IR may be more closely related to GLUT4 than to mitochondria or chaperones.

23. P16: ORAC: Explain why sentence 2, which explains completely different experimental design is “in agreement” with your results? What do you mean? That it is difficult to ‘get a significant change in ORAC’? Is it really? What other methods were used in ref 48?

24. P16 Last sentence of this paragraph: There are other possible mechanisms for the cardiac protection, not necessarily anti-oxidant capacity (see Valko et al, 2007).

25. Conclusions
The changes in oxidative stress markers are in no way remarkable, did not change in both groups and did not change in muscle.
The evidence does not support the last sentence.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Grammar:
P5: Furthermore, we aimed to investigate….

2. P9: Give precise information on the anti-bodies (catalogue number or reference number).

3. P9: Where was your oxidized BSA standard purchased?

4. Check all units: if you use a forward slash (/), then do not also use -1

5. P11: The mean and S.E. for change in body weight has great range. Please supply the actual ranges.

6. Figure 1: Fix spelling error

7. P12: Give the % changes in the chaperone responses in the text.

8. P12: Par 2: Insert words: muscle before HSP60 and serum before ORAC.
9. P13 last line: CS activity is normally assayed in a muscle homogenate, not a mitochondrial fraction. It is not necessary to state that the CS is in mitochondria, since it is a bit confusing in the context of this sentence.

10. P14, Par 2: In relation to the sentence with ref 41, also check the 2007 literature.

11. P14, Par 2: in relation to the sentence with ref 42: this is important to mention in the introduction as well and should satisfy some of the comments above about the need to explain the dual roles of chaperones/cytoprotection.

12. P14 Par 2: Grammar: “due to”; “reduces” DNA damage; “increases”

13. P14 par 2 last line: insert “and lack of exercise training”

14. P15 Par 2: A sentence starting with “Most studies” should have multiple references, not none at all.
Grammar: “decreases”

15. P16 Grammar: delete “have been used”

16. P17: Oxidative stress: remove “In other words”, because production of ROS is different from synthesis of anti-oxidant enzymes.

17. P17 Sentence 2: Should be explained in the Introduction.

18. P17 Grammar: rephrase “in this weightlifters study”

19. P17 rephrase “determining protein oxidation” – this is the term usually used for the oxidation of protein as a fuel, not for oxidation-induced protein damage.

20. References
24, 37 incomplete. If ePub, still need to provide the information.

21. Table 1:
Remove the last column – the data is in the next table anyway.
No units for BMI
Footnote: mention the statistical test used.

22. Table 2:
The abbreviation VO2maxind is not commonly used. Take out the ‘ind’
Use 3 decimal places for the P. carbonyl results – means nothing as stated.

23. Table 3:
Are these the correct units to use for ORAC?

24. Table 4;
Remove. Place data only in text. All the data is not required.
Discretionary Revisions (which the author can choose to ignore)

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests at all