

**This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.**

**Author(s):** Heikura, Ida A.; Burke, Louise M.; Bergland, Dan; Uusitalo, Arja L. T.; Mero, Antti; Stellingwerff, Trent

**Title:** Impact of Energy Availability, Health and Sex on Hemoglobin Mass Responses Following Live-High-Train-High Altitude Training in Elite Female and Male Distance Athletes

**Year:** 2018

**Version:** Accepted version (Final draft)

**Copyright:** © Human Kinetics, 2018.

**Rights:** In Copyright

**Rights url:** <http://rightsstatements.org/page/InC/1.0/?language=en>

**Please cite the original version:**

Heikura, I. A., Burke, L. M., Bergland, D., Uusitalo, A. L. T., Mero, A., & Stellingwerff, T. (2018). Impact of Energy Availability, Health and Sex on Hemoglobin Mass Responses Following Live-High-Train-High Altitude Training in Elite Female and Male Distance Athletes. *International Journal of Sports Physiology and Performance*, 13(8), 1090-1096.  
<https://doi.org/10.1123/ijsp.2017-0547>

1 **Title:**

2 Impact of energy availability, health and sex on hemoglobin mass responses  
3 following live high - train high altitude training in elite female and male distance  
4 athletes

5

6 **Submission type:** Original investigation

7

8 **Authors:**

9 Ida A. Heikura<sup>1,2</sup>, Louise M. Burke<sup>1,2</sup>, Dan Bergland<sup>3</sup>, Arja L. T. Uusitalo<sup>4,5</sup>, Antti A. Mero<sup>6</sup>, and  
10 Trent Stellingwerff<sup>7</sup>

11

12 **Institutions:**

13 <sup>1</sup> Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne,  
14 Australia 3000

15 <sup>2</sup> Sports Nutrition, Australian Institute of Sport, Canberra, Australia 2616

16 <sup>3</sup> Hypo2 High Performance Sport Center, Flagstaff (AZ), USA

17 <sup>4</sup> Department of Clinical Physiology and Nuclear Medicine, HUS Medical Imaging Center,  
18 Helsinki

19 <sup>5</sup> University of Central Hospital and University of Helsinki, Finland

20 <sup>6</sup> Biology of Physical Activity, University of Jyväskylä, Finland

21 <sup>7</sup> Canadian Sport Institute Pacific, Victoria, Canada

22

23 **Address for correspondence:**

24 Ida Heikura, M.Sc.

25 Address: Level 5, 215 Spring Street, Melbourne, VIC 3000, Australia

26 Telephone: +61 422 863 010

27 E-mail: [ida.heikura@myacu.edu.au](mailto:ida.heikura@myacu.edu.au)

28

29 **Running head:** Health status influences Hbmass response

30

31 **Word count:** 4171 words

32 **Abstract count:** 231 words

33 **Number of references:** 40

34 **Number of figures and tables:** 1 table and 3 figures

35

36

37

## ABSTRACT

38 **Background:** We investigated the effects of sex, energy availability (EA), and health status on  
39 the change in hemoglobin mass ( $\Delta\text{Hbmass}$ ) in elite endurance athletes over ~3 to 4 weeks of  
40 Live-High/Train-High altitude training (Flagstaff, AZ, 2135m; n=27 females; n=21 males; 27%  
41 2016 Olympians). **Methods:** Pre- and post-camp Hbmass (optimized CO re-breathing method)  
42 and iron status were measured, EA was estimated via food and training logs and Low Energy  
43 Availability in Females Questionnaire (LEAF-Q) and a general injury/illness questionnaire was  
44 completed. Hypoxic exposure (hours) was calculated with low (<500h), moderate (500-600h)  
45 and high (>600h) groupings. **Results:** Absolute and relative percentage  $\Delta\text{Hbmass}$  ( $\%\Delta\text{Hbmass}$ )  
46 was significantly greater in females ( $6.2\pm 4.0\%$ ,  $p<0.001$ ) than in males ( $3.2\pm 3.3\%$ ,  $p=0.008$ ).  
47 ( $\%\Delta\text{Hbmass}$ ) showed a dose-response with hypoxic exposure ( $3.1\pm 3.8$  vs  $4.9\pm 3.8$  vs  $6.8\pm 3.7\%$ ;  
48  $p=0.013$ ).  $\text{Hbmass}_{\text{pre}}$  was significantly higher in eumenorrheic vs amenorrheic females ( $12.2\pm 1.0$   
49 vs  $11.3\pm 0.5$  g/kg;  $p=0.004$ ). Although statistically under-powered,  $\%\Delta\text{Hbmass}$  was significantly  
50 less in sick (n=4,  $-0.5\pm 0.4\%$ ) versus healthy (n=44 athletes;  $5.4\pm 3.8\%$ ;  $p<0.001$ ). There were no  
51 significant correlations between self-reported iron intake, sex hormones or EA on Hbmass  
52 outcomes. However, there was a trend for a negative correlation between LEAF-Q score and  
53  $\%\Delta\text{Hbmass}$  ( $r=-.353$ ,  $p=0.07$ ). **Conclusion:** Our findings confirm the importance of baseline  
54 Hbmass and exposure to hypoxia on increases in Hbmass during altitude training, while  
55 emphasizing the importance of athlete health and indices of EA on an optimal baseline Hbmass  
56 and hematological response to hypoxia.

57 **Key words:** world-class athletes, athlete health, adaptations to altitude, altitude training,  
58 hemoglobin mass

60 Many high performance endurance athletes undertake specialized forms of altitude training.  
61 The lack of agreement regarding the effects of altitude training on hematology and performance  
62 is partially explained by various differences in the methodology of altitude training studies<sup>1</sup>. For  
63 example, there are different modalities of altitude exposure, with several common options being  
64 Live High-Train High (LHTH) or Live High-Train Low (LHTL) with hypobaric or normobaric  
65 hypoxia, or intermittent hypoxic exposure at rest (IHE) or during training (IHT)<sup>2</sup>. Nevertheless,  
66 irrespective of changes in performance, a change in hemoglobin mass (Hbmass) is considered an  
67 objective and relatively easily measured outcome of altitude exposure within a standardized  
68 altitude training protocol, with typical increase of 2–5% following a block of altitude training  
69 being reported<sup>3-7</sup>. However, the mechanisms associated with optimizing Hbmass increases are  
70 multifactorial and include the type of altitude modality, the duration and level of exposure (also  
71 termed hypoxic dose<sup>8</sup>) and possibly the initial Hbmass level<sup>9</sup>. Indeed, there is consistent  
72 evidence of a progressive increase in Hbmass with three weeks of altitude training<sup>3,7,10</sup>, with  
73 supportive factors including the adequacy of baseline ferritin concentrations and doses of iron  
74 supplementation<sup>11,12</sup>. Meanwhile, only two studies have reported that injury and/or illness tends  
75 to negatively affect Hbmass changes<sup>6,13</sup>. One aspect of athlete health is optimal energy  
76 availability (EA), which is defined as the dietary energy available to support body function once  
77 the energy cost of exercise has been deducted from daily energy intake<sup>14</sup>. Low EA has  
78 detrimental effects on many areas of health and training adaptation, including impairment of  
79 menstrual status, protein synthesis and iron status and an increased risk of illness and injury<sup>15</sup>.  
80 However, to our knowledge no study has investigated the effects of symptoms of low EA on  
81 altitude-induced hematological adaptations.

