

**RELATIONSHIP BETWEEN PHYSICAL PERFORMANCE AND STRESS DURING
10-DAY WINTER SURVIVAL TRAINING IN SOLDIERS**

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Sotilaalta vaaditaan korkeaa resilienssiä, jotta sotilastehtävät voidaan suorittaa onnistuneesti operaatioissa ja sotaharjoituksissa. Yksi oleellinen osatekijä sotilaan resilienssiä on fyysinen suorituskyky. Tämän tutkimuksen tarkoitus oli tutkia muutoksia sotilaiden fyysisessä suorituskyvyssä, subjektiivisessa stressissä ja syljen biomarkkereissa 10 päivän talviselviytymisharjoituksen aikana. Lisäksi tutkittiin yhteyksiä sotilaiden fyysisen suorituskyvyn ja stressimarkkereiden välillä.

26 Suomen puolustusvoimien varusmiespalvelusta suorittavaa miespuolista sotilasta osallistui vapaaehtoisesti tutkimukseen (keskiarvo \pm keskihajonta: ikä 20 ± 1 vuotta; pituus 180 ± 7 cm; paino 75.4 ± 10.2 kg ja painoindeksi 23.4 ± 2.5 kg/ m²). Tutkimuksen aikana tutkittavat osallistuivat 10 päivän kestoiseen selviytymisharjoitukseen talviolosuhteissa. Ensimmäiset kaksi päivää harjoituksesta oli valmisteluvaihetta, jolloin harjoiteltiin erilaisia selviytymistaitoja. Tätä seurasi 7 päivän kenttävaihe, jossa tutkittavat suorittivat haastavia sotilas- ja selviytymisharjoituksia. Fyysisen suorituskyvyn testit suoritettiin ennen (PRE), aikana (MID1, MID2) ja jälkeen (POST) harjoitusjakson. Sylkinäytteet kerättiin päivittäin kello 08:00 ja 20:00, joista kortisoli ja alfa-amylaasi analysoitiin. Subjektiivista stressiä mitattiin NASA – Task Load Index kyselyllä, jonka tutkittavat täyttivät joka aamu. Lisäksi kehonkoostumus mitattiin fyysisen suorituskyvyn mittauspäivinä, ja energiankulutus sekä energiansaanti kaikilta harjoituspäiviltä.

Kehonpaino laski 2.8 ± 3.1 kg PRE arvosta POST arvoon ($p < 0.01$). Aamusta mitatun syljen kortisolin korkein arvo mitattiin päivänä neljä (43.2 ± 20.4 nmol/ L, $p < 0.001$), joka oli 131 ± 86 % nousu PRE arvoon verrattuna. Aamusta mitatun alfa-amylaasin korkein arvo mitattiin päivänä viisi (150 ± 101 u/ mL, $p < 0.05$), joka oli 239 ± 306 % nousu PRE arvoon verrattuna. Kaikki fyysisen suorituskyvyn testien tulokset, lukuun ottamatta alaraajojen isometristä maksimivoimaa, laskivat tilastollisesti merkitsevästi PRE arvosta POST arvoon ($p < 0.05$). Myös subjektiivinen stressi nousi 95 ± 22 % valmisteluvaiheesta kenttävaiheeseen ($p < 0.01$). Korrelaatioanalyysi paljasti tilastollisesti merkitsevän käänteisen yhteyden ennen harjoitusta mitatun alaraajojen isometrisen maksimivoiman ja harjoituksen aikana mitatun kortisolin aamuarvon muutoksen välillä ($p < 0.001$, $r = 0.61$). Regressioanalyysi paljasti, että ennen harjoitusta mitattu yläraajojen isometrinen maksimivoima ennusti 14.6 % subjektiivisen stressin muutoksesta harjoituksen aikana ($p < 0.05$). Tämän tutkimuksen päälöydökset olivat, että 10 päivän talviselviytymisharjoitus aiheuttaa useita muutoksia sotilaan toimintakyvyssä, jotka ovat nähtävissä heikentyneenä fyysisenä suorituskykynä sekä syljen kortisolin ja alfa-amylaasin pitoisuuden nousuna. Lisäksi subjektiivisen stressin, NASA- TLX kyselyllä mitattuna, voidaan nähdä nousevan harjoituksen aikana. Tulosten perusteella erityisesti riittävä ala- ja yläraajojen maksimivoima on tärkeässä roolissa ehkäisemään talviselviytymisharjoituksesta aiheutuvaa kortisolin nousua ja subjektiivista stressiä.

Asiasanat: selviytymisharjoitus, armeija, resilienssi, syljen kortisoli, syljen alfa-amylaasi, maksimivoima, fyysinen suorituskyky

ABSTRACT

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To successfully execute missions and operate under demanding circumstances, the soldier needs high resilience. Physical characteristics are a major component of the resilience of a soldier. The purpose of the present study was to examine changes in soldiers' physical performance, subjective stress, and saliva biomarkers during a 10-day winter survival training. In addition, the present study investigated the relationship between soldiers' physical performance and stress markers during this training phase.

26 Finnish Army male soldiers, who performed their compulsory military service, participated in the study (mean \pm SD: age 20 ± 1 y; height 180 ± 7 cm; weight 75.4 ± 10.2 kg and BMI 23.4 ± 2.5 kg/ m²). During the study, the participants went through the 10-day survival training. The first two days consisted of the preparation phase including basic survival skill training. It was followed by the field phase which included military and survival tasks. Physical tests were done before (PRE), during (MID1, MID2), and after (POST) the survival training period. Saliva samples were collected daily at 08:00 and 20:00 from which cortisol and alpha-amylase were analyzed. The subjective stress was measured with NASA - Task Load Index questionnaire which subjects filled every morning. In addition, body composition, energy expenditure, and energy intake were measured.

The body mass decreased 2.8 ± 3.1 kg from PRE to POST ($p < 0.01$). The morning saliva cortisol peak value increased 131 ± 86 % from PRE and was measured on day 4 (43.2 ± 20.4 nmol/ L, $p < 0.001$). The morning saliva alpha-amylase peak value increased 239 ± 306 % and was measured on day 5 (150 ± 101 u/ mL, $p < 0.05$). There was a significant decrease in all physical fitness tests from PRE to POST ($p < 0.05$) except for the maximal isometric strength of the lower extremities. Also, the subjective stress increased 95 ± 22 % from the preparation phase to the field phase ($p < 0.01$). The correlation analysis showed a statistically significant inverse relationship between the maximal isometric force of the lower extremities before the training period and cortisol change during the training period ($p < 0.001$, $r = 0.61$). The regression analysis revealed that maximal isometric strength of the upper extremities before training predicted 14.6 % of the change in subjective stress ($p < 0.05$). The primary findings of this study were that 10-day winter survival training led to a decrease in physical performance characteristics and an increase in saliva cortisol and alpha-amylase. In addition to these, there was a significant increase in subjective stress measured by the NASA-TLX questionnaire from the preparation phase to the field phase. Based on the present study, the lower- and upper-body maximal strength plays an essential role to prevent stress-induced cortisol increase and subjective stress during survival training.

Keywords: Survival training, military, resilience, saliva cortisol, saliva alpha-amylase, maximal strength, physical performance

ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
AMPK	AMP-activated kinase
CBG	Cortisol binding globulin
CRH	Corticotropin-releasing hormone
GnRH	gonadotropin-releasing hormone
HPA axis	Hypothalamic-pituitary-adrenal axis
HPG axis	Hypothalamic-pituitary-gonadal axis
LC/ NE	Locus coeruleus-noradrenergic system
mC2R	Melanocortin 2 receptor
mTOR	Mammalian target of rapamycin
NPY	Neuropeptide-Y
SAM axis	Sympathetic -adreno-medullar axis
VO ₂ max	Maximal oxygen uptake

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ABSTRACT

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1 INTRODUCTION

To successfully execute missions and operate under demanding circumstances, soldiers need to have a high level of resilience. Defining resilience is not unambiguous because there is a certain difference of views between science communities. In the army context, we can define resilience as the mental, physical, emotional, and behavioral ability to adapt and cope with stressors and adversities (Szivak & Kraemer 2015). Especially physical requirements of the soldier are increasing due to changing nature of battlefields. Nowadays the battlefield requires soldiers to carry heavy equipment under intense melee conditions and tasks usually have a high anaerobic demand such as running with a heavy load or carrying the wounded off from the battlefield. (Nindl et al. 2013.) Understanding how physical fitness and resilience affect each other can give practical tools to evaluate soldiers' cumulated fatigue during training and missions.

The physical performance of the soldier could be divided into strength, power, and endurance characteristics together with motor coordination. Optimal physical performance creates the ability to physically survive and successfully execute tasks on the battlefield. (Sharp et al. 2009.) During military training and missions, physical performance is challenged. Previous studies have shown a radical decline in soldiers' strength levels after military field training lasting from a few days to weeks. (Chester et al. 2013; Hamarsland et al. 2018; Nindl et al. 1997.) It seems that there are also relationships between strength levels and immunological changes. Upper body strength can have a relationship with relative changes in the serum IGF-1 concentration and creatine kinase activity (Ojanen et al. 2018).

Operational missions and military training are often lasting for prolonged periods. Thus, there is also a need for sufficient aerobic and anaerobic performance. Military training together with energy deficit has been found to decrease $VO_2\text{max}$ due to a decline in body mass. Because the energy deficit is part of the training and cannot be eliminated, there is a need to make sure that the soldier's $VO_2\text{max}$ is high enough that partial decline won't significantly affect the soldier's operational performance. (Henning et al. 2011.)

Other physical characteristics like height, vision, detection capability, multitasking capability, anthropometry, speed, and motor skills influence performance in different occupational tasks (Maavoimien sotilaan toimintakykyvaatimukset 2019). These should be also noted when evaluating factors that can affect a soldier's ability to execute the task.

Resilience is challenged under stressful military circumstances. The stress response can be acute or chronic. It starts when a soldier is exposed to a stimulus or an event that is perceived as threatening. The internal physiological responses of the body will occur to adapt and function effectively under the new challenging situation. This is called fight-or-flight response which is automatically turned on when a stressor is faced. Most of these physiological reactions result from the activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic-adrenomedullar (SAM) axis (Kavanagh 2005, Atkinson & Hilgard 2009.)

The purpose of the present study was to examine changes in soldiers' physical performance and total stress during 10-day survival training. In addition, this study investigated the relationship between soldiers' physical performance and stress during this training phase. Review of the literature focus to cover characteristics of the soldier's physical performance and how different stress factors influence on soldier's ability to execute missions. In addition, human physiology under acute and chronic stress will be covered.

2 PHYSICAL PERFORMANCE OF THE SOLDIER

The physical performance during military training and operations is challenged due to many different factors such as high physical strain, restricted energy intake, insufficient sleep, psychological burden, and environmental extremes (Szivak 2018; Ojanen et al. 2018; Henning et al. 2011). The combination of these stressors can cause both physiological and psychological impairments increasing the risk to fail the mission (Henning et al. 2011). Physically fit soldiers have an advantage in the face of stressors not only because of improved mission performance but also because physical performance has implications for psychological factors such as mental health and the ability to cope with stress (Flanagan et al. 2012; Silverman et al. 2014; Tsatsoulis & Fountoulakis 2006). In addition, physically fit soldiers are less susceptible to injuries and illness (Silverman et al. 2014). Because of these mentioned factors, it is important to understand physiological mechanisms of strength, power, and endurance and how to improve these characteristics.

2.1 Strength, power, and injury prevention

The power and strength production of the neuromuscular system and its adaptations are based on neural and morphological factors. Morphological adaptations to strength training are based on the increased cross-sectional area of the muscle cells. In addition, morphological adaptations include changes at the cellular level such as activation of satellite cells and conversion of myosin isoforms in type II muscle cells, especially to IIA isoforms. (Folland & Williams 2007.) Neural adaptations to strength development are based on the improved coordination of agonist and antagonist muscles, increased motor unit recruitment, increased motor unit firing frequency, and decreased activity of inhibitory neurons. (Fleck & Kraemer 2014, 101–108).

Strength development should be considered important for a soldier's performance as high strength and power levels allow more efficient performance in heavy and long-lasting exercises. Nindl et al. (2013) reported how the weight of soldiers' equipment has increased over the past few decades and today's loads can vary from 28.7 to 60 kg depending on the mission. At the same time tasks have usually high anaerobic demands such as running with loads or carrying

the wounded off the battlefield (Nindl et al. 2013). It is crucial to make sure that strength and power levels in soldiers are high enough to execute these tasks. Sufficient strength levels also help to cope with psychological stress in a combat situation when there is less physical exertion. (Szivak & Kraemer 2015). These high demands of anaerobic work capacity with heavy carriage should be noted in the military training. An excessive amount of aerobic endurance training does not give positive adaptation for type II muscle cells which are the main producers of high power and strength output. Further, the typical bodyweight exercises are not optimal to stimulate the development of strength and hypertrophy. (Mala et al. 2015.) Figure 1 shows the basic idea of the “size principle”. The intensity of exercise impacts the level of motor unit recruitment. To optimize strength and power development, there must be exercises with high loads. (Kraemer & Szivak 2012.)

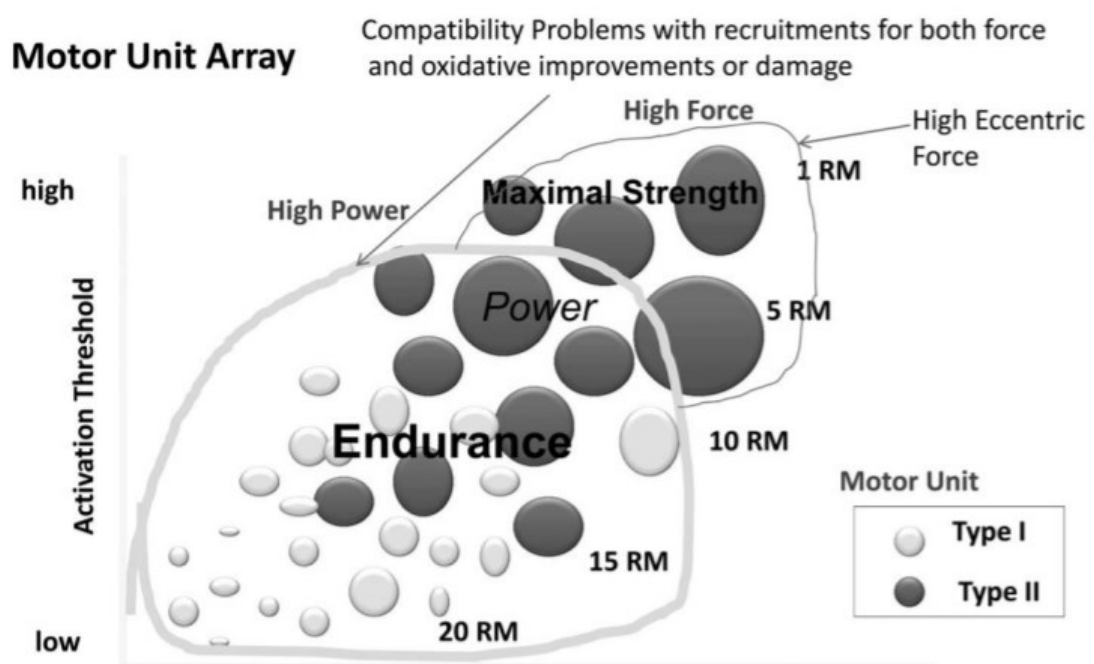


FIGURE 1. Circles represent different types and sizes of motor units. High force output is required to activate the largest type II cells (Kraemer & Szivak 2012.)

