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2 **Aerobic fitness, energy balance and BMI are associated with training load assessed by**
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4 **activity energy expenditure**
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48 **Running head:** AEE, VO₂max, energy balance and BMI
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Abstract

The present study examined whether activity energy expenditure related to body mass ($AEE \cdot kg^{-1}$) is associated with maximal aerobic fitness (VO_{2max}), energy balance and BMI during the two hardest weeks of the military basic training season (BT). An additional purpose was to study the accuracy of the pre-filled food diary (EI). Energy expenditure (EE) with doubly labeled water, EI, energy balance and mis-recording were measured from 24 male conscripts with varying VO_{2max} . $AEE \cdot kg^{-1}$ was calculated as $(EE \times 0.9 - \text{measured basal metabolic rate})/\text{body mass}$. The reported EI was lower ($p < 0.001$) than EE ($15.48 \text{ MJ} \cdot d^{-1}$) and mis-recording of the pre-filled diary was -20%. The negative energy balance ($-6 \pm 26\%$) was non-significant, however the variation was high. The subjects with low VO_{2max} , high BMI and negative energy balance were vulnerable for low $AEE \cdot kg^{-1}$. However, in the multivariate regression analysis only BMI remained in the model explaining 33% of the variation in $AEE \cdot kg^{-1}$. During wintertime BT, $AEE \cdot kg^{-1}$ is affected by energy balance, VO_{2max} and BMI. From these three factors, overweight limits the most the high-level training. Furthermore, optimal energy balance facilitates physical performance, and enables high training loads to be sustained during the BT season.

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Key Words: AEE; PAL; energy intake; underreporting; under-eating; military training

INTRODUCTION

To improve the physical fitness of new conscripts in the Finnish Defense Forces, the amount of physical training during a basic military training period (BT) was doubled in 1998 (Training division of the defence staff, 2003). In contrast, the fitness level of young men entering Finnish compulsory military service has declined, with a concomitant increase in body mass during the last 25 years (Santtila et al., 2006). It is not known whether the overall training load during military service is suitable for the current conscripts, who exhibit varying physical fitness levels.

Aerobic fitness, as measured by maximal oxygen uptake (VO_{2max}), is an important component of the physical fitness profile (ACSM, 2001). In addition, aerobic fitness has been found to be an important component of performance, and can thus influence the ability to sustain the training load during BT (Jones & Knapik, 1999), while low aerobic fitness increases the risk of injury and illness leading to limited duty days during BT (Jones & Knapik, 1999; Knapik et al., 2001). However, the measurement of training load and performance over a long period of BT, when training includes different kind of activities, is challenging. A measure of physical activity level known as PAL, an average daily multiple of basal metabolic rate (BMR), is used to classify occupational workload and leisure time physical activity according to the following criteria: sedentary (1.4 x BMR), normal (1.8 x BMR), or active (2.0 x BMR) (Nordic Nutrition Recommendations, 2004). Another way to describe physical activity is the energy cost of activity (AEE) with corrections for differences in body size (Ekelund et al., 2004). AEE includes muscular activity, including shivering and fidgeting as well as purposeful physical exercise (Poehlman, 1989), and it is affected by the intensity and duration of activity as well as by individual differences in movement efficiency (Ainsworth et al., 1993).

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4 All conscripts train together according to the same program throughout BT. Thus the physical
5 activity is not optional and the PAL should be the same regardless of the subject's fitness
6 status. From another perspective, individuals with low aerobic fitness will experience greater
7 physiological stress relative to their maximum capacity at any given absolute level of aerobic
8 work (Jones & Knapik, 1999). Thus it could be hypothesized that the less fit conscripts will
9 experience more physiological stress during physical activity because they have to work at
10 higher relative intensities, and aerobic fitness is not necessarily related to AEE.
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20 The doubly labeled water (DLW) method has been used as a gold standard for the
21 measurement of total daily energy expenditure (EE) (Schoeller et al., 1986; Westerterp et al.,
22 1995). Several studies have used the DLW method in military operations, for example during
23 rigorous field training exercise (DeLany et al., 1989; Forbes-Ewan et al., 1989; Mudambo
24 et al., 1997; Castellani et al., 2006), in extremely cold environments (Hoyt et al., 1991; Jones
25 et al., 1993), or in the garrison (Mudambo et al., 1997). In combination with the measurement of
26 basal metabolic rate, the DLW method allows the calculation of AEE and PAL over
27 prolonged periods in military environments.
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37 Energy intake (EI) has been found to influence EE and activity level (DeLany et al., 1989;
38 Klein & Goran, 1993; Thompson et al., 1995). In addition, unfamiliar stressful situations have
39 been found to affect energy intake (Popper et al., 1989). Most young men entering the
40 military service will have their first experience of demanding physical training, eating
41 outdoors, and performing overnight exercises in a forest. The common way to assess EI is a
42 self-reported food diary. However, when DLW has been used to validate EI, a discrepancy
43 between self-reported EI and measured EE has been observed, mainly due to underreporting
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2 (Goris & Westerterp, 1999). Underreporting can be divided into mis-recording and under-
3 eating; mis-recording refers to a discrepancy between EI and measured EE with no change in
4 body mass, and under-eating is a discrepancy accompanied by a decline in body mass over the
5 food-recording interval (Goris & Westerterp, 1999), representing a negative energy balance.
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10 With a description of mis-recording, an estimate of true energy intake could be obtained.
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14 No studies have examined the associations between soldiers' maximal aerobic fitness, energy
15 balance, body size, and activity during the BT season in winter conditions. Thus, the purpose
16 of this study was to determine the importance of VO_{2max} , energy balance and body size on
17 AEE related to body mass ($AEE \cdot kg^{-1}$) during the two hardest weeks of the wintertime BT
18 season. In the present study, the activity level ($AEE \cdot kg^{-1}$) equated to the training load during
19 the entire period of BT. An additional purpose was to study the accuracy of a self-reported
20 pre-filled food diary in the military environment without food weighing, but with known food
21 composition.
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31 **METHODS**