82        There are conflicting findings in the literature regarding factors which alter the Hbmass  
83 response to altitude. For example, Wachsmuth *et al.*<sup>6</sup> found no sex based differences in 45 elite  
84 swimmers in the relative Hbmass response with 3-4 weeks of LHTH over multiple camps;  
85 however the absolute change was higher in males, which they hypothesized to be due to higher  
86 baseline values. Conversely, in a meta-analysis Rasmussen *et al.* calculated lower Hbmass  
87 changes in athletes with high baseline values following various altitude training protocols<sup>9</sup>. In  
88 contrast, Heinicke *et al.*<sup>16</sup> investigated the effects of 3-weeks of LHTH altitude training at 2050m  
89 on Hbmass in 6 male and 4 female world class biathletes and reported that Hbmass improved by  
90 ~9% in both males and females despite differences in baseline levels and very low subject  
91 numbers. Athlete calibre is another factor that may affect the hematological adaptations to  
92 altitude. Indeed, while some studies have shown increased Hbmass in elite athletes<sup>6,17</sup>, others<sup>18,19</sup>  
93 have failed to do so, leading some experts to question the usefulness of altitude training in  
94 athletes with already high Hbmass levels<sup>20</sup>. Obviously, the impact of baseline Hbmass values,  
95 which are greater in males than females and in elite versus non-elite, and the subsequent hypoxic  
96 induced changes in Hbmass, is far from being completely understood. Finally, the beneficial  
97 effects of altitude training on other body systems such as angiogenesis and increased buffering  
98 capacity<sup>21</sup> are often forgotten. Indeed, even if no hematological improvements are seen after  
99 altitude training, an athlete may have benefited from the camp via improvements in non-  
100 hematological outcomes.

101        Due to lack of studies on the effects of EA and hormonal health (i.e. reproductive, metabolic  
102 and anabolic hormones) on the Hbmass response at altitude, and due to contrasting results  
103 regarding other factors that may influence this response in males vs. females and elite athletes,  
104 our aim was to investigate the changes in Hbmass following LHTH altitude training in one of the

105 largest to date single cohort of elite female and male endurance athletes (27% Olympians) over a  
106 single training camp. Specifically, we aimed to confirm previous findings on the effects of length  
107 of exposure to hypoxia on change in Hbmass. However, we also wanted to investigate whether  
108 additional factors including sex, pre-camp Hbmass, health status (illness/injuries), EA sex  
109 hormone concentrations and bone health would affect Hbmass changes. Our hypothesis was that  
110 the magnitude of increase in Hbmass would depend primarily on hypoxic exposure, and possibly  
111 also on pre-camp Hbmass levels and health status.

## 112 **METHODS**

### 113 **Participants**

114 World-class middle- and long-distance runners and racewalkers (females, n=27; males,  
115 n=21) were recruited. The inclusion criteria was 18–40 years of age and having an IAAF score  
116 (International Association of Athletics Federations Scoring Tables 2011<sup>22</sup>) of at least 1050  
117 points (corresponds to 13:45.20min and 16:00.04min in the 5000m in males and females,  
118 respectively) scored within the preceding two years prior to study (baseline IAAF score). The  
119 study protocol was approved by the Ethics Committee of University of Jyväskylä and conducted  
120 according to the Declaration of Helsinki. All participants were enrolled in, and regularly  
121 screened by, anti-doping monitoring programs. No participants have ever served any anti-doping  
122 rule violation, and thus, to the best of our knowledge, were not involved with the use of any  
123 prohibited substances.

### 124 **Study design**

125 In a non-blinded longitudinal study design, we investigated pre- (Hbmass<sub>pre</sub>) and post-  
126 altitude (Hbmass<sub>post</sub>) Hbmass, iron and health status (sex hormones, bone mineral density

127 (BMD), injury/illness frequency) during a pre-competition LHTH altitude training camp in  
128 Flagstaff, AZ (2135m altitude; spring 2016). The measurements included baseline fasted blood  
129 samples, body composition and BMD measurements via Dual-energy X-ray Absorptiometry  
130 (DXA), followed by 7-day food and training logs on the second week of the camp. Female  
131 athletes filled out a validated Low Energy Availability in Females Questionnaire (LEAF-Q<sup>23</sup>).

### 132 **Hemoglobin mass**

133 Total Hbmass was measured with the adapted two-minute carbon monoxide (CO)  
134 rebreathing protocol<sup>24</sup>. In brief, subjects rebreathed a dose of CO based on body mass (BM)  
135 (1.25 ml/kg BM for males and 1.00 ml/kg BM for females) and ~4 L pure oxygen for 2 minutes  
136 via closed circuit spirometer. A nose clip was worn and a portable CO meter (FLUKE CO-220,  
137 Everett, Washington) was used to detect possible CO leakage via the nose, mouthpiece and  
138 spirometer throughout the 2 minutes of CO rebreathing. Determination of %HbCO was  
139 measured for baseline and 6 and 8 minutes after rebreathing from capillary fingertip blood  
140 samples tested with OSM3 hemoximeter (Radiometer, Copenhagen, Denmark). Hbmass was  
141 calculated from the mean change in %HbCO before and after CO rebreathing. Measurements  
142 were conducted pre- (within ~48-72h of arrival) and post-camp (within ~48-72 h from departure)  
143 by the same technician at Hypo2 High Performance Sport Center in Flagstaff, AZ. The typical  
144 error reported for the measurement done at Hypo2 is 1.9%. Throughout this manuscript, Hbmass  
145 values are reported as absolute (absolute total Hbmass), relative (Hbmass relative to BM) and  
146 percentage (percentage change in Hbmass, % $\Delta$ Hbmass).

147 **Hematology and anthropometry**

148 Resting overnight fasted venous blood samples were collected at the beginning and at the  
149 end of the camp. Venous blood was collected into 8.5 mL SST gel tubes (BD Vacutainer,  
150 Franklin Lakes, NJ, USA) and centrifuged at 3400rpm for 10min using a Mini E Horizon  
151 centrifuge (Drucker Company, Philipsburg, PA, USA). The fasted samples were analyzed for  
152 serum iron, ferritin, testosterone and estradiol and measured via electrochemiluminescence  
153 immunoassay (ECLIA) method. Body composition and BMD was measured in a fasted state by a  
154 trained technician with DXA (GE Lunar DPX-IQ).

155 **Dietary intakes and training characteristics**

156 To avoid the possible effects of the initial altitude acclimatization on training and eating  
157 habits the athletes were asked to keep food and training logs concurrently on the second week of  
158 the altitude training camp. The principal investigator met each athlete to provide detailed  
159 instructions on how to record all food and fluid intake accurately. The participants were asked to  
160 record the time of all meals and training sessions, the type of food (brand names, flavors, etc.)  
161 and amounts. Participants were provided with kitchen scales and measurement cups to facilitate  
162 the recording process. If the participants ate out, they were asked to provide photos of meals with  
163 verbal description to facilitate cross-checking. Athletes were free to supplement with iron  
164 according to self-chosen protocols (e.g. brand and dosage) during the camp, however details of  
165 this were recorded.

166 The participants were asked to record training for seven days including total distance, time  
167 and session rating of perceived exertion (sRPE<sup>25</sup>). The use of sRPE is validated to reflect training  
168 load, and sRPE values of <4 (zone 1), 4–7 (zone 2) and >7 (zone 3) have been shown to  
169 correspond well to the heart rate and blood lactate values<sup>26</sup>.



