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34 ABSTRACT

Changes in physiological markers and physical performance in relation to paratroopers' initial body 35 36 composition were investigated during a 20-day winter military field training (MFT) and the 37 subsequent 10-day recovery period. Body composition, serum hormone concentrations and enzymatic 38 biomarkers, and physical performance of 58 soldiers were measured before, during, and after MFT. 39 Comparisons were done according to soldiers' body fat percentage before MFT between low-fat (<12% body fat) and high-fat (>12% body fat) groups. Correlations between body fat percentage 40 preceding MFT and changes in muscle mass, physical performance, and serum hormone 41 42 concentrations and enzymatic biomarkers were investigated. It was hypothesized that soldiers with a 43 higher fat percentage would have smaller decrements in muscle mass, physical performance, and serum testosterone concentration. The change in muscle and fat mass was different between groups 44 (p<0.001) as the low-fat group lost 0.8 kg of muscle mass and 2.0 kg of fat mass, while there was no 45 46 change in muscle mass and a loss of 3.7 kg of fat mass in the high-fat group during MFT. Fat 47 percentage before MFE correlated with the changes in muscle mass ($R^2=0.26$, p<0.001), serum testosterone concentration ($R^2=0.22$, p<0.001), and evacuation test time ($R^2=0.10$, p<0.05) during 48 49 MFT. The change in muscle mass was correlated with the changes in evacuation test time ($R^2=0.11$). 50 p<0.05) and countermovement jump test results (R²=0.13, p<0.01) during MFT. Soldiers with a higher 51 initial fat percentage lost less muscle mass, had smaller decrements in some aspects of physical 52 performance, as well as in serum testosterone concentration during MFT.

Keywords: body composition, military field training, strength performance, anaerobic performance, hormonal changes

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58 Environmental extremes are an important part of the operative stressors soldiers need to be prepared 59 for when optimizing performance for military operation (Nindl et al. 2013). High physical performance is especially important for special operations force (SOF) soldiers who face demanding 60 tasks in these challenging conditions. The demands of cold environment in combination with the 61 62 physical demands of the mission often lead to negative energy balance (Castellani et al. 2015, Ahmed 63 et al. 2020). Consequently, previous research has shown that military field training (MFT) lasting 64 several days is characterized by the loss of body weight, including both fat and muscle mass (Gan et 65 al. 2021, Vikmoen et al. 2020, Hamarsland et al 2018, Nindl et al. 2007, Johnson et al. 1994), the decrease in physical performance (Gan et al. 2021, Vikmoen et al. 2020, Hamarsland et al 2018, Nindl 66 et al. 2007, Johnson et al. 1994, Hoyt et al. 2006), and the changes in hormonal biomarkers such as 67 testosterone and cortisol (Øfstengin et al. 2020, Kyröläinen et al. 2007, Nindl et al. 2007). While lower 68 fat mass has been associated with better performance in several military tasks (Pihlainen et al. 2018, 69 70 Lyons et al. 2005), it has been shown that a higher fat percentage can attenuate the loss of muscle 71 mass during prolonged MFT (Gan et al. 2021, Vikmoen et al. 2020, Hamarsland et al. 2018). This 72 could be explained by the higher rate of fat oxidation per kilogram of fat free mass observed in individuals with larger quantities of fat mass, as this fat-predominant energy metabolism would 73 74 decrease the need to oxidize body's protein reserves during energy deficit (Hoyt et al. 2006). As 75 individuals with a higher fat percentage at the beginning of MFT may be less prone to the loss of 76 muscle mass, it is possible that this could also lead to smaller decrements in physical performance. 77 However, the evidence for this is controversial as there are findings supporting both a relationship 78 between muscle mass loss and a decrease in physical performance (Nindl et al. 2007, Johnson et al. 79 1994) as well as support for the null hypothesis (Vikmoen et al. 2020, Hamarsland et al. 2018). 80 Furthermore, the direct relationship between soldiers' fat percentage preceding MFT and changes in 81 physical performance have not been previously studied.

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In addition to the decrease in physical performance, the decrease in serum testosterone and the 83 84 increase in serum cortisol are common findings in soldiers participating in MFT (Øfstengin et al. 85 2020, Hamarsland et al. 2018, Szivak et al. 2018, Kyröläinen et al. 2007, Nindl et al. 2007). 86 Interestingly there also seems to be a strong relationship between soldiers' fat percentage or fat mass 87 preceding MFT and the change in serum testosterone as leaner soldiers seem to have a greater 88 reduction in serum testosterone concentrations (Berryman et al. 2022). It has also been suggested that 89 a relationship exists between the change in serum testosterone concentration and the change in muscle 90 mass during MFT (Berryman et al. 2022, Hamarsland et al. 2018) and that interventions attenuating 91 decrements in serum testosterone concentrations could possibly be used to reduce adverse changes in 92 muscle mass and physical performance (Berryman et al. 2022).