Optimal strength training can also prevent different injuries during military training. These injury-preventing mechanisms are based on strength training-related carryover effect with improved coordination, enhanced techniques (for example lifting things), strengthened

connective tissues reducing joint loads, and better psychological perception of high-risk situations (Bahr & Krosshaug 2005, Myer et al. 2005). Jones & Hauschild (2015) found that male soldiers who scored the lowest points in fitness tests were 1,6 times more likely to be injured compared to highest scored soldiers. In female soldiers, injury risks were 1,4 times higher in those who scored the lowest points (Jones & Hauschild 2015). Although, the evidence is not clear that higher strength levels could prevent injuries in military training. For example, Jones et al. (1993) found no correlation between strength levels and reported injuries during 12 weeks of military training. However, most of the studies done with athletes have found positive effects of strength training for injury prevention (Lauersen et al. 2018.).

Typical military training alone is not enough to gain strength and power. That is why programmed strength training should be added weekly to part of the physical training. (Groeller et al. 2015). Programming strength training includes manipulating different training variables such as intensity and volume (Toigo & Boutellier 2006). A large part of military training is endurance type of activity, which can have an interference effect on muscle strength and power adaptations (Hawley 2009). Vaara et al. (2015) studied the effects of added resistance training during 8 weeks of military training. The resistance training- group performed two resistance training sessions per week. They did not find improvements in strength levels. The possible reason is that a high amount of overall aerobic activities impaired strength gains. Also, the volume of strength training was possibly too low to see improvements in a short time (Vaara et al. 2015.) Interestingly, there is also conflicting results that low volume strength training (60min/ week) in military conscripts (n=290) can have positive adaptations in strength levels even if weekly endurance type of activity is high (20 hours/week) (Kilen et al. 2020).

Task-specific explosive strength training can also be used. Ojanen et al. (2020) studied strength and power adaptations when training with a traditional strength training program or with a task-specific explosive training program during military activity. Task-specific training included infantry-based exercises with the 27kg combat gear. Both groups increased significantly maximal isometric knee extension force during the 12-week training period. (Ojanen et al. 2020.)

One topic of interest is to know how soldiers' strength and power levels change during and after military survival training. Previous studies have shown a radical decline in soldiers' strength levels after different military field training practices lasting from a few days to weeks. Chester et al. (2013) observed changes in soldiers' performance after 15 days of survival-simulation training where they found a 10 % decrease in vertical jump height and an 8 % reduction in body mass after the training period. Hamarsland et al. (2018) measured a 20 % and 9 % decrease in lower- and upper-body strength and a 28 % decrease in jump height after 1 week of the Norwegian Special Forces selection course.

Ojanen et al. (2018) has also found interesting results while observing physical, hormonal, and immunological changes during prolonged (22-day) military field training. They found an association between the number of push-ups performed in 60 seconds and relative changes in the serum IGF-1 concentration and creatine kinase activity. These results showed a connection between upper body strength and physical strain during prolonged military field training. There was also a significant decline in body mass between PRE and POST. (Ojanen et al. 2018.) It has been shown that loss of body mass can affect especially the strength of the lower limbs which can be critical for the ability to move fast under heavy load (Montain & Young 2003). Thus, it is important that soldiers' physical training includes strength training for succeeding well in the different tasks and preventing negative physiological effects (Ojanen et al. 2018). The strength and power development also have an impact on submaximal high-intensity endurance performance which is important for a soldier on today's battlefield (Kraemer & Szivak 2012).

2.2 Aerobic and anaerobic fitness

Endurance performance can be determined as the ability to resist fatigue (Zatsiorsky & Kraemer 2006, 162). There are three main characteristics for endurance – maximal oxygen consumption (VO_{2max}), efficiency and lactate threshold. Maximal oxygen consumption and lactate threshold determine the level of oxygen consumption that can be sustained for an exact period. Efficiency determines the oxygen consumption level at a certain speed or power output. (Joyner & Coyle 2007.)

Depending on the duration and intensity of physical activity, different energy transfer systems are activated (McArdle et al. 2010, 459). The anaerobic energy system is predominant during short high-intensity work. It is divided into alactic and lactic components. Most of the energy is produced by anaerobic pathways when maximal intensity exercise is lasting up to 60 seconds. When intensity decreases and the duration of work is extending to 2 to 4 minutes, aerobic energy production becomes more important. The aerobic energy system has an enormous capacity to produce energy from carbohydrates and fats in the presence of oxygen and it is the predominant energy transfer system when the duration of activity is over 3 minutes. In prolonged exercises – like marching long distances – the aerobic metabolism generates more than 99% of the energy requirements. (Gastin 2001; McArdle et al. 2010, 459.)

Maximal oxygen consumption ($VO_2\text{max}$) is typically the main index of cardiorespiratory function and aerobic capacity. It depends on the capacity of the cardiovascular system to transport oxygen to working muscles and the efficiency of muscle cells to use that oxygen for energy generation. (Tecklin 2004.) The main physiological training adaptations of $VO_2\text{max}$ are enhancement of cardiac output, greater oxygen-carrying capacity, and arterial-venous oxygen differential. Increased cardiac output is based on the enhanced capacity of ventricular filling and the increased efficiency of the Frank-Starling mechanism during exercise. Greater oxygen-carrying capacity and arterial-venous oxygen differential are adaptations to increased blood hypervolemia and hemoglobin content. (Warburton & Bredin 2012; Stegemann 1981; Ekblom et al. 1968.) Aerobic exercise also causes several other adaptations. For example, increased mitochondrial biogenesis, angiogenesis, work economy, musculotendinous resiliency, bone mineral density, and endocrine downstream actions (Hackney 2019).

Because the nature of today's battlefield has changed from being dominated by being mostly aerobic to an anaerobic battlefield, the importance of anaerobic capacity has raised to be an important characteristic of a soldier's performance (Mala et al. 2015). Increased anaerobic capacity is based on increased levels of anaerobic substrates, enzymes, and increased capacity to produce higher levels of blood lactate (McArdle et al. 2010, 465). Endurance exercise training also shifts the lactate threshold to a higher exercise intensity which is largely adaptation of increased mitochondrial biogenesis coupled with improved oxygen delivery capacity (Hackney 2016).

Military training should be periodized in a way that both – aerobic and anaerobic – pathways would enhance leading to increased performance in soldiers. Studies have shown declined physical performance and increased body mass in young men entering the military in western countries. (Santtila et al. 2006; Knapik et al. 2017). Professional soldiers' performance during military operations has also been shown to decrease. Sharp et al. (2009) studied changes in performance during 13 months of operation in U.S military soldiers. Especially maintaining aerobic fitness level seems challenging during operation. They found an increase in upper- and lower body strength but a decrease in aerobic fitness level and an increase in body fat. (Sharp et al. 2009.)

The programming of military training should take into account these challenges and include both low- and high-intensity training to optimize soldiers' endurance characteristics. Santtila et al. (2010) showed that Finnish 5,5 months lasting infantry training includes approximately 400 hours of low-intensity aerobic work. High volumes of low-intensity aerobic training have a negative effect on explosive strength gains and can increase injury risks so training should be periodized carefully. Adding more high-intensity training could be a beneficial option for this. (Friedl et al. 2015; Hawley 2009.) Studies have found a positive result with moderate to high-intensity endurance training for increasing VO_{2max} for soldiers. Grant et al. (2017) found that moderate and high-intensity military training improved VO_{2max} during the first 12 weeks and decreased 2.4 km running time significantly.

2.3 Combined strength and endurance

Military occupational tasks usually require both endurance and strength characteristics. Because of this, it would seem reasonable to implement combined strength and endurance training. However, concurrent endurance and strength training over an extended period can attenuate strength and power development (Hickson 1980; Wilson et al. 2012). This attenuating effect from the concurrent training has been described as the interference effect (Doma & Deakin 2014). Studies have shown that the interference effect is concerning only strength and power gains. For endurance performance, concurrent training can result in even better adaptations compared to only endurance training. (Berryman et al. 2010.) The interference

effect is caused by the activation and deactivation of specific cellular signaling pathways (Hickson 1980; Baar 2014). The main signaling pathways which are responsible for the interference effect are mTORC1 and AMPK, of which mTORC1 is activated by mechanical stimuli of strength training and AMPK by endurance type of training. Interestingly fasting, immobilization, shifts in redox state, and aging are also activating AMPK and inhibiting mTORC1 leading to decreased muscle hypertrophy and strength adaptations. (Baar & Ellefsen 2019.) In the military environment especially the influence of fasting should be noted if the goal is to increase strength and power.

It is important to mention, that the impact of the interference effect is not entirely clear. Like mentioned earlier, there are contradicting findings of the interference effect and military training. Wilson et al. (2012) concluded that concurrent training can have a negative impact on power output but can still lead to positive strength and muscle mass adaptations. Kilen et al. (2020) showed that low volume strength training (60min/ week) combined with endurance training (60min/ week) can have positive adaptations in strength levels even if the weekly military activity is high (20 hours/ week). The possible explanation for contradicting findings might lie in the total volume of training. The clearest evidence of the interference effect has been found when strength and endurance exercises are performed in the same workout and over six times per week at high intensities (Hickson 1980, Kraemer 1995.).

To prevent these negative outcomes from the combined strength and endurance training, block-periodized training could be a good option. The block-periodization provides the possibility to focus on specific adaptations and prevent overreaching and injuries. Abt et al. (2016) studied the effect of block-periodized training consisting of three different 4-week blocks. The blocks were divided into 1) aerobic training, muscular strength, and coordination training block, 2) power and strength training and mixed endurance training, 3) power, strength, and high-intensity training. They found a significant increase in aerobic capacity, upper body muscular endurance, and total body strength. Body composition changes were also positive. (Abt et al. 2016.)

2.4 Motor skills & speed

Specific skills for the soldier such as agility to move effectively and use the gun quickly require physical characteristics like speed, motor skills, and multitasking. Understanding how stressors like pressure under mission or coldness can affect these characteristics is highly important. (Maavoimien sotilaan toimintakykyvaatimukset 2019.)

Adaptations to motor skill training are based on plasticity in the motor cortex and spinal cord including basal ganglia, cerebellum, and red nucleus. Learning a new skill increase the number of synapses in the specific area of the motor cortex. (Adkins et al. 2006.) A soldier's tactical performance highly depends on his or her motor skills, speed, and agility characteristics. The time is usually limited in intense combat situations and the soldier must react and move quickly often in life-threatening situations. The ability to handle a weapon fast and shoot under pressure is a vital motor skill. (Joseph et al. 2018.)

Challenges increase when the soldier must carry a heavy load during these high-intensity movements. (Joseph et al. 2018.) The fighting load can vary from 25 to 37 kg and effective moves including reaction to contact, pushing, and pulling object, and maneuvering quickly with load requires strength, endurance, motor skills, agility, and speed. (Mala 2015; Joseph et al. 2018.) It is critical to understand that repeated high-intensity bouts impair the ability to maintain speed and agility during tasks (Girard 2011). Developing these skills and maintaining good strength and aerobic fitness levels prevents failing in these tasks (Tomczak 2015). The temperature can also affect the ability to successfully perform different motor skills. Oksa et al. (2006) showed how weapon handling skills decrease in a cold environment. A coldness decreases the temperature of working muscles leading to a decrease in fine motor skills. They found that training specific motor skills like weapon handling in warm and cold environments was beneficial to improve weapon handling in a cold environment. (Oksa et al. 2006.)

Agility and speed are also essential for soldiers. Adding agility training to besides traditional military physical training can have superior results compared to only traditional military physical training. Lennemann et al. (2013) found that adding agility training improved agility-

specific test results, visual vigilance, and continuous memory significantly whereas traditional training – which includes running and calisthenics – did not. These positive results, not just for physical performance, but also for cognitive performance are promising (Lennemann et al. 2013).

3 ENVIRONMENTAL CHALLENGES FOR THE SOLDIER

The most important goal in military training is to prepare soldiers to face different physiological and psychological factors of the battlefield. For example, energy deficit, sleep deprivation, equipment, and other load carriage, environmental factors (cold, heat, and altitude), medical illnesses, injuries, post-traumatic stress disorder, and environmental exposure to different hazards are all significant risk factors for failing the task. (Nindl 2013; Liebermann 2016.) All these factors can influence soldiers' performance in many ways like decreased endurance and strength levels, changes in working memory, decreased body mass, increased depression, and degradations in decision making (Liebermann 2016). Figure 2 shows the primary factors that should be noted when evaluating soldiers' performance (Kyröläinen et al. 2018).

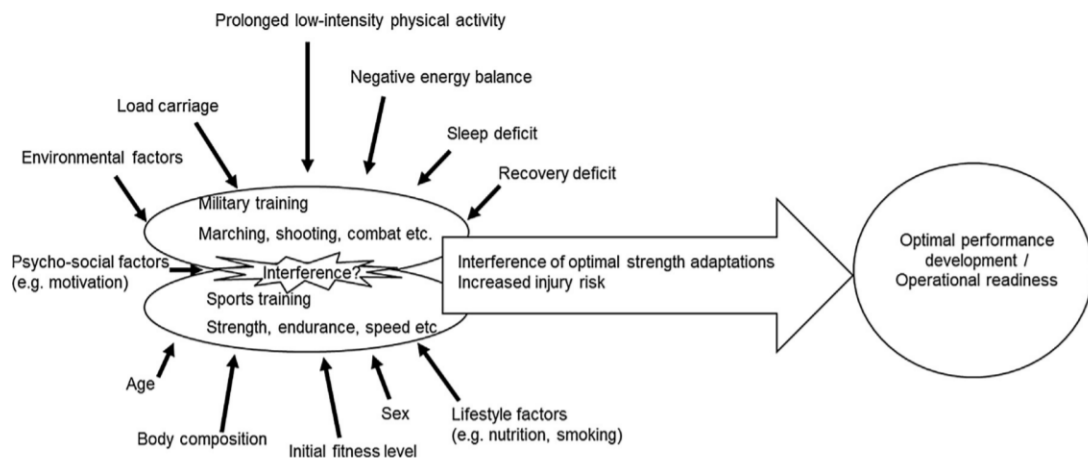


FIGURE 2. Different factors influence on soldier's performance (Kyröläinen et al. 2018).

