Reviewer’s report

**Title:** Non-invasive Muscle Contraction Assay to Study Rodent Models of Sarcopenia

**Version:** 2  **Date:** 4 June 2011

**Reviewer:** Kate Murphy

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There is a need for a reliable functional muscle assay with high throughput for preclinical rodent studies. The authors have developed a system that electrically stimulates the lower hindlimb muscles of anaesthetised rats and measures the isometric force. The system assesses muscle function with reasonable throughput (8 animals/hour) and is not associated with the voluntary factors that are problematic with other in vivo tests such as grip strength, rotarod and treadmill performance. It is also non-invasive, allowing the repetitive monitoring of muscle function in the same animal, and sufficiently sensitive to detect changes in muscle function with ageing and dexamethasone treatment. Although the development of this system represents an important contribution to the field, the validity of the data generated by this system remains uncertain due to the presentation of the results. The following revisions need to be made in order to demonstrate the validity of the system and make the manuscript acceptable for publication.

**Major compulsory revisions**

1. Throughout the manuscript, 9-11 month old rats are referred to as “young”. This is misleading as this age actually represents adult rats and this group of rats should therefore be referred to as “adult” (e.g. Brooks, S.V. & Faulkner, J.A. Journal of Physiology 404: 71-82, 1988).

2. Figure 3. In addition to the absolute mass of the plantarflexor muscle (Figure 3B), the plantarflexor muscle mass relative to body mass should also be provided. Calculations based on the information provided in Figures 3A and 3B suggest that relative plantarflexor muscle mass would be ~0.0058, 0.0049, 0.0040, 0.0038 and 0.0030 for 10-12 mo RB, 24 mo lean, 24 mo light, 24 mo fat and 24 mo heavy, respectively.

3. Figures 3 and 4. P values are given to indicate significance, but there is no information as to what group this refers to? i.e. P<0.01 vs. 10-12 mo RB? If this is the case and only differences compared to the 10-12 mo RB group are presented, were no differences found between the aged groups?

4. Figure 3C. No statistics are given but it is stated (page 13, 2nd para) that the “younger rats had the strongest muscle force, followed by the aged lean group. The heavy, light and fat groups exhibited weaker force…” Were these statistically significant differences? If so, then it is assumed that a two-way ANOVA (force,
time) was performed and this should therefore be stated in the Methods. If no statistical significance was found, then it cannot be said that one group had a higher force than the other. The same is true for the legend to Figure 3C.

5. Figure 3 and Figure 4 fatigue envelope data. The data are presented relative to that of the 10-12 mo RB group, with SEM (or SD) given, but how was the SEM (or SD) determined given that animals were not matched between groups?

6. Figure 3 legend. In reference to panel C (page 27), it is stated that, “Muscle force is shown relative to the average first contraction of the 10 mo old rats.” However, absolute force (N) is given and this statement is therefore incorrect and needs to be removed.

7. Results. Page 13, 2nd para. In reference to Figure 3D, it is stated that, “All aged rat subgroups had reduced muscle performance for all parameters, except half fatigue time, when compared to the younger adult rats.” However, according to the significance symbols placed on Figure 3D, this is only correct for slope T50. For F max, only the 24 mo light and fat groups were different from 10-12 RB for F max; for F AUC T50, only 24 mo fat was different from 10-12 RB and there was no difference between any group for F min.

8. Dexamethasone study. Page 13, last para. It is stated that, “a loss of hindlimb muscle mass proportional to the overall loss of lean mass” but the data is not shown. Please provide the absolute and relative (to body mass) plantarflexor muscle mass in the vehicle and Dex-treated mice.

9. Dexamethasone study. Page 14. The authors suggest that the “significantly reduced fatigable muscle force” in the Dex-treated rats indicates “a loss of type II fibers.” However, the authors have no immunohistochemical data to support this claim. If Fmax is expressed relative to muscle mass, then the reduction in force may simply be due to the smaller mass of the muscle. Whether this loss of mass represents a reduction in fibre cross-sectional area, or a loss of fiber number and/or a fiber-type shift has not been examined in this study and the authors’ claim that the reduction in force may be due to a loss of type II fibers is therefore pure speculation and should be removed.

10. Discussion. Page 17. There is much speculation about how specific measures of the fatigue envelope represent contributions by specific muscle fiber types. For instance, the authors suggest that the “profound loss of fatigable force and a somewhat diminished fatigue-resistance force” of the aged rats concurs with the preferential loss of type II fibers that occurs with ageing. However, the authors need to back up this data with immunohistochemical data showing the differences in fiber-type composition of the plantarflexor muscles between the different subgroups of aged rats and in comparison to the 10-12 mo RB.

Minor essential revisions

11. Background. Page 5, 1st para. “… #2 receptor activators” should instead read, “… #2-adrenoceptor activators.”
12. Methods. Dexamethasone treated rat model. Why was 11 days chosen as the time to test rats after pellet administration?

13. Methods. It is stated that a one-way ANOVA was used, but what post-hoc test was employed?

14. Results. Page 12, last para. A number of body composition types were found for the 24 month old rats, but no information is given as to the criteria used to define these different types. Please provide this criteria. Also, were different body composition types also found for the 10-12 month old rats? If not, please note this.

15. Figure 1C and Figure 1D – please provide x and y axes and titles

16. Legends to Figure 2-4. State how group data are presented (i.e. mean ± SEM).

17. Results. Page 13, 1st para. The plantarflexor muscle mass does not need to be presented in the text as it is already provided in Figure 3B. The same is also true for the legend to Figure 3.

18. Legend to Figure 3 – please provide sample size for each group. If subgroups were screened from 315 aged rats, does this suggest that each group of aged rats represented ~78 rats? Why were such a large number of aged animals screened for body composition?

19. Figure 4B shows that 48 rats were treated with vehicle and 23 were treated with Dex. Why was there such a large difference in sample size? Also, why were only 12 rats from each group assessed for body composition (Figure 4A)?

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

**Quality of written English:** Acceptable

**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