

# Anabolic Adaptations Occur in Conscripts During Basic Military Training Despite High Prevalence of Vitamin D Deficiency and Decrease in Iron Status

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**ABSTRACT** Introduction: In Estonian Defense Forces that are drawn up on the basis of the conscription model considerable numbers of young men are prematurely discharged from military service for medical reasons, but causes leading to premature dropout of conscripts have not been systematically studied. However, one of the factors involved could be relatively demanding physical training that starts at the beginning of military service in the form of basic military training (BMT). Cumulative training and nontraining stresses experienced by conscripts during BMT may exceed their physiological adaptability and increase the probability of becoming prematurely discharged. Therefore, the primary purpose of this study was to assess physiological responses to 10-week BMT in Estonian conscripts. Materials and methods: The protocol of the study confirmed to the standards set by the Declaration of Helsinki and it was approved by the Research Ethics Committee of the University of Tartu. Mean  $\pm$  SD age and body mass index of 94 conscripts studied was  $20.9 \pm 1.7$  years and  $24.2 \pm 3.0$  kg·m<sup>-2</sup>, respectively. Fasting venous blood analysis was performed four times during BMT (October to December) and once 15 weeks after the end of BMT (in March). One-factor (time) repeated measures analysis of variance was used to evaluate the differences within the variables. Statistical significance was set at  $p < 0.05$ . Where a significant main effect was observed, Tukey's honesty significant difference post-hoc analysis was used to locate differences between the means. A Pearson product moment coefficient of correlation ( $r$ ) with  $\alpha$  level set at 0.05 was applied to determine the relationship between variables. Results: Significant increases in serum testosterone concentration (60.6%), testosterone to cortisol ratio (61.1%), blood erythrocyte count (4.3%), hemoglobin concentration (3.8%) and hematocrit (2.2%), and decrease in serum ferritin concentration (39.3%) occurred between weeks 1 and 10 during BMT (in all cases  $p < 0.0001$ ). Fifteen weeks later, these parameters were still at increased or decreased levels, respectively, compared to week 1. The prevalence of vitamin D deficiency (serum 25(OH) D concentration  $< 50$  nmol·L<sup>-1</sup>) increased from 42.6% in week 1 to 80.8% in week 10 and to 91.5% 15 weeks later. Serum 25(OH)D levels did not correlate with testosterone concentrations ( $r = 0.062$ ,  $p = 0.552$  in Wk-1 and  $r = -0.079$ ,  $p = 0.448$  in Wk-25). Conclusion: These findings suggest that BMT induces anabolic physiological adaptations in conscripts despite vitamin D deficiency and decrease in iron status. However, high prevalence of vitamin D deficiency and decline in iron status may limit physiological adaptations and improvement in physical work capacity to a suboptimal level. Furthermore, as vitamin D influences a variety of functions important for health, deficiency in conscripts should be considered a major concern that needs treatment. An acknowledged limitation of the study is the lack of a control group of conscripts possessing normal vitamin D status and stable serum ferritin levels throughout the study period. Nevertheless, the research design employed enabled to determine two factors that potentially limit physiological adaptability of conscripts to military training loads in ecologically authentic environment.

## INTRODUCTION

Estonian Defense Forces are drawn up on the basis of the conscription model. Military service is compulsory for all

male citizens over 18 years of age and starting in 2013 female Estonian citizens can also complete military service on a voluntary basis. According to the National Defense Development Plan for the period of 2013 to 2022, in order to ensure acceptable operational capacity of reserve units and manning professional units, 3,200 conscripts must be trained annually. Implementation of this plan is complicated owing to small and decreasing population of the country<sup>1</sup> and considerable numbers of young men who do not qualify for military service or who are prematurely discharged from military service for medical reasons.<sup>2</sup>

Causes for the premature dropout of conscripts from military service have not been systematically studied in Estonia. However, one of the factors involved could be relatively demanding physical training that starts at the beginning of military service. Daily work-related physical activity in

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61.4% of Estonian men in the 16 to 24 years age group is low or very low and 77.6% of them are involved in leisure time physical activity lasting >30 minutes only 2 to 3 times a week or much more rarely.<sup>3</sup> Therefore, the majority of the conscripts are likely to receive their first experience of demanding systematic physical exercise during basic military training (BMT) where the training load may exceed their previous training level. Furthermore, conscripts entering military service are intensively exposed to nontraining stress because of the totally new environment and the change in their working and living conditions.<sup>4</sup> Thus, cumulative training and nontraining stresses experienced by conscripts during BMT may exceed their physiological adaptability and increase the probability of becoming prematurely discharged from military service.

Therefore, the primary purpose of this study was to assess physiological responses to 10-week BMT in Estonian conscripts. The assessment was performed on the basis of the longitudinal monitoring of blood biochemical and hematological parameters.

