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Tarja Nykänen

Nutrition, Hormonal and Metabolic Status, and Physical Performance in Different Military Contexts

UNIVERSITY OF JYVÄSKYLÄ

FACULTY OF SPORT AND
HEALTH SCIENCES

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ABSTRACT

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The overall purpose of the thesis is to investigate how nutrition is related to hormonal and metabolic status, and physical performance of soldiers in a crisis management operation in Lebanon, and in winter survival training in Lapland. During a six-month crisis management operation, dietary intake, body composition, cardiovascular risk factors, and physical activity of soldiers were measured at baseline, mid-phase, and the end of the operation. In a ten-day winter survival training, energy intake, energy expenditure, body composition, hormones, and other biomarkers, and physical fitness were assessed four times during the survival training in conscripts. Among crisis management soldiers, carbohydrate intake was below and saturated fatty acid intake above recommendations. Low fiber intake and increased body fat mass were associated with elevated total and LDL cholesterol values. Total physical activity was low. During the first two days of survival training, severe energy deficit, as a result of increased energy expenditure and restricted energy intake, and changes in body mass, body fat mass, leptin, testosterone-cortisol ratio, free fatty acids, and urea were observed. These outcomes returned towards baseline in later measurements, especially when recovery was emphasized. Energy intake, expenditure, and balance were not associated with physical fitness levels. Consuming more fiber-rich carbohydrates and less saturated fatty acids could improve quality of diet and cardiometabolic health among soldiers during garrison-like environments. Survival training with severe energy deprivation caused remarkable changes in energy metabolism. By recognizing nutritional and physiological responses to different military conditions, military performance can be optimized.

Keywords: soldier, energy, dietary intake, military condition

TIIVISTELMÄ (ABSTRACT IN FINNISH)

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Ravitsemus, hormonaaliset ja metaboliset vasteet ja fyysinen suorituskyky erilaisissa sotilasympäristöissä
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Väitöskirjan tarkoituksena oli tutkia sotilaiden ravinnonsaantia ja siihen liittyviä fysiologisia vasteita kriisinhallintaoperaatiossa Libanonissa ja selviytymisharjoituksessa Sodankylässä. Kriisinhallintaoperaatiossa sotilaiden ravinnonsaantia, kehon koostumusta, sydän- ja verisuonitautien riskitekijöitä ja fyysistä aktiivisuutta seurattiin kuuden kuukauden operaation alussa, puolivälissä ja lopussa. Selviytymisharjoituksessa varusmiesten energiansaantia- ja kulutusta, kehon koostumusta, hormoneja ja aineenvaihduntaan liittyviä biomarkkereita ja fyysisen kunnon osatekijöitä mitattiin neljä kertaa kymmenen päivän aikana. Kriisinhallintaoperaatiossa sotilaat saivat ravinnostaan liian vähän hiilihydraatteja ja liikaa tyydyttynyttä rasvaa verrattuna kansallisiin suosituksiin. Vähäinen kuidun saanti ja suurempi kehon rasvan määrä olivat yhteydessä kohonneisiin kokonais- ja LDL-kolesteroliarvoihin. Fyysisen aktiivisuuden taso oli alhainen. Selviytymisharjoituksen ensimmäisinä päivinä havaittiin merkittävä energiavaje suurentuneen energiankulutuksen ja rajoitetun energiansaannin takia. Samalla kehon paino ja rasvan määrä vähenivät sekä leptiinin, vapaiden rasvahappojen ja urean pitoisuuksissa sekä testosteroni-kortisoli -suhteessa havaittiin selviä muutoksia. Muutokset tasaantuivat harjoituksen edetessä, erityisesti kun palautumista tehostettiin. Energian saanti, -kulutus tai tasapaino eivät olleet yhteydessä kuntomuutoksiin. Kriisinhallintasotilailla kuitupitoisten hiilihydraattien lisääminen sekä tyydyttyneen rasvan vähentäminen parantaisi perusruokavalion laatua ja sotilastehtävissä suoriutumista sekä edistäisi sydänterveyttä. Selviytymisharjoitus aiheutti merkittävän energiavajeen ja muutoksia kehon energia-aineenvaihdunnassa ja hormonitasoissa. Eri sotilastehtävien ja -ympäristöjen aiheuttamien fysiologisten vasteiden tunnistaminen ja ravitsemuksen huomioiminen on tärkeää sotilaiden suorituskyvyn ylläpitämiseksi vaihtuvissa olosuhteissa.

Avainsanat: sotilas, energia, ravinnonsaanti, sotilasympäristö

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In Lappeenranta on 17.10.2023

Tarja Nykänen

ORIGINAL PUBLICATIONS AND AUTHOR CONTRIBUTION

This dissertation is based on the following four original publications, which will be referred to by their Roman numerals.

I Nykänen, T., Pihlainen, K., Santtila, M., Vasankari, T., Fogelholm, M., & Kyröläinen, H. (2019). Diet macronutrient composition, physical activity, and body composition in soldiers during 6 months deployment. *Military Medicine*, *184*(3-4), e231–e237. https://doi.org/10.1093/milmed/usy232

II Nykänen, T., Pihlainen, K., Kyröläinen, H., & Fogelholm, M. (2020). Associations of nutrition and body composition with cardiovascular disease risk factors in soldiers during a 6-month deployment. *International Journal of Occupational Medicine and Environmental Health*, *33*(4), 457–466. https://doi.org/10.13075/ijomeh.1896.01541

III Nykänen, T., Ojanen, T., Heikkinen, R., Fogelholm, M., & Kyröläinen, H. (2022). Changes in body composition, energy metabolites and electrolytes during winter survival training in male soldiers. *Frontiers in Physiology*, *13*, 797268. https://doi.org/10.3389/fphys.2022.797268

IV Nykänen, T**.**, Ojanen, T., Vaara, J. P., Pihlainen, K., Heikkinen, R., Kyröläinen, H., & Fogelholm, M. (2023). Energy balance, hormonal status, and military performance in strenuous winter training. *International Journal of Environmental Research and Public Health*, *20*(5), 4086. https://doi.org/10.3390/ijerph20054086

The author of this thesis, who is the first author of the papers mentioned above, was responsible for designing the study protocol for the dietary intake measurements with Dr. Kai Pihlainen (I, II) and Dr. Tommi Ojanen (III, IV). The author participated in data collection in Lebanon (I, II) and Lapland (III, IV), and was responsible for the assessment of food diaries in studies III and IV. The author was the lead researcher in data interpretation, preparation of manuscripts and management of the publication processes in all articles (I-IV).

ABBREVIATIONS

ATP	Adenosine-triphosphate
BF%	Body fat percent
BIA	Bioimpedance
BM	Body mass
BMI	Body mass index
BMR	Basal metabolic rate
CHO	Carbohydrate
CNS	Central nervous system
CVD	Cardiovascular disease
DLW	Doubly labeled water
DXA	Dual energy X-ray absorptiometry
EA	Energy availability
EB	Energy balance
EE	Energy expenditure
EI	Energy intake
E%	Percentage of total energy intake
FFA	Free fatty acids
FFM	Fat-free mass
FEX	Field exercise group
FM	Fat mass
HDL	High-density lipoprotein
HRV	Heart rate variability
IGF-1	Insulin-like growth factor 1
LDL	Low-density lipoprotein
MDRI	Military dietary reference intake
MET	Metabolic equivalent
MJ	Megajoule
MVPA	Moderate to vigorous physical activity
NATO	North Atlantic Treaty Organization
NNR	Nordic Nutrition Recommendations
PA	Physical activity
PRO	Protein
RDA	Recommended dietary allowance
REC	Recovery group
SB	Sedentary behavior
SNS	Sympathetic nervous system
TB	Triceps brachii
TCA	Tricarboxylic acid cycle
T/C ratio	Testosterone/cortisol ratio
TDEE	Total daily energy expenditure
VL	Vastus lateralis
VO_2max	Maximal oxygen uptake

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ORIGINAL PUBLICATIONS

1 INTRODUCTION

The role of nutrition and diet in military performance and operational readiness has been well-established in past decades. Optimal nutrition can maintain better health, prevent chronic diseases, support physical training and recovery, and promote success in military tasks (Karl et al., 2022). In addition, an improvement in diet and energy balance may contribute to better psychological resilience in soldiers (Beckner et al., 2023; Lutz et al., 2017).

Non-deployed personnel in garrisons can live like their civilian counterparts and individual health behavior is emphasized. Military personnel have access to proper dining facilities, and they can optimize their physical training and recovery (Karl et al., 2022). Despite optimal facilities, modern society may weaken the quality of diet through the availability of energy-dense food, by skipping meals or making unhealthy choices. Global trends of increasing overweight and obesity rates cannot be avoided in military populations, and these factors compounded by other behavioral (smoking, alcohol consumption, low physical fitness) and individual (age, gender, heredity) factors may result in metabolic disorders later.

On the other hand, deployed personnel in modern battlefields face multiple stressors. Many nutritional strategies have been developed to support military performance. However, these strategies cannot compensate for the complexity of missions. The intensity of military tasks varies from very low workloads in crisis management operations in the heat (Pihlainen et al., 2018) to prolonged and intense activities in survival-type trainings in the cold (Pasiakos et al., 2020). The highest energy expenditure levels were measured during a cold and high-altitude deployment of male soldiers (Tharion et al., 2005). Thus, this kind of winter training induces totally different physiological responses from deployments in the heat. In addition, other environmental factors such as terrain, humidity, depth of snow, load carriage, and psycho-social stress factors contribute to military performance and may require specific diets to avoid adverse effects.

The Finnish Defence Forces consist of obligatory military service and its reserve force, as well as a volunteer military system for women. Characteristics of military tasks vary between nations and branches, which also applies to the

Finnish Army. Additionally, diet, food culture and eating behavior differ between countries and even within Finland. The unique characteristics of the Finnish population highlights the importance of studying nutritional factors and their associations with physiological adaptations, physical performance, and cardiometabolic health among Finnish soldiers in different military conditions.

Thus, the overall purpose of this thesis was to study how nutrition is related to hormonal and metabolic state, and physical performance among soldiers in crisis management operation and winter survival training.

2 REVIEW OF THE LITERATURE

2.1 Role of nutrition in military context

Optimal military performance and operational readiness are affected by several internal and external factors, as shown in Figure 1 (modified from Kyröläinen et al., 2018). Many of these factors are related to nutrition directly (diet, negative energy balance) or indirectly (body composition, prolonged physical activity, load carriage, sleep and recovery deficit, environmental factors, psycho-social and motivational factors). Furthermore, age and gender influence body composition, dietary requirements, and training adaptations also in military (Kyröläinen et al., 2018; McClung et al., 2022).

Thus, the role of nutrition in soldiers' physical performance is complex. One way to describe associations is to divide short- and long-term effects of diet, but it is not the most optimal way in all cases. In addition to acute and chronic effects, diet also has direct and indirect effects on the health, physical performance and operational readiness of soldiers, which all have to be considered when discussing the role of nutrition in the military.

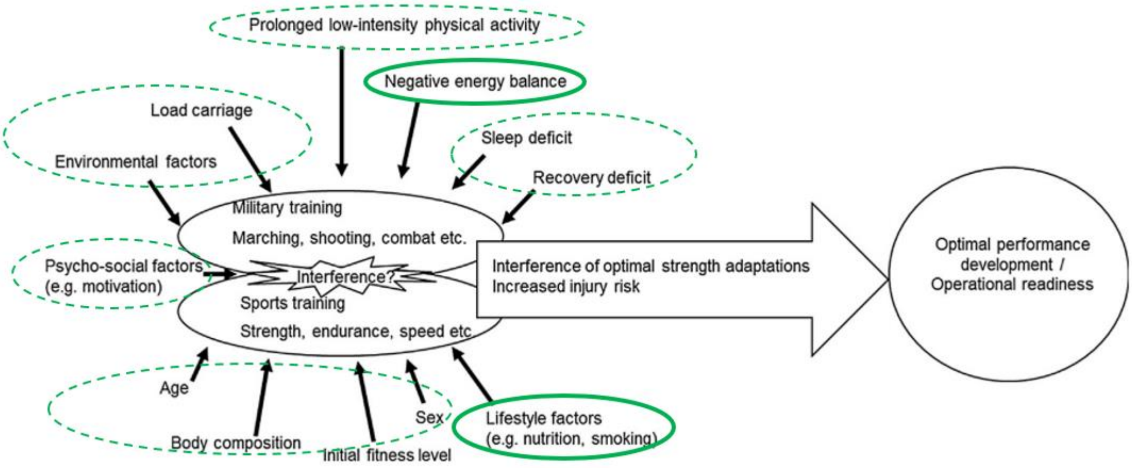


FIGURE 1. Factors affecting physical and military training, military performance, and operational readiness. (Nutrition-related direct factors in bolded lines, indirect factors in dashed lines) (Modified from Kyröläinen et al., 2018)

A soldier's diet varies depending on their duties. In garrisons, fresh food and main courses are served in dining halls, and food and fluid can be consumed *ad libitum*. In field trainings, meals can be transported to the training area, or in some cases, field kitchens are established. In the Finnish Defence Forces, food supply is organized by a catering company, which is responsible for most dining facilities and services in garrisons. It also provides food supply for field trainings. The catering company follows national nutritional recommendations, like all catering services in the public sector (Blomhoff et al., 2023). The aim of catering company services is to promote healthy eating habits and lifestyles, especially during conscript service, but also for military personnel (Leijona Catering website, www.leijonacatering.fi).

When fresh food supply is not possible, soldiers use field rations to fulfil their nutritional needs during missions. Field rations are food packages which typically contain ready-to-eat meals, snack items, and beverage powders. The rations are provided to troops during field operations or soldiers carry the rations themselves (Tassone & Baker, 2017). Nutritionally, combat rations contain all of the macronutrients and micronutrients needed in field conditions, but high total energy expenditure and underconsumption of rations typically contribute to energy and nutrient deficit during operations (Tanskanen et al., 2012; Tassone & Baker, 2017). According to a systematic review by Tassone and Baker (2017), continuous consumption of combat rations could lead to reductions in body mass and adverse effects on body composition, and these changes are more obvious in longer operations (up to 34 d). Measuring body mass and body composition before and after an operation would inform the magnitude of energy deficit, and therefore help officers, leaders and catering services optimize dietary requirements during operations.

Nutrition Science and Food Standards for Military Operations (2010) compared field rations between different North Atlantic Treaty Organization (NATO) members. The report concluded that it was challenging to offer rations which fulfill the nutritional requirements for soldiers. Minimum requirements were reached, but some rations needed supplementation to optimize nutritional intake. Furthermore, many behavioral and physical factors contribute negatively and positively to the consumption of field rations. For example, attitudes of leaders and officers, timing of eating, eating alone vs in groups, and some psychological and cultural factors (food preferences, variety of meals) can enhance or depress eating behavior.

Food supply policies, intensity and duration of missions, and environmental factors need to take into account, when discussing military nutrition. In addition, individual responses in different conditions produce the complex phenomenon to consider.

2.2 Dietary and fluid intake

2.2.1 Dietary recommendations

Dietary guidelines have been designed to optimize nutritional needs, promote health and prevent chronic diseases at the population level (Blomhoff et al., 2023; U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2020–2025). Most Western countries have modified their national recommendations to integrate the latest scientific evidence into the habits of their population and culture. Finnish nutritional recommendations are based on Nordic Nutrition Recommendations (NNR) and the latest version was published in June 2023 (Blomhoff et al., 2023).

Recommendations for macronutrient intake have been widely documented in general guidelines for sport and the military (Table 1). Most of the guidelines agree that diet should be based on fiber-rich carbohydrates and the proportional energy content of carbohydrates should be 45-60 E% (Percentage of total energy intake) (NNR) or 45-65 E% (American Guidelines). Protein content of food is recommended to be 10-35 E% for 18-30-year-olds. males in American Guidelines, but in NNR, the proportion is 10-20 E% (Blomhoff et al., 2023; U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2020-2025). Recommendation of fat intake varies from 20-35 E% (Dietary Guidelines for Americans) to 25-40 E% (NNR), but the recommendations for saturated fatty acid intake are consistent in both guidelines (< 10 E%).

General recommendations have targeted healthy and moderately active individuals. They do not apply to people with specific needs or medical conditions. Thus, special recommendations have been designed for athletes to optimize energy and nutrient supply for regular training (Jäger et al., 2017; Vitale & Getzin, 2019), and for soldiers to meet to specific nutritional requirements of military tasks and physical training (Army regulation 40–25, 2017; Pasiakos et al., 2015,).

To optimize macronutrient intake at individual level, the recommended amount is often presented as grams per kilogram of body mass. For protein intake, the recommendations vary substantially between Recommended Dietary Allowance (RDA) (0.8 g $\cdot$ kg^{-1} $\cdot$ d^{-1}) and military (1.5 g $\cdot$ kg^{-1} $\cdot$ d^{-1}). RDA is adequate for soldiers when activity level is low and energy intake is balanced with expenditure. In field conditions, metabolic demands are accelerated, and higher protein intake may diminish adverse effects on body composition (Pasiakos et al., 2015).

TABLE 1. Comparison of dietary recommendations.

	American*	**NNR**	**Sports**	**Military**
CHO	45-65 E%	45-60 E%	Based on exercise load Low 3-5 g $\cdot$kg^{-1} $\cdot$d^{-1} Moderate (~1 h $\cdot$d^{-1}) 5-7 g $\cdot$kg^{-1} $\cdot$d^{-1} High (~1-3 h $\cdot$d^{-1}) 6-10 g $\cdot$kg^{-1} $\cdot$d^{-1} Very high (>4-5 h $\cdot$d^{-1}) 8-12 g $\cdot$kg^{-1} $\cdot$d^{-1} (Thomas et al., 2016; Vitale & Getzin, 2019)	Long-term increased PA 4-8 g $\cdot$kg^{-1} $\cdot$d^{-1} (Army regulation 40–25, 2017)
Protein	10-35 E% 0.8 g $\cdot$kg^{-1} $\cdot$d^{-1} (RDA)	10-20 E%	1.2-1.7 g $\cdot$kg^{-1} $\cdot$d^{-1} (Rodriguez et al., 2009) 1.2-2.0 g $\cdot$kg^{-1} $\cdot$d^{-1} (Thomas et al., 2016; Jäger et al., 2017) 1.4 g $\cdot$kg^{-1} $\cdot$d^{-1}, 0.3 g $\cdot$kg^{-1} every 3-5 hour (Vitale & Getzin, 2019)	1.5-2.0 g $\cdot$kg^{-1} $\cdot$d^{-1} (Pasiakos et al., 2015) 0.8-1.6 g $\cdot$kg^{-1} $\cdot$d^{-1} (Army regulation 40–25, 2017)
Fat	20-35 E%	25-40 E%	Like general recommendations, not < 20 E% (Thomas et al., 2016; Vitale & Getzin, 2019)	Like general recommendations (Army regulation 40–25, 2017)

*for 18-30 yrs. males, CHO=carbohydrate, PA=physical activity, RDA= Recommended Dietary Allowance, NNR=Nordic Nutrition Recommendations

2.2.2 Dietary intake in soldiers

Macronutrient intake of soldiers has been studied in different garrison-like conditions. During a 6-month deployment in Afghanistan, PRE, MID and POST measurements revealed that CHO intake was 46, 48, and 43 E%, protein intake 16, 17, and 17 E%, and fat intake 35, 34, and 35 E%, respectively (Fallowfield et al., 2014). Beals et al. (2015) reported that Airborne Division soldiers in garrison training consumed 3.9 ± 2.0 g $\cdot$kg^{-1} $\cdot$d^{-1} (males) and 4.0 ± 2.1 g $\cdot$kg^{-1} $\cdot$d^{-1} (females) of carbohydrates, 1.4 ± 0.7 g $\cdot$kg^{-1} $\cdot$d^{-1} (males) and 1.3 ± 0.8 g $\cdot$kg^{-1} $\cdot$d^{-1} (females) of protein, and 32.4 ± 9.6 E% (males) and 29.1 ± 8.6 E% (females) of fats. Results showed underconsumption of CHOs and overconsumption of fat, which was also observed in Belgian soldiers during a special force qualification course (De Bry et al., 2021). Edwards et al. (2022b) reported similar results in British cadets: CHO underconsumption in males and females in camp, field and combined camp-field training. Protein intake was sufficient (> 1.2 $g \cdot kg^{-1} \cdot d^{-1}$) in all conditions, except for females in field training. In cold weather mountain training, mean PRO intake was 1.6 ± 0.7 $g \cdot kg^{-1} \cdot d^{-1}$ and CHO intake was 3.1 ± 1.3 $g \cdot kg^{-1} \cdot d^{-1}$ (Beals et al., 2019). In a study by Margolis et al. (2016), protein (PRO) or CHO supplements were served to two intervention groups to determine if extra supplements could help meeting requirements. Protein intake was higher in the PRO group (2.0 ± 0.3 $g \cdot kg^{-1} \cdot d^{-1}$) than CHO or control groups (1.3 ± 0.3 and 1.3 ± 0.2 $g \cdot kg^{-1} \cdot d^{-1}$, respectively). CHO intake was greater in the CHO group (5.8 ± 1.3 $g \cdot kg^{-1} \cdot d^{-1}$ 1) compared to the PRO and control groups (4.2 ± 1.0 and 4.2 ± 0.7 $g \cdot kg^{-1} \cdot d^{-1}$, respectively). Furthermore, fiber intake seems to be low in males and females (Lutz et al., 2019), and saturated fat exceeded the recommended level of 10 E% (De Bry et al., 2021). In conclusion, macronutrient intake of military or tactical personnel did not meet requirements: protein intake seemed to be at the recommended level, but fat intake exceeded the recommended range, and CHO failed to meet the reference values (MacKenzie-Shalders et al., 2021).

Reference values for protein intake varies depending on guidelines and personnel. For soldiers, recommendations are condition specific (Pasiakos et al., 2015). Garrison-like circumstances, where regular meals are available, general recommendations for protein intake could be sufficient. Physical training, especially strength training, increases protein requirements to fulfil the needs of accelerated protein turnover. Field operations typically cause energy deficit, and higher intake of protein could spare a loss of fat-free mass (Pasiakos et al., 2015).

In addition, micronutrient intake is not optimal among military personnel (Beals et al., 2015). Lutz et al. (2018) found that male and female soldiers remained under recommended levels of vitamins D and E, magnesium, potassium, α-linolenic acid, and fiber intake. Males did not consume adequate quantities of linoleic acid, and females did not meet calcium and iron recommendations. During crisis management operations, the healthy eating index 2010 decreased over 3 months, resulting in declines in vitamin D, calcium and iron levels (Farina et al., 2017). In a 12-month deployment, vitamin D intake, assessed by food frequency questionnaire, remained below reference values in the PRE- and POST-measurements (Carlson et al., 2013). Seasonal fluctuations of vitamin D status were found in 6-month deployment in Afghanistan (Fallowfield et al., 2019). Magnesium, zinc, copper, and selenium intakes were within the normal range at all three measurement points. Although, small, but significant, decreases were observed in magnesium and zinc intake (Fallowfield et al., 2014). Furthermore, garrison diet did not meet omega-3 fatty acid requirements, and substitution of eggs, poultry, and pork were recommended to achieve better n-3/n-6 proportion at the tissue level (Marriot et al., 2014).

To conclude the dietary intake studies in soldiers, underconsumption of CHO and overconsumption of fat have been demonstrated in several military studies in garrison and during training (Baker et al., 2020; MacKenzie-Shalders et al., 2021). Low CHO intake does not support physical exertion and recovery and may limit energy supply for moderate- to high-intensity tasks, even in garrison conditions (Beals et al., 2015). Although protein intake met the reference values, misconceptions about protein consumption exist among soldiers. Some soldiers believe that higher amounts of protein (> 2.0 g $\cdot$kg^{-1} $\cdot$d^{-1}) facilitate adaptations to strength training and there is no limit to the benefits, so scientific evidence and education need to be implemented (Pasiakos et al., 2015). In addition, suboptimal intake of several micronutrients was observed in military studies (Beals et al., 2015; Fallowfield et al., 2019; Lutz et al., 2019).

2.2.3 Fluid recommendations and intake in soldiers

Water is a main component of the human body. The fraction of fluid from total body mass varies from 50 to 75%, being higher in men and the young (Blomhoff et al., 2023). Fluid balance maintains normal organ function and supports thermoregulation by transporting heat via perspiration (Blomhoff et al., 2023; Luippold et al., 2018). The aim of fluid requirements is to maintain a euhydrated state, which is determined as < 2% change in body mass (Luippold et al., 2018; Thomas et al., 2016). The guiding value for fluid and water intake for adults is 1.0–1.5 L per day (Blomhoff et al. 2023). In Nordic civilians, mean fluid intake is approximately 1.0–2.0 L from ingested fluids, and 1.0–1.5 L from foods, resulting in 2.0–3.5 L of water per day (Blomhoff et al., 2023).

Recommended daily fluid intake for soldiers ranges from 1.9 to 4.7 L in thermoneutral conditions and with moderate activity (Army regulation 40–25, 2017). Insufficient hydration status was observed in 81% of aeronautical military personnel in garrison (Carretero-Krug et al., 2021), and in 33% of personnel in military course (Rogers et al., 2016). During military trainings, acute dehydration was found during marching in hot temperatures (Nolte et al., 2019), and insufficient fluid intake was assessed during demanding military course (De Bry et al., 2021),

Various trainings and environmental conditions in the military influence sweating rate, for example, work intensity, environmental factors, body armor (Luippold et al., 2018), as well as gender, physical fitness and other individual factors. Soldiers are exposed to thermal heat stress, especially during deployments in hot environments (e.g., Chad, Lebanon, Mali, Afghanistan etc.), where sufficient fluid intake is essential for maintaining physical performance and operational readiness. Blood biomarkers, such as sodium, potassium, chloride, hematocrit and hemoglobin, have been used to evaluate electrolyte balance (Na, K, Cl) and plasma volume (hematocrit, hemoglobin) (Armstrong et al., 1999; De Carvalho et al., 2007).

During a 40 km march in mean temperature of +27.1° C, *ad libitum* water intake was 0.74 ± 0.26 L $\cdot$h^{-1}, but body mass still decreased 4.0% ± 1.4% (Nolte et al., 2019). However, dehydration assessed by serum sodium concentration was not conducted (Nolte et al., 2019). When blood biomarkers were evaluated during a 16 km march in moderate temperatures (range 21.3° C - 29.4° C) and hydrated by water *ad libitum* or CHO-drink, sodium, potassium, glucose, hematocrit and hemoglobin decreased and no differences were found between groups

(De Carvalho et al., 2007). Dehydration was not observed, and some participants were overhydrated (De Carvalho et al., 2007).

Fluid is lost via sweat, urine and ventilation in hot and cold environments. Cold exposure differs from heat by blunting thirst sensation (Pasiakos, 2022). Winter uniforms, equipment, and activity in snow or difficult terrain increase workload, and, thus, sweating and ventilation. In addition, food and fluid supply to the field may be limited. Below 0° C, techniques for transportation and storage of fluids are important to prevent freezing them. Like moderate and hot environments, body fluid loss can be prevented in the cold if particular attention is paid to increasing fluid intake (O'Brien et al., 1996). Similar findings were observed in a Finnish study, where *ad libitum* fluid intake was compared to enhanced fluid intake (10 x 0.2 L dose $\cdot d^{-1}$) in winter military training. The enhanced group drank 2.2 ± 0.05 L $\cdot d^{-1}$ and the *ad libitum* group drank 1.3 ± 0.05 L $\cdot d^{-1}$ (Rintamäki et al., 2007).

2.3 Diet and cardiometabolic risk factors in soldiers

Risk factors for cardiovascular diseases (CVD) have been updated by the American College of Cardiology and the American Heart Association (Arnett at al., 2019). Primary hypercholesterolemia, metabolic syndrome, hyperlipidemia / hypertriglyceridemia, and elevated C-reactive protein increase the risk of CVD. Metabolic syndrome is diagnosed when three of the following factors exist: increased waist circumference, elevated triglycerides, elevated blood pressure, elevated glucose, and low high-density lipoprotein cholesterol.

Low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides are widely used blood lipids associating with CVD risk. High concentration of LDLs and low concentration of HDLs play a role in the progression of atherosclerosis in the endothelium of arteries. Accumulation of modified LDLs in the intima promotes lesion formation and activates inflammatory processes, which contribute to atherosclerosis (Linton et al., 2019). HDLs prevent the inflammation process and promote cholesterol efflux to reduce lesion formation (Linton et al. 2019). In addition to lipoproteins, triglycerides are used to assess the lipid profile and CVD risk. Triglycerides seem to have a causal relationship to atherosclerotic progression independent of LDLs (Tada et al., 2018). In addition, proinflammatory biomarkers such as tumor necrosis factor-α, interleukin-6 and C-reactive protein (CRP) have been shown to be associated with CVD risk factors (Libby, 2006).

Nutritional factors related to CVD risk are well-established: Dietary patterns, named as Nordic and Mediterranean diets, could be the most effective patterns to protect CVD progression (Badimon et al., 2019; Berild et al., 2017; Casas et al., 2018; Srinath Reddy & Katan, 2004). These diets, rich in fruits, berries, vegetables, whole grains, seafood, nuts, seeds and legumes, contain many bioactive compounds, such as unsaturated fatty acids, polyphenols, fiber, phytosterols, vitamins and minerals, which have anti-inflammatory, antioxidant, and antithrombotic effects to prevent the initiation and progression of CVD (Badimon et al., 2019; Casas et al., 2018). The Nordic diet had a beneficial effect on LDL cholesterol, blood pressure and inflammatory factors (Bertil et al., 2017; Lankinen et al. 2019). In addition, the many components of a healthy dietary pattern have synergistic effects on CVD risk, rather than dominant effects from a single nutrient (Casas et al., 2018).

Prevalence of CVD risk factors has been widely studied in military populations. According to a systematic review (Baygi et al., 2020), the prevalence of obesity and metabolic syndrome in military populations were 14% and 21%, respectively. Soldiers seem to have less CVD risk factors than their age-matched civilian counterparts in the U.S. (Pasiakos et al., 2012), but in some military populations, the prevalence of risk factors was even higher than their civilian peers (Baygi et al., 2020). Pooled data from over 90,000 Chinese military officers revealed that prevalence rate of hypertension was 47% (95% CI 42–52%), hyperlipidemia was 30.9% (26–36%), diabetes mellitus was 20.7% (17–26%), heart diseases was 48% (42–55%), and cerebrovascular diseases was 20% (14.8–26.9%) (Mara et al., 2018). Rates were higher with age, except for hyperlipidemia, and prevalence of hypertension and heart diseases peaked after retirement (Mara et al., 2018). When predicting CVD risk, Parastouei et al. (2021) presented that 96.5% of Iranian soldiers had low risk for CVD development in the next 10 years. Rietjens et al. (2020) demonstrated that 10-year CVD risk scores stayed unchanged after a three-month submarine deployment, but triglycerides declined and fasting free fatty acids increased with adiposity in submariners.

Hypercholesterolemia was detected in 51% of Brazilian cadets, where 24% presented high levels of LDLs and 11.2% presented low levels of HDLs (Hilgenberg et al., 2016). In Chinese officers, hyperlipidemia was observed in 21% (range 17–26%) (Mara et al., 2018). Higher than recommended levels of total cholesterol were found in 8% of U.S. recruits, LDLs in 39%, triglycerides in 5%, and HDL were below recommended levels in 33% of recruits at baseline (Pasiakos et al., 2012). In Polish soldiers, hypercholesterolemia was observed in half of the participants and abnormal LDLs in 60% (Gielerak et al., 2020). High triglycerides and low HDLs were found in 36% and 13%, respectively.

Hypertension was observed in 15% of male Brazilian cadets (Hilgenberg et al., 2016), 47% (95% CI 42–52%) of Chinese officers (Mara et al., 2018), and 21% of Polish soldiers (Gielerak et al., 2020). In addition, prevalence of hypertension increases with age in military populations (Gielerak et al., 2020; Mara et al., 2018). Combat exposure and combat injury seem to increase risk of hypertension in military veterans (Egan et al., 2020).

In military operations, inflammatory markers have been hypothesized to be positively associated with psychological stress symptoms (Groer et al., 2015) and different physical training loads, like load carriage (Pasiakos et al., 2016). Emphasizing energy intake could attenuate the inflammatory response (Pasiakos et al., 2016). Thus, the relation between inflammatory markers and CVD risk is complex and not properly studied in the military.

Few studies have examined the chronic effect of diet in preventing CVD risk in military personnel. An Iranian cross-sectional study demonstrated that diet quality had no significant relationship with CVD risk factors among male soldiers, but increased sodium intake predicted an elevated risk of CVD development (Parastouei et al., 2021). In Brazilian cadets, prevalence of CVD risk factors and dietary patterns was studied (Hilgenberg et al., 2016). High prevalence of dyslipidemia (51%) and hypertension (15%) were observed, and overconsumption of saturated fat (87%) and inadequate fiber intake (93%) were documented among young soldiers (21.5 ± 1.2 yrs. males, 21.6 ± 0.9 yrs. females), but their associations were not examined.

To conclude, military populations cannot avoid the worldwide increase of CVD risk factors. The prevalence rates seem to be higher in Asian and Latin American military populations than in Western military populations. Thus, investigation of CVD risk factors is necessary to reveal whether soldiers are at higher risk for CVD, and furthermore, promote healthy behavior to prevent progression of CVD in military populations (Baygi et al., 2020).

2.4 Energy balance and body composition in soldiers

Energy balance is calculated by energy intake (EI) minus energy expenditure (EE), usually defined as MJ $\cdot d^{-1}$ or kcal $\cdot d^{-1}$. In military studies, energy expenditure is measured with the doubly labeled water (DLW) technique (e.g., Ahmed et al., 2020; Fallowfield et al., 2014; Hoyt et al., 2006; Margolis et al., 2016), which is considered the gold standard for energy expenditure assessment. In DLW, regular sampling and the expense of isotopes limits its use. Therefore, other methods, such as accelerometers, have been widely used in field trainings and conditions where sampling and isotope storage is not possible (e.g., Charlot et al., 2022). In addition, accelerometers record data of different intensities of physical activity. Some studies have derived total energy expenditure values by measuring resting energy expenditure, calculating thermogenesis and adding exercise induced energy expenditure (e.g., Mullie et al., 2019). Also, continuous monitoring of heart rate or heart rate variability can be used to predict maximal oxygen uptake, and further, energy expenditure (Smolander et al., 2011).

Energy intake is estimated by food diaries (Fallowfield et al., 2014), food diaries with dietary weighing (e.g., Edwars et al., 2022a), dietary assessment tools (e.g., Beals et al., 2019) or a left-over method when food rations are known beforehand (e.g., Charlot et al., 2020). Some studies reported a food log method, in which a list of items was provided, and participants filled the percentage of consumed item (e.g., Margolis et al., 2016). Retrospective methods, such as a food frequency questionnaire (e.g., Carlson et al., 2013), nutrition history questionnaire (e.g., Beals et al., 2015), and 24-hour dietary recall (e.g., Øfsteng et al., 2020) have been used solely or simultaneously with other methods.

2.4.1 Energy expenditure and intake

An overall description of energy requirements for different military tasks has been published by Tharion et al. (2005). Mean energy expenditure was 19.3 ± 2.7 MJ, with a total range of 13.0-29.8 MJ $\cdot d^{-1}$. Average duration of studied period was 12.2 days (range from 2.25 to 69 days). Combat training produced higher EE rates than support tasks. Hot environments did not influence EE values, but cold and high-altitude exposures tended to increase values (Tharion et al., 2005). In the review by Charlot (2021), mean EE values were lower 17.4 ± 4.1 MJ $\cdot d^{-1}$ (10.3 – 28.3 MJ $\cdot d^{-1}$) than in the previous paper by Tharion et al. (2005), although the average duration (11.5 ± 7.7 d, range 3 – 34 d) was quite similar in both studies. Charlot (2021) summarized that mean EI values were 10.8 ± 2.9 MJ $\cdot d^{-1}$ (range 2.7 – 16.8 MJ $\cdot d^{-1}$), resulting in a mean EB of -6.7 ± 4.4 MJ $\cdot d^{-1}$ (-19.7 – 0.9 MJ $\cdot d^{-1}$) and a loss of body mass -0.25 ± 0.19 kg $\cdot d^{-1}$ (-0.85 – -0.01 kg $\cdot d^{-1}$).

Conditions in crisis management operations are somewhat comparable to conditions in garrisons. Daily duties include military tasks, like patrolling, guarding, and physical training sessions. Occupational workload can be low, but operational environments can change rapidly, and soldiers have to maintain their military readiness continuously (Pihlainen et al., 2018). In garrisons, energy expenditure levels have been reported to be 14.4 MJ $\cdot d^{-1}$ (3439 kcal $\cdot d^{-1}$) for support soldiers, 14.9 MJ $\cdot d^{-1}$ (3559 kcal $\cdot d^{-1}$) for transport units (Tharion et al., 2005), and 13.4 MJ $\cdot d^{-1}$ (3204 kcal $\cdot d^{-1}$) in support and special force soldiers (Tharion et al., 2004). These numbers are comparable to the civilian population matched by age and gender (Tharion et al., 2005). Fallowfield et al. (2014) reported that mean EE was 15.2 MJ $\cdot d^{-1}$ (3623 kcal $\cdot d^{-1}$) during deployment in Afghanistan. Edwards et al. (2022a) published that EE values were the lowest in camp training (males 4264 ± 581, females 3714 ± 132 kcal $\cdot d^{-1}$) and the highest in field exercise (males 5361 ± 539, females 4420 ± 391 kcal $\cdot d^{-1}$). Mixed camp and field training resulted in energy expenditure values close to field exercise alone (males 4371 ± 579, females 3546 ± 163 kcal $\cdot d^{-1}$) (Edwards et al., 2022a).