32 **Subjects**

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34 Voluntary male conscripts ($n=24$) from the Signal Battalion of Northern Finland with varying
35 VO_{2max} levels participated in the present 2-week study. Conscript is a definition of a person
36 who enters compulsory military service, which is strictly performed according to the standard
37 direction of the Defense Command. The subjects were selected from a total of 131 subjects,
38 who volunteered the present study. From the original subjects, 47 were discarded based on
39 cardiorespiratory or musculo-skeletal disorders, and incomplete fulfillment or willingness to
40 perform special duties. The remaining 84 were divided into three categories according to their
41 level of voluntary physical activity before military service. From each category, 20 subjects
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2 were selected, making a total of 60 randomly ordered subjects. Finally, these 60 subjects were
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4 divided into three groups of equal numbers according to their initial VO_2max ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)
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6 when entering military service: $> 45 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for group 1; $40 - 44.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for
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8 group 2; $< 39.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for group 3. From each group, 8 subjects were randomly
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10 selected making a total of 24 subjects. Subject characteristics are presented in Table 1. The
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12 subjects' physical fitness characteristics were representative of young Finnish men (Santtila et
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14 al., 2006). All subjects were fully informed of the experimental protocol and gave their
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16 written consent to participate in the study. They were also advised of their right to withdraw
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18 from the investigation at any time. The study protocol was approved by the Finnish Defence
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20 Forces, and the Ethics Committees of the University of Jyväskylä and the Kainuu region,
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22 respectively.

23 24 25 26 **Study protocol**

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28 The study took place during wintertime in Finland, when daily outdoor temperatures ranged
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30 between -6°C to -19°C , with an average of -11°C (according to the local weather station). The
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32 experimental protocol is presented in Table 2. The overall physical load of the 8-week BT
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34 season was set according to the standard direction of the Defense Command. The study took
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36 place during the last two weeks of BT, which was the most strenuous period of the BT season.
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38 Food and water intake were ad libitum during the study. The average sleeping time at the
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40 garrison was from 10 p.m. to 5:45 a.m. Military education included physically demanding
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42 activities such as marching, combat training and sport related physical training. The
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44 conscripts also carried combat gear weighing 25 kg, including clothing, particularly during
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46 marching and combat training. In addition, the training included an overnight field exercise.
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48 Garrison training involved theoretical education in classroom settings, material handling,
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2 shooting and general military education, such as close order drills. Table 2 provides a more
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4 detailed description of the other activities performed out of garrison.
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8 **Maximal oxygen uptake**

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10 To determine $VO_2\text{max}$, the conscripts performed a maximal treadmill test. The start of the test
11 involved walking for 3-min at $4.6 \text{ km}\cdot\text{h}^{-1}$ and walking/jogging at $6.3 \text{ km}\cdot\text{h}^{-1}$ (1% slope) as a
12 warm-up. Thereafter, exercise intensity was increased every 3-min to induce an increase of 6
13 $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in the theoretical $VO_2\text{max}$ demand of running (ASCM, 2001). This was
14 achieved by increasing the initial running speed of $4.6 \text{ km}\cdot\text{h}^{-1}$ by a mean of $1.2 \text{ km}\cdot\text{h}^{-1}$ (range
15 $0.6 - 1.4 \text{ km}\cdot\text{h}^{-1}$), and by increasing the initial grade of 1 deg by a mean grade of 0.5 deg
16 (range 0.0 - 1.0 deg) up to the point of exhaustion (ASCM, 2001). Pulmonary ventilation and
17 respiratory gas exchange data were measured on-line using the breath-by-breath method
18 (Jaeger Oxygen Pro, VIASYS Healthcare GmbH, Hoechberg, Germany), and mean values
19 were calculated at 1 minute intervals for statistical analysis. Heart rate was continuously
20 recorded at five-second intervals using a telemetric system (Polar810i, Polar Electro Oy,
21 Kempele, Finland). Blood lactate was determined 1 minute after completion of the exercise
22 from a fingertip blood sample using a lactate analyzer (LactatePro®, Arkray, Japan). The
23 criteria used for determining $VO_2\text{max}$ were: $VO_2\text{max}$ and heart rate did not increase despite
24 an increase in grade and/or speed of the treadmill, a respiratory exchange ratio (RER) higher
25 than 1.1, and a post-exercise blood lactate higher than $8 \text{ mmol}\cdot\text{l}^{-1}$ (ASCM, 2001). All
26 participants satisfied these criteria.
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45 **Anthropometry and body composition**

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47 Body mass was measured with an accuracy of 0.01 kg (Inbody720 body composition
48 analyzer, Biospace Co. Ltd, Korea) at the beginning (BMpre), after one week (BMmid) and at
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2 the end of the experimental period (BMpost). The measurements were performed between
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4 6:00 and 7:00 a.m. after an overnight fast and after voiding, with no exercise for 12 hours
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6 before the test. The physical activities in the daily program was planned to be light a
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8 preceding day each measurement, and fluid status was estimated to be in balance based on the
9
10 dietary records of the subjects. The subjects were barefoot and they wore T-shirts and trousers.
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12 The BMmean was presented as an individual average of BMpre, BMmid and BMpost, and the
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14 change in BM (Δ BM) was calculated as the difference between BMpost and BMpre. Body
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16 height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body mass
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18 index (BMI) was calculated as BMpre divided by the body height squared. Fat free mass
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20 (FFM) was calculated from total body water (TBW) as follows: $FFM = TBW/0.732$ (Pace &
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22 Rathburn, 1945). Fat mass (FM) was calculated as $BMpre - FFM$, and percentage of body fat
23
24 as $FM/BMpre \times 100$. TBW was measured with deuterium dilution according to the Maastricht
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26 protocol (Westerterp et al., 1995). TBW was calculated as the 2H dilution space divided by
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28 1.04, correcting for exchange of the 2H label with nonaqueous H of body solids (Schoeller et
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30 al., 1980).

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31 32 33 **Basal metabolic rate**

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35 The basal metabolic rate (BMR) was measured once, for practical reasons, approximately 19
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37 days before the DLW period. After an 11-h fast, the subject woke up at 5:30 a.m. for BMR
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39 measurements performed in a semirecumbent position, lying for 30 min in a quiet room.
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41 Oxygen consumption and carbon dioxide measurements were made with the same gas
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43 analyzer (Jaeger, Oxygen Pro, Germany) as used in the VO_{2max} test. BMR was automatically
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45 calculated from the oxygen and carbon dioxide values as follows: $EE = 1.59 \times VCO_2 + 5.68 \times$
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47 $VO_2 - 2.17 \times$ urinary nitrogen (Weir, 1949), where urinary nitrogen was assumed to be 15
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2 g/day. In the analysis, BMR was determined during the last 15 min of the 30-min
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4 measurement period.
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7 **EE, AEE, AEE·kg⁻¹ and PAL**

