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Effects of Military Training on Aerobic Fitness, Serum Hormones, Oxidative Stress and Energy Balance, with Special Reference to Overreaching

JYVÄSKYLÄN YLIOPISTO

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ABSTRACT

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In Finnish compulsory military service, all conscripts have to perform the same physical training program. This may cause some conscripts to exceed their capacity to tolerate the physical load imposed and hence contribute to overreaching (OR) in a multi-stressor military environment. Therefore, this study evaluated the training load of an 8-week Finnish military basic training (BT) period during wintertime and the incidence of OR. A further aim was to investigate the associations between physical fitness, energy balance, fatness, mood state and training load during BT and during an 8-day intensive training course (TC) with easy-to-use energy supplementation.

The results showed that the physical training load (≥ 4 MET) of BT is two hours per day with a half hour rest, such as a daily nap (≤ 1.0 MET), and is comparable to values found in persons performing strenuous work or daily competitive athletic training. During the first 4 weeks of BT, positive training responses as indicated by improved aerobic fitness and decreased oxidative stress, were observed. In the second half of BT, increased somatic symptoms of OR and several physiological and biochemical markers indicated that the training load was not well tolerated by all the conscripts, 33% of whom were classified as OR. OR subjects did not have increased fat mass or lower fitness before BT. Instead they showed increased oxidative stress and concentration of serum sex hormone binding globuline at rest. During BT, the OR subjects had higher physical activity than the noOR subjects. Moreover, the results confirm that markers of oxidative stress, serum cortisol, testosterone/cortisol -ratio at rest and maximal lactate/RPE -ratio could be useful tools for monitoring whether military training is too stressful. Of the three factors of energy balance, fitness and fatness, fatness limited the most high-level training while fitness protected from sick leave. TC did not have an adverse effect on physical performance. Although energy supplementation improved positive mood state, it did not prevent energy deficit or influence physical activity or performance. However, negative mood state affected energy intake already before TC.

In conclusion, this thesis provides new evidence of the vulnerability of the conscripts to OR and supports the promotion of optimal energy intake to enhance performance during a strenuous military training period.

Keywords: training monitoring, physical activity, biochemistry, aerobic capacity, energy balance, exercise testing, military, male, young adult

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ABBREVIATIONS

AEE	activity energy expenditure
AEE·kg^{-1}	activity energy expenditure related to body mass
BMI	body mass index
BT	8-week basic training
CHO	carbohydrate
DLW	doubly labelled water
DIT	diet-induced thermogenesis
EE	energy expenditure
EI	energy intake
GSSG	oxidized glutathione
HR	heart rate
IGF-1	insulin-like growth factor
La	blood lactate
MET	metabolic equivalents
MVPA	moderate to vigorous physical activity, ≥ 4 MET
OR	overreaching
ORAC	oxygen radical absorbance capacity
OTS	overtraining syndrome
PA	physical activity
PAL	physical activity level
RER	respiratory exchange ratio
RNS	reactive nitrogen species
RPE	rating of perceived exertion
ROS	reactive oxygen species
VLPA	very light to light activity, 1.0-3.9 MET
TGSH	total glutathione
TC	8-day training course
T/C –ratio	testosterone/cortisol ratio
URTI	upper respiratory tract infection
VO$_2$max	maximal oxygen uptake

LIST OF ORIGINAL PUBLICATIONS

This thesis in based on the following original articles, which are referred to in the text by their Roman numerals.

I Tanskanen MM, Kyröläinen H, Uusitalo AL, Huovinen J, Nissilä J, Kinnunen H, Atalay M, Häkkinen K (2011). Serum sex hormone-binding globulin and cortisol concentrations are associated with overreaching during strenuous military training. J Strength Cond Res 25:787-797.

II Tanskanen MM, Uusitalo AL, Kinnunen H, Häkkinen K, Kyröläinen H, Atalay M (2011). Association of military training with oxidative stress and overreaching. Med Sci Sports Exerc 43:1552-1560.

III Tanskanen M, Uusitalo AL, Häkkinen K, Nissilä J, Santtila M, Westerterp KR, Kyröläinen H (2009). Aerobic fitness, energy balance, and body mass index are associated with training load assessed by activity energy expenditure. Scand J Med Sci Sports 19:871-878.

IV Tanskanen M, Westerterp KR, Uusitalo AL, Häkkinen K, Atalay M, Kinnunen H, Kyröläinen H (2012). Effects of easy-to-use protein-rich energy bar on energy balance, physical activity and performance during 8 days of sustained physical exertion. PLos ONE, 7:e47771

FIGURES

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1 INTRODUCTION

The physical performance, especially cardiovascular fitness of young men entering Finnish compulsory military service, has declined, along with a concomitant increase in body mass during the last 25 years (Santtila et al. 2006). The same phenomenon has been observed, for example, in the Norwegian (Dyrstad et al. 2005) and German (Leyk et al. 2006) compulsory armies and in the USA professional army (Knapik et al. 2006). Furthermore, approximately 14% of conscripts interrupt their compulsory military service in Finland within the first month (Defence Command 2012). In Germany, the failure rates of male volunteers significantly increased after 2001, and 37 % of the subjects failed to pass the physical fitness test (Leyk et al. 2006).

In competitive sports and military training the goal of the training is to improve performance. However, both prolonged endurance and strength training at high intensity or volume together with inadequate recovery can lead to overreaching (OR) and may further lead to the development of overtraining syndrome (OTS) (Halson and Jeukendrup 2004). Both OR and OTS are characterized by an unexpected decrease or at least stagnation in performance capacity despite increased or sustained training load. The time required for recovery from OR ranges from several days to several weeks, whereas the development of OTS and recovery from it may take a much longer time.

There is some evidence to suggest that the development of OR or OTS is not solely related to the stress of physical training load, but non-training mental stressors like lack of support (Kenttä et al. 2001), lack of motivation (Kenttä and Hassmen 1998; Meehan et al. 2002), daily hassles (Meehan et al. 2002) and external expectations (Matos et al. 2011) also play a role (Figure 1a). In competitive sport, food and sleep are enjoyed ad libitum and social life is not restricted. In contrast, military training also involves the stressors of sleep deprivation and energy availability, as well as stressful environmental factors like working, living and sleeping outdoors and, occasionally, restricted social life (Figure 1b).

Conscripts entering military service are intensively exposed to non-training stressors due to a totally unfamiliar environment and change in their working conditions. This is supported by the study by Huovinen et al. (2009), in which a stress was defined as heart rate variability. They observed that the environmental stress induced by the first week of BT varied between individuals: some conscripts experienced a decrease in stress from the first day to the end of the first week, some showed no change and some experienced an increase in stress. Furthermore, many conscripts have a low fitness level at the beginning of military service which cannot meet the demands of basic military training. Most of these young men will have their first experience of demanding physical training, eating outdoors, and performing overnight exercises in a forest. In addition, the physical training involved in military service may exceed their previous training level, which could contribute to OR in the multi-stressor military service environment.

The effect of the Finnish 8-week basic military training (BT) program on physical fitness and performance among conscripts, who are known to exhibit large inter-individual variations in physical fitness levels, needs further research. Therefore, this thesis aimed to clarify the incidence of OR and the associations between conscripts' maximal aerobic fitness, energy balance, body fatness and physical activity during BT and also during a shorter, 8-day intensive training course (TC).

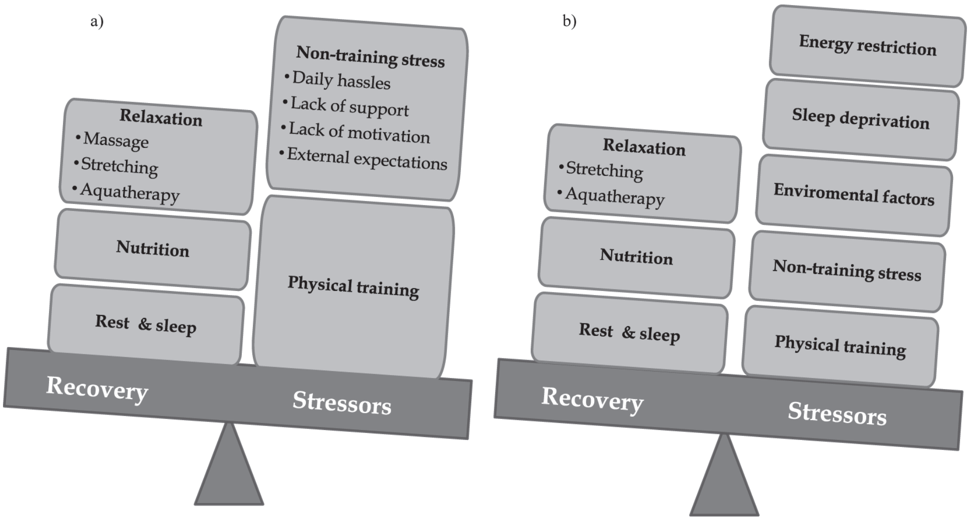

FIGURE 1 The reasons for overreaching and overtraining in a) competitive sport and b) in military training. The sizes of the boxes are for illustrative purposes only.

2 REVIEW OF THE LITERATURE

2.1 Military training and physical performance

In Finland basic military training (BT) takes up the first eight weeks of military service. The main purpose of BT is to enhance conscripts' physical and mental performance for the subsequent more demanding military training. Physical training includes physical activities such as marching, combat training and sport related physical training. At the beginning of BT, the amount of physical training is planned to be approximately two hours per day, increasing to 3-4 hours during weeks 4 to 7. The intensity of physical activity in the daily program is low during the first week and increases thereafter. During the BT period, practical skills are trained daily. Rifle marksmanship in the garrison is performed twice a week throughout the BT period. Garrison training involves theoretical education in classroom settings, material handling, shooting and general military education, such as close order drills. The conscripts also carry combat gear weighing 20 kg including clothing, particularly during marching and combat training. The BT schedule includes four longer (from two to eight hours) marching exercises with battle equipment. However, daily outdoor exercise, including transportation, often takes the form of marching. Conscripts march four times per day (approximately 5 km in total) for meals, and sleep in dormitory-type rooms. (Training Division, Finnish Defence Forces, 2004).

Finnish military science divides physical performance in the into two main components: aerobic fitness (endurance) and muscular fitness (maximal muscle strength and muscle endurance). Military duties mainly involve aerobic-type activities, and thus physical training during BT in Finland consists mainly of endurance training at a low intensity level. Muscle fitness training is mainly performed as muscle endurance-type training. (Training Division, Finnish Defence Forces, 2004). However, recent studies emphasize the role of concurrent endurance and strength training in order to improve physical performance (Kraemer et al. 2004b; Santtila et al. 2010; Santtila et al. 2008; Santtila et al. 2009a).

Finnish military training has been observed to improve aerobic (Mikkola et al. 2012a; Santtila et al. 2010) and muscular fitness (Cederberg et al. 2011; Santtila et al. 2012), reduce blood pressure (Cederberg et al. 2011) and to induce beneficial changes in body composition (Mikkola et al. 2009; Santtila et al. 2012) and lipid profile (Cederberg et al. 2011). In response to BT, some previous studies have reported improvement in aerobic fitness (Legg and Duggan 1996; Patton et al. 1980; Santtila et al. 2008), meaning that the training load has been optimal, the training program well planned and the stressors in balance with recovery. In some cases, improvement in aerobic fitness was observed only among a low initial aerobic fitness group (Dyrstad et al. 2006; Faff and Korneta 2000; Mikkola et al. 2012a; Rosendal et al. 2003; Santtila et al. 2008), indicating that the training was most effective for the least fit subjects, but too light for subjects with good fitness. In contrast, no improvement (Booth et al. 2006; Dyrstad et al. 2006; Faff and Korneta 2000; Legg and Duggan 1996) or even a decrease (Booth et al. 2006) in aerobic fitness has also been reported. This might indicate too light a training load, or imbalance between stressors and recovery, referred as OR or even OTS.

After BT, military training is continued as special training and finally unit training, both of which involve field exercises. In contrast to the BT training, the demanding military field training courses lasting from 2-3 days to 8-10 weeks, seek the upper limit of tolerance to multiple stressors. The stressors in field training vary, and include sleep deprivation, energy restriction, physical and mental loading and environmental challenges. In addition, the responses of physical performance to military field training depend on previous training experience and recovery time. Thus, for example, after a short 5-10 day field training period, aerobic fitness has been observed to show no change (Montain et al. 1997; Rognum et al. 1986), to improve (Hodgdon et al. 1991) and to decrease (Montain et al. 1997). Furthermore, anaerobic power has been reported to decrease (Nindl et al. 2002; Welsh et al. 2008) or to be unchangeable (Hodgdon et al. 1991).

2.2 OR, OTS and overload training

Multiple signs and symptoms have been associated with OR and OTS. However, there are no reliable and specific markers of impending OTS, and no knowledge on how to identify individuals who are more vulnerable to OR and OTS. Moreover, the precise mechanisms leading to OR and OTS have not yet been delineated. There are theories about the effect of cytokines (Robson 2003; Smith 2000; Steinacker et al. 2004), amino acids (Gastmann and Lehmann 1998), dysfunction of hypothalamus (Barron et al. 1985) and autonomic imbalance (Lehmann et al. 1998) on the pathophysiology of OTS. Impaired antioxidant capacity and increased oxidative stress have been associated with chronic fatigue syndrome (Fulle et al. 2000; Smirnova and Pall 2003) and may also be related to OTS (Finaud et al. 2006b; Tiidus 1998).

In order to monitor responses to training and to avoid OR and OTS, there is a clear need for reliable tools that facilitate the early diagnosis of OTS (Urhausen and Kindermann 2002). It should also be borne in mind, that physiological responses to overload training are often individual (Slivka et al. 2010; Uusitalo et al. 2000). On the other hand, well-trained athletes (Rowbottom et al. 1997; Slivka et al. 2010; Verde et al. 1992) and soldiers (Hoyt and Friedl 2006; Väänänen et al. 1997; Väänänen et al. 2004) may tolerate very high training volume and intensity without developing OTS, particularly when non-training stressors are limited.

Although the classification of OR and OTS is challenging, OTS has been documented in a wide variety of both endurance and strength training sports. Morgan et al. (1987b) reported that OTS developed in more than 48% of female elite distance runners at least once in their careers. Other prevalence studies demonstrated that OTS developed in 21% of the Australian swimming team after 6 months of training for a national competition (Hooper et al. 1993; Hooper et al. 1995). Kenttä et al. (2001) investigated 287 young Swedish elite athletes from various sports, and 37% of the athletes reported having experienced OTS (significant performance decrement due to physical training) at least once in their career in sports. In England, 29% of a sample of 376 young athletes in various sports (Matos et al. 2011) reported having been OR or OTS. The incidence was significantly higher in individual sports, participants in which also reported a higher number of training hours compared to team-sport athletes. However, training years was not a significant predictor of OR or OTS.

The following review of OR and OTS focuses on intensified overload training intervention and follow-up studies in which training volume, intensity or both of these increased during the training season or the subjects were exposed to a previously unaccustomed training load. The typical duration of overload training has been from two to four weeks after familiarization training and is coded as *"typical"*. A training period from four to ten days is only included, if it has been extremely strenuous and is coded as *"short-term"*, while more than four weeks is coded as *"prolonged"*. The observed responses to overload training depend on the duration of the intensive training period and also on the type of the training, such as endurance (Lehmann et al. 1991; Uusitalo et al. 1998a) and strength (Fry et al. 1998; Häkkinen et al. 1989; Häkkinen et al. 1985) training, which stimulate different responses. In addition, the training background of the subjects (amateur, recreational, well-trained or professional highly-trained athlete) has an influence on training responses. Since military training and duties mainly consists of endurance-type activities and occasionally strength training such as carrying extra loads, only endurance and concurrent (combined endurance and strength) overload training studies are included.

2.2.1 OR and OTS in the military environment

Only two military studies have reported on OR or OTS as an outcome. Chicharro et al. (1998) found a 24% incidence of OTS among young men who performed an 8-week BT period in the Spanish Army. The criteria for OTS were a 30% decrease in serum free testosterone/cortisol ratio, a classical criterion for OTS (Adlercreutz et al. 1986; Coutts et al. 2007a; Mäestu et al. 2005). Additionally, OTS was associated with a decrease in performance (i.e., isometric strength, vertical jump, anaerobic power) and increase in serum cortisol concentration. However, at the beginning of BT there were no differences between the OTS and noOTS subjects in VO_2max, although the OTS subjects had lower anaerobic power in the Wingate test.

Another study by Booth et al. (2006) followed a 6-week BT in the Australian Army. The prevalence of sick-leaves was 8 % during BT, and the most common reasons were muscle soreness and symptoms of upper respiratory tract infection (URTI). Based on an increase in negative psychological symptoms and a decrease in serum free testosterone/cortisol ratio, immune function and aerobic performance, the authors concluded that OTS existed.

In general, response to BT has mostly been reported on the group level and omitting the dropouts. In addition, the main purpose of strenuous military field training studies is to evaluate the upper limit of toleration to multiple stressors. Furthermore, the term 'overtraining' might have been used as a synonym for overuse injury (Bullock et al. 2011). Overuse injuries lead to sick leave and one reason for their occurrence is too exhaustive training load (Bullock et al. 2011). On the other hand, low performance is a risk factor for injury (Knapik et al. 2001a).

However, dropout rates during BT have been reported to be 14% in the Australian Army (Pope et al. 1999), 6% in the U.S. Air Force (Talcott et al. 1999), 13% in the U.S. Army (Knapik et al. 2001a) and 10% in the U.S. Marine Corps (Reis et al. 2007), which might indicate that training load has been too exhaustive and that OR or OTS exits in the military. Compared to those who completed BT, drop outs had higher injury rates and lower physical performance (Knapik et al. 2001a; Pope et al. 1999; Reis et al. 2007; Talcott et al. 1999), although the main reasons for drop out have been medical, psychological and behavioral. Interestingly, fatness is not necessarily among the major causes for discharge during the first year of active duty (Poston et al. 2002) and among high-fitness capacity soldiers low body fat was actually a risk factor for drop out during a 3-month BT period (Moran et al. 2011).

2.2.2 Physical performance and cardiovascular adaptation to overload training

A classical definition of OTS is "unplanned and unexpected decrement in performance, despite increased or maintained training load". Aerobic fitness, as measured by maximal oxygen uptake (VO_2max), is an important component of the physical fitness profile (ACSM 2001, 5-6). In addition, aerobic fitness has been found to have an influence on the ability to sustain the training load during BT (Jones and Knapik 1999), while low aerobic fitness increases the risk for injuries and illnesses leading to limited duty days during BT (Jones and Knapik 1999; Knapik et al. 2001b).

Physical performance

A decrease in VO_2max has been observed after a short-term (Hedelin et al. 2000a), typical (Halson et al. 2002; Jeukendrup et al. 1992; Snyder et al. 1995) and prolonged (Coutts et al. 2007b; Uusitalo et al. 1998b) overload training. In addition, two weeks' intensified training induced a decline in performance in a time-trial (Bosquet et al. 2001; Coutts et al. 2007c; Halson et al. 2003; Jeukendrup et al. 1992). However, in some cases progressively increased training volume leading to OR induced stagnation in VO_2max (Bosquet et al. 2001; Coutts et al. 2007c; Gastmann et al. 1998; Rietjens et al. 2005) or a decrease in VO_2max occurred after detraining (Bosquet et al. 2001; Gastmann et al. 1998). Also among race horses a 16-weeks periods of overload training was not associated with a decrease in VO_2max, although run time decreased. However, decreases in VO_2max occurred slowly during detraining (Tyler et al. 1996). Thus, time to exhaustion in an exercise test has been suggested to be a better indicator of decreased aerobic fitness than VO_2max (Coutts et al. 2007c; Halson and Jeukendrup 2004; Urhausen et al. 1998b). Furthermore, an anaerobic jump test has been found to be a sensitive indicator for detecting decrement in physical performance, when accompanied by sleep disruption and underfeeding, after short-term (Welsh et al. 2008) and prolonged military field training (Nindl et al. 1997).

Heart rate

Maximal heart rate (HR) has been observed to decrease after short-term (Hedelin et al. 2000a), typical (Bosquet et al. 2001; Bosquet et al. 2008; Jeukendrup et al. 1992; Lehmann et al. 1992; Verde et al. 1992) and after prolonged overload training (Bosquet et al. 2008; Coutts et al. 2007b; Urhausen et al. 1998b; Uusitalo et al. 1998b). In addition, short-term (Hedelin et al. 2000a), typical (Verde et al. 1992) and long-term (Uusitalo et al. 2000) intensive training decreased submaximal HR. However, the meta-analysis of HR, OR and OTS by Bosquet et al. (2008) suggests that submaximal HR cannot be considered as a valid sign of OR after short-term training. Instead, it may be useful in the detection of OR and OTS after long term training together with maximal HR and other objective signs and symptoms of OR and OTS.

However, a decrease in submaximal HR at submaximal work load and maximal HR have also been observed with concomitant improvement in VO_2max, indicating a positive training effect (Zavorsky 2000). It has been suggested that the decrease in maximal HR is due to exercise-induced hypervolemia leading to increased stroke volume and maintenance of cardiac output with lower HR or enhanced baroreceptor reflex function and reduced vasoconstriction of the blood vessels (Zavorsky 2000). In OR or OTS, the decrease in maximal HR might be due to decreased maximal work rates at the end of an incremental treadmill test (Coutts et al. 2007b), but it is also related to a decrease in sensitivity of the β-adrenoreceptors or a decrease in their number (Lehmann et al. 1998; Zavorsky 2000).

Blood lactate

Both optimal and overload training induce a right shift of the blood lactate curve, leading to possible misinterpretation of test results (Bosquet et al. 2001; Jeukendrup and Hesselink 1994; Snyder et al. 1993). A decrease in submaximal blood lactate (La) can result from decreased production or increased utilization by the muscle and other organs (Bosquet et al. 2001). The La response has been observed to be blunted with low glycogen stores, which may be the possible mechanisms for decreased La in OR and OTS (Costill et al. 1988a). However, in OR athletes a decrease in maximal La can also occur with normal muscle glycogen stores (Snyder et al. 1995). Thus, the capacity to mobilize the available substrate is a more critical factor than substrate availability (Bosquet et al. 2001).

Maximal post-exercise La has been observed to decrease after a typical overload training period (Bosquet et al. 2001; Jeukendrup et al. 1992; Lehmann et al. 1996; Snyder et al. 1993) as well after prolonged exhaustive endurance training (Urhausen et al. 1998b; Uusitalo et al. 2002). In addition, La during submaximal exercise has been found to be reduced after typical overload training (Bosquet et al. 2001; Jeukendrup et al. 1992; Lehmann et al. 1996; Snyder et al. 1995). In contrast, 6-week concurrent overload training did not change the La response to maximal exercise (Coutts et al. 2007b). Since the right shift of the La curve induced by optimal training is a result of improved lactate utilization, the main difference between the two conditions is the decrease in maximal post-exercise La during overload training (Bosquet et al. 2001).

RPE

The use of the Rating Scale of Perceived Exertion (RPE, from 6–20), established by Borg in 1970, has been described as a good indicator of physical stress and physical working capacity (ACSM 2001, 78-79). RPE has been found to increase due to typical overload training during submaximal exercise (Garcin et al. 2002; Halson et al. 2002). During an incremental maximal test Bosquet et al. (2001) found no changes in RPE after typical endurance-type overload training compared with before training. However, the differences were calculated at the relative intensities of the maximal test, and the absolute values were not reported.

La/RPE -ratio

According to the hypothesis by Snyder et al. (1993), a decrease in blood La concentration for a given exercise is accompanied by an increase in RPE during overload training, while RPE remains the same or decreases during optimal training. The La/RPE -ratio is thought to be an ideal indicator of training load, because it combines both physiological and psychological factors (Snyder et al. 1993). The maximal La/RPE -ratio after an incremental test to exhaustion has been shown to decrease after a high intensity training period (Bosquet et al. 2001; Snyder et al. 1995), but not necessarily at submaximal workloads (Bosquet et al. 2001). However, the ratio can be influenced by short-term changes in dietary carbohydrate (CHO) intake (Duke et al. 2011). Thus, it has been recommended that the La/RPE -ratio as an OR or OTS indicator should be used with a caution and together with monitoring of CHO intake (Duke et al. 2011).

2.2.3 Association of overload training with mood state and health status

Together with decreased physical performance, impaired mood state, subjective complaints and general health status have been consistently described as sensitive and early markers of OR and OTS in several reviews (Fry et al. 1991; Meeusen et al. 2006; Urhausen and Kindermann 2002; Uusitalo 2001).

Mood disturbance has been observed to increase after typical endurance-type overload training (Dupuy et al. 2010; Halson et al. 2003; Verde et al. 1992), after a short-term strenuous adventure race (Anglem et al. 2008) and in military training in association with sleep deprivation (Nindl et al. 2002) and at the stage of OTS compared to normal training (Hooper et al. 1995; Nederhof et al. 2008; Urhausen et al. 1998b). Furthermore, OR athletes have shown higher scores or *negative mood state* compared to noOR athletes (Tanskanen et al. 2010; Uusitalo et al. 1998b). Moreover, *somatic symptoms* increased with high training load (Bosquet et al. 2001; Coutts et al. 2007d) and *feelings of fatigue* after typical endurance-type overload training (Bosquet et al. 2001; Dupuy et al. 2010; Halson et al. 2003; Tanaka et al. 1997; Verde et al. 1992) and during a training season (Pierce 2002). In conclusion, mood disturbances seem to increase in a progressive manner with stepwise increases in training load. The relationship appears to be dose-dependent and reductions in training load are accompanied by improved mood state (Anglem et al. 2008; Fry et al. 1994; Hooper et al. 1995; Morgan et al. 1987a; Nederhof et al. 2008; O'Connor et al. 1989; Tanaka et al. 1997). The reasons for mood disturbances and fatigue are thought to be a consequence of increased cytokine production (Robson 2003) and changes in brain serotonin metabolism (Armstrong and VanHeest 2002). However, for cytokines the scientific evidence is lacking and for serotonin metabolism it is conflcting. Uusitalo et al. (2006) found no difference between noOR athletes and OR athletes in brain serotonin metabolism, while Budgett et al. (2010) found decreased serotonin metabolism in OR athletes compared to OR athletes.

Overload training has been reported to impair *general health status* (Anglem et al. 2008; Bosquet et al. 2001; Coutts et al. 2007d) and induce symptoms of *upper respiratory tract infection (URTI)* (Bosquet et al. 2001; Foster 1998; Mackinnon 2000; Verde et al. 1992). Furthermore, there is evidence that increased training load is a risk factor for increased incidence for URTI, while moderate training decreases the risk (Heath et al. 1991; Nieman 1994; Spence et al. 2007). The explanations for increased URTI during an intensive training period is suggested to be related to decreased total leukocyte and NK cell numbers, attenuated neutrophil function and lymphocyte proliferative capacity and salivary IgA concentration (Mackinnon 2000) as well as alteration in the concentration of stress hormones (Nieman 1994). Together with physical stress, psychological stress also alters immunity suggesting that the summative effects of both types of stress are cumulative and synergistic (Clow and Hucklebridge, 2001).

2.2.4 Association of overload training with anabolic and catabolic hormones

Although there are no specific markers of impending OTS, a potentially beneficial indicator of maladaptation is the hormonal response to training, which has been documented as a valid indicator of training status (Häkkinen et al. 1987). However, hormonal responses to training have been somewhat controversial depending on for example the study design, subject material and mode of training. Both hormone concentrations at rest (chronic adaptation) and during exercise (acute response) respond specifically to extensive endurance and strength training (Armstrong and VanHeest 2002). Generally, hormones which increase the rate of lipolysis, glycolysis, or gluconeogenesis will be elevated during exercise, and the magnitude of these hormonal alteration is not as great in trained as in untrained individuals (Bunt 1986). Furthermore, response to acute exercise depends on the intensity and duration of the exercise as well as hydration status before exercise (Hackney 2006; Maresh et al. 2006). It has been recommended that corrections for plasma volume changes should be taken into consideration, when sampling biochemical and hormonal parameters in blood following an acute bout of exercise, regardless of the exercise protocol and exercise mode performed (Kargotich et al. 1997). It has also been suggested that acute exercise-induced changes in serum hormones could be superior in monitoring training load and avoiding OTS when compared with resting hormone levels (Urhausen and Kindermann 2002; Uusitalo et al. 1998a). Finally, hormonal action depends on not only hormonal production and concentration in the blood, but also on the availability and sensitivity of target cell receptors, binding affinity and metabolic clearance rate (Bunt 1986).

Catabolic hormone: cortisol

Cortisol is a glucocorticoid secreted by the adrenal cortex and, among other functions, it increases gluconeogenesis, promotes fat mobilization and utilization, and protects the organism from an overreaction of the immune system in the face of exercise-induced muscle damage (Duclos et al., 2007). The circadian variation of cortisol has little day-to-day variation in the rate at which cortisol declines during the day within healthy, resting individuals (Thuma et al. 1995). However, there is a circadian variation in cortisol responses to exercise with responses being greater during the trough than during the peak, when calculated relative to the pre-exercise baseline (Thuma et al. 1995). Both physical and mental stress has an influence on serum cortisol concentrations; decreased cortisol levels have been found to be related to improved mood while increased levels depressed mood (Clow and Hucklebridge 2001).

Resting serum cortisol concentration has been observed to decrease after both a short-term (Hedelin et al. 2000a) and typical period of endurance-type overload training induced by an increase in training volume (Lehmann et al.

1992; Snyder et al. 1995), but not after an increase in training intensity (Lehmann et al. 1992). In contrast, military BT induced an increase in resting serum cortisol among OTS-classified soldiers (Chicharro et al. 1998) and conscripts with added strength training during BT (Santtila et al. 2009b) as well as after short-term (Hackney and Hodgdon 1991; Opstad 1992), typical (Diment et al. 2012; Kyroläinen et al. 2008) and prolonged (Nindl et al. 2007b) exhaustive military training with energy restriction and sleep deprivation. However, no change occurred in cortisol at rest during BT among noOTS soldiers (Chicharro et al. 1998) and conscripts who trained according to general command or with added endurance training (Santtila et al. 2009b). Furthermore, serum cortisol levels at rest failed to reflect OR or OTS among previously highly trained athletes after typical (Mackinnon et al. 1997; Tanaka et al. 1997) and prolonged (Coutts et al. 2007d) endurance type of overload training. Also, changes in resting cortisol levels did not differ between OR and noOR athletes during typical-endurance type (Mackinnon et al. 1997) or after prolonged concurrent overload training among highly trained rugby players (Coutts et al. 2007b) or amateur athletes (Moore and Fry 2007).

In response to acute exercise, a diminished cortisol response has been observed to maximal exercise (Halson et al. 2004; Lehmann et al. 1992) and to submaximal exercise (Halson et al. 2004; Verde et al. 1992) after a typical and prolonged (Uusitalo et al. 1998a) endurance-type overload training period induced by an increase in training volume but not intensity (Lehmann et al. 1992). In contrast, serum cortisol levels after a day-long march remained the same when compared to a preceding morning sample at rest during a 4-day march, with ad libitum food and water (Väänänen et al. 2002). The lower responsiveness to acute exercise has been hypothesized to be hypothalamus-pituitary-adrenal axis dysregulation during OTS leading to blunted response of ACTH and finally resulting in low levels of cortisol (Halson et al. 2004; Urhausen et al. 1998a).

Anabolic hormones: testosterone and IFG-1

Testosterone is a multifunctional anabolic hormone, which is synthesized and secreted by testicular under luteinizing hormone stimulation. Testosterone circulates in plasma non-specifically bound albumin and is specifically bound to sex-hormone-binding-globulin (SHBG), which in turn decreases bioavailable testosterone in plasma (Vermeulen et al. 1999). Like cortisol, testosterone also exhibits a circadian pattern, with early morning peaks and the lowest value in the evening (Veldhuis et al. 1987).

Serum testosterone concentration has been observed to decrease after concurrent overload training in amateur athletes (Moore and Fry 2007) as well as after short-term (Hackney and Hodgdon 1991; Opstad 1992), typical (Kyroläinen et al. 2008) and prolonged (Fortes et al. 2011; Nindl et al. 2007b) exhaustive military training with energy restriction and sleep deprivation. However, serum testosterone at rest remained the same during a 4-day march, with ad libitum food and water (Väänänen et al. 2002) and after military BT in

OTS-classified soldiers (Chicharro et al. 1998), while noOTS soldiers showed an increase in serum free testosterone. Furthermore, serum testosterone failed to reflect OR and OTS at rest after a typical (Gastmann et al. 1998; Lehmann et al. 1992) and prolonged (Coutts et al. 2007d; Mackinnon et al. 1997) endurance-type overload training (Lehmann et al. 1992). In addition, changes in testosterone did not differ between highly-trained OR and noOR athletes during prolonged endurance-type (Mackinnon et al. 1997) or concurrent (Coutts et al. 2007b) overload training. In response to acute maximal exercise, testosterone remained the same after a typical endurance type of overload training (Lehmann et al. 1992). In response to a 4-day march, serum testosterone decreased only after the second day of marching compared to a preceding morning sample at rest. In both studies, food and water was available ad libitum (Lehmann et al. 1992; Väänänen et al. 2002).

Insulin-like growth factor I (IGF-I) is an important metabolic biomarker, influenced by energy balance and by anabolic and nutritional status (Nindl and Pierce 2010). Previous data support the hypothesis that IGF-I can be used as a circulating biomarker for monitoring training load (Nindl et al. 2007a) and investigating OTS (Nindl et al. 2012), particularly during a physical training period of high-energy expenditure resulting in substantial alterations in body composition (Nindl and Pierce 2010; Rarick et al. 2007).

Testosterone/cortisol –ratio

Adlercreutz et al. (1986) proposed a classical criterion for OR, a 30% decrease in the serum free testosterone/cortisol ratio (T/C –ratio), which was achieved after one week of overload training. Since then, the T/C –ratio has been used to represent the balance between anabolic and catabolic activity in the body (Adlercreutz et al. 1986). This criterion has been met after prolonged endurance overload training (Mäestu et al. 2005) and after military BT compared to before BT value among 24% (Chicharro et al. 1998) and 56% (Booth et al. 2006) of soldiers as well after an 8-week period of exhaustive military training (Fortes et al. 2011). Furthermore, a decrease in the T/C –ratio has been shown during periods of heavy training and an increase during periods of less intensive training (Vervoorn et al. 1991). Although a decrease in the T/C –ratio was observed in OR athletes after 6 weeks of concurrent training, the changes did not differ significantly between the OR and noOR athletes (Coutts et al. 2007b). The same phenomenon was also observed after a typical endurance type of overload training (Mackinnon et al. 1997). Moreover, after concurrent overload training the T/C –ratio remained the same (Moore and Fry 2007).

2.2.5 Association of overload training with oxidative stress markers

Oxidative stress was earlier defined as a stage where oxidant production overwhelms the antioxidant defense mechanisms of the cells and tissues (Sies 1991). The more recent, complementary definition of oxidative stress emphasizes the role of disruption of thiol redox circuits, resulting in imbalance in cell signaling and a disruption of redox-regulation (Jones 2008). Its harmful effect results from over-production of reactive oxygen (ROS) and nitrogen species (RNS) and/or ineffective antioxidant defense (Jackson et al., 2002). Increased levels of ROS and RNS produce lipid peroxidation, DNA damage and protein oxidation and also decrease antioxidants and/or increase oxidized antioxidants (Finaud et al. 2006b). ROS may arise from both extracellular and intracellular sources and muscle cells are protected against oxidant injury by a complex network of antioxidants (Jackson et al. 2002). Specifically, enzymatic and non-enzymatic antioxidants exist in both the intra-and extracellular environments and work as complex units to remove different ROS and RNS (Jackson et al. 2002). Because there are several different sources of ROS and RNS, and they interact with many different molecules there is no consensus on the best method to assess oxidative stress (Finaud et al. 2006b). A great deal of research has been conducted on effects of a single physical exercise and chronic physical training on oxidative stress, but the variety of laboratory procedures and exercise tests used make a comparison of the results difficult (Finaud et al. 2006b).

Previously it was thought that ROS and RNS are associated only with cellular dysfunction and disease (Westerblad and Allen 2011). However, it is now clear that they are also of integral importance under normal conditions, such as assisting in beneficial adaptation in response to training (Westerblad and Allen 2011). Dillard et al (1978) introduced acute exercise-induced oxidative stress, showing that even a moderate intensity of exercise increased the content of pentane, a lipid peroxidation byproduct, in expired air. Jenkins (1984) presented that in human subjects aerobic capacity is related to antioxidant capacity, the muscle hydroperoxide enzyme activity. It has since been well documented that while a single bout of physical exercise of sufficient intensity and duration generate ROS and RNS, both aerobic (Radak et al. 2001) and anaerobic endurance training (Bloomer and Goldfarb 2004) have been shown to enhance antioxidant status and decrease the generation of ROS and RNS both at rest and induced by acute exercise.

In contrast to optimal training load, overload training can lead to an impaired antioxidant defense and lack of expected adaptations to training (Smith 2000), and distortion of the redox balance (Finaud et al. 2006b). Physical exercise can be considered a double-edged sword: if practiced in moderation, it can induce an increase in antioxidant enzymes, while if performed in large volumes on a long-term basis, it can cause oxidative damage to cellular molecules, perturbing their homeostasis (Gomez-Cabrera et al. 2008). Furthermore, extreme increases in training volumes may lead to a substantial rise in inflammation and apoptosis markers (Fatouros et al. 2006). Therefore, overload training may induce inflammation, which is also associated with increased oxidative stress.

Recent studies have provided evidence that a progressively increased training volume can increase oxidative stress and attenuate antioxidant capacity (Finaud et al. 2006c; Margonis et al. 2007; Palazzetti et al. 2003; Schippinger et al. 2002; Vollaard et al. 2006). Impaired antioxidant capacity and increased oxidative stress have been found to be related to OTS in elite athletes (Tanskanen et al. 2010). In addition, a 21-day military field training period in a cold environment increased oxidative stress (Schmidt et al. 2002). Interestingly, the dual challenge of physical and psychological stress has been found to produce higher oxidative stress than exercise alone (Huang et al. 2010), which is the case in a military operation.

2.3 Influence of energy balance and nutrient content on responses to physical training

Energy balance is a result of optimal energy intake (EI) in relation to energy expenditure (EE), the components of which are basal metabolic rate (BMR), activity energy expenditure (AEE) and diet-induced thermogenesis (DIT) (Westerterp 2010). AEE includes muscular activity, including shivering and fidgeting as well as purposeful physical exercise (Poehlman 1989) 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). AEE was observed to increase with the overall stress induced by military field training (Bahr et al. 1991) and EE with short-term cold exposure caused mainly by increased carbohydrate oxidation (Shephard 1992). Diet-induced thermogenesis (DIT) is defined as the energy-requiring process of digesting, absorbing, and assimilating food nutrients. DIT increases with increased energy intake (Belko et al. 1986; Kinabo and Durnin 1990), but is also affected by high protein intake (Belko et al. 1986; Weigle et al. 2005).

Energy balance is an important factor in sustaining training load and maintaining high performance during strenuous military training (Bell et al. 2002; Fortes et al. 2011; Montain et al. 1997). Moreover EI has been found to have an influence on voluntary physical activity, increasing with high-energy intake (Hoyt and Friedl 2006; Montain et al. 2008; Thompson et al. 1995; Westerterp 2010). Energy deficit results in a reduction of energy expenditure via a reduction in maintenance metabolism (BMR), in the thermic effect of food, and in AEE (Westerterp 2010). Furthermore, energy deficit with concomitant physical training has been found to suppress immune function (Diment et al. 2012; Kramer et al. 1997) and to alter serum anabolic (Fortes et al. 2011; Nindl et al. 2007a) and catabolic (Halson et al. 2004; Kyroläinen et al. 2008) hormone concentration and is hypothesized to be one reason for URTI (Venkatraman and Pendergast 2002). Multiple stressors such as sleep and energy deprivation with physical and psychological stress have a deleterious effect on mood state (Lieberman et al. 2005). In contrast, a combination of energy restriction and exercise training has been shown to enhance the antioxidant capacity of a judo athletes and judo competition induced the same changes in the oxidative-antioxidants status in energy restriction and energy balance athletes (Finaud et al. 2006a).

Ready-to-eat meal rations are commonly used during the military field training (Hirsch et al. 2005). However, previous military studies have shown an energy deficit during strenuous field training (Kyroläinen et al. 2008; Nindl et al. 1997), which induces loss of body mass and fat-free mass. In many military studies, energy deficit is also an intentional additional stressor (Alemany et al. 2008; Lieberman et al. 2005; Nindl et al. 1997; Tharion et al. 2005), but underconsumption is a common problem (DeLany et al. 1989; Hirsch et al. 2005; Hoyt et al. 1991). Even though unfamiliar stressful situations might decrease energy intake (Popper et al. 1989), environmental factors have an important role in determining food selection and intake (de Graaf et al. 2005), whereas food acceptability has been found to have an impact on food intake in the field (de Graaf et al. 2005). In contrast, there is evidence that well-motivated conditioned men can increase their food intake to meet increased energy demand during short-term exhaustive training (Rehrer et al. 2010; Smoak et al. 1988).

A few studies exist on deliberate energy supplementations to ensure energy balance during short-term military field training (Bell et al. 2002; Cline et al. 2000; Edwards and Roberts 1991; Fortes et al. 2011; Montain et al. 2008; Montain et al. 1997); however, none of them have used protein-rich supplement. Carbohydrate beverage (CHO) supplement has been found to increase total energy intake during short-term military field training (Bell et al. 2002; Cline et al. 2000; Montain et al. 1997), although in some cases the consumption of rations decreased (Bell et al. 2002; Montain et al. 1997). In summary, these studies support the use of supplements in order to increase energy intake.

Consumption of a high CHO diet throughout an overload training period has improved immune activity and reduced susceptibility to URTI (Costa et al. 2005), and has attenuated decrease in performance (Achten et al. 2004; Costill et al. 1988b; Halson et al. 2004) and the negative effects of overload training on symptoms of OR (Achten et al. 2004; Costill et al. 1988b; Halson et al. 2004). Although CHO supplementation minimized many of the negative symptoms associated with OR, it could not prevent its development (Halson et al. 2004). Dietary consumption of CHO has also been shown to affect the hormonal response to training and to acute exercise. Halson et al. (2004) found that an exercise-induced decrease in cortisol during typical overload training was attenuated by a high- carbohydrate diet compared to low-carbohydrate diet. In a study by Lane et al. (2010), a low-CHO diet (30% of daily intake) resulted in an increase in serum cortisol and a decrease in the free testosterone/cortisol ratio, while no changes occurred in the control-CHO group (60% of daily intake) during short term overload training. Similarly, protein supplementation resulted in reduced bacterial/viral infections during military BT (Flakoll et al. 2004). In all of these studies, the supplementation group also received higher energy intake. Therefore, it cannot be concluded whether this phenomenon occurs due to increased protein or CHO intake or simply as a result of increased energy intake.

Where energy intake matched energy expenditure, an increase in protein intake of 3.0 $g \cdot kg^{-1}$ was shown to have a beneficial role during recovery after exercise by attenuating impairments in endurance performance (Witard et al.

2011). Furthermore, high-protein diets have been found to attenuate a decrease in fat-free mass (FFM) during negative energy balance induced by a decrease in energy intake among obese individuals (Westerterp-Plantenga et al. 2006) and also among athletes (Mettler et al. 2010). In addition, amino acid supplementation attenuated a decrease in muscle strength and power after strength overload training (Ratamess et al. 2003). In contrast, high-protein intake has not been found to attenuate a decrease in FFM when the energy deficit is induced by high energy expenditure (Pikosky et al. 2008). Furthermore, increased protein intake has been found to produce favorable phenomena among obese persons during weight loss and weight control, such as satiation (Weigle et al. 2005), increased energy expenditure by the thermic effect of food (DIT), and decreased ad libitum energy intake (Weigle et al. 2005). However, these phenomena could be unfavorable during a situation where exercise-induced energy expenditure is high and high-energy intake is recommended.

3 AIMS OF THE STUDY

The goal of physical training during the military basic training (BT) period is to increase conscripts' physical fitness (aerobic and muscle fitness) in order to prepare them for demanding military tasks. However, the physical training involved in military service may exceed their previous training level, which could contribute to OR or OTS in the multi-stressor military environment. Therefore, this study evaluated the training load of a BT period in wintertime and the incidence of OR. Furthermore, the aim was to investigate the associations between physical fitness, energy balance, fatness, mood state and training load during BT and during a shorter 8-day intensive training course (TC) with easy-to-use energy supplementation.