3.1 Physical strain

High-prolonged physical activity on the battlefield, usually combined with energy deficit and sleep deprivation, creates the possibility of acute fatigue, overreaching, or even overtraining (Ojanen 2018). During operations, soldiers' physical activity is typically consisting a high amount of low-intensity work including temporal high-intensity periods (Henning et al. 2011). Pihlainen et al. (2014) studied cardiorespiratory responses in different military tasks during the military field training. They found that unloaded 4.8 km march increased VO_2 to 19.9 ± 2.7 mL/

kg/ min and average energy expenditure to 429 ± 38 kcal/ h. The respective results with loaded 13.3 km march with full combat gear (24.4kg) were 22.7 ± 3.4 mL/ kg/ min and 483 ± 61 kcal/ h. Total variation in VO_2 was between 18 and 24 mL/ kg/ min during different tasks. It was concluded that the minimum requirement of VO_{2max} is 45 to 50 mL/ kg/ min to keep that intensity for a prolonged period. (Pihlainen et al. 2014.)

Ojanen et al. (2018) found that during prolonged 22-day military field training, the average daily activity was 12165 ± 2381 steps per day and the average Borg scale of RPE was 9 ± 2 . The moderate physical activity average (MET 3-6) was 2:12:00 in the first phase and 2:48:00 during the second phase of the field training. Other changes were decreased body weight (-2.3%), increased IGF-1 (22%) and leptin (66%), and CK (88%). These results showed that prolonged low-intensity work periods during field training can impact significantly different markers of stress. Energy intake and sleep have an impact also, however, in this study decrease in weight was evaluated to be mostly from dehydration and the average sleep time was 6 hours. (Ojanen et al. 2018.)

The consequences of physical strain are depending on the intensity and duration of the training period. As found, prolonged low-intensity training can influence different stress markers (Ojanen et al. 2018). Military survival training is another field training practice that prepares soldiers to survive on the ground and increase their stress tolerance capacity (Liebermann et al. 2016). Tomczak (2015) studied the effects of survival training with military pilots of the Polish army. Survival training consisted of mountain climbing, transport of wounded companions, constructing shelters, a night march on azimuth, and crossing a rope bridge. The training lasted 36 hours with restricted sleep (2-3 hours total). The estimated total energy expenditure from that time was 8600 kcal, and the physical activity was high during the training. There was a 6% decrease in handgrip strength but an increase in the divided attention test. Also, 15-m sprint time decreased after the survival training. The author concluded that the total stress load that subjects went through was tolerable. Thus, the acute increase in physical demands seems not to decrease performance significantly after the training period even with restricted sleep and energy intake. (Tomczak 2015.) Although, other survival training studies have found a larger decrease in performance and changes in hormonal markers after military survival training. Chester et al. (2013) observed significant changes after 15 days of survival-simulation training

likewise Hamarsland et al. (2018) after 1 week of the Norwegian Special Forces selection course. It seems that these studies were long enough to cause changes in performance and hormonal markers. Although the duration of the training period is a major component, the role of intensity in the survival training should not be forgotten. For example, Rintamäki et al. (2005) did not find a significant decrease in soldiers muscular and cardiorespiratory performance after 12-day winter military field training which might be due to lower intensity compared to Chester et al. (2013) and Hamarsland et al. (2018) studies.

The intensity of the physical strain in survival training can be determined more clearly if it is compared to normal soldiers' physical activity. McAdam et al. (2018) studied the physical activity of the army's initial entry training phase. The estimated daily total energy expenditure was on average 3238 kcal. Metabolic equivalents were divided into 4 categories: light (=2 METs), moderate (3-5.99 METs), vigorous (6-8.99 METs), and very vigorous (>9 METs). The results are shown in figure 3. The average steps per day were 13569±5197. (McAdam et al. 2018.) The physical activity results from Finnish military field training (Ojanen et al. 2018) and army initial entry training phase (McAdam et al. 2018; Knapik et al. 2007) are very similar. These results can be considered as average baseline values of soldiers' physical activity.

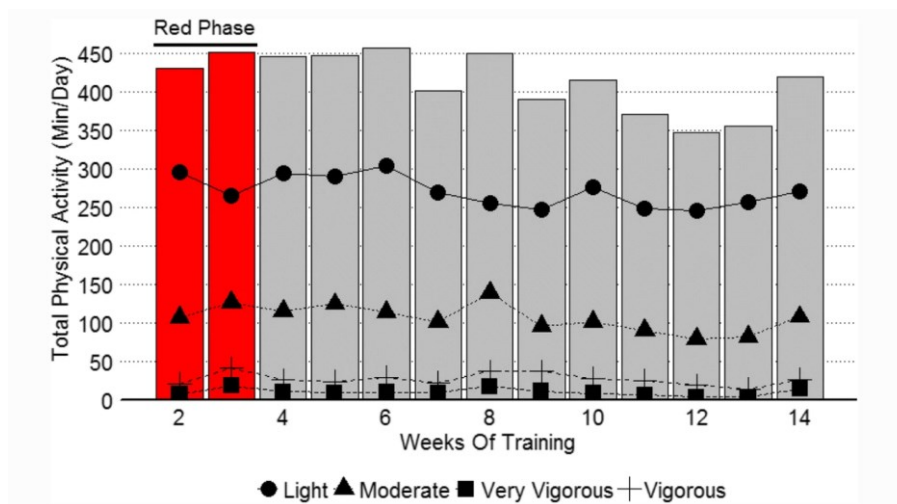


FIGURE 3. Total physical activity during 14 weeks initial entry training phase. Data is presented in minutes per day on average. The red phase describes the period where energy balance was also estimated. The average energy balance during the red phase was -595 ± 896 kcal/ day. (McAdam et al. 2018.)

3.2 Energy deficit

One typical stress factor during military operations and training is inadequate energy intake. Typical military training consists of mainly low- to moderate-intensity activity under moderate to heavy loads and restricted calorie intake due to environmental factors which can also limit the desire to eat (Murphy et al. 2018; Nindl et al. 2013). Intense field training can increase the need for energy intake even 5000-7000 kcal/ day (Kyröläinen et al. 2008). Because of the nature of survival training, the energy deficit is usually high throughout the training period.

Friedl et al. (2000) studied endocrine markers of semistarvation during the 8-week US Army Ranger course which also includes other stressors like sustained workload, restricted sleep, and thermal strain. The group had four 7- to 10-day bouts in energy restriction (average 1000-1200 kcal deficit per day). After every energy restriction period was refeeding period. The control group's energy intake was 400 kcal higher. After 8 weeks serum testosterone and IGF-1 decreased significantly and were below normal levels (testosterone 4.5 ± 3.9 nmol/ L, IGF-1 75 ± 25 µg/l). Refeeding produced fast recovery of testosterone and IGF-1, even when other stress factors continued. The author concluded that total testosterone and IGF-1 are reliable markers of acute energy deficit. (Friedl et al. 2000.)

The impact of an energy deficit on physical performance seems to cause only minimal effect. Zachwieja et al. (2001) found that 750 calories daily energy deficit did not affect strength (1RM test on leg press and shoulder press), muscle endurance (squats to failure, 5-mile run), or anaerobic capacity (Wingate test) after 2 weeks. Gutierrez et al. (2001) studied how 3 days of fasting impair physical performance. Fasting did not impair strength levels or perception-reaction time but decreased performance on maximal cycling-test called PWC₁₇₀ (Gutierrez et al. 2001). It seems that decreased performance during prolonged energy deficit is attributed to a reduction in fat-free mass (Murphy et al. 2018). Studies have concluded that less than 10% loss in body mass did not impair muscle strength and VO_{2max} (Taylor et al. 1957; Friedl 1995).

Negative protein balance increases the degradation of muscle mass leading to decreased lean body mass. The primary regulator of whole-body protein balance is energy status. Margolis et al. (2014) showed that whole-body protein turnover was 24% higher when the energy deficit increased from 2382 kcal/ day to 3390 kcal/ day. Net protein balance also decreased from -0.42 g/ kg/ day to -1.41 g/ kg/ day (Margolis et al. 2014). Increasing protein intake during severe energy deficit state might minimize lean body mass loss during the training (Pikosky et al. 2008).

3.3 Sleep

The insufficient amount of sleep during military operations can impair both physical and physiological performance. There are both acute and chronic physiological responses to restricted sleep or sleep deprivation. Factors that can affect sleep in the military environment are shown in figure 4. (Williams et al. 2014.)

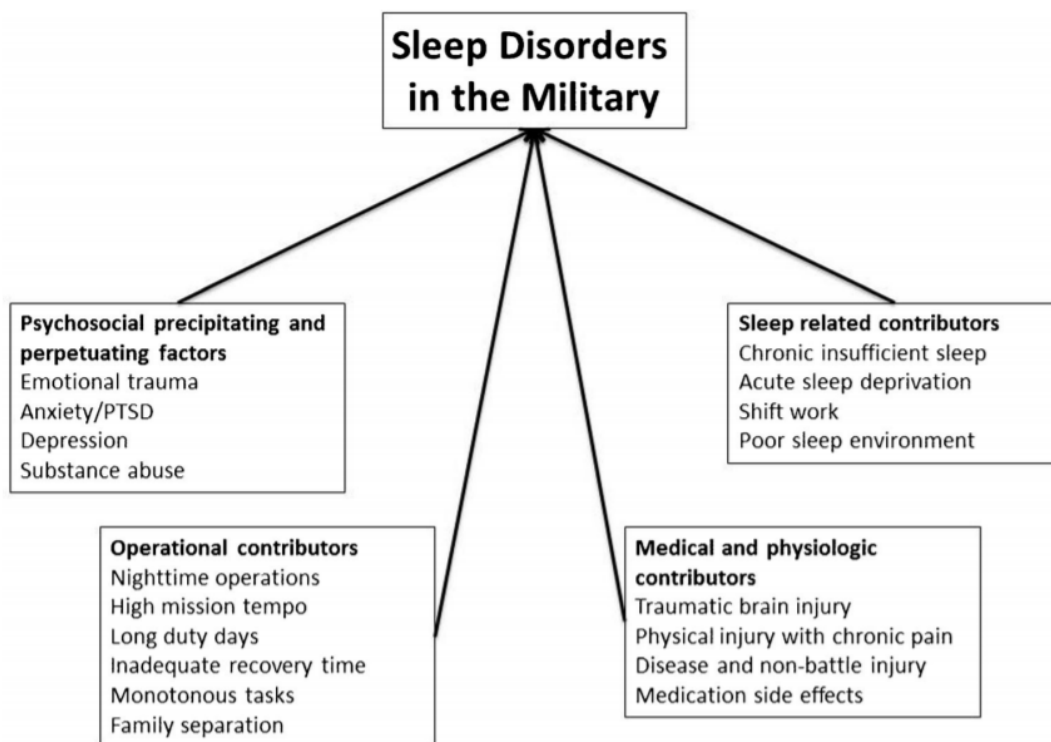


FIGURE 4. Factors related to sleep problems in the military and operational environment (Williams et al. 2014).

Vaara et al. (2009) studied the effect of 60 hours of sleep deprivation without physical activity in 20 Finnish cadets. During the period, body temperature and heart rate decreased significantly. Sleep deprivation has been shown to decrease brain activity which can lead to decreased body temperature and heart rate (Vaara et al. 2009). Sleep deprivation also declines testosterone and growth hormone levels and increases cortisol levels (Leproult & Cauter 2011; Spiegel et al. 2000).

Although sleep deprivation has effects on physiological markers, it seems that acute sleep deprivation does not affect maximal physical performance. Vaara et al. (2009) found no changes in maximal strength of the knee extensor muscles after 60 hours of sleep deprivation. Only cardiorespiratory response on submaximal workload seems to be impaired after sleep deprivation. (Vaara et al. 2009.) However, sleep deprivation seems to affect cognitive performance, especially attention and psychomotor vigilance and some behavioral responses (Killgore 2010). Skurvydas et al. (2019) studied cognitive changes in 30 young males after one night of sleep deprivation. They found weakened psychological well-being and cognitive executive function but no changes in simple reaction time, handgrip strength, or countermovement jump (Skurvydas et al. 2019).

In military field training, sleep restriction can last from days to weeks. When sleep restriction continues for days, the brain seems to adapt to restricted sleep if the sleep restriction is mild or moderate. When comparing effects of 7-, 5- and 3-hour sleep per night for 7 days, the cognitive performance declined steadily across the period in the 3-hour group but in the 5- and 7-hour groups cognitive performance decline first few days but then attenuated at a reduced level. (Belenky et al. 2003.) Those degradations in cognitive performance might be even larger in real-world situations where other stress factors are also related (Liebermann et al. 2016). Larsen (2001) found that after five days of almost complete sleep restriction during Norwegian military training, the ability to reason and make effective decisions was reduced significantly (n=62). They were told to fire humanoid dummies with real ammunition. 59 % of the subjects fired their weapon even when the target turned out to be human. 41 % of the subjects did not fire but only one of them tried to warn the others to stop firing. Severe sleep deprivation combined with strong pressure and stress seems to degrade thinking processes strongly. During the operations, this lack of cognitive performance can lead to friendly-fire incidents at worst. (Larsen 2001.)

3.4 Subjective stress

Unpredictable and ambiguous situations are part of today's military operations where the soldier is exposed to violence and threats which will lead to an acute stress reaction. To survive and execute a mission successfully soldier must have a high stress tolerance capacity. High stress tolerance helps to operate under pressure, not letting emotional and physiological stress reactions interfere with cognitive processing. (Delahajj et al. 2011.) Military survival training aims to simulate these real-world scenarios exposing soldiers to high physiological and psychological stress helping to improve stress tolerance (Vaara et al. 2020; Liebermann et al. 2016). Figure 5 shows the relationship between stress factors and how they can impact performance (Kavanagh 2005).