94 As the energy deficit, sleep deprivation, physical strain, and mental stress experienced during MFT 95 can cause a wide range of negative changes in body composition, physical performance, and hormone concentrations, the recovery from strenuous MFT has been a topic of interest in the literature. The 96 97 time span for the recovery of physical performance seems to be longer than that of hormone 98 concentrations or body composition. While hormone concentrations and body composition changes 99 are usually recovered during the first week of recovery, some aspects of physical performance can 100 take longer than two weeks to recover (Vikmoen et al. 2020, Øfstengin et al. 2020, Hamarsland et al. 101 2018). Although knowledge about soldiers' recovery after MFT has increased in the recent years, it is 102 uncertain if body composition plays a role in the time span of the recovery process.

While there seems to be a relationship between fat percentage and change in muscle mass and serum testosterone concentrations during MFT, their subsequent relationship with soldiers' physical performance is not as clear. Therefore, the primary purpose of this study was to investigate the hypothesis that there is a relationship between soldiers' fat percentage preceding MFT and the change in muscle mass, physical performance, and serum testosterone concentration during MFT, where a higher fat percentage leads to a smaller decrease in muscle mass, physical performance and serum testosterone concentration. The secondary purpose was to investigate the hypothesis that a higher fat percentage is associated with faster recovery of physical performance and serum testosteroneconcentration after MFT.

114 MATERIALS AND METHODS

116 Experimental design and procedures

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118 The present study consisted of 20 days of MFT followed by a 10-day recovery phase. The 20-day 119 MFT (Figure 1) consisted of a lighter phase (10 days) which included basic cold weather and winter 120 warfare training, followed by a demanding phase (10 days) when the soldiers applied their skills in the arctic environment performing reconnaissance tasks while skiing long distances and carrying heavy 121 122 individual equipment weighing 30-40 kg. During the demanding part of MFT the soldiers were 123 subject to heavy physical strain, cold weather, and sleep and energy deprivation. The recovery period 124 consisted of light military service and unregulated military leave. Measurements were conducted on 125 four different time points. PRE measures were collected 3 days before the start of the exercise, MID 126 measures between the light and heavy segments of MFT and, POST measures immediately after MFT. 127 Finally, RECO measurements were performed 10 days after the end of MFT. During MFT, the average ambient temperature was -10°C (range -4 - -18°C) and snow depth of 85 cm, thus practically 128 129 forcing soldiers to use skis for moving.

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131 Participants

Fifty-eight male SOF soldiers voluntarily took part in the study. There were no female SOF conscripts in the target population. The participants were divided into two groups according to their initial fat percentage (Table 1). The present study was conducted according to the provisions of the Declaration on Helsinki and was granted an ethical approval from the Ethical Committee of the University Hospital of Helsinki (HUS/1020/2019). The study was approved by the Finnish Defence Forces (AP12498). All subjects were informed of the experimental design, and the benefits and possible risks that could be associated with the study prior to signing an informed consent to voluntary participate inthe study.

142 Physical performance testing

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Soldiers' physical performance was assessed at PRE, POST, and RECO time points using a test battery consisting of six tests. The physical performance test began with a 10-minute warm-up consisting of light jogging and body-weight exercises. The tests were done in the following order: standing long jump, medicine ball throw (MBT), countermovement jump (CMJ), weighted chin-ups, sit-ups, and evacuation test (EVAC).

The standing long jump was performed on a rubber mat with an integrated measuring scale. The participants were instructed to jump as far as possible freely swinging their arms for best results. Three attempts with a short (< 1min) in-between resting period were allowed and the best jump was recorded as the final result. The standing long jump has been proven to be a valid test for explosive force production of the lower extremities with high repeatability (ICC = 0.95). (Markovic et al. 2004)

The medicine ball throw (MBT) was performed seated on the floor using a 2 kg medicine ball. The back, shoulders, and the back of the head were kept in touch with the wall and legs were kept straight on the ground during the throws. The ball was thrown off the chest using both hands. Each participant had four consecutive throws of which the longest one was recorded as the final result. MBT using this method has been proven to be a reliable method for testing upper limb explosive force production (ICC = 0.95) (Van den Tillaar & Marques 2013).