The specific aims of the study were:

1) To study the effects of an 8-week Finnish BT period on physical fitness, body composition, mood state and serum biochemical parameters, oxidative stress markers and antioxidant status. (Original papers I and II).
2) To determine the incidence of OR during BT among conscripts, and to evaluate whether the initial levels or training responses of physical fitness, body composition and serum biochemical parameters, oxidative stress markers and antioxidant status differ between the OR and noOR subjects. (Original papers I and II)
3) To determine the role of aerobic fitness (VO_2max), fatness (BMI) and energy balance on training load over two weeks during the winter BT season. An additional purpose was to study the accuracy of a self-reported pre-filled food diary in the military environment without food weighing, but with known food composition. (Original paper III)
4) To determine the effects of a protein-rich energy supplement of 4.1 $MJ \cdot d^{-1}$ on energy balance, physical activity, body composition, physical performance and subjective experiences of overall well-being during an 8-day TC period in a cold environment. An additional purpose was to define whether an ad libitum fluid intake is sufficient during winter training. (Original paper IV)

4 METHODS

4.1 Subjects

For the 8-week BT study, healthy young males entering compulsory military service in the North Finland Signal Battalion (in the Kainuu Brigade, Finland) were recruited by a questionnaire. A total of 131 conscripts responded and volunteered for the study. From this conscript population, 47 were discarded on account of cardiorespiratory or musculo-skeletal disorders, and incomplete fulfilment of or willingness to perform special duties. The remaining 84 were divided into three categories according to their level of voluntary physical activity before military service, as determined by the International Physical Activity Questionnaire (IPAQ) (Craig et al. 2003). To ensure wide variation in initial physical fitness levels, 19 conscripts were randomly selected from each category, making a total of 57 conscripts. Finally, they were divided into three groups equal in number according to their initial VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$) at entry to military service: > 45 $ml \cdot kg^{-1} \cdot min^{-1}$ for group 1; 40 - 44.9 $ml \cdot kg^{-1} \cdot min^{-1}$ for group 2; < 39.9 $ml \cdot kg^{-1} \cdot min^{-1}$ for group 3. From each group, 8 conscripts were randomly selected, making a total of 24 conscripts for the double-labelled water analysis (III). The subgroup of 35 conscripts studied in papers I and II were the conscripts who had completed all the submaximal marching tests, and undergone all the required tests and samplings.

For the TC study, 26 voluntary male conscripts (aged 20 ± 1 yrs.) from the Intelligence Company of the Kainuu Brigade were randomly divided into two groups: a control group (N = 12) and an energy supplementation group (Ebar, N = 14) of which the latter received an additional energy supplement of 4.1 $MJ \cdot day^{-1}$ during TC.

All the voluntary male conscripts were fully informed of the experimental protocol and gave their written consent to participate in the study. They were also advised of their right to withdraw from the investigation at any time. The study protocol was approved by the Finnish Defence Forces, and the Ethics

Committees of the University of Jyväskylä and the Kainuu region, respectively. The characteristics of the final subject groups at baseline in the different original papers are presented in Table 1.

TABLE 1 Characteristics of the subjects in different original publications I-IV.

	8-week BT study			8-day TC study	
	I and II		III	IV	
				Controls	Ebar
	n=57	n=35	n=24	n=12	n=14
Age (yr.)	20 ± 0	20 ± 0	20 ± 0	20 ± 0	20 ± 0
Height (cm)	178 ± 7	178 ± 8	178 ± 7	178 ± 7	179 ± 8
Body mass (kg)	80 ± 16	79 ± 18	79 ± 16	71 ± 7	71 ± 7
BMI (kg ·m^{-2})	25 ± 4	25 ± 4	25 ± 4	23 ± 2	22 ± 1
12-min running test (m)	2344 ± 365	2378 ± 324	2322 ± 363	2814 ± 208	2930 ± 178
VO$_2$max (ml ·kg^{-1} ·min^{-1})	43 ± 7	44 ± 7	43 ± 7	52 ± 5*	54 ± 1*

Values are mean ± SD. *Estimated from 12-minute running test: VO$_2$max (ml·kg^{-1}·min^{-1}) = running distance (m) - 504.9/ 44.7 (Cooper 1968).

4.2 Experimental design

4.2.1 8-week BT study (I-III)

Performance tests and physiological and psychological markers to monitor the BT training load were selected based on the existing literature on OTS and training studies. Performance tests (VO$_2$max test and submaximal test) and biochemical determinations were performed three times, and psychological markers five times, during the 8-week training period. In addition, acute responses to a submaximal test were studied. For the submaximal test, a marching test was selected, as it closely resembles the routine activities of the subjects and enables the test to be performed for a large group of subjects. Furthermore, sick leaves were followed throughout BT, as poor VO$_2$max levels have been associated with training related to sick leaves during military service (Knapik et al. 2001b). Energy expenditure by the DLW method was measured during two weeks in the latter part of BT. PA was monitored by accelerometers throughout BT. The experimental protocol is presented in Table 2.

TABLE 2 The experimental design during the 8-week military BT period (I-III).

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
VO_2max –test	X				X			X
Submaximal marching test	X			X			X	
Body composition	X			X		X	X	X
Blood samples	X			X			X	
Physical activity	X	X	X	X	X	X	X	X
DLW assessment						X	X	X
Food diary						X	X	X
Questionnaire	X			X		X	X	X

The study took place during the winter in Finland, when daily outdoor temperatures ranged from -31˚C to +1˚C, with a mean of -13˚C (data from the local weather station). The overall physical load of the 8-week BT period was set according to the standard direction of the Defense Command. The intensity of physical activity in the daily program was planned to be low in the first week of BT and increased thereafter. Food and water intake were in accordance with the standard army meal, and water intake was not restricted. However, the subjects were not permitted to use any extra nutritional supplements during the study. The average sleep time at the garrison was from 10 p.m. to 5:45 a.m. The military education included physically demanding activities such as marching, combat training and sport-related physical training. The subjects also carried combat gear weighing 20 kg, including clothing, particularly during marching and combat training. In addition, the training included an overnight field exercise. The garrison training involved theoretical education in classroom settings, material handling, shooting and general military education, such as close order drills. Subjects marched four times per day (approximately 5 km in total) for meals, and slept in dormitory-type rooms.

During the BT period, practical skills were trained daily. At the beginning of BT, the amount of physical training was approximately two hours per day, increasing to 3 to 4 hours during weeks 4 to 7. The BT schedule included four longer (from two to eight hours) marching exercises with combat equipment. However, daily outdoor exercise, including transportation, was often performed by marching. Rifle marksmanship in the garrison was performed twice a week throughout the BT period.

4.2.2 8-day TC study (IV)

The study took place during the winter in Finland, when daily outdoor temperatures ranged from -16°C to -1°C, with a mean of -4°C (data from the local weather station). During the study, the subjects completed an 8-day field training course, which consisted of a variety of military-relevant tasks and skills training, and sustained endurance exercise involving skiing and patrolling activities. During TC, subjects carried combat gear weighing of 60-70 kg inclusive of their clothes and food rations. The subjects were randomly divided into two groups: a control group (N = 12), allowed to eat only their field rations, and an energy supplementation group (Ebar, N = 14), who received an additional energy supplement of 4.1 MJ·d^{-1} during the 8-day TC. Water intake was ad-libitum. However, subjects were not permitted to use any other extra nutritional supplements during the study. Physical performance was studied at six days (-6d PRE) and one day (PRE) before, in the middle (MID) and one day (POST) and three days (+3d POST) after TC, including measurements of 3-km combat loaded field running, vertical jump, anaerobic power and handgrip strength. In addition, mood state, body composition and physical activity were studied together with energy and water intake recordings. The experimental protocol is presented in Table 3.

TABLE 3 The experimental protocol during the 8-day training course (IV).

			Intensive training course									
Day			**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**		
Test	**-6 PRE**	**PRE**	**MID**								**POST**	**+3 POST**
Body mass	X	X					X				X	X
Fat free mass		X									X	
Fat mass		X									X	
Questionnaires	X	X					X				X	X
Vertical jump	X	X					X				X	
Anaerobic power	X	X					X				X	
Hand-grip test	X	X					X				X	
3-km running test		X									X	
Physical activity			X	X	X	X	X	X	X	X		
Energy and water intake			X	X	X	X	X	X	X	X		
Energy bar supplementation			X	X	X	X	X	X	X	X		
Deuterium dose		X								X		
Urine samples		X	X							X	X	

4.3 Measurements and analysis

4.3.1 Body composition

BM was measured to within an accuracy of 0.01 kg (Inbody720, Biospace Co. Ltd, Seoul, Korea). For each subject, the repeated measurements were performed between 6:00 and 7:00 a.m. after an overnight fast and after voiding with no exercise for 12 hours before the test, except the MID measurements during TC (IV). The physical activities in the daily BT program were planned to be performed at a low intensity on the day preceding each measurement and fluid status was estimated to be in balance based on the dietary records of the subjects. The subjects were barefoot and they wore T-shirts and trousers (I-II) or shorts (IV). In papers III and IV, BM was measured at the beginning (BMpre), in the middle (BMmid) and at the end of the experimental period (BMpost). The BMmean was presented as an individual average of BMpre, BMmid and BMpost, and the change in BM (ΔBM) was calculated as the difference between BMpost and BMpre. Body height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as BM (kg) divided by height (m) squared.

In papers I and II, body composition measurements (fat-free mass [FFM], fat mass [FM] and percentage of body fat [F%]) were performed using eight-point bioelectrical impedance (Inbody720, Biospace Co. Ltd, Seoul, Korea). Recent studies have shown that eight-polar bioelectrical impedance is a reliable method to detect changes in FFM (ΔFFM) and FM (ΔFM) when performed under strict standardized conditions (Kilduff et al. 2007), as was the case in this study. Accordingly, ΔFFM and ΔFM during the BT were calculated using this method.

In papers III and IV, FFM was calculated from total body water (TBW) as follows: FFM = TBW/0.732 (Pace and Rathburn 1945). FM was calculated as BMpre – FFM, and percentage of body fat as FM/BMpre x 100. TBW was measured with deuterium dilution according to the Maastricht protocol (Westerterp et al. 1995b). Briefly, at 10:00 p.m., before the measurements and after collecting a baseline urine sample, the subjects drank a weighed mixture of ^2H_2O. Subjects consumed no foods or fluids for 10 hours after dose administration, during the overnight equilibration of the isotope with the body water. Subsequent urine samples were collected from the second and third voiding in the morning of day 1. In the TC study, the process was repeated on days 9 and 10. TBW was calculated as the ^2H dilution space divided by 1.04, correcting for exchange of the ^2H label with nonaqueous H of body solids (Schoeller et al. 1980).

4.3.2 Physical performance

Maximal aerobic fitness

To determine VO_2max (I-III) ($mL \cdot kg^{-1} \cdot min^{-1}$), the subjects performed a maximal treadmill test during week 1 (before training), at weeks 5 (wk5) and 8 (wk8) of the BT period and two weeks after BT (wk10). The follow-up tests for each subject were always performed at the same time of day between 8.30 a.m. and 5 p.m., after consumption of a similar diet before each test. The start of the test involved walking for 3 min at 4.6 $km \cdot h^{-1}$ and walking/jogging at 6.3 $km \cdot h^{-1}$ (1% slope) as a warm-up. Thereafter, exercise intensity was increased every 3 min to induce an increase of 6 $mL \cdot kg^{-1} \cdot min^{-1}$ in the theoretical VO_2max demand of running (ACSM 2001, 303). This was achieved by increasing the initial running speed of 4.6 $km \cdot h^{-1}$ by a mean of 1.2 $km \cdot h^{-1}$ (range 0.6 – 1.4 $km \cdot h^{-1}$), and by increasing the initial slope of 1 degree by a mean of 0.5 degrees (range 0.0 - 1.0

degree) up to the point of exhaustion (ACSM 2001, 117). Pulmonary ventilation and respiratory gas exchange data were gathered on-line using the breath-by-breath method (Jaeger Oxygen Pro, VIASYS Healthcare GmbH, Hoechberg, Germany), and mean values were calculated at 1-min intervals for the statistical analysis. The analyzer was calibrated before each test according to the manufacturer's specifications. Heart rate (HR) was continuously recorded at 5-s intervals using a telemetric system (Polar810i, Polar Electro Oy, Kempele, Finland). Rating of perceived exertion (RPE) was assessed at the end of each workload. The criteria used for determining VO_2max were: a lack of increase in VO_2max and heart rate despite an increase in slope and/or speed of the treadmill, a respiratory exchange ratio (RER) higher than 1.1 and a post-exercise blood lactate (La) value (determined 1 minute after exercise completion from a fingertip blood sample) that was higher than 8 $mmol·L^{-1}$ (ACSM 2001, 117). All subjects fulfilled these criteria. Technical errors of the VO_2max and HR determinations varied between 1 and 2 % based on the manufacturers' manuals. The repeatability of VO_2max and HRmax to volitional exhaustion in an exercise test among athletes has been reported to be 4% and 5%, respectively (Bingisser et al. 1997).

The 12-min running test (IV) (Cooper 1968) was performed indoors in a sporthall. Subjects were instructed to perform the 12-min run with maximal effort at a progressively increasing running speed. Subjects were allowed to stop the test at any time for safety purposes. The accuracy of the measurements was ±10 meters. Before the test, the subjects warmed up by walking 1 km.

The 3-km running test (IV) with maximal effort was performed on an outdoor track PRE and POST TC while carrying a 20-kg backpack. All subjects were instructed to complete the test in the shortest possible time. HR was continuously recorded using heart rate monitors (RS 800, Polar Electro Oy, Kempele, Finland). La was determined before and 1 minute after completion of the exercise from a fingertip blood sample. Prior to the 3-km test, a standardized warm-up consisting of walking 1 km was performed.

Submaximal aerobic fitness (I-II)

The 45-minute submaximal marching test (exercise) was performed, after one week of adjustment to military service, at the end of the first week (before training), and after four (wk4) and seven (wk7) weeks of the BT period. The time between the tests was three weeks and the follow-up test for each subject was always performed at the same time of day, between 9 a.m. and 12 p.m. HR was recorded in 5-s intervals throughout the exercise to define the mean HR. RPE was assessed three times, at 15-min intervals, using a 6- to 20-point scale. RPE was calculated as the average of these three values. The aim for determining marching speed was to divide the subjects into three groups so that each group comprised subjects marching at a similar load corresponding to approximately 70% of their individual maximal workload. Exercise of this intensity and duration have been shown to result in increased serum cortisol levels (Thuma et al. 1995) and to be tolerable for untrained subjects. First, theoretical individual maximal workload ($mL·kg-1·min^{-1}$) was calculated based on the treadmill speed and slope in the VO_2max test using the ACSM estimation formula for VO_2max in running (ACSM 2001, 303). Second, since in load carriage tasks (~20kg) the relationship between relative VO_2max and endurance time has been shown to be lower compared to an absolute VO_2max value (Bilzon et al. 2001), the theoretical individual maximal workload expressed as $mL \cdot kg\text{-}1 \cdot min^{-1}$ was converted into $L \cdot kg^{-1}$. Third, the individual speed corresponding to 70% of maximal workload when carrying the 20 kg of combat gear during the test on even terrain was determined. It was assumed that carrying the extra load required a similar level of effort as carrying extra body weight. As the ACSM formula for the estimation of VO_2max in walking yields unrealistically low values for brisk walking (above 5 $km \cdot h^{-1}$ h), a new equation for marching was determined that combines the ACSM equations for walking and running between 5 and 8 $km \cdot h^{-1}$. The equation is based on earlier findings that walking and running are metabolically equivalent at a velocity of approximately 8 $km \cdot h^{-1}$ (McArdle et al. 2001). Fourth, the individually estimated marching speeds were rounded to the closest even number. 12 subjects were assigned to group marching at 6 $km \cdot h^{-1}$, 43 subjects to a group marching at 7 $km \cdot h^{-1}$and 4 subjects to a group marching at 8 $km \cdot h^{-1}$. An accurate and even marching speed was ensured by the group leader who walked in front of the group without extra weight and wore a Polar S625 (Polar Electro Oy, Kempele, Finland) running computer, which was equipped with an individually calibrated speed measuring device.

Explosive strength of leg extensors (IV)

Explosive strength of leg extensors was assessed with a static vertical jump test using a contact mat (Newtest Powertimer, Newtest Oy, Oulu, Finland). The subjects stood on the contact mat with hands on hips and the knees flexed at an angle of 90 degrees. Instructions were given for jumps to be performed vertically with maximal effort, and for the subject to land on the mat with knees straight. The hands were kept on the hips during the jump. The subjects performed three static jumps with a 5-s rest between each jump. The mean flight time of the two best jumps was recorded and the rising height of the centre of gravity was assessed. The test-retest reliability of the measurement has been reported to be high (intraclass correlation coefficient (ICC) = 0.97) (Bosco and Viitasalo 1982). Vertical jump tests were performed -6d PRE, PRE, MID and POST TC. Before the tests each subjects was required to complete a standardized warm-up of 5-min. The follow-up tests for each subject were always performed at the same time of day after consumption of a similar diet before each testing session.

Anaerobic power (IV)

Anaerobic power was assessed during a 15-s all-out counter movement jump test on the contact mat. Power was calculated as follows: Power ($W \cdot kg^{-1}$) = $((g^2) \times (T_f \times 15))/(4n \times ((15- T_f))$, where g = gravitational acceleration (9.81 $m \cdot s^{-2}$), T_f = total flight time, n = number of jumps performed in 15 s (Bosco et al. 1983).

The test-retest reliability of the measurement has been reported to be high (r= 0.95) (Bosco et al. 1983). Anaerobic power tests were performed -6d PRE, PRE, MID and POST TC. Before the tests each subject was required to complete a standardized warm-up of 5 min. The follow-up tests for each subject were always performed at the same time of day after consumption of a similar diet before each testing session.

The maximum isometric strength of the hand and forearm muscles (IV)

Maximum isometric strength of the hand and forearm muscles was assessed in a sitting position with the static hand-grip test using a dynamometer (SAEHAN, Saehan Corp., Masa, Korea). Three maximum isometric efforts with one minute's rest was performed and maintained for about 5 s with the right and left elbow flexed at 90 degrees. No other body movement was allowed. The handle of the dynamometer was individually adjusted for each subject. The subjects were strongly encouraged to perform with maximal effort. The hand-grip results are presented as the mean of the right and left hand maximum. The test-retest reliability of the measurement has been reported to be high in young adults (ICC = 0.94) (Tsigilis et al. 2002). Tests were performed -6d PRE, PRE, MID and POST TC. Before the tests each subject was required to complete a standardized warm-up of 5 min. The follow-up tests for each subject were always performed at the same time of day after consumption of a similar diet before each testing session.

4.3.3 Questionnaire and sick leave

During BT, the subjects rated somatic symptoms of OTS on a five-point Likert scale. In addition, the question “Do you feel physically or mentally overloaded?” was asked. Furthermore, sick leave was defined as attendance / not-attendance in daily military service because of illness or injuries examined by a physician. Questionnaires were administered one day before and one week after the marching test at the same time of day in standardized conditions to avoid pre- versus post-exercise and morning versus evening variations (Meeusen et al. 2006).

The questionnaire used to assess the somatic symptoms of OR and OTS was formulated based on previously reported symptoms of OTS (Fry et al. 1991; Kenttä et al. 2001; Meeusen et al. 2006; Urhausen and Kindermann 2002). The somatic symptoms were subjective ratings of well-being: upper respiratory tract infections (Cronbach's alpha 0.82), flu-like symptoms (Cronbach's alpha 0.74), digestive disorders and reduced appetite (Cronbach's alpha 0.45), musculoskeletal disorders (Cronbach's alpha 0.70), physical complaints and sleep difficulties (Cronbach's alpha 0.61). The subjects rated how severely they experienced the items on the list of symptoms during the last week using a five-point Likert scale; 1 = not at all, 2 = one day, 3 = 2-3 days, 4 = 4-5 days, 5 = 6-7 days. The degree of symptoms was determined as the sum of their scores.

In the TC study (IV), subjects rated how they felt with respect to five dimensions:

1) Hunger on an eight-point Likert scale.
2) Physical performance on a seven-point Likert scale.
3) Positive and negative mood states on a four-point Likert scale, which contained six positive and nine negative items; the means of both sets of items were calculated (Uusitalo et al. 1998b).
4) Somatic symptoms (see above) on a five-point Likert scale during the last five days.
5) Upper respiratory tract infections and flu-like symptoms according to the Wisconsin Upper Respiratory Symptom Survey (WURSS-21) (Barrett et al. 2005).

4.3.4 Blood samples and analyses (I, II)

Blood samples were drawn from the antecubital vein after an overnight fast at rest (basal), 2 hours after a light breakfast before submaximal exercise (pre-exercise) and immediately after exercise (post-exercise). Basal samples were taken for serum total testosterone, cortisol and SHBG. Pre-exercise samples were taken for serum total testosterone, cortisol and total insulin-like growth factor-1 (IGF-1) and plasma oxidative stress and antioxidant capacity markers. Post-exercise samples were taken for serum testosterone and cortisol and plasma oxidative stress and antioxidant capacity markers. Fingertip blood lactate (La) samples were taken pre- and post-exercise. Circadian variability in blood parameters was minimized by collecting individual samples at the same time in the morning, between 6.30 a.m. and 7.30 a.m., and pre- and post-exercise samples between 9 a.m. and 12 a.m. following similar patterns of food ingestion. The standardized breakfast before the test had a total energy content of 1860 kJ, 61 g of carbohydrate (56% of total energy intake), 16 g of protein (14% of total energy intake) and 15 g of fat (30% of total energy intake). All blood samples were taken in the same position (seated). Subjects were instructed not to ingest alcohol, coffee, tea, chocolate or cola drinks since the previous evening of the measurements.

La was analyzed using a Lactate Pro® analyzer (Arkray, Japan), the coefficient of variance of which (CV) was 3%. Serum samples for the hormonal analyses were kept frozen at –80°C until assayed. Testosterone, cortisol and SHBG were analyzed by an Immulite 1000 (Diagnostics Products Corporation, Los Angeles, USA) using commercial chemiluminescent enzyme immunoassays IMMULITE®/IMMULITE 1000 (Diagnostics Products Corporation, Los Angeles, USA). The intra-assay CV for these assays were < 5.7%, < 4.8% and < 2.4%, respectively, and sensitivities 0.5 nmol$\cdot$L^{-1}, 5.5 nmol$\cdot$L^{-1}, and 0.2 nmol$\cdot$L^{-1}, respectively. IGF-1 was analyzed with a R&D duoset ELISA kit (R&D Systems, Minneapolis, MN, USA). The intra- and inter-assay CV and sensitivity for these assays were < 6.4 %, < 9.1 % and 26 pg$\cdot$ml^{-1}, respectively.

Blood samples were centrifuged at 1200 g and 4°C for 15 min immediately after collection to separate the plasma. Plasma samples were stored in multiple portions at -80°C until analysis. Protein carbonyls - markers of protein oxidative damage - were measured using the ELISA method as previously described (Oksala et al. 2007). Nitrotyrosine concentrations were determined with an ELISA method using a commercial kit (HyCult biotechnology b.v; Netherlands). Lipid peroxidation marker total malondialdehyde in plasma was measured according to the method of Gerard-Monnier (Breusing et al. 2010). Oxygen radical absorbance capacity (ORAC) was used for the measurement of antioxidant capacity, which was performed using a multi-well plate reader according to the methods described previously (Kinnunen et al. 2005). The maximal intra-assay CV for protein carbonyls, nitrotyrosine, malondialdehyde and ORAC were 5.9%, 10.0%, 6.2% and 8.1%, respectively, and the maximal inter-assay CV were 9.2%, 12.7, 10.6% and 11.3%, respectively. Total glutathione (TGSH) and oxidative stress marker oxidized glutathione (GSSG) concentrations were determined spectrophotometrically as described previously (Lappalainen et al. 2009; Sen et al. 1994). The tissues were deproteinized with metaphosphorous acid (MPA) for the TGSH and GSSG analysis. The maximal intra-assay CV for these assays was 4.8 %, and inter-assay 6.4 %. Plasma protein carbonyl results were expressed in nmol $\cdot$mg^{-1} protein. Protein carbonyls and ORAC measurements were performed in triplicate and ELISA, nitrotyrosine, TGSH and GSSG measurements in duplicates.

Submaximal exercise-induced percentage changes in plasma volume (%ΔPV) was calculated from changes in hemoglobin and hematocrit according to the method of Dill and Costill (1974). Hematocrit and hemoglobin were analyzed using a Sysmex KX 21N-analyzer (Sysmex Co., Kobe, Japan). Post-exercise values were adjusted for changes in plasma volume: Post-exercise adjusted = post-exercise + (post-exercise * %ΔPV /100). Exercise-induced relative changes were calculated using the equation (post-exercise adjusted – pre-exercise)/pre-exercise *100. Testosterone, cortisol, malondialdehyde, nitrotyrosine and ORAC post-exercise values are reported adjusted for these changes. The BT and/or exercise-induced relative changes are expressed as Δ%.

4.3.5 Training load assessed by accelerometer measured physical activity (II, IV)

Physical activity (PA) was measured by a customized version of the Polar AW200 Activity monitor that was worn on the non-dominant wrist. The AW200 has been found useful and accurate for the measurement of EE during long-term exercise (Brugniaux et al. 2008) and during intensive military training (Kinnunen et al 2012). AW200 contains a uniaxial accelerometer, the signal of which is bandpass-filtered (0.3-3.0 Hz) to reduce sensitivity to repeated low-intensity hand movements. The device counts hand movements, if acceleration exceeds 1.0 m $\cdot$s^{-2} (Kinnunen et al. 2009). Epoch length was set at one minute and a curvilinear equation was used to transform activity counts to metabolic equivalents (1 - 16 MET) which were further adjusted by body height. Periods that contained no single movement during 30 min in daytime (or during 6 hours or more at nighttime) were classified as non-wear time and excluded from the analysis.

During BT (II), PA was measured in 34 subjects. Daytime was defined as the hours from 6:00 a.m. to 9:00 p.m. and nighttime from 9:00 p.m. to 6:00 a.m. PA per minute was classified into either (1) no activity = REST ≤ 1.0 MET, (2) very light to light activity = VLPA, 1.0-3.9 MET or moderate to vigorous physical activity = MVPA ≥ 4 MET. REST (during daytime) or sleep (during nighttime) was selected if the accelerometer showed hand movements less than 50% of a 10-minute moving window. Each subject's daily and nightly data were included in the analysis only if the activity recording covered more than 80% of daytime and 90% of nighttime. Measures of PA were adjusted by the proportion of the time recorded. The activity watches were collected every evening between 9 to 10 p.m. for data download, and redistributed within 30 minutes. Out of a total of 41 days, the days and nights when at least two-thirds of subjects had enough data were taken for further analysis. In all, 33 days and 28 nights were included in the analysis. Data were pooled into two-week periods; weeks 1-2, weeks 3-4, weeks 5-6 and weeks 7-8.

During TC (IV), PA per minute was classified into either (1) rest ≤ 1.0 MET, (2) sitting = 1-2 MET, (3) standing = 2-3.5 MET, moderate activity = 3.5-6 MET, (4) vigorous activity ≥ 6 MET or (5) active time ≥ 3.5 MET (= moderate + vigorous activity). REST (i.e. sleep) was selected, if the accelerometer showed hand movements less than 50% of a 10-min moving window. Subjects were only included in the analysis, if their activity recordings covered more than 90% of the entire recording time. The activity devices were collected on day 5 for data download, and redistributed within 5:30 h:min. Data were pooled into three periods: days 1-5, days 5-8 and the total time of TC days 1-8. Data recordings lasted from 9:00 a.m. on day 1 to 20:00 p.m. on day 8.

4.3.6 Components of energy expenditure (III, IV)

Basal metabolic rate (BMR) (III, IV)

In the BT study (III), basal metabolic rate (BMR) was measured once, for practical reasons, approximately 19 days before the DLW period. After fasting for 11 h, the subject woke up at 5:30 a.m. for BMR measurements performed in a semi-recumbent position, lying for 30 min in a quiet room. Oxygen consumption and carbon dioxide measurements were conducted with the same gas analyser (Jaeger, Oxygen Pro, Germany) as used in the VO_2max test. BMR was automatically calculated from the oxygen and carbon dioxide values as follows: EE = 1.59 x VCO_2 + 5.68 x VO_2 - 2.17 x urinary nitrogen (Weir 1949), where urinary nitrogen was assumed to be 15 g/day. In the analysis, BMR was determined during the last 15 min of the 30-min measurement period.

In the TC study (IV), BMR was estimated by the equation of Schofield et al. (1985): BMR ($MJ \cdot d^{-1}$) = 0.063 * BMmean - 0.042 * height(m) + 2.953.

Energy expenditure (EE) (III, IV)

The doubly labelled water (DLW) method has been used as a golden standard for the measurement of total daily energy expenditure (EE) (Schoeller et al. 1986; Westerterp et al. 1995b). Several studies have used the DLW method in military operations, for example during rigorous field training exercise (Castellani et al. 2006; DeLany et al. 1989; Forbes-Ewan et al. 1989; Mudambo et al. 1997), in extremely cold environments (Hoyt et al. 1991; Jones et al. 1993), or in the garrison (Mudambo et al. 1997). Compared to the respirometry method, validation studies have resulted in an accuracy of 1–3% and a precision of 2–8% for EE measured by DLW (Westerterp and Plasqui 2004).

During two weeks in the latter part of BT, EE (III) was measured with DLW according to the Maastricht Protocol (Westerterp et al. 1995b). Briefly, at 10:00 p.m., before the measurements and after collecting a baseline urine sample, the subjects drank a weighed mixture of ^2H_2O (99.9 atom %) and $H_2{}^{18}O$ (10 atom %) resulting in an initial excess total body water enrichment of 150 ppm for deuterium and 300 ppm for oxygen-18. Total body water was estimated from body composition, calculated on the basis of height, weight, age and gender, with the equation of Deurenberg et al. (1991), assuming a 73% hydration of fat-free mass. Subjects consumed no foods or fluids for 10 hours after dose administration, during the overnight equilibration of the isotopes with the body water. Subsequent urine samples were collected from the second and third voiding in the morning of day 1, and from the first and second voiding in the morning of days 8 and 15. Isotope quantities (deuterium and oxygen-18) in urine were measured with a isotope ratio mass spectrometer (Optima, VG Isogas, Middlewich, UK), and CO_2 production was calculated from isotope ratios at baseline, and on days 1, 8, and 15, using the equations from Schoeller et al. (1986). CO_2 production was converted to daily metabolic rate using an energy equivalent based on the individual's dietary macronutrient composition (Black et al. 1986).

During TC (IV), EE was calculated by the method of intake/balance (Hoyt et al. 1991) as follows:

$$\text{EE} = \text{energy intake (EI)} - \text{changes in body energy stores } (\Delta\text{ES})$$

Changes in body energy stores (ΔES) were calculated from changes in FFM and FM between PRE and POST values. Change in FFM was assumed to be 27% protein and 73% water, and change in FM was assumed to be 100% fat (DeLany et al. 1989). The energy equivalents used for protein and fat were 18.4 and 39.8 $\text{kJ} \cdot \text{g}^{-1}$, respectively.

Training load assessed by activity energy expenditure (AEE), AEE $\cdot$kg^{-1} and PAL (III)

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, 121-125). Another way to describe physical activity is the energy cost of activity (activity energy expenditure, AEE) with corrections for differences in body mass (Ekelund et al. 2004).

In this study, AEE was calculated as EE x 0.9 – BMR, assuming diet-induced thermogenesis of 10% (Poehlman 1989). To remove the confounding effect of body mass (Ekelund et al. 2004), AEE was adjusted for BMmean (AEE $\cdot$kg^{-1}). PAL was calculated as EE / BMR.

4.3.7 Energy intake, energy balance and energy availability (III, IV)

To study energy intake (EI), in the BT study (III) the subjects kept a pre-filled food diary for 3-4 days in two phases during weeks 6 to 8. Altogether, habitual food intake was recorded for 7 days during the two-week study period. 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, were 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 how to report food intake. All 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 the brand names of every non-military food that they consumed, and 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 for food that was consumed in military circumstances were added to the database.

During TC (IV), the control group was allowed to eat only pre-packaged field rations. The average daily energy content of a field ration was 17.9 MJ $\cdot$d^{-1} with an average macronutrient energy composition of 14 E% protein, 58 E% carbohydrate and 28 E% fat. The subjects in the Ebar group were advised to eat five commercial energy bars per day, which equals 4.1 MJ $\cdot$d^{-1} of extra energy. The energy bars weighed 55 g/bar with an average macronutrient energy composition of 32 E% protein, 46 E% carbohydrate and 22 E% fat. The total energy provided for the Ebar group was 22.0 MJ $\cdot$d^{-1} (17 E% protein, 56 E% carbohydrate and 27 E% fat). For studying EI and water intake, the subjects completed daily pre-filled ration diaries, which included information about the ratio served, and thus the subjects were only required to record the amount of food and fluid that they consumed. The subjects had completed these diaries before the study to ensure that they were familiar with how to report food and water intake. In addition, they were advised to be as accurate as possible in recording the amount of food and fluid consumed. Furthermore, all food wraps and rations which were not consumed were collected. Daily nutritional consumption was quantified from manufacturer-supplied ingredient labels and by the computer program Nutrica®software (version 3.11, Finland) using the information from ration diaries, wraps and returned rations.

Accuracy of the food diary, estimated true energy intake and energy balance

To study the accuracy of a self-reported pre-filled food diary (III), information on true energy intake (TrueEI) was needed. Estimated TrueEI was calculated using EI and changes in body energy stores, ΔES, 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. 1995a). Underreporting, mis-recording, energy balance and TrueEI were calculated as follows:

$$\text{Underreporting } (\%) = (EI - EE) / EE \times 100$$
$$\text{Mis-recording } (\%) = [(EI - \Delta ES) - EE] / EE \times 100$$
$$\text{Energy balance } (\%) = [(\Delta ES / \text{study days}) / EE] \times 100$$
$$\text{TrueEI} = EI - \text{mis-recording} = EI - [(EI - \Delta ES) - EE)] = \Delta ES + EE$$

In the TC study (IV), energy balance was calculated as follows:

$$\text{Energy balance} = EI - EE$$

Energy availability (IV) describes the fraction of energy that is left for physical activity, after controlling for BMR. To differentiate the need of energy intake for subjects with different body mass, energy availability was calculated as follows:

$$\text{Energy availability} = EI / BMR$$

4.3.8 Water balance (IV)

Total water intake during TC was calculated from reported food and water intakes and metabolic water as follows:

$$\text{Total water intake} = \text{fluid intake} + \text{water content of food} + \text{metabolic water}$$

The amount of metabolic water was estimated from protein, fat and carbohydrate intake and from the change in body mass (BM) (Goris and Westerterp 1999). Oxidation water is 0.41 mL/g for protein, 1.07 mL/g for fat and 0.6 mL/g for carbohydrate (Fjeld et al. 1988). A change in BM of 1 kg is assumed to be a change of 0.75 kg fat mass (FM) and 0.25 kg FFM. FM is pure fat and fat-free mass is 73% water and 27% protein (Westerterp et al. 1995a). *Water loss* over TC was measured with the deuterium elimination method (Fjeld et al. 1988). After collecting a baseline urine sample, the subjects drank a weighed mixture of ^2H_2O at 10:00 p.m. before the beginning of the study. Its elimination was calculated from two urine samples after dosing (at day 1 in the morning) and two samples at the end of the training period (day 8 in the evening). *Water balance* was calculated as follows:

Water balance = total water intake - water loss

4.3.9 Criteria for overreaching (OR) (I, II)

No single symptom or a set of symptoms have been found to be associated with OR or OTS. However, a decrease in performance and an increase in perceived exertion at the same subjective work load have been found to be related to OR and OTS (Halson et al. 2002; Hedelin et al. 2000a; Uusitalo 2001; Verde et al. 1992). In addition, impaired general health status, negative mood and feelings of fatigue have been found to be related to OR and OTS (Halson et al. 2002; Meeusen et al. 2006; Uusitalo 2001; Verde et al. 1992). Therefore, the subjects in this study had to fulfill three of five criteria to be classified as OR subjects. In addition, differences between subjects who fulfilled a single OR criterion were evaluated to determine whether initial levels of physical fitness, body composition and serum biochemical parameters differed among these subjects.

Criterion 1. A reduced VO_2max of greater than 5% from the best value to the wk8 (Halson et al. 2002; Hedelin et al. 2000b; Snyder et al. 1995; Uusitalo et al. 2000; Verde et al. 1992) or did not perform the test because of illness. Absence from the test was set as an additional criterion, because all three VO_2max tests were completed by a total of 36 subjects. It was assumed that illness itself reduces performance. Furthermore, overload training has been reported as a risk factor for upper respiratory tract infections (Spence et al. 2007; Verde et al. 1992).

Criterion 2. An increase in mean RPE during the submaximal marching test greater than 1.0 (Halson et al. 2002; Hedelin et al. 2000b; Verde et al. 1992) from the lowest value at wk1 or wk4 and remaining more than 1.0 above the lowest value, or did not perform the test because of illness (see description above). All three submaximal marching tests were completed by a total of 35 subjects.

Criterion 3. An increase in somatic symptoms of OTS (Bosquet et al. 2001; Fry et al. 1991; Meeusen et al. 2006; Urhausen and Kindermann 2002) greater than 15% from wk4 to wk7, and remaining the same or increasing from wk7 to wk8. Subjects were divided into tertiles based on an increase in somatic symptoms of OTS from wk4 to wk7; 15% was the cut-off for the upper third.

Criterion 4. Admitted feeling physically or mentally overloaded at week 7 or 8 (Halson et al. 2002; Meeusen et al. 2006; Uusitalo 2001; Verde et al. 1992).

Criterion 5. Sick leave (Spence et al. 2007; Verde et al. 1992) of more than 10% of daily service. Subjects were divided into tertiles based on sick leave during BT; 10% was the cut-off for the upper third.

4.3.10 Statistical analysis

Statistical analyses were carried out using SPSS software for Windows (SPSS Inc., Chicago, IL). Values are expressed as mean ± SD. Assumptions for normality were not met for FM (I-III), protein carbonyls, malondialdehyde, nitrotyrosine (II), La before and after submaximal exercise (I-II) and La before 3-km running test (IV), and data were log transformed before statistical analysis. The untransformed values are shown in the text, and in tables and figures for more meaningful comparison, except for nitrotyrosine (II). The level of statistical significance was set at $p<0.05$.

Independent samples T-tests were used to identify significant differences between the OR and noOR subjects at baseline (I), between the controls and Ebar subjects (IV) and groups of subjects satisfying each of the single OR criteria 1, 2, 3 and 4 (I)). One-way ANOVA was used to identify significant differences between the groups in OR criterion 5 (sick leave) (I).

Physiological and/or psychological responses to the BT period (I, II) (baseline and wk4 and wk7) and acute submaximal exercise (before and after) were assessed using repeated-measures ANOVA. *Post hoc* analysis LSD (I) or Bonferroni (II) was used to identify significant differences. In addition, the effect of acute exercise and BT and their interaction were calculated.

Mixed-design factorial ANOVA (II, IV) [group (noOR vs. OR) x exercise x BT or group (control vs. Ebar) x TC (PRE, MID, POST)] was used to identify differences between and within subjects. Bonferroni as the post hoc analysis was used to identify significant differences. In addition, the effect of group and BT or TC interaction were calculated, and 95% confidence intervals (CI) were determined. If the 95% confidence interval included zero, it was concluded that there was no significant effect. Crosstabs and Pearson's correlation coefficients were used to observe associations between variables and to define whether BMI is a valid indicator of body fatness among these young male conscripts.

In the study of fitness, (VO_2max), fatness (BMI) and energy balance on training load (III), change in BM was tested by paired samples T-test. To rule out the potential effect of BM on VO_2max, FFM-adjusted values for VO_2max (VO_2max_{FFM}) were calculated according to the method described by Toth et al. (1993). To examine the effects of fitness, BMI and energy balance on AEE $\cdot kg^{-1}$, the subjects were divided into tertiles with equal numbers of subjects according to their VO_2max_{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), 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) 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_2max_{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.

5 RESULTS

5.1 8-week basic training (BT) (I-II)

5.1.1 Training load assessed by physical activity throughout BT (II)

Mean daytime MVPA during BT was 2:07 ± 0:24 h:min, LVPA 11:36 ± 0:30 h:min and REST 0:30 ± 0:18 h:min. During the nighttime between 9:00 p.m. and 6:00 a.m., the average MVPA was 0:05 ± 0:02 h:min, VLPA 1:00 ± 0:18 h:min and sleeping time 7:30 ± 0:18 h:min.

There was a main effect of BT for daytime REST and MVPA ($p<0.001$), daytime VLPA ($p<0.05$) and nighttime MVPA ($p<0.001$). Daytime MVPA was higher ($p<0.001$) and VLPA ($p<0.05$-0.001) and REST lower ($p<0.001$) during the latter part of BT compared to weeks 1-2 (Table 4). However, nighttime MVPA was higher during the first four weeks of BT compared to the latter half of BT ($p<0.05$-0.01)(Table 4). Nighttime VLPA and sleeping time remained the same throughout BT.

TABLE 4 Physical activity during BT (mean ± SD).

	Weeks 1-2	Weeks 3-4	p	Weeks 5-6	p	Weeks 7-8	p
Day rest (h:min)###	2:00±0:06	0:24±0:12	\$\$\$	0:30±0:24	\$\$\$	0:30±0:24	\$\$\$
Day VLPA (h:min)#	12:06±0:24	11:54±0:30	\$	10:18±0:42	\$\$\$, ^^^	11:36±0:36	\$\$\$, ¤¤¤
Day MVPA (h:min)	1:49±0:23	2:13±0:29	\$\$\$	2:12±0:24	\$\$\$	2:22±0:28	\$\$\$
Sleeping time (h:min)	7:24±0:12	7:24±0:24		7:36±0:36		7:30±0:30	
Night VLPA (h:min)	1:00±0:12	1:00±0:24		0:54±0:30		0:54±0:36	
Night MVPA (h:min)###	0:06±0:02	0:06±0:03		0:03±0:02	\$, ^	0:04±0:02	\$\$, ^

Difference compared to; weeks 1-2 \$\$ p<0.01, \$ p<0.05; weeks 3-4 , ^ p<0.05; weeks 5-6 ¤¤ p<0.01, ¤ p<0.05. VLPA= very low physical activity, MVPA = moderate to vigorous physical activity. Main effect of BT $^{\#\#\#}$ p<0.001, # p<0.05.

5.1.2 Body composition (I, II)

Both BM and FM exhibited a main effect of BT ($p<0.001$), decreasing from wk4 to wk7 ($p<0.001$ and $p<0.01$, respectively), with lower values compared to the baseline values ($p<0.001$). All subjects exhibited a decrease in FM from baseline to wk7 (range from -28.0 to -0.4%). In contrast, mean FFM was higher at wk7 compared with the baseline values ($p<0.05$) (Table 5).

TABLE 5 Body mass (BM), fat mass (BM) and fat-free mass (FFM) at baseline, week 4 and week 7. (n=34).

	Baseline			Week 4				Week 7					Baseline vs. week 7
	Mean	±	SD	Mean	±	SD	Δ%	Mean	±	SD	Δ%	p	Δ%
BM (kg)$^{\#\#\#}$	78.7	±	17.7	78.5	±	16.9	0	77.0	±	15.9	-1.8	$$$, ^^^	-1.8
FFM (kg)	62.6	±	9.4	62.9	±	8.8	0.7	63.0	±	9.3	0.1	$	0.8
FM (kg)$^{\#\#\#}$	16.1	±	10.7	15.6	±	10.7	-3.8	14.0	±	9.1	-7.5	$$$,^^	-12.6

Difference compared to; baseline $$$ $p<0.001$, $ $p<0.05$; week 4 ^^^ $p<0.001$, ^^ $p<0.01$.
Main effect of BT $^{\#\#\#}$ $p<0.001$.

BMI correlated positively with F% ($r=0.80$, $p<0.001$) and FM ($r=0.90$, $p<0.001$) at baseline. Crosstabs analysis further revealed that 72% of the conscripts assigned into the correct tertiles of BMI and FM, while 66% of them assigned into the correct tertiles of BMI and F%. Only 2% of the conscripts were classified into the highest group based on BMI, but they were actually in the lowest FM group.

5.1.3 Physical performance (I, II, IV)

Maximal aerobic fitness

Among the 35 conscripts who completed all three submaximal marching tests, VO$_2$max tests were completed by a total of 21 conscripts. In 65% of cases, the reason for not participating in the test was URTI. There was a main effect of BT for VO$_2$max ($p<0.001$), maximum HR ($p<0.01$) and post-exercise lactate and La/RPE -ratio ($p<0.05$) in the VO$_2$max tests. The conscripts showed positive responses to training with an 11% increase in VO$_2$max from baseline to wk5 ($p<0.001$) (Figure 2a). From wk5 to wk8, VO$_2$max did not change significantly. In addition, the individual VO$_2$max values at baseline were negatively correlated with individual Δ%VO$_2$max from baseline to wk5 ($r = -0.61$, $p<0.001$) and wk8 ($r = -0.74$, $p<0.001$). Maximum HR was lower at wk5 ($p<0.01$) and wk8 ($p<0.001$) compared to baseline (Figure 2b). Although RPE remained the same (Figure 2d), the maximal La/RPE -ratio was lower at wk8 compared to wk5 ($p<0.001$)(Figure 2a). In addition, post-exercise lactate was lower at wk8 compared to baseline ($p<0.01$) and wk5 ($p<0.05$) (Figure 2c). Among the total of 57 conscripts, all VO$_2$max tests were completed by a total of 36 conscripts. Their responses to the VO2max tests were equal compared to those of the subgroup of 21 conscripts who underwent all the submaximal and maximal tests.