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9 EE was measured with DLW according to the Maastricht Protocol. (Westerterp et al., 1995).
10 Briefly, at 10:00 p.m., before the measurements and after collecting a baseline urine sample,
11 the subjects drank a weighed mixture of ²H₂O (99.9 atom %) and H₂¹⁸O (10 atom %) resulting
12 in an initial excess total body water enrichment of 150 ppm for deuterium and 300 ppm for
13 oxygen-18. Total body water was estimated from calculated body composition, based on
14 height, weight, age and gender, with the equation of Deurenberg et al. (1991), assuming a
15 73% hydration of fat-free mass. Subjects consumed no foods or fluids for 10 hours after dose
16 administration, during the overnight equilibration of the isotopes with the body water.
17 Subsequent urine samples were collected from the second and third voiding in the morning of
18 day 1, and from the first and second voiding in the morning of days 8 and 15. Isotope
19 quantities (deuterium and oxygen-18) in the urine were measured with an isotope ratio mass
20 spectrometer (Optima, VG Isogas, UK), and CO₂ production was calculated from isotope
21 ratios at the baseline, and days 1, 8, and 15, using the equations from Schoeller et al. (1986).
22 CO₂ production was converted to daily metabolic rate using an energy equivalent based on the
23 individual macronutrient composition of the diet (Black et al., 1986). AEE was calculated as
24 EE x 0.9 – BMR, assuming diet-induced thermogenesis of 10% (Poehlman, 1989). To remove
25 the confounding effect of body size (Ekelund et al., 2004), AEE was adjusted for BMmean
26 (AEE·kg⁻¹). PAL was calculated as EE /BMR.
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Energy intake

For studying energy intake (EI), the subjects kept a pre-filled food diary for 3-4 days in two phases. Altogether, habitual food intake was obtained from 7 days during the study. The pre-filled food diary included information about the food served in the military. Details of the food and fluids that were served, including their composition, was known beforehand, and this information was written in the pre-filled diary. Thus, the subjects were only required to record the amount of food and fluid that they consumed. The subjects had completed these diaries twice before the study to ensure that they were familiar with reporting food intake. The subjects received detailed verbal and written instructions about common household measures, such as cups and tablespoons, and specific information about the quantity of the measurements. They were asked to record brand names of every non-military food that they consumed, as well as their cooking methods. In addition, they were advised to be as accurate as possible in recording the amount and type of food and fluid consumed. Any questions, ambiguities, or omissions were resolved individually and controlled via personal interviews, which included questions about whether they ate less during the recording period (under-eating) or wrote down everything they consumed (mis-recording). Daily nutritional consumption was quantified by the computer program Nutrica®software (version 3.11, Finland). The recipes of the food that was consumed in military circumstances were added to the database.

Accuracy of the food diary and estimated true energy intake

Estimated true energy intake (TrueEI) was calculated using EI and changes in body energy stores (Δ ES), which was calculated from changes in body mass, assuming that a body mass of 1 kg is equivalent to 30 MJ (75% fat mass, 25% fat-free mass, with 73% water; (Westerterp et

al., 1995). Underreporting, mis-recording, under-eating (energy balance), and TrueEI were calculated as follows:

$$\text{Underreporting (\%)} = (EI - EE) / EE \times 100$$

$$\text{Mis-recording (\%)} = [(EI - \Delta ES) - EE] / EE \times 100$$

$$\text{Under-eating (\%)} = \text{energy balance (\%)} = [(\Delta ES / \text{study days}) / EE] \times 100$$

$$\text{TrueEI} = EI - \text{mis-recording} = EI - [(EI - \Delta ES) - EE] = \Delta ES + EE$$

Statistical analysis

Changes in BM were tested by paired samples T-test. FM was log transformed to reduce skewness. To remove the potential effect of body size on $VO_{2\max}$, FFM adjusted values for $VO_{2\max}$ ($VO_{2\max_{FFM}}$) were calculated according to the method described by Toth et al. (1993). Pearson correlation coefficients were computed to determine the linear relationship between EE, AEE, $AEE \cdot kg^{-1}$, PAL, BMR and $VO_{2\max_{FFM}}$ (l/min), energy balance and body composition (BMpre, FM, FFM, BMI). To examine the effects of BMI, fitness and under-eating on $AEE \cdot kg^{-1}$, the subjects were divided into tertiles with equal numbers of subjects according to their BMI (< 22.5 $kg \cdot m^{-2}$ for LowBMI; 22.6 – 24.8 $kg \cdot m^{-2}$ for ModerateBMI; > 24.9 $kg \cdot m^{-2}$ for HighBMI), $VO_{2\max_{FFM}}$ (< 3.44 $l \cdot min^{-2}$ for LowFIT; 3.45 – 3.68 $l \cdot min^{-2}$ for ModerateFIT; > 3.69 $l \cdot min^{-2}$ for FIT) and energy balance (< 0.76 $MJ \cdot d^{-1}$ for Low; -2.16-0.75 $MJ \cdot d^{-1}$ for Moderate; > -2.17 $MJ \cdot d^{-1}$ for High under-eating). One-way ANOVA with LSD post hoc test was used to compare the differences in $AEE \cdot kg^{-1}$, energy-balance and fitness or BMI between the category groups. For further evaluation of the interaction and to identify the predictors of $AEE \cdot kg^{-1}$ from the independent variables multivariable linear regression analysis was used. The independent variables were $VO_{2\max_{FFM}}$ ($l \cdot min^{-1}$), body composition and energy balance. The final model was computed after the multicollinearity diagnostic and the assumptions for regression analysis were fulfilled. All statistical analyses were performed

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using SPSS (14.0.1. 2005; SPSS Inc., Chicago, IL). The level of statistical significance was set at $p < 0.05$.

