

Ilkka Väänänen

**PHYSIOLOGICAL RESPONSES TO DAILY REPEATED PROLONGED
WALKING**

Licentiate thesis, University of Jyväskylä
Faculty of Sport and Health Sciences
Department of Biology of Physical Activity
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ABSTRACT

Väänänen Ilkka. Physiological responses to daily repeated prolonged walking.

The purpose of this study was to describe physiological responses to daily repeated acute but non-competitive prolonged walking to the cardiorespiratory, autonomic nervous, musculoskeletal and hormone systems. The data of this quasi-experimental short-term follow-up (reversal) field trial was collected from healthy, 23 to 48 years old Finnish male soldiers in 1993 (preliminary study, $n = 6$) and the primary study ($n = 15$), in 1994, during the "International Four-Day Long-Distance March" in Nijmegen, The Netherlands. The acute cardiovascular responses were estimated by measuring the heart rate during the marches. The responses of the autonomic nervous system were estimated by measuring the heart rates during the orthostatic test. The musculoskeletal responses were estimated by measuring the perceived pains, flexibility, functional strength, use of elastic energy and oedemic changes of the lower extremities. The hormonal responses were estimated from the concentrations of serum testosterone, luteinising hormone (LH), follicle stimulating hormone (FSH) and cortisol, and the urinary excretion of catecholamines. The mean daily walking distance was 46 (preliminary) and 41 (primary study) km and the average speed from five to seven km per hour. Averaged heart rate during walking was 109 ± 9 (preliminary) and 108 ± 6 (primary study) beats per min. Morning heart rate in the supine position increased progressively through the marching period. After the first march the perceived pain increased significantly and remained at similar increased level until the end of the marching period. Leg measurements showed no signs of oedema, decreases in flexibility, or functional strength. The responses on serum testosterone and cortisol concentrations were minor. LH concentration decreased during the second and third day by 31% and remained steady on the fourth day. In FSH concentration the suppression was seen all the time after the second march. Catecholamine excretion rates indicated cumulatively increased sympathoadrenal stress. This study demonstrates that pituitary-gonadal axis, excluding the secretion of FSH and the adrenal cortex, adapted to repeated prolonged walking. However, when using the sensitive IFMA, which can detect low concentrations of gonadotropins, secretion of FSH was seen to remain reduced and no adaptation was seen. This study indicated that a daily repeated long lasting acute but non-competitive walk of intensity at approximately 60% of maximum heart rate which (100-120 beats per min) is well within the physiological capabilities of individuals with good aerobic capacity.

Key words: Hormones, functional capacity, lower extremities, muscle soreness, adaptation, recovery, walking

TIIVISTELMÄ

Väänänen Ilkka. Päivittäin toistetun pitkäkestoisen kävelyn fysiologiset vasteet.

Tämän puolikokeellisen kenttätutkimuksen tarkoitus oli kuvata sydämen ja verenkierto- sekä tuki- ja liikuntaelimestön että autonomisen hermoston ja hormonitoiminnan vasteita päivittäin toistettuun pitkäkestoiseen kävelysuoritukseen. Aineisto kerättiin Hollannin Nijmegenissä järjestettävän "Neljän Päivän Marssin" aikana terveiltä, 23 – 48 -vuotiailta suomalaisilta miessotilailta vuosina 1993 (esitutkimus, n = 6) ja 1994 (varsinainen tutkimus, n = 15). Sydämen ja verenkiertoelimestön kuormittumista mitattiin marssinaikaisella sykeseurannalla. Autonomisen hermoston toiminnan säätelyä tutkittiin ortostaattisella sykereaktiotestillä. Alaraajojen toimintakykyä mitattiin kipukyselyllä, maksimaalisilla ponnistushypyillä, nivelten liikelaajuusteilla sekä reisien ja pohkeitten ympärysmittauksilla. Hormonitoiminnan vasteina analysoitiin seerumin testosteroni-, gonadotropiini- ja kortisolipitoisuuksia sekä katekoliamiinien eritysnopeutta virtsaan. Päivittäinen kävelymatka oli ensimmäisenä vuonna 46 km ja toisena 41 km. Kävelynopeus vaihteli viidestä seitsemään kilometriä tunnissa. Aamulla mitattu leposyke nousi koko jakson ajan. Ensimmäisen päivän jälkeen kiputunteukset lisääntyivät merkittävästi ja ne säilyivät koholla koko marssitapahtuman ajan. Alaraajojen toimintakyvyssä ei tapahtunut muutoksia. Testosteroni- ja kortisolipitoisuuksien muutokset olivat pieniä, mutta luteinisoivan hormonin pitoisuus laski toisen ja kolmannen marssin jälkeen 31 % ja se säilyi neljännen päivän alentuneella tasolla. Follikkelia stimuloivan hormonin pitoisuus laski koko ajan toisesta päivästä lähtien. Katekoliamiinien eritysnopeudet osoittivat kasaantuvaa sympatoadrenaalista kuormittumista. Tämä tutkimus osoitti, että aivolisäke-kives-akseli, lukuunottamatta follikkelia stimuloivan hormonin ja katekoliamiinien eritystä, sopeutuu toistettuun pitkäkestoiseen kävelyy. Tämä tutkimus osoitti hyväkuntoisten miesten pystyvän marssimaan neljän päivän ajan noin 60 prosentin teholla maksimaalisesta syketasosta ilman merkittäviä fysiologisia haittoja.

Avainsanat: Hormonit, toimintakyky, alaraajat, lihaskipu, sopeutuminen, palautuminen, kävely

PREFACE

This study has been performed in LIKES-Research Center, Jyväskylä. This licentiate thesis is based on 3 articles (Väänänen, I., Mäntysaari, M., Huttunen, P., Komulainen, J., & Vihko, V. *The effects of a 4-day march on the lower extremities and hormonal balance*. *Milit Med* 1997; 162:118-122; Väänänen, I., Mäntysaari, M., & Vihko, V. *Soldiers' physiological and psychological loading during a 4-day march*. *Ann Med Milit Fenn* 2001; 76:59-67 and Väänänen, I., Vasankari, T., Mäntysaari, M., Vihko, V. *Hormonal responses to daily strenuous walking during 4 successive days*. *Eur J Appl Physiol*, in press).

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I dedicate this work to my son Ville, and to the loving memory of my other son; Alekski.

“Nobody knows how long he can continue until he has tried.”

Goethe

Villähde, Alekski’s Day, October 10th, 2002

Ilkka Väänänen

Aleksi in memorium

1 INTRODUCTION

“I never give up on walking, it gives me pleasure. It has given my best ideas. I don't know any bad thoughts, which don't disappear during walking.”

Sören Kirkegaard

Regular participation in physical activity results in positive health-related outcomes and benefits but there could be also risks and negative effects if the total amount of physical activity is too large. The injury risk of daily fitness walking is 0.2 and 1.3/1 000 walking hour in race walking (Parkkari, Kannus, Natri, Lapinleimu, Palvanen, Heiskanen, Vuori & Järvinen, 2001). Physical exercise causes a disturbance of homeostasis in cells and organs, which may result in decreased mechanical output of performance and fatigue. In the recovery phase homeostasis has to be re-established and the body will respond to stress in order to adapt. The regenerative processes still continue after restoration of the previous homeostatic situations, which results in an overcompensation or supercompensation (Harre, 1975; Viru, 1984). Ideally, subsequent exercise/training session should not take place until supercompensation has occurred (Harre, 1975).

Training and competitions in endurance events consist of daily long lasting sessions. There is uncountable number of extreme sustained exercises for professional and recreational sportsmen to participate in e.g., *Tour de France*, *Paris-Brest-Paris* and *Finlandia-Cycling* for cyclists, *Finlandia-Running* for runners, *International Arctic Circle Skating Week* for skaters, *Mountain-Orienteering* for orienteerers, *Päijänne-Rowing* for rowers, *Finlandia-Paddling* for paddlers, and *Raid Galoises* and *Ecochallenge* for extremers. For walkers there are more than ten multiple day ‘overlong’ walking events (e.g., Austria: *Mödling*, Belgium: *Blankenberge*, Denmark: *Viborg*, England: *Wellingborough*, Ireland: *Castlebar*, Japan: *Higashimatsuyama*, Luxembourg: *Diekirch*, The Netherlands: *Nijmegen*, Norway: *Verdal*, Switzerland: *Bern*, Taiwan: *Taipei*) in the world and even two in Finland (*Summer Night March* and *Vaasa March*) nowadays, too. These fitness events are favoured by not only among regularly training and competitive athletes but also by normal population. From practical aspect it would be important to know, what kind of strain these strenuous mass sporting events cause and what kind of persons are able to sustain the effort without dropping out.

The acute effects of a single bout of several types of physical exertions are quite well known, as well as the effects of long-term physical training, but much far less is known about the physiological responses to daily repeated strenuous physical exertions and the loading vs. recovery/adaptation. An important question is, whether there is a limit beyond which signs and symptoms of over-dosage may develop.

After ‘the jogging boom’ in the mid-1980s, walking has emerged as an alternative activity to promote health and fitness. Moreover the year 1999 was nominated as “The Year of Walking” by eight Finnish associations. Approximately two million adult Finns, 68% of the population, report walking regularly for recreation. Walking is the most popular fitness-related activity of the Finnish adult population and in Europe, too (de Almeida,

Graca, Afonso, D'Amicis, Lappalainen & Damkjaer, 1999). In year 1990 the number of exercise walkers was estimated to be approximately 66.6 million in America (Spelman, Pate, Macera & Ward, 1993).

To understand the suitability of a given exercise, we must understand the specific effects of that exercise-form from the molecular level to the effects on the total body (Edington & Edgerton, 1976, 4). Therefore, the purpose of this study was to describe the physiological responses to daily repeated acute but non-competitive prolonged walking to the cardiorespiratory, autonomic nervous, musculoskeletal and hormonal systems in healthy, Finnish male soldiers, who marched 163 to 185 km over four days.

2 REVIEW OF THE LITERATURE

2.1 Terminology

The term *load* is defined as external forces that act upon a body (Nigg, Denoth, Kerr, Luethi, Smith & Stacoff, 1984) and *overload* is a load greater than the rated load which can cause damage (International dictionary of medicine and biology, 1986, 2048). *Overloading* is the condition resulting from excessive sensory stimulation, in which the stimuli are too intense or too rapid for an individual to respond appropriately (International dictionary of medicine and biology, 1986, 2048).

Homeostasis is the relative stability or constant condition of the internal environment of an organism which is preserved through feedback mechanisms despite the presence of influences capable of causing profound changes and those processes considered collectively by normal organisms which homeostasis is maintained (Guyton & Hall, 2000, 3; International dictionary of medicine and biology, 1986, 1331).

Steady state is any condition that remains constant at a given point in time because of the presence of opposite forces on processes that cancel out one another's effects (International dictionary of medicine and biology, 1986, 2692).

Supercompensation is a state of improved work capacity, above the level of which the person has recently been capable. This has been characterised as a state of balanced homeostasis with homeostatic markers reflecting either baseline values or improvements, depending upon the nature of the variable (Fry, Morton & Keast, 1991).

Adaptation is the advantageous change or changes of behavior, physiology, or structure by which an organism modifies itself to fit into a particular environment. A nongenetic change in a organism that takes place in response to an environmental stimulus and a progressive reduction in the sensitivity of a sense organ following prolonged exposure to the same sensory stimulus are defined as *adaptation* (International dictionary of medicine and biology, 1986, 2692), too.

Physical activity (PA) is defined as any body movement produced by contraction of skeletal muscle that substantially increases energy expenditure. The dose is used to describe intensity, frequency, duration, mode/type, and purpose of PA (Bouchard & Shephard, 1994, 77; Howley, 2001; U.S. Department of health and human services, 1996). Frequency is described as the number of activity sessions per day, week, or month, and duration typically refers to the length of activity in each session. Intensity describes, in relative or absolute terms, the effort associated with the PA (Howley, 2001). Physical activity is frequently categorised by the context in which it occurs (U.S. Department of health and human services, 1996).

Leisure-time physical activity (LTPA) is a broad descriptor of the activities one participates in during free time e.g., dance, gardening, hiking, walking, etc. based on personal interests and needs. The common element is that these activities result in increased energy expenditure, although the intensity and duration can vary considerably (Bouchard & Shephard, 1994, 77). The absolute intensity of LTPA describes the actual rate of energy expenditure (Howley 2001).

Occupational physical activity (OPA) is associated with the performance of a job, usually with the time frame of an 8-h work day (Howley, 2001).

Training is the result of biological adaptations achieved after repeated exercise bouts over a period of several days, weeks, or months of exercise (Edington & Edgerton, 1976, 8).

Exercise (exercise training) is usually performed on a repeated basis over an extended period of time and it is planned, and structured to improve or maintain one or more components of physical fitness (Bouchard & Shephard, 1994, 78; U.S. Department of health and human services, 1996). Different exercises have different biological requirements and could be classified according to the speed of movement, resistance to that movement, and duration or the time over which that movement has to be repeated (Edington & Edgerton, 1976, 4-7).

Overloading training is the process of stressing an individual to provide a stimulus for adaptation and supercompensation (Fry et al., 1991).