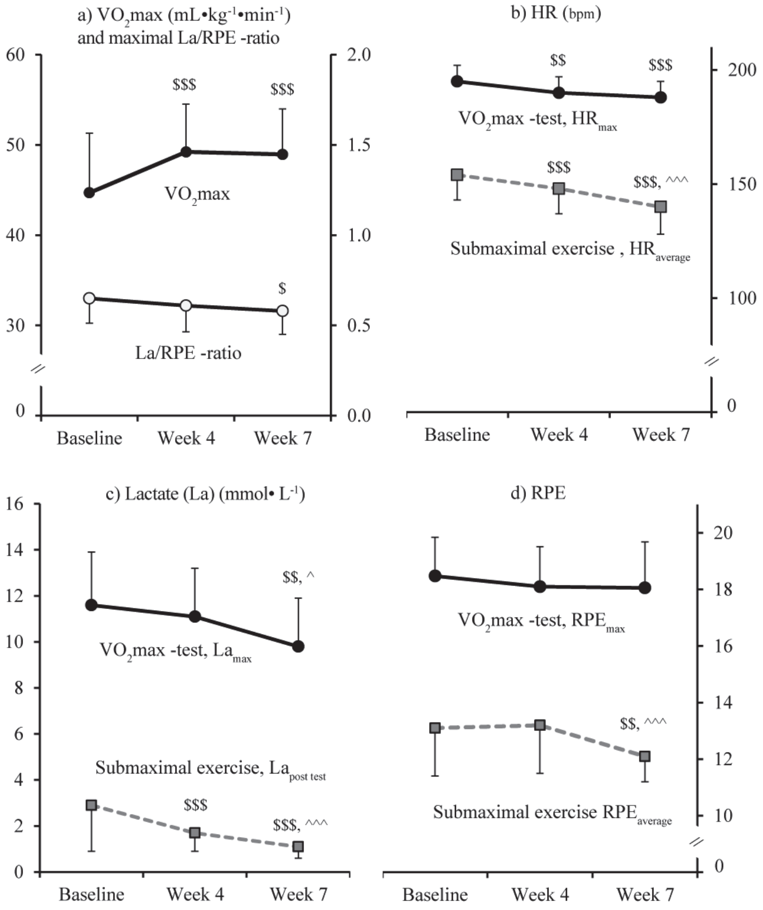

FIGURE 2 Responses (mean±SD) to the maximal VO2max test (n=21) and 45-min submaximal exercise at 70% of VO2max at baseline, week 4 and week 7 (n=35). Difference compared to: baseline \$\$\$ $p<0.001$, \$\$ $p<0.01$, \$ $p<0.05$; week 4 ^^^ $p<0.001$, ^ $p<0.05$. RPE, rate of perceived exertion.

Responses to submaximal exercise

There was a main effect of BT for HR and RPE during submaximal exercise ($p<0.001$). HR was lower at wk4 ($p<0.001$) compared with baseline values, and at wk7 compared with the wk4 and baseline values ($p<0.001$)(Figure 2b). RPE was lower at wk7 compared with the baseline ($p<0.001$) and wk4 values ($p<0.01$) (Figure 2d). Blood La exhibited a main effect of BT ($p<0.001$) and exercise ($p<0.001$), and BT and exercise interaction ($p<0.001$) by increasing due to submaximal exercise at baseline (179 ± 211 %, $p<0.001$) and at wk4 (68 ± 91 %, $p<0.001$), but not at wk7 (13 ± 49 %, n.s.). In addition, post-exercise La was lower at wk4 ($p<0.001$) and wk7 ($p<0.001$) compared with the baseline values (Figure 2c).

5.1.4 Questionnaire and sick leave (I)

The somatic symptoms associated with OTS exhibited a main effect of training ($p<0.001$), whereby symptoms increased towards the end of BT. The greatest increase was observed between wk4 and wk7 ($p<0.01$) (Figure 3a). Concurrently, the proportion of subjects who answered “yes” to the question “Do you feel physically or mentally overloaded?” increased from 41% to 80% (Figure 3b). Those who admitted to feeling physically or mentally overloaded in weeks 7 or 8 had lower VO_2max (3.3 ± 0.4 vs. 3.9 ± 0.5 L·min^{-1}, $p<0.001$), fat-free mass (61.9 ± 0.8 vs. 68.7 ± 5.6 kg, $p<0.05$) and higher HR in the submaximal marching test (152 ± 11 vs. 141 ± 10, $p<0.05$) at baseline compared to those who answered “no”.

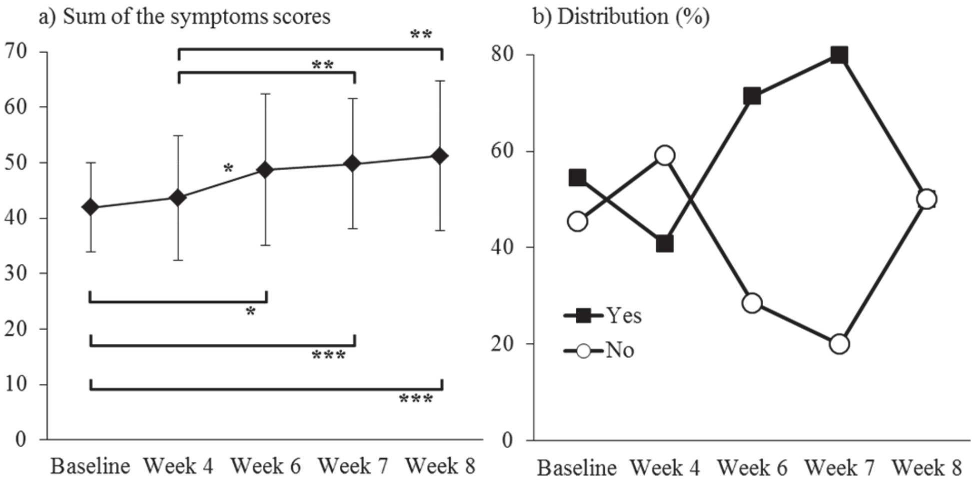

FIGURE 3 a) Mean (±SD) sum of the somatic symptoms scores associated with OTS, determined from five-point Likert scale questionnaires during the 8-week military basic training period. Significant difference; *** $p<0.001$, ** $p<0.01$, * $p<0.05$. b) The proportion of subjects who answered “yes” or “no” to the question “Do you feel physically or mentally overloaded?” (n=35).

During the 42 BT military service days, the average length of sick leave was 3 days, ranging from 0 to 10 days (0 - 24%). The subjects who had sick leave of more than 10% had lower VO_2max ($p<0.01$), basal testosterone ($p<0.05$) and T/C –ratio ($p<0.05$) at baseline compared to those who had no sick leave (Figure 4). In addition, subjects with more than 10% sick leave also had lower basal testosterone at wk4 and wk7 ($p<0.05$) (Figure 4c). In 71% of the sick leave days, the reason for sick leave was URTI, in 13% musculo-skeletal disorders, in 3% digestive disorders and in 13% miscellaneous reasons.

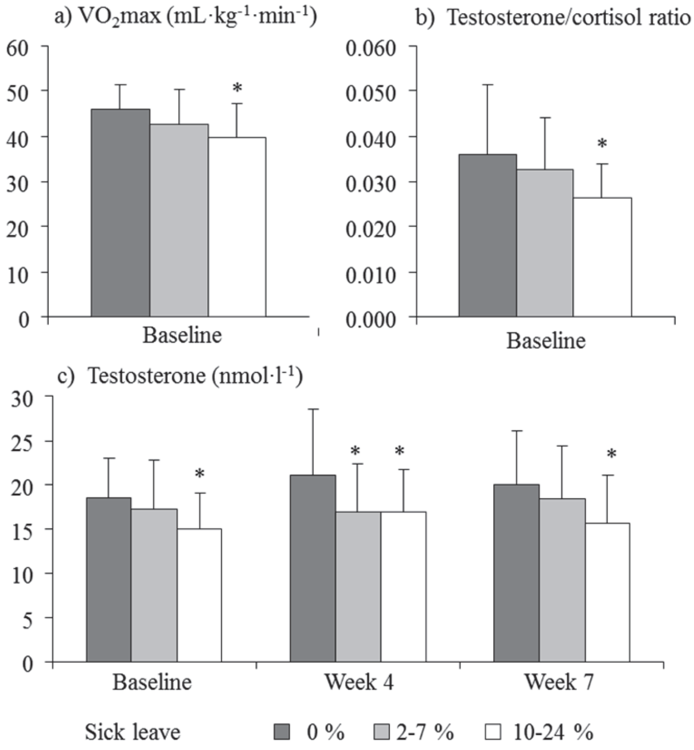

FIGURE 4 Mean (±SD) a) VO2max and b) basal serum testosterone/cortisol ratio at baseline and c) basal serum testosterone at baseline, week4 and week7 of BT in conscripts who had a sickness absence of 0%, 2-7% and 10-24% of days. Difference compared to the 0% group; * $p<0.01$. (n=57).

5.1.5 Serum hormones (I)

There was a main effect of BT for serum cortisol, SHBG and IGF-1 ($p<0.001$) and for the T/C -ratio ($p<0.05$). Basal testosterone levels were similar at all three time points (Figure 5a). Basal SHBG increased, while basal cortisol and pre-exercise IGF-1 decreased from wk4 to wk7 ($p<0.001$), and were significantly different from the baseline values ($p<0.05$ - 0.001) (Figures 5b, 5c, 5d). The serum basal T/C -ratio increased from wk4 to wk7 ($p<0.01$) (before 0.034 ± 0.013, wk4 0.037 ± 0.016, wk7 0.040 ± 0.012). However, 46% of the subjects showed an initial increase in basal testosterone during the first 4 weeks of training. Thereafter, basal testosterone decreased from wk4 to wk7 in these subjects. In contrast, 23 % of the subjects showed an increase in basal testosterone throughout the study.

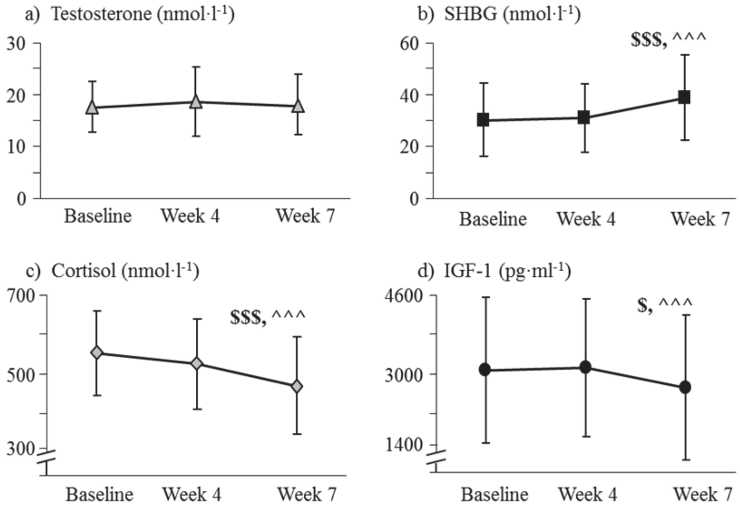

FIGURE 5 Mean (±SD) a) serum testosterone, b) SHBG, c) cortisol and d) IGF-1 concentrations at rest at baseline and at week 4 and 7 of BT. Difference compared to: baseline $$$ p<0.001, $ p<0.05; week 4 ^^^ p<0.001. (n=35).

Serum testosterone and cortisol showed a main effect for the 45-min exercise ($p<0.001$). In response to this acute submaximal exercise, serum testosterone increased significantly at each time point ($p<0.05$ - 0.001). At wk7, Δ%testosterone induced by submaximal exercise was lower compared with the baseline values ($p<0.05$) (Figure 6). Serum cortisol increased significantly only at wk4. Individual variations in testosterone and cortisol responses were large, ranging from -48 to 107 % (Figure 6).

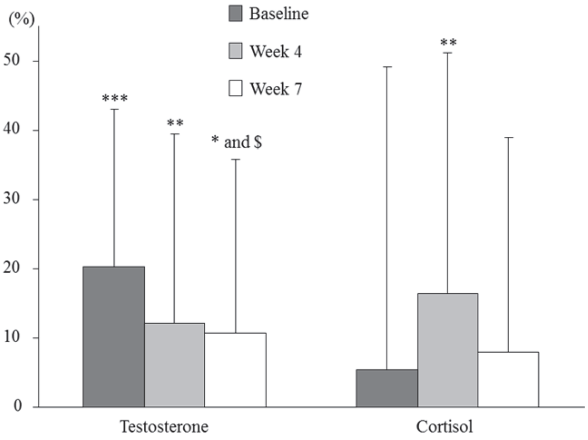

FIGURE 6 Mean (±SD) serum testosterone and cortisol responses (Δ%) to the 45-minute submaximal exercise at baseline and at week 4 and 7 of BT. Increase due to exercise; *** p<0.001, ** p<0.01, * p<0.05. Difference compared to baseline; $ p<0.05. (n=35).

5.1.6 Oxidative stress markers and antioxidant capacity (II)

There was a main effect of BT for TGSH ($p<0.05$) and ORAC ($p<0.01$), main effect of submaximal exercise for TGSH ($p<0.001$), GSSG ($p<0.001$) and the GSSG/TGSH ratio ($p<0.001$), and exercise x BT interaction for TGSH ($p<0.05$), GSSG ($p<0.001$), the GSSG/TGSH ratio ($p<0.01$) and ORAC ($p<0.05$). TGSH and ORAC (Figure 7) and protein carbonyls, malondialdehyde and nitrotyrosine at rest remained unchanged during BT. After 4 wk of BT, a decrease at rest was observed in GSSG ($p<0.01$) and an increase in GSSG and the GSSG/TGSH ratio during the latter part of BT (Figure 7).

At each time point, submaximal exercise induced a decrease in TGSH and an increase in GSSG and the GSSG/TGSH ratio (Figure 7). However, a significant submaximal exercise induced decrease in ORAC was observed only at wk4 (Figure 7). In addition, at wk4 TGSH and ORAC were lower after submaximal exercise than at baseline (TGSH $p<0.01$; ORAC $p<0.01$) and wk7 (TGSH $p<0.001$; ORAC $p<0.001$). GSSG and the GSSG/TGSH ratio after submaximal exercise were higher at wk4 (GSSG $p<0.05$; GSSG/TGSH $p<0.01$) and GSSG at wk7 ($p<0.001$) than at baseline (Figure 7). No significant changes due to the submaximal exercise were observed in protein carbonyls, malondialdehyde and nitrotyrosine (Table 7).

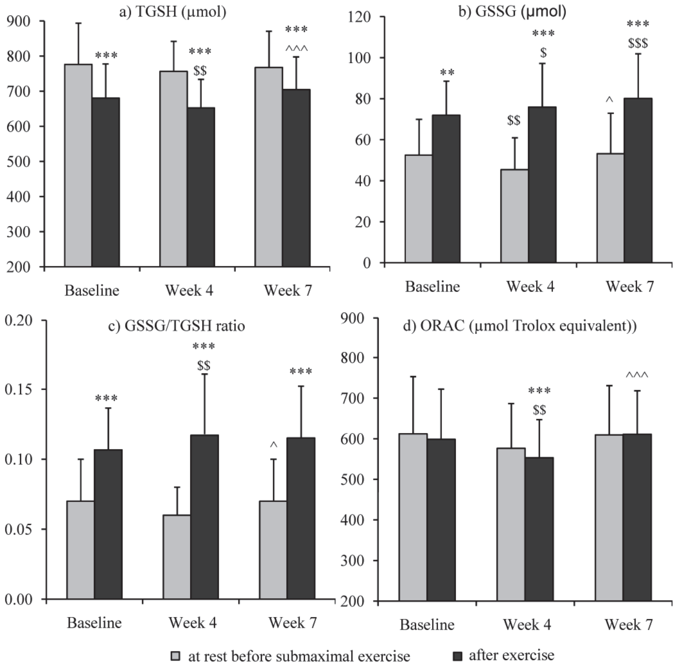

FIGURE 7 Mean (±SD) plasma a) TGSH, b) GSSG, c) GSSG/TGSH and d) ORAC concentrations before and after the 45-minute submaximal exercise at baseline, and at week 4 and 7 of BT. Difference compared to: before exercise *** $p<0.001$, ** $p<0.01$; baseline $$$ $p<0.001$, $$ $p<0.01$, $ $p<0.05$; week 4 ^^^ $p<0.001$, ^^ $p<0.01$. (n=35).

5.2 Overreaching at the end of BT (I-II)

From the total of 57 subjects, OR criterion 1 was detected in 42% of subjects, criterion 2 in 32%, criterion 3 in 13%, criterion 4 in 83% and criterion 5 in 32% of subjects. According to the measured results at baseline, the subjects who fulfilled OR criterion 1, 2 or 3 were not significantly different from those who did not fulfill these OR criterion. At least three of the five criteria were detected in 17 of the subjects, all of whom were classified as OR. Of the remainder, 34 were classified as noOR. The remaining 6 subjects lacked some questionnaire results and were not classified as either OR or noOR. Of the 17 OR classified subjects, five subjects had a decrement in VO_2max of greater than 5% from the best result to wk 8, two subjects stagnation in VO_2max (decrease less than 5%) and two subjects an increase in mean RPE during the submaximal exercise greater than 1.0 (see description on page 44). VO_2max was also measured after the BT period in week 10. Eight of those who did not perform a test or have a decrease or stagnation in VO_2max at week 8 did so on week 10. Thus in total, 15 of the 17 OR subjects showed a decrement or stagnation in performance. The remaining two subjects had absences from test to classify it, meaning that they performed VO_2max test only at baseline and at wk5. Table 6 presents the distribution of the criteria in the OR group.

TABLE 1 OR criteria distribution among OR classified subjects.

Subject	Criterion 1 ↓ VO_2max or no test	Criterion 2 ↑ RPE or no test	Criterion 3 ↑ OTS symptoms	Criterion 4 Feelings of overloading	Criterion 5 Sick leave
1	wk8 no test; -- wk10		x	x	
2	↓ wk8			x	x
3	↓ wk8	↑	x	x	
4	wk8 no test; -- wk10	↑	x	x	
5	wk8 no test; ↓ wk10		x	x	x
6	no test	no test	x	x	x
7	-- wk8		x	x	x
8	no test	no test	x	x	x
9	↓ wk8	no test			x
10	wk8 no test; ↓ wk10	↑			x
11	↓ wk8	↑		x	
12	wk8 no test; -- wk10	no test	x	x	
13	wk8 no test; ↓ wk10	↑		x	
14	↓ wk8	no test		x	
15	wk8 no test; ↓ wk10	↑		x	x
16	-- wk8	no test		x	x
17	wk8 no test; -- wk10			x	x
	15/17	12/17	8/17	15/17	10/17

↓ = decrease in VO_2max; -- = decrease in VO_2max less than 5%; ↑= increase in mean RPE during the submaximal exercise; no test = absence from a test because of sick leave.

The main reason for absence from the VO_2max and submaximal exercise tests was URTI (see page 50 for the other reasons for sick leave). Because of the study timetable and the conscripts' military service tasks, the tests could not be performed at a time when the subjects were healthy. There were no differences in sickness between the OR and noOR subjects during weeks 1-7. However, OR had a higher incidence of sick leave during the last week of BT compared to the noOR subjects (mean difference 5.5%, 95% CI 1.8 to 9.0%, $p<0.004$)(Figure 8).

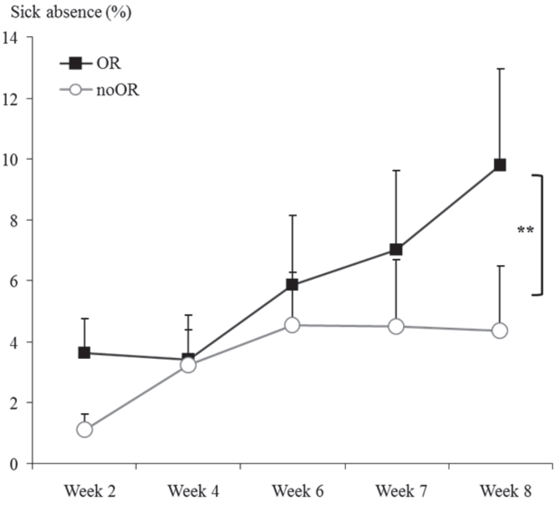

FIGURE 8 Mean (±SD) relative incidence of sick absence during the 8-week military basic training period among OR and noOR classified subjects. There was a main effect of training for sick absence ($p<0.001$).

5.2.1 Differences between the noOR and OR subjects (I, II)

At baseline. SHBG ($p<0.01$) and cortisol ($p<0.05$) exhibited a main effect of group, La/RPE ($p<0.05$) BT x group interaction and GSSG ($p<0.001$) and GSSG/TGSH group x exercise x BT interaction ($p<0.05$). At baseline, OR had higher SHBG ($p<0.01$), GSSG ($p<0.01$), GSSG/TGSH ratio ($p<0.05$) and malondialdehyde than noOR at rest (Figure 9). In OR, no response of GSSG and GSSG/TGSH to acute submaximal exercise was seen, in contrast to noOR (Figure 9). Otherwise there were no baseline differences between the two groups.

Response to BT at rest. The OR subjects had higher SHBG than noOR also at wk4 ($p<0.01$) and wk7 ($p<0.01$) (Figure 9b). However, OR exhibited decreased GSSG ($p<0.01$) and a trend towards a decreased GSSG/TGSH ratio ($p=0.058$) from baseline to wk4 (Figure 9d and 9e). Furthermore, OR had higher basal serum cortisol than noOR at wk7 ($p<0.05$), while noOR had a lower cortisol at wk7 compared to baseline ($p<0.001$) and wk4 ($p<0.001$) (Figure 9a). Moreover, OR and noOR differed in terms of the response of the basal testosterone/cortisol -ratio to training from wk4 to wk7 (OR -6 ± 23 %, noOR 24 ± 32 %$p<0.001$) ($p<0.05$). There were no differences between the groups in testosterone, IGF-1, ORAC, TGSH, protein carbonyls and nitrotyrosine at rest (Table 7), or in the relative changes at rest during BT.

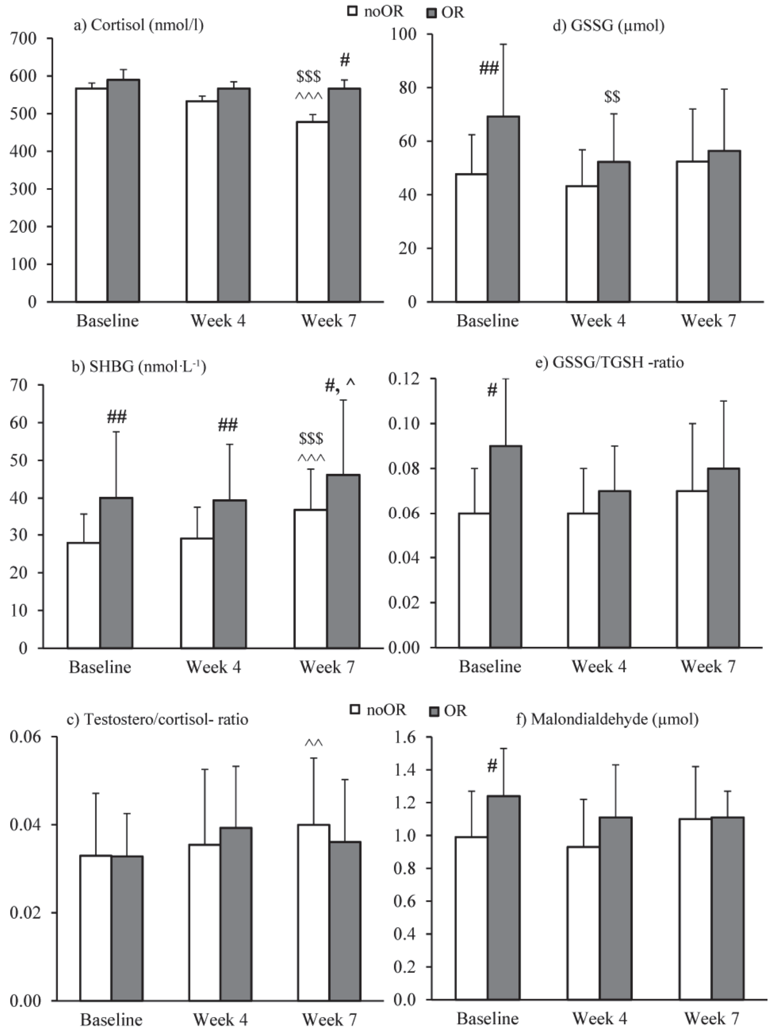

FIGURE 9 Mean (±SD) basal serum cortisol, SHBG and testosterone/cortisol –ratio (a, b, c) and plasma GSSG, GSSG/TGSH and malondialdehyde concentrations at rest (d, e, f) at baseline, and at week 4 and 7 of basic training among noOR and OR. Difference compared to: noOR ## p<0.01, # p<0.05; baseline $$$ p<0.001, $$ p<0.01; week 4 ^^^ p<0.001, ^^ p<0.01, ^ p<0.05.

Response to exercise during BT. Compared to baseline, OR had a higher relative increase in GSSG due to submaximal exercise both at wk4 (p<0.01) and wk7 (p<0.05), and in the GSSG/TGSH ratio at wk4 (p<0.05) (Figures 10d and 10e). Among noOR the exercise-induced decrease in TGSH was lower at wk7 than at wh4 (p<0.01) (Table 7). However, only OR exhibited a significant exercise-induced decrease in ORAC at wk4 (p<0.05) compared to wk7 (p<0.05) (Figure

10f). Moreover, OR and noOR differed in the response of the maximal La/RPE ratio in the VO₂max test from baseline to the end of BT (OR -30 ± 18 %, noOR -3 ± 20 %p<0.001) (Figure 10c).

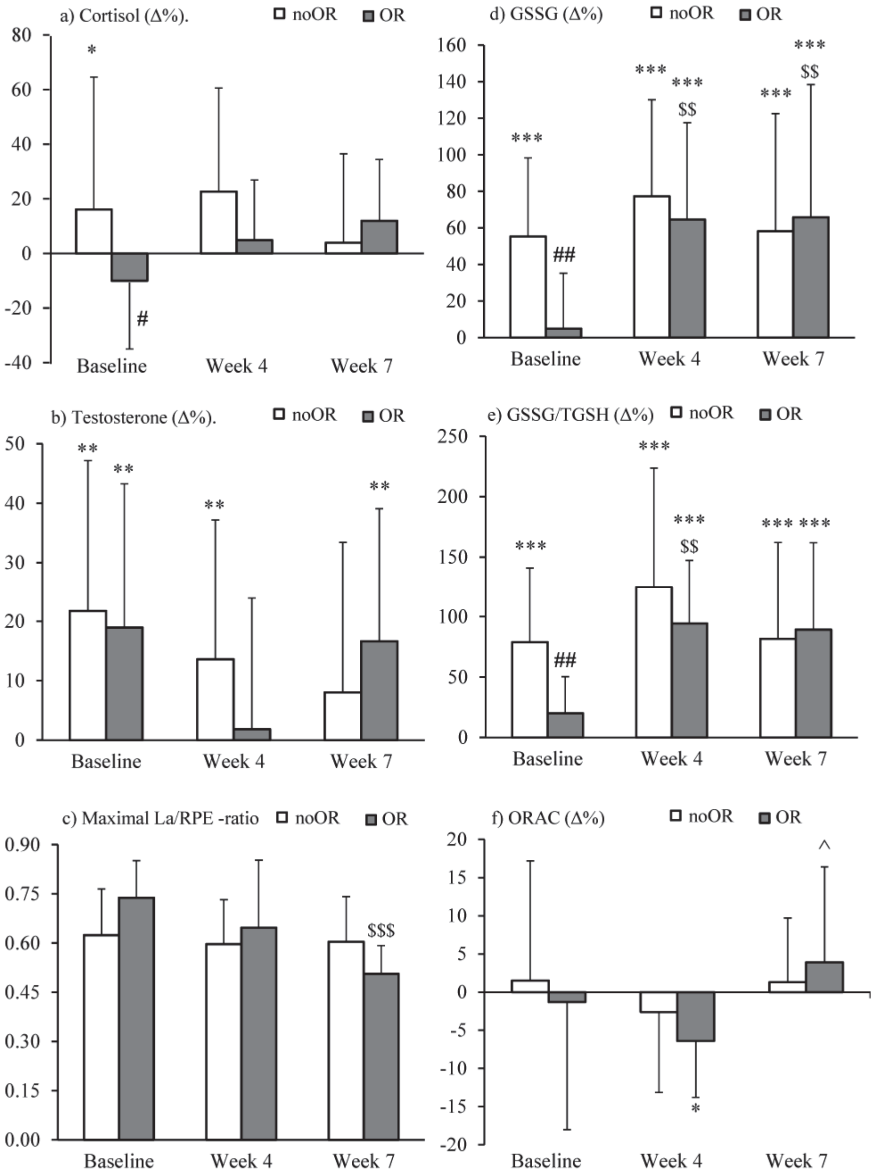

FIGURE 10 Mean (±SD) serum a) cortisol and b) testosterone and plasma d) GSSG, e) GSSG/TGSH and f) ORAC changes (%) due the 45-minute submaximal exercise and c) La/RPE ratio at the end of VO₂max test at baseline, and at week 4 and 7 of BT among noOR and OR. Difference compared to: noOR ## p<0.01, # p<0.05; baseline $$$ p<0.001, $$ p<0.01; week 4 ^^^ p<0.001, ^^ p<0.01, ^ p<0.05. Significant change due the exercise *** p<0.001, ** p<0.01, * p<0.05.

There were no differences between the groups in cortisol, testosterone, ORAC (Figure 10), TGSH, protein carbonyls and nitrotyrosine relative responses to exercise at baseline or during BT (Table 7).

TABLE 7 TGSH and oxidative stress markers among noOR (N=24) and OR (N=11) at rest and relative change due to the submaximal exercise (Δ% ex.)

			Baseline		Week 4		Week 7	
			Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
Testosterone (nmol ·L^{-1})	At rest	noOR	17,4 ± 4,8		18,1 ± 6,8		18,3 ± 6,1	
		OR	18,7 ± 4,6		21,2 ± 5,4		19,5 ± 6,5	
IGF-1 (pg ·mL^{-1})	At rest	noOR	3225 ± 1667		3334 ± 1491		2885 ± 1573	^^^
		OR	2584 ± 1431		2625 ± 1352		2315 ± 1549	
ORAC (µmol Troxol eq.)	At rest	noOR	602 ± 156		569 ± 118		605 ± 112	
		OR	634 ± 131		594 ± 113		612 ± 146	
TGSH	At rest (µmol)	noOR	773 ± 108		756 ± 83		776 ± 100	
		OR	801 ± 140		754 ± 91		767 ± 115	
	Δ% ex.	noOR	-11 ± 11	***	-15 ± 8	***	-8 ± 7	***, ^^
		OR	-12 ± 7	***	-12 ± 11	***	-6 ± 16	***
Protein carbonyls	At rest (nmol·mg^{-1})	noOR	0,09 ± 0,02		0,09 ± 0,02		0,09 ± 0,02	
		OR	0,10 ± 0,02		0,09 ± 0,02		0,10 ± 0,02	
	Δ% ex.	noOR	0 ± 5		-1 ± 4		1 ± 4	
		OR	0 ± 5		1 ± 4		1 ± 5	
Nitrotyrosine	At rest	noOR	6,53 ± 2,31		6,56 ± 2,18		6,53 ± 2,18	
		OR	6,45 ± 1,84		6,45 ± 1,78		6,52 ± 1,79	
	Δ% ex.	noOR	4 ± 15		-3 ± 11		2 ± 16	
		OR	9 ± 23		-1 ± 11		-6 ± 12	
Malondialdehyde	Δ% ex.	noOR	3 ± 24		7 ± 26		0,3 ± 23	
		OR	-11 ± 27		4 ± 26		-10 ± 21	

Difference within the group compared to week 4 ^^^ p<0.001, ^^ p<0.01. Significant change due the exercise *** p<0.001.

Physical activity among noOR and OR. PA was monitored in 9 OR and 9 noOR subjects. The OR and noOR subjects did not differ in incidence of sick leave during BT, and thus there was no need to take into account the influence of sick leave on physical activity. There were main effects of group for daytime VLPA (p<0.05) and nighttime MVPA (p<0.05). OR had higher nighttime MVPA compared to noOR during weeks 3-4 (p<0.05) and weeks 5-6 (p<0.05) (Figure 11), and less daytime VLPA during weeks 1-2 (p<0.05) and weeks 5-6 (p<0.05) (Figure 11). However, OR had higher daytime MVPA compared to noOR during weeks 1-2 (p<0.05) and weeks 5-6 (p<0.05) (Figure 11). There were no differences between the groups in REST, nighttime VLPA and sleeping time. However, REST was less during weeks 5-6 than weeks 7-8 (p<0.01; weeks 5-6 0:36 ± 0:24 h:min; weeks 7-8 0:42 ± 0:24 h:min,) in OR, but not in noOR.

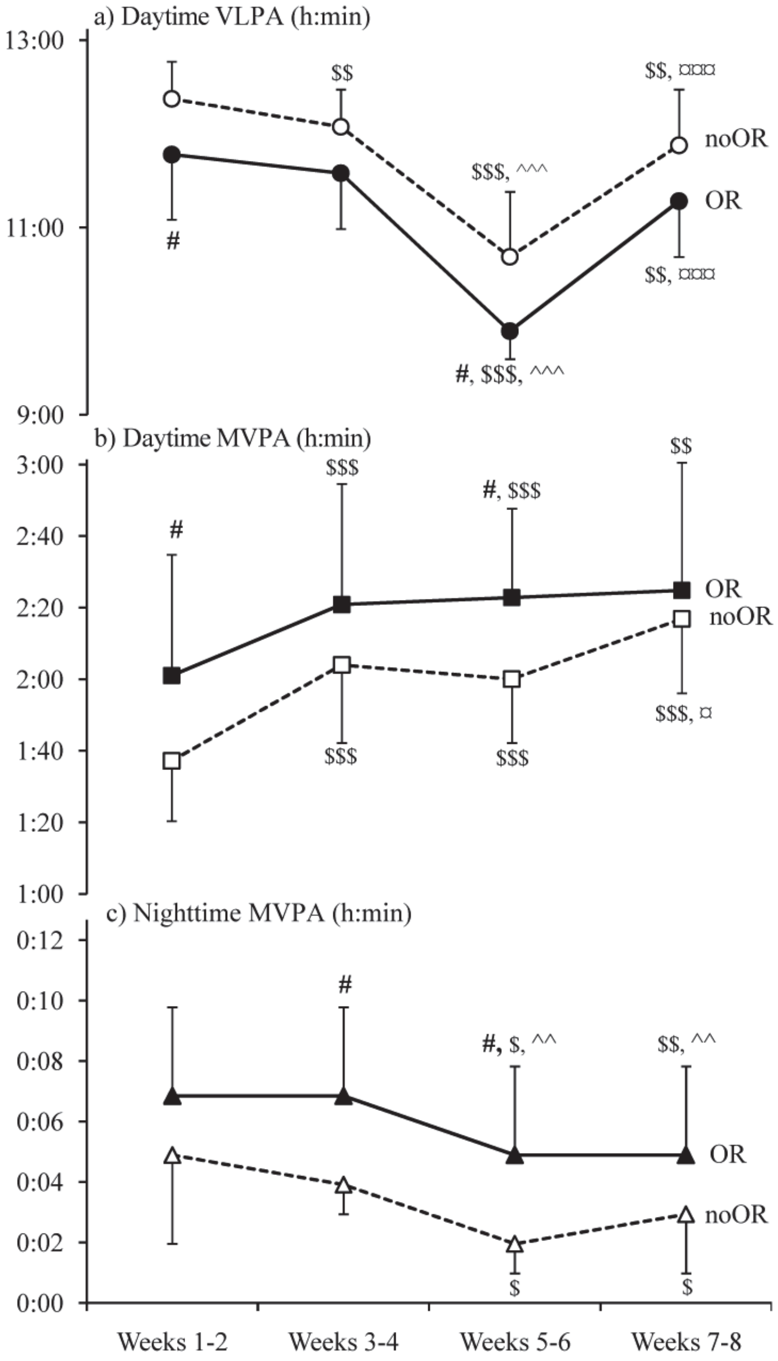

FIGURE 11 Mean (±SD) a) daytime very low (VLPA) and b) moderate to vigorous (MVPA) physical activity and c) nighttime moderate to vigorous (MVPA) physical activity during BT among the noOR- and OR-classified subjects. Difference compared to no OR # p<0.05; weeks 1-2 $$$ p<0.001, $$ p<0.01, $ p<0.05; weeks 5-6 ¤¤¤ p<0.001, ¤¤ p<0.01, ¤ p<0.05.

5.3 Training load during two weeks of BT and associations of aerobic fitness, energy balance and BMI with training load (III)

During the two-week study period at the end of BT, total EE was 15.48 ± 1.65 MJ·d^{-1} and PAL 2.03 ± 0.21. BMR (7.66 ± 0.92 MJ·d^{-1}) represented 50 ± 5% and AEE (6.26 ± 1.21 MJ·d^{-1}) 40 ± 5% of total EE. The average training load, AEE·kg^{-1} , was 0.08 ± 0.02 MJ·d^{-1}·kg^{-1}.

Energy balance and accuracy of self-reported pre-filled food diary in the military environment

The reported EI of 11.5 ± 3.2 MJ·d^{-1} was significantly lower than measured EE (15.5 ± 1.6 MJ·d^{-1}), and thus the results revealed significant underreporting (-26.0 ± 17.5%, $p<0.001$). The data on reported intake indicated that daily carbohydrate consumption in relation to total reported EI was 58 ± 4% (5.2 ± 1.6 g·kg^{-1}), fat 27 ± 4% (1.1 ± 0.4 g·kg^{-1}), protein 14 ± 2 % (1.3 ± 0.4 g·kg^{-1}), and alcohol 0.3 ± 0.9% (0.01 ± 0.04 g·kg^{-1}). When reported EI was corrected for mis-recording (-20.4 ± 26.3%), TrueEI (14.62 ± 4.17 MJ·d^{-1}), did not differ from EE (-5.6 ± 25.5%, ns.), which indicates a non-significant negative energy balance (under-eating) of the present subjects. However, Figure 12 indicates that many subjects had a negative energy balance that was negatively related to BMpre ($r = -0.73$, $p<0.001$), FM ($r = -0.83$, $p<0.001$) and BMI ($r = -0.80$, $p<0.001$).

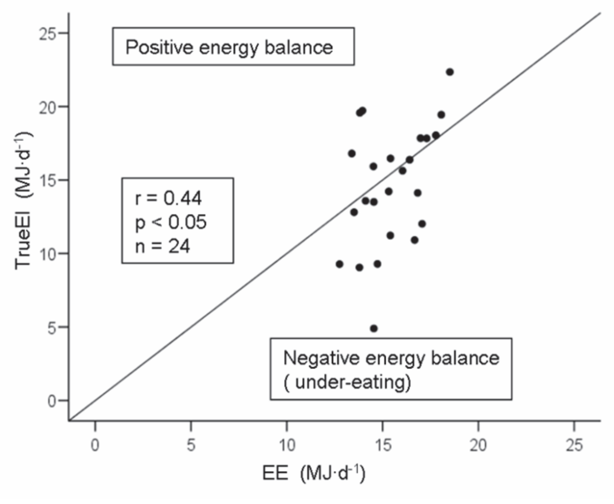

FIGURE 12 Relationship between estimated true energy intake (TrueEI) and energy expenditure (EE). The line represents the energy balance.

Associations of aerobic fitness, fatness and energy balance with training load

The subjects in the HighBMI group had lower AEE·kg^{-1} ($p<0.01$) compared with the subjects in the LowBMI and ModerateBMI groups (Figure 13b). The HighBMI subjects had also significantly higher negative energy balance (-4.2 ± 3.08 MJ·d^{-1}) compared with the subjects in the LowBMI (1.6 ± 3.23 MJ·d^{-1}, $p<0.001$) and the ModerateBMI (0.11 ± 2.30 MJ·d^{-1}, $p<0.01$) groups. The LowBMI and ModerateBMI groups did not differ in AEE·kg^{-1}, AEE·kg^{-1} and energy balance. All three BMI groups did not differ in VO_2max_{FFM}. AEE·kg^{-1} was also lower in LowFIT compared with ModerateFIT and HighFIT ($p<0.05$) (Figure 13a), but BMI and energy balance did not differ between these three fitness groups. Furthermore, AEE·kg^{-1} was lower among subjects in the High under-eating group compared with the Low and Moderate under-eating groups ($p<0.01$) (Figure 13c). These subjects also had higher BMI (27.8 ± 3.6 kg·m^{-2}) compared with subjects in the Low (21.4 ± 2.4 kg·m^{-2}, $p<0.001$) and Moderate (23.6 ± 1.9 kg·m^{-2}, $p<0.01$) under-eating groups and lower VO_2max_{FFM} (3.43 ± 0.27 l·min^{-2}) compared with those in the Moderate under-eating group (3.75 ± 0.28 l·min^{-2}, $p<0.05$). The Moderate and Low under-eating groups did not differ in AEE·kg^{-1}, BMI or VO_2max_{FFM}.

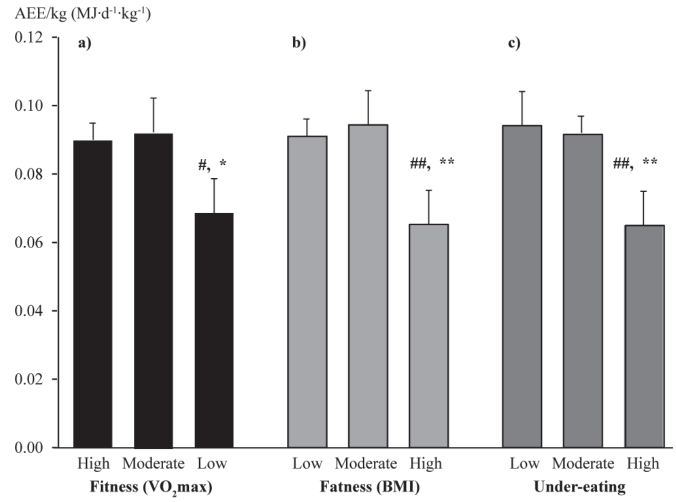

FIGURE 13 Mean (±SD) training load expressed as AEE·kg^{-1} in Low, Moderate and High fitness (a), fatness (b) and under-eating (c) groups. Significant difference as compared with: Low BMI and under-eating ## $p<0.01$, Moderate BMI and under-eating ** $p<0.01$, High fitness # $p<0.05$, Moderate fitness * $p<0.05$.

VO_2max_{FFM} was positively related to AEE and AEE·kg^{-1} ($r = 0.44$, $p<0.05$ and $r = 0.41$, $p<0.05$, respectively), but had no relationship with EE (Table 8). BMI was negatively related to AEE·kg^{-1} ($r = -0.60$, $p<0.01$), but had no relationship with EE or AEE (Table 8). In addition, in spite of the fact that BM did not change (-0.4±1.8 kg, ns.) during the two-week training period, energy balance was related to AEE·kg^{-1} ($r=0.58$, $p<0.01$) (Table 8). In the multivariate regression analysis only BMI remained in the model, explaining 33% of the variation in AEE ·kg^{-1} with a standard error of estimate (SEE) of 0.02 MJ·d^{-1}·kg^{-1}, or 25 % of mean AEE·kg^{-1}.

TABLE 8 Pearson correlation coefficients between BMI, VO$_2$max and energy balance and EE, AEE, AEE ·kg^{-1} and BMR.

	BMI (kg·m^{-2})	VO$_2$max (l·min^{-1})	VO$_2$max$_{FFM}$ (l·min^{-1})^a	Energy balance (MJ·d^{-1})
EE (MJ·d^{-1})	0.3	0.77 ***	0.29	0.29
AEE (MJ·d^{-1})	-0.7	0.44 *	0.44 *	0.21
AEE·kg^{-1} (MJ·d^{-1}·kg^{-1})	-0.60 **	-0.16	0.41 *	0.58 **
BMR (MJ·d^{-1})	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.
$^{a)}$VO$_2$max adjusted by fat-free mass by using a regression-based approach (Toth et al. 1993).

5.4 8-day training course (TC)(IV)

5.4.1 Physical activity measured by accelerometer

Mean rest during TC was 4:05 ± 0:32 h:min per day, sitting 5:05 ± 0:46 h:min, standing 9:10 ± 0:44 h:min and active time 5:38 ± 1:11 h:min per day (moderate activity 5:32 ± 1:09 h:min, vigorous activity 0:06 ± 0:03 h:min). However, a main effect of TC was observed for rest, sitting and moderate activity ($p<0.001$) and for standing and active time ($p=0.05$). Rest and standing increased during days 5-8 compared to days 1-5. On the contrary, standing, moderate activity and active time decreased. Physical activity did not differ between Ebar and the control groups during TC.