Energy intake is highly dependent on food supply for soldiers. Dining halls and restaurants in military bases serve regular meals and snacks, where soldiers have multiple choices of fresh food, main courses and desserts. Dining facilities in crisis management operations can vary, but several camps deliver daily meals and fresh food for the soldiers, like in garrison. In addition, snacks and beverages can be consumed *ad libitum*. In some situations, field rations or ready-to-eat meals are utilized, for example during patrolling, where fresh food is not available. Only a few studies have examined energy intake during crisis management operation, where mean energy intake levels varied from 1807-2529 kcal $\cdot d^{-1}$ (Lindholm et al., 2012; Fallowfield et al., 2014). In a 6-month follow-up, energy intake dropped at mid-measurement (3 months), but recovered in post (Fallowfield et al., 2014). Thus, regular screening of diet and body composition is important to reveal changes during longer deployments. Rietjens et al. (2020) estimated positive energy balance in submariners, but no significant change in body mass. Energy expenditure and intake values during crisis management operations are presented in Table 2.

TABLE 2. Energy expenditure, energy intake, energy balance and change in body mass in crisis management operations and submarine deployment.

Author, year	**Military operation, length**	**Mean energy expenditure** MJ ·d^{-1} (kcal ·d^{-1})	**Mean energy intake** MJ ·d^{-1} (kcal ·d^{-1})	**Energy balance** MJ (kcal)	**Δ in body mass**
Lindholm et al., 2012	Crisis management in Tšad, 4 mo	10.4 (2484) [b]	7.6 (1807) #	NA	-3.5%
Fallowfield et al., 2014	Crisis management in Afghanistan, 6 mo	15.2 (3623) [a]	11.0 (2529) #	NA	- 4.6 ± 3.7% (0-3 mo) +2.2 ± 2.9% (3-6 mo)
Rietjens et al., 2020	Submarine deployment, 3 mo	12.3 ± 2.1 (2937 ± 498) [a]	13.2 ± 3.3 (3158 ± 786) ##	+0.9 ± 2.1 (+221 ± 506)	−1.4 ± 3.4 kg (NS)

NS = not statistically significant, NA= not assessed, mo = month

[a] doubly labelled water, [b] accelerometer/heart rate monitor.

#food diary method, ##derived from energy expenditure and body composition.

Search was done via Pubmed by keywords "deployment /crisis management, soldier/military, energy balance / energy". Papers measuring energy intake and expenditure were included.

Energy balance during strenuous field training is typically highly negative. Several factors influence energy expenditure and/or energy intake depending on the conditions, and a great interindividual variation needs to be considered when evaluating mean values. Energy expenditure, intake and balance in survival trainings are shown in Table 3. Very high energy expenditure values were measured in arctic trainings (Castellani et al., 2006; Hoyt et al., 2006; Margolis et al., 2016) where expenditure was as high as 26.6 ± 2.0 MJ ·d^{-1} (Hoyt et al., 2006). Similar energy expenditure values were observed during the Tour de France cycling race, where mean daily energy expenditure was 25.4 MJ, and the highest mean value was as much as 32.7 MJ ·d^{-1} (Saris et al., 1989). Females were examined in two studies, where mean energy expenditure was 19.8 MJ ·d^{-1} (Castellani et al., 2006) and 21.9 ± 2.0 MJ ·d^{-1} (Hoyt et al., 2006). Duration of trainings varied from 2.25 d (Castellani et al., 2006) to 20 d (Kyröläinen et al., 2008).

In field training, soldiers often carry combat rations and fluids for the mission. Continuous use of combat rations may lead to greater negative energy balance (Tassone & Baker, 2017). Longer durations of field exercise (20–30 d) diminish the magnitude of energy deficit; daily energy intake seems to become closer to estimated energy expenditure (Tassone & Baker, 2017). If energy content of combat rations is increased from 7.5–8.9 MJ to 15.0–16.8 MJ, energy intake was 40–82% higher than in normal rations (Charlot, 2021). The compensatory effects of different supplements (CHO, PROT, CHO+PROT) on energy deficit have been examined (e.g., Castellani et al., 2017; Margolis et al., 2016; Tanskanen et al., 2012). Energy intake was increased by protein supplements (Tanskanen et al., 2012) and CHO supplements (Margolis et al., 2016).

Cold exposure increases energy expenditure by approximately 7% through thermogenesis of brown adipose tissue, but high intensity shivering in very extreme conditions can elevate resting metabolic rate +250% (McInnis et al., 2020). Ambient temperature also influences caloric intake, partially by regulation of appetite-mediating hormones. In moderately cold environments, mean increase in energy intake is +586 kJ (138 to 1124 kJ), which exceeds the increase of energy expenditure +144 kJ (−137 to 500 kJ). Therefore, cold exposure does not induce energy deficit if energy intake is not restricted and expenditure remains at a moderate level (McInnis et al., 2020; Millet et al., 2021). However, in military contexts, winter equipment, tasks in snow and/or high altitude, and load carriage contribute to energy expenditure, so the conditions are not comparable.

During survival field training, energy intake can be restricted on purpose. Hoyt et al. (2006) reported 1.43 MJ energy content for a 6-person squad for one week. Delivered food items were smoked eel (250 g), a liver paste (500 g), and half of a chicken (750 g), providing 6 g carbohydrate, 50 g protein, and 24 g fat to each person for the week (Hoyt et al., 2006). During another Norwegian survival training study, soldiers were delivered food rations according to their body mass (BM) (1050 $\text{kcal}\cdot\text{d}^{-1}$ for 56–65 kg BM, 1200 $\text{kcal}\cdot\text{d}^{-1}$ 66–75 kg BM, etc.). Rations contained egg, ham, bread and 100% whey protein powder (Øfsteng et al., 2020). Energy content of rations can be reinforced to increase energy intake in harsh conditions. In a two-week arctic expedition, soldiers were delivered daily field rations (4500 kcal) containing dried meat and cheese, energy and chocolate bars, soups, cakes and candies, and ready-to-eat items providing higher energy intake and macronutrient distribution: CHO 43 E%, PRO 12 E%, fat 44 E% (Charlot et al., 2020).

TABLE 3. Energy expenditure, energy intake, energy balance and change in body mass in strenuous and/or winter field trainings.

Author, year	**Military operation**	**Mean energy expenditure MJ ·d^{-1} (kcal ·d^{-1})**	**Mean energy intake MJ ·d^{-1} (kcal ·d^{-1})**	**Energy balance MJ (kcal)**	**Δ in body mass**
Jones et al., 1985	Arctic field exercise, 10 d	18.1 ± 3.8 (4317 ± 927) [a]	11.0 ± 2.1 (2633 ± 499) [e]	NA	-0.63 ± 0.83 kg
Castellani et al., 2006	Winter field training, 2.25 d	25.7 (6138) (males) 19.8 (4729) (females) [a]	6.0 ± 2.0 (1433 ± 478) (males) 4.8 ± 1.8 (1146 ± 430) (females) [e]	- 43.2 ± 10.4 (10342 ± 2484) (males) - 34.0 ± 6.0 (8121 ± 1433) (females) * total energy deficit over 2.25 d	-3.1 ± 0.8 kg (males) -1.6 ± 0.5 kg (females)
Hoyt et al., 2006	Ranger field exercise, 7 d	26.6 ± 2.0 (6353 ± 478) (males) 21.9 ± 2.0 (5231 ± 478) (females) [a]	0.2 to 2.2 (48 to 549) (males) 0.2 to 1.9 (48 to 454) (females) [e]	NA	-7.7 ± 1.1 kg (males) -5.9 ± 1.1 kg (females)
Kyröläinen et al., 2008	Field exercise, 3 weeks	24.3 (5800)	12.3 ± 1.9 (2 938 ± 454) [e]	-16.7 (-4 000) (1st) -1.9 (-450) (2nd) -4.2 (-1000) (3rd)	-4.2 ± 0.8 kg (-5.6 ± 0.9%)
Tanskanen et al., 2012	Winter field exercise, 8 d	20.2-23.3 (4825 -5565) [a] (range of means)	10.9-13.3 (2603-3177) [e] (range of means)	NA	NA
Margolis et al., 2014	Military task training (MTT) 4-day, Ski march (SKI) 3-day	22.9 ± 1.6 (5480 ± 389) (MTT), 28.7 ± 2.4 (6851 ± 562) (SKI) [a]	13.0 ± 1.0 (3098 ± 236) [e](MTT), 14.5 ± 2.5 (3461 ± 586) [e] (SKI)	NA	NA
Margolis et al., 2016	Arctic training, 4 d, with 3 intervention groups	25.8 (6167) (PRO) 25.7 (6181) (CHO) 25.5 (6096) (con) [a]	11.8 (2825) 13.1 (3131) 10.5 (2506) [e]	-14.2 (-3402) -12.8 (-3050) -15.0 (-3595)	NA
Castellani et al., 2017	Ski march, Study 1, 3 d, Study 2, 4 d	Study 1 28.5 ± 2.4 (6821 ± 578) Study 2 26.8 ± 2.3 (6394 ± 54) [a]	Study 1 14.5 ± 2.6 (3465 ± 622) Study 2 11.4 ± 3.3 (2714 ± 799) [e]	Study 1 −14.0 ± 2.9 (−3357 ± 691) Study 2 −15.8 ± 4.2 (−3782 ± 1001)#	NA
Beals et al., 2019	Mountain warfare, cold, 3 d	16.4 ± 1.2 (3913 ± 293) (river crossing) 22.8 ± 3.5 (5457 ± 828) (mountain patrol) [d]	11.9 ± 2.7 (2854 ± 657) (river crossing) 9.6 ± 2.8 (2289 ± 680) (mountain patrol) [h]	-4.6 ± 3.3 (-1044 ± 784) (river crossing) 13.0 ± 5.9 (-3112 ± 1420) (mountain patrol)	NA
Ahmed at al., 2020	Arctic field training, 5 d	20.6 ± 2.9 (4917 ± 693) [a]	9.95 ± 4.8 (2377 ± 1144) [e]	-10.6 ± 5.8 (-2539 ± 1396)	- 2.7%
Charlot et al., 2020	Arctic expedition, d1-7, d8-14	15 ± 9 (both periods) [b]	12.6 ± 2.1 (d1-7) 14.7 ± 3.4 (d8-14) [e]	-2.3 ± 2.4 (d1-7) -0.4 ± 3.6 (d8-14)	-1.1 ± 1.8 kg, -1.3 ± 2.2%, NS
Øfsteng et al., 2020	Strenuous field exercise, 10 d, low prot vs high prot	23.2 ± 5.5 (5536 ± 1305) 22.7 ± 4.3 (5427 ± 1029) [c]	4.9 ± 0.7 (1183 ± 168) 4.9 ± 0.7 (1174 ± 170) [g]	-18.8 ± 5.2 (-4373 ± 1250) -17.9 ± 4.5 (-4271 ± 1075)	-6.1 ± 2.4%, -5.2 ± 1.9%

CHO= carbohydrate group, PRO=protein group, con=control group, NA= not assessed, NS = not statistically significant

a doubly labelled water, b accelerometer/heart rate monitor, c calculation, d observation and heart rate-VO2 regression, food diary, f derived from energy expenditure and body composition, g questionnaire, h ASA24-assessment tool

Search was done with Pubmed using keywords “survival/winter, soldier/military, energy balance / energy”. Papers measuring energy intake and expenditure were included

In addition, energy availability (EA) has been used to estimate adequate energy intake to maximize training response, prevent hormonal disturbances, and maintain bone health (Loucks et al., 2011). Energy availability is calculated as follows:

$$\textit{Energy availability [kcal}\cdot\textit{kg FFM}^{-1}\cdot\textit{d}^{-1}\textit{]} = \textit{(Energy Intake [kcal}\cdot\textit{d}^{-1}\textit{]} - \textit{Exercise Energy Expenditure [kcal}\cdot\textit{d}^{-1}\textit{])/Fat-Free Mass [kg].}$$

The optimal level of EA for athletes has been determined as > 45 kcal ·kgFFM^{-1} ·day^{-1}, where diet and training are well-balanced, and disturbances are unlikely in body function (Loucks et al., 2011). Reduced EA is 30-45 kcal ·kgFFM^{-1} ·day^{-1}, which diminishes testosterone levels in men, and increases risks of impaired physical performance and physiological function. Low EA (< 30 kcal ·kgFFM-1 ·day^{-1}) limits the energy supply for growth, cellular function, thermoregulation, and the reproductive system.

Few studies have examined energy availability in soldiers, and EA remained low in all studies (Edwards et al., 2022a; Garron & Klein, 2023; Gifford et al., 2021; Mullie et al., 2019; Mullie et al., 2021). Associations with hormonal responses were not evaluated in these studies. In the study by Gifford et al. (2021), higher EA was related to improved 1.5 mile running time during the 11-month follow-up.

2.4.2 Body composition

Measurements of body composition (body mass, fat-free mass, fat mass, fat percent) have been used as a marker of physical training, energy balance, or physiological stress in military contexts. Body composition is typically measured by dual x-ray absorptiometry (DXA) and by bioimpedance (BIA) (Potter et al., 2022). Many commercial devices are available for BIA, based on multi-frequency or single-frequency system. Serum creatinine and cystatin C concentrations correlate with fat-free mass and fat mass, respectively (Kim et al., 2016). Skinfold measurements have been used to estimate fat percent and waist circumference for abdominal fat, which, in addition to bioimpedance methods, are suitable for field conditions.

As shown in Table 2, mean changes in body composition are controversial in crisis management operation studies: body mass decreased (Rintamäki et al., 2012; Sharp et al., 2008; Warr et al., 2013), increased (Dyrstad et al., 2007; Lester et al., 2010) and remained stable (Fallowfield et al., 2014; Farina et al., 2017; Nagai et al., 2016; Sedliak et al., 2021). Lester et al. (2010) reported increases in fat-free mass, fat mass and fat percent. In a 6-month follow-up study, body mass, fat mass and fat-free mass decreased during the first half of the period, but then recovered close to baseline values (Fallowfield et al., 2014). Sedliak et al. (2021) presented that body mass was maintained while fat percent decreased.

Deployments in submarines differ from crisis management operations as a result of limited sleep and physical activity (Gasier et al., 2016; Rietjens et al., 2020). These deployment studies illustrate the variety of military tasks and their physiological responses. Rietjens et al. (2020) found unfavorable changes in body composition, although body mass was maintained during three months of patrolling. Fat percent increased and fat-free mass decreased. In a study by Gasier (2016), non-obese (38%) and obese (62%) submariners were compared at baseline and after a 3-month deployment. Individuals with obesity lost body mass, fat mass and fat percent, while non-obese participants maintained their body composition. Energy and carbohydrate intake decreased in obese participants during the study.

In survival training, where food intake is restricted, body mass loss can be as high as -7.5 ± 1.1 kg (males) or -6.0 ± 1.3 kg (females) during a 7-day training (Hoyt et al. 2006). Fat mass reduced by -3.5 ± 0.7 kg in males and -3.4 ± 0.2 kg in females, and fat-free mass decreased 4.0 ± 1.2 kg (males) and 2.6 ± 1.1 kg (females). In a 10-day field exercise, -6.1 ± 2.4% loss of body mass was reported (Øfsteng et al., 2020). Loss of body mass during survival and winter trainings are presented in Table 3.

When military trainings last less than 3 d, decreases in body mass is mainly caused by dehydration or loss of fluid (Tassone & Baker, 2017). During longer trainings, fat mass and fat-free mass are reduced depending on the magnitude of energy deficit and duration of training. Most of the studies presented a decrease in fat-free mass and fat mass (Ahmed et al., 2020; Hamarsland et al., 2018; Hoyt et al., 2006; Nindl et al., 2007), but two studies reported a decrease in fat mass, but not in fat-free mass (Øfsteng et al., 2020; Tanskanen et al., 2012).

2.5 Energy metabolism and its regulation

The body's energy balance is maintained when energy-containing nutrients (carbohydrates, proteins, fats, alcohol) are at the same level as energy expended. Total energy expenditure is quantified as the sum of basal metabolic rate, diet-induced thermogenesis and total physical activity. Fed and fasted states dictate alternation between absorptive and post-absorptive conditions. The absorptive state occurs after meals when energy storing pathways are activated, and the post-absorptive state occurs before meals and during sleep when energy releasing pathways are engaged. In fasting, energy is converted from glycogen (muscle and liver) and from adipose tissue. When glycogen stores are depleted, thus, gluconeogenesis is activated for glucose production. These reactions are regulated by hormonal and neural pathways (Chapelot & Charlot, 2019).

2.5.1 General principles of energy metabolism

Carbohydrates play a major role in energy supply at rest and during exercise (Bergström & Hultman, 1967). CHO-containing food is degraded to monosaccharides by amylase and absorbed from the intestine (Chandel, 2021). Monosaccharides such as glucose, galactose, and fructose can be converted to glycogen, which is stored in the liver and muscle. In the postprandial state, blood glucose concentration rises, then recovers near fasted state at 5.5 mmol $\cdot L^{-1}$ in healthy individuals (Chandel, 2021). Brain function is impaired in hypoglycemia, so activation of glycogenolysis (the breakdown of glycogen) and gluconeogenesis (*de novo* glucose synthesis) occurs to ensure stable blood glucose levels (Chandel, 2021). Secretion of hormones, such as insulin and its counterregulatory hormone, glucagon, plays a role in stimulating glucose transport into muscle tissue and the liver in a fed state (Rui et al., 2014). In the liver, glucose is metabolized into pyruvate, and completely oxidized via the citric acid cycle to synthesize ATP in oxidative phosphorylation in mitochondria (Rui et al., 2014). During exercise, ATP production pathways are activated according to exercise intensity and duration (Hargreaves & Spriet, 2020).

In energy deprivation, blood glucose levels are similar to a fasted state, and insulin production is impaired by the lack of exogenous glucose in shorter fasting (Pasiakos et al., 2011) and longer energy deficit (21 d) conditions (Berryman et al., 2018). Simultaneously, increased glucagon production activates glycogenolysis and gluconeogenesis (Rui et al., 2014). Intermittent fasting is a weight reduction program, where eating is restricted for alternate days or timing (Patterson et al., 2017). Such a modification in feeding and fasting can influence glucoregulatory biomarkers, decreasing glucose and insulin levels (Patterson et al., 2017), but responses are dependent on the frequency and duration of fasting and feeding periods (Wang & Wu, 2022). Although intermittent fasting is a program for weight loss, physiological responses may be alike in military field trainings.

Fatty acids are derived from lipase-mediated hydrolysis of dietary fats, which can be absorbed in the intestine, and transported to adipose tissue in the form of triglycerides. Adipose tissue contains an immense amount of energy, but the fat oxidation pathway, β-oxidation, is complex and requires many steps and enzymes. Free fatty acids circulating in plasma are broken down from triglycerides (Kimura et al., 2020). Plasma free fatty acids are bound to albumin, so the availability of albumin limits free fatty acid levels. All mitochondria-containing cells can uptake free fatty acids and metabolize them via β-oxidation and the citric acid cycle (Kimura et al., 2020). Short-term fasting (hours) increases lipolysis and free fatty acids are released into plasma to produce ketones in the liver and kidneys as an energy supply (Wang & Wu, 2022). In longer energy deficit (21 d), free fatty acid levels increase (Berryman et al., 2018). Semi-starvation activates fat oxidation more in females than in males (Hoyt et al., 2006).

Proteins are one of the indispensable nutrients, so absorption of peptides and amino acids from the intestine and storage in muscle tissue are effective processes. During the first hours of fasting, essential and total amino acids are reduced, and in longer fasting, essential amino acids decrease more than nonessential (Wang & Wu, 2022). In a 3-week energy deficit at high altitude, protein oxidation for energy substrates was 17% in a standard protein dose group and 33% in a high protein dose group, and both groups lost body mass (Berryman et al., 2018).

Ketosis is a metabolic state where a lack of dietary carbohydrates shifts energy oxidation from glucose to fat. Manipulation of dietary macronutrients by minimizing CHO intake has been used to achieve greater body mass loss compared to traditional weight loss programs (Coleman et al., 2021). Ketogenic diets have been shown to reduce body mass and visceral fat also in military, without compromising physical performance outcomes (LaFountain et al., 2019). In addition, ketogenic diet can maintain cognitive performance better than traditional CHO based diet during sleep deprivation (Henderson et al., 2022).

2.5.2 Regulation of energy metabolism

Energy homeostasis is defined as a stable state where energy intake and expenditure are balanced. It is also an adjustable mechanism when EI and/or EE are increased or decreased (Chapelot & Charlot, 2019). Physiological pathways regulating energy homeostasis can be divided into structures which interact and overlap simultaneously: afferent neurons and hormones linking the periphery to central nervous system, hypothalamus, brainstem and nucleus tractus solitarius, cortical regions, and efferent neurons of the sympathetic nervous system (Chapelot & Charlot, 2019). In this section, regulating hormones are discussed more precisely: appetite-mediating hormones (leptin and ghrelin), substrate hormones (insulin and glucagon), and overall anabolic-catabolic hormones (testosterone and cortisol).

Leptin is a 167-amino acid peptide, which belongs to the family of long-chain helico cytokines (Münzberg & Morrison, 2015). It is produced in adipose tissue and secreted into the bloodstream. Leptin signals the "status" of adipose tissue to the brain – both the amount and stability of fat stores – but leptin regulation is disturbed in obese individuals (Rosenbam & Leibel, 2014). Mutations found in leptin or its receptors were the first genetic mutations found to cause severe obesity, but, to date, obesity is likely caused by a dysregulation of central neuronal circuits involving leptin (Münzberg & Morrison, 2015). Exogenous leptin infusion has been tested as a treatment of obesity, by activating pathways and lowering energy intake, but the response is not dose-dependent (Rosenbam & Leibel, 2014).

Ghrelin is a 28-amino acid peptide produced primarily by gastric oxyntic cells, and partially by the duodenum, ileum, cecum, and colon (Stanley et al., 2005). Plasma ghrelin levels are regulated by diurnal rhythm and food intake, so plasma ghrelin levels increase while fasting, and fall after food intake. Thus, ghrelin is an orexigenic factor (Stanley et al., 2005). Increase in ghrelin in energy deficit stimulates appetite, driving energy intake and energy homeostasis (Stanley et al., 2005).

Leptin secretion is impaired, and ghrelin is elevated in acute energy deficit (Pasiakos et al., 2011). Three groups (CHO, CHO-fat, CAL-DEPR) were studied in randomized and placebo-controlled trial where leptin, ghrelin, insulin and glucose were tested over 14 hours. Participants in caloric deprivation (CAL-DEPR) received 255 kJ energy vs. 3776 kJ (CHO) and 3744 kJ (CHO-fat) over the studied period. Leptin decreased in the CAL-DEPR group compared to other groups, while ghrelin levels stayed elevated in CAL-DEPR (Pasiakos et al., 2011). Blood-glucose and insulin peaked after meals in CHO and CHO-fat groups coinciding with normal glucose metabolism in a fed state.

Circulating testosterone and cortisol are potential markers of metabolic stress, and widely used in athletes (Lee at al., 2017). Testosterone is the male sex hormone, made and secreted by the testicles, whereas cortisol is produced by the adrenal cortex. Testosterone has anabolic effects and cortisol is catabolic. The testosterone-cortisol ratio (T/C ratio) is sensitive to multi-stressor conditions (Tait et al., 2022). In laboratory analysis, free testosterone and total testosterone can be defined. Free testosterone is based on total testosterone and testosterone-binding proteins. Energy deficit diminishes testosterone production while cortisol increases. Other anabolic hormones, such as insulin, growth hormone, insulin-like growth factor- 1 (IGF-1), and others react to stress – typically reducing under physiological strain. Energy deficit impairs IGF-1 production and diminishes plasma branched-chain amino acids and, therefore, would metabolic responses under caloric deprivation (Gomez-Merino et al., 2004). Decreases in IGF-1 and increases in cortisol were observed during severe energy restriction in males and females, but both hormones recovered to baseline values within one week (Vikmoen et al., 2020).

2.5.3 Energy metabolism in military studies

Severe energy restriction and loss of lean body mass are related to attenuation of testosterone, free testosterone, T/C ratio and increase of cortisol (e.g., Berryman et al., 2018; Hamarsland et al., 2018; Kyröläinen et al., 2008; Øfsteng et al., 2020; Szivak et al., 2018). In Berryman's study (2018), free testosterone and cortisol decreased, but mean total testosterone did not change in energy deficit and at high altitude. Therefore, low-dose testosterone supplementation has been studied to minimize loss of fat-free mass (Stein et al., 2022) and to induce responses in ghrelin and appetite (Karl et al., 2020). Circulating ghrelin levels were maintained both in testosterone supplement and control groups, with no disturbances on satiety. Stein et al. (2022) observed increases of androgenic steroid metabolites, acylcarnitines and decreases in amino acid metabolites with testosterone supplementation, and these changes were associated with beneficial changes in lean body mass. Fat mass changes were inversely associated with changes in acylcarnitines.

In a study by Szivak et al. (2018), soldiers with better physical fitness returned to baseline levels of norepinephrine and neuropeptide Y concentrations more quickly after survival training. Hamarsland et al. (2018) observed hormonal recovery in one week for testosterone, IGF-1 and thyroxine but not cortisol after a 1-week arduous military course.

Substrate oxidation and whole-body protein turnover were measured from physically active men (18-42 yrs) during energy deficit at high altitude for 21 days (Berryman et al., 2018). Participants were divided into two groups, where one group got standard protein (SP) dose (1 g $\cdot$kg^{-1} $\cdot$d^{-1}) and another high protein (HP) intake (2 g $\cdot$kg^{-1} $\cdot$d^{-1}) during exposure. Whole body FFM or FM did not differ between the groups. Relative distributions of substrates were: 40% fat, 43% CHO, and 17% protein in the SP group, while the HP group exhibited 31%, 36%, and 33%, respectively. The HP group oxidized more protein than the SP group and, furthermore, whole-body net protein balance was more negative in the HP than the SP group. Based on these findings, higher protein intake did not protect from loss of FFM in negative energy balance (Berryman et al., 2018).

During a six-month deployment in Afghanistan, leptin and ghrelin were measured three times (Hill et al., 2015). Leptin levels were correlated with body fat at all three time points, and changes in leptin and ghrelin levels were related to changes in fat percentage. Appetite-mediating hormones seem to respond to changes in body fat, rather than drive body composition changes (Hill et al., 2015). These findings were corroborated by a study in overweight submariners during a 3-month deployment (Gasier et al., 2016).

In addition to single biomarkers, metabolomics could give new insights into an individual's physiology and chemical map (Surendran et al., 2022). Metabolomics is the determination of large-scale biomarkers from different metabolic pathways. Over 900 metabolites have been identified and classified into eight metabolic subclasses: lipids (33%), amino acids (17%), xenobiotics (10%), nucleotides (3%), peptides (2%), carbohydrates (2%), cofactors and vitamins (2%), 'energy' (1%), and additional compounds (31%). Metabolomics have also been examined in military contexts (Accardi et al. 2016; Albores-Mendez et al. 2022; Gwin et al. 2022; Karl et al. 2017; Stein et al. 2022). In soldiers, objective and analytical implications of molecular nutrition could improve understanding of nutritional and health status and, thus, optimize operational readiness (Accardi et al., 2016). Accardi et al. (2016) summarized that high-resolution metabolomics platforms, which detects 61 metabolites, is suitable for evaluating amino acid, fat and mitochondrial metabolism, but further research is needed for vitamins and coenzymes.

2.6 Energy balance and physical performance in crisis management operations and survival trainings

Acute or prolonged negative energy balance leads to a decrease in body mass and loss of fat-free mass. Simultaneously, a wide range of physiological responses have been reported, e.g., impairment of hormonal status and health outcomes. Studies also present confounding outcomes for physical fitness variables (maximal strength, muscular endurance and aerobic/anaerobic capacity), which are direct and important factors of military performance (Figure 1). In addition, cognitive capacity and shooting performance contribute to overall operational readiness of soldiers. Thus, associations between energy variables or dietary factors and physical performance have not been clearly established in military populations.

Changes in physical performance during crisis management operations have previously been summarized (Pihlainen, 2021). Aerobic fitness decreased in four studies (Dyrstad et al., 2007; Lester et al., 2010; Sharp et al., 2008; Warr et al., 2013), increased in one study (Sedliak et al., 2021), and stayed unchanged in 3 studies (Fallowfield et al., 2014; Nagai et al., 2016; Warr et al., 2013). Results in strength tests are controversial as a result of the complexity of test patterns. Overall, muscular endurance was maintained or increased in four studies (Dyrstad et al., 2007; Rintamäki et al., 2012; Fallowfield et al., 2014; Sedliak et al., 2021), as well as strength and power of the upper and lower extremities in all but one (Sharp et al., 2008) of the previously mentioned studies. Dietary intake was evaluated in three studies (Fallowfield et al. 2014; Farina et al., 2017; Lindholm et al., 2012) and energy balance in two studies (Fallowfield et al., 2014; Lindholm et al., 2012), but associations and other statistical analyses between dietary intake and physical performance were not evaluated.

Survival or other arduous trainings under severe energy deficit cause decreases in muscle strength variables. An 8-week U.S. Army Ranger course implemented of intermittent caloric deprivation (-1000 kcal $\cdot$d^{-1}) following 5-6 d of recovery with overfeeding (300–500 kcal $\cdot$d^{-1}). The results found a loss of body mass from 78.4 ± 8.7 to 68.4 ± 7.0 kg (13%) and impairment in lower-body power, incremental dynamic lift strength, and vertical jump (Nindl et al., 2007). Lower-body power, but not maximal lifting strength, was associated with changes in fat-free mass. Recovery from the 8-week arduous training was completed in five weeks, when changes in body mass, hormonal responses and physical performance returned to baseline (Nindl et al., 1997).

Tanskanen et al. (2012) observed an increase of energy intake in a protein bar supplement group compared to controls in an 8-day winter military training of conscripts. However, no differences were found in energy deficit, physical activity, change in physical performance, or change in fat mass between groups. The supplement group also developed more positive moods and less hunger. Changes in performance tests were not associated with energy availability, defined as EI/estimated basal metabolic rate (Tanskanen et al., 2012).

In a 10-day military field exercise with severe energy deficit, 1 RM bench press, 1 RM leg press, counter movement jump (CMJ), and mean and peak power during Wingate test decreased. During 7-d recovery, all strength values, except for CMJ, recovered toward pre-intervention values. Supplemental protein did not affect physical performance impairment or loss of body mass in energy deficiency when comparing high and low protein groups (Øfsteng et al., 2020). However, any statistical analyses between protein intake and physical performance outcomes were not examined.

A daily 5.1 MJ nutritional supplement were delivered during an 8-week military training (Fortes et al., 2011). Beneficial effects from the energy supplement were observed in body mass, fat-free mass, fat mass, maximal dynamic lift strength, vertical jump, and explosive leg power. Testosterone, testosterone/cortisol ratio nor IGF-1 differed between supplement and control groups (Fortes et al., 2011). In a study by Ross et al. (2020), moderate or high dose of protein could enhance knee flexion and extension performance, independent of energy intake. Energy balance values were not presented, as well as associations between protein intake and strength tests. However, increasing protein intake (1.5-2.0 g·kg^{-}

$^{1}\cdot d^{-1}$) maintains muscle anabolism and other muscle tissue level metabolism in military-like energy deficiency (Pasiakos et al., 2015).

Muscle strength, measured by leg press, bench press, and jump height, reduced during a 1-week arduous course and stayed below baseline after one-week recovery (Hamarsland et al., 2018). Similar results were observed in a study by Vikmoen et al. (2020) where body composition and explosive strength returned after one week in both males and females. Physical performance responses to energy deficit were quite similar in both genders, but explosive strength (measured by CMJ) recovered faster in females than in males (Vikmoen et al., 2020).

According to U.S. Institute of Medicine's Committee on Military Nutrition Research, body mass loss of more than 3% but less than 10%, does not likely impact performance of soldiers (Tassone & Baker, 2017). When body mass loss is more than 10%, impact on performance is very likely. However, rapid weight loss, common in weight class sports, of 6% in a week was related to poorer cognitive performance (increased fatigue, tension, depression, and poor short-term memory) (Choma et al., 1998). Similar results were also found in a randomized and controlled study in soldiers, where increased fatigue and impaired self-control were reported in an energy deficit group, but not in an energy balance group (Beckner et al., 2023).

In a review by O'Leary et al. (2020), energy deficit (500-4000 kcal $\cdot d^{-1}$) was related to decrease in muscle strength and power in 11 studies. Power of the lower extremities decreased by 9–28% in six studies, and in the upper extremities in three studies. Three studies did not report a decline in lower body strength or power. Results of aerobic capacity are controversial. Impairment of aerobic capacity was observed in two studies with short-term energy deficit. Longer deployments (6-13 months) resulted maintained or decreased aerobic performance.

In a meta-analysis by Murphy et al. (2018), changes in physical performance were not related to daily energy balance or duration of military training but were associated with total energy deficit through the training period. Strength and power in lower extremities decreased approximately 2% when total energy deficit was from -5686 to -19,109 kcal, and 7-10% when total energy deficit was -39,243 to - 59,377 kcal during the entire operation. Body mass reduction under these deficit conditions were ≤ 3% and ≤ 8%, respectively (Murphy et al., 2018). These findings disagree with a systematic review by Tassone & Baker (2017) in which 3–10% reduction in body mass did not likely influence physical performance.

It is notable that aerobic capacity is often measured by maximal oxygen uptake test (VO_2max ($ml\cdot kg^{1}\cdot min^{-1}$)) and reduced body mass will compensate to response to aerobic performance. Knapik et al. (1987) found that a 3.5-day fasting did not influence aerobic or anaerobic performance, or isometric strength, indicating that energy deficit might be independent of physical performance outcomes. Sleep deficit or other compounding stressors, which are typical characteristics of military field trainings, would be dependent factors of impaired physical performance.

In conclusion, declines in physical performance observed in crisis management operations, other deployments, and especially strenuous field trainings, with energy deficit, reduce body mass and have hormonal disturbances. Direct associations between energy intake, expenditure or balance, and physical performance were not found. A threshold of energy intake, where physical performance could be maintained in military context, also has not been established. Sufficient protein intake enhances tissue-level metabolism in muscle.

3 PURPOSE OF THE THESIS

The overall purpose of the thesis was to investigate how nutrition is related to hormonal and metabolic status, and physical performance of soldiers in a crisis management operation (I, II) and in winter survival training (III, IV).

Purpose	Original paper
to describe changes in dietary macronutrient and energy intake, and total physical activity and their associations with body composition during a 6-month deployment.	I Diet macronutrient composition, physical activity and body composition in soldiers during six months deployment
to investigate associations of dietary intake and body composition with CVD risk factors and pro-inflammatory biomarkers during a 6-months crisis management operation.	II Associations of nutrition and body composition with CVD risk factors in soldiers during a 6-month deployment
to examine the effect of a 10-day winter survival training period on body composition, energy balance, appetite-mediating hormones, substrate metabolites and electrolytes, and to compare changes in biomarkers between an exercise (FEX) and a recovery (REC) group.	III Changes in body composition, energy metabolites and electrolytes during winter survival training in male soldiers
to observe energy intake, expenditure, and balance, and their associations with hormonal status, strength, endurance and shooting performance in strenuous field exercise, and to investigate effects of a 36-hour recovery on these outcomes in soldiers.	IV Energy balance, hormonal status, and military performance in strenuous winter training

The synthesis of these four studies describes the multi-dimensional role of nutrition in military performance under varying contexts. This dissertation gives new insights into diet and its association with CVD risk factors during a crisis management operation, and physiological responses of severe energy deficit during winter survival training among Finnish soldiers. The wide range of military tasks and their nutritional and physiological demands will give more comprehensive understanding to military nutrition. Documentation of physiological responses in different conditions would promote optimal education, preparation, recovery, and operational readiness of soldiers in various military contexts.

4 METHODS

4.1 Participants

This dissertation is based on two research projects: a crisis management operation in Lebanon, and winter survival training in Lapland. Finnish male soldiers, who were deployed for six months in a military crisis management operation in Lebanon, voluntarily took part into studies I and II. Number of participants, age, height, body mass and body mass index are presented in Table 4. They were informed of the study design and gave their written consent to participate. The study was planned and carried out according to the guidelines of the Ethical Committee of the Central Finland Health Care District.

Finnish conscripts were recruited to studies III and IV. Studies were part of a larger multidisciplinary research project authorized by the Finnish Defence Forces (AO1720). The ethical review of the study was granted by the Scientific and Ethical Committee of the Helsinki University Hospital Research (HUS/900/2018). All participants were informed of the experimental design, methods, benefits, and possible risks prior to signing an informed consent document. Characteristics of participants are shown in Table 4.

