Author's response to reviews

Title: Effects of a 7-day military training exercise on inflammatory biomarkers, serum hepcidin, and iron status

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Author's response to reviews: see over
Reviewer 1 (Diego Moretti)

General: This is an informative study on hepcidin response to a phase of intensive physical challenge and stress in contrast to a phase with more common training and a control phase. The methods utilized are appropriate and the manuscript is concise and well written.

There was no control group in the study, and the implications of this should be clearly stated by the authors.

Response: The authors appreciate Reviewer 1’s constructive comments. Each of the revisions suggested by the reviewer has been incorporated into the manuscript. As the reviewer correctly notes, there was no control group in the study, as the study included an observational design. The observational nature of the study is noted in the revised manuscript, and the implications of the study are included in the “Conclusions” of the Abstract and Discussion.

Major Revisions:

More details should be provided on the 'Norwegian arctic combat rations.' What were they made of and what was the rough nutritional composition?

Response: Details regarding the energy and macronutrient composition of the Norwegian Arctic Combat Rations have been provided in the revised manuscript (Table 2).

Due to the lack of the control group, the authors should only discuss the global effect of the exercise program on inflammation and hepcidin.

Response: As indicated above, the observational nature of the study is noted in the revised manuscript. The Discussion is focused on the global effect of the military task on inflammation and hepcidin, although it does include speculation regarding the longer term effects of elevated hepcidin on iron status.

Minor Revisions:

Ferritin was elevated before hepcidin, was there an explanation for that- Is ferritin more sensitive to inflammation than hepcidin?

Response: Reviewer 1 makes an interesting point; ferritin was elevated at the PRE-timepoint, whereas hepcidin was not elevated until the POST-timepoint. The authors are not aware of studies directly addressing the timecourse of elevations in ferritin/hepcidin in response to inflammation, although current data do indicate that ferritin could be more sensitive (or
respond more quickly) to inflammatory stimuli than hepcidin. In response to Reviewer 1’s comment, we have added a line to the Discussion addressing this point.

How was a change in Hb of 4% be statistically detectable is such a small number of subjects. The authors should check this.

Response: As the Reviewer indicates, the change in Hb is only 4%, yet this change is statistically significant. The authors have repeated the analysis with the same result; changes are statistically significant due to the repeated measures nature of the test and the limited variability in the data.

A line on the discussion should be added on the measurement method for hepcidin. The authors used a kit, but the comparability of the hepcidin measurement is in general hampered by lack of standardization between methods and this does not allow to compare values between studies.

Response: At the Reviewer’s request, a paragraph has been added to the Discussion addressing this point.

Reviewer 2 (Michael Hoppe)

General: This is an interesting observational study of Norwegian male soldiers measured hepcidin, IL-6 and markers of iron status and related factors during a 7-day winter training exercise.

Major Revisions:

Page 5, last sentence: What was the daily energy content of the arctic combat rations? Could it be characterized as a state of starvation, which is a kind of physiological stress in itself? This needs to be addressed.

Response: As noted in response to Reviewer 1, details regarding the energy and macronutrient composition of the Norwegian Arctic Combat Rations have been provided in the revised manuscript (Table 2). To summarize, Soldiers were provided three rations per day during the pre-march period, and four rations per day during the march, although only 66% of the rations were consumed during the ski-march. As indicated by the Reviewer, caloric deficit (although not quantified in the present study) may have contributed to the physiologic strain of the march; this point has been added to the Methods section of the revised manuscript.

Minor Essential Revisions:

Page 5, line 1-2: Incorrect statement. The effect of military training on hepcidin and iron status has been investigated earlier (reference 16).

Response: Reviewer 2 is correct; hepcidin had been investigated previously during military training, although the previous study included hepcidin measurement after 8-9 weeks of
activity, yielding a more chronic response. This point has been clarified in the revised manuscript.

Page 6, Results: Where there any measurements of hydration grade (e.g. Hematocrit)? Excessive loss of body water (i.e. dehydration) can affect serum/plasma concentrations. This needs to be addressed.

Response: Measurements of hydration grade were not conducted, as the lack of an accurate tool for the assessment of hydration status continues to plague the field. To overcome variability/inaccurate conclusions potentially introduced by alterations in body water, the authors have chosen to use multiple biomarkers for the assessment of iron status and inflammation. This issue has been addressed in the revised manuscript.

Discretionary Revisions:

Figure 1: In my opinion, this figure is unnecessary. These visualized data can be presented in table 2 together with the other biomarkers. By presenting all data together in one and the same table makes it more lucid.

Response: Figure 1 has been deleted and the data has been added to Table 3 as directed.