5.4.2 EI, EE, energy availability and water balance

The Ebar group consumed 58 ± 15% and the controls 57 ± 10% of the energy provided during TC. Energy intake from field rations did not differ between the groups (control 10.9 ± 1.7 MJ ·d^{-1} vs. Ebar 10.0 ± 1.0 MJ ·d^{-1}). However, energy availability ($p<0.05$) and total energy intake, including energy supplementation, were higher ($p<0.05$) in Ebar compared to controls (Table 9). Intake of protein was also higher in Ebar ($p<0.001$) than controls (Table 9). Energy balance was negative among all subjects and neither energy balance (control -9.2 ± 6.4 MJ ·d^{-1}, -40 ± -25%; Ebar -10.1 ± 3.7 MJ ·d^{-1}, -43 ± -12%, $p=0.056$) nor energy expenditure (control 20.2 ± 5.7 MJ ·d^{-1}; Ebar 23.3 ± 3.2 MJ ·d^{-1}, $p=0.060$) differed between the groups. Fluid intake (control 2.3 ± 0.5 L ·d^{-1}; Ebar 2.1±0.6 Ld^{-1}) and total water intake was similar in both groups, and there was no significant water deficit (Table 9).

TABLE 9 Energy intake, macronutrient composition of the consumed food and water balance during the 8-day training course among the control and Ebar groups.

	Controls (N=12)	Ebar (N=14)	p
Total energy intake ($MJ \cdot d^{-1}$)[a]	10.9 ± 1.8	13.3 ± 3.0	**0.025**
Total energy intake ($kJ \cdot kg^{-1} \cdot d^{-1}$)	157 ± 30	189 ± 43	**0.038**
Energy availability[b]	1.5 ± 0.3	1.8 ± 0.4	**0.028**
Carbohydrate ($g \cdot d^{-1}$)	359 ± 46	413 ± 84	0.063
Fat ($g \cdot d^{-1}$)	86 ± 21	99 ± 23	0.165
Protein ($g \cdot d^{-1}$)	92 ± 20	149 ± 46	**0.001**
Carbohydrate ($g \cdot kg^{-1} \cdot d^{-1}$)	5.2 ± 1.0	5.9 ± 1.2	0.108
Fat ($g \cdot kg^{-1} \cdot d^{-1}$)	1.2 ± 0.3	1.4 ± 0.3	0.196
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)	1.3 ± 0.3	2.1 ± 0.6	**<0.001**
Fluid intake ($L \cdot d^{-1}$)[c]	2.3 ± 0.5	2.1 ± 0.6	0.376
Total water intake ($L \cdot d^{-1}$)[d]	3.4 ± 0.6	3.3 ± 0.5	0.833
Water loss ($L \cdot d^{-1}$)	-3.5 ± 0.5	-3.3 ± 0.5	0.382
Water balance ($L \cdot d^{-1}$)	-0.1 ± 0.7	0.0 ± 0.7	0.584

Values are mean ± SD. [a]Estimated from food records. [b]Energy availability = energy intake / basal metabolic rate estimated by the equation of Schofield et al. (1985). [c]Estimated from fluid intake records. [d]Total water intake = fluid intake + water content of food + metabolic water.

5.4.3 Body composition and physical performance

TC induced a decrease in BM and FM in both controls and Ebar ($p<0.001$) while FFM (Figure 14) and TBW remained unchanged (control PRE 43.4 ± 4.5 L, POST 43.4 ± 4.7 L; Ebar PRE 44.6 ± 4.7 L, POST 44.9 ± 4.7 L).

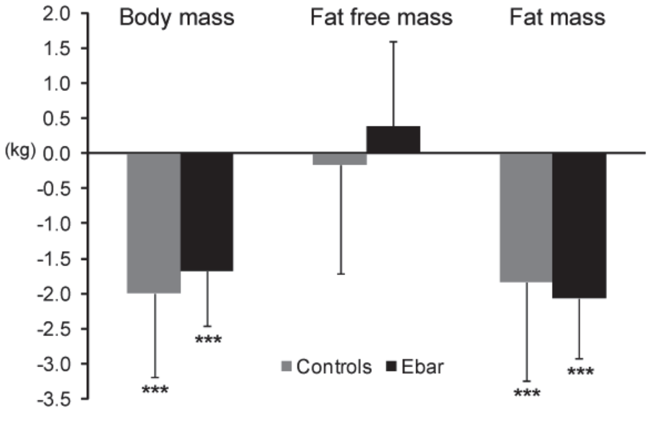

FIGURE 14 Mean (±SD) changes in body composition during the 8-day training course in the control and Ebar groups. *** p<0.001, significant change during the training course.

TC had a main effect on 3-km running time, anaerobic power and hand-grip ($p<0.001$) and group had a main effect on 3-km running time ($p<0.05$) and anaerobic power ($p<0.05$). Hand grip decreased in both groups from PRE to MID but it recovered to the initial levels POST TC. 3-km running time improved in both groups as well. However, only the controls showed an increase ($p=0.022$) in anaerobic power from MID to POST. Maximum heart rate during the 3km (control PRE 186 bpm, POST 189 bpm; Ebar PRE 191 bpm, POST 191 bpm), blood lactate after the 3km (control PRE 10.6 $\text{mmol}\cdot\text{L}^{-1}$, POST 11.9 $\text{mmol}\cdot\text{L}^{-1}$; Ebar PRE 11.5 $\text{mmol}\cdot\text{L}^{-1}$, POST 12.1 $\text{mmol}\cdot\text{L}^{-1}$), and vertical jump remained the same in both groups. Only the Ebar group improved anaerobic power before TC (6 day PRE to PRE, $p<0.001$), thus the relative change was higher in Ebar than in controls ($p<0.01$). Although Ebar had higher anaerobic power than controls in PRE ($p<0.01$), MID ($p<0.05$) and POST ($p<0.05$), there were no differences between the groups in its rate of change from PRE to POST.

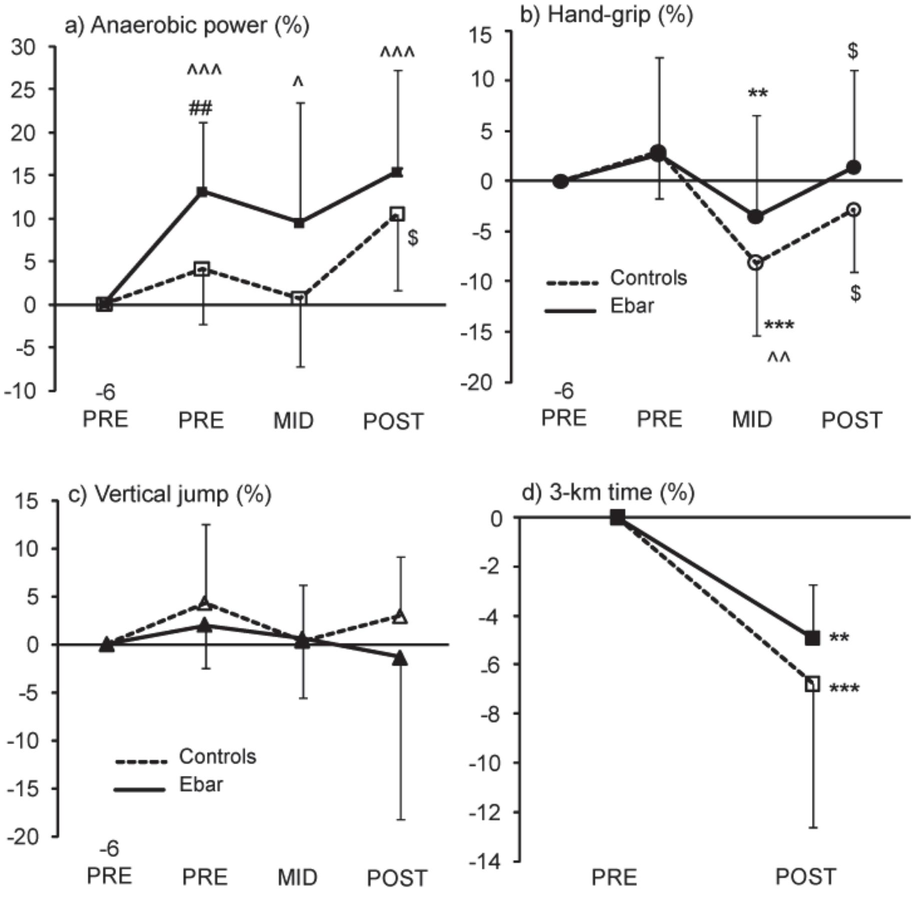

FIGURE 15 Changes (%) in physical performance in the control and Ebar groups during TC. Differences in change compared to the controls ## $p<0.01$. Differences in absolute change within the group compared to: -6 PRE ^^^ $p<0.001$, ^ $p<0.05$; PRE *** $p<0.001$, ** $p<0.01$; MID $ $p<0.05$.

5.4.4 Questionnaire

Data analysis of the questionnaire (Figure 16) revealed significant main effects for hunger ($p<0.001$; $p<0.05$, respectively), physical performance ($p<0.001$; $p<0.01$) and positive mood state ($p<0.01$; $p<0.05$) in both the TC and Ebar groups. Ebar felt less hungry than the controls POST and +3d POST ($p<0.01$). Furthermore, Ebar reported feeling less hungry +3d POST compared to MID ($p<0.001$), while controls did not. In addition, Ebar evaluated their physical performance better compared to the controls -6d PRE and PRE ($p<0.05$), and reported improved positive mood state from MID to +3d POST, while the controls did not. TC had a main effect on negative mood state and somatic symptoms ($p<0.001$), which increased from -6d PRE and PRE to MID and decreased +3d POST to the PRE levels. Symptoms of URTI did not change.

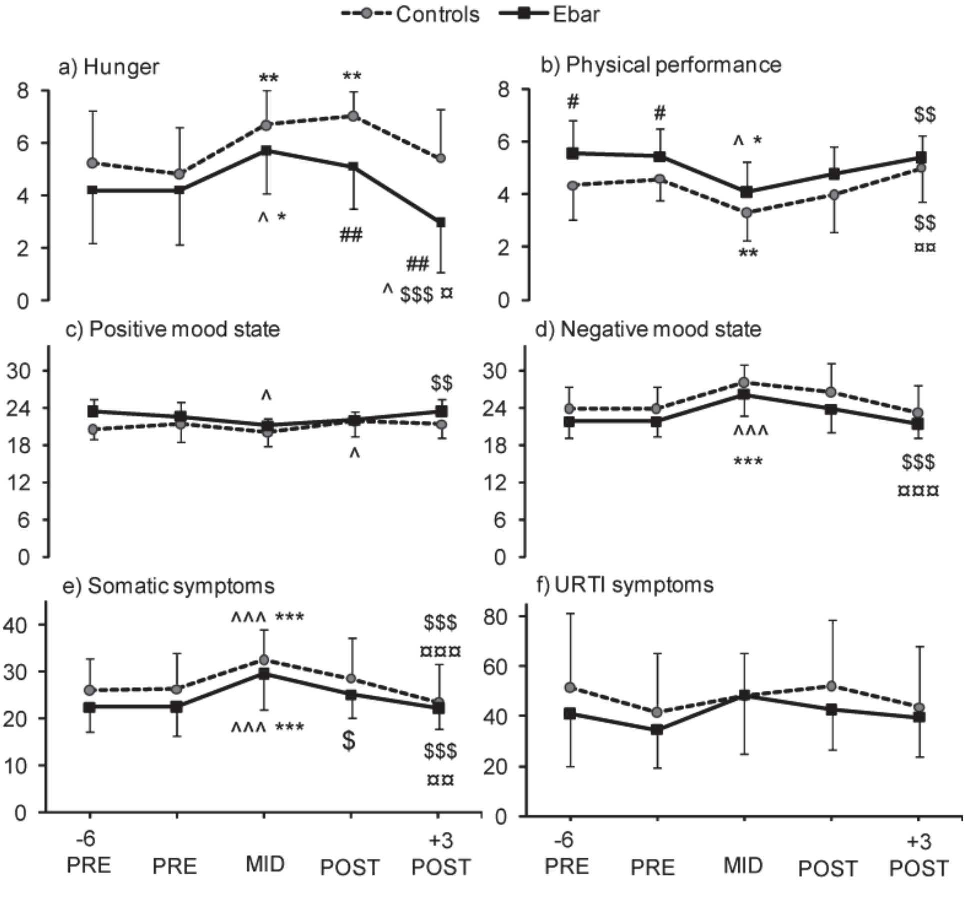

FIGURE 16 Questionnaire responses (mean ± SD) during the 8-day training course among the control and Ebar groups. Difference compared to: control ## $p<0.01$, # $p<0.05$; -6 PRE ^^^ $p<0.001$, ^ $p<0.05$; PRE *** $p<0.001$, ** $p<0.01$, * $p<0.05$; MID $$$ $p<0.001$, $$ $p<0.05$, $ $p<0.05$; POST ¤¤¤ $p<0.001$, ¤¤ $p<0.01$, ¤ $p<0.05$.

5.4.5 Association between physical activity, energy availability, water balance and performance

While there were no differences between the groups in physical activity, energy availability and water balance, the groups were pooled for correlation analysis. Correlations between physical activity and energy availability, water balance, hand-grip and anaerobic power are presented in Table 10. Energy availability correlated only negatively with sitting throughout TC. Instead, water balance correlated positively with sitting and negatively with moderate activity and active time. Water balance was not related to either energy intake or energy availability. Change in hand-grip from PRE to MID correlated positively with sitting and negatively with moderate and active time on days 1-5. Change in anaerobic power from PRE to the MID correlated negatively with vigorous activity. Changes in the performance tests were not associated with energy availability or water balance. Change in BM was not related to change in 3-km running time, but it correlated negatively with change in anaerobic power from MID to POST (r = -0.46, p = 0.021).

TABLE 10 Pearson correlation coefficient between physical activity and energy availability, water balance, hand grip and anaerobic power during the 8-day training course.

		Energy availability	Water balance	Hand grip ΔPRE-MID	Anaerobic jump ΔPRE-MID
Sitting (h:min)	Days 1-5	-0.33	**0.49***	**0.40***	-0.09
	Days 5-8	-0.35	**0.47***	-0.04	-0.09
	Days 1-8	**-0.44***	**0.53****	0.27	-0.07
Standing (h:min)	Days 1-5	0.10	**0.46***	0.14	0.30
Moderate activity (h:min)	Days 1-5	0.05	**-0.63*****	**-0.45***	-0.04
	Days 5-8	0.21	**-0.59****	-0.16	-0.11
	Days 1-8	0.14	**-0.64*****	-0.37	-0.05
Vigorous activity (h:min)	Days 1-5	-0.11	-0.10	0.25	**-0.42***
	Days 1-8	-0.20	-0.27	0.13	**-0.40***
Active time (h:min)	Days 1-5	0.04	**-0.63*****	**-0.43***	-0.07
	Days 5-8	0.21	**-0.58****	-0.18	-0.09
	Days 1-8	0.13	**-0.64*****	-0.36	-0.06

*** $p<0.001$, ** $p<0.01$, * $p<0.05$.

However, negative mood state before TC negatively associated with energy availability during TC and sleeping time during the first half of TC (Table 11). In addition, negative mood state in the middle of TC correlated negatively with active time and change in anaerobic power from PRE to MID (r = -0.40, p = 0.048). Furthermore, a negative association was observed between energy availability and somatic symptoms and URTI symptoms, and between URTI symptoms and moderate activity and active time. Moreover, URTI symptoms before, during and after TC had a positive association with water balance during TC. (Table 11).

TABLE 11 Pearson correlation coefficient between physical activity, energy availability and water balance and questionnaire data during the 8-day training course.

		Negative mood state			Somatic symptoms			URTI symptoms				
		-6 PRE	PRE	MID	-6 PRE	PRE	MID	-6 PRE	PRE	MID	POST	+3 POST
Energy availability		**-0.41***	**-0.43***	-0.26	**-0.40***	**-0.50****	**-0.45***	-0.25	**-0.40***	-0.36	**-0.49***	**-0.47***
Water balance		0.06	0.12	0.36	0.37	0.35	**0.41***	**0.44***	**0.51****	**0.60*****	**0.42***	0.27
Sleeping (h:min)	Days 1-5	-0.24	**-0.47****	-0.28	-0.15	-0.23	-0.15	0.22	0.06	0.09	-0.23	-0.16
	Days 5-8	-0.07	-0.22	0.07	0.14	0.18	0.11	**0.41***	0.35	**0.47***	0.20	0.23
	Days 1-8	-0.17	**-0.40***	-0.10	0.01	-0.01	-0.01	**0.39***	0.26	0.34	0.00	0.06
Moderate activity (h:min)	Days 1-5	-0.08	-0.03	-0.34	-0.12	-0.15	-0.25	-0.24	-0.31	**-0.48***	-0.34	-0.26
	Days 5-8	-0.18	-0.06	**-0.51****	-0.06	-0.1	-0.20	-0.40	-0.29	**-0.39***	-0.15	-0.11
	Days 1-8	-0.13	-0.05	**-0.43***	-0.11	-0.13	-0.27	-0.32	-0.33	**-0.48***	-0.30	-0.21
Active time (h:min)	Days 1-5	-0.06	-0.03	**-0.34**	-0.11	-0.14	-0.23	-0.23	-0.30	**-0.48***	-0.33	-0.25
	Days 5-8	-0.19	-0.07	**-0.51****	-0.06	-0.11	-0.22	**-0.40***	-0.30	-0.38	-0.14	-0.09
	Days 1-8	-0.12	-0.05	**-0.43***	-0.10	-0.13	-0.26	-0.32	-0.32	**-0.47***	-0.28	-0.20

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

6 DISCUSSION

The purpose of this thesis was to evaluate the training load of an 8-week Finnish military BT period among conscripts, determine the incidence of OR during BT and to evaluate the differences between the OR and noOR subjects. The specific aims of the present investigations were: first, to determine the associations between energy balance, maximal aerobic fitness, fatness and training load during two weeks of BT in wintertime; and second, to study the effects of an additional protein-rich energy supplement on energy balance, physical activity, body composition, physical performance and subjective experiences during an 8-day TC in cold climatic conditions.

The main findings showed that the physical training load in BT is comparable to that of daily competitive athletic training. During the first 4 weeks of BT, positive training responses as indicated by improved aerobic fitness and decreased oxidative stress were observed However, 33% of the conscripts were classified as OR. Markers of oxidative stress, serum cortisol, testosterone /cortisol -ratio at rest and maximal lactate/RPE -ratio could be useful tools for monitoring whether military training is too stressful. During BT, the ability to train is affected by energy deficit, fitness and fatness, of which fatness has the most limiting effect on high-level training. In contrast, during strenuous TC, physical activity seems to be primarily affected by factors other than energy balance, such as mood state.

6.1 Training adaptation to the 8-week BT

The average physical training load (≥ 4 MET) assessed by physical activity during the 8-week BT period was two hours per day with only half an hour of total rest (≤ 1.0 MET) during the daytime. Otherwise, the day included light physical activity, from sitting and standing to slow walking (1.0-3.9 MET), eleven hours per day. Furthermore, physical training load increased and light activity and rest time decreased after 2 weeks of BT, as planned in the daily program. However, according to the training schedule, the amount of physical training should have increased from two hours per day to 3 to 4 hours during weeks 4 and 7; instead it remained stable during the last 6 weeks.

Sick leave and mood state

The subjects with a "high incidence of sick leave" had lower serum testosterone and T/C -ratio and VO_2max at the beginning of BT than the "no sick leave" subjects. Furthermore, subjects who admitted to feeling overloaded demonstrated low VO_2max and fat-free mass at baseline. These observations confirm the earlier finding that among other environmental and epidemiological factors poor aerobic fitness is a risk factor for sick leave during military service (Knapik et al. 2001b) and also for mental well-being (Aberg et al. 2012).

During BT, there was a high incidence of dropouts from the second and last performance tests: only 63% of the subjects completed all three submaximal tests and 38% both the submaximal and maximal tests. The main reason for sick leave and dropout from the test was URTI, which might be a consequence of increased physiological and psychological stress. The increase in somatic symptoms of overreaching towards the end of BT, along with a concurrent increase in the number of subjects who reported being overloaded, support the increased training load during the latter part of BT compared to the training load experienced before BT (Bosquet et al. 2001; Coutts et al. 2007d; Dupuy et al. 2010; Halson et al. 2003; Tanaka et al. 1997; Verde et al. 1992). However, a possible reason for the high prevalence of URTI symptoms might also have been crowded living conditions (Gray et al. 2001). Furthermore, the present study was implemented during winter time, and URTI episodes have been observed to show seasonal variation in military training, with higher prevalence during wintertime than summertime (Juvonen et al. 2008). In addition, decreases in temperature and humidity have been found to increase the risk for URTI (Mäkinen et al. 2009).

Body composition and physical performance

For those conscripts who were able to perform all the tests, the increase in VO_2max during the first four weeks of BT indicates an improvement in aerobic capacity. Moreover, the decrease in HR, post-exercise La and serum testosterone during the 45-min submaximal marching test indicate reduced exercise-induced metabolic stress (Patton et al. 1980; Santtila et al. 2010; Wenger and Bell 1986). Furthermore, as expected, our data showed an inverse relationship between initial VO_2max and relative changes in VO_2max, indicating that the training was the most effective for the least fit subjects. This is comparable with studies performed in the Polish (Faff and Korneta 2000) and USA (Patton et al. 1980) armies as well as previous studies in Finland (Mikkola et al. 2012a; Santtila et al. 2008).

During the second half of the 8-week training period, stagnation in VO_2max was observed, which indicates that the training load may have been too exhaustive or too light. However, decreased maximal post-exercise lactate and HR (Hedelin et al. 2000a; Meeusen et al. 2006; Uusitalo 2001) support that training has been too exhaustive. Body composition remained the same during the first four weeks, but BM and FM decreased and FFM increased during the latter part of BT.

Serum hormones

A new finding of this study was that serum testosterone concentration and the T/C -ratio at the beginning of the training period had an impact on sustaining stress and maintaining health during the 8-week military training period. Low testosterone concentrations before the training season have been found to be related to decreases in performance during a competitive season among soccer players (Kraemer et al. 2004a). In addition, in well-trained strength athletes a high initial testosterone concentration was related to further improvement in strength (Häkkinen et al. 1988). Furthermore, a low saliva testosterone concentration was related to tiredness during the competitive period among rugby players (Maso et al. 2004). Therefore, testosterone levels before the training season could be a valid marker of tolerance in the forthcoming training season.

Among the 35 conscripts who performed all the tests, basal serum testosterone did not change, while basal serum IGF-1 and cortisol decreased and basal serum SHBG and T/C -ratio increased. Previously, serum total testosterone concentration has been shown to decrease in response to demanding training periods (Coutts et al. 2007b; Urhausen and Kindermann 2002; Urhausen et al. 1987), and also after 8 weeks of military training (Fortes et al. 2011). Furthermore, a 7-week intensive endurance overload training program (Urhausen et al. 1987) and concurrent overload training for 6 weeks (Coutts et al. 2006) have been found to decrease testosterone/cortisol ratios. This has also been observed during the later phases of a prolonged strenuous strength training period (Häkkinen et al. 1985). The increase in the T/C -ratio in the present study was a result of decreased cortisol concentrations, which may be a marker of overtraining, along with lower psychosocial stress (Rietjens et al. 2005; Snyder et al. 1995; Steinacker et al. 2004; Urhausen and Kindermann 2002). As in the present study, basal SHBG has been found to increase (Mäestu et al. 2005; Nindl et al. 1997) and IGF-1 to decrease (Fortes et al. 2011; Nindl et al. 2007a; Nindl et al. 2003; Nindl et al. 1997; Nindl et al. 2006) after heavy training. A decrease in IGF-1 is suggested to indicate a diminished capacity for protein deposition. In addition, IGF-1 is sensitive to both energy intake and dietary protein content, and demonstrates significant decreases with inadequate dietary intake (Fortes et al. 2011; Nindl et al. 2003; Nindl et al. 1997).

Although the increase in the T/C -ratio in response to demanding training conflicts with previous findings, the increase in SHBG and decrease in IGF-1 in this study indicate that the training load during the second half of the 8-week training period may have been too exhaustive. This is also supported by the fact that 46% of subjects showed a decrease in basal serum testosterone from wk4 to wk7, which is an indicator of overload training. However, 23 % of the subjects showed increases in basal testosterone throughout the study. This is in line with the previous studies, where marked individual differences were found in training- and overload training-induced hormonal changes (Häkkinen et al. 1988; Häkkinen et al. 1985; Uusitalo et al. 1998a).

Oxidative stress markers and antioxidant capacity

Recently, oxidative stress and antioxidant status have been found to be related to OTS in elite athletes (Tanskanen et al. 2010). However, the responses of oxidative stress markers and antioxidant capacity to basic military training have not been reported previously. In this study, day activity time increased and rest time decreased during the first 4 weeks of BT, with a concomitant increase in aerobic performance, while body mass and fat-free mass remained the same. Simultaneously, decreased oxidative stress at rest, manifested as lower glutathione oxidation (GSSG), was observed. The decrease in oxidative damage can be explained either by attenuated generation of ROS (energy restriction) or by enhancement of tissue protection and antioxidant systems, as a consequence of adaptation to regular exposure to a small amount of ROS during regular physical exercise (Radak et al. 2005). Endurance training itself has also been found to decrease oxidative stress at rest without inducing changes in body mass (Devries et al. 2008). Thus, the decreased oxidative stress observed in the present study may be a consequence of enhancement of the antioxidant defense system in response to a tolerable training load (Gomez-Cabrera et al. 2008; Margaritis et al. 1997), also among the OR-classified subjects. A high capacity to consume oxygen (enhanced VO_2max), and consequently to produce a larger amount of ROS, is accompanied by high erythrocyte glutathione peroxidase activity and a high glutathione concentration, both of which serve to protect the organism from lipid peroxidation and cell membrane damage (Laaksonen et al. 1996; Margaritis et al. 1997).

In the present study, in contrast to the decreased oxidative stress at rest, four weeks of BT increased the susceptibility to acute exercise-induced oxidative stress as evidence of higher glutathione oxidation and lower antioxidant capacity after submaximal exercise. At baseline, the subjects were not familiar with an acute physical load that can lead to increased oxidative stress. In contrast, higher exercise-induced oxidative stress was observed after four weeks training than at baseline. In previous studies, both endurance (Finaud et al. 2006b) and strength (Nikolaidis et al. 2007) training has been found to reduce post-exercise oxidative stress in performance of the same physical task. However, Vollaard et al. (2006) found that neither tapering nor overload endurance training affected the exercise-induced increase in GSSG among endurance-trained athletes. In this study, increased exercise-induced oxidative stress may indicate overreaching due the intensive BT period. In the entire group of 35 subjects and in the OR subjects, a U-shape curve of oxidative stress at rest was observed during BT: GSSG decreased at rest from baseline to wk4, returning to baseline values at wk7. Interestingly, submaximal exercise decreased ORAC at wk4. This might be explained by increased consumption of the body's endogenous antioxidant resources. When coping with oxidative stress, plasma antioxidants are mobilized into the tissue (Margonis et al. 2007). Similarly, Palazzetti et al. (2003) found decreased extracellular antioxidant capacity but enhanced plasma glutathione peroxidase activity at rest and increased exercise-induced lipid peroxidation after four weeks of endurance-type overload training in highly-trained triathletes, all of which imply the absence of concomitant responses in antioxidant parameters during OTS.

During the second half of the 8-week training period, although day activity time and rest time remained the same, GSSG and the GSSG/TGSH ratio at rest increased and submaximal exercise resulted in a higher induction of the GSSG/TGSH ratio, indicating that the training load was too strenuous and causing oxidative stress (Gomez-Cabrera et al. 2008; Margonis et al. 2007). However, ORAC did not response to submaximal exercise at the end of BT, as was the case in wk4. This could be a marker of OR, since diminished antioxidant protection has been observed among overtrained athletes (Tanskanen et al. 2010). The result also confirm the previous study reporting that antioxidant capacity and oxidative stress can fluctuate during the training season, and therefore should be considered as a biomarker to monitor the training load of athletes (Teixeira et al. 2009).

Plasma nitrotyrosine and protein carbonyls, markers of oxidative protein modification, remained the same during the entire 8-week training period, both at rest and after exercise. There were also no differences between OR and noOR. Plasma nitrotyrosine levels showed wide variation, which may partly explain the observed findings. The present results also support the previous findings that plasma nitrotyrosine level may not be a sufficiently sensitive marker to assess training or acute exercise-induced changes in oxidative stress (Rush et al. 2003; Tanskanen et al. 2010). Proteins are an important target for oxidative challenge. Reactive oxygen species modify amino acid side chains of proteins to form protein carbonyls (Levine et al. 2000). Serum protein carbonyls have been found to increase after an intensive 12-week strength training period (Margonis et al. 2007), decrease after exhaustive marches (50 and 80 kg) in well-trained soldiers (Chevion et al. 2003) and increase after an exercise test to exhaustion in athletes (Tanskanen et al. 2010). The turnover time for protein carbonyls varies from several hours to days (Chevion et al. 2003). The 8-week training period might have been too short and / or the submaximal exercise load in the present study may not have been strenuous enough to modify proteins. Exercise may also have activated a mechanism that removes oxidatively modified proteins from the circulation, or alternatively, activation of antioxidant mechanisms that remove ROS (Chevion et al. 2003). In addition, the differences between studies in oxidative stress responses to training and exercise may be explained by differences in the energy availability, training mode and length of training period, fitness levels and genetic background of the subjects. The subjects in the present study were low to moderately fit conscripts in the compulsory army, and the training consisted of both strength and endurance training.

6.2 Incidence of OR and differences between the OR and noOR subjects

Classification of OR and OTS is challenging, and is rarely implemented on a single criterion. However, a decrease in performance has been suggested to be the one and only valid criterion when combined with delayed recovery time of more than two weeks (Budgett et al. 2000; Halson and Jeukendrup 2004; Meeusen et al. 2012; Meeusen et al. 2006). In this study, to be classified as OR, subjects had to fulfill three out of five criteria, including decreased aerobic physical performance. Based on these criteria, OR was detected in 33% of the subjects. Absence from the VO_2max or submaximal exercise tests was set as an additional criterion for decreased performance. Sick leave itself can affect training responses, even resulting in a detraining effect. However, the OR and noOR subjects did not differ in sick leave during the first 7 weeks of BT and the period of sick leave lasted only a few days. Thus, the decrease in performance was mainly caused by excessive training load.

A high mean training load of two hours per day during an 8-week BT period could be a risk factor for OR among subjects with a low fitness level. Surprisingly, the OR and noOR subjects did not differ from each other in VO_2max or body composition at the beginning of BT. This suggests that fitness or fatness in this study were not strong underlying factors for overreaching. On the other hand, the OR subjects were more physically active both during the day and night time than the noOR subjects. These results support the hypothesis that the consequences of physical training are influenced not only by the intensity and duration of training, but also by the duration of recovery time. Among the OR subjects, the accumulated training load of high physical activity during the day- and nighttime during the latter part of BT may have aggravated the symptoms and indicators of OR. However, the OR and noOR subjects did not differ with respect to the tasks they performed during military service. Thus higher physical activity might more likely be an individual property rather than an external factor. Some supportive evidence for this hypothesis comes from a study by Knapik et al. (2011) in which PA was measured by a pedometer during BT, and those who had high PA had also higher risk for injuries.

The only previous study on OR/OTS among young men entering military service and performing an 8-week BT training period is that reported by Chicharro et al. (1998). They found a 24% incidence of OTS during BT, determined by absolute decreases in the free T/C -ratio. Similarly, in this study, the OR subjects differed in response to training compared to the noOR subjects. The OR subjects exhibited a decrease in the basal Δ% T/C -ratio, whereas the noOR subjects exhibited an increase. Snyder et al. (Snyder et al. 1995) defined five criteria for OR, one of which was a decreased maximal La/RPE ratio. In the present study, the OR subjects showed a decrease in the maximal La/RPE ratio during the latter part of BT, while the noOR subjects did not.

The relationship between basal serum SHBG and the incidence of OR have not been reported earlier. In the present study, the OR subjects had higher levels of SHBG, both before training and after 4 and 7 weeks of training. Although basal SHBG has been found to increase after heavy training (Mäestu et al. 2005; Nindl et al. 1997), the mechanism underlying the present findings is still not fully understood. Testosterone did not differ between the noOR and OR subjects. However, most testosterone is bound to SHBG, which in turn decreases the bioavailable testosterone concentration in serum, and may thereby have an influence on training responses. Although IGF-1 has been hypothesized to be indicator of OR/OTS (Nindl et al. 2007a; Nindl and Pierce 2010; Nindl et al. 2012; Rarick et al. 2007), in this study no differences between the OR and noOR subjects were observed. However, IGF-1 decreased in all conscripts. The limitation of the present study is that only total IGF-1 and not IGF-1 binding proteins were measured. IGF-1 binding protein 3, in particular, has been found to have associations with OR (Elloumi et al. 2005) and might also influence other stressors than energy deficit (Fortes et al. 2011).

The OR subjects had higher basal serum cortisol at the end of BT, which is in line with the OR scenario presented by Steinacker et al. (2004), where an increase in basal cortisol was observed in the OR state. Basal serum cortisol has been found to increase during extremely stressful military training periods (Nindl et al. 2007a; Nindl et al. 2006), but no change (Coutts et al. 2007b; Mäestu et al. 2005; Urhausen et al. 1987), or a decrease (Rietjens et al. 2005; Snyder et al. 1995) has been observed in endurance-based overtraining studies. However, in response to strenuous strength training, both no change (Fry et al. 1998; Kraemer et al. 2006) and an increase (Fry and Kraemer 1997) in basal serum cortisol have been reported. In addition, a clearly observable increase in serum cortisol was found during strenuous high volume combined endurance and strength training (Kraemer et al. 1995). However, increase in cortisol did not take place during low volume and low frequency combined endurance and strength training, despite the long training period of 21 weeks (Mikkola et al. 2012b).

Glucocorticoids, like cortisol, exert many beneficial effects in exercising humans: they increase the availability of metabolic substrates for the energy requirements of muscles, maintain normal vascular integrity and responsiveness, and protect the organism from an overreaction of the immune system in response to exercise-induced muscle damage (Rietjens et al. 2005; Steinacker et al. 2004). The differences between military and overtraining studies in the findings for the profile of cortisol and other hormones and in the incoherent training responses of basal serum cortisol observed in the present study may be explained by the differences in energy availability, training mode and fitness levels of the subjects. The previous military training studies were performed under conditions of nutritional and sleep deprivation in combination with high energy expenditure and physiological strain. Most of these studies involved relatively short (from 5 to 14 days) training periods, and the subjects were soldiers. In the overtraining studies, food and sleep were ad libitum, and the subjects were athletes, or at least had a good fitness level. Furthermore, the training period in the overtraining studies ranged from 2 to 9 weeks, and consisted mostly of endurance or strength training. The subjects in the present study were low to moderately fit conscripts doing compulsory military service, and the training consisted of both strength and endurance training. Another reason for the discrepancies between findings may be differences in the total training load and energetic demands of training (Gastmann et al. 1998).

Recently, it has been proposed that the best indicator of OR and OTS is the physiological response to acute stress, such as an exercise test (Meeusen et al. 2006). Because of sick absences, only 47% of the OR subjects performed all the submaximal tests. This may be the reason for the non-significant difference in cortisol responses to exercise between the OR and noOR subjects. However, among the conscripts who were able to perform all the submaximal tests, the exercise-induced increase in cortisol observed in this study was only significant after 4 weeks of training. It has been suggested that increased basal levels of cortisol contribute to exhaustion of the hypothalamic-pituitary-adrenal axis, thus preventing an adequate cortisol response to such acute stress (Fry and Kraemer 1997). In the present study, the attenuated cortisol response before training could be explained by the higher basal values, although this seems unlikely as the pre-exercise cortisol levels were not associated with cortisol responses to the 45-min submaximal marching test. According to Steinacker et al. (2004), adreno-corticotropic hormone (ACTH) and cortisol increase during a recovery period in response to metabolic stress; in turn, during overreaching, the cortisol response is blunted and this is accompanied with an increase in ACTH response. Overtraining is characterized by a decrease in both the ACTH and cortisol responses (Steinacker et al. 2004), and exercise-induced decreases in cortisol have been linked to intensive training (Urhausen and Kindermann 2002). Thus, in this study, the increased response of cortisol to acute exercise after 4 weeks of training among the 35 conscripts who completed all the tests indicates an improvement in performance, while an attenuated response after 7 weeks is indicative of overreaching.

In this study, increased oxidative stress was associated with OR. To our knowledge, this is the first study showing that increased oxidative stress prior to a strenuous 8-week combined endurance and strength training period was related to the incidence of OR during the training period. Oxidative stress markers, including GSSG, the GSSG/TGSH ratio and malondialdehyde at rest, were higher in the OR than in noOR subjects. In addition, the OR subjects showed no increase in GSSG and the GSSG/TGSH ratio in response to submaximal exercise, which might be a consequence of high resting levels. Impaired antioxidant capacity and increased oxidative stress have been found in the OTS state (Tanskanen et al. 2010). However, whether increased oxidative stress is a factor contributing to OTS or an outcome of a training protocol with inadequate recovery remains unclear.

6.3 Association of aerobic fitness, fatness and energy balance with training load (III)

In this study, the training load during a two weeks of BT in wintertime was assessed by DLW derivate PAL and AEE $\cdot kg^{-1}$. The PAL value was 2.0, which is comparable to values observed in people performing very strenuous work or daily competitive athletic training (Nordic Nutrition Recommendations, 2004, 121-125). Throughout BT, all conscripts train together according to the same program. Thus physical activity is not optional and therefore PAL should be the same regardless of the subject's fitness or fatness status. From another perspective, individuals with low aerobic fitness will experience greater physiological stress relative to their maximum capacity at any given absolute level of aerobic work (Jones and Knapik 1999). Thus, it could be hypothesized that less fit conscripts will experience more physiological stress during physical activity because they have to work at higher relative intensities, and aerobic fitness is not necessarily related to AEE $\cdot kg^{-1}$.

Contrary to this hypothesis, good aerobic fitness was related to higher training load. Our unexpected findings might partly be explained by the more intense participation and engagement of fit conscripts in military training. Moreover, in field situations the fitter 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 greater energy cost of cycling at the same relative intensity than untrained, less fit controls. This was due to the lower workload required of the less fit subjects to increase their heart rate to 75 % of maximum, and thus their energy expenditure was also low (Horton et al. 1994). In contrast, during high intensity activities, the low absolute work capacity of the less fit subjects limits their work ability.

BMI was used as an index of fatness and it was not associated with AEE. However, the higher the BMI was, the less was the training load, AEE $\cdot kg^{-1}$. Among children, it has been observed that AEE and EE tend to increase with increasing fatness (Ekelund et al. 2002). Therefore, when performing the same absolute task, fatness increased absolute AEE. However, this was not seen in the present study, in which most military tasks were performed at low-to-moderate intensity levels. This suggest that the fatter subjects were able to perform the tasks well, while their less fat counterparts performed the same tasks at higher absolute intensity, increasing their relative AEE $\cdot kg^{-1}$.

In the multivariate regression analysis, only BMI remained in the model as explaining the variation in training load, despite the fact that the correlation and one-way analysis revealed that good fitness level and positive energy balance were also related to higher training load (AEE $\cdot kg^{-1}$). First, this suggests that more active subjects exhibit greater levels of aerobic fitness. Second, this was partly explained by the fact that negative energy balance (under-eating) was negatively related to BMI. In other words, the subjects with higher BMI were more prone to lose body mass than their slimmer counterparts. Although negative energy balance was not significant (-5.6%) at the group level, the individual variation in energy balance ranged from -66.3 to 41.7%. Based on Figure 12, one-third of the subjects had a negative energy balance. However, in the present subjects, of whom one-third (n=8) had a BMI over 25, deciding acceptable tolerance limits for negative energy balance is difficult. The positive relationships observed between energy intake and energy expenditure (Figure 12), and between energy balance and AEE $\cdot kg^{-1}$ are well in line with those reported in other studies, where low energy intake has been found to be related to low energy expenditure (DeLany et al. 1989; Thompson et al. 1995), and to spontaneous physical activity (Thompson et al. 1995) when compared to a group with an adequate energy intake. Moreover, it has been shown that energy expenditure and AEE increase simultaneously with increased energy intake, which might partly reflect the influence of energy intake on BMR (Klein and Goran 1993). However, Castellani et al. (2006) came to a different conclusion. They found that during a short period (54 hours) of physical loading, recruits were able to maintain high energy expenditure and PAL despite sustaining substantial energy deficits.

Accuracy of self-reported pre-filled food diary in the military environment

An additional purpose of the present study was to investigate the accuracy of a pre-filled food diary. Despite the great care was taken to evaluate energy intake as correctly as possible without food weighing, mis-recording was as high as -20% and EI was only 74% of EE. It has been found that subjects who accurately reported their food intake at the first attempt were also able to do so at the second attempt (Goris et al. 2001). On the other hand, it is known that accuracy decreases if the minimum recording time of seven days is extended. This happens due to a decline in motivation in keeping the food diary (Goris et al. 2001). The calculation of under-eating, mis-recording and estimate of true energy intake were all based on changes in body energy stores, in which an energy content of 30 MJ corresponds to 1 kg of body mass. However, the mass ratio of 30 MJ / 1 kg for storage or mobilization of energy between FM and FFM might differ individually, thereby affecting the present results. Changes in body energy stores could have been measured according to changes in FM and FFM, but two weeks may be a too short a period to measure these parameters with sufficient accuracy. A more accurate method to estimate mis-recording and to calculate true energy intake involves a measure of water balance (Goris and Westerterp 1999), but this method still requires the recoding of food and fluid intake, which are both prone to mis-recording (Goris et al. 2001; Goris and Westerterp 1999; Tharion et al. 2005; Westerterp and Goris 2002).

6.4 Effects of protein-rich energy supplement on energy balance, physical activity, body composition, physical performance and subjective experiences during 8-day TC (IV)

The present study showed that supplementary easy-to-use protein-rich energy bars increased total energy intake and energy availability during an 8-day demanding wintertime military training course. Despite the higher energy supply, energy intake was similarly insufficient in the Ebar group as in the control group. The subjects in both groups, however, consumed only 57±13% of the energy provided during TC. The difference between this study and the previously published energy supplementation studies during short-term military field training (Bell et al. 2002; Cline et al. 2000; Edwards and Roberts 1991; Fortes et al. 2011; Montain et al. 2008; Montain et al. 1997) is the use of a protein-rich supplement. However, as in the previous studies, so too in this study the use of supplements increased energy intake, but could not prevent energy deficit.

Although energy availability was higher in the present Ebar group, it did not increase physical activity compared to controls. Furthermore, an increase in aerobic physical performance after TC was observed in both groups. The reason that the additional 4.1 MJ $\cdot d^{-1}$ was insufficient to maintain energy balance might be that Ebar also had higher energy expenditure, although the difference between the groups was not significant. Energy expenditure was calculated by a method of intake / balance, which also takes into account increased protein intake-induced thermogenesis and the 60 to 70 kg weight of the combat gear carried. The energy expenditure of physical activity was also increased by overall stress induced by TC (Bahr et al. 1991).

In the present study, increased somatic and URTI symptoms and negative mood state already before TC were related to lower energy availability during the training, which may indicate a lack of motivation for eating. In addition, increased somatic symptoms in the middle of TC were related to lower energy availability. Although Lieberman et al. (2008) found no effect of a short 2-day energy deficit on mood state in a laboratory environment, the result of insufficient energy intake despite sufficient food provision is in line with other military training studies. In a study of an 11-day cold-weather field exercise, the mean energy intake was 13.1 MJ $\cdot d^{-1}$ (3132 kcal $\cdot d^{-1}$), which was lower than the rations issued by from 17.6 to 21.8 MJ $\cdot d^{-1}$ (4200 to 5200 kcal $\cdot d^{-1}$) (Hoyt et al. 1991). During a 28-day military training period in hilly terrain, the average intake of subjects with a ready-to-eat meal was 12.4 MJ $\cdot d^{-1}$ (2960 kcal $\cdot d^{-1}$) of the issued 15.1 MJ $\cdot d^{-1}$ (3600 kcal $\cdot d^{-1}$) (DeLany et al. 1989). However, the subjects with lightweight rations consumed nearly all, with an average intake during training of 8.1 MJ $\cdot d^{-1}$ (1930 kcal $\cdot d^{-1}$). However, neither of these groups met the requisite energy needs, as evidenced by their decrease in body weight (1.1 kg and 4.3 kg, respectively)(DeLany et al. 1989). Popper et al. (1989) found that eating less than usual was more evident on the first day of the first combat situation, while the amount of eating increased during the second combat situation, although remaining on a lower level than usual. The three most likely reasons reported for this behavior in these respective situations have been lack of time to prepare and eat food and not being hungry (Popper et al. 1989). The inadequate energy intake in the present study could also be a consequence of the subjects' previous experiences that an 8-day energy deficit could be tolerated in the knowledge that they would have a rest period after TC.