Training fatigue/stress is the normal fatigue that is experienced followed by several days of heavy training associated with an overloading stimulus. This fatigue is reversed and supercompensation occurs by the last few days of a period of reduced training load (regeneration microcycle) (Fry et al., 1991; Kuipers & Keizer, 1988).

Exhaustion is the result of the body's inability to meet the exercise demands (Edington & Edgerton, 1976, 3).

Overtraining and *physical overstrain* are the non-differentiate general terms for any short- or long-term condition which indicates that the individual has been stressed by training and extraneous stressors to the extent that a person cannot perform at an optimum level following an appropriate regeneration period (Fry et al., 1991; Kuipers & Keizer, 1988; U.S. Department of health and human services, 1996).

Overreaching is the state in which an accumulation of training stress results in a short-term decrement of performance capacity with or without related physiological and psychological signs and symptoms of overtraining, and in which the restoration of performance capacity may take from several days to several weeks (Budgett, 1990; Fry et al., 1991; Fry & Kraemer, 1997; Kuipers & Keizer, 1988).

Overtraining syndrome and *staleness* are the states of the long lasting imbalance between training and recovery. *Muscular overstrain* occurs when the muscular stress tolerance is exceeded by exercise, resulting in transient local fatigue and muscle soreness and it may be considered as local overtraining (Kuipers & Keizer, 1988).

Training stressors are those resulting from the physical, physiological, and psychological stress induced by the training workloads administered during overload training. *Extraneous stressors* are those resulting from activities and psychological forces related to lifestyle (Fry et al., 1991).

2.2 Loading during physical exercise

The human body is capable, to some degree, to actively adapt to external forces. The degree of adaptation may be connected with the frequency components in the acting forces. Load is dependent on the external and internal influences, and movement. External parameters could be equipment (e.g., backpack), shoe and surface. Internal factors are the anthropometric facts and the individual situation (from both a physiological and a psychological point of view). Movement may influence the load of the human body concerning the type as well as the frequency of a certain type of movement (Nigg et al., 1984). A specific exercise will elicit a specific response in a specific individual at a specific point in time (Edington & Edgerton, 1976, 7). The attention in exercise loading focuses on factors such as frequency and length of workouts, type of exercise, speed, intensity, duration, and repetition of the activity. It also requires that the progress of the load is observed, as well as the recovery intervals (Fowler, 1983).

If the volume of the physical activity is too large or the recovery is too short training could lead to problems. There are many risk factors for training injuries which could be categorised either as intrinsic or extrinsic in nature (Jones & Knapik, 1999). Intrinsic factors are inherent characteristics of individuals (e.g., age, race, gender), anatomical characteristics (e.g., *pes cavus*, *genu valgus*), physical fitness (e.g., low cardiorespiratory or muscle endurance, high and low flexibility), and lifestyle and behavioural characteristics (e.g., physical activity, smoking). Extrinsic factors are external to the individual, such as physical training programme (e.g., high running mileage, frequent marching and running), equipment, terrain and weather conditions, which influence the risk of injury.

Foster (1998) has developed simple methods of monitoring the characteristics of training. First the participant is asked to rate the global intensity of the entire training session by Borg's RPE scale (Borg, 1973). Then this intensity score is multiplied by the duration of the training session. A single number representing the magnitude of that training session is derived and it is called the session "load". The training load during each week is summated to create a weekly training load. Additionally, the daily mean training load as well as the standard deviation of training load are calculated during each week. The daily mean divided by standard deviation is calculated as training "monotony". The product of the weekly training load and monotony was calculated as training "strain" (Foster, 1998).

2.3 The mechanisms of adaptation

Adaptation to life conditions, change in the external environment and any kind of bodily activity are always directed toward maintaining or restoring the constancy of the internal milieu of the body (Viru, 1984; Viru & Smirnova, 1995). In situations that require the activation of adaptation processes, the main events within an individual are described by Viru and Smirnova (1995) in the following manner: “The agent (stressor) acts, by various pathways, on the structures of the central nervous system. If the required intensity of the homeostatic reactions is high, or it is necessary to maintain them for a prolonged duration, the mechanisms of general adaptation (mobilisation of energy reserves and protein resources, and activation of defence faculties) will be activated.” During the recovery period after acute adaptation, and to a lesser extent also during adaptation, the dynamic reserves are used extensively for the adaptative synthesis of the enzymes and structural proteins to restore the functional capacity of cellular structures that had been highly active during acute adaptation. Depending on the intensity of the inductive stimulus of adaptive protein synthesis, it may result in the production of such an amount of proteins to warrant further development of related functional possibilities. The latter is founded on the morphological and metabolic improvement. If the action inducing such adaptive synthesis of proteins is repeated with sufficient frequency, a stable adaptation develops together with elevated levels of morphological and metabolic improvement of related cellular function. (Viru, 1984)

In Edington & Edgerton’s (1976, 10) model the daily exercise “sets up” the body so that subcellular mechanisms are stimulated to bring about those adaptive changes that characterise the “trained state” i.e. something specific about the act of the exercise stimulates the cells to adapt so as to be more prepared to protect against this same exercise stress. It is highly likely that the exercise “sets up” the cell, while the actual adaptation occurs during the recovery phase. Both short-term and long-term adaptations take place within the cells’ response to an exercise stress. Short-term adaptations, which occur during the actual exercise, mainly involve the conversion of an inactive component to active chemicals. Long-term adaptations, which account for the primary training adaptations, are mainly concerned with increased amounts of primary proteins (Edington & Edgerton, 1976, 10).

2.4 Physiological responses to exercise

When challenged with any physical task, the human body responds through a series of integrated changes in function that involve most, if not all, of its physiological systems (e.g., cardiorespiratory, nervous, musculoskeletal, hormone and immune systems).

2.4.1 The responses of the cardiorespiratory system to exercise

The primary function of the cardiorespiratory system is to provide the body with oxygen (O₂) and nutrients, rid the body of carbon dioxide (CO₂) and metabolic waste products,

maintain body temperature and acid-base balance, and transport hormones from the endocrine glands to their target organs (e.g., Guyton & Hall, 2000, 144).

The cardiorespiratory system responds predictably to increased demands of exercise. It is directly proportional to the skeletal muscle oxygen demands and oxygen uptake (VO_2) increases linearly with increasing rates of work (e.g., McArdle, Katch & Katch, 2000, 290-291). Oxygen uptake (VO_2 , $\text{ml} \cdot \text{min}^{-1}$) during walking with centrally carried load and heavy footwear has been calculated for men as follows:

$$\text{VO}_2 = 4.1m_b + 0.367(m_b + m_{load}) \cdot v^2 + 2.017m_{shoe} \cdot v^2 \text{ (Holewijn, Heus & Wammes, 1992)}$$

where m_b is body mass (kg), m_{load} is mass of centrally carried load (kg), m_{shoe} is shoe mass (kg), v is walking velocity ($\text{km} \cdot \text{h}^{-1}$).

2.4.2 The responses of the autonomic system to exercise

A large segment of the nervous system is called the autonomic system. It operates at a subconscious level and controls many functions of the internal organs, including pumping activity of the heart, movements of the gastrointestinal tract, and glandular secretion (e.g., Guyton & Hall, 2000, 4). Reduced basal (morning) heart rate is a classic effect of functional adaptation to the endurance exercise training (e.g., McArdle et al., 2000, 200, 370). On the contrary, elevated morning heart rate may be accompanied in overreaching and may reflect an early stage in the development of the overtraining state (Dressendorfer, Wade & Scaff, 1985; Dressendorfer, Hauser & Timmis, 2000; Kuipers & Keizer, 1988). At rest, the heart rate (HR) depends on complex neurohumoral interactions (Dressendorfer et al., 1985): Afferent nerve traffic from specialized receptors sensitive to pressure, volume, or chemical changes in the heart, blood vessels, lungs or kidneys; reflex and tonic discharge of cardiovascular centers in the brain stem that receive incoming afferent impulses and higher commands from the hypothalamus and cerebral cortex; the balance of efferent impulses in sympathetic (adrenergic) and parasympathetic (cholinergic) nerve fibers to the cardiac pacemaker; activity of beta-adrenergic and cholinergic membrane receptor sites; the influence of circulating catecholamines; and the intrinsic rate of pacemaker discharge. In addition, local temperature and pH, substrate utilization for energy metabolism could modify heart rate (Dressendorfer et al., 1985; Roussel & Buguet, 1982). The initial changes in HR after standing up are solely mediated by withdrawal of vagal tone (Ewing, Hume, Campbell, Murray, Neilson & Clarke, 1980).

2.4.3 The responses of the musculoskeletal system to exercise

Delayed onset muscle soreness (DOMS) is often observed in subjects unaccustomed to exercise (Appell, Soares & Duarte, 1992; Ebbeling & Clarkson, 1989; Kuipers, 1994) or related to unusually prolonged or strenuous (Dressendorfer & Wade, 1983; Miles & Clarkson, 1994) exertion. Decreased flexibility, stiffness, impaired neuromuscular performance as well as oedema and muscle soreness and pain are typical signs of DOMS (e.g., Armstrong, 1984; Cleak & Eston, 1992). The idea that an unusual increase in physical activity may be associated with muscle tissue damage prevails widely (e.g.,

Dressendorfer, Wade, Claybaugh, Cucinell & Timmis, 1991; Koplán, Powell, Sikes, Shirley & Cambell, 1982). For example, a marathon run can cause an acute loss of muscle function (Nicol, Komi & Marconnet, 1991a, 1991b; Kyröläinen, Pullinen, Candau, Avela, Huttunen & Komi, 2000; Sherman, Armstrong, Murray, Hagerman, Costill, Staron & Ivy, 1984) and increased turgidity may be found as a symptom of overreaching or short-term overtraining (Kuipers & Keizer, 1988). A sensation of discomfort is most evidenced in skeletal muscle 1 to 2 days following exercise (MacIntyre, Reid & McKenzie, 1995).

2.4.4 The hormonal responses to exercise

The hormone system integrates physiological responses and plays an important role in maintaining homeostatic conditions at rest and during exercise. It consists of eight major endocrine glands that secrete chemical substances called hormones which are transported in the extracellular fluid to all parts of the body to help regulate cellular function (Guyton & Hall, 2000, 5, 836). The acute response of a single bout of prolonged physical exercise as well as that of regular physical training on anabolic and catabolic hormones is well documented (Brisson, Quirion, Ledoux, Rajotte & Pellerin-Massicotte, 1984; Dessypris, Kuoppasalmi & Adlercreutz, 1976; Dessypris, Wäger, Fyhrquist, Mäkinen, Welin & Lamberg, 1980; Guglielmini, Paolini & Conconi, 1980; Tanaka, Cléroux, de Champlain, Ducharme & Collu, 1986; Vasankari, Kujala, Taimela & Huhtaniemi, 1993).

However, scattered information exists on the effects of daily repeated prolonged submaximal exercise. Some field exercise studies have included sleep deprivation or low energy diet, or both (Aakvaag, Benthall, Quigstad, Walistad, Rööningen & Fonnum, 1978; Aakvaag, Sand, Opstad & Fonnum, 1978; Opstad, 1992). After 20 days of running (1 100 km), testosterone and cortisol concentrations decreased (Schürmeyer, Jung & Nieschlag, 1984). After ten successive days of intensive training, serum cortisol was elevated in competitive swimmers (Kirwan, Costill, Flynn, Mitchell, Fink, Neuffer & Houmard, 1988). On the third skiing day the elevation of the cortisol concentration ceased (Fellmann, Bedu, Boudet, Mage, Sagnol, Pequignot, Claustrat, Brun, Peyrin & Coudert, 1992). However, no change was detected in serum gonadotropins using the radioimmunoassay (RIA), a far less sensitive method for detecting very low concentrations of gonadotropins than the immunofluorometric assay (IFMA) (Huhtaniemi, Ding, Tähtelä & Välimäki, 1992; Jaakkola, Ding, Kellokumpu-Lehtinen, Vaaralahti, Martikainen, Tapaninen, Rönberg & Huhtaniemi, 1990; Lucia et al., 2001).

Exercise-induced changes in testosterone concentration can be caused by a change in the production rate, altered binding or clearance (Tremblay, Chu & Mureika, 1995). Several other mechanisms (haemoconcentration, decreased hepatic blood flow) could be involved, too (Tremblay et al., 1995). During prolonged exercise, changes in serum testosterone concentration may be caused by direct testicular suppression or mediated through the hypothalamus-pituitary level (Aakvaag et al., 1978a). Tanaka et al. (1986) concluded that the cause is reduced testosterone secretion which occurs in spite of increased stimulation of the hypothalamic-pituitary unit.

The central nervous system (CNS) maintains and regulates cortisol production and secretion through the hypothalamus-pituitary-adrenal axis. Cortisol is being secreted in

bursts which are superimposed on a circadian rhythm that has its peak in the early morning hours and its nadir at the beginning of sleep (Kuhn, 1989). The response of cortisol to physical exercise is caused by a rise in adrenocorticotrophic hormone (ACTH) (Schwartz & Kindermann, 1990) and is best seen 20-30 min after the stimulus (Kuhn, 1989). Another theory for the exercise induced changes in serum cortisol was proposed by Galbo (1981), who concluded that it is removed from plasma at higher rates during work loads below 50% of maximal oxygen uptake (VO_{2max}) than at rest.