TABLE 4. Characteristics of participants in each study (mean ± SD).

Original paper	**n**	**Age (years)**	**Height (cm)**	**Body mass (kg)**	**Body mass index (kg·m^{-2})**
I	40	29.5 ± 8.4	178.7 ± 6.5	77.8 ± 8.7	24.4 ± 2.7
II	35	30.0 ± 8.7	178.6 ± 6.3	77.1 ± 8.9	24.2 ± 2.5
III, IV					
Recovery group (REC)	26	19.7 ± 1.2	181.1 ± 5.8	78.2 ± 9.6	23.9 ± 2.7
Field exercise group (FEX)	42	19.6 ± 0.8	179.4 ± 6.2	74.4 ± 10.7	23.1 ± 2.8

4.2 Experimental design

Original articles I and II were six-month observational follow-up studies of a crisis management operation. Measurements were conducted three times (0 mo, 3 mo, 6 mo) during the operation in Southern Lebanon from May to the end of November. Baseline measurements were performed after a minimum of two weeks acclimatization period in the operational area. The study was planned and carried out according to the guidelines of the Ethical Committee of the Central Finland Health Care District.

The main purpose of the crisis management operation was to monitor the cessation of hostilities and support of the Lebanese government – armed forces as well as the local population. Typical soldiers´ duties were patrolling 4-6 hours at the operational area, mainly by vehicles, and guarding the military base. The duties were performed in three shifts around the clock. The security situation was relatively calm, although tension at the operational area increased in the mid-phase of deployment. The military base was situated on a hill (775 m above the sea level) with a mean temperature of 22.3 ± 4.3° C (range 11–36° C) and mean humidity of 54 ± 17% (Thermochron, iButton, Maxim Integrated, San Jose, California, USA).

Data in articles III and IV were collected over a 10-d intervention, in which sixty-eight male soldiers volunteered for the study. They were divided into two groups (FEX = field exercise, REC = recovery) according to their platoon. The FEX group (*n* = 42) had garrison and field military training, whereas the REC group (*n* = 26) had a 36-h recovery period after the first military field training phase. Randomization was not possible given that survival training was a part of their military service and implemented by platoons. More participants were selected for the field exercise group because a higher attrition rate was expected from this group. The present study was part of a larger multidisciplinary research project, authorized by the Finnish Defence Forces (AO1720). The ethical review of the study was granted by the Scientific and Ethical Committee of the Helsinki University Hospital Research (HUS/900/2018). All participants were informed of the experimental design, methods, benefits, and possible risks prior to signing an informed consent document. Data from studies III and IV were collected as presented in Table 5. In study III, measurements were on days 1, 6, 8, and 10, and study IV collected data on days 1, 6 and 8.

TABLE 5. Study protocol in studies III and IV. REC = recovery group, FEX= field exercise group.

Days	**PRE** **Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**MID** **Day 6**	**Day 7**	**POST** **Day 8**	**Day 9**	**Day 10**
Tasks	Education in garrison			Field training, both groups		Recovery in REC Field training in FEX			Field training, both groups	
Body composition	X					X		X		X
Fitness and shooting tests	X					X		X		
Blood samples	X					X		X		X
Energy intake and expenditure	X				X		X			

4.3 Data collection

4.3.1 Dietary intake

Studies I, II: The dietary intake data from all three measurement points were estimated from food diaries recorded on 3 consecutive days. Served meals did not differ between days, except for Sundays when breakfast and lunch were combined. Participants were asked to maintain their regular food consumption and to register all food, fluid and nutritional supplements immediately after each meal by writing them down in a food diary. They were asked to estimate food weights using household measures and standard units. The research group weighed certain items (portions of meat or fish, slices of bread, fruits, vegetables) to improve accuracy of the measurements. Food diaries were reviewed and then analyzed with Nutri Flow (Flow-team, Oulu, Finland) software program.

Studies III, IV: Energy intake was estimated with pre-filled food diaries by a software program (Fineli, National Food Composition Database, Finland). When meals and food items were known beforehand, diaries were pre-filled and participants marked the time and amount of food consumed. During field training phase, restricted field rations consisted of one protein bar (226 kcal), eight crackers (306 kcal), and two lunch meal rations (mean 661 kcal per portion).

Representative days of garrison, field training and recovery phases were analyzed further in relation to the energy expenditure values.

4.3.2 Energy expenditure and physical activity

Study 1: Total physical activity (subgroup n = 29) was measured via a three-dimensional accelerometer at a frequency of 100 Hz (Hookie AM20, Traxmeet, Oulu, Finland). The participants were instructed to wear the device on the left side of their waist at all times while they were awake for ten days, except during showering, sauna or swimming. The data were analyzed and calculated for sedentary behavior (SB), e.g., standing, sitting, lying (metabolic equivalent, MET < 1.5), and for physical activity, e.g., step count, number of breaks during SB, MET mean, MET max (per min). Physical activity was further broken down into light PA (MET 1.5–3.0) and moderate to vigorous PA (MVPA, MET > 3.0) (Vähä-Ypyä et al., 2015).

Studies II, III, IV: Energy expenditure was estimated via heart rate variability measurements (Firstbeat Bodyguard 2, Firstbeat Technologies Oy, Jyväskylä, Finland). The Bodyguard 2 is a two-electrode portable device connected to the chest. Participants wore the Bodyguard 2 continuously, with the exception of short breaks to install the batteries. The accuracy of estimated energy expenditure is reported within 7–10%, using oxygen consumption during exertions of different intensities but not against DLW (Smolander et al., 2011).

4.3.3 Body composition

Body composition (body mass, body fat%, skeletal muscle mass) was evaluated via bioimpedance devices (Inbody 720/770, Biospace, Soul, South Korea) in all studies (I-IV). The measurements were done early in the morning after an overnight fast, and participants were advised to only wear underwear and to urinate before the measurement. The same device was used for each measurement to avoid variability between devices.

In study I, subcutaneous fat and muscle thickness of the vastus lateralis (VL) and triceps brachii (TB) muscles were measured via ultrasonography technique (Sonoace R3, Samsung Medison, Co & Ltd, Seoul, Korea). Measurement points of VL and TB were marked at the beginning of the study based on anatomical landmarks. The probe was moved manually with electrode gel over the marked points of the skin, avoiding compression of muscle tissue. A leg support was used to diminish compression of the tissue. Three scans were taken for later analysis. An electrical caliper and the image processing system in the ultrasound device integrated images and the average of these scans was used.

4.3.4 Blood biomarkers and blood pressure

Fasting blood samples were drawn from the ulnar vein using Terumon Venosafe (Terumo Europe, Leuven, Belgium) and were centrifuged at a speed of 3500 rpm, then serum was frozen at −20° C for later analysis (II, III, IV).

Study II: Glucose, serum high-density lipoprotein (HDL) cholesterol and triglycerides (TG) were analyzed with the Konelab 20 XTi -device (Thermo Electron Co, Vantaa, Finland) and the isolated low-density lipoprotein (LDL) cholesterol fraction was used for direct measurement of LDL-cholesterol (CHOD-PAP method). The ranges for triglycerides, HDL cholesterol, and LDL cholesterol assays were 0.1–15, 0.09–11, 0.04–2.84, and 0.3–8.9 mmol $\cdot L^{-1}$, respectively. Intra- and inter-assay coefficients of variance were 1.0% and 3.8% for TG, 3.4% and 3.9% for s-LDL, and 0.5% and 7.6% for s-HDL, respectively. Sensitivity for glucose was 0.1 mmol $\cdot L^{-1}$, and intra- and inter-assay coefficients of variance were 1.0 and 2.0%, respectively. Serum concentrations of C-reactive protein (CRP), tumor necrosis factor-alpha (TNF- α), and interleukin-6 (IL-6) were measured using commercial high sensitivity ELISA kits according to the manufacturer's instructions (Quantikine HS, R&D Systems, Minneapolis, USA). Assay specifications were 0.10 mg/L for CRP sensitivity, 0.11 pg $\cdot mL^{-1}$ for IL-6, and 0.19 pg $\cdot mL^{-1}$ for TNF- α. The maximum intra- and inter-assay CV% were 4.8 and 6.1% for CRP, 5.9% and 9.8% for IL-6, and 6.1% and 7.7% for TNF- α, respectively.

Blood pressure was measured after a 5-min rest, three times at 2-min intervals by a semiautomatic blood pressure device (Omron M6 Comfort, Omron Healthcare, Kyoto, Japan). Means of the three measurements were used for statistical analyses.

Study III: Leptin and ghrelin were determined with an ELISA-kit immunoassay system (Dynex DS 2, Dynex Technologies, Chantilly, VA, United States); creatinine, glucose and urea were determined with a photometric enzymatic method (Konelab 20 Xti Clinical Chemistry Analyzer, Thermo Scientific, Vantaa, Finland); free fatty acids were determined with an enzymatic colorimetric assay (Konelab 20 Xti Clinical Chemistry Analyzer, Thermo Scientific, Vantaa, Finland); electrolytes (Na, K, Cl) were determined with an ion selective electrode method (ISE; Konelab 20 Xti Clinical Chemistry Analyzer, Thermo Scientific, Vantaa, Finland). The sensitivity and inter-assay coefficient of variation for these assays were: 0.2 ng $\cdot mL^{-1}$ and 6.1% for leptin, 0.6 pg $\cdot mL^{-1}$ and 17.3% for ghrelin, 2.32 µmol $\cdot L^{-1}$ and 2.2% for creatinine, 0.1 mmol $\cdot L^{-1}$ and 3.8% for glucose, 1.1 mmol $\cdot L^{-1}$ and 5.8% for urea, 10 µmol $\cdot L^{-1}$ and 5.8% for free fatty acids, 100 mmol $\cdot L^{-1}$ and 0.7% for sodium, 2 mmol $\cdot L^{-1}$ and 2.5% for potassium, and 55 mmol $\cdot L^{-1}$ and 3.3% for chloride, respectively.

Study IV: Testosterone and cortisol levels were acquired via Immulite immunoassay analyzer (Siemens Immulite 2000 XPI, Siemens Healthcare, USA). The sensitivity and inter-assay coefficient of variation for these assays were: 0.2 ng $\cdot ml^{-1}$ and 6.1% for leptin, 0.6 pg $\cdot ml^{-1}$ and 17.3% for ghrelin, 0.5 nmol $\cdot L^{-1}$ and 7.8% for testosterone, and 5.5 nmol $\cdot L^{-1}$ and 6.5% for cortisol, respectively. Leptin and ghrelin were analyzed as previously described.

4.3.5 Fitness tests and shooting test

Study IV: Maximal isometric force of the upper- and lower-body extremities was measured bilaterally by a leg and bench press dynamometer (Faculty of Sport and Health Sciences, University of Jyväskylä, Finland). In bench and leg press, positions were adjusted as previously described (Häkkinen et al., 2001; Vaara et al., 2020). Participants were instructed to produce maximal force as fast as possible with verbal encouragement from the testing personnel. One trial attempt was allowed before the two test trials, with minimum 60 s of recovery between the trials. The best performance was selected for further analysis.

A standing long jump was used to measure explosive force production of the lower extremities (Bosco et al., 1983) on a specifically designed gym mat (Fysioline Co., Tampere, Finland). Before testing, the participants were instructed on the correct technique, and they performed 2–3 warm-up jumps. The participants were advised to jump (horizontally) forward as far as possible from a standing position and land bilaterally without falling backward. The best of the three jumps was utilized, measuring from the start line to the landing point.

A seated medicine ball throw was measured for assessing explosive force production of the upper body (Beckham et al., 2019). The participants sat in an upright position on the floor with their legs fully extended and back kept against the vertical wall throughout the test. The 3-kg medicine ball was held with both hands in front of their chest, with their forearms positioned parallel to the ground. The participant threw the ball vigorously as far forward as possible while maintaining their back against the wall. The distance from the wall to landing point of the medicine ball was recorded. The best result out of the three trials was used in the analysis. The participants were allowed to have at least three training throws before the test measurements.

Sit-ups and pull-ups were conducted to assess muscular endurance by counting maximal repetitions in one minute. Sit-ups were used to assess abdominal and hip flexor performance (Viljanen et al., 1991), and push-ups were used to measure performance of the arm and shoulder extensor muscles (American College of Sports Medicine, 2000). Technical advice was given before the tests and incorrect repetitions were excluded. All strength tests were transformed to z-scores and the mean of values formed the "strength index".

Endurance performance was measured by a 20-m shuttle run test (Léger & Lambert, 1982). Participants were advised to run 20 m back and forth with accelerating pace as long as possible. The test was finished when participants were not able to keep the given pace or voluntarily dropped out. Maximal oxygen uptake values were calculated from the test results, as previously described (Ramsbottom et al., 1988).

Shooting accuracy was estimated by an optical infra-red weapon system (Eko-Aims Oy, Ylämylly, Finland). Ten shots were given in prone and standing positions to the target 10 m away from the shooting line in an indoor hall. The sum of ten shots from both positions was recorded for analysis. The sum variable "shooting index" was aggregated from these results.

4.3.6 Statistical analyses

Study I: Statistical analyses were carried out with a commercial software (IBM SPSS 22.0.0, Chicago, Illinois, USA). Data were analyzed by repeated-measured ANOVA and paired t-tests. If assumptions were not met, logarithm transformations were applied (protein intake (E%, g $\cdot$d^{-1}), CHO intake (g $\cdot$d^{-1}), fiber intake (g $\cdot$d^{-1}), subcutaneous fat of VL and TB (cm)) or nonparametric Friedman's tests were utilized (water intake, PUFA). Spearman's product moment correlation coefficients were used for testing linearity of body composition, dietary intake and physical activity data. The statistical significance was met when p-values were under 0.05.

Study II: The linear correlation between nutritional or body composition variables and cardiovascular risk factors was obtained using Pearson's product-moment correlation. For triglycerides, PUFAs, and IL-6, Spearman's correlation coefficient was utilized. Linear regression analyses were conducted to examine linear relationships between nutritional or body composition parameters and cardiovascular risk factors or inflammatory markers. All analyses were carried out by a commercial software (IBM SPSS 25.0.0, Chicago, Illinois, USA). P- values <0.05 were considered statistically significant.

Studies III, IV: All statistical analyses were performed using R v. 3.6.3 (2020, R Foundation for Statistical Computing, Vienna, Austria). Pairwise comparisons were performed using a Tukey's test. Logarithmic transformations were done when the distribution was positively skewed (III: leptin, ghrelin, free fatty acids; IV: leptin, ghrelin, testosterone-cortisol ratio). Non-parametric Mann–Whitney U-tests were used to verify the linear mixed effect model when residuals were not normally distributed (body mass, leptin, glucose, sodium and chloride, III). Pearson correlations were calculated to estimate associations between baseline and differences in associations from baseline to the end of study (III), as well as between energy variables and strength, shooting index, endurance performance and hormonal status (IV). Time × group interactions were tested with F-tests based on the Satterthwaite method (Kuznetsova et al., 2017). A linear mixed effect model was used to estimate changes within and between groups over the studied periods. Values are presented as the means ± standard deviations and statistical significance was set at $p < 0.05$.

5 RESULTS

5.1 Crisis management operation (I, II)

5.1.1 Body composition and physical activity (I)

Body mass, BMI, fat mass and fat% did not change significantly during the deployment. Skeletal muscle mass (SMM) increased from PRE 38.5 ± 4.3 kg to POST 39.0 ± 4.7 kg ($p = 0.009$). Changes in body composition are shown in Table 6.

Muscle thickness of *triceps brachii* (TB) increased from PRE to MID and further to POST (3.84 ± 0.63, 3.92 ± 0.55, 4.04 ± 0.62, respectively, $p = 0.016$ MID_POST, $p = 0.013$ PRE_POST). Muscle thickness of *vastus lateralis* (VL) decreased from MID (3.81 ± 0.71) to POST (3.58 ± 0.72, $p = 0.019$). Subcutaneous fat thickness of VL first decreased from PRE (1.03 ± 0.47) to MID (0.97 ± 0.45, $p = 0.047$) but, thereafter, returned to baseline by POST (1.05 ± 0.45, $p = 0.006$ MID_POST). Subcutaneous fat thickness of TB increased from the PRE (0.67 ± 0.26) to POST deployment (0.72 ± 0.24, $p = 0.003$).

TABLE 6. Mean (± SD) body composition characteristics during the deployment (n = 40).

	PRE	**MID (3 mo)**	**POST (6 mo)**
Body mass (kg)	77.8 ± 8.7	78.0 ± 9.0	78.3 ± 9.5
Skeletal muscle mass (kg)	38.5 ± 4.3	38.7 ± 4.3	39.0 ± 4.7 p = 0.009 PRE_POST
Body fat%	13.6 ± 4.3	13.2 ± 4.4	13.3 ± 4.1
Visceral fat area (cm^2)	49.5 ± 18.3	51.8 ± 22.3	51.9 ± 20.1

Step count reduced from PRE to MID ($p = 0.010$) and from PRE to POST ($p = 0.007$). Mean daily step counts were 9835 ± 2743, 8538 ± 2699, 8388 ± 2875. MVPA and number of breaks during sedentary behavior decreased from PRE to MID and from PRE to POST.

5.1.2 Dietary intake (I)

Participants ate 4.9 ± 1.0 (PRE), 4.7 ± 0.9 (MID) and 4.4 ± 0.8 (POST) meals per day (means ± SD). Reported energy intake was 10.1 - 10.5 MJ $\cdot d^{-1}$ during deployment. Carbohydrate (CHO) intake (E%, g $\cdot d^{-1}$) increased from PRE to MID. Protein intake (E%, g $\cdot d^{-1}$) decreased from PRE to MID but then recovered. Water intake decreased from PRE to POST (Table 7).

TABLE 7. Mean (±SD) energy, dietary macronutrient and water intake during the deployment (n=40).

Nutrient	**PRE**	**MID (3 mo)**	**POST (6 mo)**
Daily energy intake MJ ·d^{-1} (kcal ·d^{-1})	10.3 ± 2.6 (2454 ± 616)	10.5 ± 2.8 (2521 ± 660)	10.1 ± 2.8 (2425 ± 676)
Carbohydrates (E%)	39.5 ± 6.9	42.6 ± 7.7 $p = 0.007$ PRE_MID	40.1 ± 9.1
Protein (E%)	21.7 ± 3.8	18.7 ± 4.6 $p < 0.001$ PRE_MID	22.3 ± 5.0 $p < 0.001$ MID_POST
Fat (E%)	35.0 ± 7.0	35.7 ± 5.0	34.9 ± 7.0
Carbohydrates (g ·d^{-1})	242.8 ± 77.5	268.5 ± 85.0 $p = 0.038$ PRE_MID	242.4 ± 84.7 $p = 0.024$ MID_POST
Protein (g ·d^{-1})	132.5 ± 40.3	117.3 ± 41.0 $p = 0.006$ PRE_MID	134.7 ± 46.2 $p = 0.002$ MID_POST
Fat (g ·d^{-1})	95.6 ± 31.6	100.4 ± 31.4	95.0 ± 35.0
Fiber (g ·d^{-1})	17.5 ± 8.4	21.3 ± 10.4 $p = 0.019$ PRE_MID	16.0 ± 7.6 $p < 0.001$ MID_POST
Saccharose (E %)	10.2 ± 5.2	11.9 ± 5.0 $p = 0.039$ PRE_MID	10.6 ± 6.0
Saturated fatty acids (E%)	13.2 ± 2.7	12.5 ± 2.5	12.3 ± 3.3
Monounsaturated fatty acids (E%)	12.6 ± 2.2	12.5 ± 2.2	11.9 ± 2.8
Polyunsaturated fatty acids (E%)	15.7 ± 54.8	16.2 ± 37.6	8.6 ± 18.6
Water (L ·d^{-1})	4.5 ± 1.6	4.2 ± 1.7	3.7 ± 1.6 $p < 0.001$ PRE_POST

5.1.3 Cardiovascular risk factors and their associations with body composition (II)

The relative prevalence of soldiers having dyslipidemia, hyperglycemia, hypertension, or a high level of inflammatory markers in the study II are shown in Table 8.

The highest prevalence of dyslipidemia was found in LDL cholesterol: 31.4%, 40.0%, and 34.3% of soldiers exceeded the reference value at 0, 3, and 6 months, respectively. A total of 68.6% of soldiers had high systolic blood pressure (> 120 mmHg) at 6 months, while the earlier results were 31.3% (0 mo) and 40.0% (3 mo).

TABLE 8. Prevalence (%) of dyslipidemia, hyperglycemia, high level of pro-inflammatory biomarkers and hypertension among soldiers (n = 35)

	0 mo	**3 mo**	**6 mo**
High total cholesterol (> 5.0 mmol ·L^{-1})[1]	28.6	31.4	28.6
Low HDL cholesterol (< 1.0 mmol ·L^{-1})[1]	22.9	22.9	8.6
High LDL cholesterol (> 3.0 mmol ·L^{-1})[1]	31.4	40.0	34.3
Hypertriglyceridaemia (> 1.7 mmol ·L^{-1})[1]	8.6	8.6	8.6
Hyperglycemia (> 6.0 mmol ·L^{-1})[2]	2.9	0	2.9
High interleukin-6 (> 3.4 ng ·L^{-1})[3]	5.9	8.8	2.9
High tumor necrosis factor-α (> 8.1 ng ·L^{-1})[3]	36.4	39.4	21.2
High systolic blood pressure			
(> 120 mmHg)[4]	31.4	40.0	68.6
(> 140 mmHg)[4]	2.9	2.9	8.6
High diastolic blood pressure			
(> 80 mmHg)[4]	20.0	22.9	28.6
(> 90 mmHg)[4]	2.9	8.6	5.7

[1] Reference values for lipids: Dyslipidemias: Current Care Guidelines 2017 (referred 24/06/2019) www.kaypahoito.fi

[2] Reference value for blood glucose: Type 2 Diabetes: Current Care Guidelines 2018 (referred 24/06/2019) www.kaypahoito.fi

[3] Reference values for pro-inflammatory markers: HUSLAB - tutkimusohjekirja (referred 24/06/2019) huslab.fi/ohjekirja/4842.html, huslab.fi/ohjekirja/4282.html

[4] Reference values for optimal blood pressure: Hypertension: Current Care Guidelines 2015 (referred 24/06/2019) www.kaypahoito.fi

5.1.4 Associations between diet, body composition, and cardiovascular risk factors (I, II)

Energy and fat intake correlated positively with SMM in all measurement points. Fat intake correlated positively with body mass in PRE, MID and POST, and energy intake with body mass in MID and POST. Protein intake did not correlate significantly with body mass, SMM or fat%.

No systematic associations between nutrient intake and cardiovascular risk factors or inflammatory markers were found at any measurement point. When considering changes between 0 and 6 mo, negative associations were found between the change in fiber intake and the changes in total and LDL cholesterol concentrations (R = -0.362, p = 0.033; R = -0.394, p = 0.019, respectively). No other associations were observed.

BF% and fat mass correlated positively with total and LDL cholesterol concentrations at all measurement points (Figure 2).

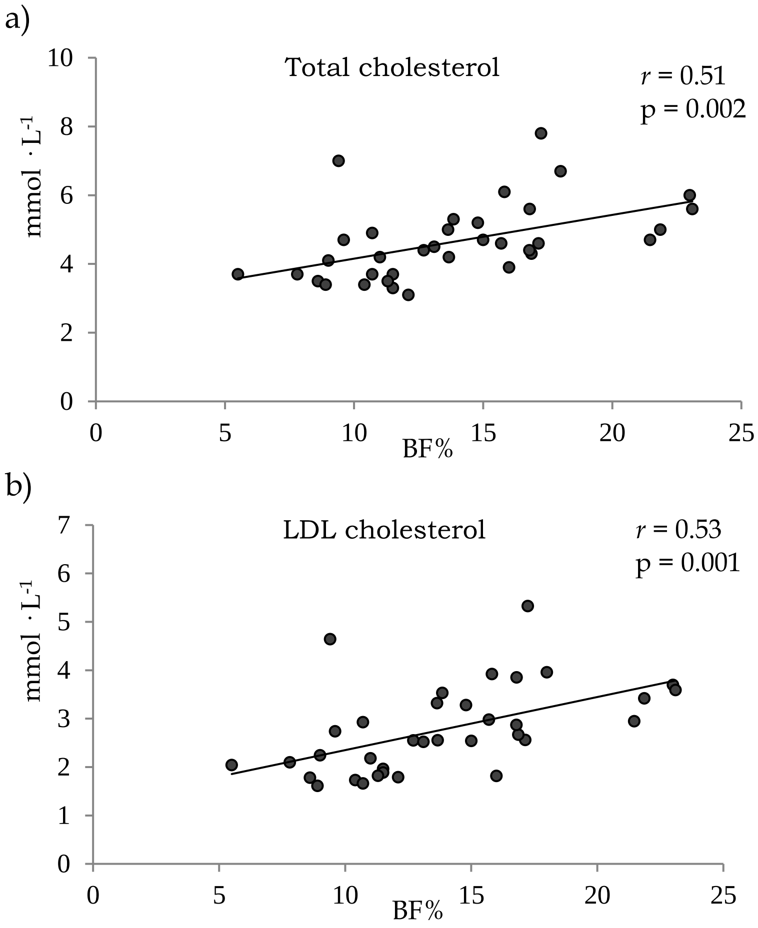


FIGURE 2. Relationship between body fat percentage (BF%) and total cholesterol (a) and LDL cholesterol (b) at baseline (n = 35).

5.2 Winter survival training (III, IV)

5.2.1 Energy balance and body composition (III, IV)

Energy intake, expenditure, and balance are shown in Figure 3. Based on the representative food diaries from both groups, energy intake on day 5 was 1.9 ± 0.9 MJ $\cdot d^{-1}$ (447 ± 245 kcal $\cdot d^{-1}$) in FEX (n = 24) and 1.5 ± 1.4 MJ $\cdot d^{-1}$ (357 ± 338 kcal $\cdot d^{-1}$) in REC (n = 20). On day 7, energy intake was 5.4 ± 3.1 MJ $\cdot d^{-1}$ (1294 ± 743 kcal $\cdot d^{-1}$) in FEX (n = 12) and 12.6 ± 3.7 MJ $\cdot d^{-1}$ (3003 ± 882 kcal $\cdot d^{-1}$) in REC (n = 21). Energy intake differed significantly between groups ($p < 0.001$) on day 7. Calculated energy balance on day 5 was – 18.1 ± 6.3 MJ $\cdot d^{-1}$ (-4323 ± 1515 kcal $\cdot d^{-1}$) in the FEX group and 19.4 ± 7.3 MJ $\cdot d^{-1}$ (-4635 ± 1742 kcal $\cdot d^{-1}$) in the REC group. On day 7, the estimated energy balance was 17.7 ± 7.6 MJ $\cdot d^{-1}$ (-4222 ± 1815 kcal $\cdot d^{-1}$) in FEX and -2.5 ± 4.6 MJ $\cdot d^{-1}$ (-608 ± 1107 kcal $\cdot d^{-1}$) in REC, which yielded a significant difference ($p < 0.001$).

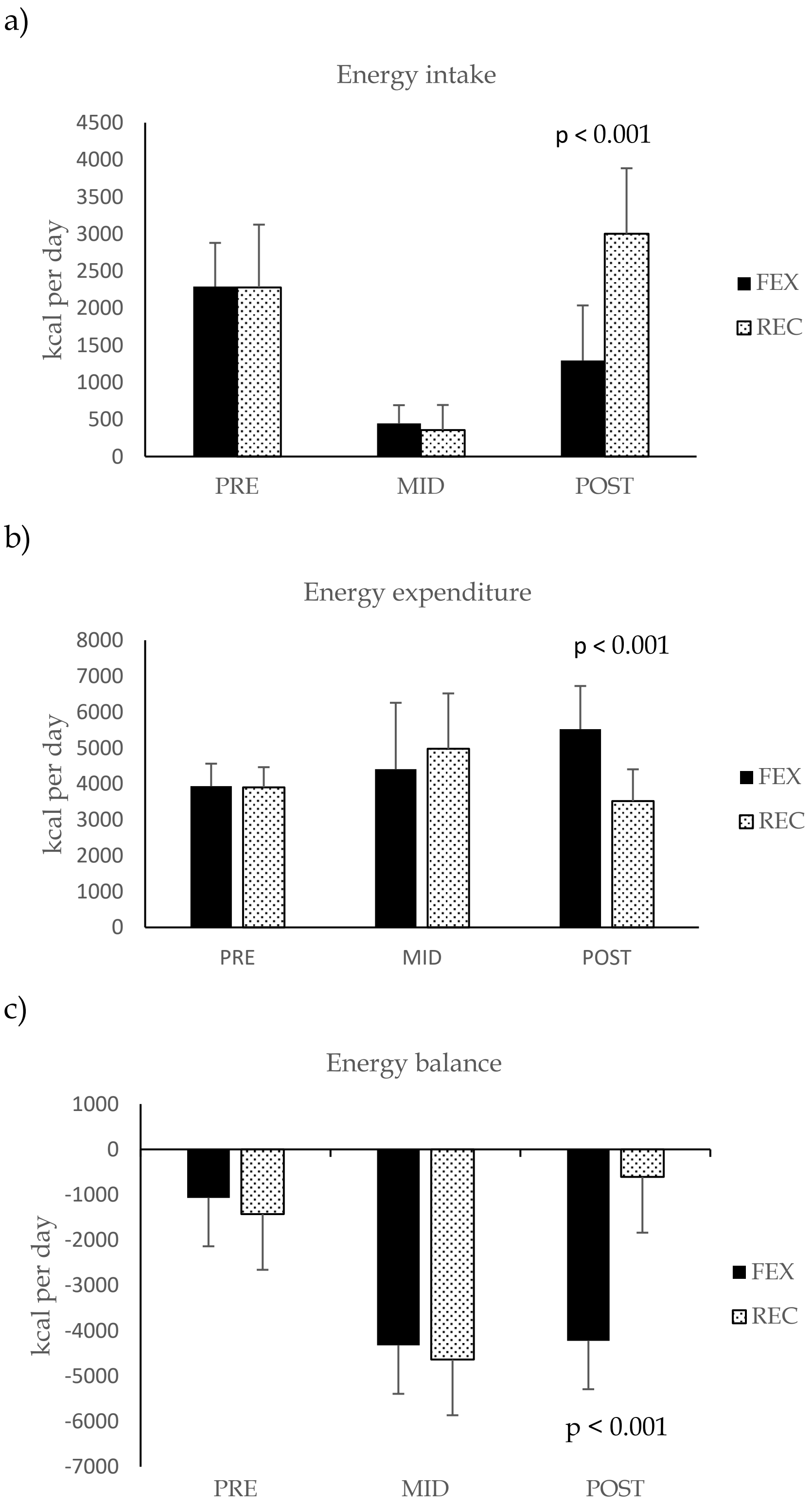


FIGURE 3. Mean (± SD) energy intake (EI), energy expenditure (EE), and energy balance (EB) during the training in the REC and FEX groups. REC = recovery, FEX = field exercise. Statistically significant between-group differences are presented.

For body composition parameters, significant time x group interactions were found for body mass ($p < 0.001$), body fat% ($p < 0.001$) and skeletal muscle mass ($p = 0.004$), but not for creatinine. Differences in model parameters within and between groups are presented in Table 9. Body mass decreased in the FEX group by day 6 and remained lower than baseline thereafter. In the REC group, body mass first decreased by day 6 but then increased by day 8, and finally decreased again by day 10. On day 8, significant differences were found in body mass ($p < 0.001$) and body fat% ($p = 0.017$) between the groups, and on day 10, body fat% also differed ($p < 0.001$) between the groups.

TABLE 9. Changes in body mass, body fat%, skeletal muscle mass and serum creatinine during the training period. REC = recovery group, FEX = field exercise group.

		Day 1	**Day 6**	**Day 8**	**Day 10**	**Δ (%) day 1-10**
Body mass (kg)	FEX	74.4 ± 10.7	72.9 ± 9.8 $p < 0.001^{\text{day1\_6}}$	72.6 ± 9.6 $p < 0.001^{\text{day1\_8}}$	72.6 ± 9.5 $p < 0.001^{\text{day1\_10}}$	-3.9 ±1.7
	REC	78.2 ± 9.7	74.6 ± 9.2 $p < 0.001^{\text{day1\_6}}$	77.1 ± 8.6 $p < 0.001^{\text{day1\_8}}$ $p < 0.001^{\text{day6\_8}}$	75.5 ± 9.0 $p < 0.001^{\text{day1\_10}}$ $p < 0.001^{\text{day6\_10}}$ $p < 0.001^{\text{day8\_10}}$	-3.0 ± 2.1
Body fat%	FEX	13.8 ± 4.8	12.8 ± 3.9 $p < 0.001^{\text{day1\_6}}$	10.4 ± 3.3 $p < 0.001^{\text{day1\_6}}$ $p < 0.001^{\text{day6\_8}}$	9.0 ± 3.5 $p < 0.001^{\text{day1\_10}}$ $p < 0.001^{\text{day6\_10}}$ $p < 0.001^{\text{day8\_10}}$	-37.0 ± 9.6
	REC	14.1 ± 5.2	11.5 ± 5.6 $p < 0.001^{\text{day1\_6}}$	10.9 ± 4.7 $p < 0.001^{\text{day1\_8}}$	10.9 ± 4.7 $p < 0.001^{\text{day1\_10}}$	-20.1 ± 11.4
Skeletal muscle mass (kg)	FEX	36.2 ± 4.6	35.9 ± 4.8 $p < 0.001^{\text{day1\_6}}$	36.9 ± 4.8 $p < 0.001^{\text{day6\_8}}$	37.3 ± 4.8 $p < 0.001^{\text{day6\_10}}$	1.4 ± 2.5
	REC	38.0 ± 3.7	37.1 ± 3.1 $p < 0.001^{\text{day1\_6}}$	38.6 ± 3.2 $p < 0.001^{\text{day6\_8}}$	38.0 ± 3.3 $p < 0.001^{\text{day6\_10}}$ $p = 0.003^{\text{day8\_10}}$	-0.2 ± 2.5
Creatinine (μmol ·L^{-1})	FEX	89.8 ± 11.4	82.4 ± 12.2 $p < 0.001^{\text{day1\_6}}$	88.4 ± 11.9 $p = 0.017^{\text{day6\_8}}$	91.9 ± 10.1 $p < 0.001^{\text{day6\_10}}$	2.2 ± 10.2
	REC	89.2 ± 9.4	85.2 ± 12.6	91.1 ± 8.4	91.6 ± 9.2 $p = 0.031^{\text{day6\_10}}$	2.1 ± 12.5

For body mass, body fat% and skeletal muscle mass n = 42, 38, 27, 25 (FEX) and n = 26, 22, 22, 20 (REC) for days 1, 6, 8 and 10 respectively
For creatinine n = 32, 26, 27, 25 (FEX, respectively) and n = 20, 18, 18, 18 (REC)

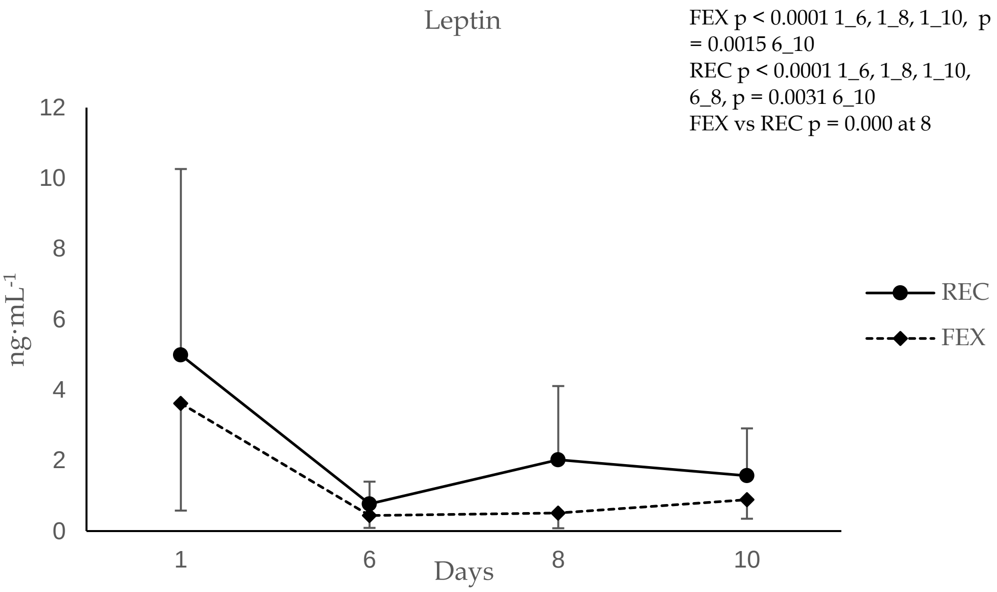
5.2.2 Blood biomarkers (III)

A time x group interaction for leptin was statistically significant ($p < 0.001$), but not for ghrelin. Serum leptin concentration decreased significantly in both groups from day 1 to day 6, while in the FEX group, it remained significantly lower until the end of the study (Figure 4a). After the recovery period, leptin concentration increased in the REC group towards baseline so that a significant difference between the groups was observed on day 8. Serum ghrelin level stayed stable throughout the study period, except for a slight increase in the FEX group from day 6 to day 10.