In the present study, no association between physical activity and energy availability was observed. This is in line with other studies among soldiers, where all subjects were expected to have a substantial energy deficit (Alemany et al. 2008; DeLany et al. 1989) but not an energy deficit that was self-selected (Thompson et al. 1995). Similarly, in a study by Mettler et al. (2010), athletes were able to maintain their training volume and intensity during the 2-week study period, despite an energy restriction of 60% of habitual energy intake and reduced well-being. Although enough energy was provided in the present study, the subjects did not utilize it, which resulted in an energy deficit. Thus, the influence of energy intake on physical activity is not precisely known. However, in our study the increased negative mood state and URTI symptoms in the middle of TC were related to a lower level of physical activity, indicating the role of motivation to perform tasks and be active. Alhough tasks were ordered to be executed as a group, soldiers often help each other out. In addition, by helping other group members in their duties, soldiers have the possibility to make several behavioral choices, consciously or not, which may clearly affect their energy expenditure in spite of being assigned the same whole-day tasks. This is supported by the wide variation, ranging from 3:50 to 7:45 h:min per day, observed in total physical activity time.

Influence of protein intake

In the present study, Ebar had higher energy intake and energy availability than the controls, and their protein intake was 2.1 g$\cdot$kg^{-1}. However, there were no differences between the groups in changes in FFM, energy deficit or physical activity, although Ebar showed a trend towards an increase rather than decrease in FFM. Among firefighters, eat-on-the-move snacks increased not only energy intake but also physical activity during a 2-d experimental period compared to ready-to-eat meal days (Montain et al. 2008). Friedl et al. (2000) have also shown the positive effects of food supplementation during US Ranger training; 400 kcal/day higher energy intake attenuated the decline in FFM in an energy deficit state in the presence of sleep deprivation, psychological stress and high physical activity. Previously, a protein intake of 1.8 g$\cdot$kg^{-1} did not prevent a decline in FFM during an energy deficit of 4.2 MJ caused by increased energy expenditure (Pikosky et al. 2008). In the present study, protein intake was sufficient to attenuate the decline in FFM during exhaustive training, even though the energy deficit was 40%. This is in line with a study of recreational athletes who trained for an average of 6 hours per week. Their protein intake was sufficient (~ 2.3 $g \cdot kg^{-1}$) to maintain lean body mass during a 40% energy restriction compared to controls (~ 1.0 $g \cdot kg^{-1}$) (Mettler et al. 2010).

Second, the Ebar group reported feeling less hungry after TC than controls. The protein bars might have induced satiation (Weigle et al. 2005) in the Ebar group, leading to their inadequate energy intake from field rations. Third, the questionnaire data revealed that the Ebar group reported better physical performance and more positive mood state than controls. The likely mediator of this potentially beneficial effect of protein feeding is perturbations in psychological symptoms of stress (Witard et al. 2011). In contrast, during weight loss among recreational athletes, no differences were observed between the low and high protein intake groups in satiety and total mood disturbance (Mettler et al. 2010).

Physical performance

The improvement in 3-km running time observed in both groups in this study was an unexpected result. It might be expected that a high activity level of over 5 hours per day with only 4 hours rest would induce decrements in physical performance. An increase in 3-km running time could in part be due to weight loss (Fogelholm 1994), but that was not the case in the present study. Possible adaptation to the testing procedure and an enhanced level of motivation and effort on the last day of the study (Nindl et al. 2002) cannot be ruled out and may partially explain the improved results. An improvement was also observed in the study by Hodgdon et al. (1991) after a nine-day field training period in cold winter weather: time over the snowshoe course improved without any change in anaerobic power. Furthermore, in the present study, the subjects carried combat gear weighing 60-70 kg during TC, whereas in the 3-km test the load was a 20-kg backpack. Thus, the improved 3-km performance might also be a consequence of the 8-day load-carrying training, which decreased the energy cost of load-carrying (Knapik et al. 2004).

Furthermore, lower body anaerobic power and vertical jump height remained the same after TC. This confirms previous reports that muscle strength and aerobic and anaerobic endurance do not appear to be adversely affected by prolonged back load carriage (Knapik et al. 2004) or the short-term (< 10 days) underfeeding (Mettler et al. 2010; Rognum et al. 1986; Zachwieja et al. 2001). In contrast, decreased lower body anaerobic power has also been found after a short period (72 h) of sustained military operations (Nindl et al. 2002) and after eight-day military field training (Welsh et al. 2008). In these studies, the time from completion of the training course to the beginning of post testing was less than three hours. In the present study, the post test was performed 1 day after the training course, which may explain the difference between the results for anaerobic performance. Also, loss of body mass was related to an improvement in anaerobic power, which is line with previous findings (Viitasalo et al. 1987). However, it has to be taken into account that a longer period of underfeeding should clearly induce decrements in physical performance (Nindl et al. 1997).

Contrary, hand-grip strength decreased in the middle of the training course in both groups and returned to the baseline levels one day after. Thus hand-grip strength might indicate acute overall physiological and mental fatigue, although it has not been found to be a sensitive indicator of muscle mass loss and energy deficit (Johnson et al. 1994).

The present results are also in line with the finding that low (~ 1.0 $g \cdot kg^{-1}$) or high (~ 2.3 $g \cdot kg^{-1}$) protein intake has no influence on changes in performance tests after hypoenergetic weight loss (Mettler et al. 2010). A daily carbohydrate intake of 5-6 $g \cdot kg^{-1}$ was lower than recommended for endurance athletes (Hawley and Burke 2010), but it was nevertheless enough to maintain anaerobic and even improve aerobic performance. For energy-restricted strength and power athletes, carbohydrate intake above 3.0 g/kg has been recommended to maintain anaerobic performance (Zachwieja et al. 2001). Furthermore, in light of recently published evidence, the improvement in 3-km running time could be a consequence of pre-test carbohydrate loading after low carbohydrate intake during a training period (Cox et al. 2010). In the present study, the subjects were able to rest 1 day after the training course before the post test, which gave them time to load their energy stores.

Water balance

In the present study, in both groups, ad libitum fluid intake was 2.2±0.5 L per day without water deficit. However, the variation in fluid intake (from 0.9 $L \cdot d^{-1}$ to 2.8 $L \cdot d^{-1}$) and water balance (from -1.7 $L \cdot d^{-1}$ to 0.8 $L \cdot d^{-1}$) was wide. The recommendation for fluid intake during military training in cold weather is 2 L per day (Rintamäki et al. 1998). The results in the present study confirm that the recommendations are enough at the group level, but not for the most active subjects, whose physical activity was related to higher water deficit (Westerterp et al. 2005). The negative correlation of water balance with physical activity partly explains the wide variation in water balance. Instead, water balance did not correlate with physical performance after the training course or with the change in FFM. In a study by Edwards and Roberts (1991), energy intake correlated positively with water intake, but water balance was not measured. In this study such a correlation was not observed and water balance was calculated by the difference between estimated water intake and water loss measured by the deuterium elimination method. Since 73% of fat-free mass is water (Westerterp et al. 1995a), it might be assumed that the change in body mass loss is through water deficit. However, in the present study water deficit and the changes in TBW and FFM were nonsignificant. Thus, there was no body mass loss through water deficit.

6.5 Methodological strength and limitations

The present data were collected during two different training periods in wintertime: 8-week basic training and 8-day field training. A strength of the present studies are the well-controlled physiological, psychological and biochemical measurements. For each subject, all the physiological tests and blood samples drawn for the biochemical measurements were always performed at precisely the same time of day to minimize circadian variability. The standardization procedure also included a similar rest time and diet before each test. Moreover, the questionnaires were administered at the same time of day in standardized conditions to avoid pre- versus post-exercise and morning versus evening variations. The only exception was the test in the middle of the field training course, the purpose of which was to evaluate the acute effects of the field training. Furthermore, all the baseline and follow-up tests were performed with the same investigator, measurement technique and calibrated measurement equipment. All these were implemented in a military environment, which ensured good repeatability and minimized the variability of the data. In civil life, this would have been challenging to accomplish.

The subjects for the basic training study were selected on the basis of their voluntary physical activity before military service. Although physical activity was determined by the subjective International Physical Activity Questionnaire (IPAQ), this ensured wide variation in the subjects' initial physical fitness levels. Otherwise, physical activity was measured by objective methods using an accelerometer and the golden standards method DLW. Furthermore, the main outcome variable, maximal aerobic fitness (VO_2max), was measured using the breath-by-breath measurement technique during an incremental treadmill test until voluntary exhaustion, which is known to be the best available method. Moreover, the training responses were measured by multiple performance and biochemical variables with concomitant evaluation of mood state. A further strength of the study is that energy expenditure and energy intake were also measured using the best available methods, and body composition was evaluated by the deuterium dilution method during the two-week training period.

A limitation of the 8-week basic training study is the lack of a control group of young civilian men of the same age. This would have yielded deeper understanding of some of the changes in the measured variables, such as URTI, during wintertime in Finland. Another limitation is that the submaximal tests were performed as a group test and that blood samples were not taken before, during or after the VO_2max test.

The 8-day field training study was performed during the darkest time of the year in Finland, in the beginning of December. Beside the energy deficit observed among all the subjects, they all improved in aerobic fitness after the training course. A strength of this study was that URTI was evaluated by a validated questionnaire and water balance measured by the deuterium elimination method. Moreover, changes in body composition were measured by the deuterium dilution method. An outcome and also a limitation of TC study was the high amount of under-eating. First, for proper evaluation of the effects of increased energy intake on PA and performance, a third group, obliged to eat all the food provided should have been studied. Second, to avoid bias, the subjects would have to be absolutely unaware of the group they are included in throughout the intervention period.

Finally, an important strength of the present studies is the large variation in the statistical methods used. For example, in comparing the role of fitness, fatness and energy balance, a regression-based approach was used to remove the confounding influences of BM and FFM, rather than the standard ratio approach where VO_2max values are expressed relative to BM (ml/kg/min). 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 VO_2max with BM was significantly different from zero ($p<0.01$), but this was not the case with FFM. Therefore, VO_2max was adjusted for FFM.

7 MAIN FINDINGS AND CONCLUSION

The main findings and conclusions of the present studies are summarized as follows:

1) Evaluation of the 8-week period of Finnish basic training

a) The average physical training during BT is two hours per day with half an hour of rest comparable to sleeping or a nap. The daily training load increased during the first 4 weeks of BT, which induced positive improvements in aerobic fitness manifested as an increase in maximal aerobic capacity and decreased submaximal exercise-induced stress. Simultaneously, decreased oxidative stress at rest was observed, while body mass and hormone concentrations remained the same. Furthermore, as expected, an inverse relationship between initial VO_2max and relative changes in VO_2max was observed, indicating that the effectiveness of the training was greatest for the least fit subjects.

b) During the second half of the 8-week BT, the accumulated training load led to stagnation of the increase in VO_2max, which indicates that the training load was not well tolerated by all the conscripts. This was also supported by the decrease in maximal post-exercise lactate and HR, increase in SHBG, decrease in IFG-1 and increased emotional and somatic symptoms of OR/OTS. Furthermore, oxidative stress at rest increased back to the baseline values.

2) Overreaching

a) Because one-third of the subjects were classified as overreached, the planning of the BT program needs to be carefully considered. In particular, the program should involve more recovery phases, both daily and periodically. If demanding training is continued after BT, overtraining syndrome may develop.

b) Subjects who were classified as overreached did not have increased fat mass or lower fitness or report less vigorous physical activity before military service. Instead, they had increased oxidative stress and SHBG at rest and different response to acute stress when compared to their noOR counterparts.

c) During BT, the OR-classified conscripts had higher physical activity which caused training load accumulation at the end of BT, possibly further contributing to the development of OR.
d) A U-shaped curve of oxidative stress at rest was observed during BT: a decrease at rest from baseline to week 4 followed by an increase culminating in a return to baseline values at the end of BT, suggesting that increased oxidative stress may be associated with OR and may also be a marker of insufficient recovery leading to OR.
e) Serum cortisol and T/C -ratio at rest and maximal La/RPE -ratio could be useful tools for monitoring whether military training is too stressful.
f) If the amount of sick leave is set as a criterion of tolerance to exercise training, these results suggest that in order to enhance physical fitness and to avoid OTS, conscripts could be divided into different training groups according to their aerobic fitness level. Initial serum basal SHBG and testosterone levels as well as T/C -ratio, would give additional information, although in practice collecting the samples from a large group of subjects is challenging. However, the use salivary sampling would be practical for large subject pools.

3) Importance of fitness, fatness and energy balance on training load

a) The subjects with low aerobic fitness, high BMI and negative energy balance showed vulnerability to low training load, but of these three factors only BMI remained in the multivariate regression model. This indicates that fatness may have the most limiting effect on high intensity physical training. These results suggest that in order to enhance physical fitness in a group of conscripts who exhibit variable fitness levels, such conscripts could be divided into training groups based on BMI alone, rather than aerobic fitness level or a combination of these parameters. However, conscripts with high BMI and low body fatness also have to be taken into account, although among the young male conscripts in this study BMI was a valid indicator of body fatness.
b) Another important issue is the role of energy balance in sustaining a high training load. The results confirm that during BT, the ability to train is affected by aerobic fitness and BMI as well as by energy balance. Initially, BMI has to be taken into consideration. Thereafter, energy balance should be optimized to facilitate physical performance, and to allow a high training load to be sustained during BT. This situation is comparable with that of athletes: without adequate energy intake, high level training is not possible. Thus, because energy balance influenced training load, conscripts should be advised to ensure adequate energy intake, especially during the most strenuous training period.

4) Effect of protein-rich energy supplement on energy balance and physical activity

An easy-to-use energy bar supplement of 4 MJ/day did not prevent energy deficit or influence on PA during the 8-day strenuous military field training course. A satiation effect of the high content of protein in the bars might have induced a decreased energy intake from field rations, since the Ebar group felt less hungry after the training course than the controls. Furthermore, Ebar showed improved positive mood state during the latter part of TC while controls did not. However, negative mood state and URTI symptoms before the training course affected energy intake during TC. Thus mood state and health status are important factors in preventing undereating. In addition, during strenuous training, physical activity seems to be primarily affected by factors other than energy supplementation, such as mood state.

5) Water balance during the military field training course in cold weather

During the 8-day military field training course in cold weather, the conscripts fulfilled the fluid intake recommendation of 2 liters per day. However, the variation in fluid intake (from 0.9 $L \cdot d^{-1}$ to 2.8 $L \cdot d^{-1}$) and water balance (from -1.7 $L \cdot d^{-1}$ to 0.8 $L \cdot d^{-1}$) was wide. The results further revealed that 2 liters per day is not enough for the most active subjects in a cold environment.

YHTEENVETO (FINNISH SUMMARY)

Varusmieskoulutuksen kuormittavuus ja ylikuormittumisen ilmeneminen; vaikutukset aerobisen kuntoon, seerumin hormonipitoisuuksiin, oksidatiiviseen stressiin ja energiatasapainoon

Palvelukseen astuvien varusmiesten fyysinen kunto on heikentynyt ja kehon paino noussut viimeisten 15 vuoden aikana. Varmuutta ei ole siitä, kuinka fyysisesti kuormittavaa varusmiespalvelus on erikuntoisille nuorille miehille. Ylikuormittuminen on seurausta vääristyneestä levon ja kokonaiskuormituksen suhteesta, jonka tunnusmerkkinä pidetään odottamatonta suorituskyvyn heikkenemistä huolimatta siitä, että harjoittelu on lisääntynyt tai säilynyt ennallaan. Aerobinen kunto on merkittävä fyysisen kunnon ja sotilaan suorituskyvyn osatekijä. Fyysisen aktiivisuuden on todettu olevan yhteydessä aerobiseen kuntoon, viitaten myös aerobisen kunnon ja energiankulutukset yhteyteen erilaisissa sotilaallisissa toiminnoissa. Toisaalta energiatasapaino on välttämätöntä, jotta harjoittelu olisi tehokasta ja laadukasta ja siten suorituskyky paranisi sotilaskoulutuksen aikana. Raskaan sotilaallisen maastoharjoituksen on havaittu aiheuttavan energiavajetta. Sen sijaan proteiinipitoisen ruokavalion on havaittu estävän kehon lihamassan katoa tilanteessa, jossa on energiavajetta.

Tämän väitöskirjatutkimuksen tarkoituksena oli selvittää talvikauden 8-viikon peruskoulutuskauden (PK) kuormittavuutta, ylikuormitusoireiden ilmenemistä sekä ylikuormittuneiden ja ei-ylikuormittuneiden varusmiesten eroa aerobisen suorituskyvyn, kehon koostumuksen ja biokemiallisten mittareiden suhteen varusmiespalveluksen alussa sekä PK:n aikana. Lisäksi tarkoituksena oli tutkia PK:n harjoituskuorman yhteyttä aerobiseen kuntoon, kehon koostumukseen ja energiantasapainoon. Näiden lisäksi testattiin proteiinipitoisen energiapatukan vaikutusta energia- ja nestetasapainoon, kehon koostumukseen, fyysiseen aktiivisuuteen, suorituskykyyn ja nälän tunteeseen sekä mielialaan kahdeksan vuorokauden sotilaallisen maastoharjoituksen aikana talviolosuhteissa.

Tutkimukseen valittiin fyysisen aktiivisuuskyselyn perusteella erikuntoisia vapaaehtoisia varusmiehiä (N=57, ikä 20 v), jotka suorittivat PK:n alussa, 4 viikon jälkeen ja lopussa maksimaalisen ja 45-min submaksimaalisen aerobisen suorituskykytestin sekä täyttivät oirekyselyn. Lisäksi mitattiin kehon koostumus. Verinäytteet otettiin levossa ennen submaksimaalista testiä ja sen jälkeen. Ylikuormittuminen määritettiin, mikäli kolme viidestä kriteeristä täyttyi: 1) maksimaalinen aerobinen suoristuskyky heikentynyt vähintään -5 %, 2) RPE:n kasvu >1 submaksimaalisessa testissä tai poissaolo testeistä sairauden vuoksi, 3) somaattisten oireiden lisääntyminen >15 %, 4) tunsi olevansa henkisesti tai fyysisesti kuormittunut ja 5) poissaolopäivät palveluksesta sairauden vuoksi yli 10 % palvelusajasta. Harjoituskuorma määritettiin fyysinen aktiivisuuden perusteella, joka mitattiin kiihtyvyysanturilla (Polar AW200) koko PK:n ajan sekä energiankulutuksen johdannaisena kaksoisleimattu vesi (DLW) menetelmää käyttäen 14-päivän ajan. Raportoitu energiansaanti kartoitettiin ravintopäiväkirjasta ja todellinen energiansaanti laskettiin energiankulutuksen, kehon energiavarastojen muutoksen ja raportoidun energiansaannin perusteella.

Maastoharjoitustutkimuksessa tutkittavat olivat vapaaehtoisia tiedustelukomppanian sissikoulutuksessa olevia varusmiehiä (N=26, ikä 19 ± 1 v), jotka jaettiin satunnaisesti kontrolliryhmään (N=12) ja energiapatukka (Ebar) ryhmää (N=14). Kontrolliryhmä käytti maastoharjoituksen aikana ravintonaan ainoastaan taistelumuonapakkausten sisältöä. Ebar-ryhmä sai pakkausten lisäksi lisäenergiaa proteiinipitoisesta energiapatukoista 4.1 MJ/vrk. Energian- ja nesteen saanti kartoitettiin ruokapäiväkirjan, ruokakääröjen ja nauttimatta jääneen ruoan perusteella. Nestehukka ja kehon nestetilavuus mitattiin deuteriummenetelmällä. Energiakulutus arvioitiin energiansaannista ja kehon energiavarastojen (rasva- ja lihasmassa) muutoksesta. Energian riittävyys laskettiin = energian saanti / arvioitu perusaineenvaihdunta. Fyysinen aktiivisuus maastoharjoituksen aikana mitattiin kiihtyvyysanturilla (Polar AW200). Fyysinen suorituskyky (3 km marssijuoksu, käden puristusvoima ja anaerobinen hyppytesti) mitattiin ja nälän tunne sekä mieliala kartoitettiin kyselyllä ennen maastoharjoitusta, sen puolivälissä ja jälkeen.

Tutkimus osoitti, että talviajan PK:n aikana keskimääräinen fyysinen harjoitteluaika on kaksi tuntia päivässä ja päivänokosiin verrattava lepoaika (≤ 1.0 MET) on 30 min päivässä. Tämä vastaa siviilioloissa säännöllisesti raskasta vapaa-ajan liikuntaa harrastavien aktiivisuutta. PK:n neljän ensimmäisen viikon harjoittelulla oli positiivisia harjoitusvaikutuksia, josta osoituksena olivat aerobisen suorituskyvyn paraneminen ja oksidatiivisen stressin väheneminen levossa. Kuten odotettua, aerobinen maksimaalinen suorituskyky parani eniten huonokuntoisilla. Neljän viimeisen viikon aikana suorituskyky ei enää muuttunut. Lisäksi useiden fysiologiset ja biokemialliset muuttujat sekä oirekyselyn tulokset osoittivat, että kaikki varusmiehet eivät sietäneet kuormitusta, sillä 33 %:a heistä luokitettiin ylikuormittuneiksi. Näillä varusmiehillä ei ollut eroa ei-ylikuormittuneisiin varusmiehiin verrattuna aerobisessa suorituskyvyssä, kehon koostumuksessa ja liikunta-aktiivisuudessa PK:n alussa. Sen sijaan ylikuormittuneilla varusmiehillä oli korkeampi plasman oxidatiivinen stressitaso ja seerumin SHBG levossa sekä heikompi oxidatiivinen stressivaste submaksimaaliseen rasitukseen. Lisäksi tulokset osoittavat, että plasman oxidatiivinen stressitaso, seerumin kortisoli, ja testosteroni/kortisoli-suhde levossa sekä maksimaalinen laktaatti/RPE-suhde voi olla hyödyllisiä mittareita sotilaallisen koulutuksen kuormittuneisuuden seurantaan. PK:n aikana kykyyn sietää harjoitusta vaikuttavat energiatasapaino, aerobinen kunto ja lihavuus. Näistä kolmesta tekijästä, lihavuus rajoittaa eniten tehokasta harjoittelua, mutta toisaalta hyvä aerobinen kunto suojaa sairastumisilta.

Maastoharjoituksen aikana keskimääräinen lepoaika oli neljä tuntia ja fyysisesti tehokasta aktiivisuutta oli lähes kuusi tuntia vuorokaudessa. Huomioitavaa oli, että Ebar-ryhmä nautti vain 58% tarjotusta energiasta ja kontrolliryhmä 57%. Vaikka energiansaanti ja -riittävyys olivat suurempi Ebar ryhmällä kuin kontrolleilla, energiavajetta oli huomattavassa määrin molemmilla ryhmillä. Kehon lihasmassassa ei ollut merkitseviä muutoksia, vaikkakin Ebar ryhmällä se hiukan lisääntyi ja kontrolliryhmällä laski. Nesteen nauttiminen oli suositusten mukaista (n. 2 litraa päivässä) molemmilla ryhmillä, mutta se ei riitä fyysisesti aktiivisimmille varusmiehille. Energiavajeesta huolimatta maastoharjoitus ei heikentänyt suorituskykyä, sen sijaan marssijuoksun aika parani molemmilla ryhmillä. Ebar-ryhmä koki vähemmän nälkää ja enemmän positiivista mielialaa kuin kontrolliryhmä. Mielenkiintoinen havainto oli negatiivisen mielialan yhteys alhaisempaan energian riittävyyteen ja fyysiseen aktiivisuuteen maastoharjoituksen aikana.

Tutkimustulokset osoittavat, että talviajan peruskoulutuskauden harjoituskuorma vastaa siviilioloissa säännöllisesti raskasta vapaa-ajan liikuntaa harrastavien aktiivisuutta. Varusmiehiä tulee valistaa riittävästä energiansaannista etenkin kuormittavien maastoharjoitusten aikana. Lisäksi tulokset osoittivat, että helposti nautittavat energiapatukat eivät estäneet energiavajetta, vaikuttaneet fyysiseen suorituskykyyn eivätkä lisänneet fyysistä aktiivisuutta kahdeksan vuorokauden sotilaallisen maastoharjoituksen aikana. Energiapatukan suuri proteiinipitoisuus saattoi aiheuttaa kylläisyyden tunteen, joka puolestaan vähensi energian nauttimista. Tämän tutkimuksen perusteella muut tekijät kuten mieliala saattavat vaikuttaa ruokahaluun ja fyysiseen aktiivisuuteen raskaan maastoharjoituksen aikana, jonka vuoksi taistelumuonapakkausten sisältöä on tarvetta tarkastaa nautittavammaksi. Lisäksi tutkimustulokset osoittavat, että hyvä fyysinen kunto ennen palvelukseen tuloa suojaa sairastumisilta ja edesauttaa sopeutumista harjoittelukuormaan ensimmäiset 4 viikkoa. Varusmiehistä 1/3 ylikuormittui, joka tulisi mahdollisuuksien mukaan jatkossa huomioida PK jakson suunnittelussa. Kuitenkaan huono fyysinen kunto tai lihavuus varusmiespalveluksen alussa eivät vaikuttaneet ylikuormittumisen ilmenemiseen. Sen sijaan lihavuus on este tehokkaalle harjoittelulle, joka tulisi ottaa huomioon jaettaessa varusmiehiä tasoryhmiin fyysisen koulutuksen osalta.

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ORIGINAL PAPERS

I

SERUM SEX-HORMONE BINDING GLOBULIN AND CORTISOL CONCENTRATIONS ARE ASSOCIATED WITH OVERREACHING DURING STRENUOUS MILITARY TRAINING

by

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SERUM SEX HORMONE–BINDING GLOBULIN AND CORTISOL CONCENTRATIONS ARE ASSOCIATED WITH OVERREACHING DURING STRENUOUS MILITARY TRAINING

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ABSTRACT

Tanskanen, MM, Kyröläinen, H, Uusitalo, AL, Huovinen, J, Nissilä, J, Kinnunen, H, Atalay, M, and Häkkinen, K. Serum sex hormone–binding globulin and cortisol concentrations are associated with overreaching during strenuous military training. *J Strength Cond Res* 25(3): 787–797, 2011—The purpose was (a) to study the effect of an 8-week Finnish military basic training period (BT) on physical fitness, body composition, mood state, and serum biochemical parameters among new conscripts; (b) to determine the incidence of overreaching (OR); and (c) to evaluate whether initial levels or training responses differ between OR and noOR subjects. Fifty-seven males (19.7 ± 0.3 years) were evaluated before and during BT. Overreaching subjects had to fulfill 3 of 5 criteria: decreased aerobic physical fitness ($\dot{V}O_2$max), increased rating of perceived exertion (RPE) in 45-minute submaximal test at 70% of $\dot{V}O_2$max or sick absence from these tests, increased somatic or emotional symptoms of OR, and high incidence of sick absence from daily service. $\dot{V}O_2$max improved during the first 4 weeks of BT. During the second half of BT, a stagnation of increase in $\dot{V}O_2$max was observed, basal serum sex hormone–binding globulin (SHBG) increased, and insulin-like growth factor-1 and cortisol decreased. Furthermore, submaximal exercise–induced increases in cortisol, maximum heart rate, and postexercise increase in blood lactate were blunted. Of 57 subjects, 33% were classified as OR. They had higher basal SHBG before and after 4 and 7 weeks of training and higher basal serum cortisol at the end of BT than noOR subjects. In addition, in contrast to noOR, OR subjects exhibited no increase in basal testosterone/cortisol ratio but a decrease in maximal La/RPE ratio during BT. As one-third of the conscripts were overreached, training after BT should involve recovery training to prevent overtraining syndrome from developing. The results confirm that serum SHBG, cortisol, and testosterone/cortisol and maximal La/RPE ratios could be useful tools to indicate whether training is too strenuous.

KEY WORDS biochemistry, training monitoring, testosterone, IGF-1, aerobic capacity

INTRODUCTION

The fitness level of young men entering Finnish compulsory military service has declined with a concomitant increase in body mass (BM) during the last 25 years (30). In order to improve the physical fitness of new conscripts entering military service, a new updated physical training program for an 8-week basic military training (BT) period was established in 1998 (35). However, the effect of the BT program on physical fitness and performance among current conscripts, who exhibit large interindividual variations in physical fitness levels, is unknown.

Conscripts entering military service are intensively exposed to nontraining stresses because of the totally new environment and the change in their working conditions. Most of these young men will have their first experience of demanding physical training, eating outdoors, and performing overnight exercises in a forest. In addition, the physical training involved in military service may exceed their previous training level, which could contribute to overreaching (OR) in the multistressor environment of the military service. Overreaching is an accumulation of training and nontraining stress resulting in short-term decrements in performance capacity, which may be accompanied by related physiological and psychological signs and symptoms of maladaptation (22). Overreaching could also be an early indicator of the development of overtraining syndrome (OTS) (22,33), which is

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characterized by an unexpected decrease in performance, despite increased or maintained training load (38). There is some evidence to suggest that the development of OTS is not only solely related to the stress of training but also to nontraining (psychological) stress (15,21).

To monitor responses to training and to avoid OR and OTS, there is still a clear need for reliable tools that facilitate the early diagnosis of OTS (36). Although there are no specific markers of impending OTS, testosterone (an anabolic hormone) and cortisol (a catabolic hormone) have often been used to represent the overall anabolic and catabolic activities in the body (2,37). In addition, serum testosterone and cortisol concentrations have been identified as reliable markers of military training stress (24), and the testosterone/cortisol ratio has been used to represent the balance between anabolic and catabolic activity (1). It has also been suggested that acute exercise-induced changes in serum hormones could be used to monitor training load and to avoid OTS (36). In particular, a diminished response of cortisol to an acute submaximal exercise (39), and reduced basal cortisol levels (31,36) may be useful indicators of exhaustive training loads. In addition, insulin-like growth hormone (IGF-1) has recently been identified as a suitable marker for monitoring training load (23) and investigating OTS. Furthermore, an impaired mood state and subjective complaints are consistently described as sensitive and early markers of OTS (36).

The purpose of the present research was (a) to study effect of an 8-week Finnish military basic training period (BT) on physical fitness, body composition, mood state, and serum biochemical parameters, (b) to determine the incidence of OR during BT among new conscripts, and (c) to evaluate whether the initial levels or training responses of physical fitness, body composition, and serum biochemical parameters differ between OR and noOR subjects.

METHODS

Experimental Approach to the Problem

Purpose 1. Performance tests and physiological and psychological markers to monitor the training load of BT training were selected based on existing literature of OTS and training studies. Performance tests (maximal aerobic fitness $\dot{V}O_2$max test and submaximal test) and physiological tests were performed 3 times, and psychological markers 5 times during the 8-week training period. In addition, acute responses to a submaximal test were studied. For the submaximal test, a marching test was selected because it closely resembles the routine activities of conscripts and enables a test to be performed for a large group of subjects. Furthermore, sick absences were followed over the entire BT, because poor $\dot{V}O_2$max levels have been associated with training related sick absences during military service (17). The experimental protocol is presented in Table 1.

TABLE 1. Experimental design during the 8-week military basic training period.

Week	1	2	3	4	5	6	7	8
$\dot{V}O_2$max–test	X				X			X
Submaximal marching test	X			X			X	
Body composition	X			X			X	
Blood samples	X			X			X	
Questionnaire	X			X		X	X	X

Purposes 2 and 3. No single symptom or set of symptoms have been found to be associated with OR or OTS. However, a decrease in performance and an increase in perceived exertion at the same subjective workload have been found to be related to OR and OTS (13,14,38,39). In addition, impaired general health status, negative mood, and feelings of fatigue have been found to be related to OR and OTS (13,22,38,39). Therefore, subjects in this study had to fulfill 3 of 5 criteria to be classified as OR subjects. In addition, differences between subjects who fulfilled a single OR criteria were evaluated to determine whether initial levels of physical fitness, body composition, and serum biochemical parameters differed among these subjects.

Criteria 1. A reduced $\dot{V}O_2$max of greater than 5% (13,31) or did not perform the test because of illness. Absence from the test was set as an additional criterion, because all 3 $\dot{V}O_2$max tests were completed by a total of 36 conscripts. It was assumed that illness itself reduces performance. Furthermore, overload training has been reported as a risk factor for upper respiratory track infections (URTIs) (32,39).

Criteria 2. An increase in mean rating of perceived exertion (RPE) during the submaximal marching test greater than 1.0 (13) from the lowest value at week 1 or week 4 and remaining more than 1.0 above the lowest value, or did not perform the test because of illness (see description above). All 3 submaximal marching tests were completed by a total of 35 conscripts.

Criteria 3. An increase in somatic symptoms of OTS greater than 15% from week 4 to week 7 and remaining the same or increasing from week 7 to week 8. Subjects were divided into tertiles based on an increase in somatic symptoms of OTS from week 4 to week 7; 15% was the upper third.

Criteria 4. Admitted feeling physically or mentally overloaded at week 7 or 8.

Criteria 5. Sick absence more that 10% of daily service (upper third tertile based on the sick absences during BT).

The study took place during winter in Finland, when daily outdoor temperatures ranged from −31 to +1°C, with an average of −13°C (data from the local weather station). The overall physical load of the 8-week BT period was set according to the standard direction of the Defense Command. The intensity level of physical activity in the daily program was low in the first week of BT and increased thereafter. Food and water intake were in accordance with the standard army meal, and water intake was not restricted. However, subjects were not permitted to use any extra nutritional supplements throughout the study. The average sleeping time at the garrison was from 10 PM to 5:45 AM. Military education included physically demanding activities such as marching, combat training, and sport–related physical training. The conscripts also carried combat gear weighing 20 kg, including clothing, particularly during marching and combat training. In addition, the training included an overnight field exercise. Garrison training involved theoretical education in classroom settings, material handling, shooting, and general military education, such as close-order drills. Conscripts marched 4 times per day (ca. 5 km in total) for meals and slept in dormitory-type rooms.

During the BT period, practical skills are trained daily. At the beginning of BT, the amount of physical training is approximately $2 \cdot h \cdot d^{-1}$, increasing to 3–4 hours during weeks 4–7. The BT schedule includes 4 longer (from 2 to 8 hours) marching exercises with battle equipment. However, daily outdoor exercises, including transportation, are often performed by marching. Rifle marksmanship in the garrison is performed twice a week throughout the BT period.

Subjects

Male conscripts ($n = 57$, aged 19.6 ± 0.3 years) from the Signal Battalion of Northern Finland participated in the present study. They were selected from a total of 131 subjects who initially volunteered to participate. From these subjects, 47 were discarded based on cardiorespiratory or musculoskeletal disorders and incomplete fulfillment or willingness to perform special duties. The remaining 84 were divided into 3 categories according to their level of voluntary physical activity before military service, which was determined by the International Physical Activity Questionnaire (7). To ensure large variations in initial physical fitness levels, 19 conscripts were randomly selected from each category, making a total of 57 subjects. All subjects were fully informed of the experimental protocol and gave their written consent to participate in the study. They were also advised of their right to withdraw from the investigation at any time. The study protocol was approved by the Finnish Defence Forces, and the Ethical Committees of the University of Jyväskylä and the Kainuu region of Finland.

$\dot{V}O_2$max Test

To determine $\dot{V}O_2$max ($ml \cdot kg^{-1} \cdot min^{-1}$), the conscripts performed a maximal treadmill test in week 1 (before training) and after 5 (week 5) and 8 (week 8) weeks of the BT period. The follow-up tests for each subject were always performed at the same time of the day between 8.30 AM and 5 PM, after having consumed a similar diet before each test. The start of the test involved walking for 3 minutes at 4.6 $km \cdot h^{-1}$ and walking or jogging at 6.3 $km \cdot h^{-1}$ (1% slope) as a warm-up. Thereafter, exercise intensity was increased every 3 minutes to induce an increase of 6 $ml \cdot kg^{-1} \cdot min^{-1}$ in the theoretical $\dot{V}O_2$max demand of running (2). This was achieved by increasing the initial running speed of 4.6 $km \cdot h^{-1}$ by a mean of 1.2 $km \cdot h^{-1}$ (range 0.6–1.4 $km \cdot h^{-1}$), and by increasing the initial grade of 1 deg by a mean of 0.5 deg (range 0.0–1.0 deg) up to the point of exhaustion (2). Pulmonary ventilation and respiratory gas exchange data were measured on-line using the breath-by-breath method (Jaeger Oxygen Pro, VIASYS Healthcare GmbH, Hoechberg, Germany), and mean values were calculated at 1-minute intervals for statistical analysis. The analyzer was calibrated before each test according to manufacturer specifications. Heart rate (HR) was continuously recorded at 5-second intervals using a telemetric system (Polar810i, Polar Electro Oy, Kempele, Finland). Rating of perceived exertion was assessed at the end of each workload. The criteria used for determining $\dot{V}O_2$max were a lack of increase in $\dot{V}O_2$max and HR despite an increase in workload of the treadmill, a respiratory exchange ratio higher than 1.1, and a postexercise blood lactate value (determined 1 minute after exercise completion from a fingertip blood sample using a lactate analyzer; LactatePro®, Arkray, Japan) that was higher than 8 $mmol \cdot L^{-1}$ (2). All participants fulfilled these criteria.

Submaximal Marching Test

The 45-minute submaximal marching test (exercise) was performed after 1 week of adjustment to military service at the end of the first week (before training), and after 4 (week 4) and 7 (week 7) weeks of the BT period. The time between the tests was 3 weeks, and the follow-up exercise for each subject was always performed at the same time of the day between 9 AM and 12 PM. Heart rate was recorded in 5-second intervals during the entire exercise to define the mean HR. Rating of perceived exertion was assessed 3 times, every 15 minutes using a 6- to 20-point scale. Rating of perceived exertion was calculated as the average of these 3 values. The aim of determining marching speed was to divide subjects into 3 groups so that each group involved subjects marching at approximately 70% of their individual maximal workload. Exercise of this intensity and duration has been shown to result in increased serum cortisol levels (34) and to be tolerable for untrained subjects. Firstly, theoretical individual maximal workload ($ml \cdot kg^{-1} \cdot min^{-1}$) was calculated based on the treadmill speed and grade in the $\dot{V}O_2$max test using the American College of Sports Medicine (ACSM) estimation formula for $\dot{V}O_2$ in running (2). Secondly, because in load carriage tasks (~20 kg) the relationship between relative $\dot{V}O_2$max and endurance time has been shown to be lower compared with an absolute $\dot{V}O_2$max value (3), theoretical individual maximal workload as $ml \cdot kg^{-1} \cdot min^{-1}$ was converted into $L \cdot kg^{-1}$. Thirdly, the individual speed corresponding to 70% of maximal workload when carrying the 20-kg combat gear during the test on even terrain was determined.

It was assumed that carrying the extra load required a similar level of effort to carrying extra body weight. Because the ACSM formula for the estimation of $\dot{V}O_2max$ in walking yields unrealistically low values for brisk walking (above 5 $km{\cdot}h^{-1}$), a new equation for marching was determined that combines the ACSM equations for walking and running between 5 and 8 $km{\cdot}h^{-1}$. The equation is based on the earlier findings that walking and running are metabolically equivalent at a velocity of approximately 8 $km{\cdot}h^{-1}$ (20). Fourthly, the individually estimated marching speeds were rounded to the closest even number. Twelve subjects were categorized into the group marching at 6 $km{\cdot}h^{-1}$, 43 subjects at 7 $km{\cdot}h^{-1}$, and 4 subjects at 8 $km{\cdot}h^{-1}$. An accurate and even marching speed was ensured by the group leader who walked in front of the group without extra weight and wore the Polar S625 (Polar Electro Oy) running computer, which was equipped with an individually calibrated speed-measuring device.

Body Composition

Body composition measurements (BM, fat-free mass [FFM], fat mass [FM], and percentage of body fat [F%]) were performed at the same time points as the marching tests using 8-point bioelectrical impedance (Inbody720, Biospace Co. Ltd, Seoul, Korea). For each subject, the measurements were performed at the same time between 6 AM and 7 AM after an overnight fast and after avoiding, with no exercise for 12 hours before the test. The physical activities in the daily program were planned to be of a low intensity on the day preceding each measurement, and fluid status was estimated to be in balance based on the dietary records of the subjects. The subjects were barefoot and wore T-shirts and trousers. Body height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as BM (kg) divided by height (m) squared. Recent studies have shown that 8-polar bioelectrical impedance is a reliable method to detect changes in FFM (ΔFFM) and FM (ΔFM) when performed under strict standardized conditions (16). Accordingly, ΔFFM and ΔFM during the study were calculated using this method.

Blood Samples and Analyses

Blood samples were drawn from an antecubital vein after an overnight fast at rest (basal), 2 hours after a light breakfast before exercise (pre-exercise) and immediately after exercise (postexercise). Basal samples were taken for serum total testosterone, cortisol, and serum sex hormone–binding globulin (SHBG); pre-exercise samples for serum total testosterone, cortisol, and total insulin-like growth factor-1 (IGF-1); and postexercise samples for serum testosterone and cortisol. Fingertip lactate (La) samples were taken pre-exercise and postexercise. Circadian variability in blood parameters was minimized by collecting basal samples at the same time in the morning between 6.30 AM and 7.30 AM and by collecting individual pre-exercise and postexercise samples at the same time of day between 9 AM and 12 AM after similar patterns of food ingestion. The standardized breakfast before the test had a total energy content of 1,860 kJ, 61 g of carbohydrate (56% of total energy intake), 16 g of protein (14% of total energy intake), and 15 g of fat (30% of total energy intake). All blood samples were taken in the same position (seated). Subjects were instructed not to ingest alcohol, coffee, tea, chocolate, cola drinks, or bananas on the morning of the measurements or the previous evening.

The percentage change in plasma volume (%ΔPV) was calculated from changes in hemoglobin and hematocrit according to the method of Dill and Costill (8). Serum postexercise values were adjusted for changes in plasma volume: Postexercise adjusted = postexercise + (postexercise × %Δplasma volume/100). Exercise-induced relative changes in serum hormonal concentrations were calculated using the equation (postexercise adjusted − pre-exercise)/pre-exercise *100, and expressed as Δ%. La was analyzed from fingertip blood samples (Lactate Pro). Testosterone, cortisol, and SHBG were analyzed by Immulite 1000 (Diagnostics Products Corporation, Los Angeles, CA, USA) using commercial chemiluminescent enzyme immunoassays IMMULITE®/IMMULITE 1000 (Diagnostics Products Corporation). The intraassay coefficients of variance for these assays were $<5.7\%$, $<4.8\%$, and $<2.4\%$, respectively, and sensitivities of variance were 0.5, 5.5, and 0.2 $nmol{\cdot}L^{-1}$, respectively. Hematocrit and hemoglobin were analyzed using a Sysmex KX 21N-analyzer (Sysmex Co., Kobe, Japan). Insulin-like growth factor-1 was analyzed with an R&D duoset enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA). The intra and interassay coefficients of variance and sensitivity for these assays were $<6.4\%$, $<9.1\%$, and 26 $pg{\cdot}mL^{-1}$, respectively.

Questionnaires and Sick Absence

The questionnaire to assess the somatic symptoms of OR and OTS was formulated based on previously reported symptoms of OTS (12,15,22,36). The somatic symptoms were subjective ratings of well-being; URTIs (Cronbach's alpha 0.82), flu-like symptoms (Cronbach's alpha 0.74), digestive disorders and reduced appetite (Cronbach's alpha 0.45), musculoskeletal disorders (Cronbach's alpha 0.70), and physical complaints and sleep difficulties (Cronbach's alpha 0.61). The subjects rated how severely they experienced a list of symptoms during the last week of training using a 5-point Likert scale; 1 = not at all, 2 = 1 day, 3 = 2–3 days, 4 = 4–5 days, and 5 = 6–7 days. The sum of the symptoms associated with OTS was determined as the sum of the symptom scores. In addition, the question "Do you feel physically or mentally overloaded?" was asked. Questionnaires were administered 1 day before and 1 week after the marching test at the same time of day in standardized conditions to avoid pre-exercise vs. postexercise and morning vs. evening variations (22). Sick absence was defined as an attendance or non-attendance in daily service because of illness or injuries examined by a physician.

Table 2. BM, FM, and FFM before and after 4 and 7 weeks of military basic training ($n = 34$).*

	Before		After 4 wks			After 7 wks				Before vs. after 7 weeks
	Mean	±*SD*	Mean	±*SD*	Δ%	Mean	±*SD*	Δ%	*p*	Δ%
BM (kg)†	78.7	17.7	78.5	16.9	0.0	77.0	15.9	−1.8	***, ¤¤¤	−1.8
FFM (kg)	62.6	9.4	62.9	8.8	0.7	63.0	9.3	0.1	*	0.8
FM (kg)†	16.1	10.7	15.6	10.7	−3.8	14.0	9.1	−7.5	***, ¤¤	−12.6

*BM = body mass; FM = fat mass; FFM = fat-free mass.
Difference compared with before ***$p < 0.001$, *$p < 0.05$; week 4 ¤¤¤$p < 0.001$, ¤¤$p < 0.01$. Main effect of training, †$p < 0.001$.