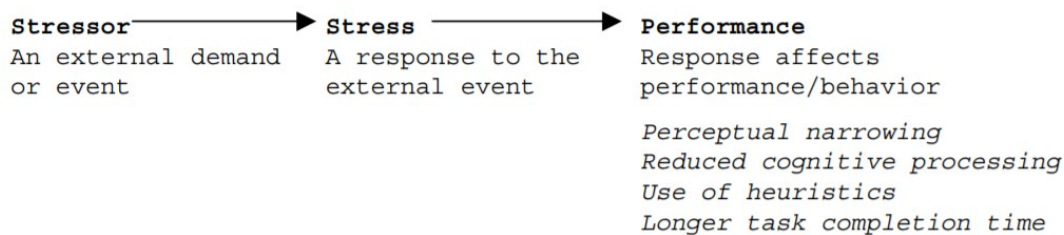


FIGURE 5. The relationship between stress factors and performance (Kavanagh 2005).

Different survival training practices are designed to increase soldiers' stress tolerance. In the Survival, Evasion, Resistance, and Escape training (SERE) soldiers are faced with a wide array of psychological and physiological stressors. It starts with survival and evasion field practice where soldiers must navigate through hostile territory and evade enemy forces. The second phase includes the capture phase where soldiers go through stressful mock interrogations. This phase is extremely demanding psychologically. (Liebermann et al. 2016.) The one main factor increasing psychological stress is a feeling of lack of control. This will elicit negative emotional reactions like fear and anxiety. The soldier must be ready to tolerate stress in situations where the lack of control is strongly present, for example, in captivity or interrogations. (Delahajj et al. 2011.)

Liebermann et al. (2016) studied the psychological responses during SERE training in the U.S Army. They found that highly psychologically stressful simulated captivity and interrogation phase degraded cognitive functions (grammatical reasoning, sustained attention, and working memory) and had a negative impact on mood (increased feeling of confusion, depression, tension, anxiety, and fatigue) (Liebermann et al. 2016). There are some predictors found that can have an impact on psychological stress. Bartone et al. (2008) found that hardiness was an important characteristic of personality to increase stress tolerance and probability of successful performance. Vaara et al. (2020) studied possible factors predicting dropout from 10-day survival training. They found that lower fitness level was associated with dropouts. The authors concluded that subjects with lower aerobic fitness levels would reach mental exhaustion faster because training included a high amount of physical activity together with energy and sleep deficit (Vaara et al. 2020.) These factors affecting stress response are called moderators (FIGURE 6) (Kavanagh 2005).

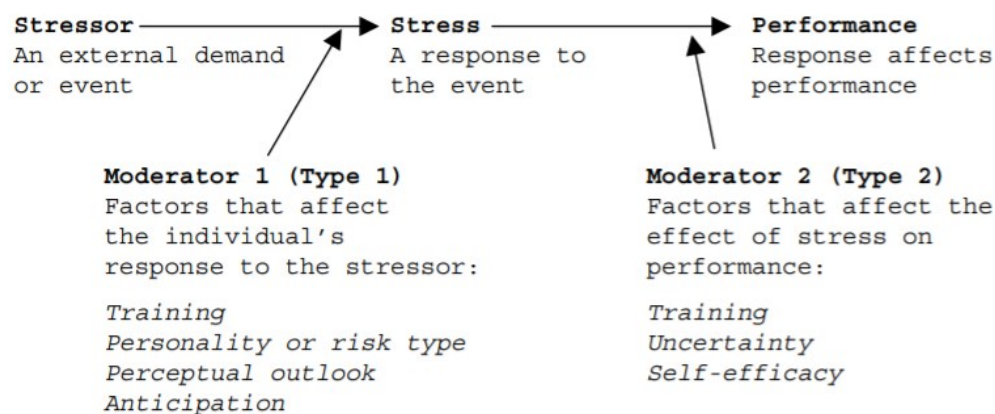


FIGURE 6. Different moderators change the relationship between stressor and performance (Kavanagh 2005).

It also seems that increased mental workload has an impact on subjective stress. It can be defined as the amount of attention the soldier must direct to a task at any given moment. The more demanding task is – leading to increased mental workload – the likelihood of failure increases. Under the pressure, the mental workload is even much higher. (Yurko et al. 2010.) Different skills that are crucial for a soldier in the operating environment like weapon

handling and tactics, must be practiced enough that mental workload is low during real combat situations. High stress during combat decreases the capacity for the mental workload so basic skills must be automated responses. (Yurko et al. 2010; Delahajj et al. 2011.)

3.5 Cold environment

Cold environments are classified as environments where the ambient temperature of the atmosphere is close to or below 0°C. (Steinach & Gunga 2015, 215). Cold exposure initiates thermoregulatory responses in the body to maintain approximately 37°C baseline core temperature (Brown et al. 2012). As human physiological thermoregulatory adaptations are limited – mainly involuntary muscle contractions and non-shivering thermogenesis by activation of brown-fat tissue – behavioral changes like wearing more clothes play an important role (figure 7.) (Steinach & Gunga 2015, 216).

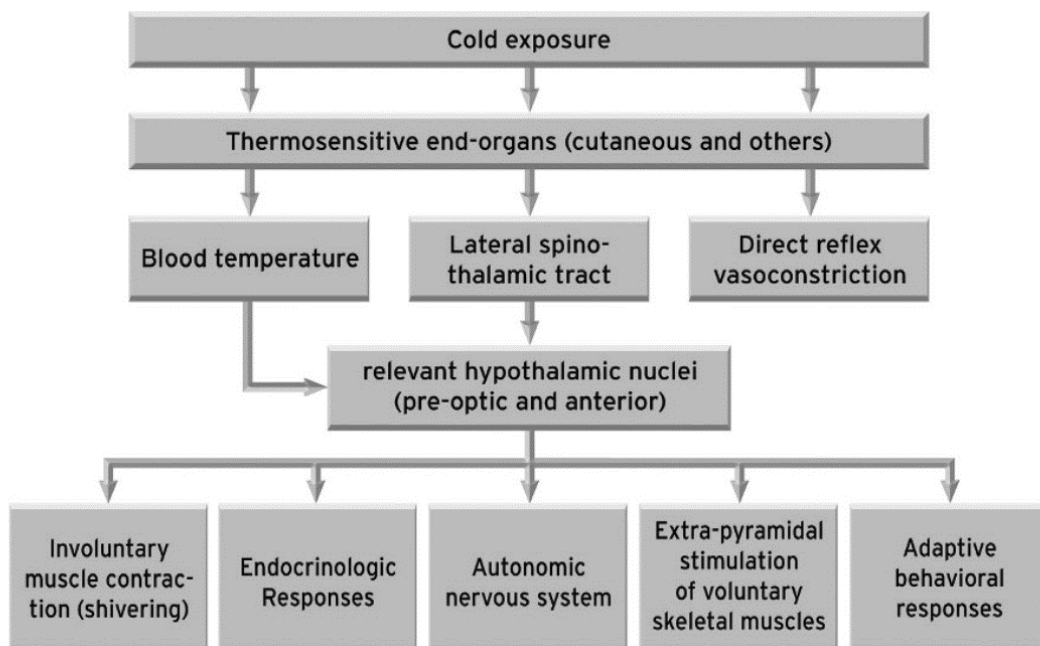


FIGURE 7. Mechanisms of the human physiology responses to cold exposure (Steinach & Gunga 2015, 217).

Military survival training under a cold atmosphere increases the physiological and psychological demands of the soldier. Hackney et al. (1985) studied cold exposure effects on

physical performance compared to a noncold environment. They found that after 4.5 days of military field training in a cold environment (-2 to -22°C), the reduction in anaerobic performance was significantly greater compared to a noncold environment (10-32 °C). (Hackney et al. 1985.) The physiological changes under cold exposure are mainly caused due to increased oxygen consumption and vasoconstrictions of peripheral blood flow (Young et al. 1996, 125-126). Thermoregulatory adaptations that produce more heat – involuntary muscle contractions and non-shivering thermogenesis – increases oxygen consumption significantly. These thermoregulatory adaptations lead to increased energy consumption and have been found to even disrupt sleep and sleep phases (Palca et al. 1986). Cold exposure together with poor sleep quality may increase cortisol levels and decrease pain tolerance (Goodin et al. 2012).

Physical activity in the cold environment increases heat production more than involuntary muscle contractions (shivering). However, physical activity also increases heat loss from the body by increasing peripheral blood flow to the working muscles. In addition, moving limbs increases convective heat loss because the layer of air at the skin surface is changing faster during the movement. As the intensity of physical activity increases, the afferent stimulus for involuntary muscle contractions decreases. At some point, intensity is high enough to prevent involuntary muscle contractions completely. This intensity level where metabolic heat production is sufficient will vary individually and is depending on the severity of cold stress. (Young et al. 1996.) Studies have been investigating whether physical fitness could influence thermoregulatory response to cold. Bittel et al. (1988) found that subjects with high aerobic capacity maintained warmer skin temperature than less fit subjects. However, this effect might be due to that subjects with high aerobic capacity have also thinner subcutaneous fat thickness and higher metabolic heat production (Bittel et al. 1988). There are however longitudinal studies indicating that endurance training strengthens cutaneous vasoconstrictor response to a cold and faster decline in skin temperature, thus providing a thermoregulatory advantage for cold exposure (Young et al. 1995; Kollias & Buskirk 1972).

Cold exposure will also affect cognitive performance and mood (Palinkas 2001). These changes are observed when core body temperature declines by 2°C or more. Decrements may be even more severe if cold exposure is combined with sleep loss, under-nutrition, dehydration, physical strain, and psychological stress which is usually the case in winter survival training. (Lieberman

et al. 2009.) Acclimation to coldness is therefore important. Oksa et al. (2006) propose that being adapted to the cold positively affects the ability to perform motor skills under cold conditions like using a gun. Functioning optimally is essential for a soldier regardless of coldness. Therefore, a cold environment could be used as a tool to increase the stress tolerance of soldiers.

4 PHYSIOLOGICAL RESPONSES TO STRESS

When a soldier is exposed to a stimulus or an event that is perceived as threatening, the internal physiological responses of the body will occur to adapt and function effectively under the new challenging situation. This is called fight-or-flight response which is automatically turned on when a stressor is faced. Most of these physiological reactions result from the activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic-adreno-medullar (SAM) axis (Kavanagh 2005; Atkinson & Hilgard 2009.) This will cause physiological and behavioral changes affecting the nervous, endocrine, and immune systems (Chu et al. 2020). During military field training and operations, stressors like energy deficit, sleep deprivation, physical fatigue, and mental strain are highly present.

4.1 The acute stress response

The physiological response to stress is controlled by the brain because it determines what is perceived as threatening. When the threat has been observed, the fight-or-flight response is turned on which triggers the activation of HPA and SAM axes. (McEwen 2007.) The first response to stress is the activation of the SAM axis. This leads to rapid improvement in alertness, vigilance, and appraisal of the environment. The second phase is the activation of the HPA axis. Responses of the HPA axis are considered sluggish compared to the SAM axis but it results in more protracted responses. Psychological and physical stressors both activate different neuronal networks and brain regions. Physical stressors activate mainly the hypothalamus and the brainstem whereas psychological stressors that are perceived in an anticipatory condition, activate mainly limbic structures and can be regulated by the reward system of the brain. However, the paraventricular nucleus of the hypothalamus and locus coeruleus (nucleus in the brainstem) are the main regions of the brain to initiate the stress response and activate the HPA and SAM axes. (Godoy et al. 2018.) Figure 8 shows a list of adaptations for the stress response (Tsigos et al. 2020; Chrousos & Gold 1992).

Behavioral adaptations	Physical adaptations
Adaptive redirection of behavior	Adaptive redirection of energy
Adaptive redirection of behavior	Oxygen and nutrients directed to the central nervous system and stressed body regions
Increased cognition, vigilance and focused attention	Altered cardiovascular tone; increased blood pressure and heart rate
Suppression of feeding behavior	Increased respiratory rate
Suppression of reproductive behavior	Increased gluconeogenesis and lipolysis
Inhibition of gastric motility	Detoxification from toxic products
Stimulation of colonic motility	Inhibition of reproductive and growth axes
Containment of the stress response	Containment of the stress response
	Containment of the inflammatory/immune response

FIGURE 8. Behavioral and physical adaptations for the stress response (adapted from Tsigos et al. 2020; Chrousos & Gold 1992).

After the stressor is recognized, the hypothalamus rapidly activates the sympathetic nervous system triggering the rapid release of acetylcholine from sympathetic splanchnic nerves which bind to receptors located in the adrenal medulla. These receptors activate exocytosis of catecholamine-filled vesicles which transport the catecholamines to the bloodstream. (Paravati et al. 2020.) The sympathetic drive also activates different brain regions like the hippocampus, the amygdala, and central catecholaminergic neurons in the brainstem to release monoamines, including norepinephrine, dopamine, and serotonin. These monoamines act through G protein-coupled specific receptors and promote different behavioral strategies to survive the initial phase of a stressful event. (Joëls & Baram 2009; Kyrou & Tsigos 2008). Especially, the locus coeruleus is the center of the central noradrenergic system. It has an important role to work as an “alarm system” for attention, excitation, and defensive response during acute stress by secreting norepinephrine. (Godoy et al. 2018.)

The main catecholamines released from the adrenal medulla are epinephrine and norepinephrine. The functions of catecholamines depend on the receptor of the target cell

(alpha- & beta-receptors). For example, catecholamines regulate blood pressure via alpha-1 receptors in smooth muscle cells. Other functions for catecholamines include enhanced cardiac muscle contractility, contraction of the pupillary dilator, relaxation of cells in the gastrointestinal tract, urinary tract, and bronchioles, increased glucagon secretion, and glycogenolysis. (Paravati et al. 2020.)

The increased activation of the HPA axis is initiated when the hypothalamus activates the sympathetic division of the autonomic nervous system by releasing the corticotropin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus. The released CRH stimulates the release of adrenocorticotropic hormone (ACTH) from pituitary corticotropes into the bloodstream. ACTH is transported through the bloodstream to the adrenal cortex where it binds to adrenal melanocortin 2 receptors (MC2Rs) to stimulate uptake of cholesterol which is a precursor for steroid hormones. After cholesterol is transported to the gland cell, it undergoes biosynthetic procedures and goes to mitochondria for hydroxylation. After this process synthesized cortisol and other glucocorticoids are released into the bloodstream (Figure 9). (King et al. 2017, Russel & Lightman 2019.)