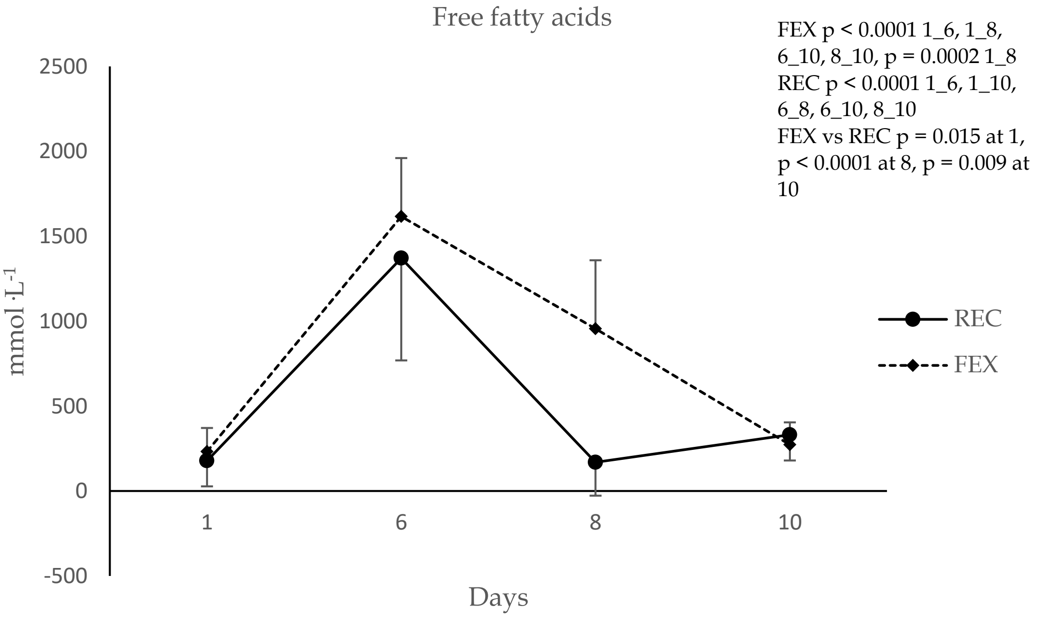
For energy substrate metabolites, significant time x group interactions were found for free fatty acids ($p < 0.001$) and urea ($p < 0.001$), but not for glucose (Figure 4b, c). Relative to baseline, free fatty acid concentration increased at day 6 in both groups, but the recovery period resulted in the return of free fatty acid concentration towards baseline in the REC group; no difference was observed between days 1 and 8, and there was a significant difference between the groups. Several significant changes were observed in urea concentration within the groups at all phases of the training, but the only significant difference between the groups was found at day 8. Significant decreases in glucose were found at day 6 in both groups, followed by a slight increase in the FEX group towards the end of the study.

For serum electrolytes, significant time x group interactions were found for sodium ($p = 0.002$) and chloride ($p = 0.03$), but not for potassium. No significant changes in sodium concentration were found, whereas in the FEX group, chloride concentration was significantly lower than baseline at days 6 and 8, and the groups differed significantly at day 8 (Figure 4d). Potassium levels decreased in both groups by day 6, and then slightly increased, but the increase was only significant in the REC group.

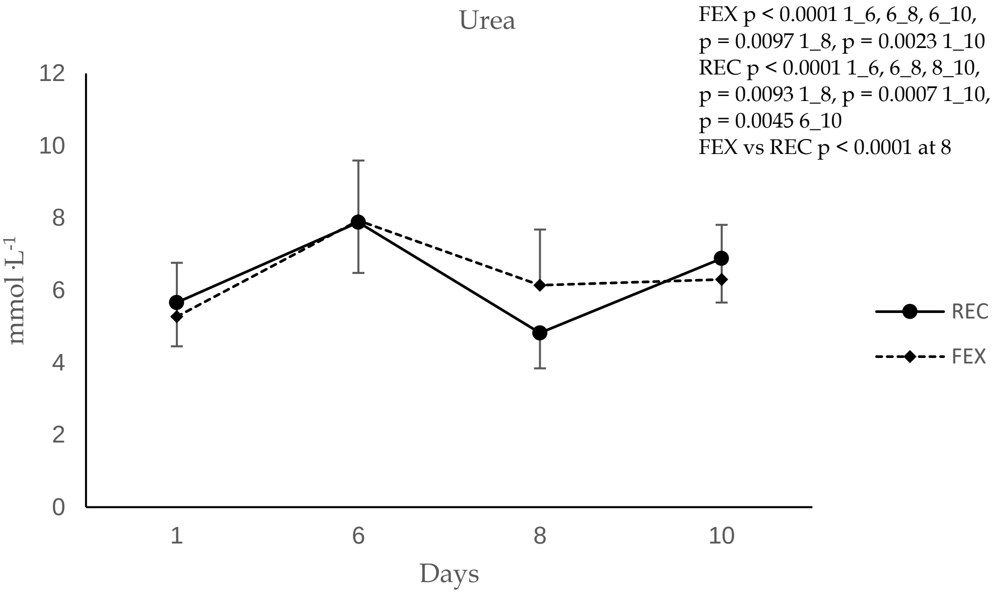
a)


Leptin
FEX p < 0.0001 1_6, 1_8, 1_10, p = 0.0015 6_10
REC p < 0.0001 1_6, 1_8, 1_10, 6_8, p = 0.0031 6_10
FEX vs REC p = 0.000 at 8
12
10
8
6
4
2
0
ng·mL-1
1
6
8
10
Days
REC
FEX

b)


Free fatty acids
FEX p < 0.0001 1_6, 1_8, 6_10, 8_10, p = 0.0002 1_8
REC p < 0.0001 1_6, 1_10, 6_8, 6_10, 8_10
FEX vs REC p = 0.015 at 1, p < 0.0001 at 8, p = 0.009 at 10
2500
2000
1500
1000
500
0
-500
mmol·L-1
1
6
8
10
Days
REC
FEX

c)



d)

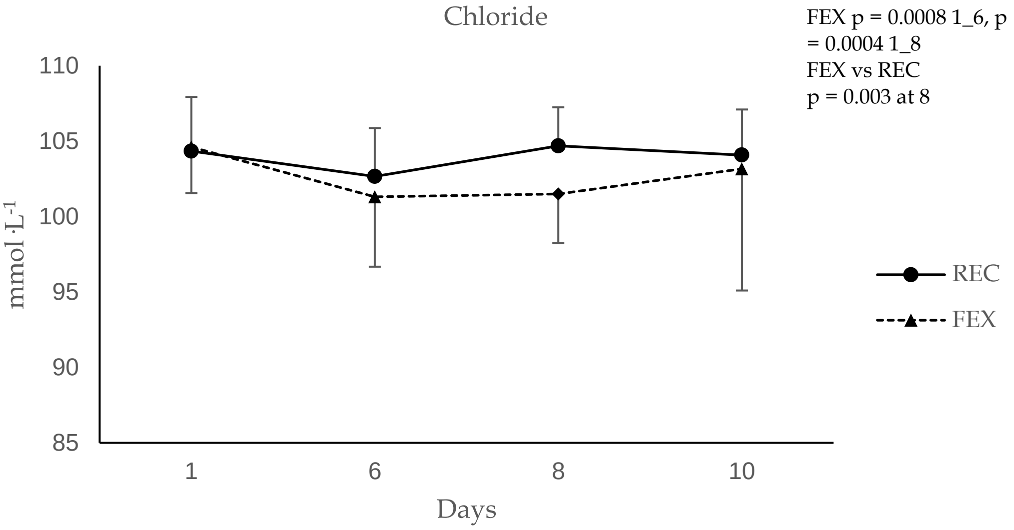


FIGURE 4. Changes in a) leptin, b) free fatty acids, c) urea and, d) chloride concentration within and between the REC and FEX groups. In the REC group n = 26, 22, 22, and 20, and in the FEX group n=42, 26, 27, and 25 for days 1, 6, 8, and 10, respectively.

5.2.3 Associations between and within body composition and biomarkers (III)

At baseline, strong positive associations were found between body mass and skeletal muscle mass (r = 0.911, p < 0.001), body mass and leptin (r = 0.573, p < 0.001), body mass and body fat% (r = 0.500, p < 0.001), and leptin and body fat% (r = 0.737, p < 0.001). When evaluating correlations between differences from day 1 to day 10, a strong inverse association was observed between skeletal muscle mass and body fat% (r = -0.682, *p* < 0.001). No other systematic associations were found within biomarkers or between changes in different biomarkers and body composition variables.

Study 4

Energy intake, expenditure and balance, and leptin and ghrelin concentrations were reported in the previous chapter. A time x group interaction was found for leptin (*p* < 0.001). Changes in testosterone/cortisol -ratio are shown in Figure 5, indicating an increase in T/C-ratio in the REC group after recovery phase.

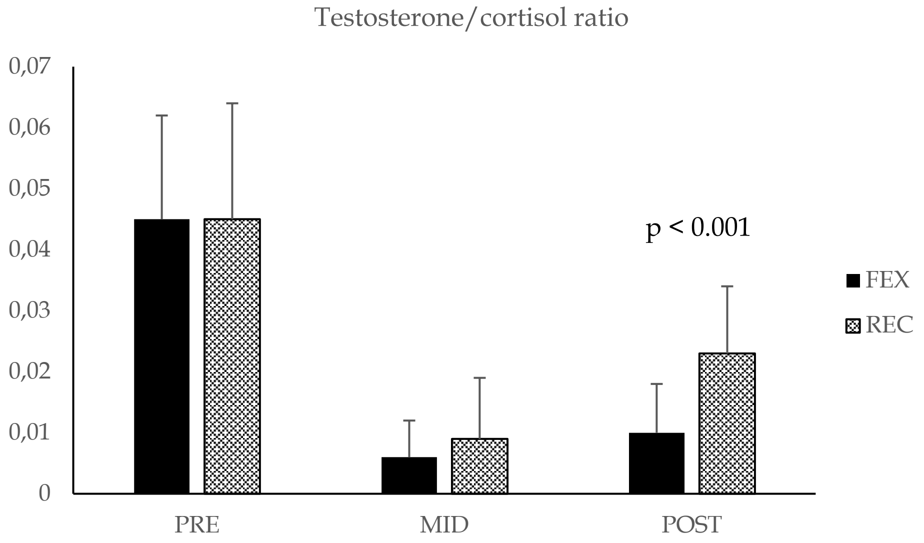


FIGURE 5. Mean (±SD) testosterone/cortisol -ratio during the training in the recovery (REC) and field exercise (FEX) groups. Between-group statistical significances are presented.

5.2.4 Strength, endurance and shooting performance (IV)

A time × group interaction was found for sum variable "strength index" (p < 0.001). Strength index decreased from PRE to MID in both groups (FEX *p* < 0.001, REC p = 0.014) and increased from MID to POST in REC (p < 0.001). Strength index differed between the groups (p = 0.025) at baseline, but the difference disappeared in later measurements. Endurance performance (20-m shuttle run) was impaired at MID in both groups (FEX $p < 0.001$, REC $p = 0.007$), but the REC group improved its performance at POST compared to MID ($p = 0.008$). The shooting index decreased only in the FEX group from MID to POST ($p = 0.0003$), but it differed between the groups at all measurement points ($p < 0.001$, $p = 0.003$, and $p < 0.001$ in PRE, MID, and POST, respectively). These changes were only seen in prone shooting results. Absolute results of physical fitness and shooting tests are shown in Table 10. Significant differences between the groups were found in the 20-m shuttle run test, where the REC group improved its performance in POST, and in prone shooting, in which the groups differed at all measurement points. Push-ups and shooting in standing position differed between groups at baseline, but the differences disappeared in MID and POST.

TABLE 10. Mean (± SD) physical fitness tests and shooting performance results during training in the recovery (REC) and field exercise (FEX) groups. Statistically significant differences between groups are presented. Number of participants are shown in brackets.

Physical and shooting performance	Group	PRE (0 d)	MID (6 d)	POST (8 d)
Maximal isometric force, upper (kg)	FEX REC	88±10 (42) 83±11 (25)	82±11 (28) 79±11 (22)	85±12 (26) 84±11 (21)
Maximal isometric force, lower (kg)	FEX REC	312±89 (42) 314±74 (25)	312±88 (28) 296±79 (22)	336±103 (26) 321±66 (21)
Standing long jump (cm)	FEX REC	225±22(41) 217±16 (24)	222±22 (27) 217±16 (22)	221±18 (24) 218±15 (21)
Medicine ball throw (cm)	FEX REC	611±74 (42) 595±58 (24)	590±68 (28) 585±51 (22)	575±68 (26) 609±47 (21)
Push ups (reps/min)	FEX REC	39±13 (41) 32±11 (24) $p = 0.029$	30±14 (27) 27±13 (22)	34±12 (24) 36±10 (21)
Sit ups (reps/min)	FEX REC	43±9 (41) 39±6 (24)	40±10 (27) 39±8 (22)	39±9 (24) 41±7 (21)
20-m shuttle run (ml ·kg^{-1} ·min^{-1})	FEX REC	46±5 (41) 45±5 (24)	39±7 (27) 41±8 (21)	36±12 (23) 45±5 (20) $p = 0.003$
Shooting, prone	FEX REC	86±10 (42) 93±6 (26) $p < 0.001$	88±7 (28) 93±3 (22) $p = 0.001$	76±9 (26) 91±6 (21) $p < 0.001$
Shooting, standing	FEX REC	62±10 (42) 69±11 (26) $p = 0.016$	63±11 (28) 68±11 (22)	64±12 (26) 69±11 (21)

5.2.5 Associations between energy variables, and hormones and physical performance (IV)

A strong positive association was found between changes in energy balance and changes in shooting index ($r = 0.786$, $p = 0.002$) in the FEX group from MID to POST, meaning that higher values in energy balance were related to better shooting performance. No other significant associations were found between energy variables and strength index, endurance performance, or shooting index.

Associations between changes in energy intake and energy expenditure and changes in hormones are presented in Table 11. Change in energy balance was not associated with any of these hormonal variables.

TABLE 11. Associations of changes in energy intake, energy expenditure, and energy with changes in leptin, ghrelin, and testosterone/cortisol ratio (T/C ratio). Statistically significant associations have been bolded. REC = recovery group, FEX = field exercise group.

		Leptin	**Ghrelin**	**T/C Ratio**
PRE-POST changes				
Energy intake	FEX	r = −0.089, p = 0.784	r = −0.003, p = 0.413	r = −0.214, p = 0.504
	REC	r = 0.099, p = 0.687	r = −0.215, p = 0.46	r = −0.035, p = 0.888
Energy expenditure	FEX	**r = −0.566, p = 0.003**	r = −0.131, p = 0.552	r = −0.231, p = 0.257
	REC	r = −0.007, p = 0.977	r = 0.028, p = 0.915	r = −0.145, p = 0.145
Energy balance	FEX	r = 0.398, p = 0.2	r = 0.104, p = 0.774	r = 0.104, p = 0.747
	REC	r = 0.197, p = 0.419	r = −0.466, p = 0.093	r = −0.387, p = 0.102
PRE-MID changes				
Energy intake	FEX	r = −0.302, p = 0.173	r = −0.35, p = 0.12	r = 0.014, p = 0.951
	REC	r = −0.384, p = 0.116	r = −0.123, p = 0.689	**r = 0.479, p = 0.044**
Energy expenditure	FEX	r = −0.059, p = 0.775	r = 0.388, p = 0.067	r = −0.366, p = 0.066
	REC	r = −0.27, p = 0.225	r = −0.001, p = 0.996	r = −0.017, p = 0.94
Energy balance	FEX	r = −0.187, p = 0.405	r = −0.337, p = 0.135	r = 0.245, p = 0.328
	REC	r = −0.181, p = 0.473	r = −0.163, p = 0.595	r = 0.15, p = 0.506
MID-POST changes				
Energy intake	FEX	r = −0.086, p = 0.814	r = 0.121, p = 0.74	**r = −0.668, p = 0.035**
	REC	**r = −0.560, p = 0.013**	r = 0.317, p = 0.269	r = −0.284, p = 0.254
Energy expenditure	FEX	**r = −0.512, p = 0.011**	r = 0.214, p = 0.326	r = −0.375, p = 0.071
	REC	r = −0.165, p = 0.269	r = −0.238, p = 0.358	r = −0.236, p = 0.303
Energy balance	FEX	r = 0.43, p = 0.215	r = −0.275, p = 0.441	r = −0.25, p = 0.485
	REC	r = −0.067, p = 0.784	r = 0.489, p = 0.076	r = −0.027, p = 0.916

6 DISCUSSION

The present studies (I, II) in the crisis management operation demonstrated that macronutrient intake did not meet the general nutritional requirements (NNR) (Blomhoff et al., 2023) or the Military Dietary Reference Intakes (MDRI) (Army Regulation 40-25, 2017). Soldiers consumed less carbohydrates and more fat, especially saturated fatty acids, than recommended. Carbohydrate intake increased and protein intake decreased at mid-phase of the operation (3 months). Reported energy intake remained low and stable during the deployment. The daily physical activity of soldiers was very low, but their body mass did not change, indicating that the soldiers actually maintained energy balance.

According to body composition measurements, skeletal muscle mass increased slightly, but subcutaneous fat thickness rose in the upper and lower extremities. Elevated LDL cholesterol (34-40% of participants) and systolic blood pressure (31-69% of participants) were observed during the operation. Changes in macronutrient intake were not associated with cardiovascular risk factors at any measurement point, but the change in fiber intake was inversely associated with changes in total and LDL cholesterol concentrations. Further, BF% and fat mass were positively associated with total and LDL cholesterol concentrations at all measurement points

The main findings of the winter survival training study were (a) the first two days of training caused severe energy deficit, which contributed to decreases in body mass, body fat%, serum leptin, glucose and potassium concentration, and increases in serum free fatty acids and urea; (b) a 2-day recovery period during survival training temporarily reversed some of the changes (body mass, leptin, free fatty acids, urea) toward baseline levels, but body mass, body fat% and leptin did not fully recover; and (c) severe energy deficit resulted from high energy expenditure and restricted energy intake. Changes in energy intake, expenditure and balance were associated with changes in leptin and testosterone/cortisol ratio, but not physical performance variables.

6.1 Nutrition, hormonal and metabolic status, and physical performance in different military contexts

6.1.1 Dietary intake

As a proportion of energy intake, low carbohydrate and high fat intake were observed in the crisis management operation (I), as seen in other studies in soldiers (Beals et al., 2017; De Bry et al., 2021; Edwards et al., 2022b; Fallowfield et al., 2014; MacKenzie-Shalders et al., 2021) and Finnish civilians (Borodulin et al., 2015). Underconsumption of CHOs is usually related to overconsumption of fat and protein, and increased fat intake is typically associated with energy-dense meals and overconsumption of saturated fat. Intake of saturated fatty acids was above the recommended level in study I. Absolute CHO intake was lower than values found by Beals et al. (2015) (range of means 242-269 $g \cdot d^{-1}$ vs 315 $g \cdot d^{-1}$), whereas absolute intake of protein was similar or higher than U.S. soldiers (range of means 117-135 $g \cdot d^{-1}$ vs 116 $g \cdot d^{-1}$). Thus, inadequate intake of CHO may impair physical training and recovery in garrison conditions. Regular training, prolonged and/or high-intensity military tasks requires CHOs as an energy substrate, and if glycogen is not available, muscle and adipose tissue are degraded to synthetize glucose (Beals et al., 2015). Furthermore, operational situations in deployments are vulnerable to acute changes, which makes good nutritional status important for continuous military readiness.

In the crisis management operation, dietary habits are highly dependent on food supply at the base camp. Regular meals were served three times a day, and extra food and snacks were able to buy from restaurants and shops nearby. Served meals contained fresh vegetables, protein (meat, poultry, fish) and carbohydrates, e.g., rice, pasta, potatoes. Same breads, margarines and drinks were served in every meal, which was beneficial for dietary recording. If soldiers had unhealthy dietary habits at home, they were likely not motivated to change their routines during the operation. Moreover, food service did not put any emphasis on serving more vegetables or fruits, and availability of certain fresh food items may have been limited. Soldiers reported monotonous life, especially in the latter part of the operation, which may have reflected their dietary habits (I). Food also has psychological and social implications, which might have been highlighted in special circumstances.

Most soldiers (and civilians) would benefit from increasing fiber-rich foods, like whole-grain, vegetables, berries and fruits. A previous study about Finnish lifestyle factors indicated that daily consumption of fresh vegetables declined from 2000 to 2017 among young men (Jääskeläinen et al., 2022). In the same time frame, overweight and obesity rates have increased. Greater fiber intake can decrease cholesterol values, prevent weight gain, improve gut microflora functions and a risk of gastrointestinal cancers (Barber et al., 2020). Additionally, fiber-rich diets contain more micronutrients, which improves the quality of diet, including in garrison-like conditions.

Fluid intake was determined in study I, where mean intake varied from 3.7 to 4.5 L $\cdot$d^{-1} in warm or hot environments. The average ambient temperature during the operation was 22.3° C and ranged 11-36° C. In survival training (III), fluid intake was asked, as is generally advised for food diaries, but inaccurate data was excluded from the further analysis. The crisis management operation was carried out under stable and calm circumstances, whereas survival training in wintertime is a strenuous environment to record fluid or dietary intake by self-filled food diaries. If fluid balance is to be studied, methods should be considered carefully, and dietary records should focus on fluid intake.

In survival training (III), dietary intake was modified and restricted by instructors. For the first two days of survival training, participants were delivered only 1847 kcal for entire two-days period. This food package contained a protein bar (226 kcal), eight crackers (306 kcal) and two lunch meal rations (661 kcal/each). One meal ration consisted of approximately 88 g carbohydrates, 27 g protein and 21 g fat. Drinking water was melted from snow. At the garrison during active recovery phase, regular meals were served by food service and *ad libitum* food intake was allowed. This indicates a huge variety of diets in the military: soldiers can be exposed to totally different environments, from free-living subjects with *ad libitum* food intake to very limited conditions with severe energy restriction (III; Castellani et al., 2006; Hoyt et al., 2006; Øfsteng et al., 2020).

When energy content of meals is so low, the distribution of macronutrients is less important. Instead, optimizing energy intake according to task and time is emphasized. Better nutritional status before survival phase might predict better performance in training. Good nutritional status can be defined as a condition, where nutrient intake, absorption and utilization are well-balanced (Larson-Meyer et al., 2018). Assessment of nutritional status can be done by using the traditional "A–E" framework of Anthropometric, Biochemical, Clinical, Dietary, and Environmental factors (Larson-Meyer et al., 2018). Based on these results, it would be beneficial to monitor soldiers' nutritional status, especially among individuals who have challenges with health or who are preparing for very extreme conditions.

Many studies have focused on the nutrient content of meals and energy balance (Tassone & Baker, 2017), but in some papers, quality of food is also discussed (Charlot, 2021). Although a soldier can meet very extreme conditions, they still have plenty of days at garrison and/or at home. In these conditions with access to more versatile foods, soldiers should focus on a well-balanced diet and healthy choices to optimize diet and physical training, which also prevents obesity and chronic diseases. Furthermore, healthy dietary patterns are associated with lower anxiety and depression rates in U.S. military (Forys-Donahue et al., 2020). In military field trainings, where food consumption is not limited, dietary intake could be reinforced by choosing more appropriate and acceptable meals in harsh conditions (Charlot et al., 2021).

Nutritional recommendations are derived from meta-analyses and reviews with strong scientific evidence behind them. However, general recommendations might not be applicable for all military contexts (Scott & Deuster, 2023).

Gonzalez et al. (2022) proposed that military personnel should follow Army Regulation 40-25 in energy and macronutrient intake. This regulation establishes nutritional standards for military feeding, operational rations and restricted rations. It also identifies the effects of environmental factors on energy and nutrient requirements (Army Regulation 40-25, 2017). Army Regulation 40-25 could be the basis of the military nutrition, but more task-specific guidelines are needed (Scott & Deuster, 2023), as seen in this dissertation.

6.1.2 Cardiovascular risk factors

Elevated LDL and total cholesterol (34-40% and 29-31% of participants, respectively) and systolic blood pressure (31-69% of participants) were observed during the operation. The results are comparable to other military studies (Gielerak et al., 2020; Hilgenberg et al., 2016; Mara et al., 2018; Pasiakos et al., 2012). The highest prevalence of hypercholesterolemia was detected in 50.7% of the Brazilian cadets (Hilgenberg et al., 2016) and the lowest in 20.7% of Chinese officers (Mara et al., 2018). The prevalence of hypertension was observed in 15% of Brazilian cadets, 47% of Chinese officers, and 21% of Polish soldiers (Gielerak et al., 2020; Hilgenberg et al.; 2016; Mara et al., 2018). Higher rate of Chinese officers could be explained by higher age of the participants, because results included retired officers, but interestingly, this was not seen in prevalence of hypercholesterolemia in the studied population (Mara et al., 2018).

Compared to Finnish civilians, soldiers had better total cholesterol values compared to civilians (29-31% vs 55-63%) (II; Borodulin et al., 2015). High diastolic blood pressure was observed in about 50 % of civilians, whereas only 20-28% of soldiers had high diastolic blood pressure (II). Both of these results were conducted in the same time frame (2012 and 2014), although, Borodulin et al. (2015) reported results from an older age group (30-59 yrs.) than the present study (II) (mean ± SD 30.0 ± 8.7 yrs). Wider age range may explain the difference.

Macronutrient intake was not related to CVD risk factors, but the change in fiber intake was negatively associated with the change in total and LDL cholesterol concentrations (II). Dietary fiber remained under recommended level 25–35 g $\cdot d^{-1}$ throughout the follow-up (I). Thus, consumption of fiber-rich foods, whenever it is possible in military bases, could improve cardiometabolic health, weight management and prevent gastrointestinal symptoms (Stephen et al., 2017).

6.1.3 Energy balance

During the crisis management operation, energy intake and expenditure were balanced, and mean body mass remained stable (I). Mean energy intake values varied from 10.1 to 10.5 MJ $\cdot d^{-1}$ (I), which agreed with previous findings (7.6–13.2 MJ $\cdot d^{-1}$) (Fallowfield et al., 2014; Lindholm et al., 2012; Rietjens et al., 2020). Total physical activity was surprisingly low during the operation (Pihlainen et al., 2018). Mean step count remained under 10 000 steps / day through the study. Crisis management operations have been regarded as physically and mentally demanding military tasks, but based on these results, sedentary behavior was predominant, and any overload symptoms were not observed (I; Pihlainen et al., 2018). Estimated energy expenditure was approximately 10.5 MJ $\cdot d^{-1}$ (unpublished data), which supports energy intake values despite inaccuracies in both methods.

These energy expenditure values were lower than in other crisis management operation studies (10.4-15.2 MJ $\cdot d^{-1}$) (Fallowfield et al., 2014; Lindholm et al., 2012; Rietjens et al., 2020). In two studies, energy expenditure was carefully measured with the DLW method (Fallowfield et al. 2014; Rietjens et al., 2020), and physical activity levels were derived as the ratio between total daily energy expenditure (TDEE) and basal metabolic rate (BMR) (Rietjens et al., 2020). In our study, accelerometers were used to estimate physical activity. This method includes inaccuracies, for example, the device does not record strength training properly or other similar movements.

Although body mass remained stable, unfavorable changes occurred in body composition (I). Subcutaneous fat increased in upper and lower extremities, revealing lack of physical training and/or imbalanced diet (I). A similar increase in adipose tissue was found in submariners in a three-month operation (Rietjens et al., 2020), whereas, during a crisis management operation in Afghanistan, body mass, muscle mass and fat mass reduced (Fallowfield et al., 2014). In a four-month operation in Chad, body mass decreased while fat mass did not change (Rintamäki et al., 2012). Other crisis management studies did not screen energy intake or expenditure (Dyrstad et al., 2007; Farina et al., 2017; Lester et al., 2010; Nagai et al., 2016; Sharp et al., 2008; Warr et al., 2013). Activity levels from the Lebanon operation can be compared to submarine deployment (Rietjens et al., 2020). However, in submarines, working environments, limited facilities for physical training and six-hour working shifts explain the low total physical activity and energy expenditure. To summarize, physical activity and energy expenditure levels can be very low in some military operations (I; II). By balancing diet according to expenditure levels and modifying physical training based on total activity of the operation, these unfavorable changes in body composition could be prevented.

In winter survival training (III; IV), high energy expenditure and low energy intake were assumed, but such a low energy intake is not commonly studied (Table 3). So far, only a few studies have reported energy intake values as low as this study III (Castellani et al., 2006; Hoyt et al., 2006; Øfsteng et al., 2020). Energy intake in our studies III and IV were 2.0 MJ $\cdot d^{-1}$ at the lowest day. Hoyt et al. (2006) limited energy intake to 0.2-2.2 MJ $\cdot d^{-1}$ for males and 0.2-1.9 MJ $\cdot d^{-1}$ for females. Other studies reported 4.9-6.0 MJ (Castellani et al., 2006; Øfsteng et al. 2020), which was similar to energy intake in the FEX group in the latter part of training (III; IV). This amount of energy and carbohydrate is not sufficient to maintain glucose needs of body, which shifts energy metabolism to use fat and protein storages.

Loss of body mass is obvious, and mainly caused by excessive energy expenditure. In these studies, III and IV, energy intake was restricted on purpose to assess soldiers' physical and mental capability in harsh environments.

Although, in practice, energy intake is not restricted on purpose, remarkable energy deficit still exists, caused by excessive energy expenditure. In addition, it is challenging to carry, prepare and consume food as much as is required in the field conditions. Energy expenditure levels in these studies III, IV (max. 23.9 MJ $\cdot d^{-1}$) are in line with previous studies presented in Table 3 (Castellani et al., 2006; Hoyt et al., 2006; Margolis et al., 2014; Margolis et al., 2016). The highest energy expenditure (28.7 ± 2.4 MJ $\cdot d^{-1}$) was measured during ski march. Compared to sports, mean daily energy expenditure in the Tour de France -cycling tour was 25.4 MJ $\cdot d^{-1}$, and the highest mean value was as much as 32.7 MJ $\cdot d^{-1}$ (Saris et al., 1989).

Military tasks and conditions in the field require lot of energy. Land navigation, activities on difficult terrain, body armor, personal equipment, lack of sleep, cold temperature, etc. increase energy expenditure, and these requirements are not adjustable, as opposed to dietary intake. Few studies have investigated carbohydrate or protein supplements (Margolis et al., 2016; Øfsteng et al., 2020), or energy bars (Tanskanen et al., 2012), but they did not provide a significant decrease in energy deficit. Thus, food availability is not necessary a predominant factor causing negative energy balance. Increasing energy density (amount of energy in a particular weight of food) might be one way to diminish energy deficit (Margolis & Pasiakos, 2023).

Interesting changes in body composition data were observed during survival training (III). Muscle mass increased after the most strenuous phase of training. These findings are logical in the REC group, for whom dietary intake was allowed *ad libitum* during the recovery phase, but muscle mass also increased in FEX group. Participants were delivered more field rations after the initial phase of survival training, but mean energy content of food remained 4.2 MJ per day, and negative energy balance still existed. Bioimpedance method is based on total body fluid, and thus, severe dehydration might have interfered with the results. Serum creatinine was also tested, which has been used to estimate muscle mass (Kim et al., 2016), but these results paralleled bioimpedance results (III). Any other explanation for these controversial results has not found, except for imbalances in body fluid and methodological inaccuracies.

6.1.4 Hormonal and metabolic status

Appetite-mediating (leptin and ghrelin), anabolic (testosterone), and catabolic hormones (cortisol) were evaluated in studies III and IV. Leptin concentration decreased during the first few days of survival training, and then recovered, whereas ghrelin was stable during training. Energy intake and expenditure were associated with leptin, but not with ghrelin. Leptin is an adipose-derived hormone, and it typically decreases in acute energy deficit (Pasiakos et al., 2011) and loss of body fat (Hill et al., 2015) as presented in study III. Ghrelin levels remained stable and differences between groups were not found, although the REC group got regular meals during recovery phase. Blood samples were collected early in the morning after overnight fast in each measurement point, which may explain stable ghrelin levels throughout the study. In a study by Pasiakos et al. (2011)

acute changes in ghrelin were compared between feeding and fasting groups in a 48-hour follow-up. In fasted group, ghrelin levels stayed elevated like in study III. High, but systematic, ghrelin concentrations were measured from a few participants of the FEX group (III), but data were included in finally results after careful consideration of sample analysis. Testosterone/cortisol ratios were determined to evaluate overall stress and recovery, and it is considered to be more sensitive to overall stress reaction than testosterone or cortisol alone (Lee et al., 2017). During survival training phase, T/C-ratio reduced in all (IV) but increased during recovery phase in the REC group. Energy intake was positively associated with T/C-ratio in the REC group in the first part of the study (PRE-MID), but then, inversely associated with the FEX group in the latter part (MID-POST). T/C-ratio aligned with predicted periods of stress given that short-time recovery returned values toward baseline and T/C-ratio values of the FEX group stayed impaired. Overall, hormonal responses, except for ghrelin, were remarkable, and the recovery phase partially returned values to baseline. However, concentrations were still lower than at baseline, indicating insufficient recovery from strenuous training (III; IV).

Biomarkers for energy metabolism (free fatty acids, urea, glucose) were evaluated in study III. Free fatty acids were released into the bloodstream as a result of energy deficit and lack of carbohydrates during the survival training phase. Decreases in body mass, body fat mass, and leptin support these findings (III). Previous studies have shown short-time (hours) and long-time (days) fasting increases lipolysis and release of free fatty acids to produce ketone bodies as fuel of brain (Kimura et al., 2020; Wang & Wu, 2022). Decrease in leptin concentration (III) indicates degradation of triglycerides, because leptin signals the "status" of adipose tissue to the brain – both the amount and stability of fat stores (Rosenbam & Leibel, 2014). Simultaneously, urea concentrations increased as a result of activated protein degradation (Haralambie & Berg, 1976). Body composition changes agreed with these metabolic responses, i.e., body mass, body fat mass, and muscle mass decreased in the same time frame (III).

Glucose concentrations were in the range of a fasting state during survival training, and even decreased after the first field phase. All samples were collected after overnight fast, but lack of dietary carbohydrates reduced glucose values even more. In the crisis management operation, blood glucose was determined as a marker of CVD risk (II). A very low prevalence of hyperglycemia (> 6.0 mmol/l) was found (2.9% of participants) at PRE and POST measurements. Hypoglycemic state was obvious in survival training (III), in which carbohydrate intake was highly limited.

Electrolytes, sodium, potassium, and chloride were examined for assessing hydration status in survival training (III). Participants were likely dehydrated during training as a result of sweating, and limited fluid intake because drinking water had to melt from snow. Acute loss of body mass supports this hypothesis. Sodium and chloride in extracellular fluid and potassium in intracellular fluid could be a response to intense training (Warburton et al., 2002), but electrolyte concentrations are well-regulated during stress. When electrolytes (and other biomarkers) are measured from blood, loss of total body water decreases extracellular water, and thus, blood volume, which may increase concentration of biomarkers (Pedlar et al., 2019). That might be the case for sodium and chloride, which are typically lost via sweating. In addition to electrolytes, other valid and reliable methods have been used to assess hydration status (Kavouras, 2002; Shirreffs, 2003). Body mass, bioimpedance, blood and urine biomarkers, blood pressure, skinfold thicknesses and heart rate have been used for evaluation (Kavouras, 2002; Shirreffs, 2003). So far, plasma and urine osmolality and urine specific gravity are the most widely used methods, in addition to body mass measurements (Kavouras, 2002). If laboratory methods are not suitable, morning urine colour could be a practical indicator in field conditions, and also in military (Kalvouras, 2002).

Instead of measuring single biomarkers, metabolomics could give a comprehensive understanding of responses in energy metabolism. Profiling can be done in hundreds of metabolites to identify changes in energy, carbohydrate, lipid and protein metabolism (Gwin et al., 2022). To date, few studies have determined metabolites in military contexts: survival training (Karl et al., 2017), sustained energy deficit (Stain et al., 2022), initial military training (Gwin et al., 2022), and overweight soldiers (Albores-Mendez et al., 2022). In some studies, increases in lipid metabolites have been associated with loss of fat mass, and amino acid metabolites have been associated with changes in muscle mass (Gwin et al., 2022; Karl et al., 2017). Thus, identifying metabolites could produce new insights into energy metabolism and mechanisms behind field conditions.

6.1.5 Nutrition and physical perfomance

In crisis management operations, outcomes of physical performance have been observed in several studies, but their relations to nutritional factors were not examined. Few studies have observed dietary intake during operation (Fallowfield et al., 2014; Rietjens et al., 2020; Rintamäki et al., 2012). In this research project, the main purpose of the study was to implement different training programs, and all training groups (strength, endurance, strength + endurance) were able to maintain or improve their physical fitness during the operation (Pihlainen et al., 2022). Only the control group had reduced muscular power of the lower extremities after the operation. Dietary factors were not analyzed in relation to physical performance variables. Energy was balanced during the operation, although, distribution of macronutrient was not optimal (I). Sufficient protein intake was observed (I), which is beneficial for strength training and muscle mass (Pasiakos et al., 2015).