## METHODS

The protocol of this longitudinal study confirmed to the standards set by the Declaration of Helsinki and it was approved by the Research Ethics Committee of the University of Tartu. A total of 109 conscripts out of 407 freshmen who started military service in the Kuperjanov Single Infantry Battalion at the beginning of October 2014 volunteered for this study and gave written informed consent. Three conscripts withdrew from participating shortly after signing the informed consent. Four conscripts participated in the study only episodically as a result of temporary illnesses and eight were prematurely discharged from military service. The mean ( $\pm$ SD) age, height, body mass, and body mass index (BMI) of the 94 participants whose complete set of data was successfully collected and included into the final analysis was  $20.9 \pm 1.7$  years,  $182.7 \pm 6.3$  cm,  $80.7 \pm 11.2$  kg, and  $24.2 \pm 3.0$  kg·m<sup>-2</sup>, respectively.

All the participants underwent 10-week BMT according to the standard program established by the Command of the Estonian Defense Forces. Food and water intake were in accordance with the standard army meal, the conscripts slept in dormitory-type rooms and the average sleeping time was 8 hours. During BMT, conscripts were daily involved in physically demanding activities like marching drills, combat training, and sport training. Conscripts passed three field camps involving overnight exercises. In addition, the BMT schedule included altogether eight longer marching exercises (from 8 to 30 km), five of which took place during field camps. During marching and combat training, the conscripts usually wore combat clothing and carry battle gear the weight of which was 10 to 11.5 kg. However, during longer marching exercises, part of the distance was covered wearing a kitbag (20–23 kg) in addition to the usual equipment. Rifle marksmanship was performed twice a week through-

out the BMT period. The cumulative physical training load increased gradually from the beginning of BMT and reached the highest level during the latest 2 weeks.

## Blood Sampling

Four venous blood samples were taken during and immediately after 10-week BMT, particularly 1 (Wk-1), 2 (Wk-2), 6 (Wk-6), and 10 (Wk-10) weeks after the beginning of BMT. An additional blood sample was drawn 15 weeks after the end of BMT, i.e., 25 weeks after the beginning (Wk-25). Each time, blood samples were taken from the antecubital vein on Monday morning, i.e., following 2 resting days, after approximately 12 hours overnight fast. Blood was collected into a 3-mL Vacutainer plastic whole blood K<sub>2</sub>EDTA tube and into 3.5-mL and 5-mL BD Vacutainer SST II Plus plastic serum tubes (Becton, Dickinson & Co, Franklin Lakes, New Jersey).

## Laboratory measures

Hematology analysis was performed from homogenized whole blood collected into EDTA-containing tubes using fluorescence flow cytometry (Sysmex XE2100D, Sysmex Corporation, Kobe, Japan). Serum urea concentration was measured using spectrophotometry (Siemens ADVIA 1800, Siemens Healthcare GmbH, Erlangen, Germany). Chemiluminescent immunoassay method was used for measurement of serum ferritin, testosterone (Siemens Centaur XP, Siemens Healthcare GmbH, Erlangen, Germany), cortisol (Immulite 2000 XP, Siemens Healthcare GmbH, Erlangen, Germany), and 25(OH)D (Liaison XL, DiaSorin S.p.A, Saluggia VC, Italy) concentrations. Serum C-reactive protein (S-CRP) concentration was determined by latex enhanced immunoturbidimetric method (Siemens ADVIA 1800, Siemens Healthcare GmbH, Erlangen, Germany).

Criteria applied for identifying iron deficiency and iron deficiency anemia were serum ferritin levels  $\leq 35$   $\mu\text{g}\cdot\text{L}^{-1}$  and blood hemoglobin concentrations  $< 120$   $\text{g}\cdot\text{L}^{-1}$ , respectively.<sup>5,6</sup> Participants exhibiting serum ferritin levels  $\leq 35$   $\mu\text{g}\cdot\text{L}^{-1}$  with blood hemoglobin concentrations  $> 120$   $\text{g}\cdot\text{L}^{-1}$  were classified as being iron deficient nonanemic.<sup>5,6</sup>

Regarding vitamin D status, the participants were classified as being vitamin D sufficient (serum 25(OH)D concentrations  $\geq 75$   $\text{nmol}\cdot\text{L}^{-1}$ ), insufficient (serum 25(OH)D concentrations  $< 75$   $\text{nmol}\cdot\text{L}^{-1}$ , but  $\geq 50$   $\text{nmol}\cdot\text{L}^{-1}$ ), or deficient (serum 25(OH)D concentrations  $< 50$   $\text{nmol}\cdot\text{L}^{-1}$ ). Similar criteria for classifying vitamin D status have been previously employed in military environment by Funderburk et al.<sup>7</sup> The cut-off value used for identifying vitamin D deficiency was that recommended by the Endocrine Society.<sup>8</sup>

## Statistical Analysis

The Statistica 12 software was used for performing statistical analysis. Data are presented as mean  $\pm$  SD. Normality of all data sets was examined using the Kolmogorov-Smirnov test.