2.5 Walking as an exercise mode

Walking is a comfortable exercise type for all adults, and it does not require special facilities or exercise equipment, except shoes. Walking campaigns (e.g., Bjärås, Härberg & Östenson, 1999; Ståhl & Laukkanen, 2000, 35-36) have been arranged to get people involved in physical activity. Walking is moving forward in such a way that the full body-mass has permanent contact with the ground, alternately via the right and the left foot. It is a rhythmic, aerobic activity involving large muscle groups that confers several benefits for fitness and health with minimal adverse effects (Morris & Hardman, 1997). In 1997 68% of Finnish population was involved in walking and the EU average was 31% (de Almeida et al., 1999).

There are several types of walking (Ståhl & Laukkanen, 2000, 5). Soldier's rhythmic walking is *marching* and wandering in nature is called *hiking*. Load carriage by backpack is familiar with these. *Pace walking* is a walking pattern where the pace varies from slow to fast, as in the interval type of training. *Power walking* has been developed to increase the intensity of walking by adding weights to the hands, wrists, ankles, and torso. Arms are kept at a 90-degree angle. *Fitness walking* and *health walking* both refer to walking programme designed to enhance fitness or health. *Nordic walking* exercise uses lightweight walking poles, similar to those used in cross-country skiing, to balance and make walking a more effective total body activity. *Snow shoe walking* with poles is a popular alternative for those willing to exercise in a wild natural surroundings during wintertime (Ståhl & Laukkanen, 2000, 5).

In research, walking has been included e.g., in the weight maintenance and weight reduction programmes (e.g., Fogelholm, Kukkonen-Harjula, Nenonen & Pasanen, 2000). In epidemiologic study (Manson, Hu, Rich-Edwards, Colditz, Stampfer, Willett, Speizer, & Hennekens, 1999) brisk walking was associated with reduction in the incidence of coronary heart disease among women. In a golf study (Parkkari, Natri, Kannus, Mänttari, Laukkanen, Haapasalo, Nenonen, Pasanen, Oja & Vuori, 2000) regular walking had many positive effects on the health and fitness of sedentary middle-aged men. Walkers during a golf game were characterised to have high adherence and low risk of injury and therefore it is considered as a form of health-enhancing physical activity (Parkkari et al., 2000).

2.6 Studies of prolonged walking with men in field conditions

Due to practical and theoretical interest to understand the effects of repeated exertions several studies (Table 1) have focused on daily repeated walking exercise with men in field conditions. Most of these studies are over ten years old and extended only a limited point of view, e.g., concentration on a few specific physiological variables. Walking distances have varied 35 to 630 km and follow-up time two to 42 days. The number of the subjects has varied between 1 to 97 and the oldest subjects have been over 70 years.

Table 1. A summary of the studies of daily repeated prolonged walking exertions with men in field conditions.

Study (authors, year)	Walk (km)	Number of walking days	Number of subjects	Measurements
Shapiro et al. 1973	110	2	26 untrained	Serum enzymes
Myles et al. 1979	204	6	25 soldiers	Self-paced work
Roussel and Buguet 1982	204	6	4 fit, young men	HR during sleep
Ross et al. 1983	35	3	15 training course men	Muscle enzymes
Davies et al. 1984	544	6	1 'non-stop' walker	Physiological, haematological and hormonal responses
Marniemi et al. 1985	344	7	10 fasted men	Biochemical parameters
Greenhaff et al. 1987	148	4	6 fasted and fed men	Metabolic response
Maughan et al. 1987	148	4	6 men	Metabolic response
Griffin et al. 1988	148	4	6 men	Plasma lipoproteins
Faber et al. 1992	630	42	11 hikers	Plasma lipoproteins
Hellsten et al. 1996	150	7	15 military trainees	Muscle damage

Shapiro, Magazanik, Sohar and Reich (1973) studied the relationship between maximal aerobic capacity and blood enzyme concentration (creatine phosphokinase, glutamic-oxaloacetic transaminase, aldolase, creatine, creatinine and sorbitol dehydrogenase) changes in 26 untrained subjects during a 110 km 2-day march. The highest enzyme elevation appeared in the subjects with the lowest maximal aerobic capacity and a moderate elevation in those with high maximal aerobic capacity (Shapiro et al., 1973).

Myles, Eclache and Beaury (1979) studied 25 infantry soldiers who marched 204 km in 6 days. The daily walking distances were 34, 34, 34, 30, 30.5, and 38.5 km at an average speed of $6 \text{ km} \cdot \text{h}^{-1}$ at the first day and at $6.5 \text{ km} \cdot \text{h}^{-1}$ during the next 5 days. An additional weight (22 to 24 kg) was assigned to each subject to ensure that they all worked at the

same percentage of their aerobic power (40%). The factor limiting performance for many of the subjects was the condition of their feet as a result of marching on the hard road surface (Myles et al., 1979).

Roussel and Buguet (1982) examined the effect of 6 days of moderate prolonged exercise on night-time heart rate during sleep in four fit, healthy young men. The length of the march was $34 \text{ km} \cdot \text{day}^{-1}$ and the speed was $6 \text{ km} \cdot \text{h}^{-1}$, with an intensity of 35% of individual $\text{VO}_2 \text{ max}$. After the exercise period the night time heart rates increased by about 10% as compared to the previous control condition, and returned to normal during the five days' recovery period. The increase was apparently not related to changes in body temperature, red blood cell content, sleep patterns, cortical adrenal or thyroid functions. The only possible explanation for the nocturnal tachycardia was related to a probable increased sympathoadrenal activity (Roussel & Buguet, 1982).

In Ross, Attwood, Atkin and Villar's study (1983) after one day's severe exercise (not detailed) and two days' walking (15 and 20 km, respectively) serum creatine kinase concentration was seven times higher than the rest level.

In Marniemi, Vuori, Kinnunen, Rahkila, Vainikka and Peltonen's (1984) study ten men hiked 344 km during seven days. The daily walking distances were from 36 to 67 km at an average speed of $3.5 \text{ km} \cdot \text{h}^{-1}$. Estimated energy consumption corresponded to $3.5 \text{ kcal} \cdot \text{min}^{-1}$ yielding in total about 84 MJ (20 Mcal). During the exercise men were allowed to drink water, mineral drinks, and juices ad libitum. Except for some natural products, no food intake was allowed. Total caloric intake during the hike was about 24 MJ (5 700 kcal) and the sleeping time was $5 \text{ h} \cdot \text{night}^{-1}$. The body mass and serum protein concentration of the subjects decreased by about 7%, on average. Serum cortisol in the evening after the daily hiking and plasma noradrenaline concentrations were significantly increased and serum testosterone levels decreased, reflecting the immediate daily response to the combined fasting and hiking. Hormonal stress adaptation was reached in three days. Decreased testosterone levels indicated the involvement of the LH-testis pathway (Marniemi et al., 1984).

Davies, Shapiro, Daggett, Gatt and Jakeman (1984) studied one male subject during 338 miles (130 h) of continuous walking and subsequent sleep deprivation. Creatine kinase and its isoenzyme levels rose throughout the walk. Catecholamine levels rose throughout the walk, with greater rises being observed in noradrenaline and dopamine. During the post-walk recovery phase, adrenaline concentration remained elevated (Davies et al., 1984).

Greenhaff, McCormick and Maughan (1987), Maughan, Greenhaff, Gleeson, Fenn and Leiper (1987), and Griffin, Skinner and Maughan (1988) studied the metabolic responses to prolonged walking on four consecutive days in fed and fasted men. Six healthy men walked 37 km per day on two occasions one month apart; during one walk they consumed a high carbohydrate (CHO) diet and during the other walk an isocaloric low CHO diet. Exercise intensity corresponded to approximately 17% of $\text{VO}_2 \text{ max}$. The first day of each walk demonstrated that the pattern of substrate mobilisation in response to this type of exercise is highly reproducible. Circulating glucose, lactate, insulin, and triglyceride concentrations remained essentially unchanged; alanine fell progressively and glycerol,

free fatty acids and 3-hydroxybutyrate rose progressively. Very low density lipoprotein cholesterol decreased and high density cholesterol increased when the subjects consumed a mixed diet. The results indicated that even in the overnight fasted state, substrate mobilisation during prolonged low intensity exercise is markedly influenced by the composition of the preceding diet.

Faber, Spinnler Benade, Celliers and Marais (1992) studied the effect of prolonged low intensity hiking over a period of six weeks on plasma lipids in 11 men. The subjects walked an average of 15 km per day, resting days included. They completed a seven-day estimated dietary record before and during the expedition. The authors concluded that increased physical activity during a hiking expedition together with drastic dietary changes (less protein and fat, and more carbohydrates as compared their habitual intake) and weight loss (73.8 to 68.2 kg) result in significant changes in plasma lipid levels (Faber et al., 1992).

Hellsten, Hansson, Jonson, Frandsen and Sjödén (1996) investigated the effect of seven days of strenuous (150 km) marching exercise on xanthine oxidase and insulin-like growth factor in skeletal muscles in 15 men. They observed an elevated expression of xanthine oxidase, insulin-like growth factor immunoreactivity and plasma creatine kinase activity after the exercise. They suggested that the increases were a result of cellular damage.

3 PURPOSE OF THE PRESENT STUDY

The aim of this work was to examine the magnitudes and the time courses of the physiological responses to daily repeated prolonged walking. The specific problems were to describe the responses of the four days' march on the

- 1) cardiovascular,
- 2) autonomic nervous,
- 3) musculoskeletal,
- 4) and hormone systems

in healthy Finnish male soldiers.

The focus was on the quantification of the amount of disadvantage caused by physical activity. Acute cardiorespiratory response was estimated by measuring the heart rate during the march. Responses of the autonomic nervous system were estimated by measuring the heart rates in an orthostatic test. Musculoskeletal responses were estimated by measuring the perceived pains, flexibility, functional strength, use of elastic energy and oedemic changes of the lower extremities. Hormonal responses were assessed using serum cortisol, testosterone and gonadotropins, and the urinary excretion of catecholamines.

The hypothesis of this study was that strenuous prolonged walking during four days would decrease the functional capacity of the lower extremities, and disturb the balance of the autonomic nervous system, and affect the secretion of hormones from adrenal cortex, and pituitary-testicular axis.

4 MATERIALS AND METHODS

The data of this quasi-experimental short-term follow-up (reversal) design (Cook & Campbell, 1979; Thomas & Nelson 1990, 311-313) were collected in 1993 (preliminary study) and 1994 (the primary study) during the “International Four-Day Long-Distance March” in Nijmegen, The Netherlands. The Ethical Committee of the University of Jyväskylä, Finland (Appendix 1) and the Defence Staff, Finnish Defence Forces, Department of the Health Care (Appendix 2) approved the arrangements of this study.

4.1 Subjects

Six (preliminary study) and 15 (primary study) physically active Finnish male soldiers (Table 2) volunteered for these studies after they had been fully informed and their signed informed consent was obtained. The subjects were required to carry a backpack weighing at least 10 kg. Apart from the participants, only one support person on bicycle was permitted per detachment. Night rest in tents commenced between 08.00 - 10.00 p.m., and ranged from 5 to 7 h in length. Consumption of food and beverages was not limited. No exercise was performed during two days before, or one week after the march.

The best result of subjects in the Cooper's 12-minute running test (Cooper, 1968) within the last two years was used to evaluate the endurance capacity of the subject. Maximal oxygen uptake and the related variables were measured from seven subjects of the primary study during an uphill walk on a treadmill to subjective maximal effort test (modified Pennsylvania State University protocol; Oja, 1973). The test began with a two-min warm-up at a five % grade setting and $5.0 \text{ km} \cdot \text{h}^{-1}$ speed. The test progression started at a five % grade for the first two minutes and continued with 2.5 % grade increases at two-min intervals up to a grade of 20 %, whereafter the speed was increased by $0.5 \text{ km} \cdot \text{h}^{-1}$ every other minute. Expired air was collected and analysed continuously with an automatic metabolic analyser (Beckmann MMC, Beckman Instruments, Illinois, USA) for successive 30-second periods. Electrocardiogram was monitored continuously and heart rate recorded at the end of each two-minute stage.

Body mass was measured, and the percentage of body fat estimated from the thickness of four skinfolds (Durnin & Rahaman, 1967) in the preliminary study seven days before (Pre7) and in the primary study one day before the first marching day (0).

Alcohol consumption and the use of analgesic drugs during the march was questioned

Table 2. Physical characteristics of the subjects (mean \pm SD).

