Reviewer's report

Title: Protein differences between human trapezius and vastus lateralis muscles determined with a proteomic approach

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Reviewer: cecilia gelfi

Reviewer's report:

1. Is the question posed by the authors well defined?
   Yes, it is

2. Are the methods appropriate and well described?
   Not at all

3. Are the data sound?
   Not completely

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   Not completely

5. Are the discussion and conclusions well balanced and adequately supported by the data?
   The discussion is well balanced, the conclusions are not supported by the data.

6. Are limitations of the work clearly stated?
   Some of the limitations are stated.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? No

8. Do the title and abstract accurately convey what has been found? Yes

9. Is the writing acceptable?
   Yes a part some topographical mistakes

Major Compulsory Revisions

Once you have done this, there are also some questions for you to answer, including one that asks your advice on publication. Please remember that it is journal policy to publish work deemed by peer reviewers to be a coherent and sound addition to scientific knowledge and to put less emphasis on interest levels, provided that the research constitutes a useful contribution to the field. Further guidance on these points follows.

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- Major Compulsory Revisions
- General comments.
- The proteomic comparison between vastus lateralis muscle and trapezious should produce differences, because those muscle are functionally different. Due to the inter-individual variability and the plasticity of the muscle, to consider the found differences reliable and being able to correlate them to muscle function, a perfect set up and a perfect experimental design accompanied with the most stringent statistical data evaluation is mandatory.

1) The experimental design is not clear: 5 subjects, 2 muscles of the same subject?
   If yes, why 5 gels including dye swap. If the author utilized the dye swap 10 gels had to be performed.

2) What does it mean alternatively labelled? The experimental design is not clear at all, as the experimental set up. A part from the long list of applied voltage and so on, already reported on user’s manual and un-necessary in this context, the essential part to show namely: the gel reproducibility, is not shown. This reproducibility is mandatory using Ettan six, known to have some problems in gel reproducibility particularly for gel N 1 and 6, due to inefficient temperature control of the running buffer.

3) Why there is such a discrepancy between detected and matched spots? My be the authors should take into consideration the second dimension which represent very often the crucial step in 2D gels.

4) Regarding the detected spots: 2447 detected 545 matched and present in all gels, 140 not applying FDR (>5%) 40 applying it (still >5 %), the number is a bit high considering FDR. To evaluating FDR which parameters did the authors apply?

5) Regarding statistical analysis: How many changes will be found if p<0.01 will be applied. I think 0.05 is too high to produce significant results.

6) Having two muscle biopsies from the same subjects a paired t-test is useful and could provide more stringent results

7) Why staining with Coomassie whit a typhoon and fluorescent staining?

8) Validation: there is not a reference and not a plot indicating the increment and
the statistical significance of this increment or decrement, in such form this validation is useless.
It is difficult to comment on discussion and conclusion sections since data are not methodologically adequately supported.