Statistical significance was set at  $p < 0.05$ . One-factor (time) repeated measures analysis of variance was used to evaluate the differences within the variables. Where a significant main effect was observed, Tukey's honest significant difference post-hoc analysis was used to locate differences between the means. A Pearson product moment coefficient of correlation ( $r$ ) with alpha level set at 0.05 was applied to determine the relationship between variables.

## RESULTS

### Body Mass and BMI

Body mass and BMI of conscripts remained stable throughout the study period (Table I).

### Serum Testosterone and Cortisol Concentrations

Rapid 33.6% increase in serum testosterone concentration occurred at the beginning of BMT between Wk-1 and Wk-2 ( $p < 0.0001$ ; Fig. 1A). Further increase in serum testosterone levels occurred at the end of BMT between Wk-6 and Wk-10 ( $p < 0.0001$ ) and within 15 weeks after the end of BMT, i.e., between Wk-10 and Wk-25 ( $p = 0.002$ ). In Wk-25 serum testosterone concentration exceeded that observed in Wk-1 by 72.9% ( $p < 0.0001$ ).

Compared to Wk-1, decreased serum cortisol concentrations occurred during the later stages of BMT, i.e., in Wk-6 ( $-8.4\%$ ;  $p = 0.0002$ ) and Wk-10 ( $-6.9\%$ ;  $p = 0.004$ ; Fig. 1B). Then, within 15 weeks after the end of BMT, serum cortisol returned to the level observed in Wk-1.

Serum testosterone–cortisol ratio (TCR) increased rapidly at the beginning of BMT between Wk-1 and Wk-2 ( $p < 0.0001$ ; Fig. 1C). Further increases were evident between Wk-2 and Wk-6 ( $15.1\%$ ;  $p = 0.0004$ ) as well as between Wk-6 and Wk-10 ( $13.2\%$ ;  $p = 0.018$ ). Within 15 weeks after the end of BMT serum TCR did not change any more and remained elevated in comparison with Wk-1 ( $p < 0.0001$ ).

### Serum Ferritin and Blood Hemoglobin Concentrations and Hematocrit

Rapid decrease ( $26.5\%$ ;  $p < 0.0001$ ) in serum ferritin concentration occurred at the beginning of BMT between Wk-1 and Wk-2 (Fig. 2A). Following the initial decrease, ferritin levels remained 30.0 to 42.6% ( $p < 0.0001$ ) lower compared to Wk-1 throughout the rest of the study period. Nevertheless, within 15 weeks after the end of BMT, i.e., between Wk-10 and Wk-25, a moderate ( $15.2\%$ ) but statistically sig-

nificant ( $p = 0.002$ ) increase in serum ferritin level was observed. In Wk-1, nine participants ( $9.6\%$ ) were classified as iron deficient on the basis of having serum ferritin levels  $\leq 35 \mu\text{g}\cdot\text{L}^{-1}$ . The prevalence of iron deficiency increased to 28.7% (27 participants) in Wk-6 and decreased thereafter to 17.0 and 18.1% (16–17 participants) in weeks 10 and 25, respectively. In seven participants ( $7.4\%$ ), persistent state of iron deficiency was identified throughout the study period.

Blood hemoglobin concentrations gradually increased during BMT between Wk-1 and Wk-10 and then remained stable for 15 weeks after the end of BT (Fig. 2B). In Wk-10, an average hemoglobin level was  $5.6 \text{g}\cdot\text{L}^{-1}$  ( $3.8\%$ ) higher ( $p < 0.0001$ ) than in Wk-1. Anemia did not occur since in all participants blood hemoglobin concentrations exceeded the level of  $120 \text{g}\cdot\text{L}^{-1}$  throughout the study period. However, seven participants ( $7.4\%$ ) steadily were classified as being iron deficient nonanemic on the basis of having serum ferritin levels  $\leq 35 \mu\text{g}\cdot\text{L}^{-1}$  and hemoglobin concentrations  $> 120 \text{g}\cdot\text{L}^{-1}$ .

Hematocrit values rapidly decreased at the beginning of BMT between Wk-1 and Wk-2 ( $2.0\%$ ;  $p < 0.0001$ ; Fig. 2C), but then gradually increased and exceeded the Wk-1 level ( $2.2\%$ ;  $p < 0.0001$ ) at the end of BMT (Wk-10). Within 15 weeks after the end of BT hematocrit remained stable.

### Serum Urea and S-CRP Concentrations

Serum urea concentrations were decreased ( $p = 0.0001$ ) in Wk-6 and increased in Wk-25 ( $p = 0.005$ ) in comparison with Wk-1 (Table II). S-CRP concentrations were decreased in Wk-6 ( $p = 0.007$ ) and Wk-25 ( $p = 0.006$ ) in comparison with Wk-1 (Table II).

### Blood Cell Counts

Erythrocyte counts gradually increased during BMT between Wk-1 and Wk-10 ( $4.3\%$ ;  $p < 0.0001$ ) and then remained stable within 15 weeks after the end of BMT (Table III).