	Study	
	Preliminary (n = 6)	Primary (n = 15)
Age (years)	36.6 \pm 7.7	27.1 \pm 3.8
Height (cm)	176.4 \pm 8.4	181.2 \pm 6.3
Mass (kg)	74.4 \pm 8.1	79.4 \pm 5.3
Body mass index (kg \cdot m ⁻²)	23.9 \pm 2.2	24.3 \pm 1.7
Body fat (%)	13.7 \pm 3.5	12.2 \pm 4.7
Training background		
- h x year ⁻¹	414 \pm 197	
- during 10 weeks preceding the march (h \cdot w ⁻¹)	9.3 \pm 4.7	6.0 \pm 2.5
Cooper's 12-minute running test		
- best result within the last two years (m)	3088 \pm 360	3177 \pm 175
VO _{2max} (ml \cdot kg ⁻¹ \cdot min ⁻¹) (n = 7)		62 \pm 5
VO _{2max} (l \cdot min ⁻¹) (n = 7)		4.7 \pm 0.6
RQ _{max} (n = 7)		1.01 \pm 0.05
HR _{max} (beats x min ⁻¹) (n = 7)		183 \pm 12

4.2 The march

The “International Four-Day Long-Distance March” in Nijmegen, The Netherlands is open to both civilian and military participants who march over 40 km on each of the four days. Walking during the march was determined as follows: “moving forward in such a way that the full body-weight has permanent contact with the ground, alternately via the right and the left feet” (Program-Magazine De 4 Daagse, 1994). As the intention of the march was not to cover the total distance within the shortest possible time, the competitive element can be considered to be minimal. Detachments marched together, two abreast. In order to avoid dehydration and energy deficit, water and standard food (soup, meat, potatoes, bread, salad, dessert) were provided. At the three main resting stops soft drinks, milk and snacks were for sale. The surface of the routes was mostly hard (asphalt or stone) and the route was almost totally flat, except on the third day. The average climate conditions during the marches in 1993 and 1994 were: air temperature 12 to 15°C early in the mornings and 18 to 30°C at noon. The subjects wore ordinary army uniforms and combat boots (1 400 g). The last 10-km of the fourth day march was walked without the backpack because of the final parade.

4.3 Measurements

The protocol of the physiological measurements and collections of the preliminary and primary study is shown in Tables 3 and 4 (p. 27). The measurements consisted of body mass, heart rate during march, orthostatic test, perceived pain, functional capacity of lower extremities. Also blood samples were taken and urine was collected during the study.

4.3.1 Body mass

Body mass was measured in the preliminary study in morning before the first marching day (PreI) and in the primary study daily (I-IV) before (Pre) and after (Post) the daily march.

4.3.2 Cardiorespiratory and autonomic nervous system

Each subject wore a heart rate monitor (Sport Tester PE 3000, Polar Electro OY, Kempele, Finland). Heart rates were recorded at 60-s intervals during march.

Orthostatic heart rate measurements were assumed to reflect the functioning of the autonomic nervous system (Piha, 1988). Recordings were taken firstly in the supine position and secondly at 30 seconds after getting up by a heart rate monitor (Sport Tester PE 3000, Polar Electro OY, Kempele, Finland). Heart rates were recorded at 15-s intervals. Measurements were performed every morning before the march (PreI-IV) and at the first day (Rec1) after the march.

4.3.3 Musculoskeletal system

Responses to the musculoskeletal system were evaluated by questions on perceived pain, assessment of functional capacity of lower extremities (the range of movement, muscle strength and the use of elastic energy, circumferences) and measurements of muscle protein (creatine kinase, CK) in serum.

Intensity of overall perceived pain was assessed using a visual analogue scale (VAS) (Haynes & Perrin, 1992). The scale was a 10-cm horizontal line ranging from "no pain" (on the far left) and "unbearable pain" (on the extreme right). The following descriptors were spread at even spaces along the line from left to right: "dull ache," "slight pain," "more slight pain," "painful," and "very painful". Pain was quantified by measuring the length of the line from the extreme left of the scale to the subject's mark. The location of different lower limb (muscle, joint, feet) and other pains, and abrasions and blisters were indicated on body charts and pain intensity was estimated with a score of descriptors ranged from 0 to 6 with terms alike in the VAS. The Finnish version of the questionnaire of the perceived pains is presented in Appendix 3.

Perceived pain was measured in the preliminary study one week before (Pre7), immediately prior to the first march (PreI) and within one hour after each march (PostI-IV). In the primary study the measurements were done in the day before the first march (0), within one hour after each march (PostI-IV), and 9 days after the last one (Rec9).

To assess the flexibility of the lower extremities, the range of movement (ROM), was measured with a standard plastic goniometer (Wang, Whitney, Burdett & Janosky, 1993). The subjects were instructed to relax the muscles during the procedure. The tightness of the knee flexors (KF) was determined by passive hip flexion with the knee extended. Subjects were positioned supine on a table and their opposite thigh and the knee to be measured were stabilised. The goniometer was placed with the stationary arm parallel to the midline of the trunk, the moving arm along the lateral midline of the thigh, and the axis over the superior half of the greater trochanter. The leg to be measured was raised to the point in the range where a small amount of pelvic movement was elicited by the investigator. The tightness of the knee extensors (KE) was measured by hip extension. Subjects were positioned supine on the table with the leg to be measured hanging over the table. The opposite hip and knee were flexed to the point where the investigator palpated that the subject's lumbar spine was flat on the table. The subjects were instructed to maintain this hip and knee position with the fingers of both hands interlocked over the anterior tibia to assist in the maintenance of the posterior pelvic tilt. The goniometer was placed with the stationary arm parallel to the lateral midline of the thigh, the moving arm along the lateral midline of the tibia, and the axis over the lateral side of the knee joint. The tightness of the hip flexors (HF) was measured with the hip extended. The procedure was the same as for the measurement of the knee extensors, except that goniometer placement was the same as that for the measurement of the knee flexors.

Functional muscle strength of the lower extremities was evaluated according to Komi & Bosco (1978) on an electric contact mat (Digitest OY, Finland). It required the average flight time of three isolated maximal voluntary vertical jumps from a static semisquatting position with no preparatory counter-movement (squat jump, SJ). The use of elastic energy of the lower extremities was evaluated according to Komi & Bosco (1978) on an electric contact mat (Digitest OY, Finland), too. It required the average flight time of three isolated maximal voluntary vertical jumps from a standing position with a preparatory counter-movement (counter-movement jump, CMJ).

Oedemic changes of the lower extremities were estimated by measuring the greatest circumference of the calves (CC), as well as the thighs (CT) 25 cm above the middle part of the patella.

The functional capacity of lower extremities was assessed in the preliminary study immediately prior to the first march (PreI) and within one hour after each (PostI-IV). In the primary study the measurements were done on the day before the first march (0) and within one hour after the last one (PostIV).

4.3.4 Blood samples and biochemical analyses

In the preliminary study blood samples were taken in the afternoons (between 1:30 to 5:30 p.m.), except PreI sample, which was taken in the morning (between 2:00 to 4:30 a.m.). In the primary study blood samples were taken in 11 time points and in the same order with respect to each subject on each day (variation ± 1 h), following a systematic time pattern: (1) a day before the first exercise session at between 2:00 to 5:00 p.m. (0 sample), (2) in the morning (between 2:00 to 5:30 a.m.) within 2 hours preceding each walking session (I - IV), (3) in the afternoon (between 2:30 to 6:30 p.m.) within 60 min after each walking session (I - IV), and (4) one (Rec1) and nine days (Rec9) after the last day of the walk (between 7:00 to 8:00 a.m. and between 1:00 to 2:00 p.m., respectively). Blood was drawn from the antecubital vein. Serum was separated and kept frozen at -20°C until assayed.

Serum creatine kinase (CK) activity and albumin (only in the primary study, $n = 10$) were analysed according to standard laboratory methods: Serum creatine kinase (Boehringer, Mannheim, Germany) and albumin (Reagenia, Kuopio, Finland) in LIKES-Research Center, Jyväskylä.

Concentrations of serum testosterone and cortisol were measured using radioimmunometric assay (RIA) kits purchased from Farnos Diagnostica (Turku and Oulunsalo, Finland) in LIKES-Research Center, Jyväskylä (in the preliminary study) and in Research Institute of Military Medicine, Helsinki (in the primary study). Concentrations of serum immunofluorometric LH and FSH in primary study were measured using time-resolved immunofluorometric assay (IFMA) kits (DELFLIA hLH Spec, DELFLIA hFSH) provided by Wallac OY (Turku, Finland) in Department of Physiology, University of Turku (LH, FSH). The intra- and inter-assay coefficients of variation were below 5 and 10%, respectively. The reference ranges for men provided by the kit manufacturer were as follows: testosterone 9 - 38 nmol \cdot l $^{-1}$, LH 1.0 - 8.4 IU \cdot l $^{-1}$, FSH 1.0 - 10.5 IU \cdot l $^{-1}$, and cortisol 244 - 727 nmol \cdot l $^{-1}$ between 7:00 and 10:00 a.m. and 110 - 418 nmol \cdot l $^{-1}$ between 3:30 and 5:30 p.m.

All urine in the preliminary study was collected. Urine prior to the first march (Rest I), during each march (I-IV) and between the marches (Rest II-IV) were collected separately. Urinary volume and collection time were measured and used to estimate urine excretion. Four ml of 6 M hydrochloric acid was added to the urine to decrease pH, and the samples were kept frozen at -20°C until assayed within six months in Department of Forensic Medicine, University of Oulu.

Urinary catecholamines were purified by an Al_2O_3 -extraction procedure. The catecholamines were extracted at a pH of 8.6 from 25 μl urine into 30 mg Al_2O_3 with 3.4 - dihydroxybenzylamine hydrobromide as an internal standard. After washing four times with 2 ml H_2O , the catecholamines were released into 100 μl 0.2 M HClO_4 - solution. A high pressure liquid chromatograph with an electrochemical detector (Esa Couluchem Multi- Electrode, model 5100 A, ESA, Inc., Chelmsford, MA, USA) was used for the determination of free noradrenaline and adrenaline. The column was an Esa Catecholamine HR-80 and a mobile phase Esa Cat-A-Phase reagent. The flow rate was 1.0 ml \cdot min $^{-1}$. The ratio of the peak height of each catecholamine standard to the peak height

4.4 Statistics

The results are presented as mean \pm SD or SE. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 9.0 for Windows). A one-way ANOVA with repeated measures was employed to examine the response of repeated march. In the case of a significant repeated march response in ANOVA, the other timepoints were compared to baseline (PreI for the morning and 0 for the afternoon); further analysis was then performed with the matched-pairs Student's t-test. A separate ANOVA for morning and afternoon hormone samples (PostI-IV, Rec9) was used because of the circadian rhythm. *A priori* P-value < 0.05 was chosen to indicate statistical significance.

5 RESULTS

5.1 Subjects and marching characteristics

None of the subjects were taking prescribed medications or had a history of cardiovascular, renal or skeletal muscle diseases. Their level of physical fitness was high and body mass was in normal proportion to their height, and they had relatively low subcutaneous fat content (Table 2, p. 23).

The characteristics of the marches are summarised in Table 5. The mean daily walking distance was 46 (preliminary) and 41 (primary study) km and the average speed from five to seven $\text{km} \cdot \text{h}^{-1}$, and the length of daily rest intervals 1.4 h. Average heart rate during walking was 109 (preliminary) and 108 (primary study) $\text{beats} \cdot \text{min}^{-1}$. The mean heart rate level was ca. 60% of maximal rate.

Table 5. Characteristics of the four days' march (days I-IV) in the preliminary (n = 6) and primary study (n = 15).

Day	I	II	III	IV	Mean	Total
Distance (km)						
Preliminary	47	42	46	50	46	185
Primary	40	40	39	44	41	163
Time (h)						
Preliminary	8.1	8.7	9.4	11.3	9.4	37.5
Primary	8.3	9.7	9.5	10.8	9.5	38.3
Average speed ($\text{km} \cdot \text{h}^{-1}$)						
Preliminary	7	5	5	5	6	
Primary	6	5	5	5	5	
Heart rate during the march ($\text{beats} \cdot \text{min}^{-1}$)*						
Preliminary	103 ± 6	109 ± 9	116 ± 10	107 ± 7	109 ± 9	
Primary	112 ± 6	107 ± 5	110 ± 10	105 ± 9	108 ± 6	
Heart rate during the march (% of maximum)*						
Primary (n = 7)	61 ± 3	58 ± 3	60 ± 5	57 ± 5	59 ± 3	

* (mean ± SD)

5.2 Heart rate during the orthostatic test

Morning heart rate in the supine position increased progressively throughout the marching period being highest ($P = 0.042$) on the day after the last march (Rec1) compared to the pre-exercise control (PreI). Heart rate in the standing position was first elevated ($P = 0.045$) on the morning of the second day (PreII) and a highly significant rise ($P = 0.006$) was seen before the fourth march (PreIV) compared to the pre-march control (PreI). The difference between the standing and supine heart rates increased before the last march (PreIV) and the day after (Rec1), respectively, compared to the pre-march control (PreI, $P = 0.001$ and 0.003) (Table 6).

Table 6. Heart rates (beats \cdot min⁻¹) in the supine and standing position and the difference between postures during the four days' march in the primary study (n = 15, mean \pm SD).

Timepoint	Supine	Standing	Difference
PreI	56.1 \pm 6.5	70.5 \pm 10.2	14.5 \pm 10.5
PreII	58.8 \pm 9.5	78.4 \pm 10.7*	19.5 \pm 9.1
PreIII	60.3 \pm 13.7	79.0 \pm 13.0	18.7 \pm 5.9
PreIV	61.6 \pm 12.5	86.8 \pm 14.4**	25.2 \pm 9.4**
Rec1	65.9 \pm 11.0*	91.5 \pm 12.4**	25.6 \pm 7.2**

* $P < 0.05$ and ** $P < 0.01$, when compared to PreI

5.3 Perceived pain and use of analgesic drugs

After the first march overall perceived pain increased ($P < 0.05$) and remained at a similar increased level until the end of the marching period (Table 7, p. 31). After the first march, pain was mainly focused (66%) on the musculature of the calves, thighs and buttocks. The intensity of the muscle pain varied from one to three ("dull ache" - "more slight pain"). During the last three days, the pain was almost completely (60 to 85%) localised to the feet. The pain intensity of abrasions and blisters of the feet varied from two to six ("slight pain" - "very painful"). Joint symptoms in the lower extremities varied from 25 to 43% of the subjects after the marches (PostI-PostIV). Almost all subjects reported pains on the scoring scale "more slight pain" on days III and IV, which were due to abrasions and friction blisters of the feet. "Other" pains were mostly abrasions caused by the straps of the backpack on the shoulders and their intensity was "dull ache".