RESULTS

BMR ($7.66 \pm 0.92 \text{ MJ}\cdot\text{d}^{-1}$) represented $50 \pm 5\%$ and AEE ($6.26 \pm 1.21 \text{ MJ}\cdot\text{d}^{-1}$) $40 \pm 5\%$ of the total EE (Table 3). The reported EI of $11.5 \pm 3.2 \text{ MJ}\cdot\text{d}^{-1}$ was significantly lower than the measured EE ($15.5 \pm 1.6 \text{ MJ}\cdot\text{d}^{-1}$), and thus the results revealed significant underreporting ($-26.0 \pm 17.5\%$, $p < 0.001$). The data of reported intake indicated that daily carbohydrate consumption in relation to the total reported EI was $58 \pm 4\%$ ($5.2 \pm 1.6 \text{ g}\cdot\text{kg}^{-1}$), fat $27 \pm 4\%$ ($1.1 \pm 0.4 \text{ g}\cdot\text{kg}^{-1}$), protein $14 \pm 2\%$ ($1.3 \pm 0.4 \text{ g}\cdot\text{kg}^{-1}$), and alcohol $0.3 \pm 0.9\%$ ($0.01 \pm 0.04 \text{ g}\cdot\text{kg}^{-1}$). When the reported EI was corrected for the mis-recording ($-20.4 \pm 26.3\%$), TrueEI did not differ from EE ($-5.6 \pm 25.5\%$, ns.), which indicates a non-significant negative energy balance (under-eating) of the present subjects. However, Figure 1 indicates that few subjects had a negative energy balance, which was negatively related to BMpre ($r = -0.73$, $p < 0.001$), FM ($r = -0.83$, $p < 0.001$) and BMI ($r = -0.80$, $p < 0.001$).

The subjects in the HighBMI group had lower $\text{AEE}\cdot\text{kg}^{-1}$ ($p < 0.01$) compared with the subjects in the LowBMI and ModerateBMI groups (Figure 2A). The HighBMI subjects had also significantly higher negative energy balance ($-4.2 \pm 3.08 \text{ MJ}\cdot\text{d}^{-1}$) compared with those subjects in the LowBMI ($1.6 \pm 3.23 \text{ MJ}\cdot\text{d}^{-1}$, $p < 0.001$) and the ModerateBMI ($0.11 \pm 2.30 \text{ MJ}\cdot\text{d}^{-1}$, $p < 0.01$) groups. The LowBMI and ModerateBMI groups did not differ according to $\text{AEE}\cdot\text{kg}^{-1}$, and both energy balance and $\text{VO}_2\text{max}_{\text{FMM}}$ did not either differ between the three BMI groups. $\text{AEE}\cdot\text{kg}^{-1}$ was also lower in LowFIT compared with ModerateFIT and FIT ($p < 0.05$) (Figure 2B), but BMI and energy balance did not differ between these three FIT groups. Furthermore, $\text{AEE}\cdot\text{kg}^{-1}$ was lower among subject in the High under-eating group compared

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Deleted: In addition, in spite of the fact that BM did not change ($-0.4 \pm 1.8 \text{ kg}$, ns.) during the two-week training period, energy balance was related to $\text{AEE}\cdot\text{kg}^{-1}$ ($r=0.58$, $p<0.01$) (Figure 2). At the same time, under-eating was negatively related to BMI. To examine whether a slim body (low BMI) has an effect on energy balance, subjects were divided into tertiles with equal numbers of subjects according to their BMI ($< 22.5 \text{ kg}\cdot\text{m}^{-2}$ for group 1; $22.6 - 24.8 \text{ kg}\cdot\text{m}^{-2}$ for group 2 ; $> 24.9 \text{ kg}\cdot\text{m}^{-2}$ for group 3). Based on these distributions, the groups displayed in Figure 2 were created. The results revealed that subjects with higher BMI were more likely to under-eat than their slimmer counterparts (Figure 2).¶

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with the Low and Moderate under-eating groups ($p < 0.01$) (Figure 2C). These subjects had also higher BMI ($27.8 \pm 3.6 \text{ kg}\cdot\text{m}^{-2}$) compared with subjects in the Low ($21.4 \pm 2.4 \text{ kg}\cdot\text{m}^{-2}$, $p < 0.001$) and Moderate ($23.6 \pm 1.9 \text{ kg}\cdot\text{m}^{-2}$, $p < 0.01$) under-eating groups and lower $\text{VO}_2\text{max}_{\text{FMM}}$ ($3.43 \pm 0.27 \text{ l}\cdot\text{min}^{-2}$) compared with the Moderate under-eating group ($3.75 \pm 0.28 \text{ l}\cdot\text{min}^{-2}$, $p < 0.05$). The Moderate and Low under-eating groups did not differ in $\text{AEE}\cdot\text{kg}^{-1}$ BMI or $\text{VO}_2\text{max}_{\text{FMM}}$.

$\text{VO}_2\text{max}_{\text{FMM}}$ was positively related to AEE and $\text{AEE}\cdot\text{kg}^{-1}$ ($r = 0.44$, $p < 0.05$ and $r = 0.41$, $p < 0.05$, respectively), but it had no relationship with EE (Table 4). BMI was negatively related to $\text{AEE}\cdot\text{kg}^{-1}$ ($r = -0.60$, $p < 0.01$) but it also had no relationship with EE or AEE (Table 4). In addition, in spite of the fact that BM did not change ($-0.4 \pm 1.8 \text{ kg}$, ns.) during the two-week training period, energy balance was related to $\text{AEE}\cdot\text{kg}^{-1}$ ($r = 0.58$, $p < 0.01$) (Table 4). Table 5 shows the multivariate regression equations for explaining variation in $\text{AEE}\cdot\text{kg}^{-1}$. Model 1 included energy balance, $\text{VO}_2\text{max}_{\text{FMM}}$ and BMI. Only BMI remained in the model by explaining 33% of the variation in $\text{AEE}\cdot\text{kg}^{-1}$ with a standard error of estimate (SEE) of $0.02 \text{ MJ}\cdot\text{d}^{-1}\cdot\text{kg}^{-1}$ or 25 % of the mean $\text{AEE}\cdot\text{kg}^{-1}$.

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DISCUSSION

To our knowledge, there are no previous reports showing a relationship between VO_2max and activity ($\text{AEE}\cdot\text{kg}^{-1}$) during the BT season. The hypothesis was that the less fit conscripts would experience more physiological stress during physical activity, and that aerobic fitness would not be related to $\text{AEE}\cdot\text{kg}^{-1}$. Our contrary findings might partly be explained by the more intense participation and engagement of fit conscripts in military training. Moreover, in field situations the fit soldiers were often called upon to perform more military tasks. In support of this theory, endurance-trained cyclists have been found to have a significantly