170 **Analysis of nutrient intake, energy expenditure and energy availability**

171 The principal investigator analyzed all dietary records with ESHA Food Processor (Oregon,  
172 US, 2016). EA was estimated from food and training diaries as energy intake minus EEE and  
173 expressed in  $\text{kcal}\cdot\text{kg}^{-1}\text{ FFM}\cdot\text{day}^{-1}$ <sup>14</sup>. Detailed information on methodology used and outcomes of  
174 these analyses is reported elsewhere<sup>27</sup>.

175 **Statistical analysis**

176 Statistical analyses were conducted using SPSS Statistics 22 (INM, Armonk, New York,  
177 USA) with data normality assessed via Shapiro-Wilk. Data were analysed for all athletes pooled,  
178 and with comparisons for sex and for female menstrual status (eumenorrhic vs. amenorrhic,  
179 defined as the absence of  $\geq$ three consecutive menses). Hypoxic dose<sup>8</sup> at 2135m was stratified  
180 into low (LOW:  $<1200\text{ km.h}$ ;  $<23\text{ days}$ ;  $n=27$ ), moderate (MOD:  $1200\text{--}1400\text{ km.h}$ ;  $23\text{--}27\text{ days}$ ;  
181  $n=13$ ) and high (HIGH:  $>1400\text{ km.h}$ ;  $>27\text{ days}$ ;  $n=8$ ) groups. For further comparison, athletes  
182 were also categorized based on hours of exposure<sup>3</sup> as follows: low ( $<500\text{ hours}$ ;  $n=18$ ), moderate  
183 ( $500\text{--}600\text{ hours}$ ;  $n=14$ ) and high ( $>600\text{ hours}$ ;  $n=16$ ). Analysis of Covariance (ANCOVA) was  
184 used to test the differences in the change in Hbmass with different hypoxic doses when  
185 controlling for  $\text{Hbmass}_{\text{pre}}$ . Athletes were divided into healthy and sick (with an illness being  
186 defined as anything that caused overall decrease in training/alteration to an athletes' training  
187 program, but excluded minor routine injuries where training could be modified or training load  
188 was not reduced due to cross-training) groups for further analysis. Baseline IAAF scores were  
189 compared to the best race performance (IAAF score) within three weeks of descent from altitude  
190 (Post IAAF score).

191 Differences in pre-camp body composition, Hbmass, iron status, EA, sex hormones and  
192 BMD between sexes, amenorrheic vs eumenorrheic females, and healthy vs sick athletes were  
193 analyzed with Student's t-test (parametric data) or Mann-Whitney U-test (nonparametric data).  
194 Changes in pre- to post-iron status, Hbmass and IAAF score were analyzed with Student's paired  
195 t-test (parametric data) or Wilcoxon signed rank test (nonparametric). Correlations were  
196 analyzed using Pearson's correlation coefficient (parametric) or Spearman's test  
197 (nonparametric). Data are presented as means±standard deviations (SD). Statistical significance  
198 was set at  $p \leq 0.05$ .

## 199 **RESULTS**

200 Table 1 summarizes athlete characteristics, dietary and training data, iron status parameters and  
201 raw Hbmass changes during the altitude camp. Pre- and post-camp Hbmass were higher in  
202 males than in females.  $\% \Delta \text{Hbmass}$  (g/kg) was  $4.9 \pm 4.0\%$  ( $p < 0.001$ ) in all athletes pooled, with  
203 significantly higher percentage ( $p = 0.008$ ) and absolute ( $p = 0.033$ ) increases in relative Hbmass  
204 values in females vs males. Relative  $\text{Hbmass}_{\text{pre}}$  was significantly higher in eumenorrheic ( $n = 20$ )  
205 vs amenorrheic ( $n = 7$ ) females ( $12.2 \pm 1.0$  vs  $11.3 \pm 0.5$  g/kg;  $p = 0.004$ ). LOW hypoxic dose  
206 ( $+3.7 \pm 3.9\%$ ;  $1013 \pm 137 \text{ km.h}$ ) increased relative Hbmass significantly less than MOD  
207 ( $+7.3 \pm 3.4\%$ ;  $1320 \pm 70 \text{ km.h}$ ;  $p = 0.018$ ) and, although not statistically significant, less than HIGH  
208 ( $4.8 \pm 3.6\%$ ;  $1563 \pm 95 \text{ km.h}$ ) groups. In contrast, when hypoxic dose was characterized as hours of  
209 exposure, there was a trend for higher response with increasing hours of exposure, and a  
210 significant difference between low and high in all athletes pooled ( $F(2,48) = 8.192$ ,  $p = 0.017$ ).  
211 However, when females and males were analysed separately, only males showed a difference in  
212 the  $\% \Delta \text{Hbmass}$  response based on hours of exposure ( $F(2,20) = 10.21$ ,  $p = 0.001$ ; Figure 2). Also,  
213 ANCOVA showed that there was a significant difference in the relative Hbmass response

214 between hours of exposure groups when controlling for Hbmass<sub>pre</sub> (F(2, 44)=4.413, p=0.018,  
215 partial eta squared=0.167). In addition, there was a strong relationship between HBmass<sub>pre</sub> and  
216 Hbmass<sub>post</sub> (partial eta squared=0.928)

217 The relative Hbmass<sub>pre</sub> negatively and significantly correlated with the %ΔHbmass (females  
218  $r=-.406$ ,  $p=0.035$ ; males  $r=-.470$ ,  $p=0.032$ ; Figure 1). In addition, hypoxic dose as km.h ( $r=.333$ ,  
219  $p=0.021$ ) and as hours of exposure ( $r=.374$ ,  $p=0.009$ ) positively correlated with %ΔHbmass.  
220 Relative Hbmass increased significantly more in healthy athletes (n=44, 26 females and 18  
221 males) compared to those who suffered from illness (n=4, 1 females and 3 males) during the  
222 camp (Figure 3;  $p<0.001$ ). Two females (%ΔHbmass +7.1 and +14.6%) suffered mild injuries  
223 but continued cross-training to maintain training load during the camp and thus, were not  
224 considered as having an illness. When these athletes were included in the analysis as  
225 “sick/injured”, the difference in the %ΔHbmass between sick and healthy athletes became non-  
226 significant ( $3.3\pm 6.3$  vs  $5.1\pm 3.6\%$ , respectively;  $p=0.11$ ). No correlations were found between  
227 baseline IAAF score, change in IAAF from baseline to post-camp or EA and %ΔHbmass. In  
228 females, LEAF-Q score showed a strong trend for a negative correlation with %ΔHbmass (g/kg;  
229  $r=-.353$ ,  $p=0.07$ ). There were no correlations or effect of self-reported iron supplementation  
230 protocols, baseline ferritin levels, sex hormones (data in our companion paper<sup>27</sup>), body  
231 composition parameters or BMD (data in our companion paper<sup>27</sup>) on Hbmass outcomes.

