September 6th, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3809-review.doc).

**Title:** Hypo-activity induced skeletal muscle atrophy and potential nutritional interventions: a review

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**Name of Journal:** *World Journal of Translational Medicine*

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated where required

2 Revision has been made according to the suggestions of the reviewer (see sections highlighted in yellow in the manuscript)

3 References and typesetting were corrected

Specifically:

**(1) Page 3, Para 1, Line 5 "Add HUT or Head down tilt"**

*Head down tilt bed-rest added to the text, as shown below:*

Muscle atrophy (decrease in muscle mass) is seen during reduced activity (e.g. sedentary behaviour, de-training) [5-8] or disuse models (e.g. immobilisation, head-down tilt bed-rest) [1, 3, 9, 10].

**(2) Page 3, Para 2, Line 9-11 "I would move this statement down. I would make sure to have the purpose of the review before getting in to details"**

*Statement simplified and the detail and reference moved to a relevant section later on. Reference moved down to Page 5, Para 2, Lines 1-4. See extract below:*

The final method of hypo-activity commonly reported in the literature is that of unilateral lower limb suspension (ULLS), a method of reducing habitual activity whilst causing lesser degree of inconvenience to the participants.

Tesch et al. [32] developed a model to study the effects of an unloaded limb in humans that allows for freely moveable joints but minimises load bearing. In this ULLS method, a sling suspends one lower leg and the contralateral shoe has an elevated sole to allow for a relaxed position of the unloaded limb.

**(3) Page 3, Para 3, Line 3 "You have to be careful about this statement. Because in some conditions similar to stroke or SCI, exercise is highly recommended"**

*Sentence re-worded to avoid ambiguity of the statement. We would draw the reviewer's attention to the fact that we are referring to the practicality and not the usefulness of exercise.*

Exercise prescription is not always a practical prescription, even when it would be recommendable, to individual's under-going immobilisation or bed-rest after trauma or illness, due to the presence of counter indications for exercise such as pain, immobilisation in a cast etc.

**(4) Page 3, Para 3, Line 12 - Page , Para 1, Line 3 "When I did the same search, it yields 62 studies. Bed-rest and immobilization (479), immobilization and atrophy (1218), bed-rest and atrophy (264). When I added 'human clinical studies', I only have 6 studies"**

*We apologise for the mistake and the sentence is now re-worded to clarify the specific searches that were run in PubMed.*

Studies were found using search terms 'bed-rest and atrophy' and 'immobilisation and atrophy' in PubMed. However, this returned over 1400 hits. To focus our search criteria, only data on healthy humans were selected through the inclusion of the 'human' and 'clinical trial' filters in the PubMed search. This resulted in 86 studies, suitable for inclusion in the present review.

**(5) Page 4, Para 2 "Apparently the duration of all these studies are different. I wonder if you can provide the range for each variable, for etc, for or 6 weeks and so on"**

*The range of study durations has now been added to the Figure 1 legend in accordance with our reviewer's recommendation*

Values are taken from the references used in the text for de-training (ACSA – 40 days and 24 weeks) [6, 8], bed-rest (ACSA – 30 days to 17 weeks) [2, 11, 18, 19] (PCSA – 20 days) [1, 9, 20] (Volume – 7 days and 32 days) [11, 21], immobilisation (ACSA – 9 days to 4 weeks) [3, 4, 12, 22-26] (Volume – 2 weeks and 4 weeks) [10, 12, 27] and ULLS (ACSA – 23 days and 4 weeks)[28-31].

**(6) Page 4, Para 2, Line 3 "I believe all these studies did not measure muscle size on weekly basis. They measured over the course of several weeks and then they divided by the number of weeks. Please clarify this point"**

*Page 3, Para 3, Line 3-6. Sentence added in the introduction to clarify the fact that the values were calculated per week to allow for homogeneity of the results despite different durations of studies.*

In order to provide some homogeneity in the results based on the variable duration of the hypo-activity, values are presented per week and where relevant the duration of the hypo-activity is provided in parenthesis.

**(7) Page 5, Para 3, Line 6 "Is this correct, knee flexor has greater decrease than extensors?"**

*Knee flexors have a greater decrease than extensors in this study and potential reasons are now speculated.*

It is generally accepted that muscle losses are greater in the knee extensors than the knee flexors after unloading in humans [34]. Akima et al. demonstrated the opposite to this, which could be due to the methodology used to determine PCSA [9]. In addition, since a muscle placed in a shortened position experiences a greater degree of atrophy than one placed in a lengthened position [35], the pattern/magnitude of disuse would therefore be expected to be modulated by both the mode of

hypo-activity and the joint angle adopted in the immobilisation.

**(8) Page 6, Para 1, Line 1 "Please state the reason of why no effect on TA."**

*A suggestion for the possible rationale for no effect on TA is now provided in the text.*

The tibialis anterior experiences lower activation during habitual physical activities than other muscles such as the plantar flexor, and as such may explain the lack of decrease in tibialis anterior muscle PCSA with bed-rest.

**(9) "I would encourage the authors to include subtitles throughout the review. To clearly follow his line of thoughts."**

*Subtitles are now included throughout the text.*

**(10) Page 6, Para 2, Line 12-13 "This makes more sense that the extensors to have greater decrease compared to the flexors. Please provide clarification why this is the case."**

*Clarification of this finding is included in the text.*

This is in agreement with the general acceptance that muscle volume is lost to a greater extent in the knee extensors compared to the knee flexors [34].