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2 greater energy cost of cycling at the same relative intensity than untrained, less fit controls.
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4 This was due to the lower required workload of the less fit subjects to increase their heart rate
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6 to 75% of maximum, and thus their energy expenditure was also low (Horton et al., 1994). In
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8 contrast, during high intensity activities, the low absolute work capacity of the least-fit
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10 subjects might limit their work ability.
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14 The strength of the present study was the use of a regression-based approach to remove the
15
16 confounding influences of BM or FFM, rather than a standard ratio approach to express
17
18 VO_2max values relative to BM (ml/kg/min). The ratio method could lead to a significant and
19
20 positive intercept, resulting automatically in higher values for lighter subjects (Toth et al.,
21
22 1993). In the present study, the intercept for the regression of VO_2max with BM was
23
24 significantly different from zero ($p < 0.01$), but with FFM it was not. The best predictor of
25
26 VO_2max (l/min) was FFM with the variation of 75% ($r = 0.87$, $p < 0.001$) compared to BM with
27
28 the respective variation of 35% ($r = 0.60$, $p < 0.01$). We therefore adjusted VO_2max for FFM.
29
30 Very few studies have used DLW to estimate the relationship between AEE or EE and
31
32 VO_2max (Ambler et al., 1998; Brochu et al., 1999). Our results are well in line with the study
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34 of Brochu et al. (1999), where they found a significant relationship between VO_2max and
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36 AEE in older individuals. The total EE in this study was similar ($15.5 \text{ MJ}\cdot\text{d}^{-1}$) to that
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38 measured on hilly terrain with temperatures ranging from -1.1 to $16.1 \text{ }^\circ\text{C}$ ($14.24 \text{ MJ}\cdot\text{d}^{-1}$)
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40 (DeLany et al., 1989), and to a group of soldiers working in the garrison (Mudambo et al.,
41
42 1997). However, the present EE was lower than that measured in jungle warfare (Forbes-
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44 Ewan et al., 1989), in a winter military training course (Hoyt et al., 1991; Jones et al., 1993),
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46 and in a commando field exercise in a hot and dry climate (Mudambo et al., 1997). The mean
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48 ($\pm\text{SD}$) total energy expenditure of all male military personnel ($N=424$) is reportedly $19.3 \pm$
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50 $2.7 \text{ MJ}\cdot\text{d}^{-1}$ across all activities, military occupational specialties, and environments (Tharion et
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1
2 al., 2005). Comparisons in EE between the present study and other studies should be made
3
4 with caution. For example, there are possible differences in the body mass of the subjects and
5
6 in the combat gear that was carried, both of which affect the total energy expenditure.
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10 In the multivariate regression analysis, only BMI remained in the model, although the
11 correlation and one-way analysis revealed that also positive energy balance and good fitness
12 level were related to higher activity ($\text{AEE}\cdot\text{kg}^{-1}$). In other words, the slimmer the subject, the
13
14 smaller the loss of BM. This also suggests that more active subjects exhibit greater levels of
15
16 aerobic fitness. This was partly explained by the fact that under-eating (negative energy
17
18 balance) was negatively related to BMI. Thus, especially the subjects with higher BMI were
19
20 more prone to lose body mass than their slimmer counterparts. Even though negative energy
21
22 balance was not significant (-5.6%) at the group level, the individual variation in energy
23
24 balance ranged from -66.3 to 41.7%. Based on Figure 1, one third of subjects had a negative
25
26 energy balance. However, in the present subjects, of whom one third ($n=8$) had a BMI over
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28 25, the acceptable tolerance limits for serious under-eating classification is critical. The
29
30 positive relationships observed between TrueEI and EE (Figure 1), and between energy
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32 balance and $\text{AEE}\cdot\text{kg}^{-1}$ are well in line with other studies, where low EI in males was found to
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34 be related to low EE (DeLany et al., 1989; Thompson et al., 1995), as well as to spontaneous
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36 physical activity (Thompson et al., 1995) when compared to an adequate EI group. Moreover,
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38 it has been shown that EE and AEE increase simultaneously with an increase in EI, which
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40 might be partly a reflection of EI to BMR (Klein & Goran, 1993). However, Castellani et al.
41
42 (2006) presented a contrary conclusion. They found that during a short period (54 hours) of
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44 physical loading, recruits were able to maintain high EE and PAL despite sustaining
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46 substantial energy deficits.
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2 The PAL value of 2.0 obtained during the present wintertime BT is comparable to values
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4 from people performing very strenuous work or daily competitive athletic training (Nordic
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6 Nutrition Recommendations, 2004). In addition, some subjects also ate less than required. If
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8 these stressors, together with other stressors, affect soldiers for a longer period, overtraining
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10 symptoms may occur (Meeusen et al., 2006). If the BMI is used as an index of body size, it
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12 was not associated with AEE, but was negatively related to $AEE \cdot kg^{-1}$ and positively to BMR
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14 (Table 4). Our findings showed further that BMR did not have any relationship with VO_2max ,
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16 but it was negatively related to $AEE \cdot kg^{-1}$. Among children it has been observed that AEE and
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18 EE tend to increase more with increasing body size than BMR (Ekelund et al., 2002).
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20 However, this was not seen in the present study, in which most military tasks were performed
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22 at low-to-moderate intensity levels. This meant that the least-fit subjects were able to perform
23
24 those tasks well.
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28 An additional purpose of the present study was to investigate the accuracy of a pre-filled food
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30 diary. Great care was taken to evaluate energy intake as correctly as possible without food
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32 weighing. In spite of the careful guidance and control, mis-recording was as high as -20% and
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34 EI was only 74% of EE. It has been found that subjects who accurately reported their food
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36 intake at the first attempt were also able to do so at the second attempt (Goris et al., 2001). On
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38 the other hand, it is known that accuracy will decrease if the minimum recording time of
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40 seven days is extended. This happens due to a decline in motivation in keeping the food diary
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42 (Goris et al., 2001). The calculation of under-eating, mis-recording and an estimate of true
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44 energy intake were all based on changes in body energy stores, in which the energy content of
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46 30 MJ corresponds to 1 kg of body mass. However, the mass ratio of 30 MJ / 1 kg for storage
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48 or mobilization of energy between FM and FFM might differ individually, affecting the
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50 present results. We could have measured changes in body energy stores based on changes in
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1
2 FM and FFM, but two weeks may be too short a period to measure these parameters with
3 sufficient accuracy. A more accurate method to estimate mis-recording and to calculate true
4 energy intake involves a measure of water balance (Goris & Westerterp, 1999), but this
5 method still requires the recoding of food and fluid intake, which are both prone to mis-
6 recording (Goris & Westerterp, 1999; Goris et al., 2001; Westerterp & Goris, 2002; Tharion
7 et al., 2005).