During survival training, physical performance was examined by strength and endurance tests, and a shooting test (IV; Ojanen et al. 2023). Energy intake, expenditure, and balance were not associated with physical fitness variables. However, a positive association was found between changes in energy balance and changes in the shooting index in the FEX group from MID to POST, but group differences were found at each measurement point (IV). Decreases in physical fitness during strenuous field trainings and declines in strength tests are well-documented (Hamarsland et al., 2018; Murphy et al., 2018; Nindl et al., 2007; O'Leary et al., 2020). Based on the existing literature and these studies, short-time energy deficit is not the only factor which contributes to a reduction in physical performance, but rather, is compounded by other stress factors – e.g., lack of physical training, sleep deprivation, prolonged physical activity, environmental factors, mental stress, and lack of motivation (Murphy et al., 2018; Margolis & Pasiakos, 2023). It has been determined that energy deprivation accounts for 39% of decline in lower-body power, and other factors, such as sleep deficit, muscle soreness, and lack of motivation contributes to 61% of lower-body performance (Margolis & Pasiakos, 2023). Energy deficit seems to have a cumulative influence on physical performance – total energy deficit (magnitude of deficit x duration of training) is related to physical performance outcomes (Murphy et al., 2018). Thus, longer trainings with energy deficit will result in more obvious reductions in strength and endurance capacity.

The winter survival training study was exceptional compared to studies outside the military. For ethical reasons, civilians are rarely exposed so severe energy and sleep deficit for days, but these kinds of field trainings are common in several armed forces (Ahmed et al., 2020; Hamarsland et al., 2018; Hoyt et al., 2006; Margolis et al., 2016; Nindl et al., 2007; Szivak et al., 2018). In all studies, soldiers were exposed to energy and sleep deficit, and moderate intensity workload, resulting in clear physiological responses.

In many physiological processes, the body is able to more readily adapt to stressful situations when it has been exposed to the stressor previously. For example, in high altitude exposure, repeated exposure seems to be beneficial by facilitating and speeding up the adaptive process (Mujika et al., 2019). The phenomenon is called hypoxic memory. Better hypoxic memory can cause moderate physiological responses while maintaining physical performance. That theory might be worth of considering in survival trainings: if soldiers are exposed to energy and sleep deficit more often, their body's physiological and psychological mechanisms adapt more easily. To be realistic, such conditions are too extreme for regular training, but theoretically, it can be discussed. In these studies, participants were young conscripts with no experience of such field trainings, and that might partially explain the greater physiological responses.

To conclude, most of military studies reported decreases in physical performance variables. Especially strength and power were impaired, but clear associations with energy variables were not identified. Short-time energy deficiency might not be essential to impaired physical performance (IV), but chronic energy deficit and reduction in body mass are probably related (Murphy et al., 2018; Tassone & Baker 2017).

6.2 Strengths and limitations

The main strength of studies I and II was the unique setting in a real-life crisis management operation. So far, no studies have been carried out in operational areas during an actual crisis management operation, and thus, the dietary results are more accurate than data collected afterwards. Similarly, winter survival training, studies III and IV, were implemented in field circumstances in late winter. Although several studies in survival-type trainings have been published (Table 3), response to a cold environment is still a current and interesting topic in military studies.

All studies (I-IV) used self-reported food diaries to estimate dietary intake. In the crisis management operation (I; II), three consecutive days were recorded for all three measurement points. Three meals were served daily, and snacks and other meals could be bought from stores and restaurants nearby. Compared to civilian life, days did not differ between weekdays and weekends, except for Sundays, when brunch was served instead of breakfast and lunch. Alcohol consumption was restricted so that max two portions of alcohol were allowed per day. Researchers ate same meals than participants, and recorded and weighed regularly-used food items, such as a piece of bread, margarines, drinks etc., which improved the accuracy of method. Participants reported monotonous life and a depressed mood state of the latter part of the study, which may have influenced the motivation and accuracy of the recording (I).

In studies III and IV, pre-filled food diaries were used because food intake was restricted and food items were known beforehand. With these procedures, the accuracy of the method might has increased slightly, although forgetfulness, biases and underreporting may have interfered the results (Capling et al., 2017). Based on our experience, pre-filled food diaries could help the recording in harsh conditions (III; IV; Tanskanen et al. 2012), and therefore, this procedure could be applicable in military settings also in future. We had two different software programs (Nutri-Flow, Fineli) to calculate energy and nutrient content of the meals, even though Nutri-Flow is based on a same national nutrient composition database (Fineli). However, both software programs are used in research, and Fineli contains a wide range of national food items which may improve the accuracy of the method. Collecting data in field conditions is always demanding, especially in the military. Vulnerable security situations in the crisis management operation, and lack of sleep and harsh environment in survival training undoubtedly influenced ability and motivation for filling food diaries.

Compared to all dietary assessment methods, dietary records represent 9% of all dietary intake methods used in military studies (Collins et al., 2020). The most common assessment was a food frequency questionnaire (45%), and 24-hour recall was used in 9% of studies (Collins et al., 2020). In our studies, traditional paper food diaries were used in both projects, but Beals et al. (2019) used a dietary assessment tool and software program to get digital data. Mobile phones could be the most practical tool for collecting daily diaries in civilian life, but in studies III and IV, participants were not able to use their own mobile phones in the field and internet connection can be limited in northern areas.

In our studies, energy expenditure was estimated by monitoring heart rate variability. Validation of this method has been done against VO_2max (Smolander et al., 2011), but not against the doubly labelled water method, which is defined as the golden standard method for daily energy expenditure estimation. DLW has been used in several military studies also in strenuous field conditions (Table 3), but sample collection and cost of isotopes may be limiting factors for using this method in harsh environments. Wearable physical activity monitors (heart rate, accelerometer) could be valid and practical methods for military settings (Siddall et al., 2019). Park et al. (2017) demonstrated that combining data from accelerometers and heart rate monitors could improve the accuracy of energy expenditure estimation.

Accelerometry data has some limitations but is an objective method for total physical activity assessment. The method is based on movements of the hip, but some activities, like resistance training, can be performed without any hip movements (Pihlainen et al., 2018). Furthermore, military tasks are often performed with body armor and other extra loads, which increase the intensity of training without extra movements.

The other systematically used method in our studies is multifrequency bioimpedance analysis for body composition assessment. Bioimpedance analysis is based on fluid content in the body. Body mass, fat mass and skeletal muscle mass are calculated by a built-in software (Aandstad et al., 2014). Its validity and reliability against DXA (dual energy X-ray absorptiometry) have been studied in military personnel, but neither BIA nor other methods (skinfold measurements, single-frequency bioimpedance) were superior to each other (Aandstad et al., 2014). Hydrostatic weighing has been traditionally the most accurate method for body composition estimation (Clasey et al., 1999), but nowadays, DXA is known as a reference method in validation studies. In our studies, theoretically, DXA could have been possible, but logistically challenging in Lapland, and Lebanon especially. A bioimpedance device could be transported to field conditions. The accuracy of the method could have been compromised from dehydration during survival training (III). According to BIA, muscle mass of participants increased after remarkable energy and fluid deficit, and creatinine concentrations reacted in a parallel manner. Previous literature does not agree with these findings. Although, a muscle sparing effect has been documented (Gagnon et al., 2020), so these controversial results could be explained by a dehydrated state and methodological bias. In the crisis management operation, participants were advised to fast overnight and standardize their physical training and sauna in all measurements. Measurements were conducted per the manufacturer's instructions, and such acute changes in hydration status were not observed. From our experience in field settings, a mobile bioimpedance device is practical with decent accuracy, especially when combined with ultrasound technique (I), analysis of creatinine (III), or anthropometric measurements (Aandstad et al., 2014).

Physical fitness tests have been widely used over previous decades in civilian (Häkkinen et al., 2001) and military settings (IV; Ojanen et al., 2020). Assessments include isometric maximal force production tests, muscular endurance tests, which are part of soldier's physical fitness tests, and endurance test. Due to a variety of strength tests, strength index was aggregated in study IV to describe physical fitness variables (strength and endurance) as an equal way. In military settings, several strength tests can be used to evaluate muscular and isometric strength, but as a maximal "time to exhaustion" test, aerobic capacity can be measured by one test. In study IV, 20-m shuttle run test was used to evaluate aerobic capacity indirectly, but due to the strenuous field training phase, some participants could not perform maximally. A direct maximal oxygen uptake test is the most accurate method for assessment of aerobic performance. As an individual treadmill test, this was impossible in our in-field circumstances with limited time frame. The 12-minute running test is an indirect test for aerobic capacity (Cooper, 1968) and has been used in Finnish Defence Forces as a regular test for decades. This data can be used as a background or predict variable (Vaara et al., 2020) to get better understanding of individual's aerobic capacity.

When assessing other methodologies, measurements of blood biomarkers are objective and accurate indicators of biological processes. Although some inaccuracies can occur during laboratory analyses, biomarkers produce important insights into physiological processes in military contexts. From one blood sample, many biomarkers can be determined, which is preferable in military field settings. Lipid profiles and inflammatory markers are routinely used for CVD risk assessment. Fasting and controlled blood samples were collected in study II. Hormone assessments give valid and reliable data about regulatory systems, and metabolic biomarkers (free fatty acids, urea, glucose) reflect metabolic pathways. However, metabolomics could give a more comprehensive understanding of energy metabolism than single biomarkers (Gwin et al., 2022). Analysis of electrolytes is accurate method for hydration status assessment, but its efficacy can be further bolstered when combined with other methods (Kavouras, 2002; Shirreffs, 2003).

The number of participants is typically limited in harsh environment settings. In the crisis management operation, almost 100 participants were recruited, but food diaries were obtained only from 40. In survival training, high drop-out rates were expected (Vaara et al., 2020), so a higher number of participants were recruited in FEX group, and a mixed model was used for statistical analysis. Nutritional studies in the military are often carried out in research projects with additional purposes (Tassone & Baker 2017) like studies I-IV in this dissertation. Compared to clinical nutrition research undertaken in laboratory, military studies are in the field and the accuracy of methods will be compromised (Tassone & Baker 2017).

6.3 Future directions

So far, military nutrition research has focused on characterizing nutritional requirements in different environments to optimize diet during operations (Karl et al., 2022). This thesis presented many nutritional and physiological aspects in two completely different military contexts. Military operations can be implemented in many other weather conditions, where temperature, humidity, altitude, and wind influence nutrition and military performance. In addition, army, navy, and air forces, have their own special operations and tasks to be considered. So, task-specific research is needed to investigate the variety of military duties.

The complexity of the topic makes generalized nutrition recommendations for soldiers problematic. Furthermore, individual responses to diet, physical training and other external stress factors vary a lot. Thus, tailored diets and physical training programs would help health promotion, disease prevention and physical fitness in the military. Scott and Deuster (2023) proposed precise nutrition recommendations of soldiers, which is defined as "a personalized set of recommendations for a small group of people or individual". Task-specific dietary guidelines could be determined for typical military tasks and operations, e.g., trainings in hot/cold/high altitude, marches, trainings with field rations, survival-type trainings. These guidelines cannot be applied to all military tasks, but this could be a next step in developing tailored military nutrition. New techniques, like holistic health records, big data analytics, and wearable sensors etc., will provide new tools to promote nutritional research and policy (Karl et al., 2022; Kyriazis et al., 2017), and this could contribute to tailored nutrition recommendations.

Finnish Defence Forces have annual fitness tests and occupational health care, but nutritional factors should be emphasized more in every level of organization: education, research, health care and practice. Conscript service is obligatory for all young Finnish men and that could be a potential period for proper nutritional education – ultimately promoting optimal military performance during service and good dietary behavior as a reservist. For military personnel, individual and regular monitoring of diet, blood biomarkers and physical fitness would help recognize factors contributing to health and physical performance of soldiers. On a practical level, food delivery systems, instructor roles, motivation and interest in dietary factors are involved in optimal dietary behavior. Choices at individual level, especially among salaried personnel, could be the most important behavior effector. Effective, evidence-based nutritional education, regular screening and healthy food choices would improve health and military performance.

These studies have been carried out only in male soldiers. In Finnish Defence Forces, conscript service is voluntary for women, and few female soldiers participated in measurements, but were excluded from data analysis in all studies (I-IV). Other armed forces have emphasized research of female soldiers, and this would be important to study in Finland. The Finnish military system has specialties depending on geographic location and structural system, and national research are needed. For example, the latest studies of Edwards et al. (2022a; 2022b) indicated that energy availability, bone health and hormonal metabolism are especially important issues for female soldiers, and health responses differ from men. If armed forces recruit more women, physiological outcomes need to be clarified and adjust the military training system also suitable for women (Santtila et al., 2019; Wardle et al., 2021).

7 MAIN FINDINGS AND CONCLUSION

The diet of soldiers during a crisis management operation did not meet nutritional guidelines and was similar to their civilian counterparts: CHO intake was lower, and fat intake was higher than recommended. Furthermore, fiber intake remained low while saturated fat intake exceeded the recommended level (I). Elevated LDL cholesterol (34-40% of participants) and systolic blood pressure (31-69% of participants) were observed during the operation. Changes in macronutrient intake were not associated with cardiovascular risk factors at any of the measurement points but change in fiber intake was inversely associated with change in total and LDL cholesterol concentration (II).

Winter survival training caused a remarkable energy deficit, and significant responses in body composition, leptin, free fatty acids, glucose, urea and potassium (III). Changes in blood biomarkers partially returned to baseline, especially when recovery was emphasized, but body mass and body fat% remained below baseline levels throughout the training period. However, energy intake, expenditure, or balance were not associated with any physical performance variables (IV).

To conclude, most of military studies reported decreases in physical performance variables during operation or training. Especially strength and power were impaired, but clear associations with energy variables were not identified. Short-time energy deficiency might not be essential to impaired physical performance (IV), but chronic energy deficit, reduction in body mass and interactions with other stressors are likely related (Margolis & Pasiakos, 2023; Murphy et al., 2018; Tassone & Baker, 2017).

Different military contexts can induce opposing metabolic responses and adaptations. As seen in studies I and II, physical activity in crisis management operation can be very low, and unbeneficial changes in CVD risk factors can occur. Such responses and increased adiposity (Rietjens et al., 2020) may weaken cardiometabolic health during deployments. Therefore, diet and regular physical training should be balanced according to requirements of deployment to maintain operational readiness. On the other hand, in survival trainings, the setting is fundamentally different: severe energy deprivation and high energy expenditure during field training can cause physiological shock by changing energy and substrate metabolism (III). Adaptative processes of this state balance the physiology and values return to baseline, especially when recovery is emphasized (III, IV). So, it is essential to recognize the variety of military contexts and to study physiological responses in these conditions to understand the comprehensive role of military nutrition (I-IV). In conditions, where workload is low and training facilities are limited, fiber-rich and energy-balanced diet should be emphasized to sustain good nutritional status, prevent diseases and support physical training. With this manner, operational readiness is maintained, and soldiers are capable of incoming missions, in which physiological demands can be totally different.

By recognizing nutritional and physiological responses to different military conditions, physical and military performance, and cardiometabolic health can be optimized.

YHTEENVETO (SUMMARY IN FINNISH)

Ravitsemus, hormonaaliset ja metaboliset vasteet ja fyysinen suorituskyky erilaisissa sotilasympäristöissä

Ravitsemus on yksi sotilaan suorituskyvyn osatekijä. Sotilastyö vaihtuvissa ympäristöissä aiheuttaa monia erilaisia vaatimuksia ravitsemukselle. Kriisinhallintaoperaatiot ovat kuukausien mittaisia, joissa olosuhteet voivat pysyä pitkään vakioina, mutta ympäristö on altis nopeille muutoksille. Selviytymisharjoituksessa olosuhteet ovat täysin erilaiset, sillä taistelijan tulee suoriutua fyysisesti ja psyykkisesti vaativissa olosuhteissa vähällä ravinnolla ja unella useiden päivien tai muutaman viikon ajan. Näiden eri toimintaympäristöjen vaikutuksia suomalaissotilaan ravitsemukseen ja fysiologiaan ei vielä tunneta tarkasti, sillä kansallinen tutkimuksemme on painottunut sotilaan fyysisen toimintakyvyn tutkimukseen.

Tämän väitöskirjan tarkoituksena oli tutkia a) suomalaissotilaiden ravinnonsaantia, kehon koostumusta ja niiden yhteyksiä sydän- ja verisuonitautien riskitekijöihin kuuden kuukauden kriisinhallintaoperaatiossa Libanonissa ja b) varusmiesten energiatasapainoa, fysiologisia vasteita ja niiden yhteyksiä fyysiseen kuntoon kymmenen päivän selviytymisharjoituksessa Lapissa.

Ravinnonsaantia tutkittiin ruokapäiväkirjojen avulla, kehon koostumusta bioimpedanssilaitteella, lipidejä ja energia-aineenvaihdunnan biomarkkereita verikokeilla ja fyysistä kuntoa voimaa ja kestävyyttä mittaavilla kuntotesteillä. Mittaukset toteutettiin kriisinhallintaoperaation aikana kolmesti (0, 3, 6 kk) ja sel-viytymisharjoituksessa neljästi (1, 6, 8, 10 pv).

Kriisinhallintasotilaiden ravinnonsaanti ei vastannut kaikilta osin kansallisia ravitsemussuosituksia, sillä hiilihydraattien saanti alitti, kun taas rasvan ja erityisesti tyydyttyneen rasvan saanti ylitti suositusarvot energiaravintoaineiden suhteellisesta saannista. Proteiinien osuus oli suosituksen ylärajalla. Fyysinen aktiivisuus oli alhaisella tasolla (< 10000 askelta päivässä). Vähäinen kuidun saanti ja suurempi kehon rasvan määrä oli yhteydessä kohonneisiin kokonais- ja LDL-kolesteroliarvoihin. Selviytymisharjoitus aiheutti merkittävän energiavajeen ensimmäisten päivien aikana, jolloin energiankulutus oli suurta ja energiansaanti rajoitettu. Samalla kehon painossa, rasvan määrässä sekä leptiinin, vapaiden rasvahappojen ja urean pitoisuuksissa tapahtui selvä muutos. Muutokset tasaantuivat harjoituksen edetessä, erityisesti kun palautumista tehostettiin. Energian saanti, -kulutus tai -tasapaino eivät olleet yhteydessä kuntomuutoksiin.

Yhteenvetona voidaan todeta, että kriisinhallintasotilailla kuitupitoisten hiilihydraattien lisääminen ja tyydyttyneen rasvan vähentäminen parantaisi ruokavalion laatua ja edistäisi sydänterveyttä. Selviytymisharjoitus aiheutti merkittävän energiavajeen ja voimakkaan fysiologisen vasteen, joiden tunnistaminen on tärkeää suorituskyvyn ylläpitämiseksi vaativissa olosuhteissa. Tämän aineiston perusteella yksittäisillä energiamuuttujilla ei ollut yhteyttä kuntomuutoksiin, vaan suorituskyvyssä tapahtuvat muutokset todennäköisesti johtuivat monen eri kuormitustekijän yhteisvaikutuksesta. Sotilaiden vaihtelevat tehtävät ja toimintaympäristöt aiheuttavat erilaisia fysiologisia vasteita, jolloin näiden tekijöiden tunnistaminen ja ravitsemuksen huomioiminen on tärkeää sotilaiden suorituskyvyn ja terveyden kannalta.

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ORIGINAL PUBLICATIONS

I

DIET MACRONUTRIENT COMPOSITION, PHYSICAL ACTIVITY, AND BODY COMPOSITION IN SOLDIERS DURING 6 MONTHS DEPLOYMENT

by

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Diet macronutrient composition, physical activity and body composition in soldiers during six months deployment

Diet composition, physical activity and body composition in soldiers during deployment

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STRUCTURED SUMMARY

Introduction

Optimal diet together with good physical fitness maintains readiness and military performance during longer deployments. The purpose of this study was to describe changes in dietary macronutrient and energy intake, total physical activity and body composition during a 6-month deployment in South Lebanon. Furthermore, associations of diet macronutrient intake and physical activity on body composition were also studied.

Materials and Methods

Forty male soldiers kept a 3-day food diary and their body composition was measured via bioimpedance and ultrasonography. Total physical activity was evaluated by accelerometers in a subgroup of participants. Measurements were conducted in the PRE-, MID- and POST-deployment.

Results

Mean carbohydrate intakes were 39.5-42.6 E%, protein intakes 18.7-22.3 E% and fat intakes 34.9-35.7 E%. Daily energy intake remained stable (10.1-10.3 MJ/D). Total physical activity was decreased during deployment (e.g. step count from 9835 ± 2743 to 8388 ± 2875 steps/day, $p=0.007$). Skeletal muscle mass and subcutaneous fat increased by 1.3% ($p=0.019$) and 1.9% ($p=0.006$), respectively. Energy and fat intake associated positively with body mass and skeletal muscle mass ($r=0.31$-0.48, $p<0.05$-0.001).

Conclusions

Carbohydrate intakes and physical activity were low, compared to the general recommendations. Protein intakes were relatively high. Skeletal muscle mass and subcutaneous fat increased. Suboptimal diet together with low level of physical activity may have a negative impact on body composition, physical performance, and cardiometabolic health. Consequently, soldiers should be encouraged to consume more fibre-rich carbohydrates and less saturated fatty acids as well as maintain a high level of physical fitness to sustain military readiness during long-term deployments.

INTRODUCTION

Military deployments are often physically and mentally demanding. Soldiers have to maintain continuously high levels of readiness and must respond to external stimuli whenever needed. Optimal nutritional status improves physical and mental performance, helps to prevent infections and enhances recovery after diseases and injury (1). However, previous studies have found fluctuations in the diet and nutritional status during prolonged deployments (2,3). For example, a worsened dietary quality, including decreased intake of calcium and vitamin D, was observed in special operators during deployment (3). Furthermore, reduced *e*nergy and food intake has been demonstrated to occur due to high temperature, stress and menu fatigue (2).

The total energy expenditure of soldiers is dependent on their military tasks in different environments. In garrisons, energy expenditure varies from 13.0 to 29.9 MJ/d (3100 -7100 kcal/d) in male soldiers (4), in field exercises 20.9-41.9 MJ/d (5000-10000 kcal/d) (5-8) and in longer deployments 10.5-15.1 MJ/d (2500-3600 kcal/d) (2,9-10). If the energy expenditure is very high, body mass often decreases, which occurs frequently in demanding military operations (2,6,8-10). For example, in a 6-month military operation in Chad, a decrease of 3.5% in body mass was found (9). Furthermore, during the first half of a 6-month deployment in Afghanistan, the body mass of soldiers decreased by 4.6%, which was partly regained (+2.2%) during the last three months (2). In another study, fat free mass decreased by 3.5 % and body fat increased by 1.9 % during 9- months' deployment in Afghanistan (9). These changes were also accompanied by decreases in aerobic capacity (-4.5%) and upper body muscle power (-4.9%).

Several studies (2, 8-10) have assessed the changes in physical fitness, strength and endurance capacity induced by military deployments, but only a few studies (11) have reported total physical activity or work load responses of deployment. Energy expenditure can partially reflect the amount of total physical activity but a more direct form of measurement is needed to describe how active or passive soldiers are in their duties and in their leisure time.

The main purpose of the present study was to describe changes in dietary macronutrient and energy intake, total physical activity and body composition during a 6-month deployment in South Lebanon. In addition, associations of dietary macronutrient intake and physical activity on body composition were investigated during deployment.

METHODS

Subjects and study design

Forty healthy Finnish male soldiers (mean ± SD age 29.5 ± 8.4 yrs., height 178.7 ± 6.5 cm, body mass 77.8 ± 8.7 kg, BMI 24.4 ± 2.7 kg/m^2) who were deployed for six months in a military operation in Lebanon, voluntarily took part in the study. They were informed of the study design and gave their written consent to participate. The study protocol and procedure were reviewed by the Ethical Committee of the Central Finland Health Care District.

All measurements were carried out three times in a military base in South-Lebanon. Initial measurements (PRE) were undertaken after a two week acclimatization period, and the respective measurements were repeated after 9 (MID) and 19 weeks (POST) after the initial measurements.

Dietary intake

The dietary intake data were estimated from 3-day food diaries on consecutive days of a week in all three measurement points. Participants were asked to maintain their regular food consumption and to register all the food, fluid and nutritional supplements immediately after each meal by writing them down to food diary. They were asked to estimate food weights using household measures and standard units. The research group weighed certain portions (meat, fish, bread, fruits, vegetables) to improve accuracy of the measurements. Food diaries were reviewed and then analyzed with Nutri Flow (Flow-team, Oulu, Finland) software program. Digital questionnaire was used in MID deployment to record the use of protein supplements.

In the camp breakfast, lunch and dinner were supplied from Mondays to Saturdays and on Sundays, only brunch and dinner were served. In addition, there was a possibility to buy beverages, snacks and meals from local restaurants outside the camp or when patrolling. Use of alcohol was restricted to two portions per day.

Physical activity

Total daily physical activity (PA) data were collected from a subgroup of participants (n=29), while the rest of the participants were obligated to their military duties. Total PA was measured via a three-dimensional accelerometer at a frequency of 100 Hz (Hookie AM20, Traxmeet, Oulu, Finland). The participants were instructed to wear the device on the left side of their waist at all times while they were awake for ten days, except during showering, sauna or swimming. The data were analyzed and calculated for sedentary behavior (SB), e.g. standing and sitting/lying (metabolic equivalent, MET < 1.5), and for physical activity, e.g. step count, number of breaks during SB, MET mean, MET max (/min), light PA (MET 1.5-3.0) and moderate to vigorous PA (MVPA, MET > 3.0) (12,13).

The participants were able to do strength and aerobic training (cycling, rowing, treadmill running) in two gyms in the camp. In addition, there was opportunity for walking, Nordic walking and running inside the military area as well as some ball games (soccer, beach volley, basketball).

Body composition

All body composition measurements were recorded in a fasting state in the morning (0530-0730, with participants wearing underwear. Body mass, skeletal muscle mass, fat mass and fat % were determined by using a segmental multi-frequency bioimpedance analysis assessment (InBody 720, Biospace, South Korea). Waist circumference was measured with a measurement tape (Seca 210, Seca, Hamburg, Germany) midway between the lowest rib and the iliac crest after light expiration. The average of three measurements was used.

Subcutaneous fat and muscle thickness of the vastus lateralis (VL) and triceps brachii (TB) muscles were measured via ultrasonography technique (Sonoace R3, Samsung Medison, Co & Ltd, Seoul, Korea). Measurement points of VL and TB were marked earlier based on anatomical landmarks. The probe was moved manually on the electrode gel over the marked points of the skin avoiding compression of muscle tissue. The leg support was used to diminish compression to the tissue. Three scans were taken for later analysis with an electrical caliper and the image processing system integrated in the ultrasound device and the average of these scans was used.

Statistical analyses

Statistical analyses were carried out by commercial software (IBM SPSS 22.0.0, Chicago, Illinois, USA). Data were analyzed by repeated-measured ANOVA and paired t-tests. If assumptions were not met logarithm transformations were applied (protein intake (E%, g/d), CHO intake (g/d), fibre intake (g/d), subcutaneous fat of VL and TB (cm)) or nonparametric Friedman’s tests utilized (water intake, PUFA). Spearman’s product moment correlation coefficients were used for testing linearity of body composition, dietary intake and physical activity data. The statistical significance was met when p-values were under 0.05.

RESULTS

Nutrient intakes

Participants ate 4.9 ± 1.0 (PRE), 4.7 ± 0.9 (MID) and 4.4 ± 0.8 (POST) meals per day (means ± SD). Reported energy intake was 10.1 - 10.5 MJ/d during deployment. Carbohydrate (CHO) intake E%, g/d) increased from PRE to MID. Protein intake (E%, g/d) decreased from PRE to MID but then recovered. Water intake decreased from PRE to POST (**Table 1**). A total of 57.5 % of participants reported using of protein supplements in the MID deployment. Alcohol intake (g/d) was 2.9 ± 6.0 (PRE), 2.3 ± 5.7 (MID) and 1.7 ± 4.6 (POST).

Total physical activity

Step count, light PA, MVPA, standing, sitting and lying and number of breaks during SB decreased from PRE to MID (**Table 2**). Step count, MET mean, MVPA and number of breaks during SB decreased from PRE to POST.

Body composition

Skeletal muscle mass (SMM) increased from PRE to POST. Waist circumference decreased from PRE to MID and further to POST. Body mass, BMI, fat mass, fat % or visceral fat area did not change statistically significantly during the deployment. Body composition data are shown in **Table 3**.

Muscle thickness of TB increased from PRE to MID and further to POST (3.84±0.63, 3.92±0.55, 4.04±0.62, respectively, p=0.016 MID_POST, p=0.013 PRE_POST). Muscle thickness of VL decreased from MID (3.81±0.71) to POST (3.58±0.72, p=0.019). Subcutaneous fat thickness of VL first decreased from PRE (1.03±0.47) to MID (0.97±0.45, p=0.047) but, thereafter, returned to baseline by POST (1.05±0.45, p=0.006 MID_POST). Subcutaneous fat thickness of TB increased from the PRE (0.67±0.26) to POST deployment (0.72±0.24, p=0.003).

Energy and fat intake correlated positively with SMM in all measurement points. Fat intake correlated positively with body mass in PRE, MID and POST, and energy intake with body mass in MID and POST. Other statistically significant correlations are presented in **Table 4**. Protein intake did not correlate significantly with body mass, SMM or fat %.

Statistically significant correlations were found between standing time (MET < 1.5) and body mass, BMI, fat mass, fat%, visceral fat area and waist circumference in the PRE and POST measurements (**Table 5**).

DISCUSSION

The present results demonstrated that the macronutrient intake did not meet the general nutritional requirements (14) or the Military Dietary Reference Intakes (MDRI) (15). Energy intake remained low and stable during the deployment. The daily physical activity of soldiers was very low, but their body mass did not change, indicating that the soldiers actually remained in energy balance. According to body composition measurements, skeletal muscle mass increased slightly. In addition, ultrasound measurements revealed that subcutaneous fat thickness increased in the upper and lower extremities, while muscle thickness increased only in the upper body. Energy and fat intake associated with skeletal muscle mass and the total time accumulated from standing with body mass, BMI, fat mass, fat%, visceral fat area, and waist circumference during the PRE and POST measurements.

Macronutrient intake

Intake of carbohydrate remained below 45 E% in all measurement points while the recommended level is 45-60 E% according to the Nordic Nutrition Recommendations (14). Carbohydrate intake increased during the first half of the deployment, but returned to the baseline levels by POST. Increased carbohydrate intake was related mainly to increased saccharose intake during the first half of the deployment. According to MDRI soldiers should consume carbohydrates < 494 g/d in operational environment (15) when mean carbohydrate intake remained only 242-269 g/d in this study. For soldiers carbohydrates are the main energy substrate for physical and military training and together with adequate energy intake they are essential for recovery (16). The average fat intake followed the Nordic Nutrition Recommendations and partly the MDRIs, where fat intake is recommended ≤ 35 E% (14,15). In addition the soldiers consumed more saturated fatty acids (12.3-13.2 E%) than is typically recommended (14). However, saturated fat intake was not as high as the Finnish general population according to National FINDIET 2012 Survey (17). Protein intake decreased during the first half of the deployment but during PRE and during POST the mean intake was more than 20 E% which is higher than the Nordic recommendation (10-20 E%) (14). Soldiers consumed protein ≥ 117 g/d in all measurement points which exceeds MDRIs in garrison (63-119 g/d) and in operation (≥ 91 g/d). Increased protein intake may be beneficial for soldiers in military operations in maintaining their lean body mass and improving recovery (18). Overall, macronutrient intake during the deployment supports the findings of National FINDIET 2012 Survey (17). Similar results were observed in soldiers of the 101st Airborne Division and in Afghanistan, who consumed less carbohydrates and more saturated fat than is recommended (18,2). In all circumstances, soldiers need to ensure that the macronutrient contents of their diet support the maintenance or development of physical performance and health. For example, carbohydrate ingestion has been found to reduce cortisol response in stressful environments and this may have potential implications in a successful performance of military operations (19).

Factors explaining changes in macronutrient intake in the present study are unclear. Breakfast, lunch and dinner were systematically supplied on six days per week, but on Sundays, only brunch and dinner were served. Other meals and snacks could be bought at local restaurants outside the military base. A total of 57.5 % soldiers reported the use of protein supplements in MID deployment, which may explain changes in protein intake. Together with progressive strength training, this could also explain increased muscle mass in the upper limbs. However, subcutaneous fat mass increased in the upper and lower limbs at the same time, which may indicate that soldiers probably got too much energy from the diet. The operational situation changed in the MID deployment in the South Lebanon, which may have negatively influenced food consumption and exercise routines. Some soldiers also reported a decreased mood-state and too monotonous life on the camp during the latter half of the operation, which might possibly have had a negative effect on their health behavior.

Physical activity

Total daily physical activity decreased during the deployment according to step count, mean MET and MVPA, while the time for sedentary behavior (MET < 1.5) increased. Step count did not meet the minimum daily health recommendation (10 000 steps) (20). One reason for the observed level of physical activity is low work load and calm security situation in the operational area (11). Secondly, the camp area was not wide that the soldiers took only few steps for their daily routines. Despite the training facilities inside the camp, the soldiers could not sustain their levels of physical activity during this deployment. Heart rate responses and stable hormonal levels support the assumption that the soldiers did not experience overload symptoms (11). Due to the nature of accelerometer movement detection, some stationary physical activity such as weight lifting was not necessarily recorded by the accelerometers (21).

Low work load combined with increased sedentary behavior needs to be monitored, especially, if the soldiers have to maintain their readiness during the deployment. While the operational duties may change rapidly, soldiers should maintain their readiness at all the times and thus, enhance their physical training during passive phases of operation.

Body composition

Total skeletal muscle mass slightly increased during the deployment but muscle thickness of TB, measured by ultrasonography, increased from PRE to MID and MID to POST while muscle thickness of VL decreased from MID to POST. However, increased skeletal muscle mass in upper limbs is a positive finding of the present study but at the same time, subcutaneous fat increased in TB (PRE-POST) and in VL (MID-POST). Total fat% or fat mass did not change during the deployment.

These results could at least partly be explained by the results of physical activity: the soldiers became more passive and subcutaneous fat increased simultaneously in their body. Despite the increase in skeletal muscle mass, excessive subcutaneous fat in the upper and lower limbs is not beneficial for soldiers. Total time, accumulating from standing, correlated positively with body mass, VFA, fat mass, fat%, waist circumference and subcutaneous fat thickness of VL in the PRE- and POST-deployment. This may indicate that soldiers' standing time increased, which diminished their physically active minutes. A previous study did not find association between sedentary behavior and fat mass but a positive association between higher physical activity and fat free mass was observed (22). Energy and fat intake (g/d) were positively correlated with body mass and skeletal muscle mass in all measurement points in the present study, when observing the amount of energy and fat eaten. Systematic literature review by Fogelholm et al suggested, nevertheless, that proportional macronutrient composition of the diet (E%) did not predict changes in body weight or waist circumference in longitudinal cohort studies (23).

Strengths and limitations of the study

A unique set of data regarding food intake straight from the deployment area was obtained under a controlled situation due to a research group in the vicinity which enabled the accurate observation of food servings during the measurements. To date, there are only a few studies (2,3,9), where nutritional information has been recorded straight from the operational area. However, food diaries often underestimate nutrient intake due to underreporting (24), which can lead to lower reported energy intake levels. Inaccuracy and forgetfulness may also distort data of food diaries (24).

The use of accelerometers is an objective method to objectively estimate daily physical activity and sedentary behavior (21). Nevertheless, the method describes better only endurance-type activities. Some strength training exercises and their intensities may not be accurately recorded by an accelerometer worn at the hip level and, for example, strength training performed standing (e.g. biceps curl) may be interpreted as sedentary behavior or light PA by the accelerometer.

Bioimpedance results only partly supported the ultrasonography data. While fat thicknesses increased in TB and VL, whole body fat mass or fat% did not change during the deployment. Bioimpedance analysis is based on fluid content of the body and despite the controlled measurements, results can be inaccurate, particularly, on the individual level. Fat free mass estimated via bioimpedance has been reported to have poor accuracy compared to DXA (25). Body composition measurement with ultrasound can also be a valid method to estimate thicknesses of muscle and subcutaneous fat (26). Landmarks by micro pigmentation were drawn to skin to make sure the same spots of measurements and the same investigator conducted the measurements throughout the study.

CONCLUSION

Inadequate carbohydrate intake, low physical activity and an increase of subcutaneous fat during long-term deployment indicate that soldiers need to modify their dietary habits and exercise routines to respond the changing circumstances. Based on the present findings, the soldiers should be encouraged to eat more healthy carbohydrates (e.g. whole grains, fruits and vegetables) and less foods with saturated fatty acids (fatty meat and dairy) whilst maintaining adequate protein consumption in their daily diet during the operation. In calm security situation the enhanced physical training program should be recommended to sustain optimal body composition, physical fitness and military readiness during deployment period.