**(11) Page 6, Para 3, Line 2-6 "Please state why or what's the reason behind this observation?"**

*Reasons for this observation are now provided.*

This is not surprising since the habitual loading of the lower extremities, because of body weight in normal ambulation and even in the absence of intended physical exertion, is far more substantial than that in the upper extremities. Understandably, this thereby affects the required threshold of decrease in muscle activity necessary to negatively impact on muscle metabolism

**(12) Page 6, Para 3, Line 6-7 "Define anatomical cross-sectional area"**

*ACSA is now defined in the muscle morphology section. Page 4, Para 2, Line 1. Definitions have been added in the table 1 legend.*

Anatomical cross sectional area (ACSA) is the cross-sectional area of the muscle at right angles to its longitudinal axis.

[Table 1. Relative change in muscle anatomical cross sectional area (ACSA), physiological cross sectional area (PCSA)

**(13) Page 6, Para 3, Line 6-7 "Define Physiological CSA"**

*PCSA is now defined in the muscle morphology section. Page 5, Para 3, Line 1. Definitions have been added in the table 1 legend.*

Physiological cross-sectional area (PCSA) is the area of the muscle at right angles to the longitudinal axis of the fibres.

[Table 1. Relative change in muscle anatomical cross sectional area (ACSA), physiological cross sectional area (PCSA)

**(14) Page 7, Para 2 "It would be acceptable to refer to other model that leads to hypo-activity similar to SCI, stroke and myopathy. Please also refer to the work of Gorgey AS and Dudley GA, Ryan AS et al., Akima et al. about muscle atrophy and intramuscular fat."**

*We would like to draw attention to the fact that the current view is directed at models of disuse in healthy humans. Therefore, the inclusion of studies in SCI, stroke etc. is beyond the scope of the current review to go in to details as this would be a departure from the focus of this review.*

**(15) Page 7, Para 3, Line 9 "Please say why or provide your own speculation?"**

*Speculation on this is now provided in the text.*

This is likely to be due to the comparatively substantial decrease in habitual weight-bearing forces applied to the lower limb during hypo-activity.

**(16) Page 8, Para 1, Lines 1-3 "I prefer to say imbalance between protein synthesis and protein breakdown"**

*Imbalance has now been used.*

The decrease in muscle mass seen with hypo-activity may be the result of an imbalance between protein synthesis and protein breakdown

**(17) Page 8, Para 1, Line 5-9 "I do not think that this is universal findings others showed that few days of immobilization trigger the catabolic signaling pathway. Please confirm"**

*Sentence has been corrected to clarify that there is some evidence of increased catabolic activity.*

A shorter period of immobilisation (21 days) provided little evidence of increases in mRNA for catabolic enzymes or increases in enzyme activity during this period [45]. However, there is some evidence to suggest that increases in catabolic potential do occur, and that this event happens very quickly (48 hours) after immobilisation [44].

**(18) "Please refer to the work of Cardozo C in animal model"**

*The authors are conscious of data related to animal models. Indeed "MuRF1 and MAFbx (otherwise known as Atrogin-1) were up-regulated in response to three different atrophy models (denervation, immobilisation and hindlimb suspension) in rats [51]. Bodine et al. also reported that mice deficient in either MuRF-1 or MAFbx/Atogin-1 were found to be resistant to atrophy [51]". However, as per the search strategy for this literature review the focus was entirely on human studies, specifically to emphasise what these models show, therefore with respect the authors do not agree to adding a section of comments referring to animal models.*

**(19) Page 8, Para 2, Line 13 "Please provide more details about metallothionein. Is this in serum or in muscle?"**

*Metallothionen is in muscle and this has been clarified in the text.*

Increased metallothionein expression in human skeletal muscle fibres has been associated with exposure to physiological stress, which results in elevated levels of reactive oxygen species (ROS) [51]. Urso et al. reported a more than two-fold increase in metallothioneins in human skeletal muscle

**(20) "It is unclear if the authors reported the decline per week. If you have done this mathematically, you need to be careful because the decline in strength is not linear."**

*Please refer to the response to referee's comment number 6*

**(21) Page 10, Para 1, Line 2-5 "Why?"**

*An explanation is now provided as to why isometric torque is affected more by immobilization than by ULLS.*

An explanation for the above observation may be that ULLS removes weight-bearing but allows for freely moveable joints (hence a degree of muscular activity) whereas immobilisation is a more rigid model that does not allow joint movement (hence a greater restriction of muscular activity).

**(22) Page 10, Para 2, Line 14-17 "Please provide explanation to your observation"**

*An explanation for this observation is now provided.*

As with the knee extensors vs. knee flexors difference in sensitivity to hypo-activity alluded to above, the plantar flexor muscles experience a greater level of recruitment during gait than the *tibialis anterior*. Thus, habitual muscle recruitment prior to hypo-activity would appear to be a large determinant of the relative magnitude of hypo-activity-induced changes.

**(23) Page 16, Para 3, Line 2 "Define"**

*ROS is previously defined in the muscle morphology section, Page 8, Para 2, Line 12-14*

Increased metallothionein expression in human skeletal muscle fibres has been associated with exposure to physiological stress, which results in elevated levels of reactive oxygen species (ROS)

Thank you again for considering publishing our manuscript in the *World Journal of Translational Medicine*.

Sincerely yours,

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