Anti-inflammatory analgesic drugs (acetylsalicylic acid, ibuprofen, ketoprofen, indomethacin, naproxen) were used by four subjects in the preliminary study during all marching days and by one subject during the last day. The average daily number of tablets (Burana[®] 400 mg, Ketorin[®] 50 mg) consumed per subject was 2, 3, 3, and 4 during the effort. In the primary study no one used anti-inflammatory analgesic drugs during the first day. During the second day there were three and last two days seven drug users and the average daily number of tablets (Aspirin[®] 500 mg, Burana[®] 400-600 mg, Ketorin[®] 50 mg,

Ibusal[®] 200-400 mg, Orudis[®] 100 mg) during the last three days was 2, 3, and 3 in the primary study. Beer was used by some of the subjects after finishing the daily marches (half to one litre per subject).

Table 7. Overall perceived pain and the frequency (fr) of lower limb pains and their location (muscle, joint, feet), other pains, and abrasions and blisters during the four days' march in the preliminary and primary study. The range of descriptors are "no pain" (0), "dull ache" (1), "slight pain" (2), "more slight pain" (3), "painful" (4), "very painful" (5) and "unbearable pain" (6).

	Timepoint										
	Pre7	0	PreI	PostI	PostII	PostIII	PostIV	Rec9	fr	range	
Preliminary (n)	6		6	6	6	6	6				
Primary (n)		14		12	14	14	11	8			
Overall perceived pain											
Preliminary	1.0 ± 1.2		0.6 ± 1.0	3.2 ± 1.0*	2.5 ± 1.1*	3.3 ± 1.5**	3.9 ± 2.7*				
Primary		0.1 ± 0.4		2.2 ± 1.7	2.6 ± 1.9	4.1 ± 1.9***###	5.0 ± 1.9	0.2 ± 0.4###			
Lower limb pains:											
Muscle	fr	range	fr	range	fr	range	fr	range	fr	range	
Preliminary	1/6	2	1/6	1	4/6	1-3	2/6	1-2	3/6	1-3	
Primary		1/14	1	6/12	1-2	9/14	1-5	9/14	1-5	6/11	2-5
Joints	fr	range	fr	range	fr	range	fr	range	fr	range	
Preliminary	3/14	1-3		3/12	1-2	6/14	1-5	4/14	3-6	3/11	2-4
Primary		0/14	0	9/12	1-3	12/14	1-5	12/14	1-6	10/11	1-5
Feet	fr	range	fr	range	fr	range	fr	range	fr	range	
Preliminary	0/6	0	0/6	0	2/6	1-3	6/6	1-3	5/6	2-5	
Primary		1/14	1	5/12	1-2	7/14	1-2	7/14	1-2	2/11	1
Other pains											
Preliminary	0/6	0	0/6	0	2/6	1-3	6/6	1-3	5/6	2-5	
Primary		1/14	1	5/12	1-2	7/14	1-2	7/14	1-2	2/11	1

* P < 0.05, ** P < 0.01, and *** P < 0.001, when compared to 0 or Prel

P < 0.001, when compared to previous measurement

5.4 Functional capacity of lower extremities

ROM measurements (Table 8) showed no impairment in the flexibility of the hip flexors, knee extensors or flexors of either leg. The march period did not affect the results gained from maximal voluntary vertical jumps (Table 9). The circumferences of the calves and thighs remained also unchanged in both studies (Table 10, p. 33).

Table 8. Range of motion of the lower extremities (the hip flexors = HF, the knee extensors = KE, and the knee flexors = KF) during the four days' march in the preliminary (n = 6) and in primary study (n = 12), (mean \pm SD).

Timepoint	0	PreI	PostI	PostII	PostIII	PostIV
HF ($^{\circ}$)						
Preliminary		-2 \pm 3	1 \pm 2	0 \pm 0	2 \pm 5	2 \pm 5
Primary	15 \pm 11					17 \pm 5
KE ($^{\circ}$)						
Preliminary		127 \pm 7	129 \pm 12	130 \pm 6	136 \pm 12	137 \pm 11
Primary	125 \pm 6					125 \pm 7
KF ($^{\circ}$)						
Preliminary		75 \pm 15	82 \pm 8	83 \pm 12	78 \pm 10	80 \pm 9
Primary	86 \pm 7					85 \pm 7

P = n.s.

Table 9. Flight time (ms) of the isolated maximal voluntary vertical jumps from a static semisquatting position with no preparatory counter-movement (SJ), and from a standing position with a preparatory counter-movement (CMJ) during the four days' march in the preliminary (n = 6) and in primary study (n = 12), (mean \pm SD).

Timepoint	PreI	PostI	PostII	PostIII	PostI
SJ (ms)					
Preliminary	490 \pm 15	486 \pm 57	478 \pm 44	484 \pm 47	482 \pm 50
Primary	519 \pm 31				521 \pm 29
CMJ (ms)					
Preliminary	517 \pm 17	507 \pm 62	515 \pm 43	517 \pm 67	511 \pm 52
Primary	539 \pm 34				547 \pm 47

P = n.s.

Table 10. Mean of the circumferences of the calves (CC) and the thighs (CT) during the four days' march in the preliminary (n = 6) and in primary study (n = 14), (mean \pm SD).

Timepoint	PreI	PostI	PostII	PostIII	PostIV
CC (cm)					
Preliminary	37.5 \pm 2.3	36.9 \pm 2.2	37.3 \pm 2.5	37.1 \pm 2.2	37.2 \pm 2.4
Primary	37.8 \pm 1.8				37.9 \pm 1.7
CT (cm)					
Preliminary	52.3 \pm 3.8	51.6 \pm 4.2	52.8 \pm 4.4	51.5 \pm 4.1	51.9 \pm 3.6
Primary	57.4 \pm 2.6				56.5 \pm 2.6

P = n.s.

5.5 Body mass and serum proteins

In the primary study the body mass in the morning before the daily walk (Pre) was unchanged during the four-day period (P = 0.6550) (Table 11). On the first and last day of the march serum total protein concentration increased by 4% (P = 0.02) after the first march (PostI) compared with the pre-exercise level (PreI) and by 3% (P = 0.04) compared with the baseline (0) measurement. After the final march (PostIV) there was also 4% increase in serum protein (P = 0.007) compared with the pre-session level (PreIV) (Table 11).

Table 11. Body mass (n=9), and serum total protein and albumin concentrations (n = 10) during the four days' march in the primary study, (mean \pm SE).

Day	Body mass (kg)		Total protein (g \cdot l ⁻¹)		Albumin (g \cdot l ⁻¹)	
	Pre	Post	Pre	Post	Pre	Post
0				76.7 \pm 1.0		42.9 \pm 1.0
I	78.4 \pm 1.8	77.7 \pm 1.8	75.7 \pm 0.8	79.0 \pm 1.0 ^{#*}	41.5 \pm 1.0	45.7 \pm 0.8 ^{###***}
II	78.4 \pm 1.9	76.9 \pm 1.7 ^{##}	75.7 \pm 0.9	77.2 \pm 1.4	43.2 \pm 1.1	46.1 \pm 1.2 ^{##**}
III	78.5 \pm 1.9	77.5 \pm 1.9 ^{##}	73.6 \pm 1.4	76.4 \pm 1.3	41.3 \pm 1.3	44.7 \pm 1.1 ^{##*}
IV	77.6 \pm 2.4	76.5 \pm 2.5 [#]	72.0 \pm 0.9 ^{###}	74.8 \pm 0.8 ^{##}	41.4 \pm 0.8	43.7 \pm 0.9 ^{##}
Rec1			75.2 \pm 0.8		41.6 \pm 0.9	
Rec9				78.0 \pm 1.3		42.7 \pm 0.9
ANOVA	0.65	0.20	0.002	0.015	0.09	0.009

[#]P < 0.05 and ^{##}P < 0.01, when compared to previous Pre

^{*}P < 0.05, when compared to 0

^{###}P < 0.01, when compared to PreI

A highly significant acute effect of marching was seen in serum albumin after each marching day ($P = 0.003, 0.001$ and 0.001 , respectively). The serum albumin (PostI-III) was increased significantly during the first three days compared to the baseline (0) measurement ($P = 0.006$ and 0.038 , respectively), too.

A highly significant repeated march response was seen in serum CK activity (Table 12) ($P < 0.001$). After the first day's march, CK was increased when compared to Pre7 and PreI ($P < 0.05-0.001$), and remained elevated (mean increase from 400 to 825%, $P < 0.05-0.001$) until the end of the marching period. On the recovery days (7 and 9) CK activity was back at the control level.

Table 12. Serum creatine kinase activity ($U \cdot l^{-1}$) during four days' march in the preliminary ($n = 6$) and primary study ($n = 10$), (mean \pm SD).

Day	Preliminary		Primary	
	Pre	Post	Pre	Post
Pre7		174 \pm 142		
0				137 \pm 76
I	125 \pm 67	641 \pm 440 ^{a#}	140 \pm 76	648 \pm 402 ^{***^{xxxx}}
II		964 \pm 737 ^{a#}	722 \pm 414 ^{###}	1142 \pm 811 ^{***^x}
III		833 \pm 625 ^{a#}	852 \pm 476 ^{###}	1265 \pm 1307 ^{**}
IV		818 \pm 409 ^{aa##\$}	707 \pm 497 ^{##}	1021 \pm 680 ^{***^{\$\$\$}}
Rec1			701 \pm 395 ^{###}	
Rec7		49 \pm 28		
Rec9				144 \pm 94 ^x
ANOVA		0.0009	< 0.0001	< 0.0001

^a $P < 0.05$, and ^{aa} $P < 0.01$, when compared to Pre7

^{**} $P < 0.01$, and ^{***} $P < 0.001$, when compared to 0

[#] $P < 0.05$, ^{##} $P < 0.01$, and ^{###} $P < 0.001$, when compared to PreI

^x $P < 0.05$, ^{xx} $P < 0.01$, and ^{xxx} $P < 0.001$, when compared to previous a.m. measurement

^{\$} $P < 0.05$, and ^{\$\$} $P < 0.01$, when compared to Rec7/9

5.6 Hormones

In the evening one week and one day prior to the march (Pre7, 0), and during the first morning of the march (PreI), all hormone concentrations were within the normal range for adult men (Tables 13-15, p. 35-37).

In the preliminary study during the first morning (PreI), testosterone (Table 13, p. 35) was significantly higher ($P < 0.05$) than in the afternoon one week prior the march (Pre7). After the marching days I, II and IV, testosterone was significantly decreased ($P < 0.05$) to less than 80% of the starting PreI value. However, using ANOVA, no statistically significant differences existed between the afternoon samples (Pre7 - PreI-IV, and PostI-IV - Rec7) in the preliminary study, but a highly significant repeated march effect was seen in morning

(Pre) ($P = 0.001$), but not in the afternoon (Post) (ANOVA, $P = 0.21$) in the primary study.

In testosterone the significant difference was seen in the primary study after the second day of walking (PostII) when testosterone was reduced by 18% ($P = 0.006$) compared with the morning level for the same day (PreII). After the first march (PostI), testosterone levels tended to decrease by 15% ($P = 0.06$) from the preceding morning concentration (PreI). However, pre- and post-march testosterone levels did not differ significantly on the third and fourth day of the walk ($P = 0.86$ and 0.50 , respectively) in the primary study, and a plateau was reached on the third day.

Table 13. Serum testosterone concentrations ($\text{nmol} \cdot \text{l}^{-1}$) during four days' march in the preliminary ($n = 6$) and primary study ($n = 15$), (mean \pm SD).

	Preliminary		Primary	
	Pre	Post	Pre	Post
Pre7		13.3 \pm 6.0		
0				15.4 \pm 9.6
I	16.0 \pm 5.1 ^a	11.2 \pm 4.3 [#]	14.7 \pm 5.1	12.5 \pm 4.6
II		13.3 \pm 6.3 [#]	16.2 \pm 5.5	12.7 \pm 6.4 ^{xxx}
III		13.7 \pm 5.0	14.4 \pm 5.2	14.2 \pm 7.4
IV		11.5 \pm 4.4 [#]	13.0 \pm 5.8	13.9 \pm 8.0
Rec1			14.2 \pm 5.5	
Rec7		13.5 \pm 6.5		
Rec9 ($n = 11$)				17.0 \pm 8.0
ANOVA		0.5	0.001	0.2

^a $P < 0.05$, when compared to Pre7, [#] $P < 0.05$, when compared to PreI

^{xxx} $P < 0.01$, when compared to previous Pre measurement

LH (Table 14, p. 36) in the primary study revealed a highly significant repeated march effect in the afternoon (Post) (ANOVA, $P = 0.0002$), but not in the morning (Pre) samples (ANOVA, $P = 0.12$). The acute effect of a single march on LH was seen after each of the first three days of walking. After the first march (PostI) there was a slight upward trend (3.9 vs. 5.0 $\text{IU} \cdot \text{l}^{-1}$, $P = 0.07$). After the second (PostII) and the third march (PostIII) LH was reduced by 31% ($P = 0.04$ and 0.001 , respectively) compared with the level after the first (PostI). After the third march LH was reduced by 37% compared with the pre-march (PreIII) sample ($P = 0.001$). However, after the fourth march (PostIV) the acute response was no longer observed ($P = 0.11$).