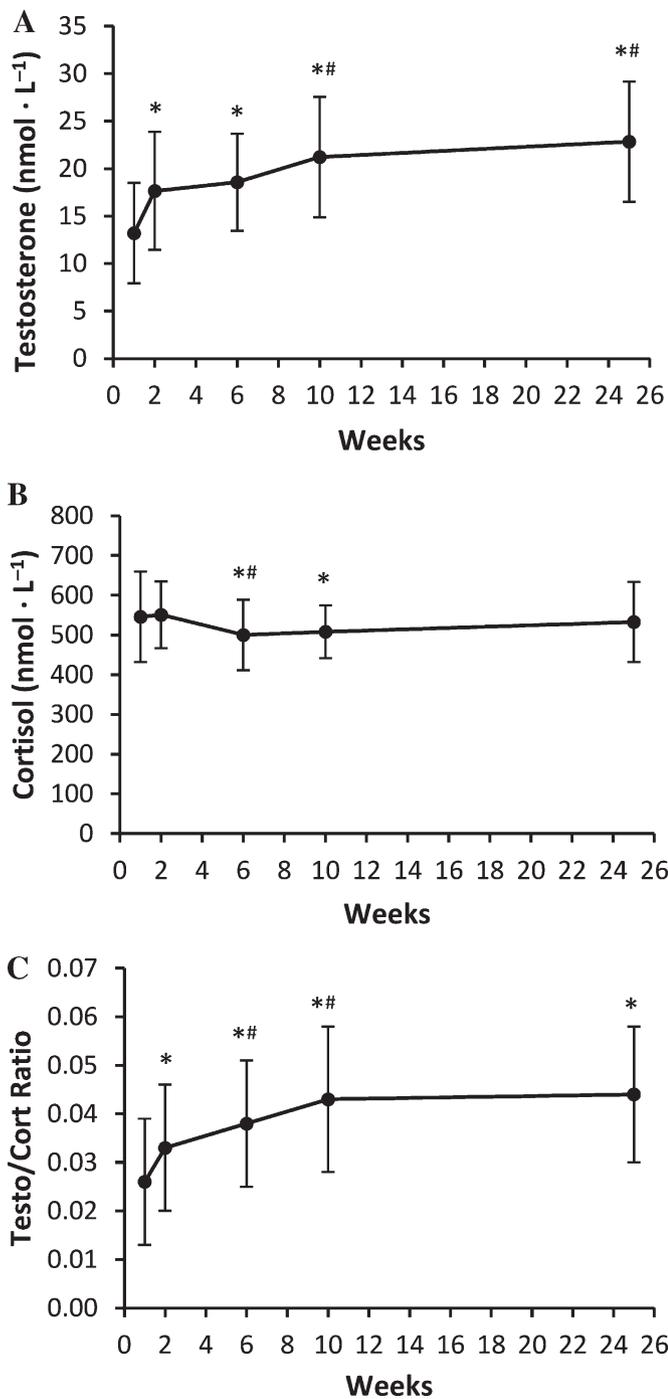
Leukocyte count was only elevated in comparison with Wk-1 at the end of BMT (Wk-10) ( $8.1\%$ ;  $p = 0.022$ ; Table III). Within 15 weeks after the end of BMT, leukocyte count returned to the level observed in Wk-1.

Rapid increase in platelet count occurred at the beginning of BMT between Wk-1 and Wk-2 ( $9.2\%$ ;  $p < 0.0001$ ; Table III). Platelet count peaked in Wk-10 ( $12.9\%$  greater than in Wk-1;  $p < 0.0001$ ) and remained elevated relative to Wk-1 throughout the rest of the study period despite  $6.5\%$  ( $p = 0.0003$ ) decrease between Wk-10 and Wk-25.

TABLE I. Body Mass and Body Mass Index

Variable	Weeks				
	Week-1	Week-2	Week-6	Week-10	Week-25
Body Mass (kg)	80.7 ± 11.2	80.6 ± 10.7	80.7 ± 9.5	80.0 ± 9.2	80.0 ± 9.0
BMI ( $\text{kg}\cdot\text{m}^{-2}$ )	24.2 ± 3.0	24.1 ± 2.9	24.2 ± 2.6	24.0 ± 2.5	24.0 ± 2.4