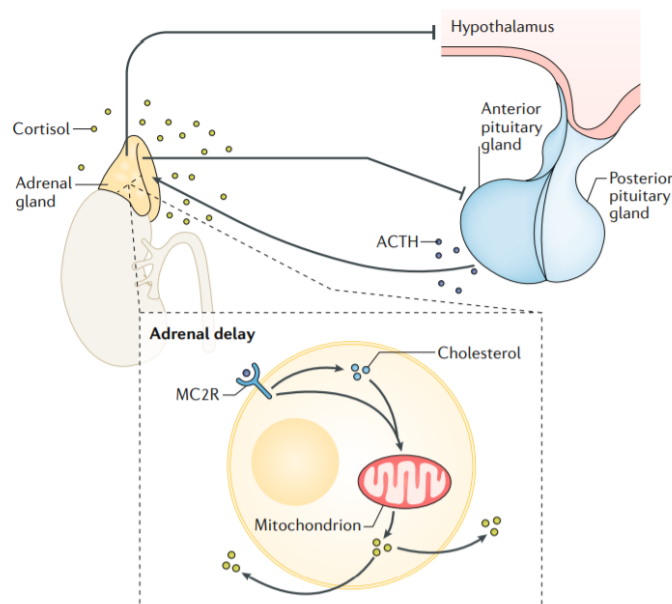


FIGURE 9. Pathway of HPA axis. Notice that there is a natural delay between processes resulting slower response to stress compared to the SAM axis (Russel & Lightman 2019).

The main human glucocorticoid released is cortisol which regulates the blood glucose and certain minerals (Atkinson & Hilgard 2009). 80-90 % of cortisol in serum is bound to cortisol binding globulin (CBG) and 5-10 % is bound to albumin. The rest of the serum cortisol is unbound and biologically active. (McEwen 2007.) The increase in cortisol levels leads to suppression of insulin secretion, increased mobilization of energy stores (gluconeogenesis, glycogenolysis, and proteolysis), suppression of the inflammatory response, and impairment of collagen synthesis. It will also increase sodium and water retention leading to an increase in blood pressure. (Khoo 2017.) Cortisol also mediates genomic and non-genomic effects in the brain. For example, cortisol can suppress the activity of the paraventricular nucleus via non-genomic signaling. (Godoy et al. 2018.) Cortisol peak levels occur between 15 to 20 minutes after the onset of the acute stress response (Russel et al. 2019).

The stress-induced changes can also be observed from the salivary alpha-amylase which is an enzyme in the oral cavity. It participates in the hydrolysis of starch and glycogen and defense against different bacteria. (Petrankova et al. 2015.) An increase in the sympathetic drive will elicit secretion of alpha-amylase from the salivary glands and the pancreas (Kiba 2017). Changes in alpha-amylase in saliva are much faster than changes in cortisol between stress-expose and rest. This is one of the benefits of using alpha-amylase as a marker of acute stress. (Takai et al. 2007.)

Like mentioned earlier, to initiate the stress response the sensory systems of the brain must first recognize the stressor (Fink 2016). Studies have found neurobiological diversity between different types of stressors. Physical stressors, like blood loss or coldness, increase the activity of the brainstem and hypothalamic regions whereas psychological stress such as pressure from executing task is engaging more brain regions that subserve emotions (the amygdala and prefrontal cortex), learning, and memory (the hippocampus), and decision making (the prefrontal cortex). (De Kloet et al. 2005; McEwen 2007.) These different responses affect also behavioral functions (Joëls & Baram 2009).

4.2 Chronic stress adaptations

Prolonged exposure to stressor leads to chronic stress. It is typically defined as a stressor that lasts a week or more. (Joëls & Baram 2009.) There are clear changes in different physiological responses between acute and chronic stress. Most of these adaptations are occurring because of prolonged hyperactivity of the HPA axis. (Fries et al. 2005.) The body must adapt to this prolonged hyperactivity. One adaptation happens in the brain by changing the ratio of arginine vasopressin and corticotropin-releasing hormone in the hypothalamus. There seems to be an increase in arginine vasopressin, which also stimulates ACTH secretion but is much weaker than CRH. (Ma et al. 1997.)

Increased arginine vasopressin secretion combined with depletion of cortisol, insufficient serum free cortisol, and glucocorticoid receptor resistance can lead to cortisol dysfunction. This can cause proinflammatory effects and disrupt the negative feedback mechanism which normally inhibits the continued release of CRH. (Hannibal & Bishop 2014.) These results increased catabolic effect in bones and muscle tissues, fatigue, depressed mood, pain sensitivity, memory impairments, sodium-potassium dysregulation, orthostatic hypotension, and impaired pupillary light reflex. (Fries et al. 2005.) During chronic stress elevated glucocorticoid levels also seems to impair synaptic plasticity and cognition performance whereas during acute stress increased glucocorticoid secretion leads to enhanced synaptic plasticity and hippocampal-dependent cognition (Fink 2016).

Chronic stress also impacts the expression of certain genes, structural alterations in specific neurons, and alterations in neuronal firing patterns in the brain regions. (Joëls & Baram 2009.) Although acute stress can lead to increases in the secretion of growth hormone, chronic stress is associated with inhibiting growth hormone secretion by CRH-stimulated somatostatin. Chronic elevation in the secretion of glucocorticoids, norepinephrine, and epinephrine affect the immune system by suppressing it. This leads to decreased activity of the cytotoxic T lymphocytes and natural killer cells which impairs the immune system responses against various infections. (Reiche et al. 2004.) Furthermore, the release of CRH suppresses gonadotropin-releasing hormone (GnRH) neurons of the arcuate nucleus. Also, glucocorticoids

have an inhibitory impact on the GnRH release, the pituitary gonadotroph cells, and the gonads. This leads to decreased secretion of luteinizing hormone and inhibited steroidogenesis. Chronic stress leads to impairment of gonadal function which can be seen in males by decreased luteinizing hormone and testosterone levels and females by menstrual disorders. (Kyrou & Tsigos 2008.) Figure 10 shows the interaction between the stress system and the hypothalamic-pituitary-gonadal axis (HPG) (Kyrou & Tsigos 2008). These effects with testosterone have been found in many military training studies. Liebermann et al. (2016) studied physiological adaptations for 14 days of survival training where subjects were exposed to multiple stressors like sleep deprivation, energy deficit, dehydration, coldness, physical fatigue, and psychological strain. They found increased cortisol, norepinephrine, epinephrine, and decrease testosterone levels. There were also negative changes in mood and a decrease in cognitive performance. (Liebermann et al. 2016.)

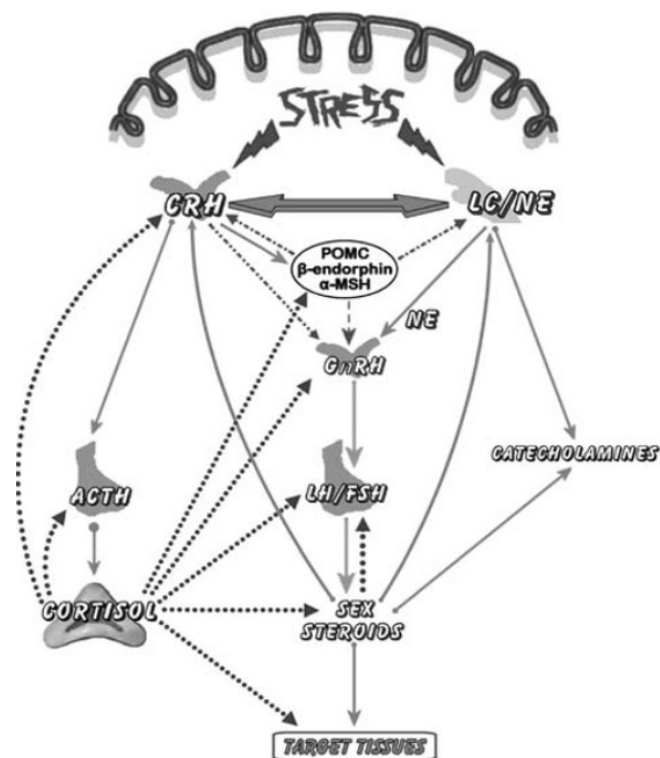


FIGURE 10. Chronic stress leads to prolonged inhibition of the HPG axis via chronic activation of the HPA axis. This leads to suppression of gonadal functions and sex hormones secretion (LC/ NE= locus coeruleus-noradrenergic system) (Kyrou & Tsigos 2008).

Interestingly, neuropeptide-Y is one major factor in improved stress tolerance. Morgan et al. (2001) reported that the U.S Army soldiers exposed to uncontrollable stress induced a significant increase in neuropeptide-Y which was associated with fewer psychological symptoms of reported dissociation and better military performance (Morgan et al. 2001). Neuropeptide-Y (NPY) is a 36-amino acid peptide that is biologically active and is found to be one the most abundant neuropeptide in the brain. NPY has several functions. For example, it regulates eating behavior, circadian rhythm, and certain cognitive functions. (Reichmann & Holzer 2016.)

It has been proposed that NPY interact with the HPA axis and possibly counteracts the biological actions of CRH especially in the amygdala (Heilig et al. 1994). NPY concentration may increase already before intensive military training as a coping strategy to prepare the body and reduce cardiovascular tone and suppress anxious behavior (Szivak et al. 2018). Szivak et al. (2018) also found that physically fitter subjects showed quicker return of norepinephrine and neuropeptide-Y to baseline after 10-day SERE training suggesting that physical fitness level could enhance recovery from military training.

5 PURPOSE OF THE STUDY

The main purpose of the present study was to examine and describe the effects of military survival training on salivary biomarkers, subjective stress, and physical performance. Additionally, the purpose was to evaluate the relationship between physical performance and stress from saliva cortisol and alpha-amylase, NASA-Task Load Index questionnaires, and physical fitness tests.

Research questions and hypotheses:

1. How the physical fitness and saliva cortisol and alfa-amylase concentrations change during the 10-day military survival training?

Hypothesis: Cumulative stress and fatigue will increase saliva cortisol and alpha-amylase concentrations and decrease physical fitness significantly through training (Chester et al. 2013; Ojanen et al. 2018; Montain & Young 2003).

2. Is the measured physical performance before training related to measured fatigue and stress during 10-day military survival training?

Hypothesis:

Better physical performance is related to lower changes in salivary cortisol, alpha-amylase, and reported subjective stress referring to better resilience (Szivak et al. 2018).

3. Are there associations between changes in physical fitness pre- and post-tests and salivary markers during 10-day survival training?

Hypothesis:

A decrease in physical performance between pre- and post-measurements is related

to a higher increase in biomarkers referring to higher stress load and fatigue (Chester et al. 2013; Szivak et al. 2018).

6 METHODS

6.1 Subjects

Twenty-six (n=26) Finnish Army male conscripts volunteered as subjects for the study. They were doing their compulsory service as the study was done. Mean (\pm SD) age, body mass, and height were 20 (\pm 1) years, 75.4 (\pm 10.2) kg, 180 (\pm 7) cm, and fat% 10.1 (\pm 3.4) respectively. All subjects completed study requirements and were considered in analyses. Subjects were informed about the experimental design and the possible risks that study participation could be associated with. They were also informed that they could cancel their participation in the study at any point without any consequences. All the subjects signed informed consent for the study and approved their participation. The present study was approved by the Finnish Defence Forces (AO1720) and ethical approval was granted by the Scientific and Ethical Committee of the Helsinki University Hospital Research (HUS/900/2018). (Vaara et al. 2020.) The present study was a part of the larger study.

6.2 Experimental design

A winter military survival training is a part of conscripts' compulsory military service in the northern parts of Finland during wintertime. The training period lasted 10 days and was divided into the preparation phase and field phase. Before this training period, subjects went through pre-measurements (PRE). Physical tests were done in the previous week before the training period. Body compositions were analyzed the day before the onset of the training period. Saliva samples were collected two times per day (08:00 & 20:00) from a day before to day nine of the training period. (Vaara et al. 2020.)

The first two days of the training phase were called the preparation phase. This phase was carried out at the garrisons and consist of basic survival skill training. At this time only saliva samples were collected. After the preparation phase started the field phase on day three. This lasted 7 days and consisted of different military and survival tasks. Through the whole field

phase, the subjects had significantly restricted energy intake and sleep duration, and high physical strain. (Vaara et al. 2020.)

Subjects were instructed to pack certain kits for the field phase. Moving between locations was done by skiing and encamping took place in temporary shelters. Through this phase, the daily diary was filled where the energy intake and subjective stress (NASA-TLX) were tracked. The field phase included two mid-tests that were done on days six and eight. Mid-tests included body composition and physical tests. The last day of the field phase was day nine of the training period when subjects came back to garrisons. The following day, post-measurements (POST) was conducted where body composition and physical tests were done. PRE, MID1, MID2, and POST were all collected at the same time of the day at 10:00 a.m. The timetable of the training period and measurements can be seen in figure 11. The weather information and distance of daily skiing were tracked through the whole survival training period. The temperature during the field training phase varied between -11 and +8 °C and the depth of snow varied between 79 and 101 cm (www.ilmatieteenlaitos.fi/havaintojen-lataus).

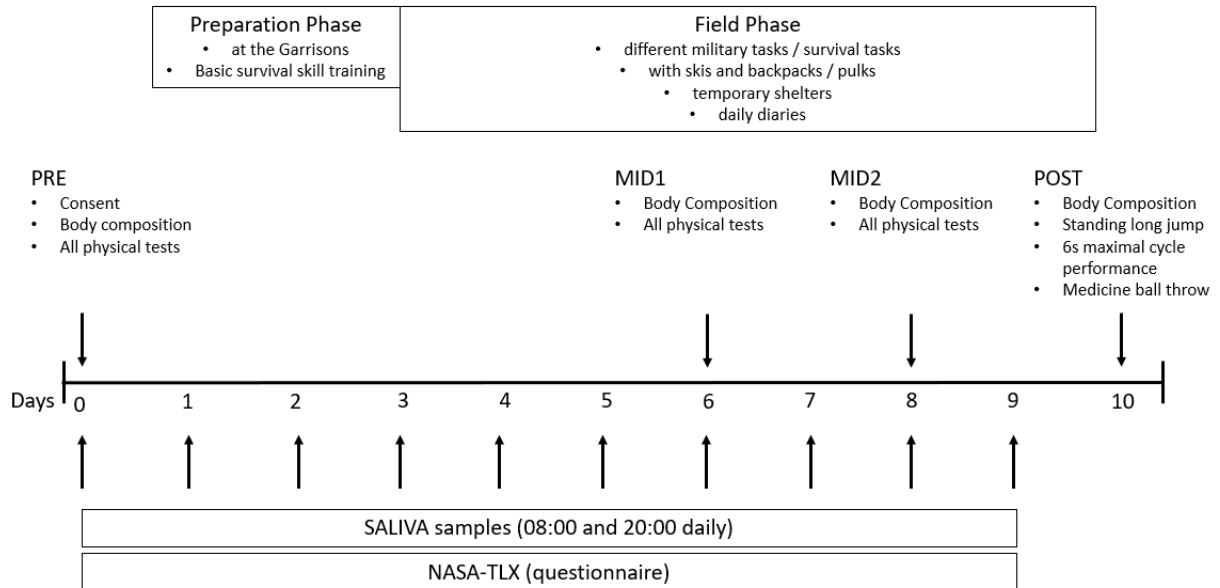


FIGURE 11. The timetable of the field training and measurements. Pre-measurement= PRE, mid-test 1= MID1, mid-test 2= MID2, post-test = POST. Notice that POST included only three of the physical tests (standing long jump, 6s maximal cycle performance, medicine ball throw).