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163 The countermovement jump (CMJ) height was calculated from the flight time recorded with a portable 164 contact mat (Newtest, Oulu, Finland). Participants were instructed to keep their hands on their waist 165 during the jump and the knees and ankles were to be extended during the landing. Three jumps were 166 performed of which the best one was recorded for statistical analyses. There was a short resting period of one to two minutes between each jump. The CMJ has been proven to be a valid test for explosive
force production of the leg extensor muscles with high repeatability (ICC = 0.98). (Markovic et al.
2004)

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171 In the weighted chin-up the participants completed maximum repetitions of chin-ups with a 10 kg 172 weight on the waist until exhaustion. Each repetition was initiated from a dead hang with straightened 173 arms and a visible pause was implemented between each repetition to minimize kipping and swinging. 174 The tip of the chin had to exceed the bar level both horizontally and vertically.

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176 In the sit-up test the participants completed as many sit-ups as possible in two minutes. The 177 participants were assisted by holding down at the ankles during the test. The upper part of the back 178 had to make contact with the ground in the bottom position and elbows had to touch the knees or the 179 upper parts of the thighs in the top position. Fingers were interlocked behind the head. If the 180 movement was stopped for more than three seconds, the test was ceased.

In the evacuation course (EVAC) two laps were run against time around a track that was 10 meters wide and 20 meters long with four cones that the participant had to go around. First lap was run without load but for the second lap the participants had to drag a dummy weighing 80 kg. A detailed description of the track is provided by Angeltveit et al. (2016) who state that the EVAC course is a valid test for anaerobic performance with good repeatability (ICC = 0.89 after familiarization) and face validity for SOF soldiers.

189 Body composition and hormone serum concentrations

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Body composition was measured, and blood samples were collected at PRE, MID, POST, and RECO time points after an overnight fast, excluding the POST time point where measures and samples were taken directly after soldiers finished MFT and a fasted state could not be ensured. Measures and samples were collected during the morning between 05:30 and 07:00.

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Body composition was analyzed using multifrequency bioimpedance analysis assessment (InBody 720/770, Biospace Co. Ltd., Seoul, South Korea) with the subjects in their underwear. While the method has been found to be reliable, it systemically overestimates muscle mass when compared to measures taken by DEXA (Dual-energy X-ray absorptiometry). Still, the correlation between DEXA and InBody analyses is high (r=0.90, p<0,001). (McLester et al. 2018.)

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Blood samples were drawn from the median cubital vein and were centrifuged (Megafire 1.0 R
Heraeus, DJB Labcare Ltd, Buckinghamshire, UK) at 2000× g for 10 min and frozen for a later
analysis. Cortisol (COR) and testosterone (TES) concentrations were analyzed using an Immulite 2000
XPI immunoanalyzer (Healthcare Diagnostics Products Ltd.). Serum creatine kinase (CK) activity was
analyzed using Indiko Plus chemistry analyzer (Thermo Fisher Scientific Ltd.). The sensitivity and
interassay coefficients of variance for these assays were 5.5 nmol/L and 6.5% for COR, 0.5 nmol/L
and 7.8% for TES, 0.08 µmol/L, and 7 U/l and 7.1% for CK.

210 Statistical analysis

Statistical analysis was performed in R (v R4.2.1). Descriptive statistics are presented as means ± SD.
Subjects were split into two groups using 12 % of fat percentage as cut-off value (n = 29 per group, n
= 58 total).

Linear mixed models for repeated measurements with restricted maximum likelihood estimation were used to examine differences between groups for response variables (body weight, muscle mass, fat mass, physical performance tests, and hormonal and enzymatic biomarkers). A random subject effect was used to account for repeated measurements. Fixed effects of time (PRE, MID, POST, RECO) and group (over 12 % body fat, under 12 % body fat), as well as their interaction term, were specified. Bonferroni post hoc tests were used to examine interactions and main effects. A pairwise Student's t test with Bonferroni correction was used to compare measurements in MID, POST and RECO Appl. Physiol. Nutr. Metab. Downloaded from cdnsciencepub.com by JYVASKYLAN YLIOPISTO on 11/24/23 For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

timepoints to measurements at PRE timepoint. A pairwise Student's t test with Bonferroni correction
was used to compare groups for age and anthropometrics at PRE. If normal distribution was not
observed a Wilcoxon signed rank test was performed with Bonferroni correction.

Linear model was performed to evaluate linear dependency between fat percentage at PRE and change
in muscle mass (PRE to POST), change in testosterone concentrations (PRE to POST), change in
physical performance (PRE to POST), as well as to evaluate dependency between change in muscle
mass (PRE to POST) and change in physical performance (PRE to POST).