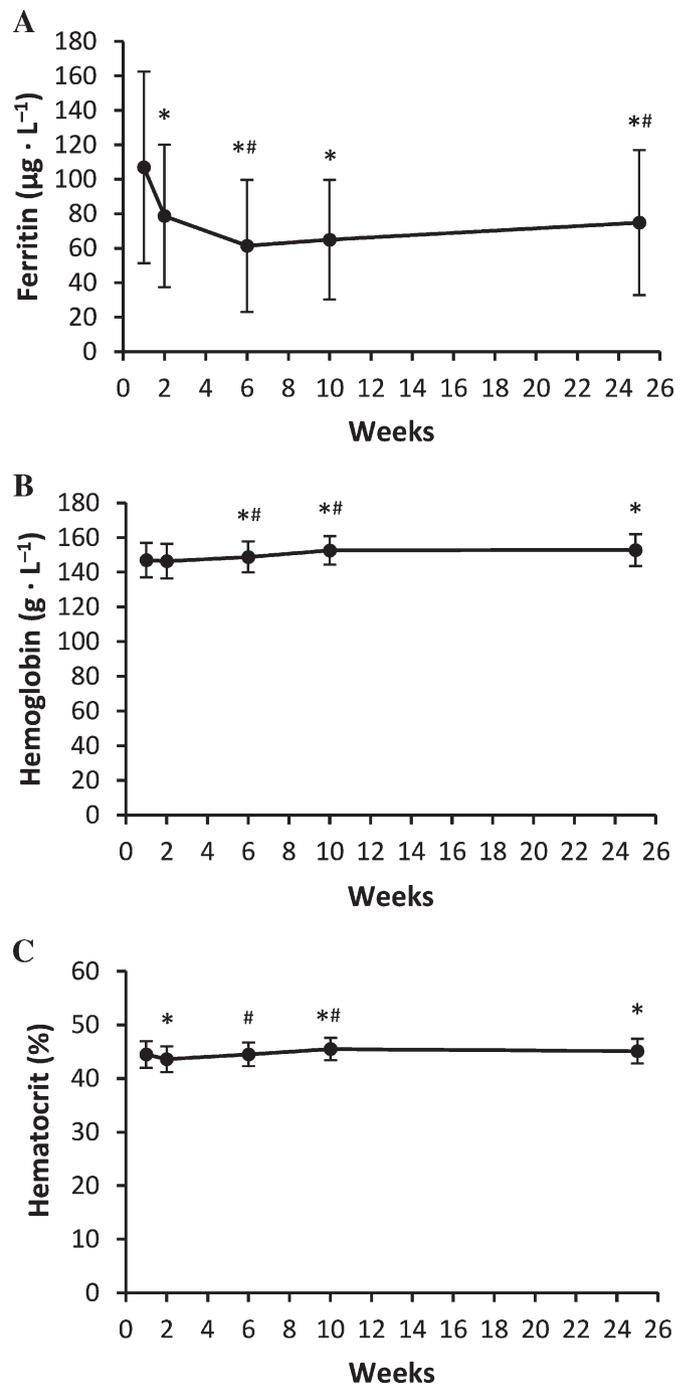
Data are presented as mean ± SD. BMI, body mass index; Week-1–Week-10, BMT; Week-25, 15 weeks after the end of BMT.



**FIGURE 1.** Serum testosterone (A) and cortisol (B) concentrations and testosterone to cortisol ratio (C) during BMT (weeks 1–10) and 15 weeks after the end of BMT (week 25). Data are presented as mean  $\pm$  SD. Significantly different ( $p \leq 0.05$ ): \* from week 1; # from previous time point.

### Serum Vitamin D Concentrations

At the beginning of BMT in Wk-1 serum vitamin D concentration was  $58.1 \pm 20.9$  nmol·L<sup>-1</sup> (Fig. 3). Gradual decrease in serum vitamin D levels occurred throughout the study period with the lowest value observed 15 weeks after the



**FIGURE 2.** Serum ferritin (A) and blood hemoglobin (B) concentrations and hematocrit (C) during BMT (weeks 1–10) and 15 weeks after the end of BMT (week 25). Data are presented as mean  $\pm$  SD. Significantly different ( $p \leq 0.05$ ): \* from week 1; # from previous time point.

end of BMT, i.e., in Wk-25 ( $30.1 \pm 11.8$  nmol·L<sup>-1</sup>). In Wk-1, 42.6% of the participants ( $n = 40$ ) were classified as vitamin D deficient on the basis of having serum 25(OH)D concentrations  $<50$  nmol·L<sup>-1</sup>, whereas in Wk-25 the prevalence of vitamin D deficiency had increased to 91.5% ( $n = 86$ ). The number of participants exhibiting vitamin D sufficiency

**TABLE II.** Serum Urea and C-reactive Protein Concentrations

Variable	Weeks				
	Week-1	Week-2	Week-6	Week-10	Week-25
Urea (mmol·L <sup>-1</sup> )	5.26 ± 1.02	5.52 ± 1.05	4.80 ± 1.00 <sup>a,b</sup>	5.55 ± 1.16 <sup>b</sup>	5.63 ± 1.12 <sup>a</sup>
CRP (mg·L <sup>-1</sup> )	4.55 ± 12.87	2.60 ± 4.14	1.30 ± 3.40 <sup>a</sup>	2.98 ± 6.18	1.28 ± 2.56 <sup>a</sup>

Data are presented as mean ± SD. CRP, C-reactive protein; Week-1–Week-10, BMT; Week-25, 15 weeks after the end of BMT. Significantly different ( $p \leq 0.05$ ): <sup>a</sup>from Week-1; <sup>b</sup>from previous time point.