## 232 **DISCUSSION**

233 This is one of the largest studies to date to investigate the contribution of hours of exposure to  
234 hypoxia, Hbmass<sub>pre</sub> and aspects of health status (e.g. outcomes of EA and illness incidence at  
235 altitude) to the Hbmass response to altitude training in a single camp and single cohort of male

236 and female world-class endurance athletes (27% Olympians). Furthermore, our large subject  
237 pool allowed for sufficient statistical power to allow a comparison of sex-based differences in  
238 responses. Our main findings were that Hbmass increased significantly in both female and males,  
239 with significantly greater relative and percentage increases in females. In addition, Hbmass<sub>pre</sub>  
240 was higher in eumenorrheic compared to amenorrheic females, and the increase in Hbmass was  
241 more prominent in athletes who remained healthy throughout. Finally, in line with previous  
242 studies, we found superior increases in Hbmass with greater hypoxic exposures and in those with  
243 lower initial Hbmass<sub>pre</sub> values.

244 Our investigation further expands the current literature on altitude training in elite athletes in  
245 which studies are commonly characterised by the collection of data over multiple time periods<sup>6</sup>,  
246 with varying altitude exposures<sup>28</sup> and/or use of simulated hypoxia<sup>11</sup>, or in the absence of  
247 measures of changes in Hbmass<sup>29-33</sup>. Unlike a few previous studies (<sup>18,19</sup>) that have failed to find  
248 an increase in Hbmass in elite athletes, and contrary to speculations on whether elite athletes  
249 with already high Hbmass benefit from altitude training<sup>20</sup>, we found significant Hbmass  
250 increases in our group of world-class distance athletes. This is in line with a recent study by  
251 Hauser et al.<sup>34</sup>, who showed increases in Hbmass after 200-230 hours of exposure to a LHTL  
252 protocol in male endurance and team sport athletes. Indeed, despite a moderate inverse  
253 relationship between baseline Hbmass and change in Hbmass, even athletes with high initial  
254 Hbmass levels (13.1g/kg in endurance athletes) showed ~4% increases following exposure to  
255 hypoxia<sup>34</sup>. Interestingly, despite similarities in the calibre of our female and male athletes, as  
256 shown by their identical baseline IAAF scores, females were more successful in improving their  
257 Hbmass over the camp (6.2 vs 3.2%; Table 1). While previous studies have failed to find a  
258 difference in Hbmass response to altitude between sexes<sup>6,16</sup>, these have generally involved

259 smaller numbers of female-to-male comparisons<sup>5,13,16,35,36</sup> or have investigated only females<sup>4,10</sup> or  
260 males<sup>18,19,34,37,38</sup>. The findings of the current study could be explained by the fact that males had  
261 significantly higher relative Hbmass<sub>pre</sub> levels (14.4 vs 12.0 g/kg; Table 1), although this is just  
262 speculation. Nevertheless, we found a negative relationship between Hbmass<sub>pre</sub> and change in  
263 Hbmass ( $\Delta$ Hbmass; Figure 1); previous investigations have also suggested that initial Hbmass  
264 play a role in the magnitude of the hematological adaptations at altitude<sup>9,20,34</sup>, although not all  
265 studies support this finding<sup>6</sup>.

266 The magnitude and length of exposure to hypoxia are crucial for altitude-induced  
267 hematological adaptations. Based on several studies, an increase of 1% per week<sup>10</sup> or 1% per 100  
268 hours of exposure<sup>3</sup> can be expected, although an exponential model of hypoxic dose has also  
269 been proposed by Garvican-Lewis and colleagues<sup>8</sup>. Indeed, we found increases of 3.7, 7.3, and  
270 4.8% at low (1013 km.h), moderate (1320 km.h) and high (1563 km.h) hypoxic doses,  
271 respectively, which resulted in a significant positive correlation between hypoxic dose and  
272  $\Delta$ Hbmass. Interestingly, comparison of changes in Hbmass with differing hours of exposure  
273 showed greater increases in Hbmass with increasing hours of exposure (3.6 vs 4.0 vs 6.2% with  
274 <500, 500-600, and >600 hours of exposure; Figure 2), which is in line with a meta-analysis  
275 showing that even shorter exposures of LHTH or LHTL are able to increase Hbmass ~3% given  
276 the athlete is free from injury/illness and has adequate iron supplementation<sup>39</sup>. Considering the  
277 same elevation for each athlete in the current study, perhaps the difference in findings between  
278 hypoxic dose and hours of exposure comparisons can be explained with different cut-off points  
279 that resulted in different categorization of athletes into low, moderate and high groups.  
280 Alternatively, our analysis between hypoxic dose groups may have been under-powered (n=8 in  
281 high hypoxic dose group). However, although exposure to hypoxia is important, our findings

282 suggest that initial Hbmass levels (Figure 1) appear to have an even greater effect on the  
283 magnitude of hematological adaptations following altitude training.

284 There have been previous indications that athlete health is associated with changes in  
285 erythropoiesis in athletes. Gough *et al.*<sup>13</sup> tracked changes in Hbmass in 15 athletes over lengthy  
286 periods (162±198 days) of training interruptions due to illness and injury, showing that reduced  
287 training and surgery (n=3) led to 2.3 and 2.7% decreases in Hbmass, respectively. Furthermore,  
288 Wachsmuth *et al.*<sup>6</sup> showed a 7.2% increase in Hbmass following 3-4 weeks of LHTH training at  
289 2320m in swimmers, while no increase was observed in ill/injured athletes (n=8). The results of  
290 our study show several new insights into the importance of health status in optimizing the  
291 response to altitude training. Principally, healthy athletes were able to increase Hbmass  
292 significantly more than athletes who became sick during the training camp (+5.4 vs -0.5%;  
293 Figure 3), which confirms the findings of previous research. While we acknowledge that our  
294 sample size of injured athletes was small and thus may have reduced the statistical power, as  
295 mentioned earlier, this finding is in line with previous studies showing an impaired response to  
296 hypoxia in athletes who were not healthy<sup>6,13</sup>. Interestingly, despite suffering minor injuries  
297 during the camp, two females who managed to maintain their training loads via cross-training  
298 did not show Hbmass erosion, with an average Hbmass increase of ~10%. This novel finding  
299 suggests that athletes suffering from minor injuries (where serious inflammation may not  
300 present) may still be able to benefit from altitude training where training volume is not  
301 compromised (via inclusion of cross-training) and where non-steroid anti-inflammatory drugs are  
302 not used (may compromise response). This is aligned with Gough *et al.*<sup>13</sup> who also showed  
303 training reductions causing decreases in Hbmass. However, these findings should be interpreted

304 with caution as we only had a very small number of athletes who developed illnesses during the  
305 camp.