16 PERSPECTIVES

17
18 The fitness level of Finnish young men has decreased with a concurrent increase in body mass
19 (Santtila et al., 2006). Consequently, some conscripts entering compulsory military service
20 have a low fitness level. However, the same program has to be performed by all conscripts in
21 the military environment. The goal of physical training during the basic training season is to
22 increase soldiers' aerobic fitness and muscle strength to prepare them for demanding military
23 tasks. This leads to the question of whether fitness or body size affects the ability to train. In
24 this study the training load was assessed by relating activity energy expenditure to body mass
25 (AEE·kg⁻¹). The subjects with low VO₂max, high BMI and negative energy balance were
26 vulnerable for low AEE·kg⁻¹, but from these three factors only BMI remained in the
27 multivariate regression model. This indicates that overweight may limit the most the high
28 intensity physical training. In order to enhance physical fitness in a group of conscripts who
29 exhibit variable fitness levels, these results suggest that conscripts could be divided into
30 training groups simply based on BMI, rather than aerobic fitness level or a combination of
31 these parameters.
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Another issue is the importance of energy balance in sustaining a high training load. The results confirm that during BT, physical performance is affected by aerobic fitness and BMI

1
2 as well as by energy balance. Initially, BMI has to be taken into consideration. Thereafter,
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4 energy balance should be optimized to facilitate physical performance, and to allow a high
5
6 training load to be sustained during the BT season. This situation is comparable with that of
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8 athletes: without adequate energy intake, high level training is not possible. Thus, because
9
10 energy balance influenced $AEE \cdot kg^{-1}$, conscripts should be advised about adequate energy
11
12 intake, especially during the most strenuous training period.

13 14 15 16 Acknowledgements

17
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19
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FIGURE LEGENDS

FIGURE 1. Relationship between estimated true energy intake (TrueEI) and energy expenditure (EE). The line represents the energy balance.

FIGURE 2. AEE·kg⁻¹ in Low, Moderate and High BMI (A), fitness (B) and under-eating (C) groups. Significant difference as compared with: Low BMI and under-eating ## p<0.01, Moderate BMI and under-eating ** p<0.01, High fitness # p<0.05, Moderate fitness * p<0.05.

PROOF

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3 The list of changes performed with regard to the manuscript (SJMSS-O-028-08.R1) entitled "Aerobic
4 fitness, energy balance and BMI are associated with training load assessed by activity energy
5 expenditure"
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8
9 We thank all reviewers' of valuable comments, which have improved greatly our manuscript and
10 concentrated our mind to present results more clearly. We appreciate the reviewers' patience and
11 hopefully the manuscript fulfills the requirement of SJSSM.
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14 15 **Reviewers' Comments to Author:**

16 17 **Reviewer: 1**

18 The original criticism of an excessive reliance on the regression-based analysis remains. Correlations
19 do not necessarily explain physiology or define causality. The response that "the correlation and
20 regression-based analysis are acceptable as far as we understand. Thus, we have done no changes in
21 this regard." is inadequate. Statements such as "The negative relationship between BMI and AEE•kg-1
22 indicates that high-level training may not be possible in overweight conscripts. Furthermore, optimal
23 energy balance facilitates physical performance, and enables high training loads to be sustained during
24 the BT season." are unsupported.
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28 **Answer:** We completely agree with your comment. Therefore, we have modified the text as you
29 suggested. We have added the one-way ANOVA analysis to examine the effects of BMI,
30 fitness and energy balance on AEE•kg⁻¹.
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33 **Reviewer: 2**

34 Reviewer 1 (My review).

35 Comment 6. Clarification on how physical training and fluid intake were kept the same was needed.
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38 The authors state that physical training and fluid intake was kept the same. This seems to be clarified.
39 However, I am puzzled how and why the investigators could keep physical training constant the day
40 preceding a measurement but at the same time did not try to control activities overall. See Comment 3
41 from Reviewer 2 where the authors agree that the more fit were doing more work than the less fit.
42 Given that some control was in place from this comment and one of the major goals of the study was to
43 see if energy balance explained AEE why then was not AEE controlled for?
44
45

46 **Answer:** We have modified the text according to your comment. "The physical activities in the daily
47 program was planned to be light a preceding day each measurement" (P 8, the paragraph
48 "Anthropometry and body composition"). However, the overall AEE was not controlled,
49 because our purpose was to evaluate whether the conscripts intake enough energy, and if not,
50 has energy balance an effect on AEE.
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53 Comment 9 and 10. Problems understanding Figure 2. The authors state the purpose of this study was
54 to determine the importance of energy balance and body size as well as VO₂ max on AEE. kg-1. The
55 response that Figure 2 illustrates better than just words is correct. However, only 33% of the variance is
56 explained. Because the correlation was significant does not make it overly meaningful. Furthermore, it
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appears that a more appropriate analysis or at least a supplementary analysis would be to do an ANOVA to see if significant differences in AEE kg^{-1} exist between the three BMI groups created. The authors make one of their main conclusions in the abstract from this correlation analysis. Correlation is not causality!

Answer: Thank you of your valuable comment. We agree and have added one-way ANOVA to compare AEE $\cdot\text{kg}^{-1}$ with BMI, fitness and energy balance groups. We had 24 subjects, which is a good number for the used expensive DLW method. If we had divided our subjects into the median of BMI and fitness and further into the basis of the median division, thus four groups (fit & fat, fit & unfat, unfit & fat, unfit & unfat) would have given only 6 subjects for each group. From the statistical point of view this would have not been enough. Thus we decided to compare BMI, fitness and energy balance groups ($n=8$ / group). In the re-revised manuscript, we have modified the text as you suggested.

When trying to come up with predictors we see from Model 1 that there is an adjusted r-square of .33 for BMI. As an aside, why is VO₂maxFFM and Energy Balance even listed since they are not contributors to this model? They should be deleted. From Model 2 we see that Energy Balance and VO₂max together have an adjusted r-square of .40. But that does not mean now that 73% of the variance in AEE $\cdot\text{kg}^{-1}$ is explained by these three factors. There are collinearity factors with these 3 predictors which is one reason I presume why all three predictors were not put into one equation. But in the abstract the authors mislead the reader because they separate out the correlations of each dependent measure. This is not correct. The authors should use the best regression equation they can come up with as the prediction equation with all the assumptions met and state that as the result. Furthermore, since they state that they are using regression to predict AEE $\cdot\text{kg}^{-1}$ (which is the main use of regression) the equation should be verified on another sample and/or developed on part of this sample and verified on a hold-out sample. Also, should the unit of measure for VO₂maxFFM ($\text{l}\cdot\text{min}^{-1}$) be per kg of FFM? The authors in my opinion did not adequately address Comments 9 and 10.

