Author's response to reviews

Title: Biomarkers of peripheral muscle fatigue during exercise

Authors:

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Author's response to reviews: see over
Ms. Ref. No.:

Dear Prof. Reeves,

Thank you for your email dating 22nd July, your and the reviewers’ valuable comments and the possibility to revise the manuscript. According to these comments the manuscript was revised with particular regard to the following points:

Reviewer #1:

1. The treatment of the biomarkers discussion does not move beyond classification and identification of by-products and responses. A more detailed characterization of the pathways from which the biomarkers are derived and some insight into expected biological variation reference ranges, and minimally important changes in biomarker activity or concentration for clinical significance would strengthen this paper immensely.

More detailed descriptions of pathways from which the biomarkers discussed are derived were provided. The biological variation of reference ranges and minimal changes in biomarker activity and their influence on clinical parameters were discussed if sufficient data were available. Whenever reported, reference limits were provided.

2. Factors that potentially impact the fatigue pathways were not discussed, although disease examples were listed in the first paragraph of the Introduction. Examples of major influences could be age, gender, disease or type of disease, and physical fitness. Each of these is likely to affect fatigue levels and most importantly, may exert an influence on some but not all of the pathways and markers mentioned in the manuscript. Moreover, the specific markers influenced may differ for each of these conditions and even for different diseases or types of diseases. Sufficient detail and depth in characterizing these influences is critical to the impact of this type of review and would enhance enthusiasm for the manuscript greatly.

Description of the single biomarkers was newly structured. The sequence of description follows the topics “pathways from which the biomarker derived”, “tissues from which the biomarker is determined”, “reference limits”, “dependency on age, sex, and fitness”, “behaviour of biomarker during exercise in healthy subjects”, and “behaviour of biomarker during exercise in diseased subjects”. Influences of these terms on the biomarker are discussed if sufficient data from the literature were available.
3. A common definition of muscle fatigue has not been established. In this manuscript the operational definition is “a reversible loss of muscle force during work over time”. A more important phenotype pertaining to muscle function is that of fatigability or the rate of loss of muscle force over time. In fact, fatigability and recovery, or the rate at which muscle function returns to baseline after becoming fatigued, are interactive determinants of the state of fatigue versus rested. While recovery is mentioned briefly, its contribution to offsetting fatigue is not discussed in the manuscript and as such creates a gap that ignores an important in fatigue severity and perhaps biomarker responsiveness. Recovery and fatigue severity are both influenced by fatigability and the degree to which muscle dysfunction is provoked. Thus a discussion on fatigability and recovery is a necessity for understanding the overall fatigue construct.

Though fatigability and recovery are important aspects of muscle fatigue, only single studies were carried out in conjunction with the biomarkers presented in the review. Whenever data about fatigability and recovery were available from the literature, they were included in the discussion. As an alternative to missing data, the chapter about “Exercise-induced fatigue” was extended and the title changed to “Exercise-induced fatigue, fatigability, tiredness, and recovery”. The terms fatigability and recovery were included in the discussion there. The influence of recovery on fatigue and the dependency of recovery and fatigue on fatigability are now discussed. Whenever data about the performance of a biomarker during recovery were available, they were included in the description.

4. Some explanation of hypothesized fatigue pathways and mechanisms was divergent with current thought. For example, under “Biomarkers Classified According to the Mechanism of Muscle Fatigue” in the second sentence of the first paragraph, the author suggests that depletion of high-energy substrate is thought to be one of the most important contributors to muscle fatigue. While it is true that there are observable decreases in muscle [ATP] and [CrP] during repetitive or prolonged contraction, a review and 2 manor book chapters (Keyser 2010; Fitts 2006; Brooks 2006) suggest that the decreases in high energy compounds are not nearly enough to result in even a small level of muscle dysfunction. Decreases in these compounds at specific locations or in association with the operation of transporters could potentially contribute to muscle fatigue but much more work is needed to establish this hypothesis. Meanwhile, even small decreases in muscle pH interfere with crossbridge binding and ATPase activity due to competitive binding and reduced enzyme function with even miniscule decreases in pH. Decreased muscle pH also impairs oxidative enzyme activity and may adversely affect ryanodine receptor function. ROS are electron scavengers, which not only cause fatigue but mitochondrial damage. Under and over expressions of selected genes believed to be interactive in mitochondrial apoptosis and genesis has been identified in association with fatigue severity. A more detailed discussion of the potential mediators of Fatigue and how the biomarkers associated with these pathways would improve the understanding of biomarker surrogacy.

In the chapter about ATP-metabolism it is now indicated that decrease of ATP and CrP during fatigue is not enough to result in muscle dysfunction. Contrary to the
controversies about ATP depletion on muscle fatigue, it has been shown and confirmed that decrease in muscle pH during fatigue results in decreased cross-bridge binding and reduced ATPase-activity. It is now also mentioned that a decrease in pH results in impairment of oxidative enzyme function and ryanodine receptor dysfunction, that fatigue may be also caused by the production of ROS (chapter about oxidative stress biomarkers), and that fatigue severity may be influenced by expression of genes involved in mitochondrial apoptosis and biosynthesis (chapter about genetic response biomarkers).