306 We were also interested to look at the effect of low EA (based on food and training records  
307 as well as physiological outcomes<sup>27</sup>) on adaptations to altitude training, since it has previously  
308 been shown to impair health and performance<sup>15</sup>, including processes such as the protein synthetic  
309 response to exercise<sup>40</sup> that are likely to be important in hemapoiesis. Our estimations from food  
310 and training logs captured during the mid-period of the altitude training camp identified a range  
311 of EA scores among both male and female cohorts spanning healthy ( $\sim 45 \text{ kcal.kg BM}^{-1}.\text{d}^{-1}$ ) to  
312 low ( $<30 \text{ kcal.kg BM}^{-1}.\text{d}^{-1}$ )<sup>14</sup>. However, we failed to identify a correlation between these  
313 estimates and Hbmass changes. This is not entirely surprising since these EA calculations are  
314 based on self-reports from a single time period of 1 week which are fraught with methodological  
315 issues, as well as not necessarily representative of earlier behaviors which may have caused  
316 chronic metabolic and hormonal perturbations<sup>27</sup>. Indeed, it is likely that athletes' eating and  
317 exercise activities during the camp were different to their habitual practices due to deliberate  
318 alterations in nutrition practices and training program to accommodate the special needs of  
319 altitude training, as well as secondary changes due to a new food environment and daily routine.  
320 These changes may have altered both the magnitude and direction of habitual EA compared to  
321 the optimal levels. Surprisingly and contrary to our hypothesis, we failed to find correlations or  
322 effects of sex hormones or BMD on Hbmass outcomes. Indeed, we assumed that low sex  
323 hormone or BMD status would negatively affect  $\Delta\text{Hbmass}$ , however this was not the case.  
324 Nevertheless, other data collected in our study which identified a high risk of chronic low EA  
325 was correlated with Hbmass responses to the altitude training. We found significantly lower  
326  $\text{Hbmass}_{\text{pre}}$  in amenorrheic vs eumenorrheic females (amenorrhea signals of chronic low EA<sup>15</sup>)

327 and a trend for higher increases in Hbmass with lower LEAF-Q scores (LEAF-Q scores of >8 are  
328 likely to be indicative of low EA in females<sup>23</sup>), which indicates that menstrual dysfunction, an  
329 indicator of long-term low EA, may influence these adaptations or their magnitude. However,  
330 despite this trend, this association between LEAF-Q and Hbmass changes at altitude requires  
331 further validation.

332 *Strengths and limitations.* The major strength of the current study is that it was conducted  
333 during the preparation period for the 2016 Olympic Games and thus, unlike several other  
334 previous studies, reflects the true training characteristics and altitude camp outcomes of elite  
335 athlete in preparation for a major competition. The sample size for the current study is one of the  
336 largest to date reported in the literature in an elite athlete population, with a single camp and a  
337 single time period protocol allowing us to detect differences that might not otherwise be  
338 detectable when using other forms of data collection. Furthermore, to our knowledge, we have  
339 highest numbers of female-to-male comparisons within these conditions. Finally, our study adds  
340 to the growing literature of the likely detrimental effects of low EA and/or menstrual dysfunction  
341 on athlete health. However, there are several limitations. First, due to the involvement of truly  
342 world-class athletes in preparation in the Olympic year, we were not able to standardise factors  
343 such as duration of altitude exposure and use or dose of iron supplementation (although all were  
344 recommended to take iron between 100 to 200mg/day). In addition, we were not able to include  
345 performance tests to provide physiological characteristics of the athletes. Therefore, it is  
346 impossible to estimate the effects of altitude exposure or changes in Hbmass on performance  
347 outcomes in these athletes. In addition, the dietary and training information was collected from a  
348 single week of the camp and may not represent habitual practices and/or the practices over the  
349 entire camp. However, since the reliability and accuracy of food records decreases with



350 increasing recording periods, and since elite athletes tend to keep their dietary intakes relatively  
351 stable over a microcycle (*personal observations*), we believe this time period was sufficient to  
352 yield an idea of the dietary patterns of these athletes. Given the study design, and the fact that  
353 altitude training tends to enhance performance, we were unable to add a sea-level control group.  
354 Finally, we acknowledge that comparing the results of the current study to the findings of  
355 previous altitude training literature, where a different study population (calibre and sport),  
356 different altitude exposure (length and elevation) and different protocol (LHTH, LHTL, IHE,  
357 IHT in normobaric or hypobaric hypoxia) make it challenging to make direct comparisons across  
358 studies. Nonetheless, we believe that our study adds novel information to the existing literature  
359 on altitude training.

360

## 361 **Conclusions**

362 These data represent one of the largest investigations to date of the effects of various factors  
363 on the Hbmass response to LHTH altitude training in world-class endurance athletes, including a  
364 robust comparison of responses in males versus females, during a pre-season preparation camp  
365 before the 2016 Olympic Games. We showed that females have significantly lower Hbmass<sub>pre</sub>  
366 than males, with a negative correlation between Hbmass<sub>pre</sub> and change in Hbmass over the camp.  
367 However, we would like to highlight the fact that despite previous expert opinions on the lack of  
368 effectiveness of altitude training in elite athletes, our cohort of world-class athletes were able to  
369 benefit from the hematological effects of the altitude camp despite being elite and possessing  
370 high initial Hbmass levels. Furthermore, our findings emphasize and confirm the previous  
371 findings on the importance of athlete health in the optimal hematological response to altitude  
372 exposure. Indeed, to our knowledge, we are the first to show that menstrual function is correlated

373 with baseline Hbmass levels and that a higher risk score for low EA in females shows a trend to  
374 correlate with less favorable changes in Hbmass following altitude training. We also found a  
375 significant difference in the Hbmass response to altitude, where healthy athletes were able to  
376 increase Hbmass on average by 5.4% compared to an average decrease of -0.5% in those who  
377 were sick during the camp, although it should be emphasized that our small sample size (n=4) of  
378 sick athletes may have reduced the statistical power. Finally, we confirm previous findings of the  
379 importance of sufficient exposure to hypoxia on hematological adaptations to altitude, where  
380 increasing hours of exposure seem to provide increasing hematological benefits independent of  
381 initial Hbmass levels.

382

383

#### **ACKNOWLEDGEMENTS**

384 The study was designed by IH, TS, AM, AU and LB; data were collected and analyzed by IH,  
385 TS, DB and LB; data interpretation and manuscript preparation were undertaken by IH, TS, AM,  
386 AU, DB and LB. All authors approved the final version of the paper. This study was financially  
387 supported by grants from the Canadian Sport Institute, Australian Catholic University/Australian  
388 Institute of Sport, Finnish Sport Institute Foundation, and Finnish Sport Research Foundation.  
389 The authors wish to thank Hypo2 High Performance Sport Center and Northern Arizona  
390 Orthopaedics and Prof. Jennifer Peat for technical analysis and statistical assistance. Finally, the  
391 authors wish to thank all the participants for volunteering for the study.

392

#### **CONFLICTS OF INTEREST**

393 The authors and funding agents do not have any conflicts of interests.

394



396

## REFERENCES

397 1. Lundby C, Robach P. Does 'altitude training' increase exercise performance in elite athletes?

398 *Exp Physiol.* 2016;101(7):783-788.

399 2. Millet GP, Roels B, Schmitt L, Woorons X, Richalet JP. Combining hypoxic methods for peak  
400 performance. *Sports Med.* 2010;40(1):1-25.

401 3. Garvican L, Martin D, Quod M, Stephens B, Sassi A, Gore C. Time course of the hemoglobin  
402 mass response to natural altitude training in elite endurance cyclists. *Scand J Med Sci Sports.*  
403 2012;22(1):95-103.

404 4. Pottgiesser T, Garvican LA, Martin DT, Featonby JM, Gore CJ, Schumacher YO. Short-term  
405 hematological effects upon completion of a four-week simulated altitude camp. *Int J Sports*  
406 *Physiol Perform.* 2012;7(1):79-83.

407 5. Wehrlin JP, Zuest P, Hallen J, Marti B. Live high-train low for 24 days increases hemoglobin  
408 mass and red cell volume in elite endurance athletes. *J Appl Physiol (1985).* 2006;100(6):1938-  
409 1945.

410 6. Wachsmuth NB, Volzke C, Prommer N, et al. The effects of classic altitude training on  
411 hemoglobin mass in swimmers. *Eur J Appl Physiol.* 2013;113(5):1199-1211.