Statistical Analyses

Purpose 1. Physiological and psychological responses to training (before, and after 4 and 7 weeks of BT) and exercise (pre and postsubmaximal marching test) were assessed using repeated-measures ANOVA. Least significance difference (LSD) as *Post hoc* analysis was used to identify significant differences. In addition, the effect of training and exercise and their interaction were calculated. Pearson product–moment correlations were used to observe associations between variables. These analyses were performed for the 35 subjects who completed all submaximal marching tests and thus had all results.

Purposes 2 and 3. Independent samples *T*-tests were used to identify significant differences between OR and noOR subjects. In addition, groups of subjects satisfying each single OR criteria (1–4) were evaluated independently by independent samples *T*-tests. For OR criteria 5 (sick absence), 1-way ANOVA was used to identify significant differences between the groups. During the study, there were 42 military service days, and the average number of sick absences was 3 days, ranging from 0 to 10 days (0–24%). Subjects were divided into tertiles based on sick absence; 0% sick absence ($n = 20$), 2–7% sick absence ($n = 19$), 10–24% sick absence ($n = 18$). All data are presented as mean ± *SD*. Where assumptions for normality were not met, the data were log transformed before statistical analysis. The untransformed values are shown in the text, tables, and figures for more meaningful comparison. Statistical analyses were performed using SPSS (Version 14.0.1. 2005; SPSS Inc., Chicago, IL). The level of statistical significance was set at $p \leq 0.05$.

Results

Purpose 1. The Effect of the Basic Training Period

$\dot{V}O_2$max. All 3 submaximal marching tests and $\dot{V}O_2$max tests were completed by a total of 21 conscripts. In 65% of cases, the reason for not participating in the test was URTI. There was a main effect of training for $\dot{V}O_2$max ($p < 0.001$), postexercise lactate ($p < 0.05$), and maximum HR in $\dot{V}O_2$max tests ($p < 0.01$). Conscripts showed positive responses to training with an 11% increase in $\dot{V}O_2$max after 5 weeks of training (before 45 ± 7 vs. week 5 49 ± 5 ml·kg^{-1}·min^{-1}, $p < 0.001$). From week 5 to week 8, $\dot{V}O_2$max did not change significantly. In addition, the individual $\dot{V}O_2$max values before

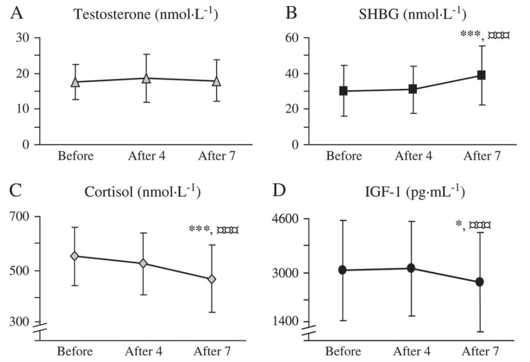

Figure 1. Mean (±*SD*) basal serum testosterone (A), serum sex hormone–binding globulin (SHBG) (B), cortisol (C), and insulin-like growth factor-1 (IGF-1) (D) concentrations before, and after 4 and 7 weeks of basic military training in 35 conscripts. Difference compared with baseline ***$p < 0.001$, *$p < 0.05$; week 4 $^{¤¤¤}p < 0.001$. There was a main effect of training for SHBG, cortisol and IGF-1 ($p < 0.001$). $n = 35$.

TABLE 3. HR, RPE, and blood La responses to the 45-minute submaximal marching test at 70% of $\dot{V}O_2$max before and after 4 and 7 weeks of military basic training ($n = 35$).*

	Before		After 4 wks				After 7 wks				Before vs. after 7 wks
	Mean	±*SD*	Mean	±*SD*	*p*	Δ%	Mean	±*SD*	*p*	Δ%	Δ%
HR ($b \cdot min^{-1}$)†	154	11	148	11	***	−4	140	12	***, ¤¤¤	−5	−9
RPE#	13.1	1.7	13.2	1.7		1	12.1	0.9	**, ¤¤¤	−8	−7
Postexercise La ($mmol \cdot L^{-1}$)†	2.9	2.0	1.7	0.8	***	−29	1.1	0.5	***, ¤¤¤	−26	−48

*HR = heart rate; RPE = rating of perceived exertion; and La - lactate.
Difference compared to: before ***$p < 0.001$, **$p < 0.01$; week 4, ¤¤¤. Main effect of training †$p < 0.001$.

training were negatively correlated with individual Δ%$\dot{V}O_2$max after 5 ($r = -0.61$, $p < 0.001$) and 8 ($r = -0.74$, $p < 0.001$) weeks of training. Postexercise lactate was lower after 8 weeks of training (9.8 ± 2.1 $mmol \cdot L^{-1}$) compared with 5 weeks of training (11.1 ± 2.1 mmol L^{-1}, $p < 0.05$) and before training (11.6 ± 2.3 $mmol \cdot L^{-1}$, $p < 0.01$). Maximum HR was lower after 5 ($p < 0.01$) and 8 ($p < 0.001$) weeks of training compared with before training values (before 195 ± 7 $b \cdot min^{-1}$, week 5 190 ± 7 $b \cdot min^{-1}$, and week 8 188 ± 6 $b \cdot min^{-1}$).

Body Composition

The initial average body height was 178.2 ± 7.9 cm, BMI 24.7 ± 4.5 $kg \cdot m^{-2}$, and F% 19.3 ± 7.5%. The values before and after 4 and 7 weeks of training for BM, FM, and FFM are presented in Table 2. From one subject, body composition measurement by 8-point bioelectrical impedance was not available at week 7; therefore, the number of the subjects is 34 in Table 2. Both BM and FM exhibited a main effect of training ($p < 0.001$), decreasing from week 4 to week 7 ($p < 0.001$ and $p < 0.01$, respectively), with lower values compared with the before training values ($p < 0.001$). All subjects exhibited a decrease in FM after 7 weeks of training compared with the before training values (range from −28.0 to −0.4%). In contrast, the mean FFM was higher after 7 weeks of training compared with the before training values ($p < 0.05$) (Table 2).

Basal Serum Hormone Concentrations

There was a main effect of training for serum cortisol, SHBG, and IGF-1 ($p < 0.001$) and for the testosterone/cortisol ratio ($p < 0.05$). Basal testosterone levels were similar at all 3 time points (Figure 1A). Basal SHBG increased, whereas basal cortisol and pre-exercise IGF-1 decreased from week 4 to week 7 ($p < 0.001$), being significantly different from the before training values ($p < 0.05$–0.001) (Figure 1B–D). Serum basal testosterone/cortisol ratio increased from week 4 to week 7 ($p < 0.01$) (before 0.034 ± 0.013, week 4 0.037 ± 0.016, week 7 0.040 ± 0.012). However, 46% of the subjects showed an initial increase in basal testosterone during the first 4 weeks of training. Thereafter, basal testosterone decreased from week 4 to week 7 in these subjects. In

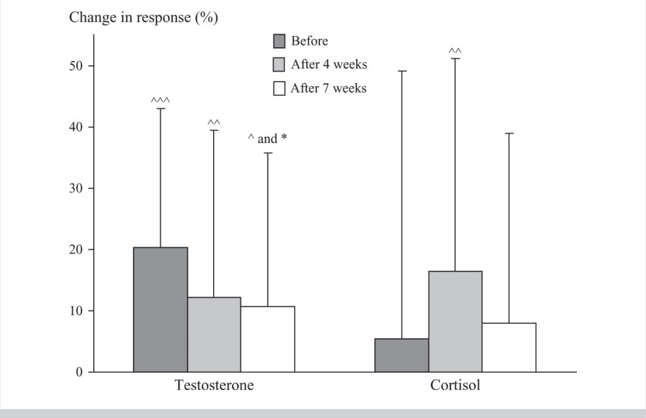

Figure 2. Mean (±*SD*) serum testosterone and cortisol responses (%) to the 45-minute submaximal marching test before and after 4 and 7 weeks of basic military training. Increase because of exercise; ^^^$p < 0.001$, ^^$p < 0.01$, ^$p < 0.05$. Difference compared with baseline; *$p < 0.05$. There was a main effect of exercise for testosterone and cortisol ($p < 0.001$). $n = 35$.

contrast, 23% of the subjects showed increases in basal testosterone throughout the study.

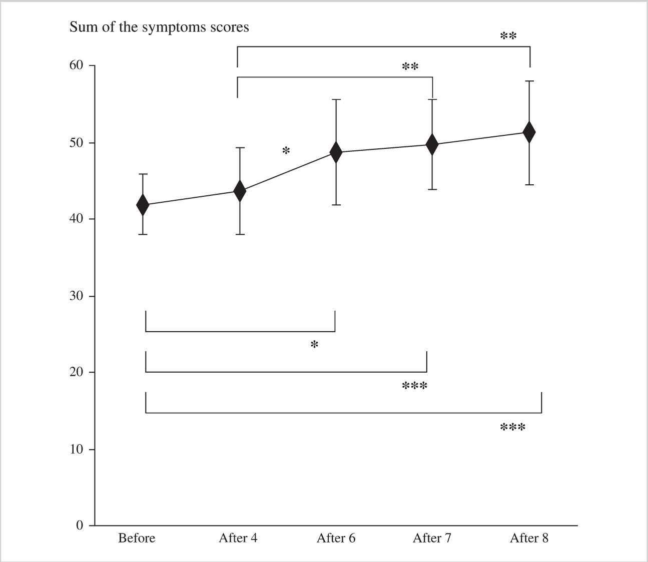

Figure 3. Mean ($\pm SD$) sum of the somatic symptoms scores associated with overtraining syndrome, defined from 5-point Likert scale questionnaires during the 8-week military basic training period. Significant difference; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. There was a main effect of training for somatic symptoms ($p < 0.001$). $n = 35$.

Responses to Submaximal Marching Tests

There was a main effect of training for HR and RPE during submaximal marching ($p < 0.001$). Heart rate was lower after 4 weeks ($p < 0.001$) compared with the before training values, and after 7 weeks of training compared with the week 4 and before training values ($p < 0.001$) (Table 3). Rating of perceived exertion was lower after 7 weeks of training compared with the before training ($p < 0.001$) and week 4 values ($p < 0.01$) (Table 3). Blood La exhibited a main effect of training ($p < 0.001$) and exercise ($p < 0.001$), and a training and exercise interaction ($p < 0.001$) by increasing because of exercise before (179 ± 211%, $p < 0.001$) and after 4 weeks of training (68 ± 91%, $p < 0.001$), but not after 7 weeks of training (13 ± 49%, n.s.). In addition, postexercise La was lower after both 4 ($p < 0.001$) and 7 ($p < 0.001$) weeks of training compared with the before training values (Table 3). Serum testosterone and cortisol showed a main effect of exercise ($p < 0.001$). In response to exercise, serum testosterone increased significantly at each time point ($p < 0.05$–0.001) (before training 20 ±23%, $p < 0.001$; week 4 12 ± 27%, $p < 0.01$; week 7 11 ± 25%, $p < 0.05$). After 7 weeks of training, Δ%testosterone induced by exercise was lower compared with the before training values ($p < 0.05$) (Figure 2). Serum cortisol increased significantly only after 4 weeks of training (before 5 ± 44%, n.s.; week 4 16 ± 35%, $p < 0.01$; week 7 8 ± 31%, n.s.). Individual variations in cortisol responses were large, ranging from −48 to 107% (Figure 2).

Questionnaire Responses

The somatic symptoms associated with OTS exhibited a main effect of training ($p < 0.001$), whereby symptoms increased toward the end of BT. The greatest increase was observed between week 4 and week 7 ($p < 0.01$) (Figure 3). Concurrently, the proportion of subjects who answered "yes" to the question "Do you feel physically or mentally overloaded?" increased from 41% to 80% (Figure 4).

Purpose 2 and 3. Incidence of Overreaching and Differences between overreaching and no-overreaching Subjects

From a total of 57 subjects, OR criterion 1 was detected among 42% of subjects, criterion 2 32%, and criterion 3 13%. According to the measured results before training, the subjects who

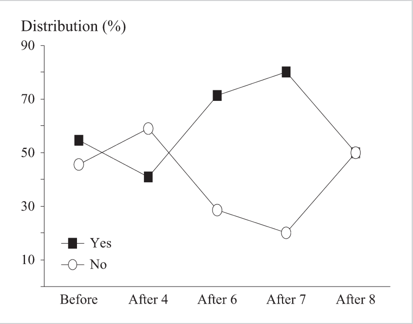

Figure 4. The proportion of subjects who answered "yes" or "no" to the question "Do you feel physically or mentally overloaded?" ($n = 35$).

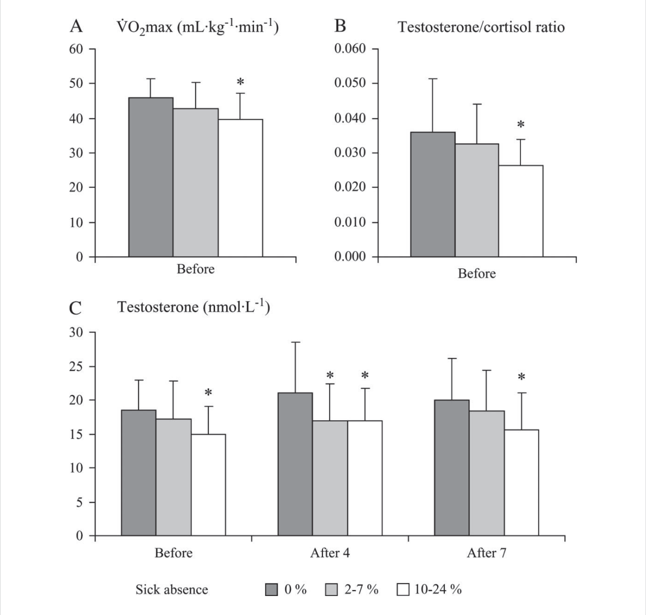

Figure 5. Mean ($\pm SD$) $\dot{V}O_2max$ and (A) basal serum testosterone/cortisol ratio (B) before training and basal serum testosterone (C) before and after 4 and 7 weeks of basic military training in conscripts who had a sickness absence of 0%, 2–7%, and 10–24% of days. Difference compared with the 0% group; $^*p < 0.01$. $n = 57$.

fulfilled OR criteria 1, 2, or 3 were not significantly different from those who did not fulfill any of these OR criteria.

Criterion 4 was detected among 83% of subjects. Those who admitted to feeling physically or mentally overloaded in week 7 or 8 had lower $\dot{V}O_2max$ (3.3 ± 0.4 vs. 3.9 ± 0.5 L·min^{-1}, $p < 0.001$), FFM (61.9 ± 0.8 vs. 68.7 ± 5.6 kg, $p < 0.05$) and higher HR in the submaximal marching test (152 ± 11 vs. 141 ± 10, $p < 0.05$) before training compared with those who answered "no."

Criterion 5 was detected among 32% of subjects. The subjects who had sick absences of more than 10% had lower $\dot{V}O_2max$ (46 ± 6 vs. 40 ± 7 ml·kg^{-1}·min^{-1}, $p < 0.01$), basal testosterone (18.6 ± 4.3 vs. 14.9 ± 4.1 nmol·L^{-1}, $p < 0.05$), and testosterone/cortisol ratio (0.036 ± 0.015 vs. 0.026 ± 0.007, $p < 0.05$) before training compared with those who had no sick absences. In addition, subjects with more than 10% sick absence also had lower basal testosterone after 4 and 7 weeks of training (21.1 ± 7.5 vs. 17.0 ± 4.7 and 20.0 ± 6.1 vs. 15.5 ± 5.6 nmol·L^{-1}, $p < 0.05$) (Figure 5A–C).

At least 3 of the 5 criteria were detected among 33% of the subjects, all of whom were classified as OR subjects. The remaining 67% were classified as noOR subjects. At the beginning of BT, only basal SHBG differed between OR and noOR subjects ($p < 0.01$) (Table 4). Similarly, OR subjects had higher basal SHBG than noOR subjects after 4 ($p < 0.01$)

Table 4. Differences between OR and noOR classified subjects before and during military basic training.*

	OR subjects		noOR subjects		
	Mean	±*SD*	Mean	±*SD*	*p*
Basal serum SHBG at baseline (nmol·L^{-1})	38.4	16.9	27.9	7.9	**
Basal serum SHBG after 4 wks (nmol·L^{-1})	38.4	14.1	28.5	8.6	**
Basal serum SHBG after 7 wks (nmol·L^{-1})	46.4	19.2	36.6	10.6	*
Basal serum cortisol after 7 wks (nmol·L^{-1})	545	145	465	97	*
Change (%) in basal testosterone/cortisol ratio from after 4 to after 7 wks	−6	23	24	32	*
Change (%) in maximal La/RPE ratio from before to after 8 wks	−30	18	−3	20	**

*OR = overreaching; noOR = no-overreaching; SHBG = serum sex hormone–binding globulin; RPE = rating of perceived exertion. Difference compared to OR; $^{**}p < 0.01$, $^*p < 0.05$.

and 7 weeks of training ($p < 0.05$). Furthermore, OR subjects had higher basal serum cortisol than noOR subjects after 7 weeks of training ($p < 0.05$). Moreover, OR and noOR subjects differed in terms of the response of the basal testosterone/cortisol ratio to training from week 4 to week 7 ($p < 0.05$), and the maximal La/RPE ratio in the $\dot{V}O_2$max test from baseline to the end of BT (Table 4).

DISCUSSION

The purpose of the present study was to evaluate the training load of an 8-week Finnish military basic training period among new conscripts, to determine the incidence of OR during BT, and to assess whether initial levels or training responses differ between OR and noOR subjects. The subjects' physical fitness characteristics in this study were representative of young Finnish men (30). However, there was a high incidence of dropouts from the performance tests; only 63% of the subjects completed all submaximal tests and 38% both submaximal and maximal tests. The dropouts did not mean that these subjects had more sick absence. However, serum testosterone, testosterone/cortisol ratio, and $\dot{V}O_2$max were lower in "high incidence of sick absence" subjects than in "no sick absence" subjects. Furthermore, subjects who admitted to feeling overloaded demonstrated low $\dot{V}O_2$max, FFM and high submaximal test HR before training. These observations confirm the earlier finding that poor aerobic fitness is a risk factor for sick absence during military service (17) and highlight the importance of good physical fitness for overall well-being. A new finding was that testosterone had an impact on sustaining stress and maintaining health during the 8-week military training period.

For those conscripts who were able to perform all the tests, the increase in $\dot{V}O_2$max indicates an improvement in aerobic capacity. In addition, decreases in HR, postexercise La, and serum testosterone during the 45-minute submaximal marching test indicate decreased exercise-induced metabolic stress (28,40) during the first 4 weeks of BT. Furthermore, as expected, our data showed an inverse relationship between initial $\dot{V}O_2$max and relative changes in $\dot{V}O_2$max, indicating that the training was most effective for the least fit subjects. This is comparable with studies performed in the Polish (9) and USA (28) armies.

During the second half of the 8-week training period, a stagnation of increase in $\dot{V}O_2$max was observed. Concurrently, basal serum IGF-1 decreased, and basal serum SHBG and testosterone/cortisol ratio increased with no change in basal serum testosterone. Furthermore, basal serum cortisol decreased with a concomitant increase in the number of subjects who felt overloaded. Previously, serum total testosterone concentration has been shown to decrease in response to demanding training periods (6,36), and during extremely stressful military training periods (23,27), and to increase with overload training (37). In contrast to the present study, basal SHBG has been found to increase (19,26) and IGF-1 to decrease (23,25–27) after heavy training. A decrease in IGF-1 is suggested to indicate a diminished capacity for protein deposition. In addition, IGF-1 is sensitive to both energy intake and dietary protein content, and demonstrates significant decreases with inadequate dietary intake (25,26). Furthermore, a 7-week intensive endurance overload training program (37) and combined endurance and strength overload training for 6 weeks (5) have been found to decrease testosterone/cortisol ratios. Even the biochemical changes of testosterone and testosterone/cortisol ratio are controversial compared with previous findings, a stagnation of increase in $\dot{V}O_2$max in this study indicates that the training load during the second half of the 8-week training period may have been too exhaustive. This is also supported by a decrease in maximal postexercise lactate and HR (14,22,38), and the fact that 46% of subjects showed a decrease in basal serum testosterone from week 4 to week 7.

Classification of overreaching (OR) or overtraining syndrome (OTS) is challenging, and it is rarely classified by a single criterion. In this study, OR subjects had to fulfill 3 of 5 criteria, and OR was detected among 33% of the subjects. The only previous study concerning OR or OTS among young men who entered military service and performed an 8-week BT training period was by Chicharro et al. (4). They found a 24% incidence of OTS during BT, determined by absolute decreases in the free testosterone/cortisol ratio, a classical criterion for OR/OTS (1,5,19). Similarly, in this study, OR subjects differed in response to training compared with noOR subjects. OR subjects exhibited a tendency toward a decrease in basal Δ% testosterone/cortisol ratio, whereas noOR subjects exhibited an increase. Snyder et al. (31) defined 5 criteria for OR, one of which was a decreased maximal La/RPE ratio. In the present study, the maximal La/RPE ratio tended to decrease during the latter part of BT in OR subjects.

To our knowledge, no previous studies have a reported relationship between basal serum SHBG and the incidence of OR. In the present study, OR subjects had higher levels of SHBG, both before training and after 4 and 7 weeks of training. Although basal SHBG has been found to increase after heavy training (19,26), the mechanism underlying the present findings is still not fully understood. Testosterone did not differ between noOR and OR subjects. However, most of the testosterone is bound to SHBG, which in turn decreases bioavailable testosterone in serum, and may thereby have an influence on training responses.

OR subjects had higher basal serum cortisol at the end of BT, which is in line with the OR scenario presented by Steinacker et al. (33), where an increase in basal cortisol was observed in the OR state. However, a decrease in basal serum cortisol during the second half of BT was observed among the 35 conscripts who performed all submaximal tests. This may be a marker of overtraining, along with lower psychosocial stress (29,31,33,36). Basal serum cortisol has been found to increase during extremely stressful military training periods (23,27), but no change (6,19,37), or a decrease

(29,31) has been observed in endurance based overtraining studies. However, in response to strenuous resistance training, both no change (11,18) and an increase (10) of basal serum cortisol have been reported. Glucocorticoids, like cortisol, exert many beneficial actions in exercising humans: increasing the availability of metabolic substrates for the energy requirements of muscles, maintaining normal vascular integrity and responsiveness, and protecting the organism from an overreaction of the immune system in response to exercise-induced muscle damage (29,33). Differences in cortisol behavior between military and overtraining studies, and incoherent training responses of basal serum cortisol in this study, may be explained by the differences in energy availability, training mode, and fitness levels of the subjects. The military training studies were performed under conditions of nutritional and sleep deprivation, in combination with high-energy expenditure and physiological strain. Most of these studies involved relatively short (from 5 to 14 days) training periods, and the subjects were soldiers. In the overtraining studies, food and sleep were ad libitum, and the subjects were athletes or at least had a good fitness level. Furthermore, the training period in the overtraining studies ranged from 2 to 7 weeks, consisting mostly of endurance or strength training. The subjects in the present study were low to moderately fit conscripts in the compulsory army, and training consisted of both strength and endurance training.

Recently, it has been proposed that the best indicator of OR and OTS is the physiological response to an acute stress, such as an exercise test (22). Only 47% of the OR subjects performed all submaximal tests because of sick absences. This may be the reason for the nonsignificant difference in cortisol responses to exercise between OR and noOR subjects. However, among the conscripts who were able to perform all submaximal tests, the exercise-induced increase in cortisol in this study was only significant after 4 weeks of training. It has been suggested that increased basal levels of cortisol contribute to exhaustion of the hypothalamic–pituitary–adrenal axis, thus preventing an adequate cortisol response to such an acute stress (10). In the present study, the attenuated cortisol response before training could be explained by the higher basal values, although this seems unlikely as pre-exercise cortisol levels were not associated with cortisol responses to the submaximal 45-minute marching test. In the compensated stage during training, adreno-corticotropic hormone (ACTH) and cortisol increase in response to metabolic stress; during overreaching, the cortisol response is blunted along with an augmented ACTH response (33). Overtraining is characterized by a decrease in ACTH and cortisol responses (33), and exercise-induced decreases in cortisol have been linked to intensive training (36). Thus, in this study, the increased response of cortisol to exercise after 4 weeks of training among the 35 conscripts who completed all tests indicates an improvement in performance and an attenuated response after 7 weeks is indicative of overreaching.

Practical Applications

The fitness level of young Finnish men has decreased with a concurrent increase in BM (30). Currently, approximately 15% of new conscripts interrupt the compulsory military service within 1 month. Consequently, many of the conscripts have a low fitness level. However, the same physical training program has to be performed by all conscripts in the military environment. In the present study, improvements in physical performance were observed during the first 4 weeks of BT, such as an increase in maximal aerobic capacity and decreased submaximal exercise-induced stress. Although general diagnostic parameters to diagnose OR or OTS have not yet been found, several changes in biomarkers and physical performance may indicate that the training load during the second half of the 8-week training period was not well tolerated by all the conscripts. Furthermore, both emotional and somatic symptoms of OR increased.

Because 33% of the subjects were classified as overreached, the end of the BT period has to be taken under careful consideration. If demanding training is continued after BT, OTS may develop. Thus, the weeks after BT should involve recovery-type training. Furthermore, if the amount of sick absence is set as a criterion for the tolerance to exercise training, these results suggest that to enhance physical fitness and to avoid OTS, conscripts could be divided into different training groups according to their aerobic fitness level. Initial serum basal SHBG and testosterone, and testosterone/cortisol levels, would give additional information, but in practice, collecting the samples from a large group of subjects is challenging. However, our results confirm that serum cortisol and the testosterone/cortisol and maximal La/RPE ratios could be useful tools for monitoring whether training is too stressful.

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II

ASSOCIATION OF MILITARY TRAINING WITH OXIDATIVE STRESS AND OVERREACHING

by

Minna M. Tanskanen, Arja L. Uusitalo, Hannu Kinnunen, Keijo Häkkinen,
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Association of Military Training with Oxidative Stress and Overreaching

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[1]Department of Biology of Physical Activity, University of Jyväskylä, FINLAND; [2]Department of Clinical Physiology and Nuclear Medicine, Helsinki University Hospital, Helsinki, FINLAND; [3]Polar Electro Oy, Kempele, FINLAND; and [4]Institute of Biomedicine/Physiology, University of Eastern Finland, Kuopio, FINLAND

ABSTRACT

TANSKANEN, M. M., A. L. UUSITALO, H. KINNUNEN, K. HÄKKINEN, H. KYRÖLÄINEN, and M. ATALAY. Association of Military Training with Oxidative Stress and Overreaching. *Med. Sci. Sports Exerc.*, Vol. 43, No. 8, pp. 1552–1560, 2011. We hypothesized that increased oxidative stress and disrupted redox balance may be predisposing factors and markers for overreaching (OR). **Purpose**: The study's purpose was to examine whether oxidative stress markers and antioxidant status and physical fitness are related to OR during an 8-wk military basic training (BT) period. **Methods**: Oxidative stress and antioxidant status were evaluated in the beginning and after 4 and 7 wk of training in 35 males (age = 19.7 ± 0.3 yr) at rest and immediately after a 45-min submaximal exercise. Physical activity (PA) was monitored by an accelerometer throughout BT. Indicators of OR were also examined. **Results**: From baseline to week 4, increased daytime moderate to vigorous PA led to concomitant decreases in the ratio of oxidized to total glutathione (GSSG/TGSH) and GSSG. After 4 wk of BT, GSSG/TGSH and GSSG returned to the baseline values at rest, whereas PA remained unchanged. At every time point, acute exercise decreased TGSH and increased GSSG and GSSG/TGSH, whereas a decrease was observed in antioxidant capacity after 4 wk of training. In the beginning of BT, OR subjects (11 of the 35 males) had higher GSSG, GSSG/TGSH, and malondialdehyde (a marker of lipid peroxidation) at rest ($P < 0.01$–0.05) and lower response of GSSG and GSSG/TGSH ratio ($P < 0.01$) to exercise than non-OR subjects. Moreover, OR subjects had higher PA during BT than non-OR ($P < 0.05$). **Conclusions**: The sustained training load during the last 4 wk of BT led to oxidative stress observable both at rest and after submaximal exercise. Increased oxidative stress may be a marker of insufficient recovery leading possibly to OR. **Key Words:** TRAINING LOAD, PHYSICAL ACTIVITY, EXERCISE TESTING, MILITARY, MALE, YOUNG ADULT

In competitive sports and military training, the goal of the training is to improve performance. However, both prolonged aerobic and strength training with high intensity or volume together with inadequate recovery could lead to nonfunctional overreaching (OR) resulting in stagnation or decrements in performance capacity (16). OR could also lead to the development of overtraining syndrome (OTS), both of which are characterized by an unexpected decrease or at least stagnation in performance despite increased or sustained training load. The time required for the recovery from OR takes several days to several weeks, whereas the development of OTS and recovery from it may take a much longer time.

Several factors and symptoms have been linked to OTS. However, there are no reliable and specific markers of impending OTS nor knowledge on how to identify individuals who are more vulnerable to OR. Moreover, the precise mechanisms leading to OR and OTS have not yet been delineated. Recently, impaired antioxidant capacity and increased oxidative stress, a state in which the production of reactive oxygen species (ROS) overwhelms antioxidant defenses, were found to be related to OTS in elite athletes (28). Animal (18,36) and human studies have provided evidence that a progressively increased training volume, which causes symptoms of OR or OTS, can increase oxidative stress and attenuate antioxidant capacity (15,35). Whereas a single bout of physical exercise of sufficient intensity and duration generates ROS, both aerobic (21) and anaerobic training (2) have been shown to enhance antioxidant status and decrease the generation of ROS. In contrast, overload training can lead to an impaired antioxidant defense and lack of expected adaptations to training (25) and distortion of the redox balance (8). Extreme increases in training volumes may lead to a substantial rise in inflammation and apoptosis markers (7). Therefore, overload training may induce inflammation, which is also associated with increased oxidative stress.

We hypothesized that increased oxidative stress and disrupted redox balance in response to heavy physical training may be predisposing factors and markers for OR. For this purpose, we aimed to study the association of oxidative

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stress markers and antioxidant status with physical activity during an 8-wk military basic training (BT) period. BT comprises the first 8 wk of military service and includes both endurance and strength types of training. A particular aim was to evaluate whether the levels of oxidative stress markers and antioxidant status and physical activity differ between OR and non-OR subjects.

METHODS

Subjects. Male conscripts (N = 35, age 19.6 ± 0.3 yr) participated in the present study. They were a part of a larger group of 60 soldiers who initially volunteered to participate and fulfilled selection criteria (29). All subjects were fully informed of the experimental protocol and gave their written consent to participate in the study. They were also advised of their right to withdraw from the investigation at any time. The study protocol was approved by the Finnish Defence Forces and the ethical committees of the University of Jyväskylä and the Kainuu region of Finland. The study took place during winter in Finland, when daily outdoor temperatures ranged from −31°C to +1°C, with an average of −13°C (data from the local weather station). The overall physical load of the 8-wk BT period was set according to the standard basic program (31). It included a total of 300 h of military training, of which 100 h was military-related physical training, 33 h was sports-related physical training such as combat training and marching, and the rest of the hours were other military training such as shooting, material handling, skill training, and general military education (23). The intensity level of physical activity in the daily program was planned to be low in the first week of BT and increased gradually thereafter. The conscripts slept from 10:00 p.m. to 5:45 a.m. in dormitory-type rooms at the garrison and marched four times per day (approximately 5 km in total) to dining. The BT schedule included four longer (from 2 to 8 h) marching exercises with a combat gear and two overnight field exercises from 1 to 3 d. Food and water intake were in accordance with the standard army meal, and water intake was not restricted. However, subjects were not permitted to use any extra nutritional supplements throughout the study.

Experimental protocol. Performance tests (maximal aerobic uptake ($\dot{V}O_{2max}$) and submaximal exercise tests) were performed, and oxidative stress and antioxidant status were determined three times, and psychological markers (questionnaires) were determined five times during the 8-wk training period. In addition, acute responses to a submaximal exercise were studied. For the submaximal exercise, a marching test with a 20-kg backpack was selected because it closely resembles the routine activities of conscripts and enables the test to be performed for a large group of subjects. The experimental protocol is presented in Table 1.

Performance tests. An incremental test until voluntary exhaustion to determine maximal oxygen uptake ($\dot{V}O_{2max}$) was carried out on a treadmill at baseline and on weeks 5 and 8 as described earlier (29). The 45-min submaximal exercise test was performed on an outdoor track at the 70% level of the subject's individual maximal workload in the beginning (baseline) of BT and on weeks 4 and 7. The follow-up tests for each subject were always performed at the same time of day ($\dot{V}O_{2max}$ test between 8:30 a.m. and 5:00 p.m., submaximal exercise between 9:00 a.m. and 12:00 noon) with the subjects having consumed a similar diet before each test and with the same test protocol. The hormonal responses to submaximal exercise have previously been reported (30).

Body composition. Body composition measurements (body mass (BM), fat-free mass, fat mass (FM), and percentage of body fat) were performed using eight-point bioelectrical impedance (InBody720; Biospace Co., Ltd., Seoul, Korea). For each subject, the repeated measurements were performed at the same time between 6:00 and 7:00 a.m. after an overnight fast and after voiding, with no exercise for 12 h before the test. The physical activities in the daily program were planned to be of a low intensity on the day preceding each measurement, and fluid status was estimated to be balanced on the basis of the dietary records of the subjects (29). The subjects were barefoot and wore T-shirts and trousers. Body height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as BM (kg) divided by height (m) squared.

Blood samples and analyses. Blood samples from an antecubital vein and fingertip were drawn 2 h after a light breakfast at rest, before submaximal exercise, and immediately after exercise. Circadian variability in blood parameters was minimized by collecting individual preexercise and postexercise samples at the same time of day between 9:00 a.m. and 12:00 noon preceded by similar patterns of food ingestion. Subjects were instructed not to ingest alcohol, coffee, tea, chocolate, or cola drinks since the evening before the measurements.

Blood samples were centrifuged at 1200g and 4°C for 15 min immediately after collection to separate the plasma. Plasma samples were stored in multiple portions at −80°C until analysis. Protein carbonyls, markers of protein oxidative damage, were measured using an ELISA method as

TABLE 1. Experimental design during the 8-wk military BT period.

Week	1	2	3	4	5	6	7	8
$\dot{V}O_{2max}$ test	X	—	—	—	X	—	—	X
Submaximal exercise test	X	—	—	X	—	—	X	—
Body composition	X	—	—	X	—	—	X	—
Blood samples	X	—	—	X	—	—	X	—
Questionnaire	X	—	—	X	—	X	X	X

previously described (19). Nitrotyrosine concentrations were determined with the ELISA method using a commercial kit (Hycult Biotechnology BV, Uden, The Netherlands). Lipid peroxidation marker total malondialdehyde in plasma was measured according to the method of Gérard-Monnier et al. (9). Oxygen radical absorbance capacity (ORAC) was used for the measurement of antioxidant capacity, which was performed using a multiwell plate reader according to the methods described previously (13). The maximal intra-assay coefficients of variation for protein carbonyls, nitrotyrosine, malondialdehyde, and ORAC were 5.9%, 10.0%, 6.2%, and 8.1%, respectively, and the maximal interassay coefficients of variation were 9.2%, 12.7%, 10.6%, and 11.3%, respectively. Protein carbonyls and ORAC measurements were performed in triplicate. Total glutathione (TGSH) and oxidative stress marker oxidized glutathione (GSSG) concentrations were determined spectrophotometrically as described previously (24). The tissues were deproteinized with metaphosphorous acid for TGSH and GSSG analysis. The maximal intra-assay and interassay coeffcients of variance were 4.8% and 6.4%. Hemoglobin and hematocrit were analyzed using a Sysmex KX-21N analyzer (Sysmex Co., Kobe, Japan). Blood lactate was analyzed from fingertip blood sample using Lactate Pro® analyzer (ARKRAY, Kyoto, Japan). Submaximal exercise–induced changes in plasma volume were calculated from changes in hemoglobin and hematocrit (6), and malondialdehyde, nitrotyrosine, and ORAC postexercise values were reported adjusted for these changes. Plasma protein carbonyl results were expressed in nanomoles per milligram of protein. The BT and/or exercise-induced relative changes are expressed as percent change.

Questionnaires and sick leave. The subjects rated how severely they experienced a list of symptoms during the last week using a five-point Likert scale: 1, not at all; 2, 1 d; 3, 2–3 d; 4, 4–5 d; and 5, 6–7 d. The somatic symptoms were subjective ratings of well-being, upper respiratory track infections, flu-like symptoms, digestive disorders and reduced appetite, musculoskeletal and physical complaints, and sleep difficulties (30). The sum of the symptoms associated with OTS was determined as the sum of the symptom scores. In addition, the question "do you feel physically or mentally overloaded?" was asked. Sick leave was defined as an attendance/nonattendance in daily service due to illnesses or injuries that were evaluated by a physician.

Physical activity. Physical activity (PA) was measured from 18 subjects with a customized version of the Polar AW200 Activity monitor (Polar Electro Oy, Kempele, Finland) that was worn on the nondominant wrist. AW200 has been found useful and accurate for the measurement of energy expenditure during long-term exercise (3). AW200 contains a uniaxial accelerometer. The acceleration signal is band-pass filtered (0.3–3.0 Hz), and the device has reduced sensitivity to repeated low-intensity hand movements. The device counts hand movements if acceleration exceeds 1.0 m·s^{-2} (12). Epoch length was set at 1 min, and a curvilinear equation was used to transform activity counts to metabolic equivalents (1–16 METs), which were further adjusted by body height. Among the same subjects, AW200 weekly physical activity energy expenditure correlated well ($r = 0.78$) to that obtained with doubly labeled water (unpublished data, see Tanskanen et al. (29)). Daytime was determined from 6:00 a.m. to 9:00 p.m., and nighttime, from 9:00 p.m. to 6:00 a.m. Periods that contained no single movement during 30 min in daytime or during 6 h or more in nighttime were classified as nonwear time and excluded from analysis. PA by each minute was classifies into 1) no activity (REST), ≤1.0 MET; 2) very light to light activity (VLPA), 1.0–3.9 METs; or 3) moderate to vigorous physical activity (MVPA), ≥4 METs. REST (during daytime) or sleep (during nighttime) was selected if the accelerometer showed no hand movements within >50% of a 10-min moving window. Each subject's daily and nightly data were included for analysis only if activity recording covered >80% of daytime and 90% of nighttime. Measures of PA were adjusted by the proportion of time recorded. The activity watches were collected every evening between 9:00 and 10:00 p.m. for data download and redistributed within 30 min. Out of a total of 41 d, we included days and nights when at least two-thirds of subjects had enough data. In all, 33 d and 28 nights were included in the analysis. Data were pooled into 2-wk periods: weeks 1–2, 3–4, 5–6, and 7–8.

Criteria for OR. In this study, subjects had to fulfill three of five criteria to be classified as OR subjects (30). Criteria 1 was a reduced $\dot{V}O_{2max}$ of >5% (11,26) or nonperformance of the test because of illness. Absence from thetest was set as an additional criterion because all three $\dot{V}O_{2max}$ tests were completed by a total of 21 of 35 conscripts. It was assumed that illness itself reduces performance. Furthermore, overload training has been reported as a risk factor for upper respiratory track infections (27,34). Criteria 2 was an increase in mean RPE during the submaximal exercise >1.0 (11) from the lowest value at week 1 or 4 until the end of BT. Criteria 3 was an increase in somatic symptoms of OTS (16,32) >15% from weeks 4 to 7 remaining the same or increasing from weeks 7 to 8. Subjects were divided into tertiles on the basis of an increase in somatic symptoms of OTS from weeks 4 to 7; 15% was the cutoff for the upper third. Criteria 4 was admitted feeling physically or mentally overloaded (16,33,34) at week 7 or 8. Criteria 5 was a sick leave >10% of daily service. Subjects were divided into tertiles on the basis of sick leave during BT; 10% was the cutoff for the upper third.

Statistical analyses. Statistical analyses were performed using SPSS (Version 16.0.1. 2005; SPSS, Inc., Chicago, IL). The level of statistical significance was set at $P < 0.05$. Assumptions for normality were not met for protein carbonyls, malondialdehyde, and nitrotyrosine, and data were log-transformed before statistical analysis. The untransformed values are shown in the text, tables, and figures for more meaningful comparison, except for nitrotyrosine. Responses to submaximal exercise (before and after) and BT period (baseline and weeks 4 and 7) were assessed using repeated-measures ANOVA. Mixed-design factorial ANOVA (group (non-OR vs OR) × exercise × BT) was used to identify differences between and within the OR and non-OR subjects. Bonferroni as the *post hoc* analysis was used to identify significant differences. In addition, the effect of exercise and BT and their interaction were calculated. Pearson product–moment correlations were used to observe associations between variables. All data are presented as mean ± SD.

RESULTS

$\dot{V}O_{2max}$ and body composition. There was a main effect of BT for $\dot{V}O_{2max}$ ($P < 0.001$) with an increase in $\dot{V}O_{2max}$ after 5 wk of BT ($P < 0.001$). From weeks 5 to 8, $\dot{V}O_{2max}$ did not change significantly (baseline = 45 ± 7 $mL \cdot kg^{-1} \cdot min^{-1}$, week 4 = 49 ± 5 $mL \cdot kg^{-1} \cdot min^{-1}$, week 7 = 49 ± 5 $mL \cdot kg^{-1} \cdot min^{-1}$). The initial mean body height was 178.2 ± 7.9 cm, BMI was 24.7 ± 4.5 $kg \cdot m^{-2}$, and percentage of body fat was 19.3% ± 7.5%. Both BM and FM exhibited a main effect of BT ($P < 0.001$), decreasing from weeks 4 to 7 ($P < 0.001$ and $P < 0.01$, respectively), with lower values compared with the pre-BT values ($P < 0.001$; BM: baseline = 78.7 ± 17.7 kg, week 4 = 78.5 ± 16.9 kg, week 7 = 77.0 ± 15.9 kg; FM: baseline = 16.1 ± 10.7 kg, week 4 = 15.6 ± 10.7 kg, week 7 = 14.0 ± 9.1 kg). In contrast, the mean fat-free mass was higher after 7 wk of BT compared with baseline ($P < 0.05$; baseline = 62.6 ± 9.4 kg, week 4 = 62.9 ± 8.8 kg, week 7 = 63.0 ± 9.3 kg).

Oxidative stress markers and antioxidant capacity. There was a main effect of exercise for TGSH ($P < 0.001$), GSSG ($P < 0.001$), and the GSSG/TGSH ratio ($P < 0.001$); a main effect of BT for TGSH ($P < 0.05$) and ORAC ($P < 0.01$); and an exercise × BT interaction for TGSH ($P < 0.05$), GSSG ($P < 0.001$), the GSSG/TGSH ratio ($P < 0.01$), and ORAC ($P < 0.05$). TGSH and ORAC (Fig. 1) and protein carbonyls, malondialdehyde, and nitrotyrosine at rest remained unchanged during BT. After 4 wk of BT, a decrease at rest in GSSG ($P < 0.01$) and an increase in GSSG and GSSG/TGSH ratio at the latter part of BT were observed (Fig. 1). At every time point, submaximal exercise induced a decrease in TGSH and an increase in GSSG and GSSG/TGSH ratio (Fig. 1). However, a significant submaximal exercise–induced decrease in ORAC was observed only at week 4 (Fig. 1). In addition, at week 4, TGSH and ORAC were lower after submaximal exercise than at baseline (TGSH, $P < 0.01$; ORAC, $P < 0.01$) and week 7 (TGSH, $P < 0.001$; ORAC, $P < 0.001$). GSSG and the GSSG/TGSH ratio after submaximal exercise at week

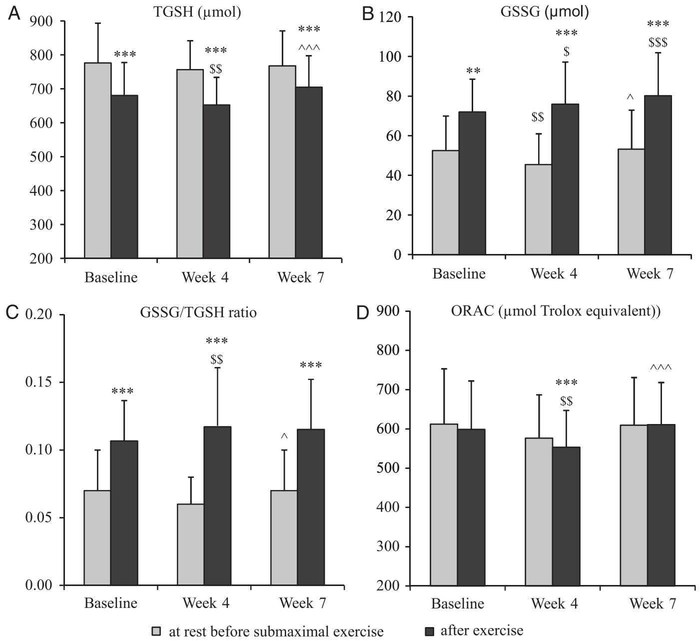

FIGURE 1—Mean ± SD plasma TGSH (A), GSSG (B), GSSG/TGSH (C), and ORAC (D) concentrations before and after the 45-min submaximal exercise at baseline and at weeks 4 and 7 of BT. Difference compared with before exercise: ***$P < 0.001$, **$P < 0.01$; baseline: \$\$\$$P < 0.001$, \$\$$P < 0.01$, ∧$P < 0.05$; week 4: ∧∧∧$P < 0.001$, ∧∧$P < 0.01$. $N = 35$.