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Table 1. Mean (±SD) energy, dietary macronutrient and water intake during the deployment (n=40).

	PRE	MID	POST
Daily energy intake (MJ/d (kcal/d))	10.3 ± 2.6 (2454 ± 616)	10.5 ± 2.8 (2521 ± 660)	10.1 ± 2.8 (2425 ± 676)
Carbohydrates (E %)	39.5 ± 6.9	42.6 ± 7.7 p=0.007 PRE_MID	40.1 ± 9.1
Protein (E %)	21.7 ± 3.8	18.7 ± 4.6 p<0.001 PRE_MID	22.3 ± 5.0 p<0.001 MID_POST
Fat (E %)	35.0 ± 7.0	35.7 ± 5.0	34.9 ± 7.0
Carbohydrates (g/d)	242.8 ± 77.5	268.5 ± 85.0 p=0.038 PRE_MID	242.4 ± 84.7 p=0.024 MID_POST
Protein (g/d)	132.5 ± 40.3	117.3 ± 41.0 p=0.006 PRE_MID	134.7 ± 46.2 p=0.002 MID_POST
Fat (g/d)	95.6 ± 31.6	100.4 ± 31.4	95.0 ± 35.0
Fibre (g/d)	17.5 ± 8.4	21.3 ± 10.4 p=0.019 PRE_MID	16.0 ± 7.6 p<0.001 MID_POST
Saccharose (E %)	10.2 ± 5.2	11.9 ± 5.0 p=0.039 PRE_MID	10.6 ± 6.0
Saturated fatty acids (E %)	13.2 ± 2.7	12.5 ± 2.5	12.3 ± 3.3
Monounsaturated fatty acids (E %)	12.6 ± 2.2	12.5 ± 2.2	11.9 ± 2.8
Polyunsaturated fatty acids (E %)	15.7 ± 54.8	16.2 ± 37.6	8.6 ± 18.6
Water (L/d)	4.5 ± 1.6	4.2 ± 1.7	3.7 ± 1.6 p<0.001 PRE_POST

Table 2. Mean (±SD) total daily physical activity and sedentary behavior data.

	PRE (n=29)	MID (n=29)	POST (n=29)
Step count	9835 ± 2743	8538 ± 2699 p=0.010 PRE_MID	8388 ± 2875 p=0.007 PRE_POST
MET mean	1.59 ± 0.16	1.55 ± 0.16	1.52 ± 0.15 p=0.046 PRE_POST
MET max (/min)	7.80 ± 1.41	7.86 ± 1.46	8.29 ± 1.87
Light PA (MET 1.5-3.0) (h:min)	1:50 ± 0:26	1:36 ± 0:22 p=0.036 PRE_MID	1:41 ± 0:28
MV PA (MET > 3.0) (h:min)	1:12 ± 0:23	1:03 ± 0:24 p=0.007 PRE_MID	1:00 ± 0:24 p=0.001 PRE_POST
Standing (h:min)	2:05 ± 0:32	1:38 ± 0:28 p<0.001 PRE_MID	1:48 ± 0:49 p=0.001 PRE_POST
Sitting and lying (h:min)	11:00 ± 1:49	10:32 ± 1:35 p=0.005 PRE_MID	11:15 ± 1:46
Number of breaks during SB	37 ± 9	33 ± 10 p=0.001 PRE_MID	31 ± 9 p=0.002 PRE_POST

MET = metabolic equivalent, PA = physical activity, MV = moderate and vigorous, SD= sedentary behaviour

Table 3. Mean (±SD) body composition characteristics during the deployment (n=40).

	PRE	MID	POST
Body mass (kg)	77.8 ± 8.7	78.0 ± 9.0	78.3 ± 9.5
Skeletal muscle mass (kg)	38.5 ± 4.3	38.7 ± 4.3	39.0 ± 4.7 p=0.009 PRE_POST
Body fat %	13.6 ± 4.3	13.2 ± 4.4	13.3 ± 4.1
Visceral fat area (cm^2)	49.5 ± 18.3	51.8 ± 22.3	51.9 ± 20.1
Waist circumference (cm)	86.2 ± 7.0	83.6 ± 6.6 p<0.001 PRE_MID	83.3 ± 6.6 p<0.001 PRE_POST

Table 4. Correlation coefficients and their p-values (n=40) between energy, CHO and fat intake and body composition.

	Body mass	SMM	Fat %
Energy intake (kcal/d)	MID: 0.31, p=0.05 POST: 0.38, p=0.02	PRE: 0.41, p=0.01 MID: 0.35, p= 0.03 POST: 0.48, p= 0.02	
CHO intake (g/d)	PRE: 0.41, p=0.01 POST: 0.48, p=0.01		PRE= -0.32, p=0.05
Fat intake (g/d)	PRE: 0.31, p=0.05 MID: 0.38, p=0.02 POST: 0.38, p=0.02	PRE: 0.42, p=0.01 MID: 0.43, p=0.01 POST: 0.41, p=0.01	

SMM=skeletal muscle mass

Table 5. Correlations and their p-values (n=36-37) between standing time and some body composition variables

Physical activity	Body mass	Fat %	VFA	WC
Standing time	PRE: 0.38, p=0.02 (n=37) POST: 0.37, p=0.03 (n=36)	0.44, p=0.01 (n=37) 0.54, p=0.01 (n=36)	0.37, p=0.02 (n=37) 0.60, p=0.01 (n=36)	0.46, p=0.01 (n=37) 0.48, p=0.01 (n=36)

VFA= visceral fat area, WC=waist circumference

II

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by

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ASSOCIATIONS OF NUTRITION AND BODY COMPOSITION WITH CARDIOVASCULAR DISEASE RISK FACTORS IN SOLDIERS DURING A 6-MONTH DEPLOYMENT

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Abstract
Objectives: This observational follow-up study investigated the associations of nutrition and body composition with cardiovascular disease (CVD) risk factors, including pro-inflammatory biomarkers, in soldiers during a 6-month deployment. **Material and Methods:** Thirty-five male soldiers were assessed at months 0, 3 and 6, and their parameters, i.e., M±SD, were as follows: age 30.0±8.7 years, height 179±6 cm, and BMI 24.2±2.5 kg/m^2. Three-day food diaries were used for monitoring macronutrient intake. Body composition was estimated using bioimpedance. Fasting blood samples for lipids and pro-inflammatory biomarkers were collected, and blood pressure measurements were performed. **Results:** Carbohydrate intake increased and protein intake decreased at month 3 ($p = 0.034$, $p < 0.001$), while body composition remained stable. Systolic blood pressure increased at month 6, while other CVD risk factors remained within the reference values. Fat mass and body fat percentage were associated positively with total and low density lipoprotein (LDL) cholesterol concentrations at all measurement points. A negative association was found between the change in fiber intake vs. the change in total ($r = -0.36$, $p = 0.033$) and LDL cholesterol ($R = -0.39$, $p = 0.019$). **Conclusions:** Lower fiber intake and a greater amount of body fat were associated with high total and LDL cholesterol concentrations. Nevertheless, the measured CVD risk factors remained within the reference values, except for the higher systolic blood pressure. A regular screening of body composition and a higher consumption of fiber-rich foods may promote cardiometabolic health in soldiers. Int J Occup Med Environ Health. 2020;33(4):457–66

Key words:
blood pressure, cholesterol, soldier, body fat, fiber intake, crisis management

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INTRODUCTION

Cardiovascular diseases (CVDs) are primary causes of mortality in western countries [1]. The CVD risk factors include behavioral (smoking, diet, physical activity, alcohol consumption), physiological (blood cholesterol, hypertension, blood glucose, BMI), and metabolic disorders [1].

Soldiers are typically young and physically active but their CVD risk can be increased as well, especially when they are overweight or obese [2,3]. Compared to civilian populations, soldiers generally have a low incidence of CVD risk factors, which can be explained by regular exercise, high aerobic fitness and/or heredity. However, the globally increasing overweight and obesity trends cannot be avoided in military populations. For example, 44.2% of individuals, upon commencing their service in the U.S. Army, have been reported to be overweight or obese [3]. Overweight and obese soldiers are at a higher risk of elevated levels of serum lipids and other CVD risk factors [3,4]. A low socio-economic position and an inadequate nutrient intake are also associated with an elevated CVD risk [5,6]. On the other hand, military work can improve cardiometabolic health by lowering the levels of lipids and blood glucose, provided that diet and exercise are well-balanced during military training [6,7].

The associations of dietary fat intake and CVD risk factors have been widely investigated and the results are quite clear: a shift from saturated fatty acids (SFAs) towards unsaturated fatty acids is beneficial for human health [8]. Thus, dietary guidelines have summarized that a restriction of SFAs, and an increase in mono- and polyunsaturated fatty acids (MUFAs, PUFAs), may promote cardiometabolic health [9].

The limitation of refined carbohydrates with a high glycemic load is also highly recommended to prevent CVDs [10]. The quality of carbohydrate sources may be as important in preventing dyslipidemia as the quality of fats [10]. An increased protein intake from plant sources might reduce blood pressure [11], and a replacement of refined carbohydrates with protein may reduce the low density lipoprotein (LDL) cholesterol and triglyceride (TG) levels [12].

It was found that U.S. soldiers, when staying in a military base, consumed fewer carbohydrates and more fat than recommended [13]. In addition, U.S. soldiers, consuming >3 servings of beverages or other sugar-containing juices, had a greater risk of elevated TG levels than those who did not [3]. In Brazilian cadets, high intakes of saturated and unsaturated fatty acids were observed [5]. The study performed during a military operation revealed that soldiers consumed 43–46% of their total energy (E%) from carbohydrates, 34–35 E% from fats, and 16–17 E% from protein [14], i.e., their dietary patterns were close to the general population. Overall, soldiers might have a higher risk for cardiometabolic disorders, both while staying in a base and during operations, if their diet includes low amounts of fiber-rich carbohydrates and high amounts of SFAs.

Negative changes in body composition have also been shown to predict CVDs in U.S. Army soldiers [2]. Diagnosed overweight or obesity may subsequently lead to hypertension or the prevalence of other CVD risk factors. While BMI is commonly used as a measure of overweight or obesity, its role is unclear as a predictor of CVDs [15]. Compared to BMI, percent body fat (BF%) or waist circumference are more accurate measures of fat mass and fat distribution, thus they may be more suitable predictors of cardiometabolic disorders [16]. Soldiers in military tasks and operations might replace their fat free mass with fat [14,17,18], which may subsequently lead to metabolic problems.

Inflammation is associated with most of the cardiovascular events in the development of CVDs [19]. Pro-inflammatory biomarkers, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP), have been shown to associate with CVD risk factors [19]. In soldiers, war zone experiences and combat stress can cause low-grade chronic inflammation [20], which may also promote vascular events together with other risk factors.

Military personnel may serve several months or years in deployments, being exposed to stressful environments. Thus, physiological, nutritional, and behavioral aspects need to be considered to minimize cardiometabolic disorders. The aim of this study was to investigate the associations of dietary intake and body composition with CVD risk factors and pro-inflammatory biomarkers during a 6-month deployment for a crisis management operation.

MATERIAL AND METHODS

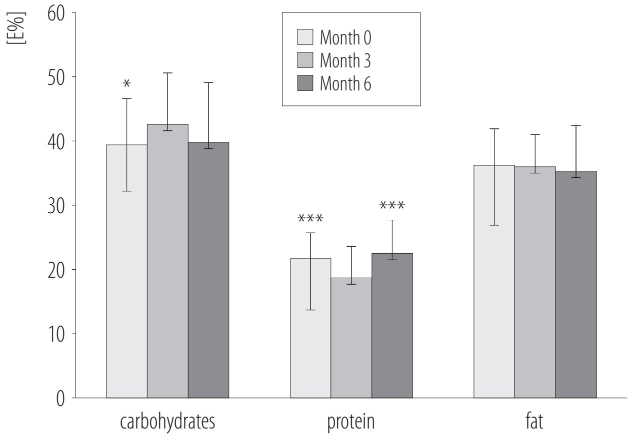
Thirty-five male crisis management soldiers volunteered for this study during the preparation period, and their parameters, i.e., mean±standard deviation (SD), were as follows: age 30.0±8.7 years, height 179±6 cm, BMI 24.2±2.5 kg/m^2. The study was planned and carried out according to the guidelines of the Ethical Committee of the Central Finland Health Care District. Measurements were conducted 3 times (at months 0, 3 and 6) during a 6-month operation in Southern Lebanon. Baseline measurements were performed after an acclimatization period lasting min. 2 weeks.

The main purpose of the crisis management operation was to monitor the cessation of hostilities and support the Lebanese government, armed forces as well as local population. Typical soldiers' duties included patrolling the operational area for 4–6 h, mainly by vehicles, and guarding the military base. The duties were performed in 3 shifts, around the clock. The security situation was relatively calm, although tension in the operational area increased in the mid-phase of the deployment. The military base was situated on a hill (775 m above sea level), the mean temperature of the military camp was 22.3±4.3°C (a range of 11–36°C) and the mean humidity was 54.0±17.2% (Thermochron, iButton, Maxim Integrated, San Jose, California, USA).

Food diaries were collected for the calculation of nutrient intake at months 0, 3 and 6 during the operation. The participants recorded all the food, fluid, and supplements consumed for 3 days using household measures and standard units. The soldiers were advised to record their intake immediately after each meal and to keep their diet as normal as possible. Items consumed daily, such as bread, drinks and spreads, were weighed and recorded by the research group. Breakfast, lunch, and dinner were served daily in the canteen of the military base, except on Sundays when only brunch and dinner were served. The participants were able to buy some meals and snacks in restaurants and shops outside the camp. The data from food diaries were analyzed using Nutri Flow (Flow-Team, Oulu, Finland) software.

Body composition was estimated using bioimpedance (Inbody 720, Biospace, Soul, South Korea). The assessments were performed after an overnight fast, with the soldiers voiding before the measurement, and wearing only underwear.

Blood samples were collected from the ulnar vein using Terumo Venosafe tubes (Terumo Europe, Leuven, Belgium), and were centrifuged at a speed of 3500 rpm. Glucose, serum high density lipoprotein cholesterol (HDL) and TG were analyzed with the Konelab 20 XTi device (Thermo Electron Co, Vantaa, Finland), and the isolated LDL fraction was used for the direct measurement of LDL cholesterol with the cholesterol oxidase phenol 4-amino-antipyrine peroxidase (CHOD-PAP) method. The ranges for TG, HDL cholesterol, and LDL cholesterol assays were 0.1–15, 0.09–11, 0.04–2.84, and 0.3–8.9 mmol/l, respectively. Intra- and interassay coefficients of variance were 1.0% and 3.8% for TGs, 3.4% and 3.9% for serum LDL, and 0.5% and 7.6% for serum HDL, respectively. Sensitivity for glucose was 0.1 mmol/l, and intra- and interassay coefficients of variance were 1.0% and 2.0%, respectively. Serum concentrations of CRP, TNF-α, and IL-6 were measured using commercial high sensitivity ELISA kits according to the manufacturer's instructions (Quantikine HS, R&D Systems, Minneapolis, USA). Assay specifications were 0.10 mg/l for CRP sensitivity, 0.11 pg/ml for IL-6, and 0.19 pg/mL for TNF-α. The maximum intra-



* $p < 0.05$; *** $p < 0.001$.

Figure 1. Macronutrient intake [E%] of soldiers (N = 35) during a 6-month deployment in Southern Lebanon, in the study conducted in June–November 2014

and interassay coefficients of variance were 4.8% and 6.1% for CRP, 5.9% and 9.8% for IL-6, and 6.1% and 7.7% for TNF-α, respectively.

Blood pressure was measured after a 5-min rest, 3 times at 2-min intervals, by a semiautomatic blood pressure device (Omron M6 Comfort, Omron Healthcare, Kyoto, Japan). The means of the 3 measurements were used for statistical analyses.

Data are presented as M±SD. A paired t-test was used to examine differences in the means between measurement points, and Bonferroni adjustments were used when needed.

The linear correlation between nutritional or body composition variables and CVD risk factors was obtained using Pearson's product-moment correlation. For TG, PUFAs, and IL-6, Spearman's rank correlation coefficient was utilized.

Linear regression analyses were conducted to examine linear relationships between nutritional or body composition parameters and CVD risk factors or inflammatory markers. All the analyses were carried out using commercial software (IBM SPSS 25.0.0, Chicago, Illinois, USA), and p-values of <0.05 were considered statistically significant.

RESULTS

The variation in macronutrient distribution is shown in Figure 1. Carbohydrate intake (E%) increased between months 0 and 3 (39.4 E%, 42.6 E%; $p = 0.034$), while protein intake (E%) first decreased (21.7 E%, 18.7 E%; $p < 0.001$) but then recovered (22.5 E%; $p < 0.001$). Fiber intake decreased between months 3 and 6 (21.4 g/day vs. 16.5 g/day, $p = 0.002$). The intake of SFAs was 12–13 E% and PUFAs 8–12 E% during the operation. The mean energy intake was 10.5±2.8 MJ/day. Body composition remained stable during the study period (Table 1).

The relative prevalence of soldiers having dyslipidemia, hyperglycemia, hypertension, or a high level of inflammatory markers in the present study is shown in Table 2. The highest prevalence of dyslipidemia was found in LDL cholesterol: 31.4%, 40.0%, 34.3% of soldiers exceeded the reference value (at months 0, 3 and 6, respectively). A total of 68.6% of soldiers had high systolic blood pressure (>120 mm Hg) at month 6, while the earlier results were 31.3% (at month 0) and 40.0% (at month 3).

The mean concentrations of total, HDL and LDL cholesterol, TG and glucose are presented in Table 3. Lipid and glucose concentrations did not change between the measurements and the mean values were within the reference values during the deployment. In addition, TNF-α decreased by 8% at month 6, compared to month 0 and 3; CRP was measured but, due to analytical problems, the number of samples remained low (N = 15); and the M±SD values were 3.87±7.59, 1.80±1.09, 1.73±3.14 at months 0, 3, and 6. Systolic blood pressure was the highest at month 6, compared to months 0 and 3.

No systematic associations between nutrient intake and CVD risk factors or inflammatory markers were found at any measurement point. When considering changes between months 0 and 6, negative associations were found between the changes in fiber intake and the changes in to-

Table 1. Body composition of soldiers (N = 35) during a 6-month deployment in Southern Lebanon, in the study conducted in June–November 2014

Variable	Body composition (M±SD)		
	month 0	month 3	month 6
Body mass [kg]	77.1±8.9	77.1±9.0	77.5±9.6
Skeletal muscle mass [kg]	38.0±4.2	38.1±4.1	38.4±4.7
Fat mass [kg]	10.8±4.1	10.6±4.1	10.6 ±3.8
Body fat [%]	13.8±4.4	13.5±4.3	13.5±4.0
Visceral fat area [cm^2]	49.7±17.5	51.7±22.8	50.3±20.5

No statistically significant changes in any variable.

Table 2. Dyslipidemia, hyperglycemia, high level of pro-inflammatory biomarkers and hypertension of soldiers (N = 35) during a 6-month deployment in Southern Lebanon, in the study conducted in June–November 2014

Variable	Prevalence [%]		
	month 0	month 3	month 6
Cholesterol			
high total [>5.0 mmol/l][a]	28.6	31.4	28.6
low HDL [<1.0 mmol/l][a]	22.9	22.9	8.6
high LDL [>3.0 mmol/l][a]	31.4	40.0	34.3
Hypertriglyceridemia [>1.7 mmol/l][a]	8.6	8.6	8.6
Hyperglycemia [>6.0 mmol/l][b]	2.9	0	2.9
High interleukin-6 [>3.4 ng/l][c]	5.9	8.8	2.9
High tumor necrosis factor α [>8.1 ng/l][c]	36.4	39.4	21.2
High blood pressure			
systolic			
>120 mm Hg[d]	31.4	40.0	68.6
>140 mm Hg[d]	2.9	2.9	8.6
diastolic			
>80 mm Hg[d]	20.0	22.9	28.6
>90 mm Hg[d]	2.9	8.6	5.7

[a] Reference values for lipids based on [21].
[b] Reference value for blood glucose based on [22].
[c] Reference values for interleukin-6 based on [23].
[d] Reference values for tumor necrosis factor-α based on [24].

tal and LDL cholesterol concentrations (R = –0.362, p = 0.033; R = –0.394, p = 0.019, respectively). No other associations were observed.

Scatter plots of the positive correlations between BF%, total and LDL cholesterol concentrations at baseline are shown in Figures 2a and 2b. It was found that BF% and fat

Table 3. Cardiovascular risk factors and pro-inflammatory biomarkers in soldiers (N = 35, except tumor necrosis factor α [TNF-α], N = 34; abnormal values), during a 6-month deployment in Southern Lebanon, in the study conducted in June–November 2014

Variable	Cardiovascular risk factors and pro-inflammatory biomarkers (M±SD)		
	month 0	month 3	month 6
Cholesterol [mmol/l]			
total	4.64±1.11	4.71±1.13	4.71±1.32
HDL	1.26±0.30	1.27±0.31	1.30±0.29
LDL	2.27±0.90	2.83±0.92	2.87±1.08
Triglycerides [mmol/l]	1.05±0.58	1.03±0.66	1.09±0.83
Glucose [mmol/l]	4.97±0.45	4.92±0.44	5.11±0.41
Interleukin-6 [ng/l]	1.83±1.14	1.63±1.26	1.49±1.15
TNF-α [ng/l]	8.00±1.77	7.97±1.37	7.34±1.36[a]
Blood pressure [mm Hg]			
systolic	118±9	118±11	123±12[b]
diastolic	76±8	75±8	76±8

No statistically significant changes in other variables except TNF-α and systolic blood pressure: [a] months 0–6: p = 0.01, months 3–6: p = 0.006; [b] months 0–6: p = 0.008, months 3–6: p = 0.008.

mass correlated positively with total and LDL cholesterol concentrations at all measurement points.

DISCUSSION

Carbohydrate intake increased and protein intake decreased at month 3, but these did not have an association with CVD risk factors or pro-inflammatory biomarkers at any measurement points. The change in fiber intake was inversely associated with the change in total and LDL cholesterol concentrations. In addition, BF%, fat mass, skeletal muscle mass, and body weight were stable during the operation, but BF% and fat mass associated positively with total and LDL cholesterol concentrations at all measurement points. When considering CVD risk factors, the mean systolic blood pressure increased at month 6 over the optimal level. The highest prevalence of dyslipidemia was found in the LDL cholesterol concentration at month 3 where 40% of the participants had values exceeding the recommended level.

Fiber intake of the participants remained below the recommended levels (25–35 g/d) [9] throughout the study, and the change in fiber intake was negatively associated with the change in total and LDL cholesterols concentrations. The negative association between dietary fiber and total and LDL cholesterol concentrations has been well established [25]. Likewise, low fiber intake is associated with a higher CVD risk [26]. During military education, low fiber intake was found in 92.7% of Brazilian cadets and hypercholesterolemia in 50.7% of them [5]. In submariners, fiber intake remained <25 g/d in obese and non-obese participants during their 3-month patrolling, but modest improvements in serum lipids were observed, mostly related to reduced body weight and body fat mass [7]. Overall, the increased intake of fiber-rich foods, like whole grain products, fruit and vegetables, could improve cardiometabolic health, insulin sensitivity, and weight management, as well as prevent gastrointestinal disorders [26].

According to the Nordic Nutrition Recommendations, fat intake should range 25–40 E% [9]. The quality of dietary fat seems to be more important than its quantity for lowering the CVD risk [8]. Replacing SFAs by unsaturated fatty acids, especially PUFAs, lowers the CVD risk [27]. In this study, fat intake ranged 35–36 E% and the intake of SFA 12–13 E%. The consumption of butter, high-fat dairy and meat products can explain the high amount of SFAs during the operation. Replacing butter with soft spreads and using vegetable oils in cooking and salad dressing might lower the intake of SFAs and increase the intake of PUFAs in all military personnel living in a camp.

Fat mass and BF% were associated positively with total and LDL cholesterol concentrations, which has also been well documented in previous studies [15,16,28]. Although obesity is a clear risk factor for diabetes and CVDs [15, 28], fat distribution, and especially visceral fat, may be more accurate for the evaluation of the CVD risk than fat mass or BF% [16]. The amount of abdominal fat, which can be measured by waist circumference or the visceral fat area, may predict abnormalities in cardiometabolic health [15,16]. In this study, waist circumference and the visceral fat area were measured, but these data were excluded due to some inaccuracies of the measurements. Altogether, body fat and BF% are associated with hyperlipidemia, also in soldiers. The follow-up of body composition during a military operation could be useful for evaluating the CVD risk and for adjusting physical training.

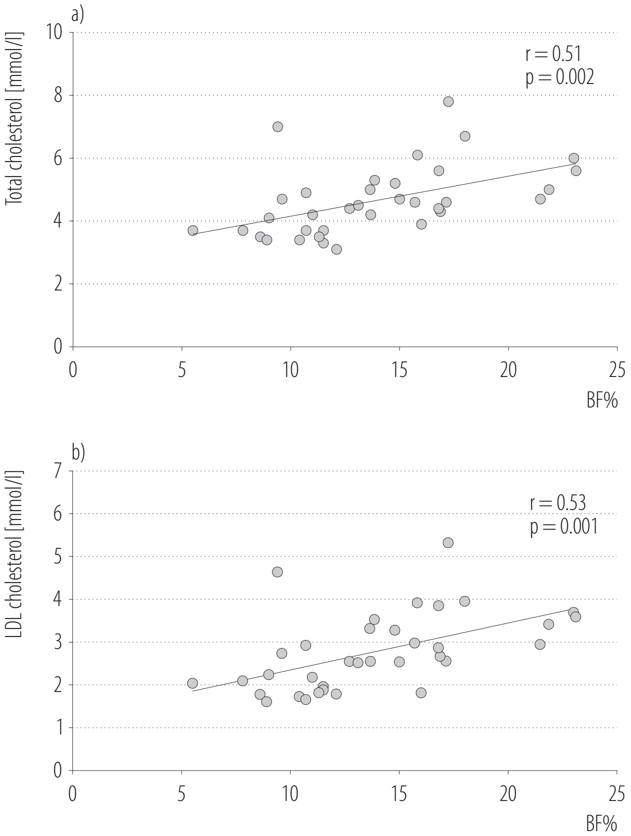


Figure 2. Relationship between body fat percentage (BF%) and a) total cholesterol, b) LDL cholesterol concentration at baseline of soldiers (N = 35) during a 6-month deployment in Southern Lebanon, in the study conducted in June–November 2014

Systolic blood pressure increased between months 0 and 3, and month 6, and the mean value exceeded the recommended level, but associations with dietary intake or body composition were not found. Previously, it has been demonstrated that a diet low in total and saturated fats, and high in fruit and vegetables and low-fat dairy products, reduces blood pressure and LDL cholesterol [12]. A partial replacement of refined carbohydrates with protein or unsaturated fat can further decrease blood pressure [11,12]. It is well known that psycho-social factors like occupational stress, personality, mental health, and sleep quality, may contribute to the development of hypertension [29]. In a separate questionnaire (data not shown), the participants reported monotonous life and depressive symptoms, especially during the second half of the deployment, which may partly explain their elevated systolic blood pressure. Therefore, a regular monitoring of blood pressure might be reasonable for those soldiers whose blood pressure was elevated at the end of the study.

Low-grade systemic inflammation can predict cardiovascular events [19,30]. Higher concentrations of TNF-α and IL-6 can promote this kind of inflammation, which might increase the CVD risk. In this study, TNF-α was decreased at month 6 and IL-6 remained stable. While CRP exceeded the normal range at baseline, these values cannot be generalized due to a small sample size. When considering all 3 inflammatory markers together, a moderately decreasing trend over the time-course of the operation was observed. Dietary intake and body composition did not correlate with pro-inflammatory biomarkers in these data, but a recent study has shown that dietary fats and carbohydrates could relate to inflammatory processes, yet the pathways are still unclear [30]. The individual variability in inflammatory responses, as well as the validity of biomarkers, must be considered when interpreting the values [30]. High concentrations of inflammatory markers have been measured in soldiers after stressful combat training, high-pressure operational situations, and in post-traumatic stress reactions [20,31]. The tension in military operations has been found to increase shortly during mid-phase measurements, but this cannot be seen in inflammatory markers. To conclude, clear explanations for the decreasing trend of inflammatory biomarkers were not found in this study. Thus, more studies are needed to clarify the inflammatory responses in military environments, as well as acute and chronic effects of such responses on the health of soldiers.

Regular exercise has multiple positive effects on CVD risk factors [32]. The physical activity of this operation has been reported elsewhere [33]. The results indicated that total physical activity, estimated by accelerometers, was very low throughout the study and stress hormone responses did not show any overload symptoms. Associations between physical activity and CVD risk factors have not been observed yet.

The data were collected on site during the deployment, which makes this study unique compared to other military operation studies [14,18]. Controlled methods were used for all variables, and the sample was representative in terms of age, sex, body composition and physical fitness. A limitation is that the sample size remained small and the statistical power was rather low. A bioimpedance analysis is not as valid as dual-energy X-ray absorptiometry, but it is a simple and portable method for estimating body composition in standardized circumstances [34]. In food diaries, underreporting, forgetting, and motivational problems may have an effect on the accuracy of reporting [35]. However, most of the consumed food items were familiar to the participants and found in the database embedded in the software. The rapid changes in operational situations were reflected in diet, physical training and sleep, especially at month 3.

CONCLUSIONS

Low fiber intake and a greater amount of body fat were associated with high total and LDL cholesterol concentrations, while a higher systolic blood pressure was measured at the end of the study. These results show that soldiers are exposed to the same CVD risk factors as civilians although they are not regarded as a risk group for cardiometabolic disorders. A systemic follow-up of body composition and adherence to a fiber-rich diet during operations might be useful to optimize cardiometabolic health in soldiers. Inflammatory responses to military work and its contribution to the CVD risk are an interesting area for future research.

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III

CHANGES IN BODY COMPOSITION, ENERGY METABOLITES AND ELECTROLYTES DURING WINTER SURVIVAL TRAINING IN MALE SOLDIERS

by

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Changes in Body Composition, Energy Metabolites and Electrolytes During Winter Survival Training in Male Soldiers

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The aim of this study was to examine changes in body composition, energy metabolites and electrolytes during a 10-day winter survival training period. Two groups of male soldiers were examined: the REC group ($n = 26$; age 19.7 ± 1.2 years; BMI 23.9 ± 2.7) had recovery period between days 6 and 8 in the survival training, whereas the EXC group ($n = 42$; age 19.6 ± 0.8 years; BMI 23.1 ± 2.8) did not. The following data were collected: body composition (bioimpedance), energy balance (food diaries, heart rate variability measurements), and biomarkers (blood samples). In survival training, estimated energy balance was highly negative: −4,323 ± 1,515 kcal/d (EXC) and −4,635 ± 1,742 kcal/d (REC). Between days 1 and 10, body mass decreased by 3.9% (EXC) and 3.0% (REC). On day 6, free fatty acid and urea levels increased, whereas leptin, glucose and potassium decreased in all. Recovery period temporarily reversed some of the changes (body mass, leptin, free fatty acids, and urea) toward baseline levels. Survival training caused a severe energy deficit and reductions in body mass. The early stage of military survival training seems to alter energy, hormonal and fluid metabolism, but these effects disappear after an active recovery period.

Keywords: military training, energy deficit, fat mass, biomarkers, recovery

INTRODUCTION

In military survival training, soldiers are exposed to multiple physiological, psychological and environmental stressors for several days. In a physiological point of view, military survival training is associated with high-energy expenditure, restricted energy intake and limited sleep, and can cause remarkable changes in body composition, energy metabolism, hydration status and endocrinological stress function. Negative energy balance has been shown to lead to decreases in body mass, fat mass and fat free mass (Hoyt et al., 2006; Nindl et al., 2007; Hamarsland et al., 2018). Furthermore, many studies have observed a decline in physical performance during survival training (Nindl et al., 2007; Margolis et al., 2014; Hamarsland et al., 2018; Różański et al., 2020).

Military survival training has also been found to disturb hormonal regulation (Lieberman et al., 2016; Szivak et al., 2018). Leptin is an adipose-derived hormone, which regulates appetite and energy metabolism (Pasiakos et al., 2011). Leptin concentration decreases during starvation (Ahima et al., 1996; Chan et al., 2003) and in military training that involves severe energy restriction (Pasiakos et al., 2011). During a 6-month crisis management operation, leptin concentration has been reported to correlate positively with body fat% (Hill et al., 2015). Another appetite-regulating hormone, ghrelin, is secreted from the gut and duodenum, and stimulates appetite (Pasiakos et al., 2011). *In vivo* ghrelin circulates in acylated and unacylated forms, and the latter form accounts for over 90% of total circulating ghrelin (Kojima et al., 1999). During an acute energy deficit, ghrelin concentration typically increases, but is not related to perceived satiety (Pasiakos et al., 2011).

When energy deficit lasts for several days, lipolysis and protein degradation may occur to maintain substrate metabolism at an appropriate level (Cahill, 2006). Glucose is an essential substrate for the brain, but when carbohydrate intake is insufficient, gluconeogenesis is upregulated to ensure adequate blood glucose level and glucose availability for the cells (Cahill, 2006). Degradation of adipose tissue provides energy for skeletal muscles, especially when glycogen stores are empty and carbohydrates are not available. Thus, loss of skeletal muscle is common during military training due to energy deprivation (Hoyt et al., 2006; Nindl et al., 2007; Hamarsland et al., 2018). In addition, loss of whole-body protein has been observed (Margolis et al., 2014). Serum creatinine is a biomarker that typically indicates renal function, but as a product of muscle catabolism, it has also been used to estimate the volume of muscle mass (Kim et al., 2016; Delanaye et al., 2017).

A rapid decrease in body mass can be explained by dehydration and/or fluid loss. In military survival training, hydration status may change rapidly, whereby restricted fluid and food intake, combined with continuous exercise, cause fluid loss. Blood biomarkers, such as sodium, have been used to evaluate hydration status (Nolte et al., 2019). In prolonged exercise, hypernatremia may occur due to a loss of total body water. A more severe sodium imbalance, hyponatremia, often results from decreased sodium and potassium, a relative excess of total body water, or a combination of both.

Metabolic changes that occur during strenuous prolonged exercise can also be estimated *via* blood parameters (Warburton et al., 2002). Only a few studies have explored these metabolic responses in a military training context (Hill et al., 2015; Karl et al., 2017). Furthermore, physiological responses of recovery period during survival trainings are poorly understood. Thus, the purpose of the present study was (a) to examine the effect of a 10-day winter survival training period on body composition, energy balance, appetite-mediating hormones, substrate metabolites and electrolytes; and (b) to compare changes in biomarkers between an exercise (EXC) and a recovery (REC) group.

MATERIALS AND METHODS

Sixty-eight male soldiers participated in the study, and they were divided into two groups: REC (n = 26) and EXC (n = 42). The division was done according to the platoons of the participants, which facilitated the planning of education. More soldiers were directed to the EXC group because a higher drop-out rate was anticipated. In the REC group 20 participants passed through the training and in the EXC group 25. The most common reasons for drop-outs were musculoskeletal disorders and upper respiratory tract infections. Three female soldiers were excluded in the EXC group, since there were no women in the REC group. Basic characteristics of the participants are presented in **Table 1**.

The present study was approved by the Finnish Defence Forces (AO1720) and the ethical approval was granted by the Scientific and Ethical Committee of the Helsinki University Hospital Research (HUS/900/2018). All participants were informed of the experimental design, the methods, the benefits and possible risks prior to signing an informed consent document to voluntarily participate in the study. The study was a part of a larger multidisciplinary research project.

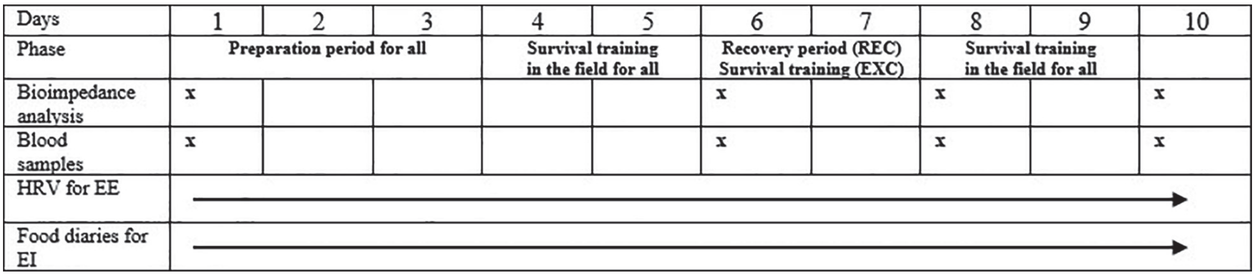
Study Protocol

The 10-d winter survival training, which included a 3-day preparation period, 6 days of survival training for the EXC group and a 1-day follow-up (for the REC group day 6 and 7 of the training was replaced with recovery) was carried out in March-April north of the Arctic Circle. The study protocol and measurements are presented in **Figure 1**. The first measurements (baseline, day 1) were conducted from 05:30 am (body composition, blood samples). Recordings of heart rate variability (HRV) and food diaries were also started later in the same morning. After the baseline measurements, a three-day preparation period began in the garrison. The participants slept in the garrison, and ate their breakfast, lunch, dinner and evening meal in a canteen. On day 4, all participants started their field training period. They performed different military tasks, slept in temporary shelters and carried their personal equipment in backpacks and pulks (extra load 23–32 kg). Participants moved with military cross-country skis in the field. Some of the military tasks were performed in subgroups including land navigation. Deep and soft snow made skiing harder than normal, and it was impossible to move without skis. The distance covered by skiing was on average 19.3 ± 1.7 km/day (min. 3.9 km; max. 25.8 km) during the field training period for the whole group. Daily variation of skiing distances occurred due to military tasks and a performance of land navigation. Education during the field training was identical for both groups, although some of military tasks were performed in smaller sub-groups. On day 6, participants were moved back to the

TABLE 1 | Basic characteristics of the participants.