6.3 Measurements

6.3.1 Physical fitness

The physical fitness tests included power, strength, and endurance measurements. Explosive force production of the lower extremities was tested with the standing long jump. The test was performed on a gym mat which was specifically designed for this purpose (Fysioline Co., Tampere, Finland). The subjects were first instructed by the correct technique. They performed a general warm-up and several warm-up jumps. After the warm-up, they were instructed to jump horizontally as long as possible with bilateral landing. The best jump from three attempts was measured as a test result. (Vaara et al. 2020.) The standing long jump has been proved to have high validity and reliability to measure changes in lower body power. Reid et al. (2017) tested the reliability of the standing long jump in track and field athletes and found that the interclass reliability coefficient was $r=0.99$ and the intraclass reliability coefficient $ICC=0.99$. Also, the validity of the standing long jump test is high (Rahman et al. 2021). Explosive force production of the upper extremities was measured with a seated medicine ball throw. The subjects were instructed to sit on the floor, keeping their legs fully extended and back against the wall throughout the test. The medicine ball was kept with both hands with the forearms positioned parallel to the ground. The medicine ball was thrown vigorously as far as possible while maintaining the back against the wall. The distance of the throw was measured from the wall to the landing point of the medicine ball. The longest throw from three throws was marked as a result of the test. (Vaara et al. 2020.) The reliability of the seated medicine ball throw has been shown to be high ($r=0.97-0.99$) (Beckham et al. 2019).

Muscle endurance was tested with sit-up and push-up tests where the subjects were instructed to perform as many repetitions as they could in a minute. Tests were performed with the correct technique. First, the sit-up test was done. In the starting position of the sit-up test, the participant first laid on his back while the legs were supported from the ankles. The knee angle was kept at 90° and fingers were crossed behind the head. A repetition was counted when the subject brought elbows to the knee-level. The correct technique in the push-up test was also instructed precisely. At the start, a subject laid down on the floor with face pointing down and feet parallel

at the pelvis to shoulder width hand position. The subjects were instructed to extend their arms from the starting position and keep the feet, trunk, and shoulder in the same line throughout the test. Repetition was counted when the torso was lowered back to the floor by flexing arms to a 90° elbow angle, respectively. (Vaara et al. 2020.)

The maximal isometric force was measured bilaterally in a sitting position by an electromechanical dynamometer manufactured by the University of Jyväskylä, Finland. The lower extremities maximal isometric test was done in the leg press and for the upper extremities in the bench press. The knee and hip angles were standardized to 107° and 110° in the leg press based on the study of Häkkinen et al (1985). For the isometric bench press, the equipment was adjusted for each participant differently. The feet were kept flat on the floor, the arms parallel to the floor, and the elbow angle was 90°. Every participant has a trial attempt and after that, the two trials were done for both movements. The instructions were given to participants to produce maximal force as fast as possible. (Vaara et al. 2020.)

The 6 seconds maximal anaerobic power cycle ergometer test (Wattbike Ltd., Nottingham, UK) was used to measure peak power of the lower extremities. The subject was seated stationary at the start with the dominant leg initiating the first downstroke. The air and magnetic resistance were set based on the body weight, which was taken before the test. The test started following 5 seconds countdown by verbal command. (Vaara et al. 2020.) The 6 seconds maximal anaerobic power cycle ergometer test has been concluded to be a valid measure of peak power output compared to 30 seconds Wingate anaerobic test (Herbert et al. 2015).

Maximal endurance capacity was tested with the 20-meter shuttle run test. In the test, subjects are running between two lines (the distance between lines is 20 meters) controlled by the test sound. Subject must get from line to line before the test sound. The test sound frequency is increased so that the required speed from line-to-line increases 0.5 km/ h after each 20-meter sprint. The test is over when the subject missed two required sprint times in a row. The last failed run between lines is not counted in the result. Mayorga-Vega et al. (2015) meta-analysis studied the validity of the 20-m shuttle run test and found a moderate-to-high validity for estimating maximum oxygen uptake ($r_p = 0.66-0.84$). Aandstad et al. (2011) evaluate the

validity and reliability of the 20-m shuttle run test in military personnel. They found a good reliability (ICC & Pearson $r=0.95-0.96$) and moderate validity (ICC= 0.8, Pearson $r=0.82$) for the test (Aandstad et al. 2011).

6.3.2 Saliva samples, body composition & energy expenditure

Saliva samples were collected twice a day. The first samples were collected on the same day with PRE tests. After that, the saliva samples were collected during the training period from day one to day nine. The samples were collected at 8:00 a.m. and 8:00 p.m. (Figure 10.) The saliva cortisol and alpha-amylase were analyzed from the samples.

Saliva samples were collected in tubes and centrifuged for 10 minutes at 3500 rpm. After centrifuging, the samples were stored at -20°C until analysis. Saliva cortisol was analyzed with chemiluminescence immunoassay (Immulite 2000xpi, IBL Hamburg) which is based on the competition principle. The measuring range of the chemiluminescence immunoassay is 0.43-110 nmol/l for saliva cortisol. The coefficient of variation in the pooled sample was 7.2 % in control measurements of the University of Jyväskylä laboratory. Saliva cortisol assessment has been validated to a reliable method for measuring stress induced cortisol changes without the need for blood sampling (Izawa & Suzuki 2007). Saliva alpha-amylase was analyzed with immunoturbidimetry assay (Konelab, 20Xti, Thermo Electron Corporation, Vantaa, Suomi) by using commercial reagents (Thermo Scientific, Vantaa, Suomi). The analytical limit of detection for immunoturbidimetry is 11.65 IU/l and the coefficient of variation is 3.6 % based on control measurements of the University of Jyväskylä laboratory.

Body composition was measured before the physical tests in the morning after an overnight fast. Body mass and fat percentage were measured by using the segmental multi-frequency bio-impedance method (BIA) (InBody 720, Biospace Co. Ltd., Seoul, South Korea). The body height was measured by a stadiometer at the beginning of their service. The energy expenditure was tracked from heart rate variability continuously during the training period (Firstbeat Ltd., Jyväskylä, Finland). Estimating energy expenditure from heart rate variability (Firstbeat

Bodyguard 2 HRV device) has been shown to correlate strongly ($r=0.75-0.98$) with indirect calorimetry (Robertson et al. 2015).

6.3.3 Questionnaires

Energy intake and subjective stress were collected by questionnaires. These variables were tracked in the diary which subjects filled every morning. Energy intake was analyzed from the self-filled food diary (<https://finelli.fi>, Finnish Institute of Occupational Health). The subjective stress was measured by the NASA-Task Load Index (TLX) questionnaire which consists of multiple factors and subjectively evaluates soldier's demands for a variety of tasks. It has been used across different professions to quantify how demanding and stressful the job feels from professional. (Hart & Staveland 1988.) It rates workload across six dimensions: mental demand, physical demand, temporal demand, performance, effort, and frustration. The raw scores were used in the analyses. NASA-Task Load Index has been shown to have good validity and reliability to assess subjective workload (Said et al. 2020; Xiao et al. 2015)

6.4 Statistical analysis

The data was analyzed and graphed by using IBM SPSS Statistics v.28 computer software (SPSS Inc., Chicago, IL, USA). Normally distributed data were analyzed for the difference between timepoints by using repeated measures ANOVA. Estimated marginal means were used to compare main effects and Bonferroni was used for confidence interval adjustment.

Pearson's correlation coefficients \textcircled{R} were calculated for physical fitness tests and saliva markers. The saliva markers and subjective stress scores for the correlation analysis were calculated by creating mean variable from days of the preparation phase and field phase. The preparation phase average value was calculated by taking the mean value from days 0, 1, and 2. The field phase average value was calculated by taking the mean value from days 3 to 9. Due to time, location, and technical problems, not all subjects completed all testing. The number of subjects taking each test is marked in each figure and table. For the regression analysis, the

linear regression model was used. The criterion for significance was $p < 0.05$ in all statistics. All values in the results are reported as mean \pm SD.

7 RESULTS

Body composition. During the 10-day training period, the body mass decreased by 2.8 ± 3.1 kg ($p < 0.01$). There was also a 3.4 ± 1.2 kg decrease in fat mass ($p < 0.01$) and a 4.2 ± 1.2 % decrease in the percentage of fat ($p < 0.01$). There was no significant decrease in muscle mass except between PRE and MID1 measurements (1.1 ± 0.8 kg, $p < 0.05$). The results of body composition measurements are shown in table 1.

TABLE 1. Mean (\pm SD) body composition values in different measurement points. *= Significantly changed from pre ($p < 0.05$); **= Highly significantly changed from pre ($p < 0.01$).

	PRE-TEST (PRE)	MID-TEST 1 (MID1)	MID-TEST 2 (MID2)	POST-TEST (POST)
Body mass (kg)	75.4 ± 10.4	72.6 ± 10.0 **	72.6 ± 9.8 **	72.6 ± 9.5 **
Fat mass (kg)	10.0 ± 3.4	9.1 ± 3.1 *	7.6 ± 3.0 **	6.7 ± 3.0 **
Fat %	13.2 ± 3.5	12.3 ± 3.4 *	10.4 ± 3.9 **	9.0 ± 3.5 **
Muscle mass (kg)	37.1 ± 5.1	36.0 ± 4.9 *	36.9 ± 4.9	37.3 ± 4.7

Saliva biomarkers. There were significant changes in saliva cortisol and alpha-amylase values during the training phase ($n=15$). The lowest saliva morning cortisol value during the training period was 14.5 ± 5.3 nmol/ L and it was measured on day three. The highest morning cortisol (43.2 ± 20.4 nmol/ L) was measured on day four. (Figure 12.) The highest saliva evening cortisol was 26.8 ± 17 nmol/ L and it was measured on day seven. The lowest saliva evening cortisol was measured at PRE which was 3.9 ± 1.6 nmol/ L (Figure 13). The highest morning saliva alpha-amylase concentration value was 150 ± 101 u/ mL, which was measured on day five, and the lowest value 44 ± 33 u/ mL was measured at the PRE (Figure 14). Saliva alpha-amylase evening peak value was measured on day three (134 ± 87 u/ mL), and the lowest value on day eight (62 ± 47 u/ mL) (Figure 15).

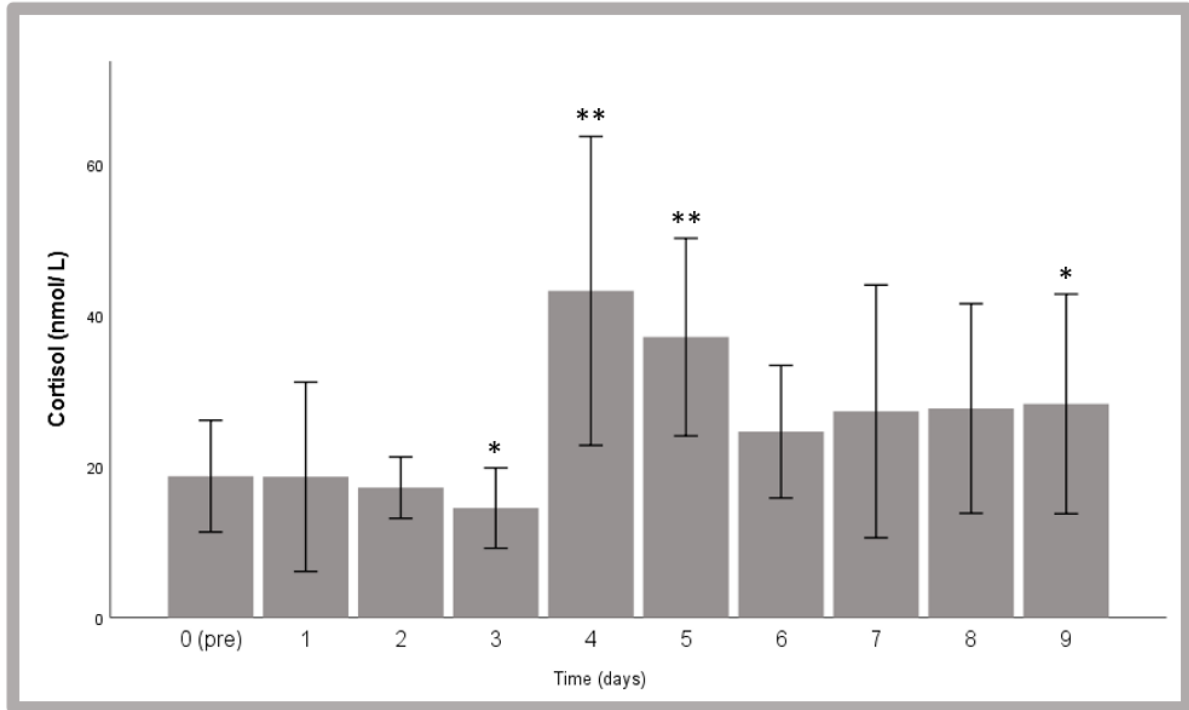


FIGURE 12. Mean (\pm SD) saliva morning cortisol values throughout the training period. *= Significantly changed from pre ($p < 0.05$); **= Highly significantly changed from pre ($p < 0.01$).