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Appropriate transformations of variables were performed when a normal distribution was not observed
 for parametric statistical testing. Pearson correlation coefficients were used to determine correlation
 between variables. A 2-tailed P value < 0.05 was considered significant.

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238 RESULTS

Body weight, muscle mass, and fat mass at all time-points are presented in Table 2 for each group 240 241 separately. There was a significant main effect of time for body mass (p < 0.001), muscle mass 242 (p<0.001) and fat mass (p<0.001) and a significant main effect of group for muscle (p<0.05) and fat 243 mass (p<0.001). While there was no significant group \times time interaction for the change of body mass, 244 there was a significant group \times time interaction for muscle (0.001) and fat mass (p<0.001) and post-245 hoc analysis indicated that the low-fat group had greater changes in muscle mass (p<0.001) and 246 smaller changes in fat mass (p < 0.001) than the high-fat group from PRE to POST. The low-fat group 247 lost 0.8 ± 0.9 kg of muscle mass (p<0.01) and 2.0 ± 1.2 kg of fat mass (p<0.001). For the high-fat 248 group, no significant change was detected for muscle mass, but a 3.7 ± 1.4 kg decrease of fat mass 249 (p<0.001) was observed. After a 10-day recovery period, body mass and body composition had 250 returned to PRE-values apart from fat mass for the low-fat group as there was a statistically significant

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increase of 0.8 ± 1.1 kg of fat mass (p<0.01) from PRE to RECO. Figure 2 shows the changes in body mass, muscle mass, and fat mass from PRE to POST (A) and their level of recovery (ratio of RECO and PRE measures) at RECO (B).

255 Physical performance

There was a significant main effect of time for all physical performance tests. Performance decreased from PRE to POST in all of the tests used (Figure 3). Greatest reduction in performance appeared in the EVAC-course time where increases of 23.8% and 18.6% were observed in the total time for the low-fat and the high-fat groups, respectively. Physical performance was still suppressed at RECO in most of the tests used but a return to PRE measurement values was observed in sit-up test for the lowfat group and in EVAC-course for the high-fat group. There was no significant group \times time interaction in any of the physical performance tests.

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Hormonal and enzymatic changes

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267 There was a significant main effect of time (p < 0.001) for serum testosterone concentration and a 268 significant group \times time interaction (p<0.001). Serum testosterone concentrations decreased by 78.8% 269 and 65.0% from PRE to POST in the low-fat and the high-fat groups, respectively. There was a 270 significant main effect of time (p < 0.001) for serum cortisol concentration (p < 0.001) and a decrease of 271 21.4% (p<0.01) in serum cortisol concentrations from PRE to POST for the high-fat group was detected. There was no significant group \times time interaction. There was a significant main effect of 272 273 time (p<0.001) for CK activity (p<0.001), and an increase in CK activity was observed from PRE to 274 POST. There was no significant group × time interaction All biomarkers returned to PRE 275 measurement values during RECO. Table 3 shows the serum hormone concentrations and creatine-276 kinase activity at different time points and the average relative change from PRE, if statistically 277 significant.

279 Correlations between fat percentage, muscle loss, changes in physical performance, and changes in280 blood biomarkers

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Fat percentage at PRE was positively correlated with the change in muscle mass from PRE to POST ($R^2=0.26$, p<0.001) and the change in serum testosterone concentration from PRE to POST ($R^2=0.22$, p<0.001). The change in soldiers' muscle mass from PRE to POST was correlated with the respective changes in CMJ performance ($R^2=0.13$, P<0.01) and changes in EVAC time ($R^2=0.11$, p<0.05), while fat percentage at PRE was only correlated with changes in EVAC time ($R^2=0.10$, p<0.05). Scatter plots and correlations are presented in figure 4.

291 DISCUSSION

293 The purpose of this study was to investigate the relationship between soldiers' fat percentage and 294 changes in muscle mass, physical performance, and blood biomarkers during a 20-day MFT in the 295 arctic environment and the following 10-day recovery period. The main findings were that soldiers 296 with a higher initial body fat percentage lost less muscle mass than those with a lower body fat 297 percentage, and greater losses of muscle mass were correlated with greater decreases in performance 298 in the EVAC test and the CMJ. In addition, a relationship between soldiers' body fat percentage 299 before MFT and the change in physical performance was observed in the EVAC test, where soldiers 300 with a lower fat percentage had a greater reduction in performance after MFT. Soldiers' body fat 301 percentage was also positively correlated with changes in serum testosterone concentrations during 302 MFT.