(serum 25(OH)D concentrations  $\geq 75$  nmol·L<sup>-1</sup>) in Wk-1 was 19 (20.2%), but none of the participants met this criterion in Wk-25. Serum 25(OH)D levels did not correlate with testosterone concentrations ( $r = 0.062$ ,  $p = 0.552$  in Wk-1 and  $r = -0.079$ ,  $p = 0.448$  in Wk-25).

## DISCUSSION

The main findings of this study were: (1) shift toward higher anabolic hormonal state during BMT; (2) decline in iron status during BMT; and (3) high prevalence of vitamin D deficiency that occurred at the beginning of BMT and further increased throughout the whole study period.

Serum TCR that is considered to reflect the dynamic balance between anabolic and catabolic processes in the body<sup>9–11</sup> may be used as a marker of the actual physiological strain of exercise training.<sup>12</sup> Recent studies in elite canoe polo athletes<sup>13</sup> and trained wrestlers<sup>14</sup> revealed that 3- to 4-week training program modifications, which induced an increase in resting TCR also provoked improvements in various performance parameters. On the contrary, in participants who followed routine training program for the same period, both TCR and performance parameters remained unchanged.<sup>13,14</sup> Regarding military environment, the findings of several studies<sup>4,15,16</sup> suggest that monitoring TCR may be a useful tool for detecting too heavy military training loads which may lead to maladaptation. In Spanish special military unit recruits a decline in the TCR  $\geq 30\%$  occurred in association with a decrease in physical performance during 8-week demanding military training program.<sup>15</sup> Similarly, in soldiers involved in 8-week U.S. army ranger training course that induced marked decreases in body mass and various indices of physical work capacity, significant decreases and increases in circulating testosterone and cortisol, respectively, were observed.<sup>16</sup> Finnish conscripts, classi-

fied as overreached on the basis of five clearly defined criteria, exhibited no increase in TCR, whereas in their counterparts, classified as nonoverreached, TCR increased during 8-week BMT.<sup>4</sup> In light of the data referred to above,<sup>4,13,14</sup> rapid increase in resting serum TCR at the beginning of BMT, further elevation during later stages of BMT and maintenance of the elevated level during 15 weeks after the end of BMT suggest sustained anabolic adaptations in our participants.

In the current study, the elevated TCR mainly arose from 34 to 73% increases in serum testosterone concentrations. Relatively small (7–8%) decreases in cortisol levels only occurred in Wk-6 and Wk-10. Similar findings, i.e., occurrence of markedly elevated resting testosterone concentrations in combination with a small decrease<sup>14</sup> or no changes<sup>13,17,18</sup> in cortisol levels have been reported in well-trained athletes during periods of increased training stress. Similar hormonal responses to BMT in our participants and to periods of markedly increased training loads in well-trained athletes suggest that physical training involved in BMT acts as a strong stimulus inducing marked anabolic adaptations in conscripts.

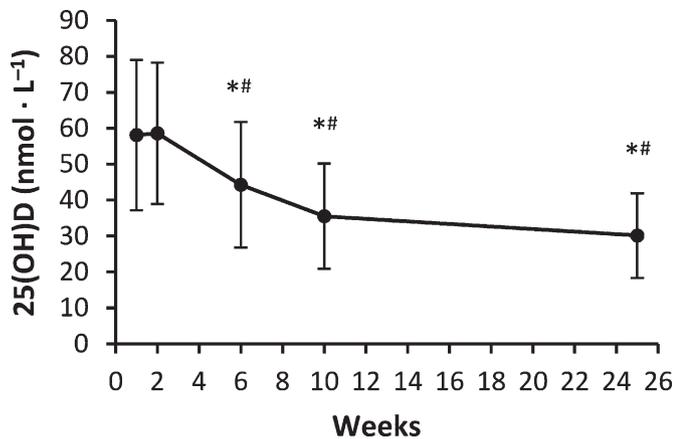
From the perspective of biochemical monitoring of sports training, morning serum urea levels  $<7.5$  mmol·L<sup>-1</sup> are considered the index of optimal recovery from preceding training loads.<sup>19</sup> Monday morning serum urea concentrations steadily remained below 7.5 mmol·L<sup>-1</sup> in our participants, suggesting that they started each consecutive week of BMT being in well recovered state.