Table 14. Serum luteinizing (LH) and follicle stimulating hormone (FSH) concentrations during four days' march in the primary study (n = 15, n_{Rec=9} = 11, mean ± SE).

	LH (IU · l ⁻¹)		FSH (IU · l ⁻¹)	
	Pre	Post	Pre	Post
0		4.2 ± 0.5		3.4 ± 0.5
I	3.9 ± 0.3	5.0 ± 0.6	3.2 ± 0.5	3.2 ± 0.5
II	4.1 ± 0.5	2.9 ± 0.3*	3.0 ± 0.4	2.6 ± 0.3*
III	4.6 ± 0.3 [#]	2.9 ± 0.3**	2.9 ± 0.3	2.5 ± 0.3*
IV	3.9 ± 0.3	4.0 ± 0.4	2.6 ± 0.3 [#]	2.5 ± 0.3*
Rec1	4.8 ± 0.5		2.8 ± 0.3	
Rec9 (n = 11)		3.7 ± 0.6		2.6 ± 0.4
ANOVA	0.1	0.0002	0.012	0.0002

*P<0.05 and **P<0.01, when compared to 0

[#]P<0.05, when compared to PreI

FSH (Table 14) in the primary study revealed a highly significant repeated march response on both morning (Pre) and afternoon (Post) samples (ANOVA, P = 0.01 and 0.0002, respectively). After the first march (PostI) there was no acute effect (P = 0.43). The acute response of single march in FSH was seen after the three last days of walking. After the second (PostII), third (PostIII) and fourth (PostIV) march FSH was significantly lower compared with the concentration of the day before the march (0 p.m.) (P = 0.02, 0.02 and 0.03, respectively). The concentration of serum FSH was reduced by 19% (P = 0.02) in morning sample before the fourth march (PreIV) when compared with the morning before the first march (PreI).

In the case of cortisol (Table 15, p. 37), the early morning value (PreI) in the preliminary study was significantly higher (P < 0.05) than the afternoon control value Pre7. After the second march in the preliminary study (PostII), cortisol was significantly lower (P < 0.05) than the early morning value (PreI). As in the case of testosterone, ANOVA showed no statistically significant differences between the afternoon samples in the preliminary study, but in the primary study a highly significant repeated march effect was seen in the morning (Pre) and afternoon (Post) (ANOVA, P = 0.01 and 0.0011, respectively). The acute response of a single march on cortisol was only seen during the first day when there was a 60% increase (296 vs. 474 nmol · l⁻¹, P = 0.003) after the first march (PostI) and after that a downward trend in afternoon samples (PostII-IV) (mean decrease from 8 to 19%, P = 0.09, 0.34 and 0.22, respectively) compared with the previous Post sample.

Table 15. Serum cortisol (C) concentrations ($\text{nmol} \cdot \text{l}^{-1}$) during four days' march in the preliminary ($n = 6$) and primary study ($n = 15$), (mean \pm SD).

	Preliminary		Primary	
	Pre	Post	Pre	Post
Pre7		282 \pm 54		
0				296 \pm 142
I	465 \pm 105 ^a	333 \pm 90	379 \pm 150	474 \pm 137**
II		225 \pm 92 [#]	514 \pm 82 [#]	386 \pm 150
III		336 \pm 161	488 \pm 102 [#]	354 \pm 106
IV		290 \pm 120	479 \pm 115 [#]	314 \pm 121
Rec1			450 \pm 153	
Rec7		275 \pm 69 [#]		
Rec9 ($n = 11$)				379 \pm 121**
ANOVA		0.2	0.014	0.001

^a $p < 0.05$, when compared to Pre7

[#] $p < 0.05$, when compared to PreI

** $p < 0.01$, when compared to 0

5.7 Catecholamine excretion

In ANOVA significant repeated march effect was not seen in the urinary excretion of catecholamines (Table 16, p. 38). But the acute response of single march was seen on the third day when excretion of noradrenaline during march (III March) was increased significantly by 233% ($P = 0.027$) when compared to the exertion during the first exercise (I March) and compared to the preceding night (I Rest) it was 300% ($P = 0.025$). Also the excretion of noradrenaline in the night (Rest) tended to increase during the experiment being 2.1-fold ($P = 0.08$) during the last night (Rest IV) compared to the night prior to the march (I Rest), but during the fourth march (IV March) there was no more increase when compared to the excretion of the first day (I March) ($P = 0.17$).

During the night preceding the first march (I Night) the urinary excretion of free adrenaline was below the detection limit of the method. It increased to $3.2 \text{ nmol} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ ($P = 0.018$) during the rest period prior to the last (IV Night) march. During the first three days the excretion of adrenaline during the march was significantly ($P = 0.02$, 0.40 and 0.001 , respectively) faster than the previous rest excretion. The largest individual increase in the daily excretion during the march in the case of adrenaline was 1000%, occurring during the third 24-h period of the experiment (IV March). The difference between the previous rest excretion and march excretion was no more significant ($P = 0.09$) after the third day.

Table 16. The urinary excretion of free noradrenaline and adrenaline ($\text{nmol} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$) during four days' march (March) and during the nights between the marches (Night) in the preliminary study ($n = 6$, mean \pm SD).

Day	Noradrenaline		Adrenaline	
	Night	March	Night	March
I	16.3 \pm 6.7	21.6 \pm 12.5	xxxxxxx	4.9 \pm 2.9 [‡]
II	18.1 \pm 4.2	37.1 \pm 17.5	1.7 \pm 1.2	6.9 \pm 2.1 [‡]
III	17.7 \pm 7.0	72.1 \pm 52.9 ^{#‡}	0.9 \pm 0.8*	9.9 \pm 4.4 ^{‡‡}
IV	34.5 \pm 21.8*	56.2 \pm 43.1	3.2 \pm 2.6*	10.6 \pm 7.2
ANOVA	0.2	0.2	0.3	0.1

xxxxxxx = below detection limit

[#]p < 0.05, when compared to I March

*p < 0.05, when compared to I Night

[‡]p < 0.05 and ^{‡‡}p < 0.01, when compared to previous night value

6 DISCUSSION

In the preliminary and primary study, six and 15, respectively, male subjects were followed during the "International Four-Day Long-Distance March" under true field conditions in Nijmegen, The Netherlands. The subjects were healthy, Finnish male soldiers, who marched 163 to 185 km over four days, corresponding to a total of 200 000-250 000 steps (Sekiya, Nagasaki, Ito & Furua, 1996). The purpose was to describe the responses of cardiorespiratory, autonomic nervous, musculoskeletal and hormonal systems to daily repeated acute but non-competitive prolonged walking during four days' march to the. The focus was on the quantification of the amount of disadvantage caused by physical activity. The hypothesis of this study was that strenuous prolonged walking during four days would decrease the functional capacity of the lower extremities, and disturb the balance of the autonomic nervous system, and the secretion of hormones from adrenal cortex, and pituitary-testicular axis.

Optimal regular exercise is known to lead to improvements in physical fitness and performance, but chronic exercise overtraining syndrome (e.g., Kuipers & Keizer, 1988; Fry et al., 1991) could unfortunately also occur in physically active persons. Exercise overtraining syndrome found to result in behavioural, emotional and physical symptoms (overstress syndrome) which are very similar to those observed in soldiers who are overstressed (e.g., Aakvaag et al., 1978a, b; Opstad, 1978; Opstad, 1992).

In exercise training overreaching occurs on a short-term basis during several days (Budgett, 1990; Fry et al., 1991; Fry & Kraemer, 1997; Kuipers & Keizer, 1988). It may be a planned phase of the training programme since it is believed to contribute to subsequent improved performances (Fry & Kraemer, 1997). But it has been also proposed that overreaching is actually an early stage of overtraining. If left unchecked, overreaching develops into overtraining (Fry et al., 1991). Training instructions for army recruits have been written by e.g., Lange (1980) and Rudzki (1989). Strategies used to minimise training monotony (and thus training strain), primarily multiple "easy" days within each week, may allow a given training load to be accomplished with comparatively fewer negative outcomes (Foster 1998).

In walking the physiological problems arise from the external (equipment, shoe and surface) and internal (anthropometric facts and individual situation) variables and movement characteristics (frequency) which influence the load and may be connected with pain and injuries (Nigg et al., 1984). All these factors influence a person's walking ability. Also the intensity and the amount of walking affects the loading. Finnish military personnel's field competence and physical working ability includes performance capacity in which shooting ability and the ability to move in all kinds of combat environments in accordance with a given task during different seasons and under varying weather conditions are combined with physical fitness. The goal for marching ability of under 55-year-old officers is to be able to march 25 km with light equipment (gun, backpack, camouflage suit, and combat shoes or boots) in six hours (Defence Staff, Finnish Defence Forces, Department of Education, 1999).

Recognition and prevention of the adverse effects of acute large and unaccustomed increments in exercise and training load on the functional capacity is an important practical aspect of sport and military medicine. External loads up to 10.4 kg would not limit the endurance time of walking (Holewijn, 1990). The predicted external load (kg) at which the limit speed of the endurance time of walking is reached at a speed of $1.3 \text{ m} \cdot \text{s}^{-1}$ ($4.8 \text{ km} \cdot \text{h}^{-1}$) for 40-year-old men is 17 kg (Holewijn, 1990).

6.1 Study design and limitations

Applied research, such as this, tends to address immediate problems, to so-called real-world settings, and to have limited control over the research settings but to give results that are of direct value to practitioners (Thomas & Nelson, 1990, 5).

A field trial is not without limitations. The preliminary study before gathering the primary study was emphasised to diminish the methodological problems (Thomas & Nelson, 1990, 155). Further, all the field measurements were performed by the same technicians.

The purpose of quasi designs is to fit the design to settings more like the real world while still controlling as many of the threats to internal validity as possible. The purpose in reversal design with time series is to determine a baseline measure, evaluate the treatment (e.g. exercise), return to baseline, evaluate the new treatment, return to new baseline, and so on (Thomas & Nelson, 1990, 297-319). The quasi-experimental reversal design is depicted as follows:

$$O_1 O_2 T_1 O_3 O_4 T_2 O_5 O_6 \text{ (Thomas \& Nelson, 1990, 313)}$$

where O signifies observation or test (subscripts refer to the order of testing) and T (treatment) signifies that exercise is applied.

Repeated measures designs, as this one, have many advantages (Cook & Campbell, 1979; Thomas & Nelson, 1990, 154-155). Firstly, they provide the experimenter the opportunity to control for individual differences among subjects, probably the largest source of variation in most studies. Secondly, the variation of individual differences can be identified and separated from the error term, in repeated measures designs, thereby reducing it and increasing power. Thirdly, they are more economical in that fewer subjects are required, and finally, they allow to study a phenomenon across time which is particularly important in studies of change. However, repeated measures designs have several problems, too (Cook & Campbell, 1979; Thomas & Nelson, 1990, 155-156). Carryover (treatments given earlier influence those given later), and practical effects (the dependent variables get better as a result of repeated trials in addition to the treatment; also called the testing effect), fatigue (subject's performance is adversely influenced by fatigue or boredom), and sensitisation (subject's awareness of treatment is heightened because of repeated exposure).

In experimental and quasi-experimental studies internal validity means controlling all variables so that rival hypothesis as explanations for the observed outcomes can be eliminated. External validity means generalisation of the results (Cambell & Stanley, 1963; Cook & Campbell, 1979; Thomas & Nelson, 1990, 297-306).

There are eight variables, which are relevant to internal validity and which might produce effects confounded with the effect of the experimental stimulus if not controlled in the experimental design (Cambell & Stanley, 1963): 1) history, the specific events occurring between the first and second measurement in addition to the experimental variable, 2) maturation, processes within the respondents operating as a function of the passage of time per se, 3) testing, the effects of taking a test upon the scores of a second testing, 4) instrumentation, in which changes in the calibration of a measuring instrument or changes in the observers or scores used may produce changes in the obtained measurements, 5) statistical regression, operating where groups have been selected on the basis of their extreme scores, 6) biases resulting in differential selection of respondents for the comparison groups, 7) experimental mortality, or differential loss of respondents and 8) selection-maturation interaction, etc., which in certain of the multi-group quasi-experimental designs might be mistaken for the effect of the experimental variable.

The factors jeopardising external validity are: 1) the reactive or interaction effect of testing, in which a pretest might increase or decrease the respondent's sensitivity or responsiveness to the experimental variable and thus make the results obtained for a pretested population unrepresentative of the effects of the experimental variable for the unpretested universe from which the experimental responders were selected, 2) the interaction effects of selection biases and the experimental variable, 3) reactive effects of experimental arrangements, which would preclude generalisation about the effect of the experimental variable upon persons being exposed to it in nonexperimental settings, 4) multiple-treatment interference, likely to occur whenever multiple treatments are applied to the same respondents, because the effects of prior treatments are not usually erasable (Cambell & Stanley, 1963; Cook & Campbell, 1979).