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On average the soldiers in the low-fat group lost 2.0 kg of their fat mass and 0.8 kg of their muscle mass. In the high-fat group the loss of fat mass was 3.7 kg with no statistically significant change in muscle mass, and a correlation between the fat percentage at PRE and change in muscle mass during

307 MFT was observed (r=0.51, p<0.001). Similar observations have been done by Vikmoen et al. (2020) 308 and Hamarsland et al. (2018), though the correlations in their studies were even stronger (r=0.75, 309 p < 0.05 and r = 0.78, p < 0.001, respectively). In a study by Gan et al. (2022) an 18 % energy deficit was 310 observed by recording food intake objectively and measuring energy expenditure by the doubly-311 labelled water method during a 62-day Singapore Ranger course in a hot humid environment. Soldiers with higher fat percentage (fat% > 15) at PRE lost more fat mass and gained rather than lost muscle 312 mass during the course as compared to leaner soldiers. Previous studies suggest that fat oxidation 313 314 could be more efficient in individuals with a higher fat mass when the subjects are of normal weight 315 and fit. (Hoyt et al. 2006.) This could lead to the effect observed in the present study, where 316 participants of the high-fat group seem to have used more of the body fat reserves for energy production, leading to a greater decrease in fat mass and sparing of lean mass during energy deficit. 317 318 The importance of this is highlighted by the fact that soldiers tend not to consume all of the available 319 food during MFT (Margolis et al. 2014) and interventions with increased protein consumption have 320 not been able to spare body mass nor muscle mass during MFT (Øfsteng et al 2020), making the most 321 obvious and accessible means of sparing muscle mass during MFT ineffective. In addition to the 322 maintenance of muscle mass fat mass can also help the soldiers to maintain safe core temperatures 323 when operating in cold temperatures (Savastano et al. 2009).

325 Soldiers' body mass, fat mass and muscle mass were fully recovered at RECO but there was a 326 significant overshoot of fat mass in the low-fat group as the group's fat mass at RECO exceeded the 327 fat mass at PRE by 13%. Similar findings with soldiers participating in strenuous MFT with negative energy balance have been reported by Nindl et al. (1997) and Friedl et al. (2000) on US Army 328 329 Rangers. This overshoot of fat mass has previously been found to be larger in individuals with lower 330 fat percentages (Dulloo et al. 2017). Data from the Minnesota semi-starvation experiment suggests 331 that the overshoot in fat mass during recovery from semi-starvation is at least partly a product of over-332 eating until muscle mass has been fully recovered to levels preceding the energy restriction (Dulloo et 333 al. 1997), which could explain why no overshooting of fat mass was not observed in the high-fat group 334 as they did not lose muscle mass during MFT.

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336 As the decrease in physical performance during sustained military occupational stress is at least partly 337 linked to the energy deficit (Guezennec et al. 1994), it could be argued that the cold environment 338 during winter MFT could result in greater changes in physical performance through increased energy 339 expenditure and thus a larger energy deficit. If the soldiers' core temperature decreases during MFT, 340 this could induce shivering thermogenesis increasing resting energy consumption up to five-fold 341 (Haman & Blondin 2017) and an increase in the activity of brown adipose tissue (Van der Lans et al. 342 2014), which could increase heat production by 17 % on average among lean individuals (Claessens-343 Van Oojien et al. 2006). Even if core temperature can be maintained through physical activity and 344 clothing, energy expenditure can still be increased as every kilogram of clothing increases energy it by 345 approximately 3 % and every layer of clothing by 4 % during physical activity (Rintamäki 2007). Hackney et al (1991) compared the effects of MFT in warm and cold environments and found that 346 347 soldiers' anaerobic performance had greater decrements after cold-weather MFT, though there were 348 no differences in weight loss between the two types of MFT, which suggests that there was no 349 difference in soldiers' energy balance. However, direct comparison between different environmental 350 conditions is problematic as the contents of military training could vary significantly between the field 351 exercises. In the present study, the changes in body weight and body composition were not as marked 352 as ones reported in most previous research on MFT of similar duration (Vikmoen et al. 2020, Øfsteng 353 et al 2020, Hamarsland et al. 2018) which could suggest that the cold exposure during MFT did not 354 dramatically increase energy expenditure. Yet as mentioned previously, comparison of military field 355 exercises is problematic as the operative stressors (e.g., workload, recovery, sleep, energy and fluid balance, stress level, environmental factors) of MFT can vary widely. Furthermore, the comparison is 356 357 distorted by the fact that soldiers in the present study used skis during MFT, as the energy expenditure 358 of skiing and marching soldiers most likely differ (Ainsworth et al. 2011). Nevertheless, it can be 359 stated that even though taking winter conditions into consideration during the planning of MFT is 360 understandably important, specific measures to combat increased energy expenditure might not be 361 necessary.