412 7. Garvican-Lewis LA, Halliday I, Abbiss CR, Saunders PU, Gore CJ. Altitude exposure at 1800  
413 m increases haemoglobin mass in distance runners. *J Sports Sci Med.* 2015;14(2):413-417.

414 8. Garvican-Lewis LA, Sharpe K, Gore CJ. Time for a new metric for hypoxic dose? *J Appl*  
415 *Physiol (1985).* 2016;121(1):352-355.

- 416 9. Rasmussen P, Siebenmann C, Diaz V, Lundby C. Red cell volume expansion at altitude: A  
417 meta-analysis and monte carlo simulation. *Med Sci Sports Exerc.* 2013;45(9):1767-1772.
- 418 10. Clark SA, Quod MJ, Clark MA, Martin DT, Saunders PU, Gore CJ. Time course of  
419 haemoglobin mass during 21 days live high: Train low simulated altitude. *Eur J Appl Physiol.*  
420 2009;106(3):399-406.
- 421 11. Govus AD, Garvican-Lewis LA, Abbiss CR, Peeling P, Gore CJ. Pre-altitude serum ferritin  
422 levels and daily oral iron supplement dose mediate iron parameter and hemoglobin mass  
423 responses to altitude exposure. *PLoS One.* 2015;10(8):e0135120.
- 424 12. Garvican-Lewis LA, Govus AD, Peeling P, Abbiss CR, Gore CJ. Iron supplementation and  
425 altitude: Decision making using a regression tree. *J Sports Sci Med.* 2016;15(1):204-205.
- 426 13. Gough CE, Sharpe K, Garvican LA, Anson JM, Saunders PU, Gore CJ. The effects of injury  
427 and illness on haemoglobin mass. *Int J Sports Med.* 2013;34(9):763-769.
- 428 14. Loucks AB, Kiens B, Wright HH. Energy availability in athletes. *J Sports Sci.* 2011;29 Suppl  
429 1:S7-15.
- 430 15. Mountjoy M, Sundgot-Borgen J, Burke L, et al. The IOC consensus statement: Beyond the  
431 female athlete Triad—Relative energy deficiency in sport (RED-S). *British Journal of Sports*  
432 *Medicine.* 2014;48(7):491-497.
- 433 16. Heinicke K, Heinicke I, Schmidt W, Wolfarth B. A three-week traditional altitude training  
434 increases hemoglobin mass and red cell volume in elite biathlon athletes. *Int J Sports Med.*  
435 2005;26(5):350-355.

- 436 17. Garvican LA, Pottgiesser T, Martin DT, Schumacher YO, Barras M, Gore CJ. The  
437 contribution of haemoglobin mass to increases in cycling performance induced by simulated  
438 LHTL. *Eur J Appl Physiol.* 2011;111(6):1089-1101.
- 439 18. Gore CJ, Hahn A, Rice A, et al. Altitude training at 2690m does not increase total  
440 haemoglobin mass or sea level VO<sub>2</sub>max in world champion track cyclists. *J Sci Med Sport.*  
441 1998;1(3):156-170.
- 442 19. Pottgiesser T, Ahlgrim C, Ruthardt S, Dickhuth HH, Schumacher YO. Hemoglobin mass  
443 after 21 days of conventional altitude training at 1816 m. *J Sci Med Sport.* 2009;12(6):673-675.
- 444 20. Robach P, Lundby C. Is live high-train low altitude training relevant for elite athletes with  
445 already high total hemoglobin mass? *Scand J Med Sci Sports.* 2012;22(3):303-305.
- 446 21. Gore CJ, Clark SA, Saunders PU. Nonhematological mechanisms of improved sea-level  
447 performance after hypoxic exposure. *Med Sci Sports Exerc.* 2007;39(9):1600-1609.
- 448 22. IAAF Scoring Tables 2011. Accessed on 31 Jan 2016. Retrieved from  
449 <https://www.iaaf.org/about-iaaf/documents/technical>
- 450 23. Melin A, Tornberg AB, Skouby S, et al. The LEAF questionnaire: A screening tool for the  
451 identification of female athletes at risk for the female athlete triad. *Br J Sports Med.*  
452 2014;48(7):540-545.
- 453 24. Schmidt W, Prommer N. The optimised CO-rebreathing method: A new tool to determine  
454 total haemoglobin mass routinely. *Eur J Appl Physiol.* 2005;95(5-6):486-495.

- 455 25. Foster C. Monitoring training in athletes with reference to overtraining syndrome. *Med Sci*  
456 *Sports Exerc.* 1998;30(7):1164-1168.
- 457 26. Seiler KS, Kjellerud GO. Quantifying training intensity distribution in elite endurance  
458 athletes: Is there evidence for an "optimal" distribution? *Scand J Med Sci Sports.* 2006;16(1):49-  
459 56.
- 460 27. Heikura IA, Uusitalo AL, Stellingwerff T, Berglund D, Mero AA, Burke LM. Low energy  
461 availability is difficult to assess but outcomes have large impact on bone injury rates in elite  
462 distance athletes. *Int J Sport Nutr Exerc Metab* In press
- 463 28. Rodriguez FA, Iglesias X, Ferlic B, et al. Altitude training in elite swimmers for sea level  
464 performance (altitude project). *Med Sci Sports Exerc.* 2015;47(9):1965-1978.
- 465 29. Woods AL, Garvican-Lewis LA, Rice A, Thompson KG. 12 days of altitude exposure at  
466 1800 M does not increase resting metabolic rate in elite rowers. *Appl Physiol Nutr Metab.*  
467 2017;42(6):672-676.
- 468 30. Stray-Gundersen J, Chapman RF, Levine BD. "Living high-training low" altitude training  
469 improves sea level performance in male and female elite runners. *J Appl Physiol (1985).*  
470 2001;91(3):1113-1120.
- 471 31. Chapman RF, Karlsen T, Resaland GK, et al. Defining the "dose" of altitude training: How  
472 high to live for optimal sea level performance enhancement. *J Appl Physiol (1985).*  
473 2014;116(6):595-603.

- 474 32. Sharma AP, Saunders PU, Garvican-Lewis LA, et al. The effect of training at 2100-m  
475 altitude on running speed and session rating of perceived exertion at different intensities in elite  
476 middle-distance runners. *Int J Sports Physiol Perform.* 2017;12(Suppl 2):S2147-S2152.
- 477 33. Sperlich B, Achtzehn S, de Mares M, von Papen H, Mester J. Load management in elite  
478 german distance runners during 3-weeks of high-altitude training. *Physiol Rep.*  
479 2016;4(12):10.14814/phy2.12845.
- 480 34. Hauser A, Troesch S, Steiner T, Brocherie F, Girard O, Saugy JJ, Schmitt GP, Wehrlin JJP.  
481 Do male athletes with already high initial haemoglobin mass benefit from 'live high-train low'  
482 altitude training? *Exp Physiol.* 2017;Oct11 doi: 10.1113/EP086590. [Epub ahead of print]
- 483 35. Woods AL, Sharma AP, Garvican-Lewis LA, Saunders PU, Rice AJ, Thompson KG. Four  
484 weeks of classical altitude training increases resting metabolic rate in highly trained middle-  
485 distance runners. *Int J Sport Nutr Exerc Metab.* 2017;27(1):83-90.
- 486 36. Gough CE, Saunders PU, Fowlie J, et al. Influence of altitude training modality on  
487 performance and total haemoglobin mass in elite swimmers. *Eur J Appl Physiol.*  
488 2012;112(9):3275-3285.
- 489 37. Garvican-Lewis LA, Schumacher YO, Clark SA, et al. Stage racing at altitude induces  
490 hemodilution despite an increase in hemoglobin mass. *J Appl Physiol (1985).* 2014;117(5):463-  
491 472.