4 (GSSG, $P < 0.05$; GSSG/TGSH, $P < 0.01$) and GSSG at week 7 ($P < 0.001$) were higher than at baseline (Fig. 1). No significant changes were observed because of the submaximal exercise in protein carbonyls, malondialdehyde, and nitrotyrosine.

Differences between non-OR and OR subjects. At least three of the five criteria were detected in 31% ($n = 11$ of 35) of the subjects, all of whom were classified as OR subjects. The remaining 69% ($n = 24$) were classified as non-OR subjects. From OR subjects, nine subjects fulfilled criteria 1; six, criteria 2; eight, criteria 3; nine, criteria 4; and six, criteria 5. There was a group × exercise × BT interaction only for GSSG ($P < 0.001$) and GSSG/TGSH ratio ($P < 0.05$). At baseline, GSSG ($P < 0.01$) and the GSSG/TGSH ratio ($P < 0.05$) were higher in OR than those in non-OR at rest (Figs. 2A, C, respectively). In OR, no response of GSSG and GSSG/TGSH to acute submaximal exercise was seen, in contrast to non-OR subjects (Figs. 2B, D, respectively). Furthermore, OR subjects had higher malondialdehyde at baseline ($P < 0.05$) than non-OR

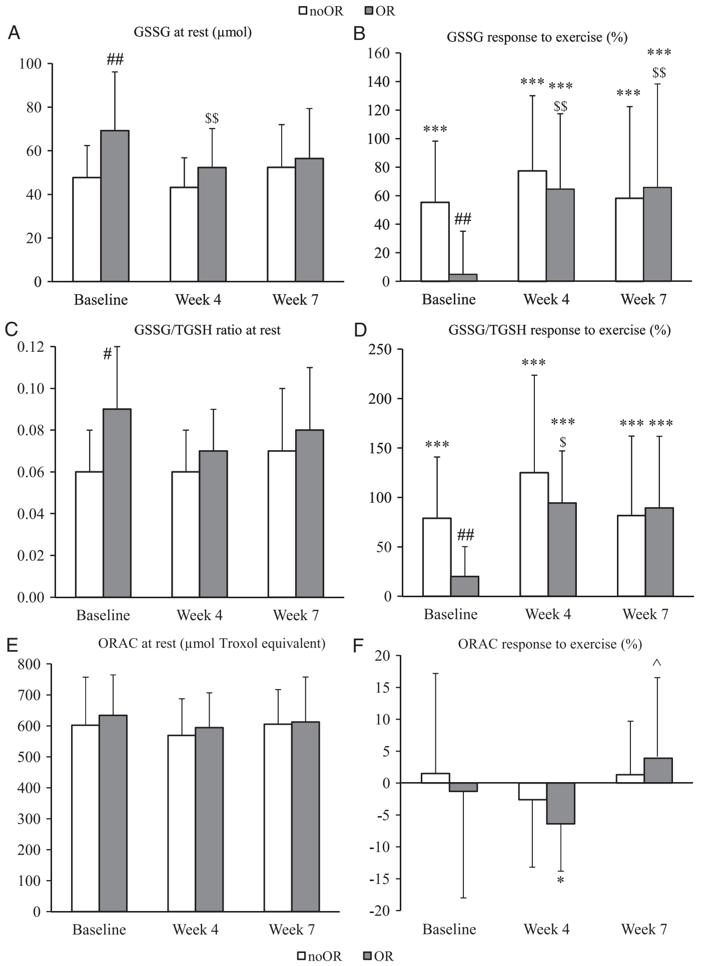

FIGURE 2—Mean ± SD plasma GSSG, GSSG/TGSH, and ORAC concentrations at rest (A, C, E) and changes (%) due to the 45-min submaximal exercise (B, D, F) at baseline and at weeks 4 and 7 of BT among non-OR ($n = 24$) and OR ($n = 11$) subjects. Difference compared with non-OR: ##$P < 0.01$, #$P < 0.05$; baseline: $$$P < 0.01$, $$P < 0.05$; week 4: ∧$P < 0.05$. Significant change due to the exercise: ***$P < 0.001$, *$P < 0.05$.

(Table 2). $\dot{V}O_{2max}$ and body composition did not differ between the groups.

In response to BT at rest, OR subjects exhibited decreased GSSG ($P < 0.01$) and a trend of decreased GSSG/TGSH ratio ($P = 0.058$) from baseline to week 4 (Figs. 2A, C). Compared with baseline, OR had a higher relative increase in GSSG due to submaximal exercise both after 4 ($P < 0.01$) and 7 ($P < 0.05$) wk of BT, and in the GSSG/TGSH ratio, after 4 wk of BT ($P < 0.05$) (Figs. 2B, D). Among the non-OR subjects, exercise-induced decrease in TGSH was lower after 7 wk of BT than after 4 wk of BT ($P < 0.01$) (Table 2). However, only the OR subjects exhibited a significant exercise-induced decrease in ORAC after 4 wk of BT ($P < 0.05$), which was significantly lower compared with week 7 ($P < 0.05$) (Fig. 2F). There were no differences between the groups either in TGSH, ORAC, protein carbonyls, and nitrotyrosine at rest or in the relative responses to exercise at baseline or during BT (Table 2). The relative changes at rest during BT in TSGH, GSSG, GSSG/TGSH ratio, ORAC, protein carbonyls, malondialdehyde, or nitrotyrosine also did not differ between the groups. However, OR had a higher incidence of sick leave only during the last week of BT compared with the non-OR subjects (mean difference = 5.5%, 95% confidence interval = 1.8%–9.0%, $P < 0.004$).

PA. During the entire BT period, the average daytime MVPA was 2 h 7 min ± 24 min; VLPA, 11 h 36 min ± 30 min; and REST, 30 ± 18 min. During the nighttime between 9:00 p.m. and 6:00 a.m., the average MVPA was 5 ± 2 min; VLPA, 1 h ± 18 min; and sleeping time, 7 h 30 min ± 18 min. There was a main effect of BT for daytime MVPA ($P < 0.001$), VLPA ($P < 0.05$), REST ($P < 0.001$), and nighttime MVPA ($P < 0.001$). Daytime MVPA was higher ($P < 0.001$) (Fig. 3), and VLPA ($P < 0.001$–0.05) (Fig. 3) and REST were lower ($P < 0.001$) during the latter part of BT compared with weeks 1–2 (REST: weeks 1–2 = 2 h ± 6 min, weeks 3–4 = 24 ± 12 min, weeks 5–6 = 30 ± 24 min, weeks 7–8 = 30 ± 24 min). However, nighttime MVPA was higher during the first 4 wk of BT compared with the latter half of BT ($P < 0.01$–0.05) (Fig. 3). Nighttime VLPA and sleeping time remained the same during the entire BT.

PA among non-OR and OR subjects. PA was monitored from nine OR and nine non-OR subjects. These subjects did not differ in incidence of sick leave during BT; thus, there was no need to take into account the influence of sick leave on physical activity. There were main effects of group for daytime VLPA ($P < 0.05$) and nighttime MVPA ($P < 0.05$). OR had higher nighttime MVPA compared with non-OR during weeks 3–4 ($P < 0.05$) and 5–6 ($P < 0.05$) (Fig. 3) and less daytime VLPA during weeks 1–2 ($P < 0.05$) and 5–6 ($P < 0.05$) (Fig. 3). However, OR had higher daytime MVPA compared with non-OR during weeks 1–2 ($P < 0.05$) and 5–6 ($P < 0.05$) (Fig. 3). There were no differences between the groups in REST, nighttime VLPA, and sleeping time. However, REST was less during weeks 5–6 than 7–8 ($P < 0.01$; weeks 5–6 = 36 ± 24 min, weeks 7–8 = 42 ± 24 min) in OR but not in non-OR.

DISCUSSION

In this study, we showed that increased oxidative stress is associated with OR. To our knowledge, this is the first study showing that increased oxidative stress before the strenuous 8-wk combined aerobic and strength training period was related to the incidence of OR during the training period. Oxidative stress markers, including GSSG, the GSSG/TGSH ratio, and malondialdehyde at rest, were higher among OR than in non-OR subjects. In addition, OR subjects had no increase in GSSG and GSSG/TGSH ratio in the submaximal exercise, which might be a consequence of high resting levels. Recently, we have reported that impaired antioxidant capacity and increased oxidative stress among athletes at the state of OTS compared to non-OTS athletes (28). However, whether increased oxidative stress is a cause for OTS or an outcome of a training protocol with inadequate recovery remains unclear.

TABLE 2. TGSH and oxidative stress markers among non-OR ($n = 24$) and OR ($n = 11$) subjects at rest and relative change due to the submaximal exercise (Δ% ex.).

			Baseline		Week 4		Week 7	
			Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
TGSH	At rest (μmol)	Non-OR	773 ± 108	—	756 ± 83	—	776 ± 100	—
		OR	801 ± 140	—	754 ± 91	—	767 ± 115	—
	Δ% ex.	Non-OR	−11 ± 11	***	−15 ± 8	***	−8 ± 7	∧∧,***
		OR	−12 ± 7	***	−12 ± 11	***	−6 ± 16	***
Malondialdehyde	At rest (μmol)	Non-OR	0.99 ± 0.28	—	0.93 ± 0.29	—	1.10 ± 0.32	—
		OR	1.24 ± 0.29	#	1.11 ± 0.32	—	1.11 ± 0.16	—
	Δ% ex.	Non-OR	3 ± 24	—	7 ± 26	—	0.3 ± 23	—
		OR	−11 ± 27	—	4 ± 26	—	−10 ± 21	—
Protein carbonyls	At rest (nmol·mg^{-1})	Non-OR	0.09 ± 0.02	—	0.09 ± 0.02	—	0.09 ± 0.02	—
		OR	0.10 ± 0.02	—	0.09 ± 0.02	—	0.10 ± 0.02	—
	Δ% ex.	Non-OR	0.03 ± 5	—	−1 ± 4	—	1 ± 4	—
		OR	0.32 ± 5	—	1 ± 4	—	1 ± 5	—
Nitrotyrosine	At rest	Non-OR	6.53 ± 2.31	—	6.56 ± 2.18	—	6.53 ± 2.18	—
		OR	6.45 ± 1.84	—	6.45 ± 1.78	—	6.52 ± 1.79	—
	Δ% ex.	Non-OR	4 ± 15	—	−3 ± 11	—	2 ± 16	—
		OR	9 ± 23	—	−1 ± 11	—	−6 ± 12	—

Difference: compared with non-OR, # $P < 0.05$; within the group compared with week 4, ∧∧ $P < 0.01$.
Significant change due to exercise: *** $P < 0.001$.

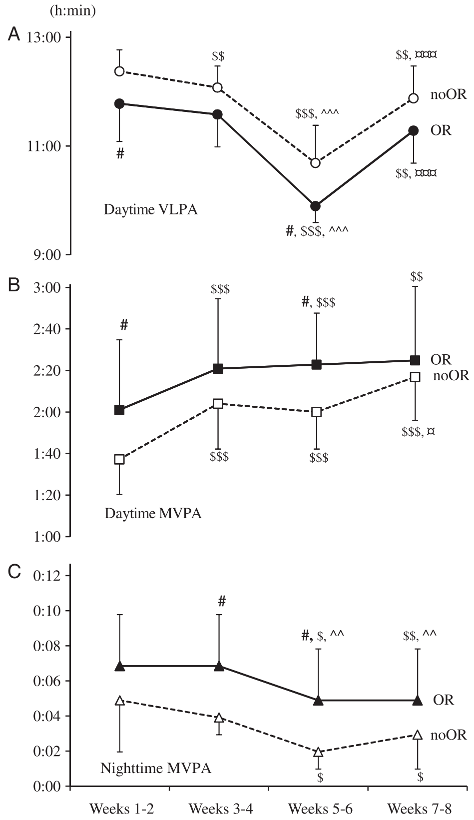

FIGURE 3—Mean ± SD daytime VLPA (A) and MVPA (B) and nighttime MVPA (C) during the BT period among non-OR and OR subjects. Difference compared with non-OR: #$P < 0.05$; weeks 1–2: $$$$P < 0.001$, $$$P < 0.01$, $$P < 0.05$; weeks 3–4: ∧∧∧$P < 0.001$, ∧∧$P < 0.01$, weeks 5–6: ¤¤¤$P < 0.001$; ¤$P < 0.05$.

The average training load during the 8-wk BT was 2 $h \cdot d^{-1}$ with only half an hour of total rest during the daytime. Otherwise, the day included low physical activity from sitting and standing to slow walking. This kind of high training load could be a risk factor for OR for subjects with a low fitness level. Surprisingly, OR and non-OR subjects did not differ from each other according to $\dot{V}O_{2max}$ or body composition at the beginning of BT. This suggests that fitness and fatness in this study are not strong underlying factors for overtraining. On the other hand, OR were more physically active both during the day- and nighttime than non-OR. These results support the hypothesis that the consequences of physical training are influenced not only by the intensity and duration of training but also by the duration of recovery time. Among the OR subjects, high physical activity during the day- and nighttime increased the accumulated training load at the end of BT, which may have contributed to the symptoms and indicators of OR. However, the OR and non-OR subjects did not differ regarding to tasks of the military service. Thus, higher physical activity might more likely be an individual property rather than an external factor.

Classification of OR/OTS is challenging, and it is rarely classified by a single criterion. In this study, OR subjects had to fulfill three of five criteria, including a decrease in aerobic physical performance. Absence from $\dot{V}O_{2max}$ tests or submaximal exercise was set as an additional criterion for a decreased performance. In 71% of the cases, the main reason for sick leave during BT was an upper respiratory track infection. Sick leave itself could affect training responses, even resulting in a detraining effect. However, OR and non-OR did not differ according to sick leave during the first 7 wk of BT. Thus, the decrease in performance probably was mainly caused by an excessive training load. Furthermore, we have recently reported that these OR subjects had higher basal sex-hormone–binding globulin than non-OR subjects at baseline and also after 4 and 7 wk of training (30). In addition, OR subjects had higher basal serum cortisol than non-OR subjects after 7 wk of training (30). Moreover, OR subjects had a decrease in the testosterone/cortisol ratio at rest from weeks 4 to 7 and in the maximal blood lactate/RPE ratio in the $\dot{V}O_{2max}$ test from baseline to the end of BT, whereas non-OR subjects did not (30).

In this study, day activity time increased and rest time decreased during the first 4 wk of BT, with a concomitant increase in aerobic performance, whereas BM remained the same. Among all 35 subjects, decreased oxidative stress at rest, evident as lower oxidized oxidation (GSSG), was observed after the first 4 wk of training. The decreased oxidative damage can be explained either by attenuated generation of ROS (caloric restriction) or by enhancement of tissue protection and antioxidant systems because of adaptation to regular exposure to a small amount of ROS (such as from exercise) (20). Aerobic training itself has been found to decrease oxidative stress at rest also without changes in BM (5). Thus, the decreased oxidative stress observed in this study may be a consequence of an enhanced antioxidant defense system in response to a tolerable training load (10,14), also among OR subjects. The high capacity to consume oxygen (enhanced $\dot{V}O_{2max}$) and, consequently, to produce a larger amount of ROS is accompanied by high erythrocyte glutathione peroxidase activity and high glutathione concentration, which both serve to protect the organism from lipid peroxidation and cell membrane damage (14).

In contrast to the decreased oxidative stress at rest, 4 wk of BT increased the susceptibility to acute exercise–induced oxidative stress evident as higher glutathione oxidation and lower antioxidant capacity after a submaximal exercise. At baseline, the subjects were not familiar with an acute physical load that may lead to increased oxidative stress. In contrast, we observed a higher exercise-induced oxidative stress at week 4 than at baseline in the trained subjects. In previous studies, both aerobic (8) and strength (17) training has been found to reduce postexercise oxidative stress at the same physical task. However, Vollaard et al. (35) found that neither tapering nor overload endurance training affected the exercise-induced increase in GSSG among endurance-trained athletes. Our results may indicate OR due to the intensive BT period. In the entire group of 35 subjects and in the OR subjects, a U-shaped curve of oxidative stress at rest was observed during BT; GSSG decreased at rest from baseline to week 4, increasing back to baseline values at week 7. Interestingly, submaximal exercise decreased ORAC at week 4. This might be explained by the increased consumption of the body's endogenous antioxidant resources. While coping with oxidative stress, plasma antioxidants are mobilized into the tissue (15).

During the second half of the 8-wk training period, although day activity time and rest time remained the same, GSSG and the GSSG/TGSH ratio at rest increased, and submaximal exercise resulted in a higher induction of GSSG/TGSH ratio. Also, stagnation in $\dot{V}O_{2max}$ was observed and all the subjects had a decrease in FM. All these findings indicate that that training load was too strenuous during the second half of BT causing oxidative stress (10,15). However, ORAC did not respond to submaximal exercise at the end of BT, as was the case at week 4. This could be a marker of OR because diminished antioxidant protection has been observed among overtrained athletes (28).

Plasma nitrotyrosine and protein carbonyls, markers of oxidative protein modification, remained the same during the entire 8-wk training period, both at rest and after exercise. There were also no differences between the OR and non-OR subjects. Plasma nitrotyrosine levels had a wide variation, which may partly explain the observed findings. The present results also support the previous findings that plasma nitrotyrosine level may not be a sufficiently sensitive marker to assess training or acute exercise–induced changes in oxidative stress (22,28). Proteins are an important target for oxidative challenge. ROS modify amino acid side chains of proteins to form protein carbonyls (1). Serum protein carbonyls have been found to increase after an intensive 12-wk resistance training period (15), to decrease after exhaustive marches (50 and 80 kg) in well-trained soldiers (4), and to increase after an exercise test to exhaustion in athletes (28). The turnover time for protein carbonyls varies from many hours to days (4). The 8-wk training period might have been too short, and submaximal exercise load in the present study may not be strenuous enough to modify proteins. Exercise may also have activated a mechanism that removes the oxidatively modified proteins from the circulation or, alternatively, activated antioxidant mechanisms that remove the ROS (4). In addition, differences among studies in oxidative stress responses to training and exercise may be explained by the differences in energy availability, training mode and length of the training period, fitness levels, and genetic background of the subjects. The subjects in the present study were low to moderately fit conscripts in the compulsory army, and training consisted of both strength and endurance training.

In conclusion, the present results suggest that increased oxidative stress may be associated with OR. Although the training had favorable effects on oxidative stress markers during the first 4 wk, sustained heavy training blunted this effect at the latter part of BT. At the end of the study, military training increased oxidative stress at rest back to pretraining levels and beyond the pretraining levels in response to submaximal exercise. Therefore, we may speculate that oxidative stress may also be a marker of insufficient recovery resulting in OR. Because one-third of the subjects were classified as overreached, the variability of the BT program has to be taken into careful consideration. Especially, the program should involve more recovery phases, both daily and periodically.

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III

AEROBIC FITNESS, ENERGY BALANCE, AND BODY MASS INDEX ARE ASSOCIATED WITH TRAINING LOAD ASSESSED BY ACTIVITY ENERGY EXPENDITURE

by

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Aerobic fitness, energy balance, and body mass index are associated with training load assessed by activity energy expenditure

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

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 the 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 [American College of Sports Medicine (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 × BMR), normal (1.8 × BMR), or active (2.0 × 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).

All conscripts train together according to the same program throughout BT. Thus, the physical activity is not optional and the PAL should be the same regardless of the subject's fitness status. From another perspective, individuals with low aerobic fitness will experience greater physiological stress relative to their maximum capacity at any given absolute level of aerobic work (Jones & Knapik, 1999). Thus, it could be hypothesized that the less fit conscripts will experience more physiological stress during physical activity because they have to work at higher relative intensities, and aerobic fitness is not necessarily related to AEE.

The doubly labeled water (DLW) method has been used as a gold standard for the measurement of total daily energy expenditure (EE) (Schoeller et al., 1986; Westerterp et al., 1995b). Several studies have used the DLW method in military operations, for example during rigorous field training exercise (DeLany et al., 1989; Forbes-Ewan et al., 1989; Mudambo et al., 1997; Castellani et al., 2006), in extremely cold environments (Hoyt et al., 1991; Jones et al., 1993), or in the garrison (Mudambo et al., 1997). In combination with the measurement of BMR, the DLW method allows the calculation of AEE and PAL over prolonged periods in military environments.

Energy intake (EI) has been found to influence EE and activity level (DeLany et al., 1989; Klein & Goran, 1993; Thompson et al., 1995). In addition, unfamiliar stressful situations have been found to affect EI (Popper et al., 1989). Most young men entering the military service will have their first experience of demanding physical training, eating outdoors, and performing overnight exercises in a forest. The common way to assess EI is a self-reported food diary. However, when DLW has been used to validate EI, a discrepancy between self-reported EI and measured EE has been observed, mainly due to under-reporting (Goris & Westerterp, 1999). Under-reporting can be divided into mis-recording and under-eating; mis-recording refers to a discrepancy between EI and measured EE with no change in body mass, and under-eating is a discrepancy accompanied by a decline in body mass over the food-recording interval (Goris & Westerterp, 1999), representing a negative energy balance. With a description of mis-recording, an estimate of true EI could be obtained.

No studies have examined the associations between soldiers' maximal aerobic fitness, energy balance, body size, and activity during the BT season in winter conditions. Thus, the purpose of this study was to determine the importance of VO_{2max}, energy balance, and body size on AEE related to body mass (AEE/kg) during the 2 hardest weeks of the wintertime BT season. In the present study, the activity level (AEE/kg) equated to the training load during the entire period of BT. An additional purpose was to study the accuracy of a self-reported pre-filled food diary in the military environment without food weighing, but with known food composition.

Methods

Subjects

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

Study protocol

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

Table 1. Physical characteristics of the subjects ($N = 24$)

	Mean ± SD	Range
Age (years)	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_{2max} (L/min)	3.6 ± 0.6	2.4–4.5
VO_{2max} (mL/kg/min)	47 ± 7	30–58

VO_{2max} refers to maximal oxygen uptake.

Table 2. The experimental protocol

	Days before DLW	Days during the DLW assessment period															
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BMR measure	− 28 ... − 1																
VO_{2max} 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				

BMR, basic metabolic rate; DLW, doubly labeled water.

a more detailed description of the other activities performed out of garrison.

VO_{2max}

To determine VO_{2max}, the conscripts performed a maximal treadmill test. The start of the test involved walking for 3 min at 4.6 km/h and walking/jogging at 6.3 km/h (1% slope) as a warm-up. Thereafter, exercise intensity was increased every 3 min to induce an increase of 6 mL/kg/min in the theoretical VO_{2max} demand of running (ACSM, 2001). This was achieved by increasing the initial running speed of 4.6 km/h by a mean of 1.2 km/h (range 0.6–1.4 km/h), and by increasing the initial grade of 1° by a mean grade of 0.5° (range 0.0–1.0°) up to the point of exhaustion (ACSM, 2001). Pulmonary ventilation and respiratory gas exchange data were measured on-line using the breath-by-breath method (Jaeger Oxygen Pro; Viasys Healthcare GmbH, Hoechberg, Germany), and mean values were calculated at 1-min intervals for statistical analysis. Heart rate was continuously recorded at 5-s intervals using a telemetric system (Polar810i; Polar Electro Oy, Kempele, Finland). Blood lactate was determined 1 min after completion of the exercise from a fingertip blood sample using a lactate analyzer (LactatePro®, Arkray, Japan). The criteria used for determining VO_{2max} were: VO_{2max} and heart rate did not increase despite an increase in the grade and/or the speed of the treadmill, a respiratory exchange ratio (RER) higher than 1.1, and a post-exercise blood lactate higher than 8 mmol/L (ACSM, 2001). All participants satisfied these criteria.

Anthropometry and body composition

Body mass was measured with an accuracy of 0.01 kg (Inbody720 body composition analyzer; Biospace Co. Ltd., Seoul, Korea) at the beginning (BM_{pre}), after 1 week (BM_{mid}), and at the end of the experimental period (BM_{post}). The measurements were performed between 6:00 and 7:00 a.m. after an overnight fast and after voiding, with no exercise for 12 h before the test. The physical activities in the daily program were planned to be light on the day preceding each measurement, and fluid status was estimated to be in balance based on the dietary records of the subjects. The subjects were barefoot and they wore T-shirts and trousers. The BM_{mean} was presented as an individual average of BM_{pre}, BM_{mid}, and BM_{post}, and the change in BM (ΔBM) was calculated as the difference between BM_{post} and BM_{pre}. Body height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as BM_{pre} divided by the body height squared. Fat-free mass (FFM) was calculated from total body water (TBW) as follows: FFM = TBW/0.732 (Pace & Rathburn, 1945). Fat mass (FM) was calculated as $BM_{pre} - FFM$, and percentage of body fat as $FM/BM_{pre} \times 100$. TBW was measured with deuterium dilution according to the Maastricht protocol (Westerterp et al., 1995b). TBW was calculated as the ^2H dilution space divided by 1.04, correcting for exchange of the ^2H label with non-aqueous H of body solids (Schoeller et al., 1980).

BMR

The BMR was measured once, for practical reasons, ~ 19 days before the DLW period. After an 11-h fast, the subject woke up at 5:30 a.m. for BMR measurements performed in a semi-recumbent position, lying for 30 min in a quiet room. Oxygen consumption and carbon dioxide measurements were made with the same gas analyzer (Jaeger Oxygen Pro) as that used in the VO_{2max} test. BMR was automatically calculated from the oxygen and carbon dioxide values as follows: $EE = 1.59 \times VCO_2 + 5.68 \times VO_2 - 2.17 \times$ urinary nitrogen (Weir, 1949), where urinary nitrogen was assumed to be 15 g/day. In the analysis, BMR was determined during the last 15 min of the 30-min measurement period.

EE, AEE, AEE/kg, and PAL

EE was measured with DLW according to the Maastricht Protocol. (Westerterp et al., 1995b). Briefly, at 10:00 p.m., before the measurements and after collecting a baseline urine sample, the subjects drank a weighed mixture of ^2H_2O (99.9 at%) and $H_2^{18}O$ (10 at%), resulting in an initial excess TBW enrichment of 150 p.p.m. for deuterium and 300 p.p.m. for oxygen-18. TBW was estimated from calculated body composition, based on height, weight, age, and gender, using the equation of Deurenberg et al. (1991), assuming a 73% hydration of FFM. Subjects consumed no foods or fluids for 10 h after dose administration, during the overnight equilibration of the isotopes with the body water. Subsequent urine samples were collected from the second and the third voiding on the morning of day 1, and from the first and the second voiding on the morning of days 8 and 15. Isotope quantities (deuterium and oxygen-18) in the urine were measured using an isotope ratio mass spectrometer (Optima, VG Isogas, Middlewich, UK), and CO_2 production was calculated from isotope ratios at the baseline, and days 1, 8, and 15, using the equations of Schoeller et al. (1986). CO_2 production was converted to daily metabolic rate using an energy equivalent based on the individual macronutrient composition of the diet (Black et al., 1986). AEE was calculated as EE × 0.9 − BMR, assuming diet-induced thermogenesis of 10% (Poehlman, 1989). To remove the confounding effect of body size (Ekelund et al., 2004), AEE was adjusted for BM_{mean} (AEE/kg). PAL was calculated as EE/BMR.

EI

For studying 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, were 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 conditions were added to the database.

Accuracy of the food diary and estimated true EI

Estimated true EI (TrueEI) was calculated using EI and changes in body energy stores (ΔES), which was calculated from changes in body mass, assuming that a body mass of 1 kg is equivalent to 30 MJ (75%FM, 25% FFM, with 73% water; Westerterp et al., 1995a). Under-reporting, mis-recording, under-eating (energy balance), and TrueEI were calculated as follows:

$$\text{Under-reporting}\,(\%) = (\text{EI-EE})/\text{EE} \times 100$$

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

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

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

Statistical analysis

Changes in BM were tested by a paired samples *t*-test. FM was log transformed to reduce skewness. To remove the potential effect of body size on VO_{2max}, FFM-adjusted values for VO_{2max} ($VO_{2maxFFM}$) 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/kg, PAL, BMR, and $VO_{2maxFFM}$ (L/min), energy balance, and body composition (BMpre, FM, FFM, and BMI). To examine the effects of BMI, fitness, and under-eating on AEE/kg, the subjects were divided into tertiles with equal numbers of subjects according to their BMI ($<22.5\,kg/m^2$ for LowBMI; 22.6–24.8 kg/m^2 for ModerateBMI; and $>24.9\,kg/m^2$ for HighBMI), $VO_{2maxFFM}$ ($<3.44\,L/min^2$ for LowFIT; 3.45–3.68 L/min^2 for ModerateFIT; and $>3.69\,L/min^2$ for FIT), and energy balance (>0.76 MJ/day for Low; -2.16–0.75 MJ/day for Moderate; and <-2.17 MJ/day for High under-eating). One-way ANOVA with the LSD *post hoc* test was used to compare the differences in AEE/kg, energy balance, and fitness or BMI between the category groups. For further evaluation of the interaction and to identify the predictors of AEE/kg from the independent variables, multivariable linear regression analysis was used. The independent variables were $VO_{2maxFFM}$ (L/min), 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 using SPSS (14.0.1. 2005; SPSS Inc., Chicago, Illinois, USA). The level of statistical significance was set at $P<0.05$.

Results

BMR (7.66 ± 0.92 MJ/day) represented 50 ± 5% and AEE (6.26 ± 1.21 MJ/day) 40 ± 5% of the total EE (Table 3). The reported EI of 11.5 ± 3.2 MJ/day was significantly lower than the measured EE (15.5 ± 1.6 MJ/day), and thus the results revealed significant under-reporting ($-26.0 \pm 17.5\%$, $P<0.001$). The data of reported intake indicated that daily carbohydrate consumption in relation to the total reported EI was 58 ± 4% (5.2 ± 1.6 g/kg), fat 27 ± 4% (1.1 ± 0.4 g/kg), protein 14 ± 2% (1.3 ± 0.4 g/kg), and alcohol 0.3 ± 0.9% (0.01 ± 0.04 g/kg). When the reported EI was corrected for the mis-recording

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

	Mean ± SD	Range
EE (MJ/day)	15.48 ± 1.65	12.75–18.51
AEE (MJ/day)	6.26 ± 1.21	4.18–8.94
AEE/kg (MJ/day/kg)	0.08 ± 0.02	0.04–0.13
PAL*	2.03 ± 0.21	1.68–2.47
BMR (MJ/day)	7.66 ± 0.92	5.50–9.44
EI (MJ/day)***	11.52 ± 3.23***	6.73–17.77
Estimated true EI (MJ/day)	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

*Calculated as EE/BMR.

***Difference with EE ($P<0.001$).

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

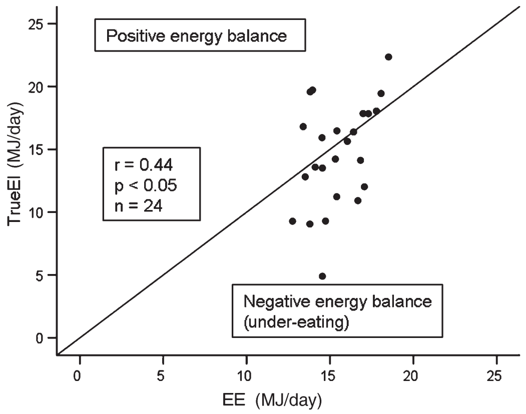

Fig. 1. Relationship between estimated true energy intake (TrueEI) and energy expenditure (EE). The line represents the energy balance.

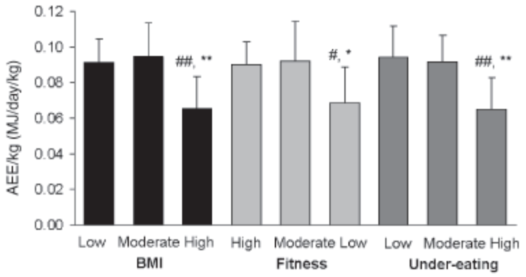

Fig. 2. AEE/kg in Low, Moderate, and High BMI (a), fitness (b), and under-eating (c) groups. Significant difference as compared with: Low BMI and under-eating $^{\#\#}P<0.01$, Moderate BMI and under-eating $^{**}P<0.01$, High fitness $^{\#}P<0.05$, Moderate fitness $^{*}P<0.05$. BMI, body mass index; AEE, activity energy expenditure.

(−20.4 ± 26.3%), TrueEI did not differ from EE (−5.6 ± 25.5%, NS), which indicates a non-significant negative energy balance (under-eating) of the present subjects. However, Fig. 1 indicates that few subjects had a negative energy balance, which was negatively related to BM_{pre} ($r = -0.73$, $P<0.001$), FM ($r = -0.83$, $P<0.001$), and BMI ($r = -0.80$, $P<0.001$).

The subjects in the HighBMI group had lower AEE/kg ($P<0.01$) compared with the subjects in the LowBMI and ModerateBMI groups (Fig. 2a). The HighBMI subjects also had a significantly higher negative energy balance (−4.2 ± 3.08 MJ/day) compared with those subjects in the LowBMI (1.6 ± 3.23 MJ/day, $P<0.001$) and the ModerateBMI (0.11 ± 2.30 MJ/day, $P<0.01$) groups. The LowBMI and ModerateBMI groups did not differ according to AEE/kg, and both energy balance and $VO_{2maxFFM}$ did not differ between the three BMI groups either. AEE/kg was also lower in LowFIT compared with ModerateFIT and FIT ($P<0.05$) (Fig. 2b), but BMI and energy balance did not differ between these three fitness groups. Furthermore, AEE/kg was lower among subjects in the High under-eating group compared with the Low and Moderate under-eating groups ($P<0.01$) (Fig. 2c). These subjects also had higher BMI (27.8 ± 3.6 kg/m^2) compared with subjects in the Low (21.4 ± 2.4 kg/m^2, $P<0.001$) and Moderate (23.6 ± 1.9 kg/m^2, $P<0.01$) under-eating groups and lower $VO_{2maxFFM}$ (3.43 ± 0.27 L/min^2) compared with the Moderate under-eating group (3.75 ±0.28 L/min^2, $P<0.05$). The Moderate and Low under-eating groups did not differ in AEE/kg, BMI, or $VO_{2maxFFM}$.

$VO_{2maxFFM}$ was positively related to AEE and AEE/kg ($r = 0.44$, $P<0.05$ and $r = 0.41$, $P<0.05$, respectively), but it had no relationship with EE (Table 4). BMI was negatively related to AEE/kg ($r = -0.60$, $P<0.01$) 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 ± 1.8 kg, NS) during the 2-week training period, energy balance was related to AEE/kg ($r = 0.58$, $P<0.01$) (Table 4). Table 5 shows the multivariate regression equations for explaining variation in AEE/kg. The model included energy balance, $VO_{2maxFFM}$, and BMI. Only BMI remained in the model by explaining 33% of the variation in AEE/kg, with a standard error of estimate (SEE) of 0.02 MJ/day/kg or 25% of the mean AEE/kg.

Table 4. Pearson correlation coefficients between BMI, VO_{2max}, and energy balance and EE, AEE, AEE/kg, and BMR

	BMI (kg/m^2)	VO_{2max} (L/min)	$VO_{2maxFFM}$ (L/min)*	Energy balance (MJ/day)
EE (MJ/day)	0.30	0.77***	0.29	0.29
AEE (MJ/day)	−0.70	0.44*	0.44*	0.21
AEE/kg (MJ/day/kg)	−0.60**	−0.16	0.41*	0.58**
BMR	0.58**	0.66***	−0.10	−0.27

$P<0.05$. $^{**}P<0.01$. $^{***}P<0.001$.

*VO_{2max} adjusted by FFM using a regression-based approach (Toth et al., 1993).

EE, energy expenditure, AEE, activity energy expenditure; AEE/kg, activity energy expenditure related to body mass; BMR, basal metabolic rate; FFM, fat-free mass.

Table 5. Multivariate regression model for explaining the variation in AEE/kg (MJ/day/kg)

Prediction equation	Adjusted-R^2	SEE	P
AEE/kg = 0.165 − (BMI × 0.003)	0.33	0.02	0.002

AEE/kg, activity energy expenditure related to body mass.

Discussion

To our knowledge, there are no previous reports showing a relationship between VO_{2max} and activity (AEE/kg) 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 AEE/kg. 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 greater energy cost of cycling at the same relative intensity than untrained, less fit controls. This was due to the lower required workload of the less fit subjects to increase their heart rate to 75% of maximum, and thus their EE was also low (Horton et al., 1994). In contrast, during high-intensity activities, the low absolute work capacity of the least-fit subjects might limit their work ability.

The strength of the present study was the use of a regression-based approach to remove the confounding influences of BM or FFM, rather than a standard ratio approach to express VO_{2max} values relative to BM (mL/kg/min). 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 VO_{2max} with BM was significantly different from 0 ($P<0.01$), but with FFM it was not. The best predictor of VO_{2max} (L/min) was FFM, with a variation of 75% ($r=0.87$, $P<0.001$) compared with BM with the respective variation of 35% ($r=0.60$, $P<0.01$). We therefore adjusted VO_{2max} for FFM. Very few studies have used DLW to estimate the relationship between AEE or EE and VO_{2max} (Ambler et al., 1998; Brochu et al., 1999). Our results are well in line with the study of Brochu et al. (1999), where they found a significant relationship between VO_{2max} and AEE in older individuals. The total EE in this study was similar (15.5 MJ/day) to that measured on a hilly terrain with temperatures ranging from $-1.1\,°C$ to $16.1\,°C$ (14.24 MJ/day) (DeLany et al., 1989) and to a group of soldiers working in the garrison (Mudambo et al., 1997). However, the present EE was lower than that measured in jungle warfare (Forbes-Ewan et al., 1989), in a winter military training course (Hoyt et al., 1991; Jones et al., 1993), and in a commando field exercise in a hot and dry climate (Mudambo et al., 1997). The mean ($\pm$ SD) total EE of all male military personnel ($N=424$) is reportedly 19.3 ± 2.7 MJ/day across all activities, military occupational specialties, and environments (Tharion et al., 2005). Comparisons in EE between the present study and other studies should be made with caution. For example, there are possible differences in the body mass of the subjects and in the combat gear that was carried, both of which affect the total EE.

In the multivariate regression analysis, only BMI remained in the model, although the correlation and one-way analysis revealed that positive energy balance and good fitness level were also related to higher activity (AEE/kg). In other words, the slimmer the subject, the smaller the loss of BM. This also suggests that more active subjects exhibit greater levels of aerobic fitness. This was partly explained by the fact that under-eating (negative energy balance) was negatively related to BMI. Thus, especially the subjects with a higher BMI were more prone to lose body mass than their slimmer counterparts. Even though negative energy balance was not significant (-5.6%) at the group level, the individual variation in energy balance ranged from -66.3% to 41.7%. Based on Fig. 1, one-third of the subjects had a negative energy balance. However, in the present subjects, of whom one-third ($n=8$) had a BMI over 25, the acceptable tolerance limit for serious under-eating classification is critical. The positive relationships observed between TrueEI and EE (Fig. 1), and between energy balance and AEE/kg are well in line with other studies, where a low EI in males was found to be related to low EE (DeLany et al., 1989; Thompson et al., 1995), as well as to spontaneous physical activity (Thompson et al., 1995) when compared with an adequate EI group. Moreover, it has been shown that EE and AEE increase simultaneously with an increase in EI, which might be partly a reflection of EI to BMR (Klein & Goran, 1993). However, Castellani et al. (2006) presented a contrary conclusion. They found that during a short period (54 h) of physical loading, recruits were able to maintain high EE and PAL despite sustaining substantial energy deficits.

The PAL value of 2.0 obtained during the present wintertime BT is comparable with values from people performing very strenuous work or daily competitive athletic training (Nordic Nutrition Recommendations, 2004). In addition, some subjects also ate less than required. If these stressors, together with other stressors, affect soldiers for a longer period, overtraining symptoms may occur (Meeusen et al., 2006). If the BMI is used as an index of body size, it was not associated with AEE, but was negatively related to AEE/kg and positively to BMR (Table 4). Our findings further showed that BMR did not have any relationship with VO_{2max}, but it was negatively related to AEE/kg. Among children it has been observed that AEE and EE tend to increase more with increasing body size than BMR (Ekelund et al., 2002). However, this was not seen in the present study, in which most military tasks were performed at low-to-moderate intensity levels. This meant that the least-fit subjects were able to perform those tasks well.

An additional purpose of the present study was to investigate the accuracy of a pre-filled food diary. Great care was taken to evaluate EI as correctly as possible without food weighing. In spite of the careful guidance and control, mis-recording was as high as −20% and EI was only 74% of EE. It has been found that subjects who accurately reported their food intake at the first attempt were also able to do so at the second attempt (Goris et al., 2001). On the other hand, it is known that accuracy will decrease if the minimum recording time of 7 days is extended. This happens due to a decline in motivation in keeping the food diary (Goris et al., 2001). The calculation of under-eating, mis-recording, and an estimate of true EI were all based on changes in body ES, in which the energy content of 30 MJ corresponds to 1 kg of body mass. However, the mass ratio of 30 MJ/1 kg for storage or mobilization of energy between FM and FFM might differ individually, affecting the present results. We could have measured changes in body ES based on changes in FM and FFM, but 2 weeks may be too short a period to measure these parameters with sufficient accuracy. A more accurate method to estimate mis-recording and to calculate true EI involves a measure of water balance (Goris & Westerterp, 1999), but this method still requires the recoding of food and fluid intake, which are both prone to mis-recording (Goris & Westerterp, 1999; Goris et al., 2001; Westerterp & Goris, 2002; Tharion et al., 2005).

Perspectives

The fitness level of Finnish young men has decreased with a concurrent increase in body mass (Santtila et al., 2006). Consequently, some conscripts entering compulsory military service have a low fitness level. However, the same program has to be performed by all conscripts in the military environment. The goal of physical training during the basic training season (BT) is to increase soldiers' aerobic fitness and muscle strength to prepare them for demanding military tasks. This leads to the question of whether fitness or body size affects the ability to train. In this study, the training load was assessed by relating activity EE to body mass (AEE/kg). The subjects with a low VO_{2max}, a high BMI, and a negative energy balance were vulnerable to low AEE/kg, but out of these three factors only BMI remained in the multivariate regression model. This indicates that overweight may limit the most the high-intensity physical training. In order to enhance physical fitness in a group of conscripts who exhibit variable fitness levels, these results suggest that conscripts could be divided into training groups simply based on BMI, rather than aerobic fitness level or a combination of these parameters.

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 as well as by energy balance. Initially, BMI has to be taken into consideration. Thereafter, energy balance should be optimized to facilitate physical performance, and to allow a high training load to be sustained during the BT season. This situation is comparable with that of athletes: without adequate EI, high-level training is not possible. Thus, because energy balance influenced AEE/kg, conscripts should be advised about adequate EI, especially during the most strenuous training period.

Key words: AEE, PAL, energy intake, under-reporting, under-eating, military training.

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IV

EFFECTS OF EASY-TO-USE PROTEIN-RICH ENERGY BAR ON ENERGY BALANCE, PHYSICAL ACTIVITY AND PERFORMANCE DURING 8 DAYS OF SUSTAINED PHYSICAL EXERTION

by

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Effects of Easy-to-Use Protein-Rich Energy Bar on Energy Balance, Physical Activity and Performance during 8 Days of Sustained Physical Exertion

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Abstract

Background: Previous military studies have shown an energy deficit during a strenuous field training course (TC). This study aimed to determine the effects of energy bar supplementation on energy balance, physical activity (PA), physical performance and well-being and to evaluate ad libitum fluid intake during wintertime 8-day strenuous TC.

Methods: Twenty-six men (age 20±1 yr.) were randomly divided into two groups: The control group (n = 12) had traditional field rations and the experimental (Ebar) group (n = 14) field rations plus energy bars of 4.1 MJ•day^{-1}. Energy (EI) and water intake was recorded. Fat-free mass and water loss were measured with deuterium dilution and elimination, respectively. The energy expenditure was calculated using the intake/balance method and energy availability as (EI/estimated basal metabolic rate). PA was monitored using an accelerometer. Physical performance was measured and questionnaires of upper respiratory tract infections (URTI), hunger and mood state were recorded before, during and after TC.