There was a 10-20 % decrease from PRE to POST in physical performance in all used assessment 363 364 methods. Significant relationship was found between change in muscle mass and change in physical 365 performance in the EVAC task (r=-0.33, p<0.05) and in the CMJ (r=0.36, p<0.01). Previously Johnson 366 et al (1994) and Nindl et al. (2007) have observed a similar relationship between loss of muscle mass 367 and decrease in physical performance in US Army Rangers. While fat percentage at PRE was correlated with changes in muscle mass during MFT, fat percentage at PRE was correlated with only 368 369 the change in performance time on the EVAC task (r=-0.31, p<0.05). Although this relationship was 370 only observed on the EVAC task, it can be stated that the EVAC task is the most task-specific out of 371 the tests used, hence the most relevant for special force soldiers. Overall, the results suggest that a 372 higher fat percentage could be beneficial for soldiers taking part in prolonged MFT as this could lead to attenuated decrements in some aspects of physical performance, possibly through the preservation 373 of muscle mass. It should be noted that only about 10 % of the variation in the change in physical 374 375 performance in the EVAC-task was explained by the change in muscle mass. Therefore, it is important to consider the possible methods to combat muscle loss during MFT and the possible benefits and 376 377 drawbacks of their use.

379 Interestingly, the significant group difference observed at PRE in lower body explosive force 380 production (standing long jump, counter movement jump) diminished by the end of MFT. Even 381 though significant decreases were observed in these variables in both groups, the performance gap 382 between the groups narrowed during the follow-up. Thus, higher body fat content may have protected 383 the loss of lower body power during MFT. On the other hand, it is also possible that group differences in explosive force production dynamics are more related to differences in muscle mass changes which 384 385 is supported by the observed correlation between the changes in muscle mass and counter movement 386 jump (r=0.36). This would seem logical since maximal strength and explosive force production are 387 dependent on neural activation and cross-sectional area of muscle (Kraemer et al. 2015, Häkkinen et 388 al. 1985). It is important to note though, that the high-fat group did not surpass the performance of the 389 low-fat group in any physical performance test at any time point. In fact, the low-fat group out-390 performed the high-fat group in four out of six physical performance tests at PRE. This in mind, favoring soldiers with higher fat percentages does not seem advisable. Therefore, it should be examined whether a period of high calorie intake before MFT could help maintain the optimal body composition and physical performance during MFT. It should be noted that the soldiers in this study were very lean even in the high-fat group and excess body fatness for soldiers is not recommendable. The optimal quantity of fat mass remains to be defined in future studies.

397 Soldiers' physical performance was not fully recovered at RECO in most of the tests used. A return to 398 PRE-values was observed only in the sit-up test for the low-fat group and in the EVAC-course for the 399 high-fat group. Recovery was deficient especially for explosive force production of the lower and 400 upper extremities. Similar findings have been made previously on the recovery of lower extremities, 401 but the recovery of explosive force production of upper extremities has usually been faster (Vikmoen 402 et al 2020, Hamarsland et al. 2018). The slower recovery time could be caused by the use of poles 403 while skiing, increasing the demand of upper body musculature when compared to marching soldiers. 404 As full recovery was reached in all the body composition and hormonal and enzymatic markers, it 405 seems that their isolated use for the assessment of soldiers' recovery from MFT is questionable since 406 they do not represent the complete recovery of physical performance.

408 It has been well-established that serum testosterone concentrations decrease, and cortisol 409 concentrations increase during demanding MFT. (Vikmoen et al. 2020, Øfsteng et al. 2020, 410 Hamarsland et al. 2018, Szivak et al. 2018, Kyröläinen et al. 2007) In the present study, serum 411 testosterone decreased throughout MFT at MID and at POST. There was a positive correlation between fat percentage at PRE and the change in serum testosterone concentrations (r=0.47, p<0.001). 412 413 Wong et al. (2019) have noted in their review that during prolonged energy restriction serum 414 testosterone concentrations decrease mainly due to central processes that decrease the amplitude and 415 frequency of the pulsating secretion of luteinizing hormone. They also suggest that leptin could be a 416 factor in suppressed testosterone levels, as leptin supplementation seems to reverse starvation-417 associated gonadal axis suppression. As serum leptin levels are positively correlated with body fat 418 percentage (Smith et al. 2006), it seems plausible that in the current study individuals with higher

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419 body fat percentages had an attenuated response to starvation-induced gonadal suppression i.e., 420 decrease in serum testosterone concentration, partly because of their higher leptin levels. It is 421 important to note though that leptin concentrations were unfortunately not measured during the present 422 study.