- 492 38. McLean BD, Buttifant D, Gore CJ, White K, Kemp J. Year-to-year variability in  
493 haemoglobin mass response to two altitude training camps. *Br J Sports Med.* 2013;47 Suppl  
494 1:i51-8.
- 495 39. Gore CJ, Sharpe K, Garvican-Lewis LA, Saunders PU, Humberstone CE, Robertson EY,  
496 Wachsmuth NB, Clark SA, McLean BD, Friedmann-Bette B, Neya M, Pottgiesser T,  
497 Schumacher YO, Schmidt WF. Altitude training and haemoglobin mass from the optimised  
498 carbon monoxide rebreathing method determined by a meta-analysis. *Br J Sports Med.*  
499 2013;47(S1):i31-39.
- 500 40. Areta JL, Burke LM, Camera DM, et al. Reduced resting skeletal muscle protein synthesis is  
501 rescued by resistance exercise and protein ingestion following short-term energy deficit. *Am J*  
502 *Physiol Endocrinol Metab.* 2014;306(8):E989-97.
- 503

## FIGURE LEGENDS

**Figure 1.** Correlation between pre-camp hemoglobin mass ( $Hbmass_{pre}$ ) and the relative change in Hbmass ( $\% \Delta Hbmass$ ) in females (A) and males (B). *Open circles*, low hypoxic dose group (<1200km.h); *open triangles*, moderate hypoxic dose group (1200-1400 km.h); *open squares*, high hypoxic dose group (>1400 km.h).

**Figure 2.** Differences in the percentage hemoglobin mass response ( $\% \Delta Hbmass$ ) to altitude in low (LOW: <500 hours of exposure, corresponds to <21 days at 2135 m), moderate (MOD: 500-600 hours of exposure, corresponds to 21-25 days at 2135 m) and high (HIGH: >600hours of exposure, corresponds to >25 days at 2135 m) hypoxic exposure groups in females and males. \*  $p < 0.05$ , \*\*  $p < 0.01$  significant difference between groups.

**Figure 3.** The magnitude of percentage change in hemoglobin mass ( $\% \Delta Hbmass$ ) in athletes who were not sick or injured during the altitude camp (healthy athletes; *white bar*) and athletes who were sick or injured during the camp (*black bar*). \*\*\*  $p < 0.001$  significant difference between groups

## TABLES

Table 1. Athlete characteristics, dietary and training data, iron status parameters and Hbmass outcomes in elite female and male distance athletes. Values are means  $\pm$  SD.

	Females (n=23)	Males (n=15)
<i>Athlete characteristics</i>		
Age (yr)	26.0 $\pm$ 3.2	27.2 $\pm$ 4.1
Height (m) ***	1.68 $\pm$ 0.05	1.80 $\pm$ 0.06
Weight pre (kg) ***	54.1 $\pm$ 4.5	68.0 $\pm$ 5.9
Weight post (kg) ***	53.7 $\pm$ 4.5	68.2 $\pm$ 5.8
Body fat (%) ***	11.7 $\pm$ 2.7	6.7 $\pm$ 1.2
Baseline IAAF score	1113 $\pm$ 39	1109 $\pm$ 45
Post IAAF score # \$\$	1090 $\pm$ 39	1072 $\pm$ 67
<i>Altitude camp activities</i>		
EA (kcal/kg FFM/day)	33 $\pm$ 7	36 $\pm$ 6
Iron supplement (mg elemental iron)	110 $\pm$ 61	142 $\pm$ 68
Dietary iron (mg.d <sup>-1</sup> ) **	16.6 $\pm$ 5.1	24.7 $\pm$ 8.6
Running (km·wk <sup>-1</sup> ) *	94 $\pm$ 27	114 $\pm$ 30
TRIMP (AU)	1998 $\pm$ 601	2363 $\pm$ 1424
Hypoxic dose (km.h <sup>-1</sup> ) *	1180 $\pm$ 193	1038 $\pm$ 235
<i>Iron status parameters</i>		
Pre serum iron	121 $\pm$ 42	112 $\pm$ 31
Post serum iron	134 $\pm$ 44	113 $\pm$ 58
Pre serum ferritin	87 $\pm$ 50	106 $\pm$ 37
Post serum ferritin ###	83 $\pm$ 45	82 $\pm$ 24
<i>Hbmass parameters</i>		
Hbmass <sub>pre</sub> (g) ***	646 $\pm$ 57	979 $\pm$ 103
Hbmass <sub>post</sub> (g) *** ### \$\$\$	681 $\pm$ 67	1013 $\pm$ 109
Hbmass <sub>pre</sub> (g/kg) ***	12.0 $\pm$ 1.0	14.4 $\pm$ 1.1
Hbmass <sub>post</sub> (g/kg) *** ### \$\$\$	12.7 $\pm$ 0.9	14.9 $\pm$ 1.0
$\Delta$ Hbmass (g)	36 $\pm$ 25	34 $\pm$ 28
$\Delta$ Hbmass (g/kg) *	0.7 $\pm$ 0.5	0.4 $\pm$ 0.4
% $\Delta$ Hbmass (g) *	5.5 $\pm$ 3.8	3.4 $\pm$ 3.0
% $\Delta$ Hbmass (g/kg) **	6.2 $\pm$ 4.0	3.2 $\pm$ 3.3

Baseline IAAF score, IAAF score (International Association of Athletics Federations scoring table 2011) prior to the camp; Post IAAF score, race IAAF score in the three-week post-camp period; EA, energy availability; TRIMP, training impulse; AU, arbitrary unit; Hbmass, hemoglobin mass;  $\Delta$ Hbmass, absolute change in Hbmass; % $\Delta$ Hbmass, relative change in Hbmass. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  significant difference between females and males;  $^{\$}$   $p < 0.05$ ,  $^{\$\$}$   $p < 0.01$ ,  $^{\$\$\$}$   $p < 0.001$  significant difference from pre to post in females;  $^{\#}$   $p < 0.05$ ,  $^{\#\#}$   $p < 0.01$ ,  $^{\#\#\#}$   $p < 0.001$  significant difference from pre to post in males

# FIGURES

Figure 1.