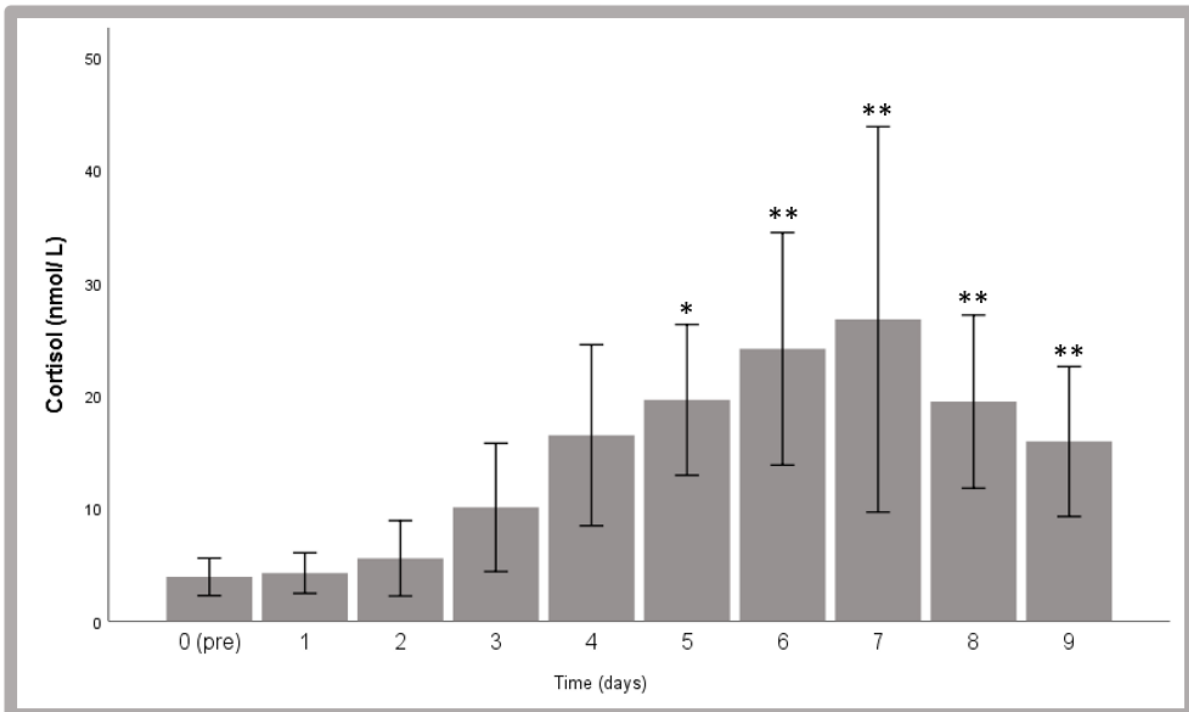


FIGURE 13. Mean (\pm SD) saliva evening cortisol values throughout the training period. *= Significantly changed from pre ($p < 0.05$); **= Highly significantly changed from pre ($p < 0.01$).

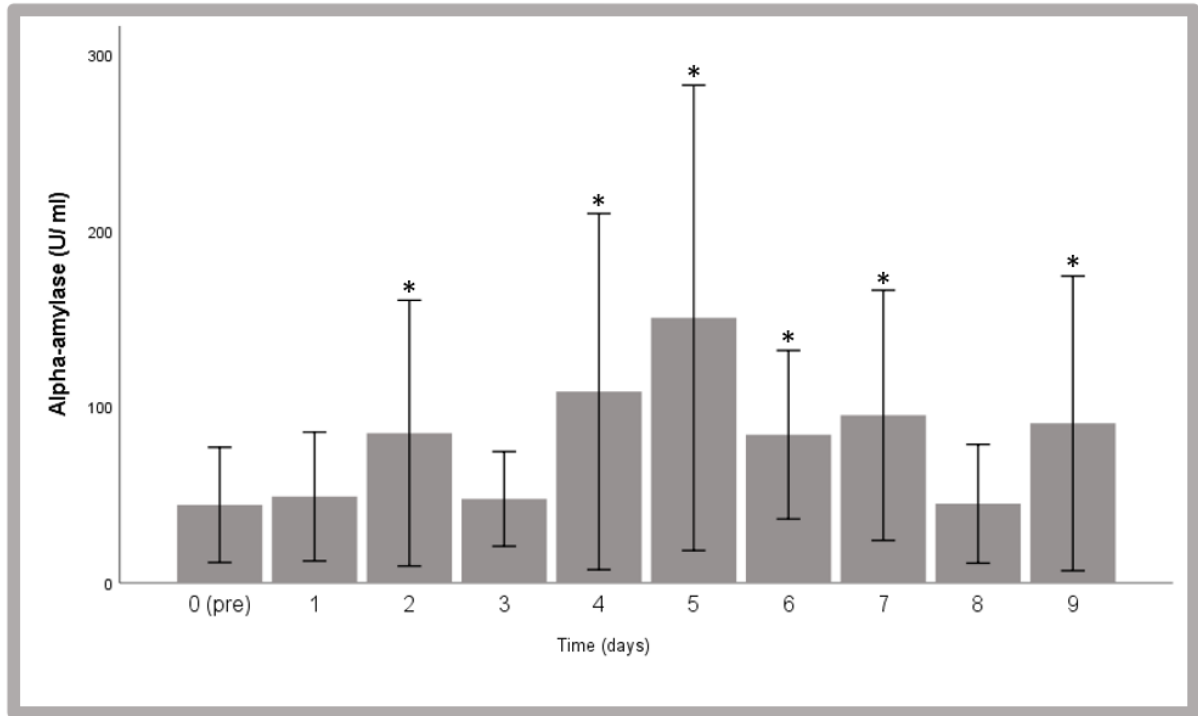


FIGURE 14. Mean (\pm SD) saliva morning alpha-amylase values throughout the training period.
 *= Significantly changed from pre ($p < 0.05$).

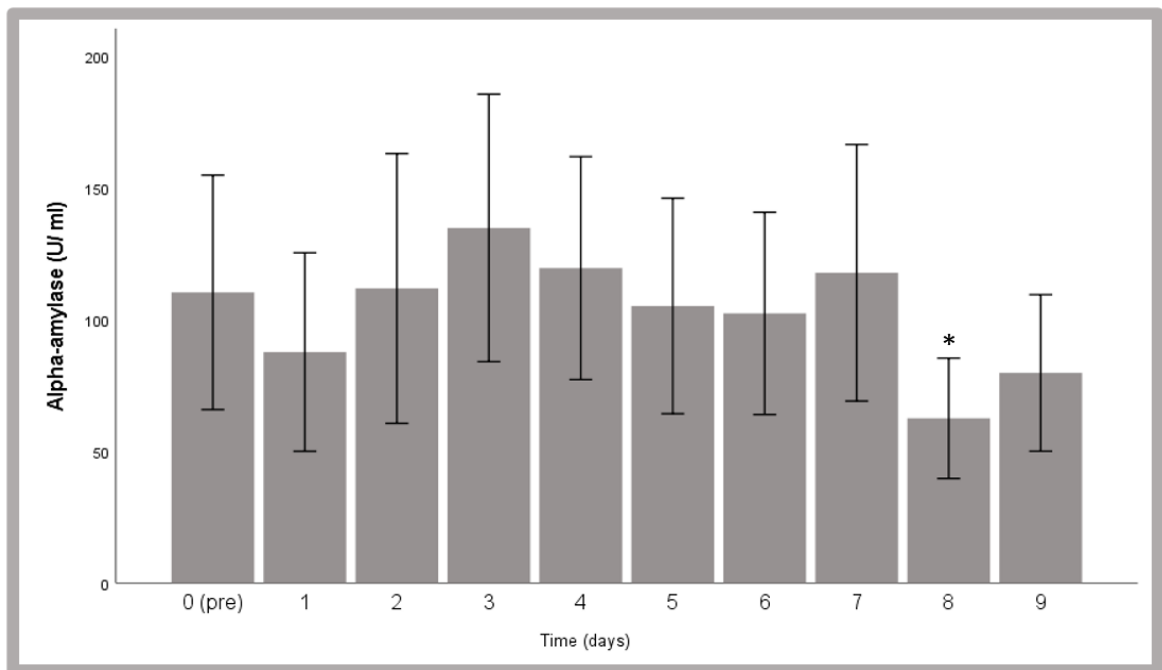


FIGURE 15. Mean (\pm SD) saliva evening alpha-amylase values throughout the training period.
 *= Significantly changed from pre ($p < 0.05$).

Physical fitness & subjective stress. Physical fitness tests were performed at four different timepoints. Not all the tests were taken at every time point. There is a small dispersion in sample size between different tests. All physical fitness tests, except maximal isometric strength of the lower extremities, changed significantly from PRE to MID2 and POST. There was also a significant change between PRE and MID1 in all physical tests except maximal isometric strength of the lower extremities and the standing long jump. (Table 2.) The subjective stress was measured by the NASA-Task Load Index questionnaire (N=24). The average score in the preparation and field phase was 39 ± 14 and 75 ± 14 points. Thus, the relative increase was 95 ± 22 % from the preparation phase to the field phase ($p < 0.001$). (Figure 16.)

TABLE 2. Mean (\pm SD) physical fitness test results in different measurement points. * = Significant difference from pre ($p < 0.05$). † = Significant difference from MID1 ($p < 0.05$).

	PRE-TEST (PRE)	MID-TEST 1 (MID1)	MID-TEST 2 (MID2)	POST-TEST (POST)
Maximal isometric strength, lower extremities (kg), n=26	330 ± 86	317 ± 88	336 ± 103	N/A
Maximal isometric strength, upper extremities (kg), n=26	92 ± 18	$83 \pm 19^*$	$84 \pm 18^*$	N/A
Standing long jump (cm), n=22	230 ± 19	223 ± 23	$221 \pm 18^*$	$217 \pm 25^*$
Push-ups (reps/min), n=24	40 ± 13	$31 \pm 15^*$	$34 \pm 12^*$	N/A
Sit-ups (reps/min), n=24	46 ± 8	$41 \pm 10^*$	$39 \pm 9^*$	N/A
6 s maximal cycle performance (max W), n=23	829 ± 113	823 ± 123	$798 \pm 118^*$	$797 \pm 107^*$
Medicine ball throw (cm), N=24	628 ± 72	$540 \pm 70^*$	$573 \pm 70^*\dagger$	$577 \pm 69^*$
20-m shuttle run test (final level), n=23	75 ± 16	$52 \pm 25^*$	$43 \pm 34^*\dagger$	N/A

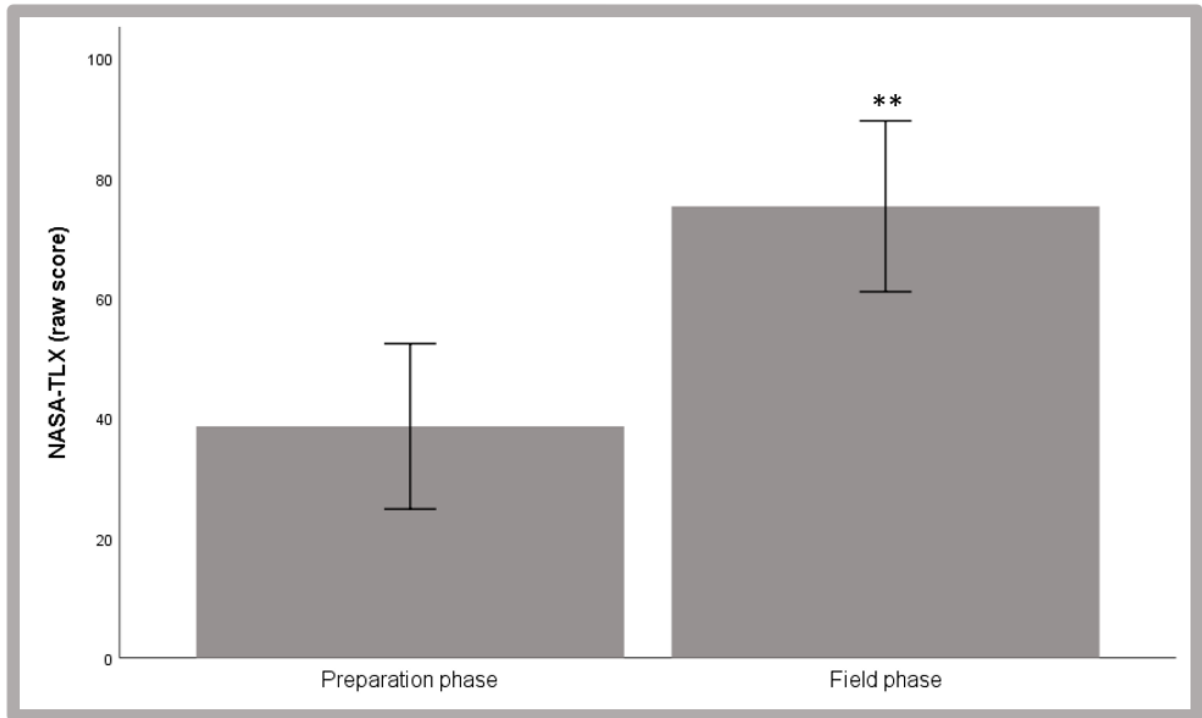


FIGURE 16. Mean (\pm SD) raw scores from the NASA-TLX questionnaire averaged from the preparation phase (days 0-2) and field phase (days 3-9). **= Highly significantly greater than pre ($p < 0.01$).

Correlations. The correlation analysis between physical fitness test results and changes in saliva biomarkers revealed a statistically significant correlation between maximal isometric strength of the lower extremities before training and change of cortisol from the preparation phase to the field phase ($n=26$, $r= 0.61$, $p < 0.001$) (Figure 17). No other correlations were found between physical fitness tests results and different markers of fatigue.

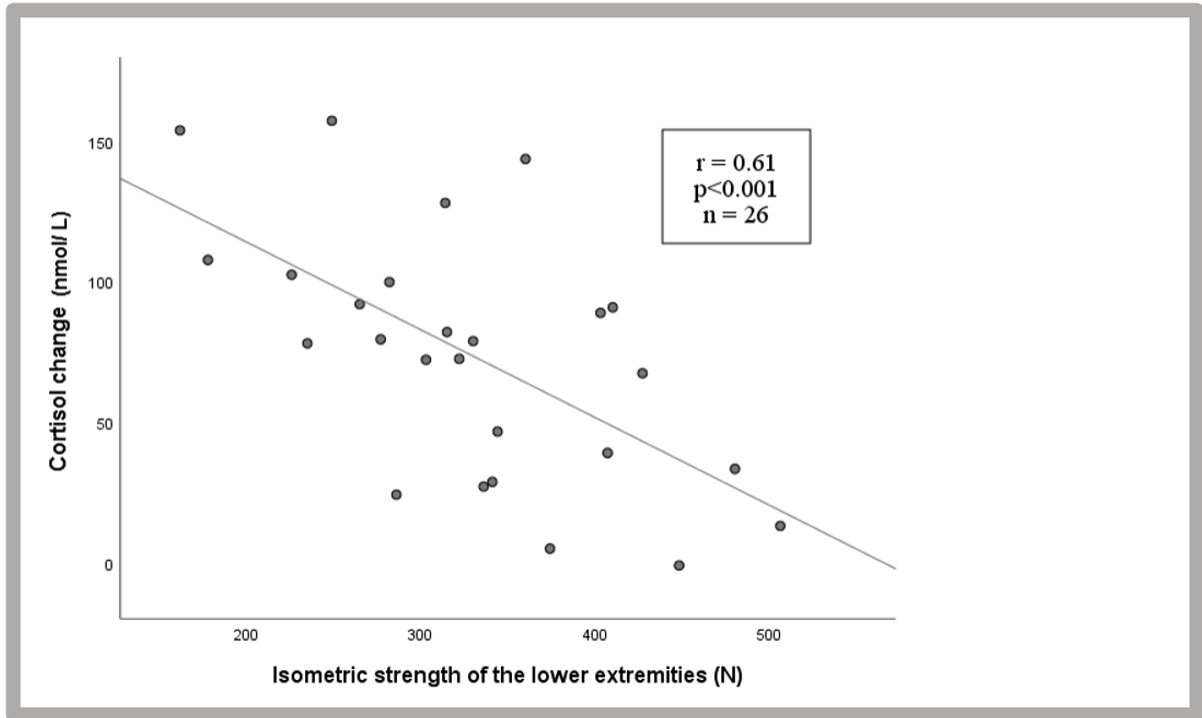


FIGURE 17. The correlation between cortisol change from the preparation phase to the field phase and maximal isometric strength of the lower extremities before the training period ($r=0.61$, $p<0.001$).