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424 There was also an expected rise in cortisol concentrations from PRE to MID but unexpectedly the 425 lowest cortisol concentrations were measured immediately after the most demanding part of MFT at 426 POST. This finding is contrary to most other findings on the subject. As negative changes in soldiers' 427 testosterone levels and physical performance were marked, it seems unlikely that this contradictory 428 finding is because of low levels of stress, both physical and mental, experienced during MFT. A 429 possible explanation for the low cortisol concentration may be the disturbances in soldiers' circadian 430 rhythm (Opstad et al. 1994). Severe physical stress combined with sleep and energy deprivation has 431 been shown to diminish the normal daily variation of cortisol and thus, it is possible that during the 432 blood sampling at POST the normal biological increase in cortisol was absent, leading to lower 433 concentration levels than expected. Although there lies uncertainty in what caused the low cortisol 434 levels measured at POST, the results still suggest that cortisol concentration is not always a reliable 435 biomarker for measuring stress load during MFT.

437 Serum CK levels were elevated at MID reaching peak levels at POST with no observed between-438 group differences. From the elevated CK levels we can determine that MFT caused a noticeable 439 amount of muscle damage. Hamarsland et al. (2018) suggested that muscle damage could in part 440 explain the discrepancy between recovery of body composition and hormonal and enzymatic markers 441 compared to the recovery of physical performance. While muscle mass itself was fully recovered, it is 442 possible and even likely that exercise-induced muscle damage still persisted at RECO.

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The results of the present study should be interpreted identifying the following limitations. Body composition was determined by bioimpedance method, which may overestimate muscle mass and underestimate fat mass and fat percentage when compared to dual-energy X-ray absorptiometry 447 measurement method (Antonio et al. 2019). However, no differences were observed in the changes in 448 these variables between the two-abovementioned methods. The bioelectrical impedance analysis is 449 based on different conductivity of electricity in extracellular and intracellular water (Ling et al. 2011). 450 Thus, acute changes in these water compartments, for example as a result of dehydration, may 451 confound the body composition results, especially for muscle mass and fat mass quantification. 452 However, bioimpedance method is currently the most practical objective body composition 453 assessment method for field studies such as the present one and, the same method has been used in 454 other similar studies (Vikmoen et al. 2020, Hamarsland et al. 2018). Another limitation to this study 455 was that energy balance was not quantified. Quantification of energy intake and expenditure would 456 have provided more information for the interpretation of body composition changes, but unfortunately 457 the nature of MFT did not enable these measurement methods. Lastly, the lack of reproducibility is a 458 limitation seen in many similar studies for the large amount of randomness inherent to military field 459 training. Weather conditions, and the lack of control of energy balance and the amount of sleep can 460 affect the demands of MFT and as consequently affect the response seen in partaking soldiers. While 461 energy balance and the amount of sleep could be controlled to a satisfying degree, weather conditions 462 could still affect the demands of MFT especially in snowy conditions when long distances are covered 463 on skis.

465 CONCLUSION

This study brought supporting evidence to the hypothesis that soldiers' fat mass helps preserve muscle mass during prolonged MFT which in turn preserves soldiers' physical performance, and a correlation between fat percentage at PRE and muscle mass change from PRE to POST was observed. To our knowledge this study is the first one to observe a direct relationship between soldiers' fat percentage and the change in physical performance, as fat percentage at PRE was correlated to the change in performance in the EVAC task.

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474 Recovery from MFT was not markedly different between the two groups as significant difference
475 between PRE and RECO measurements were detected in all physical performance test for both groups
476 except for the sit-up test for the low-fat group and the EVAC-test for the high-fat group. All hormonal
477 and enzymatic markers were recovered back to PRE-measurement levels at RECO in both groups.