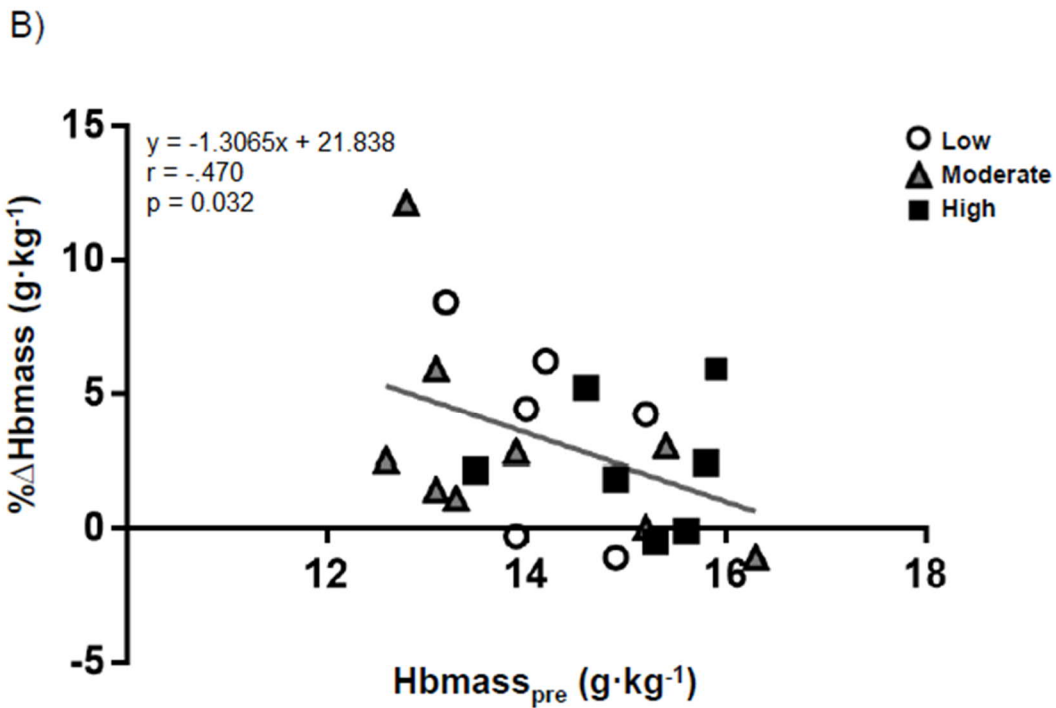
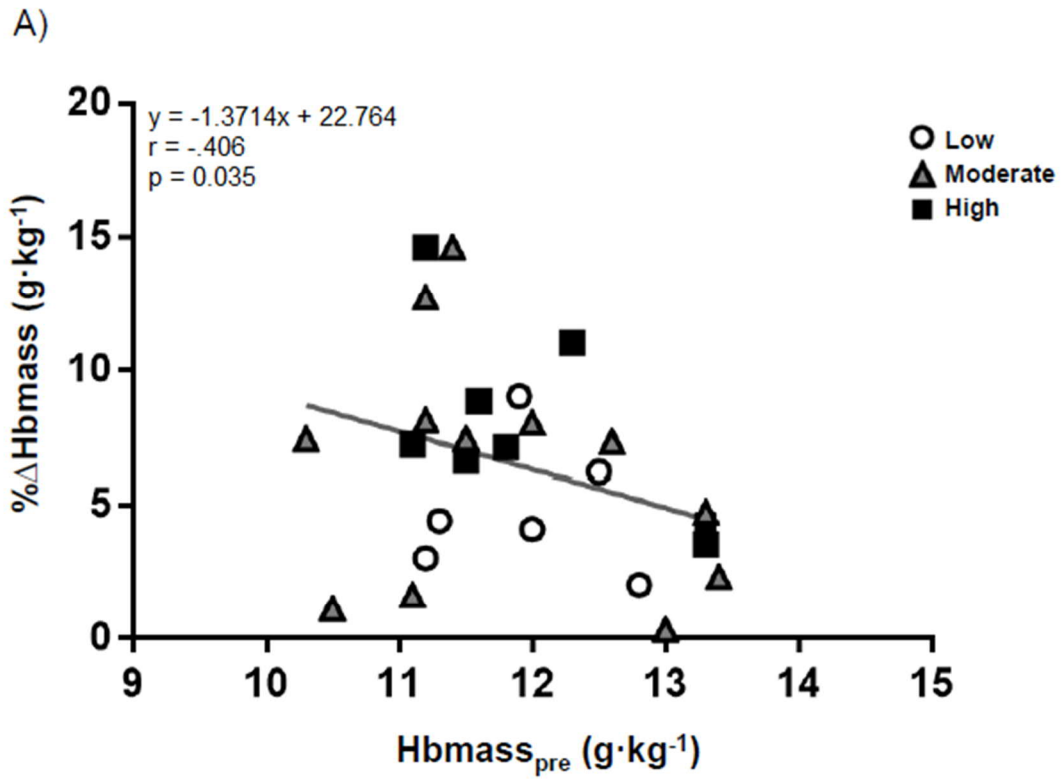


Figure 2.

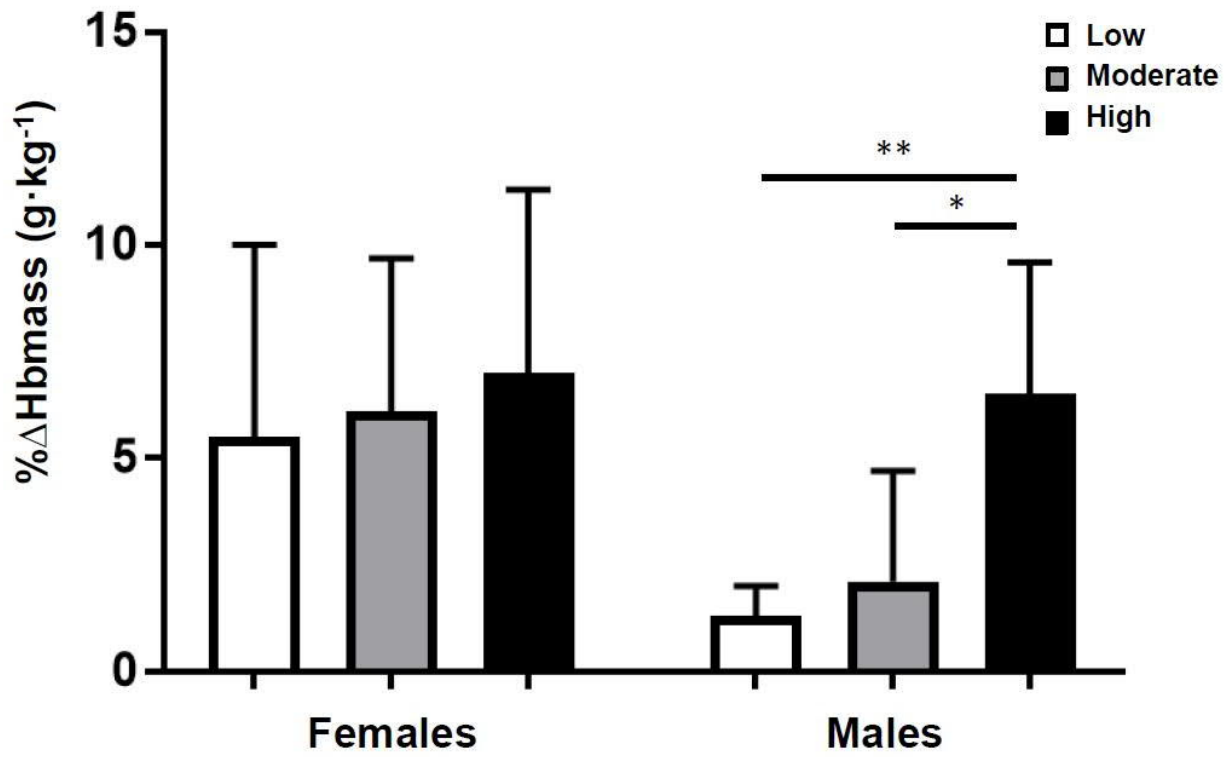


Figure 3.