Answer: You are right. We have rewritten Table 5 and deleted VO₂maxFFM and Energy Balance from Model 1 since they are not contributors to this model. There really were collinearity factors with 3 predictors in Model 2, which was the reason why all three predictors were not forced into one equation. Thus, we deleted this model. In our study, the regression was used to identify the predictors of AEE $\cdot\text{kg}^{-1}$ as well as to evaluate the interaction of the variables on AEE $\cdot\text{kg}^{-1}$. That is why there was no need for verifying the equation. We have also modified the text as you suggested and changed the text in the abstract not to mislead the reader.

The strength of the present study was the use of a regression-based approach to remove the confounding influences of BM or FFM. The ratio method could lead to a significant and positive intercept, resulting automatically in higher values for lighter subjects (Toth et al., 1993). In the present study, the intercept for the regression of VO₂max (l/min) with FMM was not significantly different from zero ($p>0.05$) (Discussion, page 13). Thus, indeed we could have used VO₂max $\text{ml}/\text{FFMkg}/\text{min}$. However, to be as precise as possible, we decided to use FFM adjusted VO₂max (l/min), which is suggested by Toth et al. (JAP1993: 75: 2288-2292) and used in previous studies when energy expenditure has been evaluated (Brochu et al. J Clin Endocrinol Metab. 1999: 84: 3872-3876.).

Reviewer 1 (Other Reviewer's Review).

Comment 1. Author's overstate their conclusions. Recommendation to authors is that they temper their conclusions.

Authors made no changes. This is an unacceptable response to this well-founded recommendation. As this reviewer mentions and I mention above, the authors' conclusions are based on correlations. Correlations are not causes. The sentence in the abstract "During wintertime BT AEE . kg-1 is affected by" is attributing cause to correlation results. This is not appropriate and bothersome that the authors did not take this criticism more constructively.

Comment 3. Comment was that more fit soldiers did more work. Authors acknowledge this point, but one of the main conclusions was that high level training may not be possible in overweight conscripts. The authors ignore the point this reviewer was making. From what I understand, perhaps the less fit soldiers chose to do less or were assigned to do less work. Given that work intensity was not controlled for, as the authors acknowledge, how can they determine what was a limit as opposed to what just happened or what work level was assigned to various individuals. It also seems suspect that the investigators had some control over what was performed (see Author's response to Reviewer 1's Comment 6) but overall work level was not controlled for. The overall impact of this is that again as this reviewer states, the conclusions are too far-reaching for the results of these analyses.

Answer: We completely agree with your comment. Therefore, we have modified the text of the abstract, conclusion and perspectives. We have added the one-way ANOVA analysis to examine the effects of BMI, fitness and energy balance on AEE·kg⁻¹.

TABLE 1. Physical characteristics of the subjects (N=24). VO₂max refers to maximal oxygen uptake.

| | Mean | ± | SD | Range |
|---|------|---|------|--------------|
| Age (yr) | 19.6 | ± | 0.2 | 19.1 - 20.1 |
| Height (cm) | 171 | ± | 7.0 | 155 - 178 |
| Body mass (kg) | 77.6 | ± | 14.9 | 56.3 - 111.3 |
| Body mass index (kg·m ⁻²) | 24.3 | ± | 3.8 | 18.3 - 32.2 |
| Fat free mass (kg) | 59.7 | ± | 9.4 | 42.4 - 72.4 |
| Fat mass (kg) | 18.0 | ± | 9.7 | 5.5 - 44.0 |
| Fat % | 22.1 | ± | 7.9 | 9.4 - 39.5 |
| VO ₂ max (l·min ⁻¹) | 3.6 | ± | 0.6 | 2.4 - 4.5 |
| VO ₂ max (ml·kg ⁻¹ ·min ⁻¹) | 47 | ± | 7 | 30 - 58 |

TABLE 2. The experimental protocol. BMR refers to basic metabolic rate.

| | <i>Days before DLW</i> | <i>Days during DLW assessment period</i> | | | | | | | | | | | | | | | | |
|--------------------------|----------------------------|--|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | |
| BMR measure | -28... -1 | | | | | | | | | | | | | | | | | |
| VO ₂ max test | - 9 ... - 5 | | | | | | | | | | | | | | | | | |
| DLW dose | | x | | | | | | | | | | | | | | | | |
| Urine sample | | x | x | | | | | | | x | | | | | | | x | |
| Body composition | | x | x | | | | | | | x | | | | | | | x | |
| Dietary record | | | | x | x | x | | | | x | x | | x | | | x | x | |
| Overnight field exercise | | | | x | x | x | x | | | | | | | | | | | |
| Skiing march | | | | | | | | | | x | | | | | | | | |
| Shooting exercise | | | | | | | | | | x | | | | | | | | |
| Combat shooting exercise | | x | x | | | | | | | | | | | | | | | |
| Combat training | | | | | | | | | | | | | | | | | | x |
| March test | | | | | | | | | | | | | x | | | | | |
| Weekend leave | | | | | | | | | | | | | | x | x | | | |

TABLE 3. Energy expenditure (EE), its components and energy intake (EI) variables in 24 conscripts.

| | Mean | ± | SD | Range |
|--|-------|---|---------------------|---------------|
| EE (MJ·d ⁻¹) | 15.48 | ± | 1.65 | 12.75 – 18.51 |
| AEE (MJ·d ⁻¹) | 6.26 | ± | 1.21 | 4.18 – 8.94 |
| AEE·kg ⁻¹ (MJ·d ⁻¹ ·kg ⁻¹) | 0.08 | ± | 0.02 | 0.04 – 0.13 |
| PAL ^a | 2.03 | ± | 0.21 | 1.68 – 2.47 |
| BMR (MJ·d ⁻¹) | 7.66 | ± | 0.92 | 5.50 – 9.44 |
| EI (MJ·d ⁻¹) ^{***} | 11.52 | ± | 3.23 ^{***} | 6.73 – 17.77 |
| Estimated true EI (MJ·d ⁻¹) | 14.62 | ± | 4.17 | 4.90 – 22.35 |
| Underreporting (%) | -26.0 | ± | 17.5 | -52.4 – 5.6 |
| Under-eating = energy balance (%) | -5.6 | ± | 25.5 | -66.3 – 41.7 |
| Mis-recording (%) | -20.4 | ± | 26.3 | -82.9 – 29.5 |

AEE, activity energy expenditure; AEE·kg⁻¹, activity energy expenditure related to body mass; PAL, physical activity level; BMR, basal metabolic rate.

^a calculated as EE/BMR.