Characteristics	REC (n = 26)	EXC (n = 42)
Age (years)	19.7 ± 1.2	19.6 ± 0.8
Height (cm)	181.1 ± 5.8	179.4 ± 6.2
Body mass (kg)	78.2 ± 9.6	74.4 ± 10.7
BMI (kg/m^2)	23.9 ± 2.7	23.1 ± 2.8

Values are presented as means ± SD. No statistical differences between groups were found at baseline. REC, recovery group; EXC, exercise group, BMI, body mass index.



Days	1	2	3	4	5	6	7	8	9	10
Phase	Preparation period for all			Survival training in the field for all		Recovery period (REC) Survival training (EXC)		Survival training in the field for all		
Bioimpedance analysis	x					x		x		x
Blood samples	x					x		x		x
HRV for EE	→	→	→	→	→	→	→	→	→	→
Food diaries for EI	→	→	→	→	→	→	→	→	→	→

FIGURE 1 | Study protocol. The REC group had 2 days recovery period, whereas the EXC group was in the field all the training. HRV, heart rate variability; EE, estimated energy expenditure, EI, energy intake.

garrison for the measurements, after which the REC group had a 2-day supervised recovery period while the EXC group returned to field training. On day 8, measurements were repeated before all participants returned to the field for the last 2 days, where circumstances were as previously described. Participants returned to the garrison late on day 9. In the morning of the day 10, post measurements were performed. All the measurements were carried out at the same protocol and the same timing for each measurement point.

Field Conditions

In this survival training, energy intake was restricted on purpose. Modified field rations were delivered to the participants for the first 2 days of the field phase, consisting of a protein bar (226 kcal), eight crackers (306 kcal) and two lunch meal rations. Mean energy content of meal rations was 661 kcal, and one portion consisted of approximately 88 g carbohydrates, 27 g protein and 21 g fat. Total energy content of these items was 1,847 kcal. Drinking was allowed *ad libitum*, but the drinking water had to melt from snow. One educational theme was how to get food from nature, thus extra meals were prepared from reindeer meat, salmon and beard moss lichen (*Bryoria fuscescens*). Beard moss lichen was collected from the surface of spruce trees, then dissolved in water with sodium bicarbonate (baking soda) for a few hours and boiled for an hour. After 2 days, extra rations were delivered to the EXC group, whereas the REC group was evacuated for 2 days and given temporary accommodation in the training area. During the recovery period, they were given normal meals and some extra snacks (candies, beverages, cookies, ice cream). They had the possibility to go to the sauna and shower, and sleeping facilities were like those in a garrison. During the recovery period the participants had light supervised physical activity (ball games, stretching) and various psychological therapy sessions.

Self-reported sleeping times varied between 0.5 and 4 h per day in the field (Vaara et al., 2020). In the garrison and during the recovery period, 7–8 h of sleep per day was possible.

The weather was typical for late winter in Northern Finland, as temperatures increased by several degrees from night (min −10.5°C) to day (max 5.4°C). The mean temperature varied between −0.3°C and −4.7°C. The average depth of snow was between 80 and 100 cm. Overall description of the study protocol and field conditions has been published previously (Vaara et al., 2020).

Blood Samples

Blood samples for leptin, unacylated ghrelin, creatinine, glucose, urea, free fatty acids and electrolytes (Na^{2+}, K^{+}, Cl^{-}) were drawn from the antecubital vein after overnight fasting. Samples were collected into VenoSafe plastic tubes (VenoSafe®, Terumo Europe, Leuven, Belgium) containing silica gel. Blood samples were centrifuged (3,500 rpm, 10 min) and serum was frozen at −20°C for later analysis. Leptin and ghrelin were determined with an ELISA-kit immunoassay system (Dynex DS 2, Dynex Technologies, Chantilly, VA, United States); creatinine, glucose and urea with a photometric enzymatic method (Konelab 20 Xti Clinical Chemistry Analyzer, Thermo Scientific, Vantaa, Finland); free fatty acids with an enzymatic colorimetric assay (Konelab 20 Xti Clinical Chemistry Analyzer, Thermo Scientific, Vantaa, Finland); and electrolytes (Na, K, Cl) with an ion selective electrode method (ISE; Konelab 20 Xti Clinical Chemistry Analyzer, Thermo Scientific, Vantaa, Finland).

The sensitivity and inter-assay coefficient of variation for these assays were: 0.2 ng/ml, 6.1% for leptin; 0.6 pg/ml, 17.3% for ghrelin; 2.32 μmol/l, 2.2% for creatinine; 0.1 mmol/l; 3.8% for glucose; 1.1 mmol/l, 5.8% for urea, 10 μmol/l, 5.8% for free fatty acids; 100 mmol/l, 0.7% for sodium; 2 mmol/l, 2.5% for potassium and 55 mmol/l, 3.3% for chloride.

Body Composition Assessment

Body composition variables (body mass, body fat%, skeletal muscle mass) were evaluated *via* bioimpedance devices (Inbody 720/770, Biospace, Soul, South Korea). The measurements were done early in the morning after an overnight fast, and participants were advised to only wear underwear and to urinate before the measurement. The same device was used for each measurement to avoid variability between devices.

Estimated Energy Expenditure and Intake

Energy expenditure was estimated *via* heart rate variability measurements (Firstbeat Bodyguard 2, Firstbeat Technologies Oy, Jyväskylä, Finland). The Bodyguard 2 is a two-electrode portable device connected to the chest. Participants wore the Bodyguard 2 continuously, with the exception of short breaks to install the batteries. The accuracy of estimated energy expenditure is within 7–10% (Smolander et al., 2011). Pre-filled food diaries were used to estimate energy intake. Since food intake was restricted during the field training on purpose and delivered food items were known beforehand, participants were given pre-filled food diaries, where they wrote the time and amount of food consumed, including also extra food delivered in the field. Nevertheless, some of the diaries were too inaccurate, were lost or got wet in the field, so only representative diaries from days 5 and 7 were included for further analysis. Energy intake was calculated according to the nutrition value of each food item and energy content of extra food, which were analyzed by software program for obtaining individual total energy intake (Fineli, National Food Composition Database, Finland).

Statistical Analysis

Time × group interactions were tested with F-tests based on the Satterthwaite method using the lmerTest R-package (Kuznetsova et al., 2017). A linear mixed effect model was used to estimate changes within and between groups over the studied period. Since military survival training is strenuous, failure and drop-out rates are relatively high. Therefore, a linear mixed effect model was chosen instead of repeated measures ANOVA to maximize observations at each time point. Pairwise comparisons were performed using Tukey's test and logarithmic transformations were done when the distribution was positively skewed (leptin, ghrelin, free fatty acids). All data were examined quantitatively and graphically. Non-parametric Mann–Whitney U-tests were used to verify the linear mixed effect model when residuals were not normally distributed (body mass, leptin, glucose, sodium and chloride). Spearman correlations were calculated to estimate associations at baseline and differences in associations from baseline to day 10. All statistical analyses were performed using R v. 3.6.3 (2020, R Foundation for Statistical Computing, Vienna, Austria). Data are presented as means ± standard deviations and statistical significance was set at $p < 0.05$.

RESULTS

Estimated energy expenditure was high in both groups during the training. In **Table 2**, energy expenditure values from day 2 to day 9 are presented for both groups. On days 1 and 10, an entire 24-h recording was not possible, so these values were excluded.

Based on the representative food diaries from both groups, energy intake was 447 ± 245 kcal/d (EXC, $n = 24$) and 357 ± 338 kcal/d (REC, $n = 20$) on day 5, and 1,294 ± 743 kcal/d (EXC, $n = 12$) and 3,003 ± 882 kcal/d (REC, $n = 21$) on day 7. Energy intake differed statistically significantly between groups ($p < 0.001$) on day 7. Calculated energy balance was −4,323 ± 1,515 kcal/d in the EXC group and −4,635 ± 1,742 kcal/d in the REC group on day 5. On day 7, the estimated energy balance was −4,222 ± 1,815 kcal/d (EXC) and −608 ± 1,107 kcal/d (REC), and the difference was statistically significant ($p < 0.001$).

For body composition parameters, significant time × group interactions were found for body mass ($p < 0.001$), body fat% ($p < 0.001$) and skeletal muscle mass ($p = 0.004$) but not for creatinine. Differences in model parameters within and between groups are presented in **Table 3**. Body mass decreased in the EXC group by day 6 and remained lower than baseline thereafter. In the REC group, body mass first decreased by day 6 but then increased by day 8, and finally decreased again by day 10. On day 8, significant differences were found in body mass ($p < 0.001$) and body fat% ($p = 0.017$) between the groups, and on day 10, body fat% also differed ($p < 0.001$) between the groups.

The time × group interaction for leptin was statistically significant ($p < 0.001$), but not for ghrelin. Serum leptin concentration decreased significantly in both groups from day 1 to day 6, while in the EXC group, it stayed at the lower level until the end of the study (**Figure 2A**). After the recovery period, leptin concentration increased in the REC group toward baseline so that a significant difference between the groups was observed on day 8. Serum ghrelin level stayed stable throughout the studied period, except for a slight increase in the EXC group from day 6 to day 10 (**Figure 2B**).

For energy substrate metabolites, significant time × group interactions were found for free fatty acids ($p < 0.001$) and urea ($p < 0.001$) but not for glucose. Results for these biomarkers are presented in **Figure 3**. Significant decreases in glucose were found at day 6 in both groups, followed by a slight increase only in the EXC group toward the end of the study. Relative to baseline, free fatty acid concentration increased at day 6 by 670% (REC) and 597% (EXC), but the recovery period resulted in the return of free fatty acid concentration toward baseline in the REC group (no difference between days 1 and 8 and a significant difference between the groups). Several significant changes were observed in urea concentration within the groups at all phases of the training, but the only significant difference between the groups was found at day 8.

For serum electrolytes, significant time × group interactions were found for sodium ($p = 0.002$) and chloride ($p = 0.03$) but not potassium. Concentrations of these electrolytes (Na, K, and Cl) are shown in **Figure 4**. No significant changes in sodium concentration were found, whereas in the EXC group chloride concentration was significantly lower than baseline at days 6 and 8, and the groups differed significantly at day 8. Potassium levels decreased in both groups by day 6, and then slightly increased, but the increase was only significant in the REC group.

At baseline, strong positive associations were found between body mass and skeletal muscle mass ($r = 0.911$, $p < 0.001$), body mass and leptin ($r = 0.573$, $p < 0.001$), body mass and body fat% ($r = 0.500$, $p < 0.001$), and leptin and body fat% ($r = 0.737$, $p < 0.001$). When evaluating correlations between differences from day 1 to day 10, a strong inverse association was observed between skeletal muscle mass and body fat% ($r = -0.682$, $p < 0.001$). No other systematic associations were found within biomarkers or between changes in different biomarkers and body composition variables. Correlation matrices at baseline and in differences from day 1 to 10 for the whole group are presented in **Supplementary Digital Content 1**.

TABLE 2 | Mean ± SD daily values of energy expenditure (kcal/d) in the exercise (EXC) and recovery (REC) groups.

	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
EXC	3,369 ± 670 *n* = 40	5,469 ± 1,593 *n* = 40	4,389 ± 2,008 *n* = 28	4,407 ± 1,850 *n* = 31	5,717 ± 1,162 *n* = 26	5,521 ± 1,204 *n* = 27	5,113 ± 1,592 *n* = 28	3,570 ± 1,202 *n* = 25
REC	3,690 ± 646 *n* = 25	5,546 ± 1,318 *n* = 25	4,340 ± 1,936 *n* = 23	4,978 ± 1,540 *n* = 22	4,998 ± 1,406 *n* = 22	3,517 ± 887 *n* = 22 ‡‡‡	5,621 ± 1,192 *n* = 23	4,162 ± 1,005 *n* = 21

‡‡‡*$p < 0.001$, between groups.*

TABLE 3 | Changes in body mass, body fat%, skeletal muscle mass and serum creatinine during the training period.

		Day 1	Day 6	Day 8	Day 10	Δ (%) day 1–10
Body mass (kg)	EXC	74.4 ± 10.7	72.9 ± 9.8 $p < 0.001^{\mathrm{day1\_6}}$	72.6 ± 9.6 $p < 0.001^{\mathrm{day1\_8}}$	72.6 ± 9.5 $p < 0.001^{\mathrm{day1\_10}}$	−3.9 ± 1.7
	REC	78.2 ± 9.7	74.6 ± 9.2 $p < 0.001^{\mathrm{day1\_6}}$	77.1 ± 8.6 $p < 0.001^{\mathrm{day1\_8}}$ $p < 0.001^{\mathrm{day6\_8}}$	75.5 ± 9.0 $p < 0.001^{\mathrm{day1\_10}}$ $p < 0.001^{\mathrm{day6\_10}}$ $p < 0.001^{\mathrm{day8\_10}}$	−3.0 ± 2.1
Body fat%	EXC	13.8 ± 4.8	12.8 ± 3.9 $p < 0.001^{\mathrm{day1\_6}}$	10.4 ± 3.3 $p < 0.001^{\mathrm{day1\_6}}$ $p < 0.001^{\mathrm{day6\_8}}$	9.0 ± 3.5 $p < 0.001^{\mathrm{day1\_10}}$ $p < 0.001^{\mathrm{day6\_10}}$ $p < 0.001^{\mathrm{day8\_10}}$	−37.0 ± 9.6
	REC	14.1 ± 5.2	11.5 ± 5.6 $p < 0.001^{\mathrm{day1\_6}}$	10.9 ± 4.7 $p < 0.001^{\mathrm{day1\_8}}$	10.9 ± 4.7 $p < 0.001^{\mathrm{day1\_10}}$	−20.1 ± 11.4
Skeletal muscle mass (kg)	EXC	36.2 ± 4.6	35.9 ± 4.8 $p < 0.001^{\mathrm{day1\_6}}$	36.9 ± 4.8 $p < 0.001^{\mathrm{day6\_8}}$	37.3 ± 4.8 $p < 0.001^{\mathrm{day6\_10}}$	1.4 ± 2.5
	REC	38.0 ± 3.7	37.1 ± 3.1 $p < 0.001^{\mathrm{day1\_6}}$	38.6 ± 3.2 $p < 0.001^{\mathrm{day6\_8}}$	38.0 ± 3.3 $p < 0.001^{\mathrm{day6\_10}}$ $p = 0.003^{\mathrm{day8\_10}}$	−0.2 ± 2.5
Creatinine (μmol/l)	EXC	89.8 ± 11.4	82.4 ± 12.2 $p < 0.001^{\mathrm{day1\_6}}$	88.4 ± 11.9 $p = 0.017^{\mathrm{day6\_8}}$	91.9 ± 10.1 $p < 0.001^{\mathrm{day6\_10}}$	2.2 ± 10.2
	REC	89.2 ± 9.4	85.2 ± 12.6	91.1 ± 8.4	91.6 ± 9.2 $p = 0.031^{\mathrm{day6\_10}}$	2.1 ± 12.5

For body mass, body fat% and skeletal muscle mass n = 42, 38, 27, 25 (EXC) and n = 26, 22, 22, 20 (REC) for days 1, 6, 8 and 10 respectively. For creatinine n = 32, 26, 27, 25 (EXC, respectively) and n = 20, 18, 18, 18 (REC). Significant differences are presented within the groups.

DISCUSSION

The main findings of this study were (a) winter survival training caused severe energy deficit, which contributed to decreases in body mass and body fat%, serum leptin, glucose and potassium concentration, and increases in free fatty acids and urea; (b) a 2-day recovery period during survival training temporarily reversed some of the changes (body mass, leptin, free fatty acids, urea) toward baseline levels, but body mass, body fat% and leptin did not fully recover.

Estimated energy balance was highly negative in both groups during the training and recovery periods, as also evidenced by a decrease in body mass. Participants in the REC group were able to eat and drink *ad libitum* during the 2-day recovery period but still their energy balance stayed negative. It should be noted that energy expenditure was estimated *via* continuous monitoring of heart rate variability and energy intake *via* self-reported food diaries, which both may feature inaccuracies (Capling et al., 2017; Fuller et al., 2020). The REC group had still high energy expenditure values during their recovery period, partly explained by the stress of cardiovascular and autonomic nervous system. Compared to previous literature, Kyröläinen et al. (2017) reported an energy deficit of ∼4,000 kcal during the first week of military field exercise. In Norwegian soldiers, the energy deficit was approximately 2,900 kcal/d during winter military training (Margolis et al., 2014), and in Naval Special Warfare SEAL Qualification Students the deficit was 1,044–3,112 kcal/d (Beals et al., 2019). Survival training in the present study differed from previous studies whereby food intake was deliberately restricted, causing remarkable physiological and psychological stress to participants. Without dietary manipulation, military training often produces a negative balance, which needs to be acknowledged and minimized, if possible. According to Beals et al. (2019), focusing on macronutrient supply and providing extra snacks for recovery periods will compensate for the lack of energy in intense military training. Even in harsh

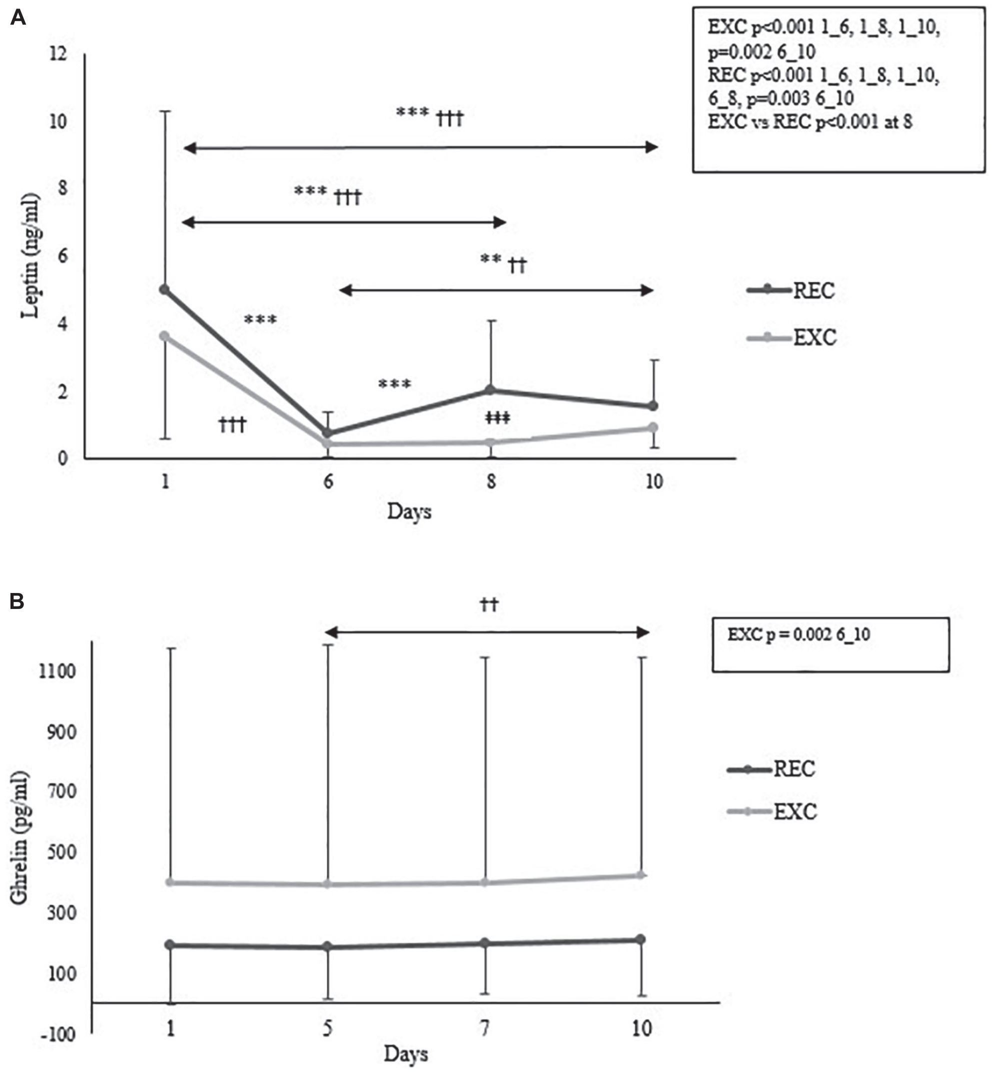


FIGURE 2 | Changes in serum **(A)** leptin and **(B)** ghrelin concentrations within and between the REC and EXC groups. For leptin, a number of subjects in the REC and EXC groups were daily 26, 22, 22, 20 and 42, 26, 27, 25, respectively. For ghrelin $n = 17$ (on all days) in the REC group and $n = 23, 23, 23, 22$ in the EXC group for days 1, 6, 8, 10 respectively. *REC, †EXC, and ‡Between groups. $^{**,\dagger\dagger}p < 0.01$; $^{\dagger\dagger\dagger,\ddagger\ddagger\ddagger,***}p < 0.001$.

circumstances (Greenland expedition), energy balance can be maintained if the food supply is appropriate and well planned (Gagnon et al., 2011; Charlot et al., 2020).

As hypothesized, body mass and body fat% decreased during the training period. In the EXC group, body fat% declined throughout the 10-day period but in the REC group body fat% remained stable between days 6 and 10. Loss of body mass is typical in survival training. For example, Hoyt et al. (2006) reported a loss of 7.7 ± 1.1 kg and a decrease in fat free mass in male cadets during a 7 day ranger field exercise. Hamarsland et al. (2018) noted a 5.3 ± 1.9 kg decrease in muscle mass during Norwegian Special Forces' "hell week," after which body mass returned to baseline within 1 week. Magnitude of weight loss is associated with the intensity and duration of exercise, the magnitude of energy and fluid deprivation, and the length of field training. In all the above-mentioned studies, body mass at baseline was approximately the same (78 kg) and BMI was in the normal range, making higher losses of body mass more critical for lean soldiers.

Interestingly, skeletal muscle mass (estimated by bioimpedance) decreased by day 6 but then increased toward the end of the study in both groups. Previous findings indicate that fat free mass typically decreases in severe energy deficit (Hoyt et al., 2006; Nindl et al., 2007; Hamarsland et al., 2018). Bioimpedance measurements were performed in the mornings after an overnight fast, but we suspect that the dehydrated state of participants may have interfered with the results. Therefore, we also measured serum creatinine to clarify the body composition results. Creatinine is a biomarker of renal function, but it can also be used as a proxy for the amount

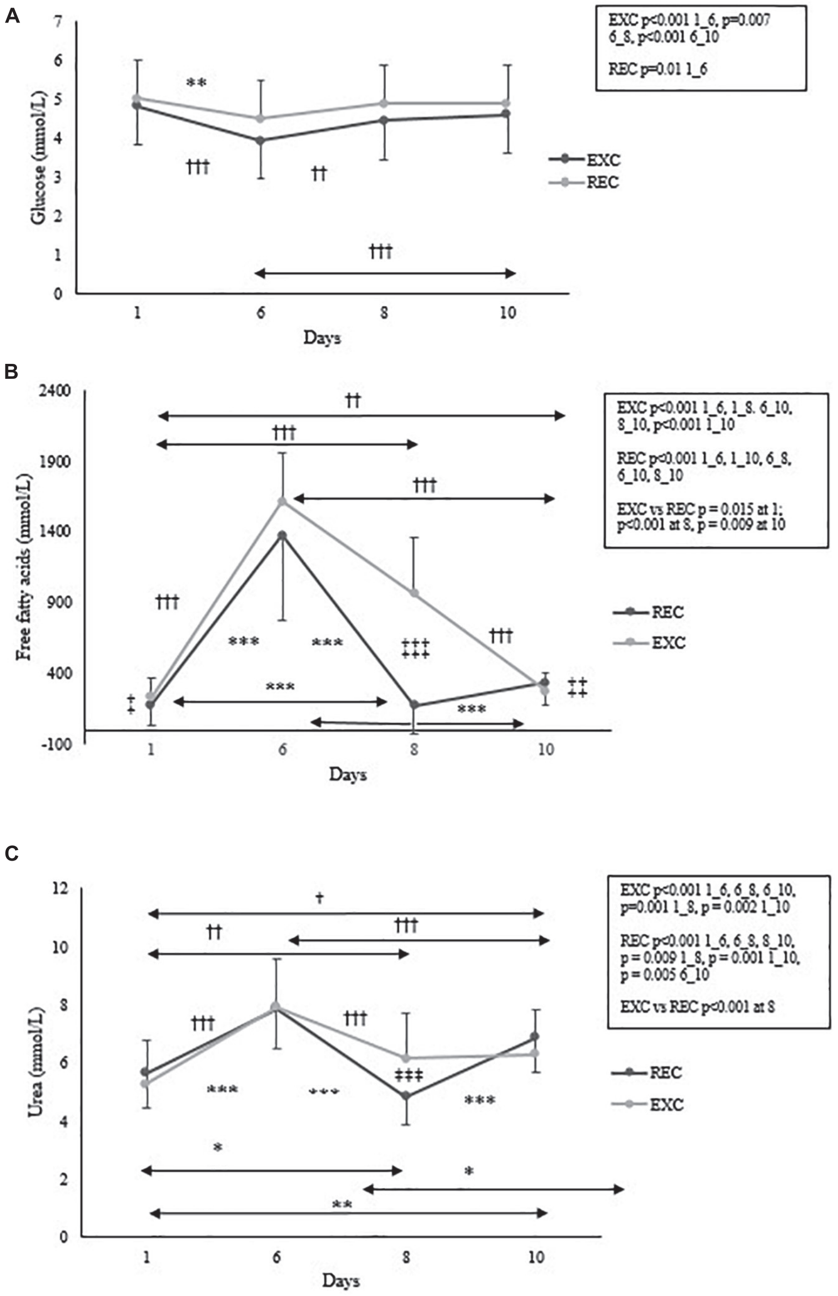


FIGURE 3 | Changes in **(A)** glucose, **(B)** free fatty acids and **(C)** urea concentrations within and between the REC and EXC groups. In the REC group, $n = 26, 22, 22, 20$ and in the EXC group $n = 42, 26, 27, 25$ for days 1, 6, 8, 10 respectively. *REC, †EXC, and ‡Between groups. *,†,‡$p < 0.05$; **,††,‡‡$p < 0.01$; ***,†††,‡‡‡$p < 0.001$.

of muscle mass (Kim et al., 2016; Delanaye et al., 2017). In this study, creatinine reacted in the same way as skeletal muscle mass, whereby its concentration increased from day 6 to day 10. The REC group did consume a higher amount of energy during their recovery period, but the EXC group also got additional energy from day 6 onward. A remarkable energy deficit still existed. One explanation for these inverse muscle mass results could be a protective metabolic mechanism during prolonged energy deprivation, where cells uptake all of the amino acids available for protein synthesis, and lipids become the primary energy substrate (Gagnon et al., 2020). Ocobock (Ocobock, 2017) suggested that the amount of body fat may protect muscle mass during a state of negative energy balance, since females gained muscle mass but males lost it in their study. Another explanation for the results is that, the used methodologies were interfered by dehydrated

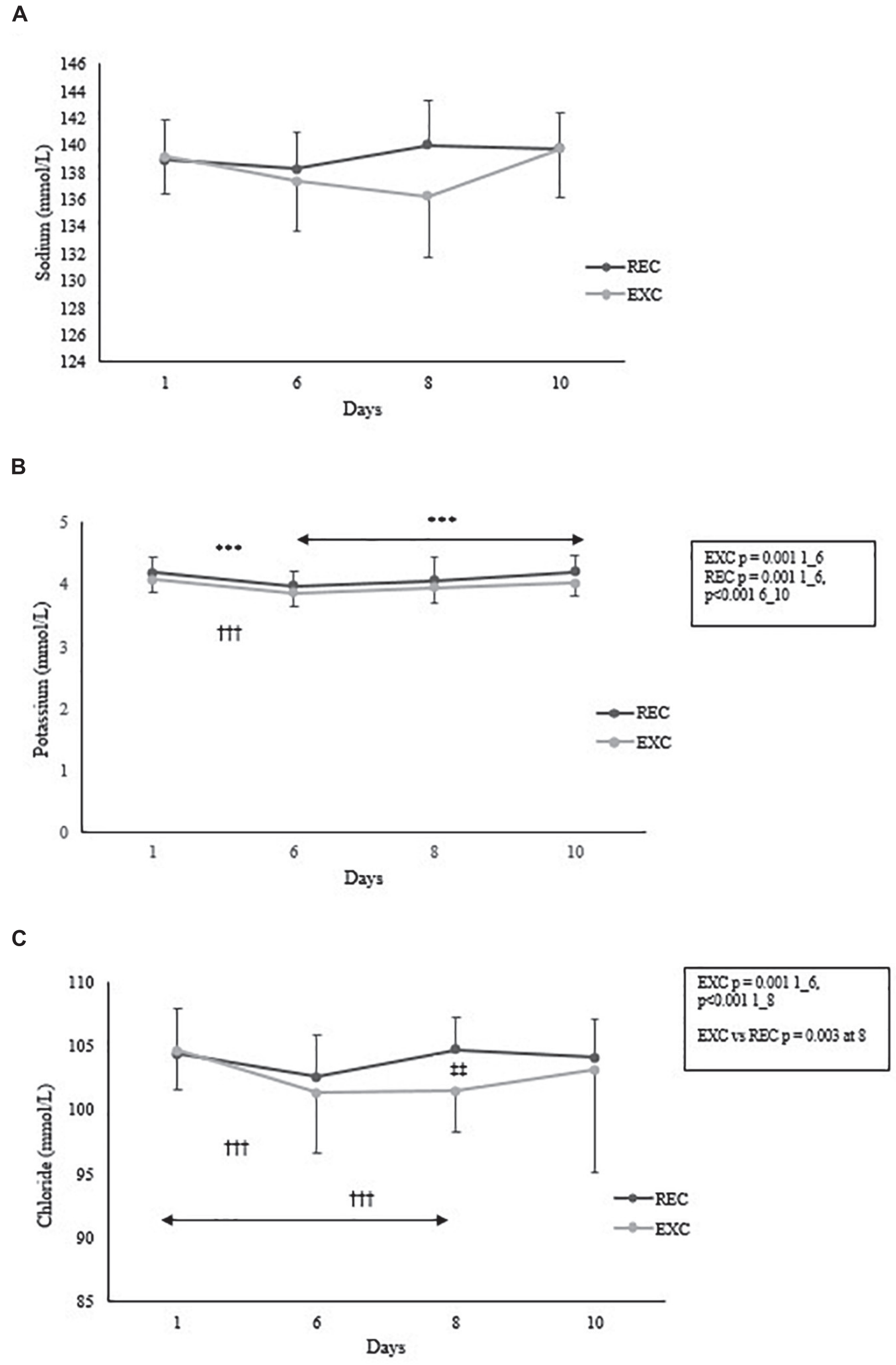


FIGURE 4 | Changes in **(A)** sodium **(B)** potassium and **(C)** chloride concentration within and between the REC and EXC groups. In the REC group *n* = 26, 22, 22, 20 and in the EXC group *n* = 42, 26, 27, 25 for days 1, 6, 8, 10 respectively. *REC, †EXC, and ‡Between groups. ‡‡$p < 0.01$; ***,†††$p < 0.001$.

and unfed state of participants and factually muscle mass did not increase.

Severe energy deficit, loss of body mass and especially loss of fat free mass have detrimental consequences for soldiers, since they disturb muscle function, the endocrinological system and military performance (Pasiakos, 2020). During arduous training, macronutrient supplementation (protein, carbohydrates) has been used to help maintain fat free mass and to provide extra energy (Pasiakos, 2020). Some novel methods have also been examined, for example, the use of ketones for extra fuel (Margolis and O'Fallon, 2020) and low-dose testosterone supplementation for preventing hormonal disturbances (Pasiakos et al., 2019), but more studies are needed to clarify the safety, dose, timing and side effects of these supplements.

A decline in leptin concentration is typically associated with acute and severe energy deficit (Pasiakos et al., 2011). In the present study, a clear decrease in serum leptin was observed at day 6 in both groups. At baseline, leptin was positively associated with body mass and body fat%, as observed in previous studies (Pasiakos et al., 2011; Hill et al., 2015). Although leptin concentration is associated with adipose tissue, the regulation of leptin concentration may alter *via* glucose metabolism (Pasiakos et al., 2011). *In vitro* and *in vivo* studies have demonstrated that blood glucose regulates leptin gene expression, but short-term starvation does not necessarily mediate leptin gene expression; it merely decreases circulating leptin concentration (Kolaczynski et al., 1996; Pasiakos et al., 2011). In this study, fasting blood glucose levels were reduced at day 6 in both groups. The training led to low glucose concentrations for several days, which could diminish leptin gene expression and circulating leptin concentration, although no associations were found.

Ghrelin is an appetite-regulating hormone and in the current study, serum ghrelin concentration remained stable throughout the training, except for a small increase in the EXC group from day 6 to day 10. These findings agree with those of a previous study, where ghrelin concentration was assessed during short-lasting starvation (Pasiakos et al., 2011): ghrelin concentration was elevated and stable during energy deficit, whereas it decreased acutely in response to feeding. In the present study, although the REC group consumed food and drinks *ad libitum* during their recovery period, this did not affect ghrelin concentration, likely because of inappropriate timing of blood samples. Hill et al. (2015) reported that ghrelin levels were elevated in soldiers who lost the greatest amount of body mass during military deployment, but this phenomenon was not seen in the current study. A few particularly high values of ghrelin were measured in the EXC group, but they were included in the statistical analysis, since elevated values were found from the same participants at all four measurement points systematically.

Blood urea concentration indicates the breakdown of nitrogen containing compounds, which typically means degradation of protein (Haralambie and Berg, 1976). During the first 2 days in the field, when energy intake was severely restricted, glycogen stores were likely depleted and muscle protein degraded. Urea concentration slightly increased, as well as free fatty acids, whereas blood glucose concentration decreased at day 6 in both groups. Free fatty acid levels peaked at day 6 in both groups and these increases were as high as 670% (REC) and 597% (EXC). According to Cahill (2006), starvation modifies fuel metabolism to ensure continuous glucose transportation to the brain. In a post-absorptive state, blood glucose and glycogen are utilized and if starvation lasts for several days, gluconeogenesis is activated. At the same time, free fatty acids are released into the bloodstream, which indicates lipolysis in adipose tissue. In the current study, the most dramatic change in energy metabolism occurred after 2 days of field training, where only a small amount of energy (357–447 kcal/d) was consumed. The lack of dietary carbohydrate shifted the primary fuel source from glycogen to fat. When additional energy was given, the biomarkers of energy substrates recovered toward baseline and muscle mass slightly increased, implying that muscle protein was spared and that all dietary protein was utilized for protein synthesis. In a study by Karl et al. (2017), a large variety of metabolites were measured during a military cross-country ski march that induced a severe energy deficit. Clear increases in fat metabolism were observed, as well as moderate increases in tricarboxylic acid cycle intermediates and branched chain amino acid metabolites. The magnitudes of changes in protein metabolites were not as high as for lipid metabolites. In the present study, only a few biomarkers of energy metabolism were used, but the findings agree with the previously described fuel metabolism results during starvation.

Rapid weight loss is usually explained by fluid loss. Sodium is the major cation in the intracellular space (Warburton et al., 2002). Hyponatremia is diagnosed when blood sodium concentration is below 135 mmol/l, and a concentration below 130 mml/l indicates severe hyponatremia. In the EXC group, sodium concentration was almost hyponatraemic at day 8 (136 mmol/l). Statistically significant changes were not observed within or between groups, mainly due to individual variation and high SD of the samples. In ultra-endurance events, hyponatremia is the principal electrolyte disorder, and commonly found in athletes seeking medical help after prolonged strenuous exercise. Loss of sweat and overhydration may be the main factors responsible for hyponatraemia. The present participants were likely dehydrated due to high sweat loss and restricted fluid intake, and this is supported by the observed rapid loss of body mass. Dehydration lowers the extracellular fluid volume, which may compensate for the sodium concentration in the blood (Pedlar et al., 2019).

Potassium concentration decreased during the first 6 days of training in both groups. Potassium is the major electrolyte in intracellular fluid, and it is released to the extracellular space during exercise in direct proportion to exercise intensity (Warburton et al., 2002). Hyperkalaemia is an acute response to exercise, but reuptake of potassium into the muscle may further lead to a hypokalaemic state. In the current study, an acute effect was not observed, and the training was longer than a typical ultra-endurance event. Nonetheless, we assume that part of the participants was heading to hypokalemia after the most strenuous phase of training at day 6.