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610 TABLES

611	Table 1. Ag	ge, anthrop	ometrics,	and body	compositio	on of study	participants at I	PRE.
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	Fat% < 12	Fat% > 12	All
	(n=29)	(n=29)	(n=58)
Age (years)	19.4 ± 0.8	19.5 ± 0.8	19.4 ± 0.8
Stature (cm)	183.4 ± 5.1	$180.2 \pm 6.0*$	181.8 ± 5.7
Body mass (kg)	79.8 ± 8.3	77.3 ± 6.5	78.5 ± 7.5
Fat mass (kg)	7.3 ± 1.9	10.7 ± 1.5***	9.0 ± 2.4
Muscle mass (kg)	41.7 ± 4.4	38.1 ± 3.5**	39.9 ± 4.3
Fat%	9.2 ± 2.0	13.9 ± 1.5***	11.5 ± 3.0

Significantly different from the low-fat group: *, p < 0.05; **, p < 0.01; ***, p < 0.001

	Group	PRE	MID	POST	RECO
Body mass (kg)	Low-fat	79.8 ± 8.3	79.4 ± 8.2	76.7 ± 8.1***	80.0 ± 8.2
	High-fat	77.3 ± 6.5	77.0 ± 6.4	74.4 ± 5.8 ***	77.8 ± 6.4
Muscle mass (kg)	Low-fat	41.7 ± 4.4	41.9 ± 4.5	$40.9 \pm 4.5 **$	41.3 ± 4.5
	High-fat	$38.1\pm3.5^{\dagger\dagger}$	$38.7\pm3.3^{***\dagger}$	$38.4\pm3.3^{\dagger}$	$38.5\pm3.7^{\dagger}$
Fat mass (kg)	Low-fat	7.3 ± 1.9	6.6 ± 1.7 **	$5.3 \pm 1.6^{***}$	8.2 ± 2.2**
	High-fat	$10.7\pm1.5^{\dagger\dagger\dagger}$	$9.5 \pm 1.8^{***\dagger\dagger\dagger}$	$7.0 \pm 1.9^{***^{\dagger\dagger}}$	$10.3\pm1.8^{\dagger\dagger\dagger}$

Table 2. Body mass, muscle mass, and fat mass at all measuring points for both groups.

614 Significantly different from PRE: *, p < 0.05; **, p < 0.01; ***, p < 0.001

615 Significantly different from the low-fat group: t, p < 0.05; tt, p < 0.01; ttt, p < 0.001

616 Table 3. Serum testosterone and cortisol concentration, and creatine kinase activity at different time

617 points and the statistically significant average relative change from PRE.

	Group	PRE	MID	POST	RECO
Testosterone (nmol/L)	Low-fat	17.73 ± 3.84	14.40 ± 2.90 (-17.0 %)***	3.56 ± 2.28 (-78.9 %)***	17.43 ± 3.56 (n/a)
	High-fat	15.94 ± 2.57	13.31 ± 3.46 (-16.7 %)***	5.60 ± 3.55 (-65.0 %)***†	15.16 ± 3.79 (n/a)
Cortisol (nmol/L)	Low-fat	431 ± 56	475 ± 49 (+11.7 %)*	371 ± 172 (-15.4)	417 ± 56 (n/a)
	High-fat	437 ± 49	479 ± 71 (+10.0 %)**	335 ± 98 (-21.4 %)**	410 ± 75 (n/a)
Creatine kinase (IU)	Low-fat	209 ± 77	469 ± 270 (+131 %)***	3562 ± 1603 (+1843 %)***	200 ± 109 (n/a)
	High-fat	174 ± 65	417 ± 170 (+149 %)***	4037 ± 2966 (+2247 %)***	157 ± 65 (n/a)

619 Significantly different from PRE: *, p < 0.05; **, p < 0.01; ***, p < 0.001. N/a = not applicable.

620 Significantly different from the low-fat group: t, p < 0.05

621 FIGURE CAPTIONS

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623 Figure 1: Study design and measurement points.

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Figure 2. A: Change in body mass, muscle mass, and fat mass from PRE to POST. B: Recovery of
body mass, muscle mass, and fat mass as presented by the ratio of RECO/PRE with 1 being fully
recovered. Low-fat group = black bar, high-fat group = white bar.

628 *Significantly different from PRE: *, p < 0.05; **, p < 0.01; ***, p < 0.001*

629 Significant difference between groups: t, p < 0.05; tt, p < 0.01; ttt, p < 0.001

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Figure 3. Results of the physical performance tests at PRE, POST, and RECO for the low-fat (dashed blue line) and high-fat (solid red line) groups. Standing long jump, MBT, CMJ, Weighted chin-up, Sit-ups, EVAC-course. There was a significant main effect of time for all physical performance tests (p<0.001) and performance was decreased from PRE to POST. Significant difference between PRE and RECO were detected in all physical performance test for both groups except for the sit-up test for the low-fat group and the EVAC-test for the high-fat group.

Figure 4. Correlations between A: change in muscle mass and fat % at PRE, B: change in testosterone
concentration and fat % at PRE, C: change in EVAC time and fat % at PRE, D: change in EVAC time
and change in muscle mass, E: change in CMJ and change in muscle mass.