^{***} difference with EE (p<0.001).

TABLE 4. Pearson correlation coefficients between BMI, VO₂max and energy balance and EE, AEE, AEE·kg⁻¹ and BMR.

| | BMI (kg·m ⁻²) | VO ₂ max (l/min) | VO ₂ max _{FMM} (l/min) ^a | Energy balance (MJ·d ⁻¹) |
|--|------------------------------|--------------------------------|--|--|
| EE (MJ·d ⁻¹) | 0.30 | 0.77 *** | 0.29 | 0.29 |
| AEE (MJ·d ⁻¹) | -0.70 | 0.44 * | 0.44 * | 0.21 |
| AEE·kg ⁻¹ (MJ·d ⁻¹ ·kg ⁻¹) | -0.60 ** | -0.16 | 0.41 * | 0.58 ** |
| BMR | 0.58 ** | 0.66 *** | -0.10 | -0.27 |

p<0.05, ** p<0.01, ***p<0.001

EE, energy expenditure, AEE, activity energy expenditure; AEE·kg⁻¹, activity energy expenditure related to body mass; BMR, basal metabolic rate.

^a VO₂max adjusted by fat free mass by using a regression-based approach (Toth et al., 1993).

TABLE 5. Multivariate regression model for explaining the variation in $AEE \cdot kg^{-1}$ ($MJ \cdot d^{-1} \cdot kg^{-1}$).

| Prediction equation | Adjusted R^2 | SEE | p |
|--|----------------|------|-------|
| $AEE \cdot kg^{-1} = 0.165 - (BMI \times 0.003)$ | 0.33 | 0.02 | 0.002 |

$AEE \cdot kg^{-1}$, activity energy expenditure related to body mass.

PROOF

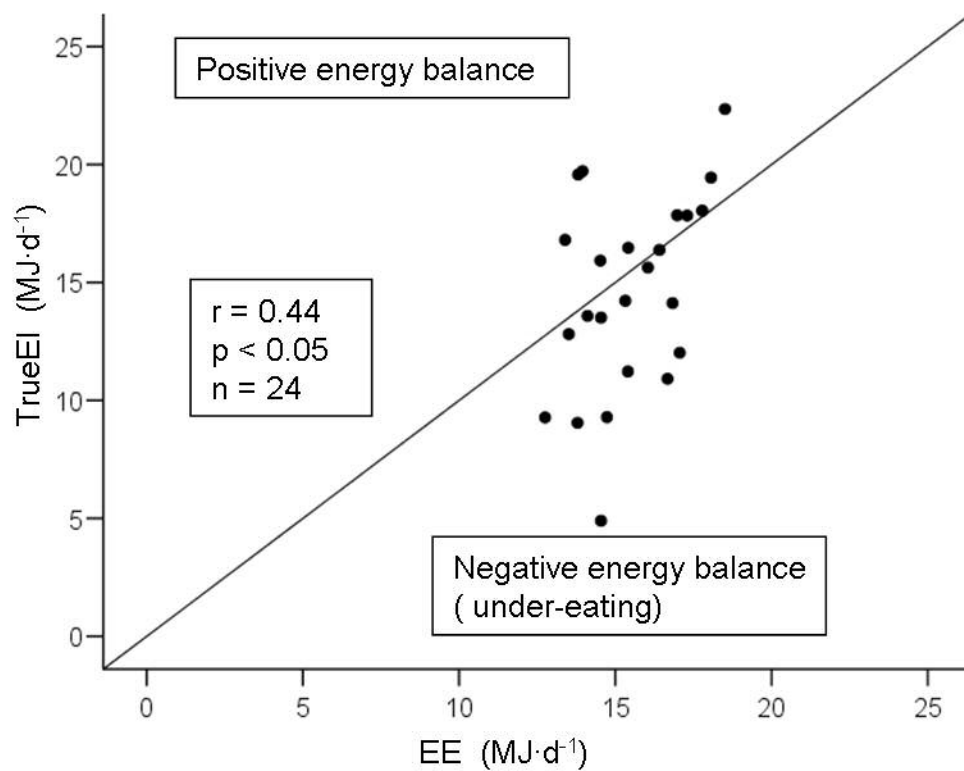


FIGURE 1. The relationship between estimated true energy intake (TrueEI) and energy expenditure (EE). The line represents the energy balance.

132x105mm (150 x 150 DPI)

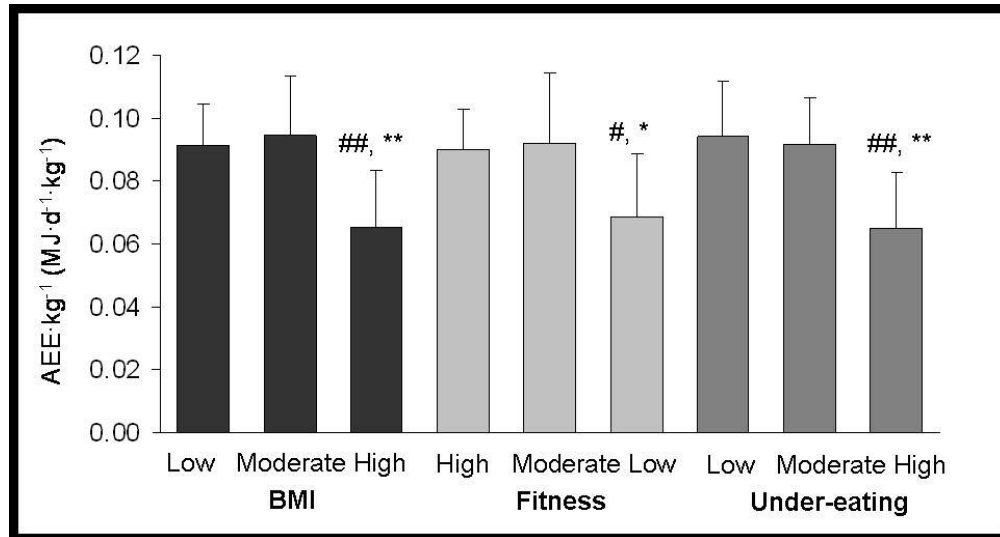


FIGURE 2. AEE \square kg⁻¹ in Low, Moderate and High BMI (A), fitness (B) and under-eating (C) groups. Significant difference as compared with: Low BMI and under-eating ## p<0.01, Moderate BMI and under-eating ** p<0.01, High fitness # p<0.05, Moderate fitness * p<0.05.

162x87mm (150 x 150 DPI)