Positive associations have been observed between serum testosterone and 25(OH)D levels in elderly men.<sup>20,21</sup> Unlike for 25(OH)D, Nimptsh et al<sup>21</sup> did not find any seasonal variation in testosterone levels, whereas Wehr et al<sup>20</sup> reported that 25(OH)D and testosterone followed similar seasonal

**TABLE III.** Erythrocyte, Leukocyte, and Platelet Counts

Variable	Weeks				
	Week-1	Week-2	Week-6	Week-10	Week-25
Erythrocyte ( $\times 10^{12}$ ·L <sup>-1</sup> )	4.93 ± 0.32	4.90 ± 0.29	4.99 ± 0.27 <sup>a,b</sup>	5.14 ± 0.29 <sup>a,b</sup>	5.19 ± 0.29 <sup>a</sup>
Leucocyte ( $\times 10^9$ ·L <sup>-1</sup> )	6.75 ± 1.85	6.86 ± 1.99	6.82 ± 1.34	7.30 ± 1.75 <sup>a</sup>	6.79 ± 1.54 <sup>b</sup>
Platelet ( $\times 10^9$ ·L <sup>-1</sup> )	236.7 ± 51.0	258.5 ± 57.2 <sup>a</sup>	262.1 ± 51.0 <sup>a</sup>	267.3 ± 50.5 <sup>a</sup>	250.0 ± 50.0 <sup>a,b</sup>

Data are presented as mean ± SD. Week-1–Week-10, BMT; Week-25, 15 weeks after the end of BMT. Significantly different ( $p \leq 0.05$ ): <sup>a</sup>from Week-1; <sup>b</sup>from previous time point.



**FIGURE 3.** Serum 25(OH)D concentration during BMT (weeks 1–10) and 15 weeks after the end of BMT (week 25). Data are presented as mean  $\pm$  SD. Significantly different ( $p \leq 0.05$ ): \* from week 1; # from previous time point.

pattern. Furthermore, Pilz et al<sup>22</sup> demonstrated significant increases in serum testosterone levels concomitant with increases in 25(OH)D concentrations as a result of vitamin D supplementation in healthy middle-aged overweight men undergoing a weight reduction program. In light of these data,<sup>20–22</sup> it is noteworthy that there was no association between testosterone and 25(OH)D levels in our participants. Furthermore, marked increases in serum testosterone concentrations occurred despite significant decreases in 25(OH)D levels across the study period. Thus, it appears that positive association exists between serum testosterone and 25(OH)D levels in elderly and middle aged,<sup>20–22</sup> but not in young men (current study). Furthermore, low and continually declining vitamin D status does not prevent occurrence of anabolic adaptations in conscripts involved in BMT in autumn and winter seasons. Nevertheless, within limitations of the current study, it remains obscure whether observed anabolic adaptations would have been more pronounced in vitamin D sufficient conscripts or not.

Testosterone stimulates erythropoiesis directly and by stimulating erythropoietin secretion,<sup>23</sup> which consequently leads to increases in hemoglobin concentrations and hematocrit levels. Therefore, increases in erythrocyte counts, hematocrit levels, and hemoglobin concentrations that occurred during BMT and maintenance of elevated levels of these parameters for the subsequent 15 weeks may be induced by marked increases in testosterone levels in our participants. The irreplaceable role of hemoglobin in oxygen delivery to tissues makes it an important physiological determinant of physical work capacity.<sup>24</sup> Enoki et al<sup>25</sup> have demonstrated that increases in testosterone levels, by up-regulating the function of lactate transporter proteins in skeletal muscle, facilitate the oxidation of lactate during exercise. Thus, increases in testosterone levels observed in our participants may have induced adaptations in both oxygen delivery system and skeletal muscle oxidative metabolism.

Platelet count increased gradually and peaked at the end of BMT at 13% higher level than that observed in Wk-1. Considering that Singh et al<sup>26</sup> have reported 13% higher platelet numbers in well-trained male cyclists compared to untrained young men, increased platelet counts in our participants may represent adaptations to training loads concomitant with BMT.

Iron, being a constituent of oxygen carrying proteins and oxidative enzymes, is vital for energy metabolism. Ferritin is considered the major iron storage protein<sup>27</sup> and serum ferritin concentration correlates with body iron stores<sup>28</sup>. In our participants, marked decrease in serum ferritin concentrations occurred during BMT. Similar findings, i.e., a decrease in serum ferritin levels indicating degraded iron status, have been reported by other researchers in both male and female soldiers during military training.<sup>29–31</sup> In our participants as well as in female soldiers studied by McClung et al,<sup>30</sup> a decrease in serum ferritin levels was coupled with an increase in blood hemoglobin concentrations. Such a pattern of changes in these two parameters may reflect an increased mobilization of stored iron for synthesis of hemoglobin. However, others<sup>29,31</sup> reported decreases in both ferritin and hemoglobin levels. Interestingly, Yanovich et al<sup>31</sup> reported significant decline in iron status during 10-week basic combat training course despite daily iron intake as high as 188% of the Recommended Dietary Allowance at the beginning of the course and further significant increases in iron intake during the course in male soldiers. These findings of Yanovich et al<sup>31</sup> suggest that activities associated with military training may contribute to decline in iron status to a greater extent than nutritional factors. Potential factor that may link increased training load concomitant with BMT with decreases in iron status is an inflammatory response to exercise. According to Gaffney-Stomberg and McClung,<sup>32</sup> inflammation associated with exercise induces increases in hepcidin levels which may lead to sequestering of iron in enterocytes and macrophages. However, S-CRP data and leukocyte counts do not indicate the occurrence of inflammatory processes in our participants.