In addition to sodium, chloride is another ion that can be lost *via* sweating in the form of sodium chloride (Emenike et al., 2014). At the cellular level, sodium and chloride ions usually move in the same direction from extracellular fluid into the cells. Thus, sodium and chloride concentrations tend to be correlated, but this was not the case in this study. Chloride concentration was lower in the EXC group at day 8, which may indicate a loss of electrolytes due to prolonged exercise. However, Gagnon et al. (2020) did not find any significant changes in electrolytes (Na^{2+}, K^{+}, Cl^{-}) after a 14-day canoeing expedition (compared to the control group), although they did not compare to baseline values and the study protocol was not comparable.

Based on the findings of this study, soldiers were exposed different physiological stress factors during survival training, which disturbed body's homeostasis, especially during the first days of strenuous training. Short recovery period had a positive effect on part of the measured biomarkers, which can improve military performance of soldiers. To optimize performance in survival training, energy and fluid refueling, adequate sleep as well as mental preparation before the training, may enhance the body's ability to adjust to arduous circumstances.

Strengths and Limitations

Our study protocol had some limitations, which must be acknowledged. Evaluation of energy expenditure was based on continuous heart rate recordings. Although it was reported that the accuracy of this procedure is 7–10% (Smolander et al., 2011), validation was not done with double-labeled water or direct calorimetry. We did ask about fluid intake but the diaries were not sufficiently valid to report these results. This data could have helped in the interpretation of changes in body mass, muscle mass and electrolytes. Bioimpedance analysis is a simple method of assessing body composition outside of the laboratory environment, and it is known that it may overestimate fat free mass and underestimate fat mass in civilians (Sillanpää et al., 2014) and in a military context (Langer et al., 2016). Since the method is based on anthropometry and the fluid content of the body, rapid changes in hydration status may interfere with impedance values. However, changes in creatinine concentration occurred in parallel with bioimpedance results, which is exceptional in training that involves energy deprivation. A valid biomarker for protein metabolism was missing due to analytical problems with free amino acid concentrations. Thus, more accurate studies are needed to clarify protein and substrate metabolism during energy deficit. A high number of dropouts is typical for arduous training (Vaara et al., 2020), but by using a mixed statistical model, we were able to use the maximum number of observations. Despite the high dropout rate, an adequate number of participants finished the survival training in both groups. Acute and severe sleep deprivation may associate with part of these results, since glucose and appetite regulation (e.g., leptin, ghrelin) are influenced by sleep (Leproult and Van Cauter, 2010). Unfortunately, the importance of sleep deprivation has not been investigated in the present study, but further study is needed, especially in military population. It is still worth emphasizing the unique protocol and extraordinary caloric and sleep deprivation, which may not be possible among civilians.

Conclusion

The present study showed that the most remarkable changes in metabolism occurred in the first 2 days of survival training, where the energy balance was highly negative. Changes in blood parameters partly recovered, especially when recovery was emphasized, but body mass and body fat% remained below baseline levels throughout the training period. A 2-day active recovery period in the middle of strenuous training may be sufficient to normalize body function and physical performance of soldiers.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the study was performed in line with the principles of the Declaration of Helsinki. The ethical approval was granted by the Scientific and Ethical Committee of the Helsinki University Hospital Research (HUS/900/2018). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TN, TO, MF, and HK conceived and designed the research. TN and TO conducted experiments. TN, TO, and RH analyzed data. TN wrote the manuscript. All authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.797268/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

IV

ENERGY BALANCE, HORMONAL STATUS, AND MILITARY PERFORMANCE IN STRENUOUS WINTER TRAINING

by

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Article

Energy Balance, Hormonal Status, and Military Performance in Strenuous Winter Training

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Abstract: Severe energy deficit may impair hormonal regulation and physical performance in military trainings. The aim of this study was to examine the associations between energy intake, expenditure, and balance, hormones and military performance during a winter survival training. Two groups were studied: the FEX group ($n = 46$) had 8-day garrison and field training, whereas the RECO group ($n = 26$) had a 36-h recovery period after the 6-day garrison and field training phase. Energy intake was assessed by food diaries, expenditure via heart rate variability, body composition by bioimpedance, and hormones by blood samples. Strength, endurance and shooting tests were done for evaluating military performance. PRE 0 d, MID 6 d, POST 8 d measurements were carried out. Energy balance was negative in PRE and MID (FEX -1070 ± 866, -4323 ± 1515; RECO -1427 ± 1200, -4635 ± 1742 kcal·d^{-1}). In POST, energy balance differed between the groups (FEX -4222 ± 1815; RECO -608 ± 1107 kcal·d^{-1} ($p < 0.001$)), as well as leptin, testosterone/cortisol ratio, and endurance performance ($p = 0.003$, $p < 0.001$, $p = 0.003$, respectively). Changes in energy intake and expenditure were partially associated with changes in leptin and the testosterone/cortisol ratio, but not with physical performance variables. The 36-h recovery restored energy balance and hormonal status after strenuous military training, but these outcomes were not associated with strength or shooting performance.

Keywords: energy deficit; physical performance; survival training; soldier

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1. Introduction

Winter survival training is one of the most demanding forms of field training for soldiers, in which they are exposed to various internal and external stressors. Energy deficit and sleep deprivation, combined with mentally demanding tasks in harsh environments, impair hormonal regulation and physical performance [1–5]. Energy expenditure is elevated due to prolonged low- to high-intensity military tasks, personal load carriage, limited sleep, and extreme weather conditions in the field [6–8]. Energy intake is often decreased because of insufficient energy content of field rations, intentional restriction of food intake, menu fatigue, or bad mood [6,9]. Furthermore, logistical problems or an extra load of food rations can diminish food supply in field trainings [7]. Thus, imbalance in energy intake and expenditure cause negative energy balance, which can occur for several days or weeks in military field trainings [6].

Severe energy deficit impairs hormonal regulation and disturbs homeostasis in harsh environments [10,11]. Appetite-mediating hormones, leptin and ghrelin, react in acute caloric deprivation [12] and in response to loss of body mass [13]. Catabolic hormones, such as cortisol, are increased and anabolic hormones (e.g., testosterone) are decreased in a negative energy balance, but hormonal changes can be recovered during training, when more energy is available [14]. Hamarsland et al. [2] observed that serum testosterone and cortisol concentrations stayed imbalanced after 24- and 72-h recovery, and cortisol levels were still elevated after 1 week of arduous military course. Nevertheless, studies have not clearly demonstrated, if a short and active recovery period could normalize hormonal status enough to maintain military performance in the field.

Declines in lower-body power and strength after arduous military training were associated with energy deficit and duration of training, while greater total negative energy balance was related to the impairment of lower-body performance [3]. Fewer studies have examined endurance capacity, but impairment of aerobic, but not anaerobic, performance has been observed [7]. The 7-d arduous military course, with energy and sleep deficit, resulted in a decrease in maximal strength [2]. After a 2-d recovery, no significant changes were observed in strength tests, but after 7 d of recovery, maximal strength values, except for counter movement jump, recovered close to pre-values, indicating hormonal and physical recovery [2,15]. In addition, energy deficit has been shown to alter shooting performance during training, especially in a standing position [16]. It is still unclear, whether energy variables are associated with a decline in physical and military performance in strenuous field training.

Thus, the purpose of the present study was (a) to observe energy intake, energy expenditure, and energy balance in strenuous field exercise; (b) to study associations of these energy variables with hormonal status (leptin, ghrelin, testosterone/cortisol ratio (T/C ratio)), and strength, endurance and shooting performance; and (c) to investigate effects of a 36-h recovery on these outcomes in soldiers.

2. Materials and Methods

Sixty-eight male soldiers volunteered for the study, and they were divided into two groups (FEX = field exercise, RECO = recovery) according to their platoon. The FEX group (n = 42) had garrison and field military training, whereas the RECO group (n = 26) had a 36-h recovery period after the military field training phase. Randomization was not possible, given that survival training was a part of their military service and implemented by platoons. For the field exercise group, more participants were selected, because a higher attrition rate was expected from this group. The present study was part of a larger multidisciplinary research project, authorized by the Finnish Defence Forces (AO1720). The ethical review of the study was granted by the Scientific and Ethical Committee of the Helsinki University Hospital Research (HUS/900/2018). All participants were informed of the experimental design, methods, benefits, and possible risks prior to signing an informed consent document. Characteristics of participants are shown in Table 1.

Table 1. Characteristics of participants in the RECO and FEX groups at baseline. Values are the means ± SD. No statistically significant differences between the groups were found.

Group	n	Age (Years)	Height (cm)	Body Mass (kg)	BMI (kg/m^2)
RECO	26	19.7 ± 1.2	181 ± 6	78.2 ± 9.6	23.9 ± 2.7
FEX	42	19.6 ± 0.8	179 ± 7	74.4 ± 10.7	23.1 ± 2.8

The study protocol is presented in Table 2. The training was divided into three phases: (1) 3-day preparation period at the garrison; (2) 2-day survival training in the field; (3) 2-day survival training (FEX) or recovery (RECO) period.

The study was conducted in Lapland, north from the Arctic Circle (location 67°24′54″ N, 26°35′26″ E) in early April. Measurements were carried out at three time-points: at baseline (PRE), after phase 2 (MID), and after phase 3 (POST). During phase 1, participants were educated on the theory and practice of survival training. The workload was moderate, 3–4 meals were served at the canteen, and sleeping was arranged similar to normal service. During phase 2, all participants started their field training period where the workload was high, while energy intake and sleep were restricted. Additionally, military tasks were stressful and arduous, partially due to snow (depth 80–100 cm). Changes in temperature were typical for late winter: mean daily temperature varied from −0.3 °C to −4.7 °C; min −10.5 °C at night; max 5.4 °C in the daytime. Since participants had started their conscript service earlier, they were adapted to local weather conditions. Moreover, the temperatures were not extreme considering any location in Finland during wintertime. Personal equipment was carried by backpacks and pulks (extra load 23–32 kg). During phase 3, the FEX group continued their field training as described, but the RECO group was moved to a temporary accommodation, where facilities were similar to the garrison (3–4 meals served, extra snacks, good sleeping facilities, bathroom, and sauna). In addition, low-intensity activities (ball games and stretching) were supervised to help recovery.

Table 2. Study protocol and the used method for each variable below. x refers to measurement day.

Days	**PRE Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**MID Day 6**	**Day 7**	**POST Day 8**
Task	Education in garrison			Field exercise		Recovery in RECO Field exercise in FEX		
Energy expenditure Heart rate variability	x				x		x	
Energy intake Pre-filled food diaries	x				x		x	
Blood samples Immunoassay	x					x		x
Body composition Bioimpedance	x					x		x
Fitness tests Strength and endurance	x					x		x
Shooting test Prone and standing	x					x		x

Body composition and blood samples were carried out in a fasting state, early in the morning, with the same protocol for each measurement point. A segmental multi-frequency bioimpedance analysis (Inbody 720/770, Biospace, Soul, Republic of Korea) was used to evaluate body mass and fat-free mass. Participants wore underwear during the assessment, and they were advised to urinate before the measurement.

For leptin, ghrelin, testosterone, and cortisol analysis, blood samples were collected into VenoSafe plastic tubes (VenoSafe®, Terumo Europe, Leuven, Belgium) containing silica gel. Samples were centrifuged (3500 rpm, 10 min), then the supernatant (serum) was collected and frozen for later analysis. Serum leptin and ghrelin levels were determined with an ELISA-kit immunoassay system (Dynex DS 2, Dynex Technologies, Chantilly, VA, USA). Testosterone and cortisol levels were acquired via Immulite immunoassay analyzer (Siemens Immulite 2000 XPI, Siemens Healthcare, Malvern, PA, USA). The sensitivity and inter-assay coefficient of variation for these assays were: 0.2 ng/mL and 6.1% for leptin; 0.6 pg/mL and 17.3% for ghrelin; 0.5 nmol/L and 7.8% for testosterone; and 5.5 nmol/L and 6.5% for cortisol.

Total energy expenditure and exercise energy expenditure were estimated via continuous heart rate variability recording (Firstbeat Bodyguard 2, Firstbeat Technologies Oy, Jyväskylä, Finland). Participants wore a two-electrode portable device during the study period. For energy availability, days 5 and 7 were chosen to get entire 24-h data and to synchronize energy expenditure with energy intake values. Exercise energy expenditure was objectively determined from heart rate variability data by the manufacturer's analysis.

Continuous heart rate monitoring has been reported to be sufficiently accurate in light- and moderate-intensity activities [17]. A strong correlation (r = 0.85–0.98; $p < 0.05$) between gold-standard energy expenditure measurement and energy expenditure evaluation has been observed in graded tests [18].

Energy intake was estimated with pre-filled food diaries by a software program (Fineli, National Food Composition Database, Finland). Meals served at phase 1 and 3, as well as food items at phases 2 and 3, were known beforehand, so diaries were pre-filled and participants marked the time and the amount of food consumed. During phase 2, restricted field ratios consisted of one protein bar (226 kcal), eight crackers (306 kcal), and two lunch meal rations (mean 661 kcal/portion). Representative days (5 and 7) were analyzed further in relation to the other energy variables.

Maximal isometric force of the upper- and lower-body extremities was measured bilaterally by a leg and bench press dynamometer (Faculty of Sport and Health Sciences, University of Jyväskylä, Finland). In bench and leg press, positions were adjusted as previously described [19,20]. Participants were instructed to produce maximal force as fast as possible with verbal encouragement by the testing personnel. One trial attempt was allowed before the two test trials, with minimum 60 s recovery between the trials. The best performance was selected for further analysis.

A standing long jump was used to measure explosive force production of the lower extremities [21] on a specifically designed gym mat (Fysioline Co., Tampere, Finland). Before testing, the participants were instructed on the correct technique, and they performed 2–3 warm-up jumps. The participants were advised to jump (horizontally) forward as far as possible from a standing position and land bilaterally without falling backward. The best of the three jumps was utilized, measuring from the start line to the landing point.

A seated medicine ball throw was measured for assessing explosive force production of the upper body [22]. The participants sat in an upright position on the floor with their legs fully extended and back kept against the vertical wall throughout the test. The 3-kg medicine ball was held with both hands in front of their chest, with their forearms positioned parallel to the ground. The participant threw the ball vigorously as far forward as possible, while maintaining their back against the wall. The distance from the wall to landing point of the medicine ball was recorded. The best result out of the three trials was used in the analysis. The participants were allowed to have at least three training throws before the test measurements.

Sit-ups and pull-ups were conducted to assess muscular endurance by counting maximal repetitions in one minute. Sit-ups were used to assess abdominal and hip flexor performance [23], and push-ups were used to measure performance of the arm and shoulder extensor muscles [24]. Technical advice was given before the tests and incorrect repetitions were excluded. All strength tests were transformed to z-scores and the mean of values formed “strength index”.

Endurance performance was measured by a 20-m shuttle run test [25]. Participants were advised to run 20 m back and forth with accelerating pace as long as possible. The test was finished when participants were not able to keep the given pace or voluntarily dropped out. Maximal oxygen uptake values were calculated from the test results, as previously described [26].

Shooting accuracy was estimated by an optical infra-red weapon system (Eko-Aims Oy, Ylämylly, Finland). Ten shots were given in prone and standing positions to the target 10 m away from the shooting line, in an indoor hall. The sum of ten shots from both positions was recorded for analysis. The sum variable “shooting index” was aggregated from these results.

Physical and shooting performance, as well as blood biomarkers, were set as dependent variables, time × group interaction and energy variables as independent variables, and body mass as the confounding factor. Time × group interactions were tested with F-tests based on the Satterthwaite method, using the lmerTest R-package. A linear mixed effect model was used to estimate changes between groups over the studied period. Sample size varied depending on the variables tested. In addition, the specific sample sizes are being reported in the tables and figures. Pairwise comparisons were performed using a Tukey's test. Logarithmic transformations were done when the distribution was positively skewed (leptin, ghrelin, testosterone-cortisol ratio). Non-parametric Mann–Whitney U-tests were used to verify the linear mixed effect model when residuals were not normally distributed (strength index, testosterone-cortisol ratio). Pairwise Pearson correlations were calculated to estimate associations between energy variables and strength, and the shooting index, endurance performance and hormonal status. All statistical analyses were performed using R v. 3.6.3 (2020, R Foundation for Statistical Computing, Vienna, Austria). Values are presented as the means ± standard deviations and statistical significance was set at $p < 0.05$.

3. Results

The mean body mass reduced in the FEX group from PRE to MID ($p < 0.001$), and was then maintained at POST (74.4 ± 10.7, 72.9 ± 9.8, 72.6 ± 9.6 kg, respectively), but in RECO, body mass first decreased in MID ($p < 0.001$), but then recovered towards the baseline ($p < 0.001$) (78.2 ± 9.7, 74.5 ± 9.2, 77.1 ± 8.6 kg, respectively). No differences were found between the groups. Skeletal muscle mass dropped in MID and increased in POST in both groups (FEX 36.2 ± 4.6, 35.9 ± 4.8, 36.9 ± 4.8 kg; RECO 38.0 ± 3.7, 37.1 ± 3.1, 38.6 ± 3.2 kg; $p < 0.001$ PRE–MID, MID–POST both). Fat mass decreased in the FEX group at all measurement points (10.6 ± 5.0, 9.4 ± 3.5, 7.6 ± 3.0 kg; $p < 0.001$ PRE–MID, MID–POST). In the RECO group, fat mass first reduced and then stabilized during the recovery period (11.4 ± 5.4, 9.0 ± 5.5, 8.7 ± 4.7 kg; $p < 0.001$ PRE–MID, MID–POST). More results have been presented in the previous study [10].

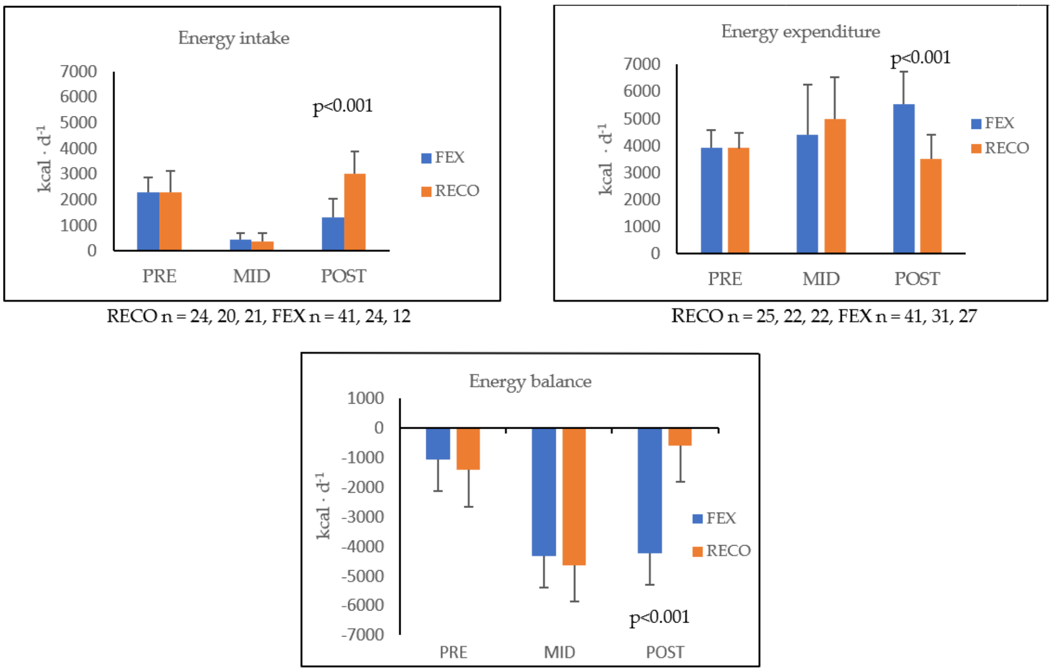
Energy intake was low, especially in the MID measurement. The RECO group received more energy than the FEX group in the POST measurement. At that time, the RECO group was transferred into an accommodation, where energy expenditure lowered and meals were served similar to the garrison. Energy expenditure values exceeded the energy intake values throughout the study, even in garrison-like circumstances. These two outcomes caused a negative energy balance in all measurement points. All results of the energy variables are shown in Figure 1. Hormonal responses are presented in Figure 2. Differences between the groups were found in leptin at MID and POST, and also in the testosterone/cortisol ratio at POST.

Absolute results of physical fitness and shooting tests are shown in Table 3. Significant differences between the groups were found in the 20-m shuttle run test, where the RECO group improved its performance in POST, and in prone shooting, in which the groups differed at all measurement points. Push-ups and shooting in standing position differed at baseline, but the differences disappeared in MID and POST between the groups.

Sum variable "strength index" decreased from PRE to MID in both groups (FEX $p < 0.001$, RECO $p = 0.014$), and increased from MID to POST only in RECO ($p < 0.001$). Strength index differed between the groups ($p = 0.025$) at baseline, but the difference disappeared in later measurements. Endurance performance (20-m shuttle run) impaired at MID in both (FEX $p < 0.001$, RECO $p = 0.007$), but the RECO group improved its performance at POST compared to MID ($p = 0.008$). The shooting index decreased only in the FEX group from MID to POST ($p = 0.0003$), but it differed between the groups at all measurement points ($p < 0.001$, $p = 0.003$, and $p < 0.001$ in PRE, MID, POST, respectively), as seen in prone shooting results alone.

The time × group interactions were found for leptin ($p < 0.001$) and strength index ($p < 0.001$), but not between any other variables. Furthermore, energy expenditure ($p = 0.038$) and energy intake ($p = 0.028$) were significant predictors for leptin.

A strong, positive association was found between changes in energy balance and changes in the shooting index ($r = 0.786$, $p = 0.002$) in the FEX group from MID to POST, whereas no other significant associations were found between energy variables and the strength index, endurance performance, or shooting index. Statistically significant associations between changes in energy intake and energy expenditure, and changes in hormones are presented in Table 4. The change in energy balance was not associated with any of these hormonal variables.

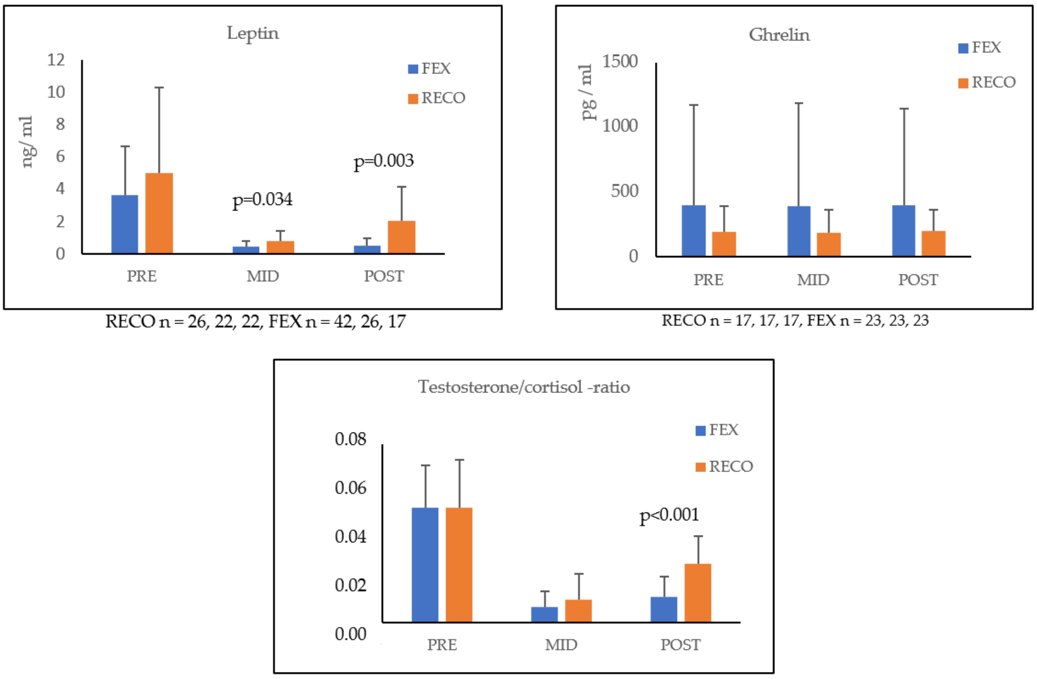


RECO n = 24, 20, 21, FEX n = 41, 24, 12

RECO n = 25, 22, 22, FEX n = 41, 31, 27

RECO n = 23, 10, 21, FEX n = 39, 24, 12

Figure 1. Mean (± SD) energy intake (EI), energy expenditure (EE), and energy balance (EB) during the training in the RECO and FEX groups. RECO = recovery, FEX = field exercise. Statistical significances are presented between the groups.



RECO n = 26, 22, 22, FEX n = 42, 26, 17

RECO n = 17, 17, 17, FEX n = 23, 23, 23

RECO n = 26, 22, 22, FEX n=42,26,27

Figure 2. Mean (±SD) leptin, ghrelin, and testosterone/cortisol ratio concentrations during the training in the recovery (RECO) and field exercise (FEX) groups. Statistical significances are presented between the groups.

Table 3. Mean (±SD) physical fitness tests and shooting performance results during the training in the field exercise (FEX) and recovery (RECO) groups. Statistical significances are presented between the groups. Number of participants are shown in brackets. VO_2max refers to maximal oxygen uptake.

Physical and Shooting Performance	**Group**	**PRE**	**MID**	**POST**
Maximal isometric force, lower (kg)	FEX	88 ± 10 (42)	82 ± 11 (28)	85 ± 12 (26)
	RECO	83 ± 11 (25)	79 ± 11 (22)	84 ± 11 (21)
Maximal isometric force, upper (kg)	FEX	312 ± 89 (42)	312 ± 88 (28)	336 ± 103 (26)
	RECO	314 ± 74 (25)	296 ± 79 (22)	321 ± 66 (21)
Standing long jump (cm)	FEX	225 ± 22(41)	222 ± 22 (27)	221 ± 18 (24)
	RECO	217 ± 16 (24)	217 ± 16 (22)	218 ± 15 (21)
Medicine ball throw (cm)	FEX	611 ± 74 (42)	590 ± 68 (28)	575 ± 68 (26)
	RECO	595 ± 58 (24)	585 ± 51 (22)	609 ± 47 (21)
Push-ups (reps/min)	FEX	39 ± 13 (41)	30 ± 14 (27)	34 ± 12 (24)
	RECO	32 ± 11 (24) $p = 0.029$	27 ± 13 (22)	36 ± 10 (21)
Sit-ups (reps/min)	FEX	43 ± 9 (41)	40 ± 10 (27)	39 ± 9 (24)
	RECO	39 ± 6 (24)	39 ± 8 (22)	41 ± 7 (21)
20-m shuttle run for VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	FEX	46 ± 5 (24)	39 ± 7 (27)	36 ± 12 (23)
	RECO	45 ± 5 (41)	41 ± 8 (21)	45 ± 5 (20) $p = 0.003$
Shooting, prone (points)	FEX	86 ± 10 (24)	88 ± 7 (28)	76 ± 9 (26)
	RECO	93 ± 6 (26) $p < 0.001$	93 ± 3 (22) $p = 0.001$	91 ± 6 (21) $p < 0.001$
Shooting, standing (points)	FEX	62 ± 10 (42)	63 ± 11 (28)	64 ± 12 (26)
	RECO	69 ± 11 (26) $p = 0.016$	68 ± 11 (22)	69 ± 11 (21)

Table 4. Associations of changes in energy intake, energy expenditure, and energy with changes in leptin, ghrelin, and testosterone/cortisol ratio (T/C ratio). Statistically significant associations have been bolded.

		Leptin	**Ghrelin**	**T/C Ratio**
		PRE-POST changes		
Energy intake	FEX	r = −0.089, p = 0.784	r = −0.003, p = 0.413	r = −0.214, p = 0.504
	RECO	r = 0.099, p = 0.687	r = −0.215, p = 0.46	r = −0.035, p = 0.888
Energy expenditure	FEX	**r = −0.566, p = 0.003**	r = −0.131, p = 0.552	r = −0.231, p = 0.257
	RECO	r = −0.007, p = 0.977	r = 0.028, p = 0.915	r = −0.145, p = 0.145
Energy balance	FEX	r = 0.398, p = 0.2	r = 0.104, p = 0.774	r = 0.104, p = 0.747
	RECO	r = 0.197, p = 0.419	r = −0.466, p = 0.093	r = −0.387, p = 0.102
		PRE-MID changes		
Energy intake	FEX	r = −0.302, p = 0.173	r = −0.35, p = 0.12	r = 0.014, p = 0.951
	RECO	r = −0.384, p = 0.116	r = −0.123, p = 0.689	**r = 0.479, p = 0.044**
Energy expenditure	FEX	r = −0.059, p = 0.775	r = 0.388, p = 0.067	r = −0.366, p = 0.066
	RECO	r = −0.27, p = 0.225	r = −0.001, p = 0.996	r = −0.017, p = 0.94
Energy balance	FEX	r = −0.187, p = 0.405	r = −0.337, p = 0.135	r = 0.245, p = 0.328
	RECO	r = −0.181, p = 0.473	r = −0.163, p = 0.595	r = 0.15, p = 0.506
		MID-POST changes		
Energy intake	FEX	r = −0.086, p = 0.814	r = 0.121, p = 0.74	**r = −0.668, p = 0.035**
	RECO	**r = −0.560, p = 0.013**	r = 0.317, p = 0.269	r = −0.284, p = 0.254
Energy expenditure	FEX	**r = −0.512, p = 0.011**	r = 0.214, p = 0.326	r = −0.375, p = 0.071
	RECO	r = −0.165, p = 0.269	r = −0.238, p = 0.358	r = −0.236, p = 0.303
Energy balance	FEX	r = 0.43, p = 0.215	r = −0.275, p = 0.441	r = −0.25, p = 0.485
	RECO	r = −0.067, p = 0.784	r = 0.489, p = 0.076	r = −0.027, p = 0.916

4. Discussion

Energy balance was negative during survival training as a result of low energy intake and high energy expenditure. Changes in energy variables were associated with changes in leptin and testosterone/cortisol ratio, but not in physical performance. Energy and hormonal status were partially normalized during the 36-h recovery period.

A negative energy balance is a typical characteristic of survival training [6,27]. The magnitude of energy deficit depends on the duration of training, the amount of daily energy deficit, food intake, and total physical activity. In the PRE-measurement, participants lived in a garrison and prepared for the field training phase. Despite the conditions, energy balance was negative in PRE-measurement, probably as a result of biases in energy intake and expenditure estimation. Energy intake was analyzed via self-reported food diaries, so underestimation could at least partially explain the negative energy balance. The preparation included low-intensity tasks with long working hours, which shortened the sleeping time. It is notable that sleep deprivation increases energy expenditure, because wakefulness maintains energy metabolism activated, even in a habitual day [28]. This phenomenon was enhanced during the field training phase, where participants were not able to sleep for more than 1–3 h per day due to military tasks [20].

Energy intake differed at the POST-measurement, because the RECO group received normal meals in their accommodation. This led to differences in energy intake, expenditure and balance at the last measurement point. Although the RECO group should have had rest in their recovery period, their estimated energy expenditure stayed elevated (over 3500 kcal/day). They were instructed to do light activities to enhance their recovery, and sleeping times were normal. Energy expenditure values have been analyzed by heart rate variability measurements. This method has been reported to be valid in treadmill tests, compared to the golden standard method [18]. Hinde et al. [29] agreed that the HRV monitor is also accurate for military purposes, but some biases exist. When energy expenditure estimation is based on the heart rate, changes in the heart rate may interfere with the results. Resting and submaximal heart rate values may be elevated due to short-term overreaching symptoms after strenuous exercise [30]. In this case, survival training, with limited sleep and increased arousal time, may have elevated energy expenditure levels. Despite methodological inaccuracies, a negative energy balance during survival training was confirmed by a decrease in body mass from PRE to MID in both groups. An increase in body mass after the recovery phase in RECO was obvious, when more energy was consumed.

Strength and endurance performance were impaired after the survival training phase, but no associations were found in changes of energy intake, expenditure, or balance with changes in physical performance. However, the 20-m shuttle run test, which assessed endurance performance, differed between the groups in POST, as expected. Although associations with energy variables were not found, overall fatigue and lack of maximal effort may partially explain the difference between the groups and the decrease in aerobic capacity in the FEX group. Participants in the FEX group were exhausted after the survival training phase, and physical fitness tests in an indoor hall gave them the option to relax or even fall asleep between the tests.

An energy deficit exists in military conditions, but the associations with physical or military performance are unclear. We did not find any associations, which can be partially explained by the duration of fitness tests. Most of the tests were completed in a few seconds, which was mainly covered by the ATP and CP storages, and less energy was required from glycogen storages. That could also explain the difference in the 20-m shuttle run test, where the RECO group, in a fed state, could maintain its performance. However, the relation was not found with any of the energy variables. According to a meta-regression of Murphy et al. [3]., muscle performance was not related to a daily energy balance, but was proportional to the total energy balance, taking into account training duration and daily energy balance. O'Leary et al. [7] presented that decreases in muscle strength and power performance were observed in most military studies, but not all. Based on these results and previous literature, the role of energy deficit in physical performance cannot be identified yet. In military conditions, duration of trainings and the magnitude of energy deficit vary, as well as physical fitness test patterns. Furthermore, military tasks often cause neuromuscular fatigue, sleep deficit, and mental stress, which impair physical performance and energy-related factors simultaneously. The latest studies have measured energy availability in a military context, to clarify the energy needs of soldiers, and all of them indicate that soldiers do not reach the optimal level of energy availability [31,32]. For future research, estimating energy availability, together with energy balance, could provide important insights at the individual level, by advancing our understanding of energy metabolism in soldiers.

The shooting index was positively associated with energy balance in the FEX group in the latter part of the study. Group differences occurred in the prone shooting at all measurements, and also in the standing shooting at PRE. Shooting performance is a complex skill, where anthropometric, technical-coordinative, physiological, and psychological factors contribute to the shooting accuracy [33], not only energy-related factors. Overall and neuromuscular fatigue will affect the readiness and alertness of participants. Furthermore, shooting with an infra-red weapon may diminish the focus during shooting compared to real weapons in an outdoor shooting range. Previous literature indicates that shooting performance could be maintained in survival trainings, despite the overall stress of the training [34]. Additionally, sleep deficit can be eliminated with caffeine to improve sighting and triggering in shooting performance [35]. Ojanen et al. [16] found that prone shooting did not change in a 3-w military field training, but positive associations were observed between changes in strength of the lower- and upper-body with changes in standing shooting.

In our study, energy intake and expenditure were related to changes in leptin, ghrelin, and the T/C ratio. Leptin and ghrelin are appetite-mediating hormones, which react in fasted and fed states. Leptin concentration decreases in starvation, whereas hunger and appetite stimulate ghrelin levels [12]. The T/C ratio describes the relation of anabolic and catabolic hormone levels; thus, the ratio increases when the anabolic state is activated and vice versa [36]. Most of the results agree with existing literature [12,37], except for the inverse association between the change in energy intake and the change in T/C ratio. Physiologically, it is well known that if more energy is consumed, more anabolism is observed in the body. Our controversial finding demonstrates that the T/C ratio decreases when more energy has been given. This finding was from the FEX group during the last phase of the study, and in this situation, the overall stress may affect the participants. Therefore, despite the small increase in energy intake, the T/C ratio was impaired. Sleep deprivation can disturb the interpretation of these results, since restricted sleep (4 h) alone resulted in a decrease in leptin and an increase in ghrelin, without energy deficit [37]. Thus, more research is needed to differentiate stress factors and their contribution to military performance in harsh environments.

A 36-h recovery seemed to increase leptin and the T/C ratio at POST, indicating hormonal recovery. Values did not reach baseline levels, which agrees with previous studies, where total recovery was observed in 7 days [2,15]. Short-time recovery improved endurance performance and the strength index in the RECO group. Such a short and active recovery may be a potential model for maintaining military performance in long-term military exercises.

Some challenges exist in measuring the energy intake, energy expenditure, and body composition: an accurate method for energy expenditure estimation is expensive or time-consuming (e.g., doubly labeled water technique), and energy intake measurement by food diaries often include biases (i.e., underestimation, inaccuracies, and forgetfulness) [38]. Thus, inaccuracies in energy intake and expenditure estimation produce errors in energy balance values. Body composition assessment by bioimpedance analysis may overestimate fat-free mass [39]. Furthermore, most of these measurements should be carried out in a laboratory to get the most accurate data, but in military conditions, this may not be possible. For military purposes, it is valuable to get data from the field and from the unique study protocol.

5. Conclusions

An 8-d survival training caused a remarkable energy deficit and response in hormonal status, but energy intake, expenditure, or balance were not related to the physical performance of soldiers. A 36-h recovery restored the energy balance and hormonal status after strenuous military training, but these outcomes were not associated with strength or shooting performance. A short recovery can return the energy and hormonal balance to near-normal levels, which can yield a better performance of military tasks. Most recent studies in this area have examined the short-term effects of strenuous military training, but the long-term physiological responses of intermittent energy deficit and recovery in military deployment require further research [7].

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.

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