Reviewer #2:

The paper describes several types of conditions as muscle fatigue. This causes confusion. The first is a sense of continuously feeling tired, even before exercise is performed, and the other skeletal muscle fatigue that develops with ongoing contractile activity of the muscle as occurs during exercise. I think it is important to clearly distinguish these two ‘fatigues’ throughout the manuscript. There is even a third issue and that is that skeletal muscle may be more fatiguable in disease states, adding further to the confusion. Maybe the author could subdivide the manuscript into three sections dealing with each of these types of fatigue. In the section of inflammation I have the impression that the author is speaking about muscle weakness rather than muscle fatigue. These things need all to be clearly distinguished in the manuscript! References not always correct. Please check carefully throughout the manuscript.

The feeling of fatigue without previous exercise was not topic of the review and statements which could have suggested such an impression were omitted. When describing a specific biomarker, fatigue during exercise was discussed in healthy controls and, if available, in various disease conditions, which could influence the fatigue response to exercise (see response to point 2 of reviewer 1). Subdividing the manuscript into fatigue at rest, fatigue during exercise, and fatigue in disease would confuse the reader and would not be in line with the intentions of the review. The section about inflammatory biomarkers intends to deal with fatigue during or after exercise but not with muscle weakness. Statements which could evoke such associations were omitted.

In the abstract say that the biomarker must be WITHOUT appreciable diurnal variations.

It is now mentioned in the abstract that a biomarker must be without diurnal fluctuations.

Page 3 Exercise section: The first sentence can be deleted.

The first sentence was deleted.

Page 4 Exercise-induced muscle fatigue: Opening sentence: although a muscle may still produce appropriate force during prolonged activity, fatigue may still be there as the capacity to generate force is diminished. I would say: Fatigue is the decreased ability to
generate force or power during ongoing contractile activity. Power is maybe more important than force.
The definition of fatigue was changed accordingly.

This section also does treat muscle fatigue and a sensation of tiredness as one and the same thing. Please note that also during submaximal aerobic exercise muscle fatigue develops that is not necessarily reflected by a sensation of tiredness, but a reduced ability to develop force and power. I think that most of the factors that contribute to fatigue in Table 1 are factors that do actually cause a sensation of tiredness, rather than muscle fatigue. It is in many of those cases that muscle fatigue does occur earlier during contractile activity, so you could say that the muscle is more fatiguable or less fatigue resistant. So may be you could change the heading into: ‘Factors that contribute to an earlier onset of muscle fatigue’ or ‘Factors that contribute to feeling tired’.
The header of the section “Exercise-induced muscle fatigue” was changed to “Exercise-induced muscle fatigue, fatigability, tiredness, and recovery”. In this section it is now clearly indicated that fatigue is not the same as tiredness. The heading of table 1 was change to “Factors that contribute to feeling tired”.

Page 4 section ‘Fatigue versus muscle damage’ I do not think ref 6 is appropriate to reflect that muscles contain different fibre type. I suggest to refer to e.g. one of the reviews from the 80’s or 90’s by D Pette, which will also discuss the myosin ATPase method. Further a good section!
Reference 6 was replaced by “Pette D. Adaptation of skeletal muscle to increased neuromuscular activity as induced by chronic low frequency stimulation. Scand J Rehabil Med Suppl 1994;30:7-18.“

Section on page 6 and 7 also mixes up damage and muscle fatigue. While ATP depletion may cause fatigue it is more often a problem of Pi accumulation as [ATP] may be constant when force decreases (see e.g. review by Jones DA J Physiol 2009). The inflammatory changes themselves may not necessarily cause muscle fatigue, but maybe more a reflection of damage that occurred during exercise.
It is now mentioned that local inflammatory changes to exercise not necessarily cause muscle fatigue, but may also reflect muscle damage that occurred during exercise. It was also added that cross bridge detachment is unaffected by fatigue, that cross-bridge attachment is reduced during fatigue, and that ATP may remain constant with decreasing force while phosphorus accumulates.

Page 8: I doubt whether serum lactate indicates an inability to convert oxygen into energy. I think it is more reflecting that the aerobic ATP generation is insufficient for the generation of the required ATP and needs to be supplemented with anaerobic ATP generation. After exercise the aerobic energy metabolism is adequate again.
The statement that serum lactate indicates the inability to convert oxygen into energy was replaced by the statement that lactate increase reflects that aerobic ATP generation is insufficient for generation of the required ATP and needs to be supplemented with anaerobic ATP generation.
Line 10 of ‘Lactate’ section: what is the 70-90% a percentage off? VO2max?
We mean the percentage of the maximal workload.

Second paragraph of ‘Lactate’ section. Lactate, you say, does also increase in patients. I guess what you want to say here is that it increases at lower (relative) workloads.
It is now mentioned that lactate increases at lower relative workloads as compared to controls.

Page 21: Could you specify what the sensitivity/specificity refer to: is the to diagnose the mitochondrial disorder or the fatiguability of the muscle?
Sensitivity/specificity on p21 refers to the lactate stress test.

There is a rather simple way to assess fatigue and that is to determine the maximal force a muscle (group) can generate after contractile activity compared the force/power the muscle could generate before the onset of contractile activity. This is the usual way of determining fatigue resistance (See e.g. Burke et al., J Physiol, 1973).
This method to determine fatigue resistance was added.

The biomarker IL-15 was excluded from the discussion since too few data were found to meet the reviewer requests. It is now only mentioned as possible biomarker of fatigue in table 4. A number of references as added to support the discussion about physiological influences on a biomarker’s performance.

We hope that all these changes adequately meet all objections raised by the reviewers and the Editor and that readability and content have improved from these changes.

Please confirm receipt of the manuscript.

Sincerely Yours,

Vienna, 13th August 2012

J. Finsterer, MD, PhD