If the number of subjects is small, as it was especially in the preliminary study, only the analysis of variance (ANOVA) for repeated measures test can be used (Thomas & Nelson, 1990, 155). Repeated measures ANOVAs had been thought to require the assumption, called compound symmetry when all the variables within a group must have equal variances, all the correlations among variables must be equal, and the covariance matrices of all groups must be equal. These assumptions are seldom met in repeated measures studies, and they have been shown to be unnecessarily strict. The correct assumption in this kind of study as this one is called sphericity where the repeated measures, when transformed by a set of orthonormal weights, are uncontrolled with each other and have equal variances (Cook & Campbell, 1979; Thomas & Nelson, 1990, 155).

In this study the lack of a control group meant that many other factors (e.g., testing environment, substance use, nutritional status, stress level, sleep deprivation, previous activity, age, sex, diseases) apart from the exercise may have had a confounding effect e.g., on the hormone results. In addition, the sample collection procedure (specimen collection, data manipulation, and analysis) may include confounding factors e.g. posture, circadian and rhythmical variation, specimen collection and storage, choices of specimens, analytical and biological variation, descriptive statistics and inference (Tremblay et al., 1995). Most of these factors were standardised in this design. However, the variation of obtain the blood sample after the exercise, and the systematic time pattern between the days could have influenced the results. Nevertheless, what was interesting about this study was that it took place during a prolonged real situation that could not have been reproduced in a laboratory.

It is easier to establish reliability of measurement in research than validity e.g., stability, alternate forms, and internal consistency (Thomas & Nelson 1990, 352). The coefficient stability was determined by the test-retest method on separate days. The alternate-forms method of establishing reliability involves the construction of two tests, both supposedly sample the same material. An internal consistency reliability coefficient can be obtained by e.g., same-day test-retest, the split-half method, the Kuder-Richardson method, of rational equivalence, and the coefficient alpha technique (Thomas & Nelson, 1990, 353). For instance, the error of testing jumps, when compared with film analysis has been reported to be in the order of $\pm 2\%$ (Komi & Bosco, 1978) and the reliability coefficients for the repeated measures of flexibility of the lower extremities have been reported to vary from 0.90 to 0.98 (Wang et al., 1993).

6.2 Responses of the cardiorespiratory system to prolonged walking

Cardiorespiratory loading in this study was at the same level as has been measured during brisk walking speed ($4.8\text{-}6.4 \text{ km} \cdot \text{h}^{-1}$) when heart rate of middle-aged men on a level surface has been estimated to be 40 to 60% of maximal aerobic power and 50 to 70% of maximal heart rate (Rodgers, Vanheest & Schachter, 1995; Vuori, 1982). Energy consumption during the marches in the present study, estimated by the average walking speed, the body mass of the subjects, and the mass of the shoes, was ca. $1,220 \text{ kJ} \cdot \text{h}^{-1}$ ($290 \text{ kcal} \cdot \text{h}^{-1}$) (McArdle et al., 2000, 170), which means a total energy cost of 2,320 kcal during an 8-h daily activity. Oxygen uptake was approximately $17 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (4.8 METs) (Holewijn et al., 1992) which was 27% of $\text{VO}_{2\text{max}}$ and 23% of oxygen uptake reserve ($\text{VO}_2\text{R} = [(\text{VO}_{2\text{exercise}} - 1 \text{ MET}) / (\text{VO}_{2\text{max}} - 1 \text{ MET})]$) (Howley 2001). The mean heart rate level was ca. 60% of the maximal heart rate. At this level energy is produced aerobically, especially through the oxidation of fatty acids. The intensity of endurance-type exercise has been defined to be light if % VO_2R is from 20 to 39, % HR_{max} 50-63, and $\text{VO}_{2\text{max}}$ 27-44 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (3.2-5.3 METs) (Howley, 2001). The physiological threshold of 'comfort' represents 70% of maximum heart rate (Morris & Hardman, 1997). De Wild, Peeters, Hoefnagels, Oeseburg and Binkhorst (1997) studied 97 over 70-year-old men, who completed the 1993 Nijmegen Four-Day long-distance March ($30 \text{ km} \cdot \text{day}^{-1}$ on four consecutive days). The mean velocity was $5 \text{ km} \cdot \text{h}^{-1}$ and the mean relative exercise intensity was 52% of $\text{VO}_{2\text{max}}$ and 70% of HR_{max} , $\text{VO}_{2\text{max}}$ was the most important predictor of the variation in self-selected velocity.

6.3 Responses of the autonomic nervous system to prolonged walking

At rest, the heart rate setting depends on complex neurohumoral interactions (Dressendorfer et al., 1985), who suggests that a valid marker of insufficient physiological recovery during excessive training is the elevated morning heart rate that is persistently more than 10% above the normal baseline, as happened in this study. During six days' moderate prolonged exercise period heart rates increased by about 10% in the night time, compared to the previous control condition (Roussel & Buguet, 1982). It was apparently not related to changes in body temperature, hematocrite, sleep patterns, cortical adrenal or thyroid functions. The only possible explanation for the nocturnal tachycardia was related to probably increased sympathoadrenal activity (Roussel & Buguet, 1982).

6.4 Responses of the musculoskeletal system to prolonged walking

In 1993 and 1994 35 101 and 33 834, respectively, walkers started Four-Day March in Nijmegen, and 2 747 and 2 858 (7.83 and 8.45%), respectively, did not finished it. The overall dropout rate in 1993 for first time participants was 15.4% (Program-Magazine De 4 Daagse, 1994). None of the subjects in this study perceived musculoskeletal or other health problems serious enough to prohibit marching or to necessitate medical care during or immediately after the event.

The level of perceived pains (VAS-scale) was significantly higher already after the first marching day, and more pronounced after the marches III and IV. A similar time profile was separately found also for lower limb pains, which focused on musculature, joints and the feet. The rest of the perceived pains were mainly due to abrasions on the shoulders caused by the straps of the backpacks. When these two studies (the preliminary and primary) are compared a little more acute muscle pains reported in the primary study. The proportion of subjects suffering from leg muscle pains remained approximately the same (50%) but the mean scoring of pains increased (score ranges "slight pain - painful") until the end of the last march. Unfortunately pain scoring and location data were collected using only a structured questionnaire. Therefore it cannot be exactly located the pains to specific muscles, but only to the local muscle groups. The majority (ca. 65%) of muscle soreness was experienced in the anterior and posterior thigh muscle groups (quadriceps femoris and hamstrings), and the rest (ca. 35%) was located in the calf and gluteus muscles.

Blisters and abrasions on the feet are some of the major problems usually encountered during walking e.g., during Exercise Fastball in France, where soldiers marched 204 km in six days, all of the injuries were due to foot disorders, such as blisters (Myles et al., 1979). The pains located in the hip, knee and ankle area might be such types as tendonitis, periostitis, or hydrophysis, which are very common medical problems during marching (Hedman, 1988; Rudzki, 1997b). Feet pains originating from friction blisters and abrasions were experienced by almost every subject. The locations of the abrasions were those areas, which were evidently most exposed to pressure and strong friction. The most important factors for producing abrasions were the constant repetitive pressure on the sole of the foot during walking as well as the friction forces exerted between the skin, socks and shoe soles. These shearing forces generate mechanical fatigue in epidermal cells, leading probably to the loss of cell to cell integrity and hence to the development of blisters. Tobacco use, ethnicity, foot type (*pes planus*), a sickness in the last 12 months and no previous activity duty military service are blister risk factors in cadet basic training (Knapik et al., 1999) but e.g., abnormalities of the foot are not significant factors in the development of the injury during recruit training (Rudzki, 1997c). The subjects wore similar boots (weight 1 400 g) and cushioned them individually. The hard surfaces (asphalt and stone covered roads) provides soft, cushion-soled footwear to allow mobility of the foot and ankle as well as to dampen the tight, prominent impact forces. On hard ground a boot is inferior to running shoes in preventing problems due to walking or running (Cavanagh, 1980; Jones, 1983). In addition, inside black boots the temperature will rise to very high levels while marching in sunshine and this effect especially when combined with limited perspiration is probably an additional factor responsible for abrasions, especially if the speed is high (Hedman, 1988). For example, Hedman (1988)

has studied the treatment of feet abrasions during a 160-km march (four days) and he conjectured that about 50% of the treated cases ($n = 39$) would probably have been forced to stop the march if they had not had access to hydrocolloid treatment. Of the 527 soldiers, 150 (28%) consulted a doctor in the course of the Four-Day March in Nijmegen in 1996 (Hysing & Fretland, 1997).

Discomfort in the hip and shoulder areas could be reduced by putting more padding in the pack harness in the areas of the iliac crest and the shoulders. The pelvis rotates in the frontal plane opposite to the shoulder girdle during most of the stride cycle. When walking at a speed of $6.5 \text{ km} \cdot \text{h}^{-1}$, each foot will be lifted about 30 cm, and accelerated to twice the average velocity of the body, and then decelerated to zero velocity again (Holewijn, 1990; Holewijn et al., 1992). When this movement is impeded due to the 10.4-kg load supported by the shoulder, the trapezius muscle has to generate a 17 N extra absolute force level (above walking with no load) per shoulder in order to overcome this (Holewijn, 1990). Lightening and reconfiguring the load to move it closer to the body and improving load distribution have been recommended in attempt to alleviate symptoms associated with carrying heavy loads (Johnson & Knapik, 1995).

In contrast to a previous study of strenuous road marching (Knapik, Reynolds & Barson, 1992), low back problems were not a major problem in this study. The reason for this difference may be the light weight of the load carried and the non-maximum walking speed. When biomechanical and metabolic effects of varying backpack loading on simulated marching was studied (Quesada, Mengelgoch, Hale & Simon, 2000) notable declines were observed for knee extension moment peaks suggesting that the knee may be effecting substantial compensations during backpack loaded marching. On the contrary, kinetic data indicated that such knee mechanics were not sustained, and suggested that excessive knee extensor fatigue may occur during prolonged loaded walking (Quesada et al., 2000).

An optimum method of load carrying should induce stability, bring the center of gravity of the load as close as possible to that of the body and rely on the use of large mass muscles (Legg, 1985). The concept of distributing the load mass more evenly around the center mass of the body has both positive and negative aspects (Knapik et al., 1997). Feet and shoulder problems could most probably be completely eradicated by means of considerations and technical solutions. The musculoskeletal loading during prolonged exercise could be adjusted by changing the gait and carrying technique.

Military basic recruit training is known to be associated with an increased risk of overuse injuries (e.g., Jordaan & Schwellnus, 1994; Ross, 1993a, b). The overall incidence of injuries in military recruits undergoing basic training was 1.8/1000 training hours (Jordaan & Schwellnus, 1994) but a much higher rate of injuries (13-15/1000 h) was found in Rudzki's (1997a) study where field training was not included. Overall, most injuries treated in US Army outpatient clinics were lower extremity training-related injuries (Jones & Knapik, 1999). The highest incidence of injuries was recorded in weeks one to three and week nine of training, which were weeks characterised by marching (> 77% of the training time). The amount of over-use injuries can be diminished significantly, if the possible overpronation is diagnosed and orthoses are used (Lehti & Rehnunen, 1992), and training is modified (Jordaan & Schwellnus, 1994). The lower marching volume did not lessen morbidity (Giladi, Milgrom, Danon & Aharonson, 1985).

Flexibility is one component of the health-related physical fitness because its importance in avoiding injury due to sudden strains or movements (Bouchard & Shephart, 1994). Unexpectedly, no significant changes in the functional capacity of the lower limbs (vertical jumps, range of motion, circumferences) were observed after the four days' march in the present study. The high volume of walking was assumed to decrease the functional capacity of the lower extremities because, because for example, even a single marathon run has caused an acute loss of muscle function (Kyröläinen et al., 2000; Nicol et al., 1991a; Nicol et al., 1991b; Sherman et al., 1984). Hence, the discrepancies between our results and those seen after marathon running are evidently caused by the extremely heavy and competitive nature of those events. In walking compared to running have been experienced 3.6-fold lower ground reaction forces (Voloshin, 1988). Limitations of the range of motion of any foot joints will disrupt gait mechanics and have been found to be associated with an increased risk of ulceration (Sumpio, 2000).

Serum CK activity has widely been used to estimate "muscle damage", even in a quantitative sense, and its increase is often related to a pathologic state in a muscle. However, some studies (e.g., Evans & Cannon, 1991) make such a relationship uncertain. In the present study, a relatively moderate increase (400 to 825%) was observed in serum CK throughout the marching period. An increase of this magnitude can well be caused by facilitated protein transfer via the lymphatics from muscle interstitium, and not necessarily from the myocellular compartment, into intravasal compartments (Komulainen, Takala & Vihko, 1995). Therefore, great care must be taken when interpreting such small changes as in this study (max. increase 9.2-fold) and e.g., Ross et al.'s (1983) seven times higher than the rest level to mean serious pathophysiological phenomena in a muscle. In contrast to, for instance, changes after eccentric bench-stepping exercise when CK increases may be 350-fold (Newham et al., 1983).