Energy expenditure. The evaluation of the total daily energy expenditure revealed high rates of daily expenses and low energy intake between days two and nine of the training period. The average daily expenditure was 4610 kcal and the average energy intake was 884 kcal. The daily energy intake and expenditure are shown in table 3.

TABLE 3. Mean (\pm SD) energy intake and expenditure between days two and nine.

	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Energy intake (kcal), n=5	N/A	667 \pm 0	405 \pm 71 ^b	423 \pm 145	761 \pm 456	1195 \pm 256 ^c	1819 \pm 596	918 \pm 817
Energy expenditure (kcal), n=19	3053 \pm 484	5380 \pm 1293 ^a	4232 \pm 2141	4672 \pm 1565 ^a	5439 \pm 1197 ^a	5372 \pm 1068 ^a	5289 \pm 836 ^a	3446 \pm 988 ^{abcdef}

^aSignificantly different from day 2, ^bSignificantly different from day 3, ^cSignificantly different from day 5, ^dSignificantly different from day 6, ^eSignificantly different from day 7, ^fSignificantly different from day 8.

Regression analysis. The regression analysis showed an association between maximal isometric strength of the upper extremities and subjective stress. Maximal isometric strength of the upper extremities before the training period predicted 14.6% of the change in subjective stress from the preparation phase to the field phase ($p < 0.05$). (Table 4.)

TABLE 4. The results of the regression analysis. NASA-TLX= NASA-Task Load Index, r = correlation, * = $p < 0.05$.

DEPENDENT VARIABLE	PREDICTOR VARIABLE	ADJUSTED R SQUARE	SIGN.	N
Change in subjective stress (NASA-TLX)	Maximal isometric strength of the upper extremities (PRE) $r=0.429$	0.146	0.041*	23

8 DISCUSSION

The primary findings of the present study were: 1) Body and fat mass decreased significantly during the training period, 2) Saliva biomarkers and subjective stress increased significantly during the training period, 3) Significant decrease in physical performance characteristics except for the maximal isometric strength of the lower extremities, 4) Significant correlation between maximal isometric strength of the lower extremities and cortisol change from the preparation phase to the field phase, and 5) maximal isometric strength of the upper extremities was inversely associated to subjective stress explaining 14.6 % of the difference in subjective stress between subjects.

The absolute changes in the body and fat mass are comparable to previous studies which have been observing body composition changes during survival training (Hamarsland et al. 2018; Szivak et al. 2018). The results showed a higher decrease in fat mass (3.4kg, respectively) than total body mass (2.8kg, respectively) which might be due to the error rate of InBody 720 body composition measurement. Nevertheless, a significant decrease in body mass and fat mass was expected due to a drastic energy deficit throughout the training period. Interestingly, there was no significant change in muscle mass between PRE and POST measurements. One possible explanation for this could be that length of the training period was short enough to prevent significant muscle loss. Hamarsland et al. (2018) found that an initial fat mass of about 10kg measured by InBody 720 could protect against muscle mass loss during survival training if the total reduction in body mass is under 6 kg. In the present study, the initial fat mass was 10.0 (\pm 3.4), thus supporting Hamarsland etl. (2018) finding.

There was a high alteration in saliva cortisol during the whole training period. The saliva morning cortisol mean peak value was highest on day four where the average morning cortisol concentration was 43.2 nmol/ L. On the previous day, the energy expenditure was on average 5380 kcal and the distance moved by skiing was 24.6 km. Based on these, day three has the highest physical stress which could partly explain the high cortisol concentration on the next morning. While there is certainly some error in these measurements, the saliva cortisol assay still has over 90% mean specificity and sensitivity on average (Zhang et al. 2013). We could

conclude that it is a valid estimate of cortisol concentration when we are comparing group averages and the most practical way to measure cortisol changes during survival training. However, it should be noted that multiple variables can affect a single saliva measurement such as acute subjective stress before the measurement or eating frequency before the measurement (Pritchard ym. 2017; Ljubijankić ym. 2008).

The significant increase in saliva cortisol during the survival training is supporting earlier findings (Szivak et al. 2018; Vikmoen et al. 2020). Fellman et al. (1992) found that during a 6-day Nordic ski race, the concentration of cortisol was almost twice as high during the first two days of competition compared to a pre-race level. Likewise, the study of an 8-week military training course showed that saliva cortisol concentration increased by over 100% from the first week to the fourth week (Bernton et al. 1995). The high resting cortisol concentration is an indicator of adrenal stress and can cause catabolic hormonal changes (Nindl et al. 2007). Also, the cortisol values can indicate the ability of the soldier to execute the mission. The impaired response of the adrenal gland to the stressful situation has an impact on soldiers' readiness and resilience and hyper-responsivity of the adrenal cortex can be a sign of dysregulated response to the stressor. Thus, resting cortisol values set the stage for the soldier's response to stressful situations and can be monitored to evaluate the soldier's performance. (Szivak et al. 2018.) Cortisol can also impact memory by blocking memory retrieval and enhancing memory consolidation. The memory consolidation effect is often stronger for emotionally arousing content (Wolf 2009). One goal for survival training is to prepare a soldier for extremely stressful situations in combat. Following cortisol response and memory, functions could be useful to monitor a soldier's executive functions during a stressful situation. Future studies should focus to investigate how survival training impact memory and cortisol together since most of the studies done by memory function and cortisol has been done with civilians (Wolf 2009).

During recent years saliva alpha-amylase has become a valid and reliable marker of autonomic nervous system activity in stress (Ali & Nater, 2020). This still quite new noninvasive marker of stress has been introduced in several studies. Oliveira et al. (2010) found that monitoring saliva alpha-amylase concentration is an efficient tool for determining exercise intensity. The results of saliva alpha-amylase reactivity are promising as some studies have found that it would have a better sensitivity to acute stress compared to salivary cortisol (Brown et al. 2013). In the

present study, a significant increase in salivary alpha-amylase was found between pre-measurement and days 4, 7, 8, 9. However, no associations between saliva alpha-amylase and other markers of stress or physical fitness tests were observed. There was a drastic variance between subjects in daily measurements of saliva alpha-amylase which reduce the accuracy of it as an indicator of stress. In conclusion, there is still a need for future studies to evaluate the accuracy of current methods to measure saliva alpha-amylase.

As demonstrated by the NASA-TLX, there was a severe increase in subjective stress from the preparation phase to the field phase. This 95 % increase was highly significant and demonstrates the impact of high physical demands, caloric deprivation, coldness, and restricted sleep on psychological stress. The analysis of subjective stress from the survival training is beneficial for future training plans and task designs. It gives more understanding, together with physiological changes, how soldiers' total stress load is cumulating during the training period. To the best of our knowledge, there are no studies relating the survival training and NASA-TLX previously. Thus, comparing results to previous studies is limited. However, other studies have been used other questionnaires to evaluate subjective stress (Liebermann et al. 2016; Chester et al. 2013). Chester et al. (2013) study from the 1-week survival training revealed a 137 % increase in perceived fatigue and a 40 % increase in Kessler-10 total scores which is designed to assess subjective distress. These results are indicating that subjective stress questionnaires are potential measurements to evaluate stress load from the survival training.

The evidence of physical strain was observed from decrements in physical fitness tests between timepoints. The only exception was maximal strength of the lower extremities where the strength levels were maintained across timepoints. The decline in physical fitness is expected because of the nature of survival training. As the results showed, there was a considerable energy deficit during the training days due to high physical activity and restricted energy intake. As the previous studies have shown, the energy deficit itself is not a major factor influencing physical performance (Zachwieja et al. 2001; Gutierrez et al. 2001). The decrements in physical performance during prolonged energy deficit are attributed because of the reduction in fat-free mass (Murphy et al. 2018) and studies have concluded that less than 10% loss in body mass did not impair muscle strength or VO_{2max} (Taylor et al. 1957; Friedl 1995). Based on the present study, severe energy deficit combined with high physical strain had a considerable impact on

physical performance even without 10% loss in body mass. This finding gives more insight into the severe energy deficit combined with physical strain and how it might impact on soldier's performance.

The unexpected finding was the maintained maximal isometric strength of the lower extremities throughout the measurements. The possible explanation might be that subjects failed to reach maximum effort in the PRE without a separate familiarization phase. Another reason could be that most of the moving was done by skiing which also requires upper-body work, thus decreasing the amount of work required from legs compared to walking. For example, Hamarsland et al. (2018) observed a 20 % decrease in leg press after 1-week survival training where the main activity was walking with a backpack. In contrast to walking, skiing does not include similar eccentric contractions to absorb shocks from the contact to the ground which leads to increased muscle damage (Eston et al. 1995). It might be that due to this, the amount of muscle damage of the lower extremities was small, thus decreasing the time to need to recover before the next physical fitness test measurement. It should also be noted that there were some limitations in physical tests. First, the number of subjects that performed each of the dependent measures varied due to factors outside of research staff control. Second, not all the physical tests were done at every timepoint. This makes it harder to compare changes between PRE and POST since there was a different timepoint for POST depending on the specific test.

Investigation of the relationships between physical performance and stress revealed a correlation between maximal isometric strength of the lower extremities and cortisol change from the preparation phase to the field phase. This inverse correlation was only moderate ($r=0.61$), but it gives a good indication of where the target should be in the soldier's strength training. Other studies have also highlighted the importance of maximum strength on the lower extremities for the soldier. Nindl et al. (2013) summarize that today's battlefield requires the soldier to carry heavy equipment under anaerobic demands. The essential part to execute tasks at a high level is the ability to wear personal protective equipment and carry other equipment at the same time. Orr (2010) found that the average load carried by soldiers in Afghanistan was around 45kg. Also in our study, subjects were required to carry heavy loads. Orr et al. (2019) showed that the strength of the lower body predicts load carriage performance and is an important factor for load carriage ability. This partly explains the relationship between

isometric strength of the lower extremities and cortisol change in the present study since there were harsh requirements to carry heavy equipment throughout the field training period. Thus, we can assume that higher strength levels of the lower extremities would help to survive with lower stress levels measured from cortisol. This is especially important because the cortisol change has been found to correlate with perceived stress and fatigue (Tavares et al. 2017; Schlotz et al. 2004).

The strength of the upper extremities should not be forgotten as there was also an association between maximal isometric strength of the upper extremities and subjective stress. The subjective stress increased by 95 % respectively from the preparation phase to the field phase. Based on the present study, maximal isometric strength of the upper extremities was inversely associated with subjective stress explaining 14.6 % of the difference in subjective stress between subjects. Other studies have also found increased demands of mental and psychological functioning and decreased mood during survival training (Liebermann et al. 2016; Chester et al. 2013). This finding underlines the importance of strength of the upper extremities for a soldier. By combining these findings between the strength of upper-body and subjective stress and the strength of lower-body and cortisol, we can conclude that whole-body strength can have a remarkable role in the performance and cumulative stress of soldiers during survival training. As we know, maximum strength has also other benefits, for example, injury prevention (Bahr & Krosshaug 2005; Myer et al. 2005). Because the evidence for the positive outcomes of the strength training for a soldier begins to be convincing, strength training should be taken seriously when planning the military physical training plan. Typical military training itself is not sufficient to produce gains in strength and power so there is a need for programmed weight training (Kyröläinen et al. 2018; Ojanen et al. 2020; Vaara et al. 2021; Groeller et al. 2015).

Interestingly the regression analysis also showed that the standing long jump and medicine ball throw results before the training period predicted 11 % of the change in average fitness test results between PRE and POST. Nevertheless, this finding did not reach statistical significance ($p=0.12$) and for that reason, it is not reported in the result section. It is still supporting earlier findings that explosive strength capacity is an essential characteristic for the soldier. (Pihlainen et al. 2017; Chester et al. 2013). Earlier studies have shown an association between explosive

strength and soldier's performance in military simulation test, sprint time in the combat load, and anaerobic military task course performance (Pihlainen et al. 2017; Mala et al. 2015).

Thus, future studies should replicate measurements of the present study with a larger sample size to conclude whether explosive strength could also predict total change in physical fitness during survival training. About evaluating stress adaptations, future studies should also consider implementing more broad scale of biomarkers to assess physiological changes during the survival training. For example, neuropeptide-Y and testosterone could be useful to create a more reliable conclusion about physiological stress adaptations during the survival training period (Szivak et al. 2018). Also, the muscle biopsies would be interesting to include for the evaluation of the amount of muscle damage in response to the survival training (Hamarsland et al. 2018).

Conclusions & practical implications

The present study demonstrates physiological and psychological changes during 10-day winter survival training. There were substantial decrements in physical performance except for the isometric strength of the lower extremities. Also, the subjective stress, saliva cortisol, and alpha-amylase increased significantly during the training period. The results between physical performance and stress markers indicate that strength of the lower extremities before the training period is associated with cortisol change during the training period. In addition, the strength of the upper extremities before training predicted lower subjective stress during the training period.

These results give more profound information about human physiology under survival training in the winter environment which is needed to help optimize military training and performance. Especially the maximum strength should be emphasized based on the findings that sufficient strength of the lower- and upper-body is protecting from stress measured by cortisol and subjective stress. Previous studies from the last decade have also noticed the importance of the maximum strength and explosive power for a soldier (Groeller et al. 2015; Pihlainen et al. 2017; Chester et al. 2013).

Another practical implication is concerning the relationship between severe energy deficit and physical performance. The severe energy deficit combined with high physical strain led to decrements in physical performance. Future training plans should evaluate energy intake during the survival training with care to prevent excessive decrements in physical performance. Severe decrements in physical performance can also influence the length of the recovery period after strenuous survival training. All these aspects should be considered when planning the survival training.

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