Seven participants were steadily classified as being iron deficient nonanemic during the study period. Although the negative impact of anemia, defined as subnormal blood hemoglobin concentration, on physical work capacity is well established,<sup>24,33</sup> the influence of iron deficiency without anemia on performance is less clear<sup>34,35</sup>. Nevertheless, recent meta-analysis<sup>6</sup> confirmed that iron treatments improve both iron status and maximal oxygen uptake in iron deficient nonanemic endurance athletes. This finding is strong evidence suggesting that iron deficiency without anemia may have detrimental impact on physical work capacity. Furthermore, marginal iron deficiency without anemia has been shown to impair physiological adaptations to aerobic training in previously untrained women,<sup>36,37</sup> whereas increased maximal oxygen uptake<sup>38</sup> and improved 15-km running performance have been demonstrated as a result of oral iron

supplementation in iron deficient nonanemic athletic subjects.<sup>39</sup> Thus, decline in iron status indicated by decreased serum ferritin levels may limit physiological adaptations and improvement in physical work capacity during BMT in conscripts.

Marked 48.2% decrease in serum 25(OH)D concentrations that occurred in our participants since the beginning of October (commencement of BMT) until the second half of March (15 weeks after the end of BMT) is consistent with the seasonal variations in vitamin D status. Such a variation has been brilliantly demonstrated in a population-based study carried out in Great Britain by Hyppönen and Power.<sup>40</sup> Estonia is located in northern latitudes similar to that of the northern part of Great Britain. The seasonal variation in vitamin D status in Estonian population has been described by Kull et al<sup>41</sup> who reported an increase in the prevalence of suboptimal vitamin D status (defined as serum 25(OH)D concentration  $<75$  nmol·L<sup>-1</sup>) from 87% in September to 97% during winter months (January–March). According to the same criterion, suboptimal vitamin D status occurred in 77.7 and 100% of our participants at the beginning of October and in the second half of March, respectively. During the same time interval, the prevalence of vitamin D deficiency (defined as serum 25(OH)D concentration  $< 50$  nmol·L<sup>-1</sup>) increased from 42.5 to 91.5% in our participants.

Considering the importance of vitamin D for bone health,<sup>42</sup> normal immune,<sup>43</sup> cardiovascular,<sup>44</sup> lung,<sup>45</sup> and skeletal muscle function,<sup>46,47</sup> high prevalence of vitamin D deficiency among conscripts should be treated as a major concern. Ruohola et al<sup>48</sup> have demonstrated positive correlation between low vitamin D status and occurrence of bone stress fractures during military training in Finnish male conscripts. Similarly, Burgi et al<sup>49</sup> reported inverse dose-response gradient between serum 25(OH)D levels and risk of stress fracture in U.S. navy female recruits. Regarding physical work capacity, low vitamin D status has been shown to be associated with reduced muscle strength<sup>50</sup> and peak torque<sup>51</sup> in highly trained male athletes and adolescent girls, respectively. Positive correlation have been demonstrated between vitamin D status and maximal oxygen consumption,<sup>52–54</sup> although this has not been a consistent finding<sup>55,56</sup>. Vitamin D supplementation has been shown to increase 10-m sprint time and vertical jump height in young-trained athletes exhibiting low serum 25(OH)D levels before supplementation,<sup>57</sup> but in athletes with higher baseline vitamin D status (mean serum 25(OH)D levels 51–53 nmol·L<sup>-1</sup>) sprint performance or indices of leg musculature strength and power did not change following supplementation despite significant increases in serum 25(OH)D concentrations<sup>58</sup>. However, Barker et al<sup>59</sup> reported that vitamin D supplementation enhanced recovery of skeletal muscle strength following intense exercise in active adults with a sufficient vitamin D status before supplementation. Thus, although the available data regarding the influence of vitamin D status on

physical work capacity are not fully conclusive, they still suggest that high prevalence of vitamin D deficiency may limit physiological adaptations and improvement in performance in conscripts during BMT. Therefore, further study is imperative to determine the potential impact of vitamin D status on physiological adaptations and physical work capacity in conscripts.

An acknowledged limitation of the study is the lack of a control group of conscripts possessing normal vitamin D status and stable serum ferritin levels throughout the study period. Nevertheless, the research design employed in this study enabled to determine two factors that potentially limit physiological adaptability of conscripts to military training loads in ecologically authentic environment.

## CONCLUSION

The findings of this study reveal occurrence of anabolic adaptations in male Estonian conscripts during BMT carried out in autumn and winter seasons. High prevalence of vitamin D deficiency in autumn–winter period is of major concern that needs treatment through elaboration of practical measures for improving vitamin D status in conscripts. Declining iron status and approximately 7% prevalence of iron deficient nonanemic state among conscripts warrant further studies which should employ methods enabling more detailed assessment of body iron stores and address the mechanism(s) responsible for the decline in iron status in conscripts during BMT.

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