When the present two studies are compared (the preliminary and primary), a little higher CK activity increase (7.7 vs. 9.2 U · l⁻¹) was found in the primary study. According Hortobagyi and Denahan (1989) the reason for the small difference may be the higher training background volume during 10 weeks preceding the march executed by the preliminary study subjects. This explanation suggests that muscle protein leak out can be at least reduced, if not prevented, by proper earlier preparation. The subjects in the primary study were younger than those in preliminary and they might not have been so accustomed to the strain as the older and more experienced men.

6.5 Responses of the hormone system to prolonged walking

Responses of blood hormones to exercise has been divided into three groups: fast responses, responses of a modest rate, and delayed responses (Virtanen 1992). The fast response is characterised by a rapid increase in the concentration of hormones in blood plasma within the first few minutes of an exercise. Modest responses are characterised by a gradual increase in the hormone concentration, which may continue up to the end of exercise or even longer. On the other hand, a gradual increase during the first period of exercise may be followed by levelling-off to a constant level or by a trend to a decline in the hormone concentration (Virtanen 1992). The mechanism for a rapid activation of the endocrine function is connected with the functions of nervous centers and a high rate transfer of the nervous influences to endocrine glands. The mechanism for a delayed activation is dependent on some effects of exercise which are cumulative. This mechanism of a delayed activation determines the final hormone levels and the magnitude of hormone changes depends on it (Virtanen 1992).

After the first and second days of walking, the concentration of serum testosterone decreased when compared with the pre-march baseline, but not after the third and the fourth day. There was also a significant decrease after the second day compared with the morning level. Hence, secretion of testosterone appears to adapt to repeated prolonged (8 to 11 h) low intensity (57 to 61%) walking within three days. Although the concentrations of anabolic hormones (testosterone, LH, FSH) before the event were within the limits of the reference ranges, they were quite low. Therefore it is presumable that changes in initially lower hormone concentrations will be smaller than in higher levels. Endurance trained men, such as these subjects, who had trained for six to nine hours per week, have been reported (Gulledge & Hackney, 1996; Hackney, Sinning & Bruot, 1990; Wheeler, Wall, Belcastro & Cumming, 1984) to have a lower basal serum testosterone concentration than control subjects.

The concentration of serum LH and, to a lesser extent, FSH appeared to decrease (19 to 31%) during this prolonged acute exercise when sensitive detection methods (IFMA) were used. LH tended to increase during the first day and significant decreases were seen after the second and the third day when compared to the pre-march baseline. The decrease was also seen after the third day compared with the morning level, but there were no changes during the fourth day. Hence, the acute responses at the pituitary level of the hypothalamic-pituitary-testicular axis (excluding secretion of FSH) also seem to disappear within four days. No acute march response on FSH was seen during the first day, but thereafter FSH declined during the last three days and the pre-march concentration of FSH did not rise significantly between the end of the third and the beginning of the last exercise session. Therefore, no adaptation to repeated low intensity prolonged walking was seen in FSH. The difference in these results compared with studies that have detected no decrease in gonadotropins after prolonged exercise (Dessypris et al., 1976; Lucia et al., 2001; Schürmeyer et al., 1984) may partly be explained by the improved precision and accuracy of the analytical method (IFMA) used in this study (Huhtaniemi et al., 1992; Jaakkola et al., 1990).

Earlier studies have reported a higher concentration of serum FSH in trained subjects, which is considered to be a sign of compensating hypogonadism due to several years of physical training or dysfunction of the Sertoli cells (Vasankari et al., 1993). These conclusions are in accordance with the present results. The secretion of FSH is unlikely to adapt to repeated prolonged exercise.

It was interesting to note that the only acute response of the adrenal cortex was measured after the first exercise session, despite the peak value of this being in the morning (Marniemi et al., 1984). During the last three days both pre- and post-concentrations of serum cortisol gradually decreased towards normal resting values. Serum cortisol was no longer elevated after the last walk or on the following morning after it when compared with the baseline samples taken before the first march. A significantly elevated cortisol level was still detected after nine days of recovery, but this could have been due to the circadian rhythm of cortisol secretion since the recovery sample was taken earlier than the Post samples following the marches (1:00 to 2:00 vs. 2:30 to 6:30 p.m.).

It is generally accepted that during prolonged severe exercise the secretion and therefore blood levels of cortisol are progressively increased (Marniemi et al., 1984). Lowered or suppressed cortisol responses to subsequent exercise have been speculated to represent a maladaptation or pathology in the athletes (Hackney & Styers, 1999; Lehmann, Foster, Dickhuth & Gastmann, 1998).

Viru et al. (2001) found two different types of resetting of the regulation of pituitary-adrenocortical activity to subsequent exercise after prolonged (2 h) continuous running: One involved an intensified mobilisation of pituitary-adrenocortical function while the other reflected the inhibition of activity within this system produced by the fatigue. The only acute response of the adrenal cortex in this study was measured after the first march, despite the peak value of cortisol being in the morning. The maximal increase was reached on the first day, suggesting either that overall hormonal stress adaptation occurs in about a day for the present type of prolonged walking or that fatigue induced by the prior exercise may have modified the hormone response by provoking a feedback suppression as demonstrated by Brandenberger et al. (1984). The suppressed response reported here was more rapid than that seen in earlier studies (for example, Fellmann et al., 1992; Marniemi et al., 1984) in which stabilisation occurred over three days.

An average body mass reduction after each walking session (1 to 2%) indicated slight dehydration, which was, however, compensated for by the next morning. However, possible haemoconcentration caused by dehydration could not induce the reduced postexercise serum concentration of testosterone, LH or FSH. On the contrary, possible haemoconcentration could result instead in increased concentrations. Sleep deprivation or a low-energy diet, which could have a major influence on the hormonal results, were not included in this study, and all subjects were healthy adult men. The hormonal comparison were made with the baseline samples (PreI samples and 0 p.m. samples) which makes the interpretation of the changes possible.

Urinary catecholamines were assayed in order to "quantitatively" estimate sympathoadrenal stress. Both adrenaline and noradrenaline excretion rates showed cumulative sympathoadrenal stress during the marching period, seen not only as cumulatively increasing excretion during the successive marches, but also, interestingly, as a tendency for cumulatively increasing night excretion. Due to a lack of reference data, the

evaluation of the usefulness of especially night excretion of catecholamines as an estimate of general sympathoadrenal stress remains open. Similar type of cumulative sympathoadrenal loading response was found in the study of Vuori et al. (1979) where the basal noradrenaline plasma levels were increased during the first days of a ski-hike. However, in four days, a plateau was reached.

6.6 Recommendations for future research

When effects of physical activity on the human body are examined, specific physiological requirements of specific exercises regulate specific biological responses. An exercise stress will elicit varied responses in different subjects, and frequently the identical exercise stress will elicit varied responses in the same person at different times. This is called individual specificity of exercise (Edington & Edgerton, 1976, 4) and therefore the results of field studies are situational and the background of the subjects and exertions must be well described. Different exercises have different biological requirements. Exercises could be classified according to the speed of movement, resistance to the movement, and duration or the time over which the movement has to be repeated. These three classification components are simultaneous and the additional variables affecting the activity, including environmental factors, must be taken into consideration. Other considerations – such as mental and social pressures, and dietary considerations must be added to the total consideration for exercise classifications (Edington & Edgerton, 1976, 4-7).

In this study the type of exercise was walking but many other endurance exercise types could be very prolonged, for example cycling, golf, orienteering, paddling, rowing, running, skating, skiing and swimming, and provided a field of investigating. The physiological responses of these kinds of long lasting aerobic exercises should also be studied with normal population (between men and women, young vs. older people), not only in regularly training and competing athletes because the responses could be different. It would be important to find the limit beyond which signs of over-dosage may develop. The critical borderline to physiological overload during daily repeated exercise still remains open and further investigations are needed to apply the results of this study to other popular types of physical activity e.g., skiing.

The present findings can be generalised partly to work physiology e.g., in the army and other physically strenuous occupations. In the soldier's action competence model (Toiskallio, 1998) four elements can be determined, i.e. physical fitness/performance and psychological, social and ethical competence. A soldier in action is part of the situation and the environment where he acts. The action is contextual, thus military research should be multidisciplinary.

Mental strength is an important ability and it can be seen as stamina, determination, bravery and the will to win (Defence Staff, Finnish Defence Forces, Department of Education 1999). From the holistic aspect, the same elements could be seen in sports, too. When functional capacity and working ability or athlete's training state is under evaluation or research in a field environment, other components than physical fitness/performance are worth of remembering and researching.

7 SUMMARY AND CONCLUSIONS

During the Four-Day March the long duration and submaximal strain provoked a challenge to the homeostatic regulatory mechanisms of the human body. This study indicates, overall, that repeated long-lasting submaximal walking evokes small, variable and unsustained changes in serum testosterone and cortisol concentrations. The acute response of the pituitary-gonadal axis (excluding the secretion of FSH) and adrenal cortex disappeared within four days of repeated prolonged walking and no dramatic longlasting changes occurred despite this major four-day effort. However, when using the sensitive IFMA, secretion of FSH remained reduced and no stability was seen. Cumulative acute response was found in the resting heart rate levels and in urinary excretion of catecholamines.

The results of this study indicated that daily repeated long lasting walk of intensity at approximately 60% of maximum heart rate which (100-120 beats per min) is well within the physiological capabilities of individuals with good aerobic capacity.

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APPENDICES

Appendix 1. The permission of the Ethics Committee of the University of Jyväskylä, Finland.



JYVÄSKYLÄN YLIOPISTO

Eettisen toimikunnan lausunto tutkimuksesta:

"Neljän päivän marssin aiheuttamat vaikutukset sotilaiden psykofyysisiin ominaisuuksiin"

Tutkimus ei poikkea LIKES -tutkimuskeskuksessa tapahtuvasta normaalista tutkimustoiminnasta. Tutkimuksen koehenkilöiltä pyydetään suostumus tutkimuksen suorittamiseen sekä tutkimuksessa tarvittavien tietojen hankkimiseen. Tutkimuksessa ja sen raportoinnissa kunnioitetaan henkilötietosuojaan vaatimuksia ja tutkittavat saavat henkilökohtaisen palautteen.

Eettinen toimikunta toivoo, että tutkittaville tiedotetaan tahosta, joka on hankkinut tarvittavat vakuutukset.

Eettinen toimikunta katsoo, että tutkimus täyttää sille asetettavat eettiset kriteerit.

Jyväskylässä 6.5.1994

Puheenjohtaja, vararehtori

Sihtööri, koulutuspäällikkö

Marjatta Marin

Timo Helosuo

Appendix 2. The research permission of the Defence Staff, Finnish Defence Forces,
Department of the Health Care.

P Ä Ä E S I K U N T A
Terveysthuolto-osasto

ILMOITUS

100 /Hh III

Helsinki

27.6.1994

1 (1)

LitM Ilkka Väänänen
LIKES-tutkimuskeskus

Ilkka Väänänen tutkimuslupa-anomus 21.4.1994

TUTKIMUSLUPA KADETTIEN MARSSIA KOSKEVALLE TUTKIMUKSELLE

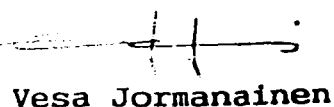
Pääesikunnan päällikkö on esittelyssä
23.6.1994 hyväksynyt tehtäväksi tutkimuksen
"Neljän päivän marssin aiheuttamat vaikutukset
sotilaiden psykofyysisiin ominaisuuksiin"
esittelymuistiossa mainituin ehdoin. (Esitte-
lyasiakirja on liitteenä.)

Tutkimuksen yksityiskohdista tutkija sopii
Maanpuolustuskorkeakoulun perustutkinto-osas-
ton kanssa.

Hallintoylilääkäri
Lääkintäeverstiluutnantti Ari Peitso



Hallintolääkäri
Lääkintäluutnantti



Vesa Jormanainen

LIITE

PE:n asiak nro 1262/Tervh-os/Hh III/23.6.1994
(ilman liitteitä)

TIEDOKSI

PEkoul-os
MpKK
KSS/Sotlääkl

5/AP/AP

Appendix 3. The Finnish version of the questionnaire of the perceived pains.

Nimi
Päiväys
Kello

1. Arvioi niitä heikoilla numeroilla kivun määrää, ja merkitse seuraavalle kipujennalle tilannesta parhaan kuvaavalle kohdalle gustorviiva.

EI KIPUA	Hieman kipua	jonkin verran kipua	melko paljon kipua	paljon kipua	erittäin paljon kipua	SIETÄMÄTÖN KIPU
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2. Mihin kehon osiin kipu on paikallistunut? Aseen kivun voimakkuutta kuvaava numero kehon osan keskellä olevalle viivalle. (0=ei kipua, 1=hieman kipua, 2=jonkin verran kipua, 3=melko paljon kipua, 4=paljon kipua, 5=erittäin paljon kipua, 6=sietämätön kipu)

—	pää/niiska	—	vasa
—	vasen hartia	—	yläselkä
—	oikea hartia	—	alaselkä
—	vasen yläraja	—	pakarit
—	oikea yläraja	—	vasen alaraaja
—	rinta	—	oikea alaraaja

3. Rastita allaolevaan kuvaan ne kehon osat, joissa on kipua.