Results: Ebar had a higher EI and energy availability than the controls. However, decreases in body mass and fat mass were similar in both groups representing an energy deficit. No differences were observed between the groups in PA, water balance, URTI symptoms and changes in physical performance and fat-free mass. Ebar felt less hunger after TC than the controls and they had improved positive mood state during the latter part of TC while controls did not. Water deficit associated to higher PA. Furthermore, URTI symptoms and negative mood state associated negatively with energy availability and PA.

Conclusion: An easy-to-use protein-rich energy bars did not prevent energy deficit nor influence PA during an 8-day TC. The high content of protein in the bars might have induced satiation decreasing energy intake from field rations. PA and energy intake seems to be primarily affected by other factors than energy supplementation such as mood state.

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Competing Interests: One of the authors (Hannu Kinnunen) works in the research department of Polar Electro Oy and for this article he has worked as a scientist concerning accelerometer data. Polar Electro Oy and its subsidiaries develop, manufacture and sell heart rate and activity products. Polar Electro Oy partly funded this study, and the following Polar Elector Oy products were used: the Polar AW200 Activity Monitor and RS 800, Polar Electro Oy. There are no further patents, products in development or marketed products to declare. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

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Introduction

Ready-to-eat meal rations are commonly used during the military field training [1]. However, previous military studies have shown an energy deficit during a strenuous field training course [2,3], which induces loss of body mass and fat-free mass. In many military studies energy restriction is caused by a purpose as an additional stressor [3–6], but also underconsumption is a common problem [1,7,8]. Even though unfamiliar stressful situations might decrease energy intake [9], environmental factors has an important role in determining food intake and choice [10] and food acceptability has been found to have an impact on food intake in the field [10]. Moreover, multiple stressors of sleep and caloric deprivation with physical and psychological stress have deteriorated effect on mood state [5].

Energy intake (EI) itself has been found to have an influence on voluntary physical activity, increasing with high-energy intake [11,12]. Furthermore, energy balance is an important factor in sustaining training load and maintaining high performance during strenuous military training [13–15]. Demanding training is risk factor for upper respiratory tract infection (URTI) among athletes [16] and soldiers [17], and energy deficit has been hypothesized to be one reason for URTI [18], however, scientific evidence is lacking. In a study by Costa et al. [19] consumption of a high carbohydrate diet throughout a strenuous training period had a favorable effect on markers of immune activity and, thereby, reduced susceptibility to URTI. Similarly in another study by Flakoll et al. [20], post exercise protein supplementation resulted in reduced bacterial/viral infections during military basic training period. In both of these studies, the supplementation group also received higher energy intake. Therefore, it cannot be concluded whether this phenomenon occurs due to increased protein or carbohydrate intake or just a result of increased energy intake. On the other hand, high-protein diets have been found to attenuate a decrease in fat-free mass (FFM) during negative energy balance induced by a decrease in energy intake among obese individuals [21] and also among athletes [22]. However, high-protein intake has not been found to attenuate a decrease in FFM when energy deficit is induced by high energy expenditure [23].

There are few published energy supplements studies during military field training in purpose to assure energy balance [12,14,15,24–26], however, none of this have used protein-rich supplement. Thus the aim of this study was to determine the effects of an additional easy-to-use protein-rich energy bar supplement of 4.1 $MJ\cdot d^{-1}$ on energy balance, physical activity, body composition, physical performance and subjective experiences of hunger, URTI, mood state and overall well-being during the 8-day military training course (TC) in a cold environment. In the present study, the aim was not to cause energy restriction and neither force the conscripts to eat the all provided food. Instead of that the aim was to observe the effect of military training in terms of self-selected diet. An additional purpose was to define whether an ad libitum fluid intake is sufficient during winter training. We hypothesized that energy supplement of 4.1 $MJ\cdot d^{-1}$ may increase the total energy intake, improve ability to maintain physical training load and sustain FFM during TC. Furthermore, we hypothesized that an energy supplement will sustain physical performance, enhance mood state and overall well-being and decrease symptoms of URTI.

Methods

Ethics Statement

All participants were fully informed of the experimental protocol and gave their written consent to participate in the study. They were also advised of their right to withdraw from the investigation at any time. The study protocol was approved by the Finnish Defence Forces, and the Ethical Committees of the University of Jyväskylä and the Kainuu region of Finland.

Participants. Twenty-six men (aged 20±1 yr.) volunteered to participate in the present study during their 8-day field training course (TC). The participants were randomly divided into two groups: the control group (n = 12) were allowed to eat only traditional field rations and the experimental (Ebar) group (n = 14) field rations plus energy bars of 4.1 $MJ\bullet day^{-1}$. The two groups did not differ according to the baseline characteristics (Table 1).

Experimental Design

The study took place during the winter in Finland, when daily outdoor temperatures ranged from −16°C to −1°C, with an average of −4°C (data from the local weather station). During the study, the participants completed 8-days field training, which consisted of a variety of military-relevant tasks and skills training, sustained aerobic exercise from skiing and patrolling activities. During TC, participants carried combat gear weighing 60–70 kg including their clothes and food rations. The participants were randomly divided into two groups: a control group (N = 12) and an energy supplement group (Ebar, N = 14) of which the latter received an additional energy supplement of 4.1 $MJ\bullet day^{-1}$ during the 8-day TC. Water intake was ad-libitum. However, participants were not permitted to use any other extra nutritional supplements throughout the study. Physical performance was studied before [six days before (-6d PRE); one day before (PRE)], during (MID) and after [one day after, POST; three days after (+3d POST)] TC. The measurements included a 3 km combat loaded field running, vertical jump, anaerobic power and handgrip strength. The participants have already done intensive military training for six months and were familiar with the stressors during TC. In addition, they were familiarized with the performance tests, and the jump and hand-grip strength tests were performed twice before (-6 PRE and PRE) the training course for minimizing the changes due to learning. In addition, mood state, body composition and physical activity were studied together with energy and water intake recordings. The experimental protocol is presented in Table 2.

Table 1. Physical characteristics of the control and Ebar groups before the training course.

	Control (N = 12)	Ebar (N = 14)
Age (years)	20.2±1.6	19.6±0.5
Height (cm)	177.8±6.6	178.9±7.9
Body mass (kg)	71.3±7.4	71.2±7.4
Fat mass (kg)	12.1±3.8	10.4±1.9
Fat-free mass (kg)	70.5±7.4	70.5±7.3
Fat%	16.8±4.5	14.6±2.4
BMI ($kg\cdot m^{-2}$)	22.6±2.1	22.2±1.1
TBW (L)	43.4±4.5	44.6±4.7
12-minute running test (m)	2814±208	2930±178
VO_2max ($mL\cdot kg^{-1}\cdot min^{-1}$)[aa]	52±5	54±1

Values are mean ± SD.

[aa]Estimated from 12-minute running test: VO_2max ($ml\cdot kg^{-1}\cdot min^{-1}$) = running distance (m) - 504.9/44.7 [57].

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Anthropometry and Body Composition

Body mass (BM) was measured with an accuracy of 0.01 kg (Inbody720 body composition analyzer, Biospace Co. Ltd, Seoul, Korea). The measurements were performed between 6:00 and 7:00 a.m. after an overnight fast and after voiding with no exercise for 12 hours prior to the test commencing, except the measurements in MID of TC. The participants were barefoot and they wore T-shirts and shorts. The BMmean (an individual average of PRE, MID and POST) was used for calculation energy intake variables per BM. Body height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Fat-free mass (FFM) was calculated from total body water (TBW) as follows: FFM = TBW/0.732 [27]. Fat mass (FM) was calculated as BM – FFM, and percentage of body fat as FM/BM x 100. TBW was measured with deuterium dilution according to the Maastricht protocol [28]. Briefly, at 10:00 p.m., before the measurements and after collecting a baseline urine sample, the participants drank a weighed mixture of ^2H_2O. Participants consumed no foods or fluids for 10 hours after dose administration, during the overnight equilibration of the isotope with the body water. Subsequent urine samples were collected from the second and third voiding in the morning of day 1. On study days 9 and 10 the process was

Table 2. The experimental protocol.

			Intensive training course									
Day	**-6 PRE**	**PRE**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**POST**	**+3 POST**
Test												
Body mass	X	X					X				X	X
Fat-free mass		X										
Fat mass		X									X	
Questionnaires	X	X					X				X	X
Vertical jump	X	X					X				X	
Anaerobic power												
Hand-grip test												
	X	X					X				X	
	X	X					X				X	
3 km running test		X									X	
Physical activity			X	X	X	X	X	X	X	X		
Energy and water intake			X	X	X	X	X	X	X	X		
Energy bar supplement			X	X	X	X	X	X	X	X		
Deuterium dose		X								X		
Urine samples		X	X							X	X	

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repeated. TBW was calculated as the ^{2}H dilution space divided by 1.04, correcting for exchange of the ^{2}H label with nonaqueous H^{+} of body solids [29].

Energy and Water Intake and Water Loss

The average daily energy from a field ration was 17.9 MJ·d^{-1} with the average macronutrient composition in energy of 14 E% protein, 58 E% carbohydrate and 28 E% fat. The participants in the Ebar group were advised to eat five commercial energy bars per day totaling 4.1 MJ·d^{-1} extra energy. The energy bars weighed 55 g/bar with the average macronutrient composition in energy of 32 E% protein, 46 E% carbohydrate and 22 E% fat. The total provided energy for the Ebar group was 22.0 MJ·d^{-1} (17 E% protein, 56 E% carbohydrate and 27 E% fat). For studying energy intake (EI) and water intake, the participants kept daily pre-filled ration diaries. The pre-filled food diary included information about the ration served, and thus the participants were only required to record the amount of food and fluid that they consumed. The participants had completed these diaries before the study to ensure that they were familiar with reporting food and water intake. In addition, they were advised to be as accurate as possible in recording the amount food and fluid consumed. Furthermore, all the food wraps and rations that were not consumed were collected. Daily nutritional consumption was quantified from manufacturer-supplied ingredient labels and by the computer programme Nutrica®software (version 3.11, Finland) using the information from ration diaries, wraps and returned rations.

Total water intake was calculated from reported food and water intakes and metabolic water as follows: Total water intake = fluid intake + water content of food + metabolic water. The amount of metabolic water was estimated from protein, fat and carbohydrate intake and from the change in BM. Oxidation water is 0.41 mL•g^{-1} for protein, 1.07 mL•g^{-1} for fat and 0.6 mL•g^{-1} for carbohydrate [30]. A change in BM of 1 kg was assumed to be a change of 0.75 kg FM and 0.25 kg FFM. FM was assumed to be pure fat and FFM 73% water and 27% protein [31].

Water loss over TC was measured using the deuterium elimination method [30]. Deuterium elimination was calculated from two urine samples after dosing (at day 1 in the morning) and two samples at the end of the training period (day 8 in the evening). Water balance was calculated as follows: Water balance = total water intake – water loss.

Energy Expenditure, Energy Balance and Energy Availability

Total energy expenditure (EE) was calculated by a method of intake/balance using EI and changes in body energy stores (ΔES); EE = EI – ΔES [8]. ΔES were calculated from changes in FFM and FM between PRE and POST values. Energy equivalents used for protein and fat were 18.4 and 39.8 kJ·g^{-1}, respectively [31].

Energy balance was calculated as follows: Energy balance = EI – EE. To differentiate the need of energy intake for subjects with a different body mass, energy availability was calculated as follows: EI/BMR, where BMR = basal metabolic rate estimated by the equation of Schofield et al. [32]. Energy availability describes the fraction of energy that is left for physical activity, while BMR has been taken into account.

Physical Activity

Physical activity (PA) was measured using a customized version of the Polar AW200 Activity monitor that was worn on the non-dominant wrist. AW200 has been found useful and accurate for the measurement of EE during long term exercise [33]. AW200 contains a uniaxial accelerometer, of which the signal is bandpass filtered (0.3–3.0 Hz) reducing sensitivity to repeated low-intensity hand movements. The device counts hand movements if their acceleration exceeds 1.0 m·s^{-2} [34]. Epoch length was set at one minute and a curvilinear equation was used to transform activity counts to metabolic equivalents (1–16 MET), which were further adjusted by body height. Among the participants in the military environment, weekly energy expenditure estimated with AW200 correlated well (r = 0.78) to that obtained with doubly labeled water (unpublished data, see [13]. Periods that contained no single movement during 30 min were classified as non-wear time and excluded from the recordings. PA by each minute was classified into either (1) rest ≤1.0 MET, (2) sitting = 1–2 MET, (3) standing = 2–3.5 MET, moderate activity = 3.5–6 MET, (4) vigorous activity ≥6 MET or (5) active time ≥3.5 MET (= moderate + vigorous activity). REST (i.e. sleep) was selected, if accelerometer showed no hand movements within more than 50% of a 10- min moving window. Participants were only included for analysis, if their activity recordings covered more than 90% of the entire recording time. The activity devices were collected on day 5 for data download, and redistributed within 5:30 h:min. Data was pooled into three periods: days of 1–5, days of 5–8 and total time of the TC days of 1–8. Data recordings lasted from 9:00 a.m. on day 1 to 20:00 p.m. on day 8.

Physical Performance

The vertical jump test, anaerobic power test and handgrip strength were performed -6d PRE, PRE, MID and POST the TC. Prior to these tests each participant was required to complete a standardized warm-up of 5-min. After warm-up, the participant performed either hand-grip or jump test with 5 min rest between the tests. The follow-up tests for each participant were always performed in the same order and at the same time of day. A standardized warm-up of 1 km walking for 3-km running test started immediately after completion the hand-grip and jump tests in PRE and POST having consumed a similar diet before each testing session.

Leg explosive power was assessed with a static vertical jump test using a contact mat (Newtest Powertimer, Newtest Oy, Oulu, Finland). Jump test has been found a sensitive indicator for detecting decrements in physical performance after short [35] and long period of military field training [3]. The participant stood on the contact mat with hands on the hips and the knees flexed at an angle of 90 degrees. Instructions were given for jumps to be performed vertically with the maximal effort, and to land on the mat with knees straight. Hands were kept on the hips during the jump. The participants performed three static jumps with 5-s rest between each jump. The mean flight time of the two best jumps were recorded and the rising height of the center of gravity was assessed. After two min rest, anaerobic power was assessed during a 15-s all-out counter movement jump test on the contact mat. Power was calculated as follows:

Power ($W \cdot kg^{-1}$) = $((g^2)\ x\ (T_f\ x\ 15))/(4n\ x\ ((15- T_f))$, where g = acceleration of the gravity (9.81 $m \cdot s^{-2}$), T_f = total flight time, n = number of the jumps performed in 15 s [36].

The maximum isometric strength of the hand and forearm muscles was assessed with the static *hand-grip test* using a dynamometer (SAEHAN, Saehan Corp., Masa, Korea). Three maximum isometric efforts with one minute rest was performed and maintained for about 5 s with the right and left hand with shoulder adducted and neutrally rotated, elbow flexed to 90 degrees and forearm in a neutral position. No other body movement was allowed. The handle of the dynamometer was individually adjusted for each participant. The participants were strongly encouraged to perform with maximal effort. The hand-grip results are presented as the mean of the right and left hand maximum.

The 3-km running test with maximal effort was performed on an outdoor track PRE and POST TC while carrying a 20 kg backpack. All participants were instructed to complete the test in the shortest possible time. Heart rate (HR) was recorded continuously using hear rate monitors (RS 800, Polar Electro Oy, Kempele, Finland). Blood lactate was determined before and 1 minute after completion of the exercise from a fingertip blood sample using a lactate analyzer (LactatePro®, Arkray, Japan).

Questionnaires

The participants rated how they experienced their feelings utilizing five different aspects: 1) hunger on an eight-point Likert scale, 2) physical performance on a seven-point Likert scale and 3) positive and negative mood states on a four-point Likert scale, which included six positive and nine negative items, and the means of both items were calculated [37]. 4) Somatic symptoms were analyzed on a five-point Likert scale; 1 = not at all, 2 = one day, 3 = 2–3 days, 4 = 4–5 days, 5 = 6–7 days. The symptoms were subjective ratings of well-being; digestive disorders and reduced appetite, musculo-skeletal, physical complaints and sleep disturbances [38]. The degree of symptoms was determined as the sum of their scores. 5) URTI symptoms were evaluated by the Wisconsin Upper Respiratory Symptom Survey (WURSS-21) [39].

Statistical Analyses

All data is presented as mean ± SD. The level of statistical significance was set at $p<0.05$. If assumptions for normality were not met, data were log-transformed before statistical analysis. The untransformed values are shown in the text, tables and figures for giving more meaningful values. Responses to TC, and differences between and within the controls and Ebar participants, were assessed using the mixed-design factorial ANOVA [group (control vs. Ebar) x TC (PRE, MID, POST). Bonferroni as the *Post hoc* analysis was used to identify any significant differences. In addition, the effect of group and TC interaction was calculated, and 95% confidence intervals (CI) were determined. If 95% confidence interval included zero, it was concluded that there was no significant effect. Pearson product–moment correlations were used to observe associations between variables. All statistical analyses were performed with PASW Statistics software (Version 18.0.0. 2009; SPSS Inc., Chicago, IL).

Results

Energy Intake and Energy Availability

The Ebar group consumed 58±15% and the controls 57±10% of the provided energy during TC. The estimated energy intake from the field ration did not differ between the groups (Ebar 10.0±1.0 $MJ \cdot d^{-1}$, $P = 0.23$ vs. control 10.9±1.7 $MJ \cdot d^{-1}$). However, the estimated total energy intake and energy availability were higher ($P = 0.038$ and $P = 0.028$, respectively) in Ebar compared to the controls (Table 3). Intake of protein was also higher among Ebar ($P<0.001$) than in the controls (Table 3). Energy balance was negative among all participants and energy balance (Ebar −10.1±3.7 $MJ \cdot d^{-1}$, −43± −12%, $P = 0.056$; control −9.2±6.4 $MJ \cdot d^{-1}$, −40± −25%) and estimated energy expenditure (Ebar 23.3±3.2 $MJ \cdot d^{-1}$, $P = 0.060$; control 20.2±5.7 $MJ \cdot d^{-1}$) did not differ between the groups. BM and FM decreased in both groups (−2.1% and −16.5%, respectively, $P<0.001$) during TC while FFM (Figure 1) and TBW remained the same (Ebar 44.9±4.7 L; control 43.4±4.7 L). The estimated fluid intake (control 2.3±0.5 $L \cdot d^{-1}$; Ebar 2.1±0.6 Ld^{-1}) and total water intake were similar in both groups, and there was no significant water deficit (Table 3).

Physical Activity

Physical activity did not differ between the groups during TC. The mean rest during TC was 4:05±0:32 h:min per day (range from 3:00 to 6:48 h:min), sitting 5:05±0:46 h:min (3:32–

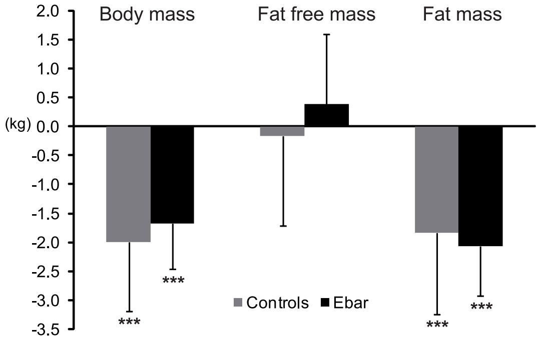

Figure 1. Changes in body composition during the 8-day training course in the control and Ebar groups. *** P<0.001, significant change during the training course.
doi:10.1371/journal.pone.0047771.g001

6:53 h:min), standing 9:10±0:44 h:min (7:54–10:31 h:min), moderate activity 5:32±1:09 h:min (3:46–7:34 h:min), vigorous activity 0:06±0:03 h:min (0:00–0:14 h:min) and active time 5:38±1:11 h:min (3:50–7:45 h:min) per day. However, a main effect of TC ($P<0.001$) was observed for rest, sitting, and moderate activity as well as for standing and active time ($P = 0.027$). Rest and sitting increased during days 5–8 compared to the first days 1–5. On the contrary, standing, moderate activity and active time decreased.

Physical Performance

Relative changes in physical performance are presented in Figure 2. The following main effects were observed: the running time of 3 km, anaerobic power and hand-grip ($P<0.001$) of TC and the running time of 3-km ($P = 0.05$) and anaerobic power ($P = 0.026$) of the group. Hand grip decreased in both groups from PRE to MID but it recovered to the initial levels POST TC. The running time for 3 km improved in both groups as well. However, only the controls had an increase ($P = 0.022$) in anaerobic power from the MID to POST. Maximum heart rate during the 3 km (Ebar PRE 191 bpm, POST 191 bpm; control PRE 186 bpm, POST 189 bpm), blood lactate after 3 km (Ebar PRE 11.5 mmol·L^{-1}, POST 12.1 mmol·L^{-1}; control PRE 10.6 mmol·L^{-1}, POST 11.9 mmol·L^{-1}), and vertical jump remained the same in both groups. While the Ebar group improved anaerobic power before the TC (6 day PRE to PRE, $P<0.001$), the relative change was higher than in the controls ($P = 0.007$). Nevertheless, Ebar had higher anaerobic power than the controls in PRE ($P = 0.009$), MID ($P = 0.046$) and POST ($P<0.028$), there were no differences between the groups in its change during TC from PRE to POST.

Questionnaire

Questionnaire data analysis (Figure 3) revealed significant main effects for both the TC and group for hunger ($P<0.001$; $P = 0.016$, respectively), physical performance ($P<0.001$; $P = 0.009$) and positive mood state ($P = 0.002$; $P = 0.014$). The Ebar group felt less hungry than the controls POST and +3d POST ($P<0.01$). Furthermore, Ebar felt themselves less hungry +3d POST compared to the MID ($P<0.001$), while the controls did not. In addition, Ebar evaluated that their physical performance was better compared to the controls -6d PRE and PRE ($P<0.05$), and they had an improved positive mood state from MID to +3d POST, while the controls did not. Negative mood state and somatic symptoms had a main effect of TC ($P<0.001$) by increasing from -6d PRE and PRE to MID and decreasing +3d POST to the PRE levels. Symptoms of URTI did not change.

Table 3. Energy intake, macronutrient composition of the consumed food and water balance during the 8-day training course among the control and Ebar groups.

	Control (N = 12)	**Ebar (N = 14)**	**P**
Total energy intake (MJ·d^{-1}) [a]	10.9±1.7	13.3±3.0	**0.025**
Total energy intake (MJ·kg^{-1}·d^{-1})	0.16±0.30	0.19±0.43	**0.038**
Energy availability [b]	1.5±0.3	1.8±0.4	**0.028**
Carbohydrate (g·d^{-1})	359±46	413±84	0.063
Fat (g·d^{-1})	86±21	99±23	0.165
Protein (g·d^{-1})	92±20	149±46	**<0.001**
Carbohydrate (g·kg^{-1}·d^{-1})	5.2±1.0	5.9±1.2	0.108
Fat (g·kg^{-1}·d^{-1})	1.2±0.3	1.4±0.3	0.196
Protein (g·kg^{-1}·d^{-1})	1.3±0.3	2.1±0.6	**<0.001**
Fluid intake (L·d^{-1}) [c]	2.3±0.5	2.1±0.6	0.376
Total water intake (L·d^{-1}) [d]	3.4±0.6	3.3±0.5	0.833
Water loss (L·d^{-1})	−3.5±0.5	−3.3±0.5	0.382
Water deficit (L·d^{-1})	−0.1±0.7	0.0±0.7	0.584

Values are mean ± SD.
[a] Estimated from food recods.
[b] Energy availability = energy intake/basal metabolic rate estimated by the equation of Schofield et al. [32].
[c] Estimated from fluid intake records.
[d] Total water intake = fluid intake + water content of food + metabolic water.
doi:10.1371/journal.pone.0047771.t003

Associations with physical activity, energy availability, water balanced and physical performance. While there were no differences between the groups in physical activity, energy availability and water balance, the groups were pooled for correlation analysis. Correlations between physical activity and energy availability, water balance, hand-grip and anaerobic power are presented in Table 4. Energy availability associated only negatively with sitting during the entire TC. Instead, water balance associated positively with sitting and negatively with moderate activity and active time. Change in hand-grip from PRE to MID correlated positively with sitting and negatively with moderate and active time on days 1–5, while change in anaerobic power from PRE to the MID correlated negatively with vigorous activity. Changes in performance tests did not associate with energy availability and water balance. Either change in BM was not related to change in the 3 km running time, but correlated negatively with change in anaerobic power from MID to POST ($r = -0.46$, $P = 0.021$). However, negative mood state before TC associated negatively with energy availability during TC and sleeping time during the first half of TC (Table 5). In addition, negative mood state in the middle of the TC correlated negatively with active time and change in anaerobic power from PRE to MID ($r = -0.40$, $P = 0.048$). Furthermore, negative association was observed between energy availability and somatic symptoms and URTI symptoms and between URTI symptoms and moderate activity and active time (Table 5).

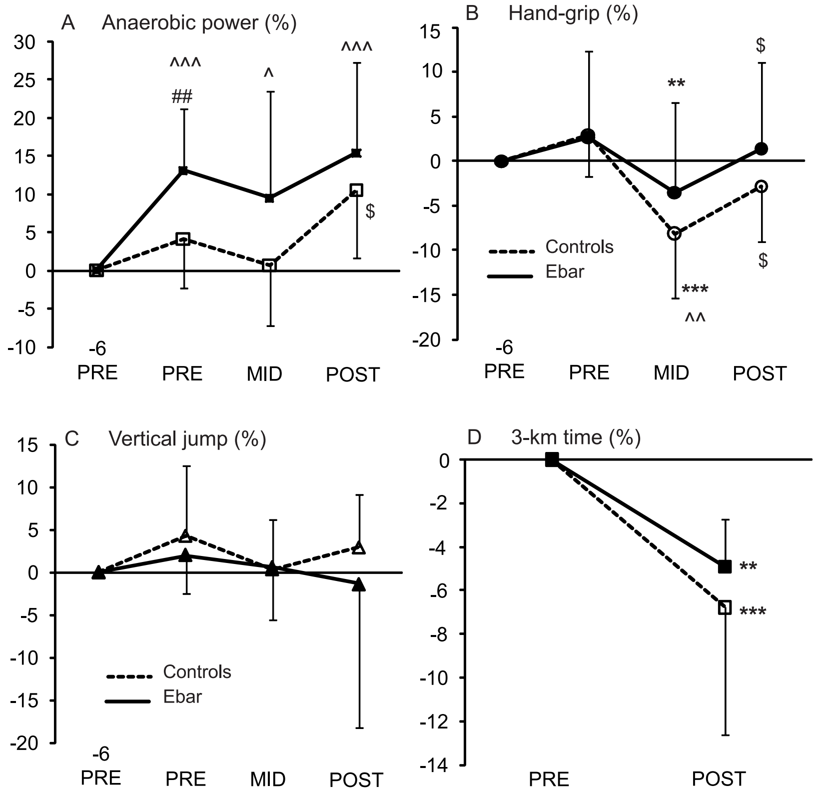

Figure 2. Changes (%) in physical performance in the control and Ebar groups during the study. Differences in change compared to the controls ## $P<0.01$. Differences in absolute change within the group compared to: -6 PRE ^^^ $P<0.001$, ^ $P<0.05$; PRE ***$P<0.001$, **$P<0.01$; MID $ $P<0.05$.
doi:10.1371/journal.pone.0047771.g002

Discussion

The present study showed that additional easy-to-use protein-rich energy bars increased total energy intake and energy availability during a demanding wintertime military training course. Despite a higher energy supply, energy intake was insufficient in the Ebar group similarly to the control group. The participants in both groups consumed, however, only 57±13% of the provided energy during TC. The difference of this study to the previously published energy supplements studies during short-term military field training [12,14,15,24–26] is the use of protein-rich supplement. However, like the previous studies, also in this study the use of supplements increased energy intake, although it could not prevent from energy deficit. Even energy availability was higher in the present Ebar group, it did not increase physical activity compared to the controls. Furthermore, an increase in aerobic physical performance after TC was observed in both groups. The reason that the additional 4.1 $MJ\bullet day^{-1}$ could not maintain energy balance might be that Ebar also had higher energy expenditure, although the difference between the groups was not significant. Energy expenditure was calculated by a method of intake/balance, which also takes into account the increased thermic effect of food of a higher protein intake and the carried combat gear of from 60 to 70 kg. Energy expenditure of physical activity was also increased by overall stress of TC [40].

In this study, increased somatic and URTI symptoms and negative mood state already before TC was related to lower energy availability during the training, which may indicate the lack of motivation for eating. In addition, increased somatic symptoms in the middle of TC were related to lower energy availability. Although Lieberman et al. [41] have found no effect of short 2-day caloric deprivation on mood state in laboratory environment, the result of insufficient energy intake despite sufficient provided food is in line with other military training studies. In a study of an 11-day cold-weather field exercise, the mean energy intake was 13.1 $MJ\cdot d^{-1}$ (3132 $kcal\cdot d^{-1}$), which was lower than rations offered from 17.6 to 21.8 $MJ\cdot d^{-1}$ (4200 to 5200 $kcal\cdot d^{-1}$) [8]. During the 28 day military training in hilly terrain, the average intake of participants with a ready-to-eat meal was 12.4 $MJ\cdot d^{-1}$ (2960 $kcal\cdot d^{-1}$) but they did not consume all of the 15.1 $MJ\cdot d^{-1}$ (3600 $kcal\cdot d^{-1}$) that they had [7]. However, the participants with lightweight rations consumed nearly all and the average intake of them during the training was 8.1 $MJ\cdot d^{-1}$ (1930 $kcal\cdot d^{-1}$). Both of these groups lost weight (4.3 kg vs. 1.1 kg, respectively) [7]. Popper et al. [9] found that eating less than usual is more obvious on the first day of the first combat situation, while the amount of eating increased on the second combat situation, but it was still less than

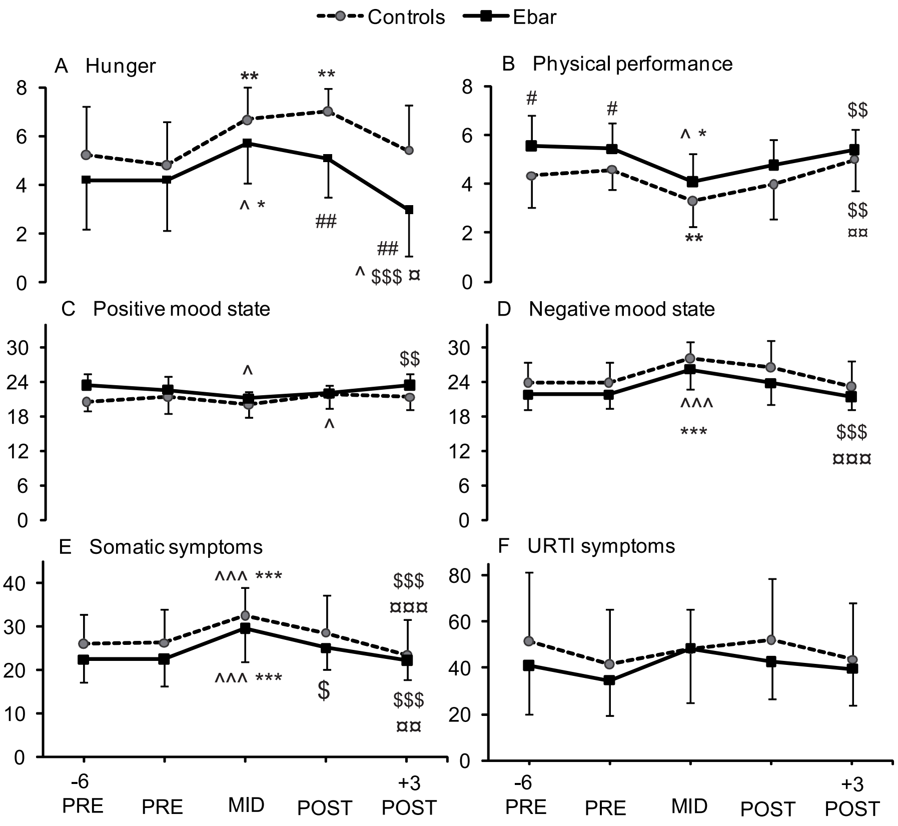

Figure 3. Questionnaire responses during the 8-day training course among the control and Ebar groups. Difference compared to: control ## P<0.01, # P<0.05; -6 PRE ^^^ P<0.001, ^ P<0.05; PRE ***P<0.001, **P<0.01, *P<0.05; MID $$$ P<0.001, $$ P<0.01, $ P<0.05; POST ¤¤¤ P<0.001, ¤¤ P<0.01, ¤ P<0.05.
doi:10.1371/journal.pone.0047771.g003

Table 4. Pearson correlation coefficient between physical activity and energy availability, water balance and changes (Δ) in hand grip and anaerobic power during the 8-day training course.

		Energy availability	Water deficit	Hand grip ΔPRE-MID	Anaerobic jump ΔPRE-MID
Sitting (h:min)	Days 1–5	−0.33	**0.49***	**0.40***	−0.09
	Days 5–8	−0.35	**0.47***	−0.04	−0.09
	Days 1–8	−**0.44***	**0.53****	0.27	−0.07
Standing (h:min)	Days 1–5	0.10	**0.46***	0.14	0.30
Moderate activity (h:min)	Days 1–5	0.05	−**0.63*****	−**0.45***	−0.04
	Days 5–8	0.21	−**0.59****	−0.16	−0.11
	Days 1–8	0.14	−**0.64*****	−0.37	−0.05
Vigorous activity (h:min)	Days 1–5	−0.11	−0.10	0.25	**−0.42***
	Days 1–8	−0.20	−0.27	0.13	**−0.40***
Active time (h:min)	Days 1–5	0.04	**−0.63*****	**0.43***	−0.07
	Days 5–8	0.21	**−0.58****	−0.18	−0.09
	Days 1–8	0.13	**−0.64*****	−0.36	−0.06

doi:10.1371/journal.pone.0047771.t004

Table 5. Pearson correlation coefficient between physical activity, energy availability and water balance and questionnaire data during the 8-day training course.

		Negative mood state			Somatic symptoms			URTI symptoms				
		-6 PRE	PRE	MID	-6 PRE	PRE	MID	-6 PRE	PRE	MID	POST	+3 POST
Energy availability		* **−0.41**	* **−0.43**	−0.26	* **−0.40**	** **−0.50**	* **−0.45**	−0.25	* **−0.40**	−0.36	* **−0.49**	* **−0.47**
Water balance		0.06	0.12	0.36	0.37	0.35	* **0.41**	* **0.44**	** **0.51**	*** **0.60**	* **0.42**	0.27
Sleeping (h:min)	Days 1–5	−0.24	** **−0.47**	−0.28	−0.15	−0.23	−0.15	0.22	0.06	0.09	−0.23	−0.16
	Days 5–8	−0.07	−0.22	0.07	0.14	0.18	0.11	* **0.41**	0.35	* **0.47**	0.20	0.23
	Days 1–8	−0.17	* **−0.40**	−0.10	0.01	−0.01	−0.01	* **0.39**	0.26	0.34	0.00	0.06
Moderate activity (h:min)	Days 1–5	−0.08	−0.03	−0.34	−0.12	−0.15	−0.25	−0.24	−0.31	* **−0.48**	−0.34	−0.26
	Days 5–8	−0.18	−0.06	** **−0.51**	−0.06	−0.10	−0.20	−0.40	−0.29	* **−0.39**	−0.15	−0.11
	Days 1–8	−0.13	−0.05	* **−0.43**	−0.11	−0.13	−0.27	−0.32	−0.33	* **−0.48**	−0.30	−0.21
Active time (h:min)	Days 1–5	−0.06	−0.03	−0.34	−0.11	−0.14	−0.23	−0.23	−0.30	* **−0.48**	−0.33	−0.25
	Days 5–8	−0.19	−0.07	** **−0.51**	−0.06	−0.11	−0.22	* −0.40	−0.30	−0.38	−0.14	−0.09
	Days 1–8	−0.12	−0.05	* **−0.43**	−0.10	−0.13	−0.26	−0.32	−0.32	* **−0.47**	−0.28	−0.20

***$P<0.001$,
**$P<0.01$,
*$P<0.05$.
doi:10.1371/journal.pone.0047771.t005

usual. The three most likely reasons for this behavior in respective situations has been reported to be lack of time to prepare and eat food as well as not being hungry [9]. The inadequate energy intake in this study could be also a consequence of their previous experiences that an 8-day energy deficit could be tolerated because participants knew they would have a rest period after TC.

In the present study, association between physical activity and energy availability was not observed. This is in line with other studies among soldiers, where all subjects were expected to have a substantial energy deficit [6,7] but not with studies where energy deficit was an option or self-selected [11,13]. Similarly in a study by Mettler et al. [22], athletes were able to maintain their training volume and intensity during the 2-week period, despite an energy restriction of 60% of habitual energy intake and reduced well-being. Although enough energy was provided in this study the participants did not utilize it, which resulted in an energy deficit. Thus, the influence of energy intake on physical activity is not known exactly. However, in our study the more was negative mood state and URTI symptoms in the middle of TC, the less was physical activity indicating the role of motivation to perform tasks and be active. Even though the tasks were ordered to do as a group, often soldiers help each others in various tasks. In addition, by helping other group members in their duties, soldiers have possibility to make several behavioral choices, consciously or not, which may clearly affect their energy expenditure in spite of the same whole-day tasks assigned to them. This is supported by a wide variation in total physical activity time from 3:50 to 7:45 h:min per day.

While the energy expenditure is extremely high, 1.8 $g \cdot kg^{-1}$ intake of protein has been recommended [23]. In the present study, protein intake among Ebar was 2.1 $g \cdot kg^{-1}$. An easy-to-use protein-rich energy bar was thought to increase energy intake and thus decrease loss of FFM during strenuous TC. Ebar had a higher energy intake and energy availability compared to the controls. However, there were no differences between the groups in changes in FFM, energy deficit and physical activity, even Ebar had a trend for an increase in FFM, rather than a decrease. Among firefighters, eat-on-move snacks increased not only energy intake but also physical activity during a 2-d experimental period compared to ready-to-eat meal days [12]. Friedl et al. [42] have also shown the positive effects of using food supplementation during US Ranger training; 400 $kcal \cdot day^{-1}$ higher energy intake attenuated decrease FMM in energy deficit state in the presence of sleep deprivation, psychological stress and high physical activity. Previously, a protein intake of 1.8 $g \cdot kg^{-1}$ has been found to be ineffective in preventing a decline in FFM during the energy deficit of 4.2 MJ caused by increased energy expenditure [23]. In the present study, protein intake was sufficient to attenuate the decline in FFM during the exhaustive training, even though the energy deficit was 40%. This is in line with a study of recreational athletes who trained an average of 6 hours per week. Their protein intake was sufficient (~ 2.3 $g \cdot kg^{-1}$) to maintain the lean body mass during the 40% energy restriction compared to the control group (~ 1.0 $g \cdot kg^{-1}$) protein intake [22].

Increased protein intake has been found to produce satiation [43], increase energy expenditure by thermic effect of food, and decreased ad libitum energy intake [43] among obese, which are favorable phenomena during weight loss and maintenance. However, these phenomena could be unfavorable during a situation where exercise-induced energy expenditure is high and high-energy intake is recommended. In the present study, high-protein bars might have induced satiation in the Ebar group, leading to their inadequate energy intake from field rations, despite energy being sufficiently provided. This interpretation is supported by the question of hunger. Namely, the Ebar group felt themselves less hungry after TC than the controls. On the other hand, even while energy intake matched energy expenditure, an increased protein intake of 3.0 $g \cdot kg^{-1}$ has been shown to have a beneficial role during recovery after exercise by attenuating impairments in endurance performance [44]. The likely mediator of this potentially beneficial effect of protein feeding is perturbations in psychological symptoms of stress [44]. On the contrary, during weight loss among recreational athletes, no differences were observed between the low and high protein intake groups in satiety and total mood disturbance [22]. In the present study, even though there were no differences in physical performance between the Ebar and control groups, the questionnaire data revealed that Ebar group felt their physical performance and positive mood state better than the controls.

It would be assumed that the high activity level over 5 hours per day with only 4 hours rest would have induced decrements in physical performance. An improvement in the 3 km running time in both groups could be partly due to weight loss [45], but that was not the case. Possible adaptation to the testing procedure and an enhanced level of motivation and effort on the last day of the study [46] cannot be ruled out and may explain partly an increase in the 3 km running time. Improvement has also been observed in a study of Hodgon et al [47] after nine-day field training in cold winter weather: the time of the snowshoe course improved without any change in the anaerobic power test. During TC, participants carried combat gear weighing 60–70 kg, but the load carried in the 3 km test was much lighter, a 20 kg backpack. The subjects felt that this lighter load was much easier to carry during the 3 km test and they felt that they were "flying" during the running. Improved 3 km performance might also be a consensus of 8-day load carrying training, which decreased the energy cost of load-carrying [48]. Furthermore, the present findings confirm that lower body anaerobic power and vertical jump do not appear to be adversely affected by prolonged back load carriage [48]. The results also confirm the previous findings among athletes and military persons that the short-term (<10 days) consequences of underfeeding have a limited impact on muscle strength and aerobic and anaerobic endurance [22,49,50]. In contrast, also decreased lower body anaerobic power has been found after short period (72 h) of sustained operations [46] and after eight-day military field training [35]. In these studies, the time from a completion of the training course to the beginning of post testing was less than three hours. In the present study, the post test was performed 1 day after the training course, which may explain the difference between the results of anaerobic performance. Also loss of body mass was related to improvement in anaerobic power [51]. However, it has to take into account that longer period of underfeeding should definitely induced decrements in physical performance [3]. Contrary, hand-grip strength decreased in the middle of the training course in both groups and returned to the baseline levels one day after. Thus hand-grip strength might indicate an acute overall physiological and mental fatigue, even though it has not been found as a sensitive indicator of muscle mass loss and energy deficit [52].

Also the present results are in line with finding that low ($\sim$1.0 $g \cdot kg^{-1}$) or high ($\sim$2.3 $g \cdot kg^{-1}$) protein intake has no influence on changes in performance tests after hypoenergetic weight loss [22]. A daily carbohydrate intake of 5–6 $g \cdot kg^{-1}$ was lower than recommended for endurance athletes [53], but it was still enough to maintain anaerobic performance and even improve aerobic performance. For energy restricted strength and power athletes, above the 3.0 $g \bullet kg^{-1}$ carbohydrate intake has been recommended to maintain anaerobic performance [49]. Furthermore, improvement in 3 km running time could be a consequence of recently published evidence that after low carbohydrate intake during training period aerobic performance is improved after carbohydrate loading before the test [54]. In this study participants were able to rest 1 day after the training course before post test and load their energy stores.

The recommendation for fluid intake during military training in cold weather is 2 liters per day [55]. In both groups of the present study, ad libitum fluid intake was 2.2 ± 0.5 L per day without water deficit. This confirms that the recommendations of 2 L per day is enough at group level, but is not enough for the most active participants while the activity was related to higher water deficit [56]. Even the variation in fluid intake (from 0.9 $L \cdot d^{-1}$ to 2.8 $L \cdot d^{-1}$) and water balance (from -1.7 $L \cdot d^{-1}$ to 0.8 $L \cdot d^{-1}$) was wide, water balance did not correlate with physical performance after the training course nor with change in FFM. In this study water balance was calculated by the differences between estimated water intake and water loss measured by deuterium elimination method. While 73% of the fat-free mass is water (Westerterp et al. 1995a), it could assume that the change in body mass loss is trough water deficit. However, in this study changes in TBW and FFM were nonsignificant and water deficit did not exist. Thus, there was no body mass loss through a water deficit.

Conclusion

Based on the present study, an easy-to-use energy bar supplement of 4 $MJ \bullet day^{-1}$ did not prevent energy deficit or influence on PA during an 8-day strenuous military TC. A satiation effect of the high content of protein in the bars might have induced a decreased energy intake from field ration, since the Ebar group felt themselves less hungry after TC than the controls. However, negative mood state and URTI symptoms affected energy intake already before the training course. Thus mood state and health status are important factors in preventing undereating. In addition, during strenuous TC, PA seems to be primarily affected by factors other than energy supplementation such as mood state. The outcome and also a limitation of this study was the tremendous undereating. For evaluation the effects of increased energy intake on PA and performance, we should have had a third group which was forced to eat all provided food. Secondly to avoid biases, the subjects should be absolutely unaware of the group at they were included during the whole intervention period. In future studies, a special attention should be paid for the taste and variability of the food as well to maintain positive mood state in order to enhance energy intake during prolonged training.

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Author Contributions

Conceived and designed the experiments: MMT KRW ALU MA KH HOK HK. Performed the experiments: MMT KRW MA KH HOK HK. Analyzed the data: MMT KRW MA HOK. Contributed reagents/materials/analysis tools: MMT KRW ALU MA KH HOK HK. Wrote the paper: MMT KRW ALU MA KH HOK HK.

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